Call for tender for the presentation of intervention proposals for the Creation of Enlarged Partnerships extended to Universities, Research Centres, Enterprises and funding basic research projects to be funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 funded from the European Union - NextGenerationEU.

Annex 1 - Project proposal
(Article 10, paragraph 3 and Article 12 of the Call)

(This attachment must be completed and digitally signed by the legal representative of the proposing entity)

HEAL ITALIA

H*ealth* ***E****xtended* ***AL****liance*
for
I*nnovative* ***T****herapies,* ***A****dvanced* ***L****ab-research, and* ***I****ntegrated* ***A****pproaches*
of Precision Medicine

SECTION A

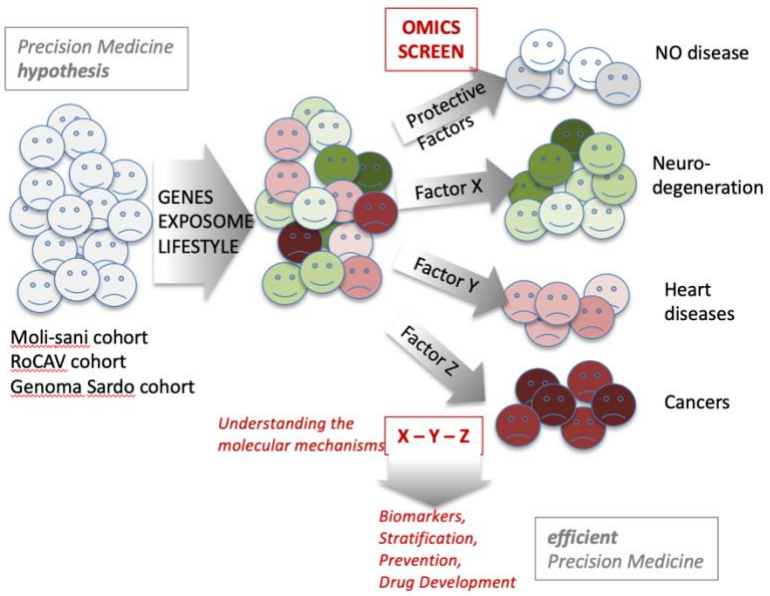
SCIENTIFIC QUALITY

A. SCIENTIFIC QUALITY

The **HEAL ITALIA** partnership aims at creating a **H**ealth **E**xtended **AL**liance for **I**nnovative **T**herapies, **A**dvanced **L**ab-research, and **I**ntegrated **A**pproaches of Precision Medicine. HEAL ITALIA is based on a multidisciplinary network of laboratories, clinical research centers and enterprises, sharing knowledge and technologies to reach results with timeliness, to increase the quality of health services to ultimately carry our NHS into a contemporary era of Precision Medicine. In this context, the HEAL ITALIA program is designed with a holistic interdisciplinary vision, joining together fundamental and translational research with technology transfer, taking advantage of the capacities of major players from the academic, clinical and private sectors. By a **One Health** approach, HEAL ITALIA will empower research teams and infrastructures to face the challenge in identifying new phenotypes, analyzing the complexity of a wide spectrum of environmental, lifestyle and genotypic determinants of multigenic/multifactorial diseases, thus responding to the health needs of more vulnerable populations.

Research Needs, Challenges, and Strategy

The definition of the factors for the development and progression of distinct diseases in individual patients is allowing Precision Medicine to progressively reaching a variety of clinical settings combining precision diagnostics with targeted therapeutics. This represents a challenge for the rapid evolution of the technologies in omic diagnostics and for the progressive introduction into the market of more targeted therapeutics. There is therefore a strong need to control/govern this tumultuous evolution enabling a patient-oriented omic, artificial intelligence (AI) and virtual reality approach combining more performing data management with the introduction of faster and reliable preclinical models capable of validating the identified prognostic and predictive markers to then generate novel diagnostic and therapeutic tools having a broader impact for patients. Applied to all medical disciplines, this approach is empowering NHS for safer and more effective individual approaches generating areas, for example, like precision surgery and precision radiotherapy. HEAL ITALIA wants to consolidate and innovate results achieved in oncology and govern additional emerging frames fostering fundamental research generating approaches of prevention, screening, risk stratification, early diagnosis and precision therapies tailored for newly defined disease phenotypes. The **originality** of the project relies on the analysis of prospective observational cohort studies (started since 2005) from **large normal healthy population** slowly progressing towards distinct diseases, to perform a large multi-omics screening to identify **factors** relevant for the progression, or protection, towards specific diseases. Understanding the underlying molecular mechanisms of these factors will allow their use as biomarkers, patient's stratification, preventive approaches and will constitute the bases for innovative drug development and therefore therapeutic intervention (see the scheme below). To accomplish this vision, HEAL ITALIA has been ambitiously organized into **8 biomedical research thematic spokes** generating a workflow from data acquisition and model development to precision diagnostics, innovative therapeutics and prevention strategies, to ultimately generate a clinical exploitation based on new devices and technologies. Strongly inter-related spokes are driving their deliverables towards different conditions, such as cancer, cardiovascular, metabolic and rare diseases, with the long-term vision of fulfilling the right of each person to homogeneously receive effective, tailored and sustainable healthcare services in respect of privacy and data protection for the benefit of the whole community.



Background

Complex diseases, including monogenic (rare diseases), polygenic pathologies (cardiovascular and metabolic disease), and cancer are the leading causes of mortality in the world. Despite the intense effort to address these diseases and the unceasing development of innovative treatment strategies, their incidence and related mortality rate are estimated to increase in the coming decades. The complexity, and the etiopathogenetic and prognostic heterogeneity of the pathologies makes the application of precision medicine imperative, addressing individualized pathways for diagnosis and treatment, to achieve an optimal clinical outcome. The existence of a causal link between rare metabolic disorders, metabolic syndrome or its components with cardiovascular and cancer development and their-related mortality is supported by a large body of evidence. Indeed, a huge number of studies evidenced how overweight and obesity induce in the organism a condition of chronic inflammation that, as a risk factor, is an imbedded mechanism favoring the developed of cardiovascular diseases, a pro-coagulation state, atherosclerosis, metabolic syndrome, insulin resistance, cancer or diabetes mellitus.

Therefore, these diseases need to be addressed as systems, by understanding the associated non-modifiable and modifiable risk factors (age, sex, behavioral, lifestyle, socioeconomic, occupational) and the basic etio-pathogenic mechanisms (genetics, epigenetics, signalling, tumour microenvironment, and immune factors), and integrating this knowledge actively. To provide an example: obesity increases the risk of several other diseases. This becomes significantly more worrying when looking at the profound changes in the lifestyles and eating habits of our populations, that, associated with the increasingly widespread sedentary lifestyle and consequent reduced energy requirements, have caused a radical change in the epidemiological scenario of these pathologies. The results of such changes have caused a significant increase in the incidence rate of chronic non-communicable diseases, especially in urban areas, with a dramatic increase in the percentage of overweight and obese individuals, especially in childhood and adolescence.

The cell pathways involved in maintaining stem cell homeostasis and in the proper performance of morphogenetic programs are often deregulated in both polygenic and cancer diseases. Indeed, in spite of the remarkable diagnostic and therapeutic progress achieved in recent years, mortality in cardiovascular, metabolic, neoplastic and rare diseases remains high due to the inability of an early precise diagnosis and current therapies to prevent the emergence of resistant cell clones. Therefore, given the correlation between metabolic and rare disorders and the increasing mortality caused by cancer and cardiovascular diseases, it is necessary to study the molecular mechanisms that link these diseases together, identifying potentially prognostic and pathogenic disease biomarkers to develop innovative strategies for personalized measure of prevention, prediction, diagnosis, monitoring and for precision therapeutic planning, for individuals at risk of developing and affected by these disorders.

Our project network is built to identify and reduce inequalities (extremely wide between North and South Italy) by developing a roadmap of cross-regional collaboration to define evidence-based pathways that are readily usable for clinical practice. The present project offers a great opportunity to undertake the development of a specific procedural framework for the generation, integration, analysis, and evaluation of evidence for the implementation of Precision Medicine technologies within the health service, able to both adequately manage the peculiarities, barriers, and challenges of Precision Medicine technologies and support their rapid introduction in the healthcare practice.

Objectives, Activities and Outcome/Products

The overall objective of the project is to deliver new, cost-effective, and evidence-based predictive and non-invasive diagnostic pathways for faster, earlier, more precise, accessible, and affordable prediction, detection, and monitoring of monogenic (rare diseases), polygenic (cardiovascular and metabolic) disorders, and cancer, as well as to identify innovative and effective therapeutic approaches. The project will allow to apply precision medicine approaches by developing *risk-based stratification algorithms*, and to provide scientific open-access evidence to health policy makers, thus *overcoming the concept of "one gene, one disease, one drug"*.

HEAL ITALIA will take advantage of two existing population cohorts started since 2005, expanded by a third cohort, to follow the progression of normal healthy people towards distinct diseases. Here, we will apply multi-omics screening strategy and analysis to identify factors relevant for disease progression. Samples will be collected, analyzed according to the adjacent scheme in order to produce compelling data that will allow the development of modern competitive Precision Medicine.

The project has the fundamental commitment of building permanent thematic networks functional to research in the field of precision medicine:

- A nationally controlled system of biobanks capable of homogeneously processing the material and dataset including diagnostic imaging collection according to standardized protocols and allowing the realization of prospective studies for the identification of prognostic biomarkers, off-label drug

screening and prevention of the risk of pathologies. Within the system, omics technologies will be developed such as metabolomics, lipidomics, proteomics which, together with the application of systems biology, will allow the definition of prognostic and therapeutic profiles for precision medicine;
- A competitive and open access to high quality Cell Factory and Preclinical Research Infrastructures to support and benchmark the quality of the activities of scientists focused on precision medicine;
- Construction of a platform with a common computer language that can facilitate clinical practice access to the genetic information obtained in the context of characterizing the profile of individual patients.
- Realization process of transferring the results stemming from scientific and technological translational research to marketplaces.

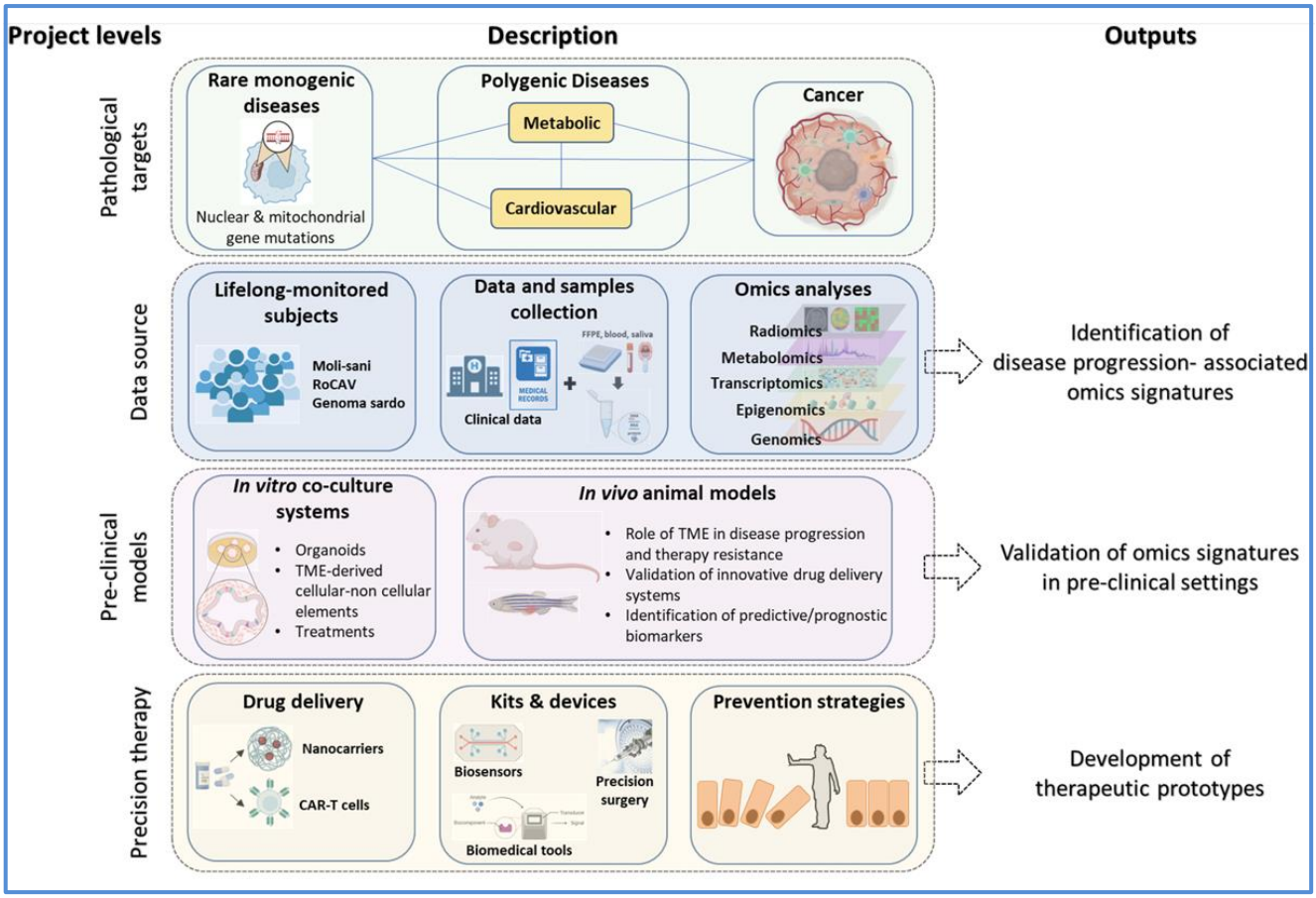
These networks will last and therefore, at the end of the three-year investment period, the outcomes of the HEAL ITALIA program will impact society in all sectors (academic, clinical, enterprises, national health system) involved in its realization leading to the above system networks.

The specific arms of the project are articulated into distinct spokes, highly inter-related between each other both at technical and translational level, all pointing to all diseases investigated, as depicted in cartoon below:

1. **Holistic Nosology**. *From patients to molecules and back:* mapping the omic landscape of clinical to molecular environment, to identify, classify, and refine the phenotypes of multifactorial diseases;
2. **Intelligent Health.** *Health Data Science:* Data management and development of advanced methods, algorithms, and machine learning approaches integrating health big data;
3. **Prediction models**: Advanced prediction models for prognosis and therapeutic response based on comprehensive data treatment;
4. **4D Precision Diagnostics.** Precision medicine integrating clinical and imaging biomarkers for a "*precise in space and time*" diagnosis;
5. **Next-Gen Therapeutics.** *From silico to bedside:* design and validation of innovative tailored and personalized therapeutic strategies;
6. **Healthy Toolbox:** Development of innovative devices for precision diagnosis and personalized therapy;
7. **Prevention Strategies:** Integrated and gender medicine approaches for prevention strategies based on environmental, lifestyle and clinical biometric data;
8. **Clinical Exploitation:** Clinical validation and implementation of innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches, based on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols.

Rationale, Methodology and Outcome

Multi-omics (genomics, proteomics and metabolomics) will be applied for the **molecular characterization** of an already available large population cohort of healthy subjects (**The Moli-sani study**, www.Moli-sani.org) collected since 2005. The cohort includes 24,325 participants, aged ≥ 35 from the general population by probability sampling in Southern Italy. A number of exposures have been measured with standardized and validated methods and 15 years follow-up made available information on incident cases on mortality and major chronic disease (cardiovascular, neurodegenerative disease, cancer). Radiomic and exposome variables have also been collected. A biobank in liquid nitrogen stores over 1 million of biological samples (serum, plasma, buffy coats, DNA, urine) immediately available to perform "omic" studies. Results from Moli-sani study could be extended to and validated in the second cohort, **the RoCAV study** (http://epimed.uninsubria.eu/) a population cohort of about 4,000 samples collected in Northern Italy since 2013 by the EPIMED center of the Insubria University of Varese, with methods comparable with the Moli-sani study. A follow -up on mortality, a major chronic disease and a biobank of biological samples are also available. A third cohort, **The Genoma Sardo study**, will further expand the database of the normal population. The strength of our proposal derives from the availability of shareable clinical and instrumental data and biological samples of populations, with related **Bio-Banks**, followed for years and/or cohorts of patients affected by complex polygenic pathologies (diabetes, metabolic syndrome), monogenic/rare or cancer followed by national and international scientific networks composed by medical, computer, statistician and data analysts' scientists that are an integral part of the project.



The main technological derivable of the project will be the platform that will allow to analyze, integrate and interpret the integrated diagnostic data, including genetic risk markers, liquid biopsy derived molecular biomarkers, imaging biomarkers, digital pathology tools, and computational algorithms (**AI-empowered and network medicine**). This will be achieved through the creation of a multisectoral network that can allow to reach a critical mass of complementary skills, of adequate patient cohort size and to share advanced technological resources in an efficient and cost-effective way. Data will be shared while maintaining absolute respect for privacy and confidentiality of data at the level of individual care units through the adoption of the "*Swarm Learning*" that uses blockchain to decentralize machine learning-based systems. This will allow the creation of an infrastructure capable not only of maintaining the databases at maximum security, but also of providing a very high level of redundancy, even in the event of failure of an entire infrastructure site. This unveils to create a database of actionable mutations e/or alterations in computational tools based on scientific knowledge, in order to: i) guide therapeutic choices based on the patient's mutational status (prescription database), ii) accelerate the enrollment based on genomic, proteomic and metabolomic profiles of patients in clinical trials, iii) validate the clinical utility of the re-defining classification of patients to prospectively enroll them in cohorts.

The consortium, through advanced computation approaches, will develop Machine Learning **models for prediction of disease initiation and progression**: *lab-on-chip and organ-on-chip*. Simulating mutations on cell structures to organelle morphologies (3D approaches with multicellular spheroids), flash irradiation and specific target molecules will be investigated at the single cell level. Micro-patterned solid-liquid interfaces will be developed as artificial niches of single cells for high-throughput drug screening and rare cells capture devices from small volume biological samples and will be integrated in microfluidic systems. Complex functional (pre-programmed or adaptative) biological structures will be fabricated by integration of stimuli responsive biomaterials with cells and 3D bioprinting. For instance, the **optimization of an experimental model** of highly aggressive tumors would give to the whole partnership the opportunity to learn about the molecular mechanisms underlying resistance to standard therapies on a large cohort of individuals affected by these pathologies.

Along with the project, blood and saliva as an alternative matrix, will be subjected to analyses aimed at defining the state of the pathways involved in the processes of proliferation, self-renewal and survival. Whilst the classic liquid biopsy has limitations deriving from the amount of genetic material needed, the study plans to design screening tests based on the evaluation of the methylation status of genes and, at single cell level, a biological mechanism that identifies when and where certain genomic instructions are carried out in the body and which evaluates some hotspots, called CpG sites, as indicators of the state. of methylation. Integrating histological and omics data with the parameters extracted from a large number of features from medical images using data-characterization algorithms (Radiomics) will pave the way for designing highly **personalized diagnostic and therapeutic, radiotherapeutic and pharmacological paths**.

The project will also move towards **newly tailored therapies**: ***i)*** Re-enabling correct functioning of genetic cascade from transcription to translation and correct functioning; ***ii)*** Optimizing immunotherapy and CAR T strategies; ***iii)*** Re-educating the microbiome to ameliorate therapeutic responses; ***iv)*** Identifying of new therapeutic targets by screening and drug repositioning.

Innovative **devices** for Precision Medicine will be developed along two main directions: diagnostics or therapy. The "devices' category will be declined in a broad sense, encompassing technologies that range from nanoscale systems for targeted drug delivery to miniaturized biosensors, from robots for precision surgery to innovative radiotherapy hardware. With respect to the biosensors, they will be designed to operate with electrochemical or optoelectronic transduction and will be implemented by advanced materials and interfaces and also under the form of point-of-care assays and even wearable devices. Systems will be prototyped with integrated microfluidics and/or new readout electronics. We will develop a large portfolio of biosensing platforms, to target biomarkers that range in size from nucleic acids and proteins to extracellular vesicles and whole cells. Our vision is to facilitate the transition towards cost- and time-effective, ultrasensitive sensing tools for enabling both early detection and frequent screening of the patients, two of the cornerstones of Precision Medicine approaches. As for devices devoted to **therapy**, we will work along three lines. The *first* set of devices will be devoted to therapy via **precision surgery**: to this end, we will develop advanced imaging materials and instrumentation, as well as both software and hardware solutions for robotics, all in view of more efficient and patient-centered minimally invasive surgery. A *second* target will be materials and instrumentation for **precision therapy**: on one hand, we will develop new scaffolds, implants and *nanostructures for regenerative medicine*; on the other, we will focus our efforts on fabrication and validation of prototypes for *flash radiotherapy*, a novel revolutionary technique for cancer treatment. The *last* set of devices for therapeutics will feature *nanosized materials*: spanning the whole chain from rational design to synthesis, characterization and validation in preclinical models, we will develop both smart drug-delivery systems and stimuli-responsive nanotherapy agents to target pathologies that range from cancer to rare diseases. Besides state-of-the-art methods for assessing the efficacy of these nanodrugs, we will develop ad hoc technological solutions for monitoring the response to therapy in cell and animal models through organic bioelectronics, exploiting the extensive know-how possessed by several of the applicants, who belong to the Heal Italia consortium. Development of innovative devices for precision diagnosis and personalized therapy will modify disease and patient-specific outcomes. The consortium will identify a synthetic method for obtaining biocompatible CD-NT with paramagnetic properties, with red and NIR emission and with magnetic resonance contrast properties sensitive to the tumor microenvironment (pH, temperature, reducing environment): 1) Optimizing of large-scale CD-NT multicomponent nanosystems and implementation of contrast properties in MRI by doping with gadolinium complexes; 2) Development of nanosystems such as: i) molecular thermometers in MRI and IF for the measurement of the cell temperature in the tumor site during photothermal treatment and ii) for the measurement of the pH of the tumor microenvironment by means of MRI and IF for monitoring and personalization of anticancer therapy.

Omics markers with machine learning algorithms, will potentiate the predictive value of already available polygenic scores for disease risk assessment. This may lead to personalized prevention, where primary prevention strategies for major chronic diseases will be specifically tailored on each individual subject. The approach has as its cornerstone the recent advances in omics sciences, molecular biology, and bioinformatics that support the evaluation and treatment of disorders, focusing on four main 4P principles: prediction, prevention, personalization, participation. Genomic predictive tests, genetic screening may be the basis to develop large-scale prevention programs tailored to individual characteristics versus the unique environmental exposure of each person. Given the network of highly qualified clinical research units, rapid transfer of results to the **clinical practice** is expected. Particular attention will be given to the innovative therapeutic approaches that will be obtained from the scientific experimentation of the research program. The development of bio-molecular research projects points to early diagnosis, treatment and follow up of rare and metabolic disorders, cardiovascular disease and cancer. The information obtained from the strategies faced by the consortium will

lead to the use of databases accessible for all Italian laboratories to optimize the insertion of patients with similar mutational or alteration profiles into specific experimental treatment protocols. Animal toxicology and GMP production is now ready for the first-in-human phase I clinical testing trial named **ORIENTATE**: tail**O**red d**R**ug repurpos**I**ng of d**E**citabine in KRAS-depende**N**t refrac**T**ory p**A**ncrea**T**ic canc**E**r. Therefore, the implementation of the project will enable the creation of a national working group, coordinated by reference experts, which will interface with the national collaborative groups in order to program clinical research and optimize the use of precision medicine for each individual pathology. Attention is also dedicated to **drug repurposing** for complex or rare diseases and faster phase III **clinical trials**.

The strengths of proposal are to have all the tools to successfully tackle the issue, including population data, patient cohorts and all the experimental and computational approaches required to discover effective non-invasive monitoring tools, from disease early onset to disease progression status. Integrating clinical, imaging and multi-omics we aim to develop precision diagnostics and therapeutic strategies, supported by computational medicine. Considering the "know-how", skills and facilities of the academic and institutional partners involved in the project, we aim to provide a substantial contribution to the improvement of scientific knowledge. This will result in generating technological innovations that, translated and marketed, will be an important competitive edge, from an industrial point of view. The comprehensive vision of the project and the involved structures implies a synergy of research laboratories and hospital/care institutions for the characterization and the development of innovative diagnostic and therapeutic tools. To choose the partner network, particular attention has been given to those institutions (industries and hospitals) that will benefit from the mutual scientific exchange with high-level scientific research laboratories. The entire project implies a continuous interchange of human resources among the high scientific institutions involved. The scientific expertise of the participants will enable an effective multidisciplinary approach to achieve the goals of the work plan. The initiative is consistent with the medium-term development strategies of the Proponent Institutions, which include the commercial inclusion of their innovative products in the anti-cancer drugs sectors that companies have long been pursuing through research activity in different directions. Business activities are widely documented in scientific and patent literature. This initiative will strengthen the research capabilities of proposers as it is foreseen the creation of new research units for research in the areas of investigation explored by the project. Compared with Community programming and in line with the **"Health" theme**, the project aims to improve the health of citizens of the European Union. Specifically, we intend to enhance the development of research aimed at studying the activities of molecules with potential therapeutic applications in the field of precision medicine. The partnership network, formed by companies and research centers, will help to enhance the competitiveness of healthcare companies by strengthening the research field that can produce new therapeutic solutions. The activities and achievements of this proposal, aimed at acquiring new knowledge, are also useful for the development of new processes and new products. The intervention also achieves an excellent **synergy between public and private** actors specialized in health science research, succeeding in making the project an adequate scale and a positive impact on all the expected productive sectors and capable of encouraging the attraction of foreign-source investments. Within this context, a standardized **health technology assessment** approach will guide the introduction of such technologies in clinical practice.

Capacities of the partnership

All Institution involved are of the highest quality, with strong collaboration between them as well as at international level, demonstrating clear scientific and managerial skills of each participant also in terms of innovation and technology. An **International Scientific Advisory Board** will supervise the work that will be exposed at annual **International Meetings**. Finally, education and teaching will be at PhD, post-doc and Publishing (www.nature.com/cdd) level. HEAL ITALIA has a strong intersectoral structure where entities from both the **private** and **clinical research sectors** are affiliated to the thematic spokes established at entities from the **academic sector**. Details of Spoke/affiliate's capacities in terms of management of research, innovation and technology transfer can be found below.

UNIVERSITÀ DEGLI STUDI DI PALERMO – Proposer, Hub founder & Spoke 3 Leader
(Short Name: UNIPA)

General information

The University of Palermo, officially founded in 1806, is at present an internationally acknowledged public research organization which covers almost all main fields of study, fostering an interdisciplinary approach.The University welcomes on average on a yearly basis about 500 enrolled foreign students and 351 incoming ERASMUS students and is active in 186 international agreements for interuniversity cooperation. In 2012 the University of Palermo has been acknowledged by the European Commission among the institutions which respect the principles of the European Charter and Code for Researchers and has been awarded the HR Excellence in Research logo. The University of Palermo can rely, too, on a large research infrastructure called 'ATeN – Advanced Technologies Network Center', which is regionally acknowledged and is part of the 2021-2027 PNIR – National Plan of Research Infrastructures. ATeN Center is one of the few R&D centers in Europe in the field of Biotechnology applied to human health to offer laboratories that range from the synthesis of materials to in vivo tests. Equipped with over one hundred items of scientific equipment spread over 3,000 square meters, it is a competence centre attracting new project ideas and technology transfer activities for researchers and companies in the Mediterranean basin. The University has recently set up its 'Centre for Sustainability and Ecological Transition', led by a Scientific Committee of professors and researchers with acknowledged expertise in the 17 SDGs of UN 2030 Agenda. The centre will develop an ongoing dialogue with the stakeholders through a Regional Forum in order to find out innovative and sustainable solutions and mitigate energy and environmental impacts connected with productive processes, coherently with EU Green Deal policy.

National and international project management skills

As regards funding for research and innovation projects, the University of Palermo is quite active in the management and implementation of projects funded under the Structural Funds, both at national and regional level (R&I National Operative Plan, Business and Competitiveness National Operative Plan, ERDF Regional Operative Plan, European Territorial Cooperation programmes); within the 2007-2013 and 2014-2020 programming periods, more than **242 projects** have been funded for an overall amount of over **156 million euro**. Indeed, the University of Palermo has also significantly improved its performance in getting directly managed European funding in the 2014-2020 programming period: In the framework of Horizon 2020 programme, 48 projects have been funded for an overall amount of 17 million euro (being ranked 26° among the Italian Universities for funding achievements), as compared to 33 funded projects in the 7th Framework Programme for R&D. Other funds have been obtained from the Italian Ministry for University and Research, particularly within PRIN – Projects of National relevance Calls for Proposals, as well as from other public institutions and private foundations.

U.E Funds

- HORIZON 2020: **n. 48** projects (**4 of which as coordinator**), for a total amount of **€ 16.638.358,36** and a success rate of 10,34%
- Joint Undertakings (Ecsel, IMI2, SESAR): **n. 6** projects, for a total amount of **€ 1.927.310,73**
- Other EU-funded projects (3HP, ISFP, JUST, REC, LIFE): **nr. 12** for a total amount of **€ 1.399.769,56**
- Joint initiatives (ERA-NET, JPI, PRIMA): **nr. 6** projects, for a total amount of **€ 307.285,36**

National Funds

National research projects: **nr. 24** PRIN 2015 projects for a total amount of **€ 1.478.568,00** (**5 funded projects as coordinator**) and **nr. 61** PRIN 2017 projects for a total amount of **€ 7.889.111,00** (**13 funded projects as coordinator**), **nr. 13** research projects funded by other Ministries for a total amount of **€ 1.171.373,91**

European Structural Funds:

- **nr. 19** national projects funded by the University and Research Ministry (PON RI 2014-2020 – Avviso 1735/2017) for a total amount of **€ 8.956.873,04**
- **nr. 8** national projects funded by the Economic Development Ministry (PON I&C 2014-2020) for a total amount of € **4.089.659,37**
- **nr. 29** regional projects (PO FESR Sicilia 2014-2020) for a total amount of **€ 17.705.762,12**
- **nr. 11** projects funded under the regional and national Rural Development Plan for a total amount of € 1.544.722,05 and 5 regional projects (PO FEAMP Sicilia) for a total amount of **€ 537.035,50**
- **nr. 22** Territorial and cross-border cooperation projects (ENI CBC MED, Italie-Tunisie, Italy-Malta, Interreg Med) for a total amount of **€ 6.908.793,73**
- **nr. 15** projects financially supported by Foundations for a total amount of **€ 1.278.665,72**

Projects funded in the health sector:

- Horizon 2020/JU/JPI: **nr. 9** projects, total amount **€ 2.061.533,70**
- National structural funds 2014-2020: **nr. 5** projects, total amount **€ 1.536.071,17**
- Regional structural funds: **nr. 7** projects, total amount **€ 4.365.104,30**
- PRIN 2017: **nr.11** projects, total amount **€ 1.548.121,00**
- National structural funds 2007-2013: **nr.7** projects**,** total amount **€ 34.084.719,88**
- Territorial and cross-border cooperation projects: **nr.2** projects (as Coordinator) total amount **€ 1.570.982,68**
- Other funding sources (Foundations, Associations, PNR-FISR): **nr. 16** national projects, total amount **€ 1.324.280,86, nr**.**1** AAL-funded project, art.169 EU treaty, for an amount of **€ 266.042,00**

Technology transfer capacity - patents - spin-off

The scientific outputs registered in the institutional repository on December 31, 2021, were 115.601 (81,37% with international relevance), nr. 816 publications on Q1 ranked journals (45,92%), nr. 413 outputs assessed in A level journals (22,46%), nr. 795 publications on scientific journals (42,33%). 134 patents have been granted from 2004 to 2021. 35 spin-off companies have been started up and officially acknowledged till 2021. The University of Palermo have all the professional expertise necessary to achieve the aims proposed along the project. In particular, the University of Palermo was among the first to isolate stem cells from colon and thyroid cancer which is, a branch of biomedical research, which in the last 15 years received particular interest in the scientific-academic world. The general research activity of the core unit involved in the project brought to light the mechanisms involved in the regulation of cancer cell survival and resistance to conventional therapies identifying a plethora of biomarkers of therapeutic response. These results contributed in a fundamental manner to oncological research consenting the core unit to formulate patents that contributed to the development of new precise neoadjuvant cancer therapies for the treatment of colon, breast and thyroid tumors. UniPA research groups published a series of articles in the field of precision medicine in top scientific journals such as Cell Stem Cell, Nature Communications, Gastroenterology, Gut and PNAS. These publications, which are particularly innovative and original, have had recognition on an international level. Furthermore, the University of Palermo stood out in the oxadiazole derivatives, as medicaments in the treatment of diseases associated with the presence of a nonsense mutation in the gene or a premature stop codon in the mRNA. Some University's facilities offers cutting-edge next generation sequencing technology in order to obtain high quality data and analyses. The obtained information can be further processed using bioinformatics tools (SNP discovery, detection of structural variants, genome-wide measurement of mRNA transcripts levels). UNIPA has enforced a sound collaboration network at regional, national and international level in the past 10 years, implementing a I3 approach (international, interdisciplinary and intersectoral). It participates in 68 public-controlled bodies with private legal status (8 Research consortia, 21 National Interuniversity consortia, with equity participation, 19 Associations, 14 Foundations, 6 Limited liability consortium companies, i.e. technology districts). It counts 175 international interuniversity agreements for cultural and scientific collaboration. It is member of 21 CIR Interdepartmental and Interuniversity Research Centres.

Participation in national and international neworks

UNIPA is member of the following networks and associations:

- APEnet - Rete degli Atenei e degli Enti di Ricerca per il Public Engagement
- RUS - Rete delle Università sostenibili
- UNIMED -Unione delle Università del Mediterraneo
- EUA European University Association
- SDSN Mediterranean branch - United Nations Sustainable Development Solutions Network
- APRE – Agenzia per la Promozione della Ricerca Europe
- CRUI Conferenza dei Rettori delle Università Italiane
- CUIA Consorzio Universitario Italiano per l'Argentina
- NETVAL - Network per la Valorizzazione della Ricerca
- PNI Cube – Associazione Italiana degli Incubatori Universitari e delle Business Plans Competitions
- FORTHEM Alliance – European University Alliance

UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA" - Hub founder & Spoke 1 Leader
Short Name TOR VERGATA

General information

Tor Vergata University of Rome is a non-economic, public university, governed by Public Law, and the Rector is the Legal representative of the University. The Rector appoints a Prorector (Prorettore vicario) who acts as his substitute in case of absence or impossibility to exercise his functions. The other Central bodies involved in the decision-making processes are the Academic Senate, the Board of Directors, the Board of Auditors, the Evaluation board and The General Director.
The mission of Tor Vergata University is to provide higher education, research and third mission activities to domestic and international stakeholders. The Central Governing Bodies, the "Presidio di Qualità" and the "Nucleo di Valutazione" oversee and implement quality Management at the university. http://pqa.uniroma2.it/normativa-e-documentazione-di-riferimento/documentazione-ateneo/.
The University of Rome Tor Vergata recognizes the value that diversity brings and so the University aims at recruiting, developing, retaining and motivate an increasingly diverse workforce. The gender equality plan is available here while the gender balance is displayed here. Main departments involved are: 1) *Department of Experimental Medicine* has several research lines covering different biological and medical aspects: cell death apoptosis, structural biology, stem cells, viral oncogenesis, antiviral agents and drug resistance, chronic infections, microbiome analysis. In the department there is also the Torvergata Oncoscience Center, a centre of excellence in cancer research; 2) *Department of Biology* has developed a multidisciplinary platform to address the key issues at the forefront of modern biology: from molecular mechanisms at the basis of the immune system in response to infections and on mechanisms of inflammation and in the regulation of the processes of tumorigenesis, apoptosis and autophagy. The Department has different facilities including the Confocal Microscopy Center; 3) *Department of Systems Medicine* promotes the integration between advancement of knowledge and excellence in the treatment of chronic-degenerative diseases, in particular the interaction between diseases (medicine of complexity) and therapeutic approaches (polytherapy); 4) *Department of Biomedicine and Prevention* has the mission to study new methods and prevention procedures through biomedical research and their effective implementation in the National Health Service; 5) *Department of Chemical Science and Technology* is highly interdisciplinary and its research covers most aspects of modern chemistry and its applications, including design of innovative drugs, study of mechanisms of action and structure-activity relationships of bioactive compounds; 6) *Department of Enterprise Engineering* carries out internationally leading research across a range of areas in business engineering. The research streams span the theoretical and applied, often crossing traditional discipline boundaries, entailing collaborations between research groups, as well as with other departments at Tor Vergata University and external academic, institutional and commercial partners.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine".
In ***spoke 1*** researchers from departments of Experimental Medicine, Systems Medicine, Biology, Biomedicine and Prevention and Chemical Science and Technologies (mean H-index 52, mean Scopus citations 17097, mean number of publications in the last 10 years 118) will focus on mapping the omic landscape of the clinical and molecular environment of the emergence of the disease, to identify, classify, and refine the phenotypes of multifactorial diseases. In ***spoke 2*** researchers from departments of Biomedicine and Prevention and Enterprise Engineering (mean H-index 25.5, mean Scopus citations 3211, mean number of publications in the last 10 years 118) will focus on the development of advanced algorithms and machine learning approaches integrating electronic health records (EHR) with imaging and pre-clinically validated high-throughput data to develop and deploy a dedicated digital inter-operable platform for both data, algorithms and models sharing among the partners of the Consortium. In ***spoke 3*** researchers from Experimental Medicine (mean H-index 54, mean Scopus citations 17683, mean number of publications in the last 10 years 130) will focus on the development of advanced prediction models for prognosis and therapeutic response based on comprehensive data treatment. In ***spoke 8*** researchers from the Department of Biomedicine and Prevention and Systems Medicine (mean H-index: 35.75, mean Scopus citations: 5016, mean number of publications in the last 10 years: 105) will contribute to Clinical validation and implementation of innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches, based on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols.

Key elements/strengths

UTOV has a strong experience in managing and supervising research projects. UTOV has managed a portfolio of more than 245 projects financed by EU programs (FP7, H2020) and other public and private funding organizations. UTOV is also involved in several international network such as EUA, UNICA, YERUN VIU. 5 JU (FCH Fitup, LOLIPEM, ECSEL, M2O, Harmony) and in 1 KIC (HEInnovate). Scientists from the *Departments of Experimental Medicine, Biology, Systems Medicine, Biomedicine and Prevention, Chemical Science and Technologies and Enterprise Engineering* have been involved in several collaborative projects including *HORIZON 2020 Eurobench,* to develop a benchmarking solution for specific scenarios including one or more outcomes of testbed, software routines and/or experimental datasets. *HORIZON 2020 COGIMON*, dealing with the problem of interaction requiring active and adaptive regulation of motion and behavior of both the human and the robot and involves whole-body variable impedance actuation, adaptability, prediction, and flexibility. *AMARSI FP7-ICT* aiming at a qualitative jump toward biological richness of robotic motor skills. *MINDWALKER FP7-ICT* combining physiological, clinical and robotic expertise to develop an integrated Brain-Machine system which undergoes a clinical evaluation process with spinal-cord injured patients. *FP7-FLORINASH* dealing with the effects of gut microbiome on metabolic disorders along aging (see Nat Med. 2018 Jul;24(7):1070-1080); *FP7-EURHYTHDIA* studying Chronotherapeutic lifestyle intervention for diabetes and obesity to reset the circadian rhythm and improve cardiometabolic risk in the European working population. *IMI2 SOPHIA* studying new approaches to Stratification of obese phenotypes to optimize future obesity therapy. *Long Live the Elderly!* to scale a Community-based pro-Active Monitoring Program (CAMP) to mitigate the impact of frailty on citizens' quality of life and use of care services. *VIGOUR*, to support care authorities in progressing the transformation of their health and care systems and to provide sustainable models for integrated care. *TENDER H2020 AFFECTIVE BASED INTEGRATED CARE FOR BETTER QUALITY OF LIFE*, dealing with NGF system and its interplay with endocannabinoid signaling, from peripheral sensory terminals to the brain.

At national level, research collaborations are carried out across actions launched by the Ministry of University and Research (MUR), such as Italian National Plan for Research (PRIN). For the promotion of industrial innovation and the enhancement of patents, the University collaborates with the MISE, while for the implementation of projects aimed at achieving results of effective interest for regional companies, the main organization is the Lazio Region. As regards the implementation of the priority research development objectives in strategic sectors such as the study of drugs, rare diseases and oncological pathologies, health protection, the reduction of accidents, major collaborations can be referred, for example, to AIFA, Ministry of Health, INAIL, Telethon and AIRC. Other research agreements are related to space and aerospace fields: ASI (Italian Space agency) in the fields of engineering, biomedicine and space applications. Collaborations with ENEA also focus on issues of common interest, ranging from energy efficiency to technologies for cultural heritage, earthquake protection, food safety and climate change. Scientists from the *Departments of Experimental Medicine, Biology, Systems Medicine, Biomedicine and Prevention, Chemical Science and Technologies and Enterprise Engineering* are involved in international collaboration with several top-level institutions and research centers, such as Imperial College London, Inserm, The International Progressive Multiple Sclerosis Alliance, The MOXFO Initiative.

ALMA MATER STUDIORUM –UNIVERSITÀ DI BOLOGNA - Hub founder & Spoke 2 Leader
Short name UNIBO

General information

Alma Mater Studiorum –Università di Bologna (UNIBO) is one of the largest and most active Italian universities in research and innovation. UNIBO is organized in a multi-campus structure (Bologna, Cesena, Forlì, Ravenna and Rimini), with 32 Departments and 5 Schools. It offers 243 Degree programmes (a.y. 2021/2022) of which 96 international ones, and 56 delivered in English and 48 PhD programmes (approx. 1819 candidates). The total number of enrolled students is 90.291. (A.Y. 2021/2022), of which 7.062 are international.. *Organization skills*
The UNIBO community is composed of 5882 permanent employees, of which 2917 professors and researchers, and 2965 staff

National and international project management skills

UNIBO is also very active in innovation and technology transfer, with 520 patented titles, 37 spinoffsand 12 start-ups,7 Interdepartmental Centres for Industrial Research (CIRI), manyagreements and collaborations with industry.The university is rooted in the local innovation ecosystem with a constant European and global perspective, taking part to the most important R&I networks at both national and EU level, both thematic and institutional ones (including UNAEuropa Allianceand TheGuild of Europeanresearch-intensive universities). It is committed to SDGs as well as to promoting ethics at all levels, through the adoption of policies on diversity (among which the GEP) and research integrity. The University of Bologna is in top positions at national level in all main relevant international rankings. For instance, in 2022 it is ranked 172nd in the Times Higher Education (THE)World University Rankings(20th in Impact Rankings and 126-150th in World Reputation Rankings).Research activities are carried out in Departments and Inter-departmental Centres, all staffed with research manager profiles.

Technology transfer capacity - patents - spin-off At central level, the Research Services Division (ARIC) oversees activities related to institutional research and competitive funding. The Industrial Relations, Third Mission and Communication Division (ARTEC) has developed a full range of services related to a structured strategy of collaboration with industry, knowledge transfer (including patent portfolio management), exploitation of research results, also through spin-off and start-ups, and related collaborations with the local, national and international innovation ecosystem.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine" In research, UNIBO is very active in all research domains, with more than 10.000 publications per yearand outstanding results in attracting research competitive funding at both European and national level.First in Italy, 14 Departments have been awarded as Departments of Excellence. At regional level, UNIBO accounts for more than 214 funded projects (33 M€).

Key elements/strengths

To develop and achieve the main aims of PE6, UNIBO exploits scientific skills provided by an interdisciplinary network and research experience from eleven departments: 1) Dept of Experimental, Diagnostic and Specialty Medicine (DIMES); 2) Dept of Medical and Surgical Sciences (DIMEC); 3) Dept ofElectrical, Electronic, and Information Engineering "Guglielmo Marconi" (DEI); 4) Dept of Statistical Sciences "Paolo Fortunati" (STAT); 5) Dept of Biomedical and Neuromotor Sciences (DIBINEM); 6) Dept of Pharmacy and Biotechnology (FABIT); 7) Dept of Computer Science and Engineering (DISI); 8) Dept of Industrial Engineering (DIN); 9) Dept of Chemistry "Giacomo Ciamician" (CHIM); 10) Dept for Life Quality Studies (QuVi); and 11) Dept of Physics and Astronomy "Augusto Righi" (DIFA). UNIBO expresses excellence at the international level in aspects relevant to the theme of Precision Medicine, including clinical and laboratory data management in digital format and their processing with in-silico medicine techniques. UNIBO has recently started a collaborative project with the three IRCCS of Bologna to realize a common IT infrastructure called AlmaHealthDB. This infrastructure will allow all participants to i) extract clinical data from primary hospital systems such as EHR, PACS, sequencing services, analysis laboratories, etc.; ii) integrate this data with research data collected during clinical trials; iii) secure management and pseudo-anonymization of data; iv) support the processing of these large collections of clinical data also with special computing resources (HPC); v) full compliance with current privacy legislation.AlmaHealthDB will also provide technical support for the implementation of local nodes for the national Health Big Data infrastructure project and is ready for integration with the EuropeanHealth Data Space (EHDS), including the possibility to provide data to the new Data Analytics and Real World Interrogation Network (DARWIN EU) of the European Medicines Agency (EMA).Furthermore, UNIBO can take advantage

of the presence of core facilitiesand specific wet laboratories for translational research.We have full access to the Center for Applied Biomedical Research (CRBA) of Bologna University https://centri.unibo.it/crba/en/instrumentation, where the following instrumentation is available: CELLSEARCH and DEPArray instrumentation (Menarini Silicon Biosystems); IncuCyte platform for live-cell analysis (Essen BioScience); NextSeq500 (Illumina) platform, IonS5 sequencing system (ThermoFisher).We also offer cutting-edge laboratoriesof Biomedical Engineering(Health 4.0 lab, BIOLAB and eDIMESlab)for bioengineering and AI based diagnostic.The IRCCS AOUBO/University of Bologna hosts more than 60 disease registries and numerous datasets for observational and clinical studies. In addition, the IRCCS AOUBO / University of Bologna hosts biological research collectionswith > 40000 samples from different sources (whole blood, plasma, serum, stools, tissue biopsies, organoids, cell lines, DNA, RNA, Proteins) spanning different medical fields, in particular: solid tumors (digestive cancers, skin tumors), hematologic tumors, rare cancers, gastrointestinal functional disorders, rare diseases, pediatric diseases, infectious diseases, dermatologic diseases. The total number of multicentre clinical trialsin the last five years is 346, with a total number of patients recruited in 2020 equal to 2.546. IRCCS AOUBO/University of Bologna is part of the Alliance Against Cancer network (ACC) and 12 European reference networks (ERN): adult rare solid tumors -EURACAN, rare lung diseases -ERN-LUNG, rare endocrine conditions -Endo-ERN , rare and undiagnosed skin disorders -ERN-Skin, rare congenital malformations and rare intellectual disabilities -ITHACA; -rare genetic syndromes at risk of cancer -ERN GENTURIS; Urogenital Diseases -ERN eUROGEN; rare kidney diseases -ERKNet; Rare Hepatological Diseases -ERN-RARE Liver; Rare Hematological Diseases -ERN-EuroBloodNet; rare and low prevalence heart disease -ERN Guard Heart; rare metabolic diseases -MetabERN.

Innovation and technology transfer.

UNIBO is very active also in technology transfer with 520-patented titles, 37 spinoffs and 12 startups, 8 Interdepartmental Centers for Industrial Research (CIRI), including Life Sciences and Health Technologies. In the Life Sciences & Medicine domain, UNIBO has developed several Patents: 11to Research, Clinical, Surgical Methods and Processes, 15to Therapeutics approaches,9to Diagnostics methods, 6to Sensors and Biomaterials, 3 topharmaceutical chemical development.

Research Division: with more than15 years' experience and about 50 people assisting the research groups in the whole project lifecycle, strictly cooperating with the Knowledge Transfer Office, for the Innovation Management, IP protection and exploitation, and the take up and commercialization of project results. Overall, in Horizon 2020, UNIBO has so far been involved in 356 funded projects (98 as coordinator) and 26 PI ERCwith more than 149 M€ of funding. In the framework of the Societal Challenge 1 –Health, Demographic Change & Wellbeing, the Innovative Medicine Initiative and related funding frameworks, UNIBO is participating in 31 projects (6 under IMI-2 JTI) with a total EU contribution of over 14 M€, becoming the 1stItalian University for H2020 SC1 participations, among them, several projects on precision medicine. Main Horizon 2020 projects related to PE6: PROPAG-AGEING(beneficiary);CARBALIVE(beneficiary); HARMONY(beneficiary);ORTHOUNION(beneficiary);PAPA-ARTIS(beneficiary);LIVERHOPE(beneficiary); TRANSMIT (coordinator); MCDS-Therapy; AFIBROTIC(coordinator); LightDyNAmics(beneficiary); PAIN-Net(beneficiary); SiNBioSys(coordinator);VOSTARS(beneficiary); FURTHER(beneficiary); MOBILISE-D(beneficiary); ConcePTION(beneficiary);ONCORELIEF(beneficiary);VEO(beneficiary);DISCOvERIE(beneficiary);DECISION(beneficiary); ISW (coordinator); GenoMed4All(beneficiary); ENLIGHTENme(coordinator); ESCAPE(beneficiary); RETENTION(beneficiary); HARMONY plus (beneficiary). In Horizon Europe, in the framework of the Cluster1 –Health, UNIBO has been involved in 3 funded projects (1 as coordinator) with more than 2 M€ of fundingincluding TRIGGER(coordinator); GOLIAT(beneficiary); PsychSTRATA(beneficiary).At national level, UNIBO is currently involved in about 200 PRIN projects, (30 M€ funding), of which 62 as national coordinator, and15 AIRC IG projects and 4 AIRC MFAG projects.

UNIBO joins International Networks and signs Cooperation Agreements with Universities from Countries across the world, to promote education and scientific cooperation and create mobility and exchange programmes for teachers, researchers and students.Unibo is member of relevant networks at European level, such as European Health Telematics Association (EHTEL), European Technology Platform for Nanomedicine, European Paediatric Translational Research Infrastructure (JRU EPTRI), Virtual Physiological Human Institute for Integrative Biomedical Research (VPH Institute).Strategic national, European and other international scientific collaborations have been developed by participating in national and European projects (see above), in addition to the following Research Infrastructures and Networks, namely: ELIXIR, BBMRI-ERIC, ECRIN–ERIC, EU-IBISBA, EPTRI, FNH-RI, VPHI (Virtual Physiological Human Institute).

UNIVERSITÀ DEGLI STUDI DI ROMA "LA SAPIENZA" - Hub founder & Spoke 4 Leader
Short name SAPIENZA

General information on the following key aspects

Sapienza Università di Roma (Magnifica Rettrice, Prof. Antonella Polimeni) – UNIROMA1

Brief presentation of your organization. UNIROMA is strongly involved in the frame of Precision Medicine with one department (Dept of Translational and Precision Medicine, N. 50 researchers) dedicated to the topic, an interdisciplinary Research centre named STITCH (Sapienza information-based Technology InnovaTion Center) who has a long-lasting collaboration with international networks such as the Network Medicine Institute Corp., the Foundation European Institute of Network Medicine and Harvard Medical School. Many research groups involved in cancer, metabolic, cardiovascular and rare diseases are actively working, by following the principles of Precision Medicine. In UNIROMA1 virtually all the experimental in vitro approaches to personalized medicine are available including patient-derived 3D and 4D cell cultures, co-coltures of cross-talking tissues, microfluidic and organ-on-chip devices, single-cell omics, experimental animal model of cancer, metabolic and vascular diseases. Finally, thanks to the interdisciplinary collaboration with engineers, computer scientists and data analysts, platforms integrating clinical data with omics data are available (BIG DATA).

Organization skills. UNIROMA1owns outstanding numbers and a complex administrative structure to handle these numbers: almost 117.000 enrolled students, 10.000 of them from abroad, 11 faculties which cover any kind of scientific and social area with 58 departments, 3 university hospitals and more than 3.300 professors and researchers.

National and international project management skills. UNIROMA1 is involved actively in high quality national and international projects with 123 collaborative projects, 67 MSCA actions and 35 ERC grants funded under Horizon 2020 and even more successful projects funded through national calls such as PRIN, National Technological Cluster, Competence Centers. UNIROMA1 hosts the "Area Supporto alla Ricerca e Trasferimento Tecnologico" and jointly with the AOU Policlinico Umberto I the Clinical Trial Center to assist researchers in application processes to ethical committee and grant application in either clinical trial and translational research.

Technology transfer capacity - patents - spin-off. The Technology Transfer (TT) structure supports the protection, management, and enhancement of intellectual property on the research results generated by its scientific network. TT is managed centrally but in daily liaison with the departments and research groups to evaluate the opportunities for protecting individual results, and for the management and enhancement of the IPR portfolio and patents, also in terms of international extensions and maintenance of licenses. The office promotes and collects all the request for establishing academic spinoffs and start-ups, looking also after their growth and future actions. UNIROMA1 can rely on 39 patents and 5 start-ups capable of boosting its innovation potential towards industrial stakeholders.

Experience in the field of "Diagnostica e terapie innovative nella medicina di precisione" PE 6: At least 20 Years of experience in carrying out preclinical and clinical research with important translational implications. Several national and international publications by the affiliated members of the Department of Translational Medicine and Precision Medicine and of other UNIROMA1 Departments. Dedicated resources: facilities offering a wide range of instruments to investigate the experimental and clinical aspects of the proposed research and commitment of human resources to implement projects.

Key elements/strengths

a. At least 20 PATIENT REGISTRIES are active, N. 150 clinical trials in progress, many active national and international research networks related to cancer, metabolic, cardiovascular, and rare diseases including ERN. Patient associations are involved in research and clinical network; biobanks of tissues and biologic samples; production of cells for human clinical trials with large experience in regenerative medicine of organs and tissues.

b. Active research NETWROKS with patient registry: ENSCA (international network study of cholangiocarcinoma); Sapienza University Mortality and Morbidity Event Rate study in diabetes" and the aggregate "Gargano Mortality Study"; Consorzio Italiano Tumori Ereditari alla Mammella; Italian Thyroid Cancer Observatory; Neuromed studio "Moli-sani"; Consortium of Investigators of Modifiers of BRCA1 2; Breast Cancer Association Consortium; European Network of Multidisciplinary Research and Translation of Autophagy knowledge (Cost Action CA15138); European Network of Chemistry and Molecular Sciences and

Technologies (Cost Action CM1407); European Pooled Analysis Consortium - Circulating Lung cancer cells; European Pooled Analysis Consortium - Circulating Breast cancer cells. ERNs: ERN-RITA; ERN ReCONNET; ERN Rare Liver; ERN EuroBloodNet; Endo-ERN; ERN BOND; MetabERN.
c. At least 100 multicentre national and international translational ONGOING TRIALS and projects dedicated to Precision Medicine are ongoing, funded by national and international grants and this has been detailed in each of the proposed projects.
UNIROMA1gained a pivotal role in supporting the entire research and innovation value chain on national and regional initiatives, many of them in cooperation with other universities and companies, with a specific focus on precision medicine. The most relevant summarized as follows: Regional Strategic Projects on Lazio S3, funded in 2019, are based on university networks in support of R&I projects developed by SMEs in collaboration with research institutions on 3 strategic domains: Aerospace, Green Economy and Life Science, the latter being coordinated by UNIROMA1. UNIROMA1 is committed and experienced in the implementation of open science practices and addressing gender aspects in research and innovation.
UNIROMA1 stands out in the national and international panorama for the promotion of digitalization and technological innovation applied to healthcare in education. UNIROMA1 promotes initiatives aimed at advancing a multidisciplinary, inter-professional and integrated vision of precision medicine, with a focus to technological applications. From here an Interfaculty Degree Course in High Technology Medicine and Surgery born, organized by the Faculty of Medicine and Dentistry with the contribution of professors from the Faculties of Medicine, Information Engineering, Computer Science and Statistics. The degree course was created to foster and encourage an education oriented to disease prevention, rehabilitation and health promotion within the community and territory, with a strong interest in the principles of "precision medicine".
Diagnostica e terapie innovative nella medicina di precisione". Spoke 4, coordinated by UNIROMA1 brings together 10 complementary academic entities, well-positioned to tackle the challenging objectives of PE6. The partners in Spoke 4 have been selected for their clinical & research capacity. The centres have access and will provide cutting-edge infrastructure essential for the advancement of the project including to advanced imaging units, molecular and genetic laboratories with innovative devices and digital laboratories supported by AI tools. The clinical centres will implement data collection for the development of the infrastructure in Spoke 2 and will bring their clinical expertise. UNIROMA1 and UNIMIB will join forces to discover and validate new integrated bioimaging for early diagnosis of polygenic diseases. For the advanced biological analysis for diagnosis and monitoring of diseases, UNIROMA1, UNIFG, UNIMORE, UNIBO, UNIPA, and UNICT will collaborate, building on their long-standing expertise in this field. Clinicians, pathologist, engineers, computer scientist, will come together from UNIROMA1, UNIVR, UNIMORE and UNIPA to cooperate on the standardization of acquisition and analysis of digital images for AI-based solutions. For the computational models for risk prediction and data-driven precision-medicine, expert computer scientists' and healthcare professionals from UNIROMA1, UNIMIB and UNICA will work closely together. With this capacity convened by the partners, Spoke 4 is ideally positioned to reach the project objectives. The centres have proven experience in collaborating on projects together, which has contributed to the establishment of good personal relationships, based on a solid teamwork concept and a strong ethical commitment, which is expected to be valuable for a quick 'jump-start' of the project

UNIROMA1 has established framework agreements with the most promising and relevant organisations and IRCCS active in the health domain such as IFO, IIT (Italian Institute of Technology), IRCCS Lazzaro Spallanzani, AIRC (Italian association for cancer research), ISS (Italian Health Institute). In addition, UNIROMA1 plays an active role within the National Technological Cluster funded by the Ministry of University and Research, with a specific reference to those for life sciences and ambient assisted living.

UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA - Hub founder & Spoke 5 Leader
Short name-UNIMIB-

General information

UniMiB was founded in 1998: its mission is to be research- and innovation-oriented, providing excellent teaching and enhancing a merit-based access policy for students. UniMiB is a dynamic university: it is part of the European Universities Association (EUA) and included in relevant networks together with top universities, research centers and big corporations. With its 33,000 enrolled students, it ranks among medium-big Universities (between 20,000 and 40,000 students). It consists of fourteen Departments, active in the fields of ICT, Educational Sciences, Economics, Business Administration and Management, Law, Mathematics, Physics and Natural Sciences, Medicine, Psychology and Sociology. In its more than twenty years of activity, UniMiB has achieved high national and international prestige, and it is ranked as the 2nd highest Italian university among those comparable in size by ANVUR (the National agency for the rating of university quality and research), and 82nd among the best 250 world-wide young universities (less than 50 years old), by 'THE Times Higher Education'. Eleven out of the fourteen departments are labelled as "Departments of excellence" by the Italian Ministry of Research (MIUR – Ministero dell'Istruzione, dell'Università e della Ricerca). These are recognized as departments that stand out for the quality of their research. The Departments of Biotechnology and Biosciences, Materials Science, and the School of Medicine and Surgery, are among them. Through our broad and rich educational offer, we aim to allow students to find their real vocation, advance their own interests, take a critical look, and acquire skills and a solid know-how to build their profession. Excellent teaching, excellent research, and high-quality service place focus on the individual, as the pillars of a modern, future-oriented university. At our university, students are taught to seize the world's opportunities and not to be frightened to confront them and put themselves out there. It is an inclusive university which shares, respects and protects diversity, as one of its main values.

Organization skill

The Government Bodies of UniMiB are detailed as follows: Rector: Giovanna Iannantuoni Academic Senate: contributes to defining UniMiB policies, strategies and development plans. It formulates mandatory proposals and opinions on matters relating to teaching, research and student services. It coordinates and works with Departments and any teaching coordination bodies, as well as ruling on any disputes. **Board of Governors**: The Board of Directors is responsible for matters relating to the strategic direction and development of the University, with consideration to the proposals and opinions of the Academic Senate and in respect to the prerogatives of teaching and research bodies.

General Director: In accordance with the guidelines of the Governing Board, the General Director is responsible for the overall management and organization of the University's services, resources, and technical and administrative staff.

The functions of technical and administrative staff include support to teaching, human resources management and budget. Research service include the identification and pursuing of funding opportunities and the continuous maintenance and enhancement of the relations with the scientific and industrial network, both on the territory and abroad, adopting an international viewpoint

National and international project management skills

At UniMiB, a dedicated Grant Office & Tender Service carries out the following activities: ● dissemination of funding notices, ● support for grant writing, ● administrative and management support for the presentation of funding applications,

● participation of the university in tenders and tenders as an economic operator,
● support to project management,
● monitoring and reporting of funded

Technology transfer capacity - patents - spin-off Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

The University supports scientific research, managing all aspects with a specific focus on Intellectual Property Protection (IP) in all its forms, from the technical achievement (Industrial Properties/patents) to cultural products (protected under copyright laws). The protection and valorization of knowledge as a result of the academic research is made through actions such as technology transfer that also includes the management of IP. On behalf of the Athenaeum, the Area promotes the development of the interactions with the productive eco-system, it supports and sustains the creation of structures that connect Academia and Industry and

encourages and facilitates technology transfer directly to companies.
Publication: formal document published on the institution's website about the topic. Motta et al. Nanoscale, 2021, 13; Marongiu et al. Sci Signal. 2021 Mar 30; Piazza et al. Nat Commun. 2018 Jun 6; Santus et al. Sci Immunol. 2017 Sep 22; Colombo et al. Nat Commun. 2016 Dec 19; Piazza et al. Nat Genet. 2013 Jan; Marktel et al. Nat Med. 2019 Feb; Aiuti et al. N Engl J Med. 2009 Jan 29; Gambacorti-Passerini et al. Blood. 2015 Jan 15; Boria et al. Int J Gynecol Cancer. 2021 Sep; Chacon et al. Int J Gynecol Cancer. 2022 Feb; Gandini et al. Nat Nanotechnol. 2020 Jun; Poli et al. Immunity 2022, Feb 8; Notarbartolo et al. Science Immunology 2021, Aug. 10; Broggi et al. Science 2020, Aug 7; Bouzin et al. Nat. Comm. 2019, 10; Bellini et al. Small 2020, Aug 28; Mazzucchelli et al. Angewandte Chemie - International Edition, 2013, Feb 5; Ongaro et al. Nanomedicine 2022, Feb;
Dedicated resources: commitment of human resources to implement projects. UniMiB has obtained a total funding of 116.800.000€ for 1179 projects, 139 as coordinator, from different funding agencies (EU, Regione Lombardia, Foundations, Ministries), including one ERC-CoG Grant https://www.UniMiB.it/ricerca/finanziamenti-alla-ricerca/finanziamenti-internazionali UniMiB is coordinator of 4 KIC RAW MATERIAL projects out of 27 funded projects. Research projects have been valorized with 148 filed patents

Key elements/strengths

The University departments are complemented in their research activities by more than 30 university and inter-university Research Centers, together with interdepartmental platforms and facilities hosted at the University premises. Relevant examples of these are the Plasma Prometeo, the Eurocold Lab, Biotechnium, Unidata Center and the Interdepartmental Platform for Mass Spectrometry. Centres of Excellence, Research Centres and Consortia have been created to manage large multidisciplinary research activities and are managed according to high scientific standards.
UniMiB has a prominent role in European Research Infrastructures, being part in and collaborating with a total of 9 ESFRI RIs, having also coordinated (ISBE) and currently coordinating (BBMRI) the national nodes of two of them. Solid relationships with local industries resulted, over the years, in the filing of several national and international patents, with 55 currently active patent families, together with the foundation of 18 spin-offs companies (initiated by university staff), exploiting opportunities from a broad range of technological sectors, namely in nanomedicine and biotechnology, therapeutic and diagnostic, energy and environment, smart materials and ICT driver mutations identification through using Next-Generation-Sequencing techniques (whole-exome, whole-genome, panels, ultradeep sequencing). Single-cell RNA-Seq analyses (scRNA). Spatial transcriptomics. Advanced confocal microscopy. Fluorescence-Activated Cell Sorting (FACS). Gene fusion detection using whole-transcriptome data. CRISPR knock-out libraries. Mass spectrometry Multiplexing; CAR-T cell platform; SPF mouse facility; Biostatistics. Bioinformatics. Computational Systems Biology. Artificial Intelligence. Molecular and cellular biology. Atomistic simulations and modelling.
As of 2021, UniMiB is part of the Italian network core facilities BicOMICs, an integrated platform for precision medicine using OMICS techniques to address prevention, diagnosis and treatment of diseases, as well as understanding their molecular mechanisms.
Shortlisted National and international collaborations:
Scientists working at UniMiB have established long-term national, transnational and international collaborations with many universities and research institutes: including, among others, Harvard University and Harvard Medical School (USA), Cornell University (USA), ASST Spedali Civili di Brescia (Italy), Norwegian University of Science and Technology (Norway), Hôpital Saint-Louis (France)

UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA – Hub founder and Spoke 6 Leader Short Name-UNIMORE

General information

The University of Modena and Reggio Emilia (UNIMORE) was founded in 1175. UNIMORE has over 27,000 students, including 3,500 postgraduates, 900 faculty members and 17 graduate (PhD) courses. UNIMORE ranks amongst the top Italian universities for its high-level research and educational programs. UNIMORE offers state-of-the-art facilities with internationally supported research activities and, through the technopoles of Modena, Mirandola and Reggio Emilia, is an essential component of the Emilia-Romagna High Technology Network. UNIMORE departments drive research and training, with 4 large biomedical Departments highly connected with the Hospital of Modena and the clinical settings.

Organization skillsNational and international project management skills

UNIMORE has been involved in 249 international projects, 119 financed by the Horizon 2020 and Horizon Europe programs and 130 fundend by other public and private entities. UNIMORE PI participate in 312 national grants. UNIMORE plays the role of coordinator in 59 international projects and 132 in national projects. Specifically, UNIMORE is partner in 144 international and 180 national projects. As third party, UNIMORE is involved in 46 international projects. The total budget of international grants is 57,62 Mil € with 28,13 Mil € from the H2020 program. Other international funding bodies (FP7, foundations, other EU programs, etc.) provide a total budget of 25,20 Mil €. National projects from grant by Ministries and other financial entities, such as regional bodies, generated 48,39 Mil €.

Technology transfer capacity - patents - spin-off

UNIMORE has 120 patents with 71 regarding to the medical area, under the supervision of the Industrial Liason Office. The current active spin-off are 33. UNIMORE signed significant agreement with pharma companies (i.e. Chiesi) and with innovation hub (Technopole of Mirandola) dedicated to medical devices where a spin-off incubator recently started.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

UNIMORE has lasting experience in biomedical research including precision diagnostics and therapeutics. Two medical Departments obtained grants from the Ministry of University "Dipartimenti Eccellenti 2017" award on the similar topics. Clinical and laboratory research with trial offices, equipped laboratories and shared facilities are multidisciplinary combined around a variety of bio-medical disciplines with very relevant impact in terms of granting, patents and publications. The University Hospital of Modena established omics platform for a variety of diseases research and diagnosis. UNIMORE is additionally hosting the national Cochrane Center providing methodological support in clinical trials and a national center of Artificial Intelligence (AI). Heads of both the omics platform (Dominici) and the Cochrane Center (D'Amico) are co-PI within the Heal Italia proposal

e.g., Publication: formal document published on the institution's website about the topic. www.unimore.it; www.nanomedicine.unimore.it/site/home.html; www.cgr.unimore.it/organization/; www.cell-therapy.unimore.it/site/home.html.

e. g., Dedicated resources: commitment of human resources to implement projects. Total staff employed (dated on 01/01/2022): 843. Of those 232 are full prof, 388 associate prof, 84 assistant prof, 79 research fellows (type b) and 60 research fellows (type a). Staff potentially involved in PE6 activities are a total of 209 with full prof: 56; associate prof: 97; assistant prof.: 26; research fellows (type b): 15 and research fellows (type a): 15.

Key elements/strengths

The University has a strategic commitment to promote research in precision medicine account an existing expertise in: i) nano- and emerging materials, devices and systems for sustainable technologies (including sensoring and bioeng.), ii) genomic and molecular medicine, iii) cell and gene therapies, iv) AI. To support these goals below a list of strengths relevant for the project:

Multidisciplinary teams: highly nationally and internationally connected research teams combining translational research and clinical activities in the main area of the project, formulating diseases-driven working hypotheses addressed by multidisciplinary team including PhD, MD, Engineers, Chemists, and Physics;

State-of-the art equipped laboratories with shares facilities of genomic (NGS, functional genomics, mRNA profiling, single cells multiomic). Equipped animal facilities. Bioinformatics, biostatistics, electron and confocal microscopy, mass spectrometry resources facilities are available at CIGS (https://www.cigs.unimore.it/index.php);
Strong clinical trials vocation with over 300 trial/yearly, two third of them in Oncology with over 500 patients enrolled/yearly; cell and gene therapy trials are already on going for COVID19 and cancer;
Patient registries: liver diseases (Fabry disease, primary biliary cholangitis, viral hepatitis, Gaucher), muscular dystrophies (FSHD), International registry on stress and depression in women with cardiovascular diseases, Italian registry in systemic sclerosis, Registry for hereditary colon cancers;
National and international research networks: ERN for rare liver diseases, ERN for scleroderma, national center for myeloproliferative neoplasms, Italian clinical network for FSHD, international nephropathy network, computational drug repurposing network, Foundation for the promotion of health and biomedical research, European Technology Platform in Nanomedicine, Trialect Corporation Program on Mol Biology of gonadotropins, Women in cardiology network, World scleroderma foundation;
Patient associations connections; ARiAE (hepatology), Unione Italiana distofia muscolare, Conacuore, Amici del Cuore, Gruppo italiano Sclerodermia, Cancer Patients association (A.Serra, LILT, AIL);
Biobanks: oncology/hematology/nephrology/neurology/rheumatology
Innovation and technology transfer.

a. UNIMORE has so far active 30 Spin-off
b. UNIMORE is in proximity with the largest biomedical devices district in Europe developing tools for health care (https://distrettobiomedicale.it). This, together with the academical strength, represents an asset in HEAL Italia development. In addition, it will bring solid innovation and technology transfer opportunities for the HEAL Italia Project, also thanks to the presence of a newborn start-up incubator
For this reason, UNMORE is the Spoke coordinating multidisciplinary activities on devices.
c. UNIMORE is a member of the ALISEI Cluster (Advanced Life Science in Italy), the National Life Sciences Technological Cluster that promotes interaction between the multidisciplinary research system, the pharmaceutical-biomedical industry and the public health care sector.
d. UNIMORE is member of "Clust-ER Industrie della Salute e del Benessere", an Emilia-Romagna Region-recognized association composed of large companies, SMEs, High Technology Network laboratories, universities and research centers to facilitate the tech transfer.

UNIMORE has been involved in a variety of national and international projects specific to the medical area. Internationally, UNIMORE has a total of 33 projects, acting as coordinator in 9 of them for a total budget of 8,47 Mil €. On national projects specifically dealing with medicine, UNIMORE counts 92 projects coordinating 52 of them with a total budget of 20,89 Mil €. This indicates the ability of UNIMORE to manage and coordinate a Spoke on the ambitious proposal of HEAL Italia

The list of all existing collaborations for UNIMORE researchers involved in HEAL Italia can be found here: www.pe6.collaboration.unimore.it;

UNIVERSITÀ POLITECNICA DELLE MARCHE – Hub founder and Spoke 7 Leader
Short Name: UNIVPM

General information*Organization skills*
UNIVPM has 4 Medical Departments of which 3 (Dept. of Scienze Cliniche e Molecolari; Scienze Biomediche e Sanità Pubblica; Scienze Cliniche e Odontostomatologiche) actively involved in this proposal. The small size of these Departments, all placed at walking distance from the University Hospital, favors active daily interactions and collaborations, from bench to bedside. Patients' biological samples of scientific interest can be immediately processed and stored in the Biobank, placed in the same campus, and subsequently analyzed in the laboratories.
National and international project management skills
UNIVPM has increased its presence at European level due to a high standard in research and industrial collaborations for research and development and innovation. In the previous European Research and Innovation Framework, FP7 and Horizon2020, and in other European research programs (*PRIMA, COST, LIFE, DG ENV, ERANET, AAL, JPI MYBL, JPI WATER, JPI OCEANS, INTERREG PROGRAMME, MED PROGRAMME*) UNIVPM has secured funding for over 150 EU projects with a total budget of 36M€ and participated in 25 projects with the role of coordinator. In the current European Research and Innovation Framework, Horizon Europe, UNIVPM has 12 funded projects, in which it plays the role of partner with a total budget of 3,5M €.
Technology transfer capacity - patents - spin-off
UNIVPM has an office dedicated to this task, with a scientific committee composed of members from each Department. There are currently 100 active patents, which led to 59 spin-off activities.
Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"
The 3 UNIVPM Departments involved in the proposal are mainly focused on Precision Medicine and Disease Prevention Strategies. To this end, two integrated collaborative platforms have been developed since 2019 (see description below) for accurate profiling of clinical phenotypes of patients affected by chronic diseases, rare diseases, onco-hematological diseases with unmet medical ne*eds.*
Decennial experiences of single research groups have been merged since 2019 for the goal of implementing precision medicine at UNIVPM
A multitude of publications has been produced in recent years, as witnessed by the selection of 2 departments involved in this proposal as Departments of Excellence by VQR-ANVUR of Italian Ministry of Research
A specific research programme of Marche Region *(POR MARCHE FESR 2014/2020-ASSE 1-OS 2-AZIONE 2.1 -INTERVENTO 2.1.1- SOSTEGNO ALLO SVILUPPO DI UNA PIATTAFORMA DI RICERCA COLLABORATIVA NEGLI AMBITI DELLA SPECIALIZZAZIONE INTELLIGENTE AREA TEMATICA: "Medicina personalizzata, farmaci e nuovi approcci terapeutici"- COLLABORATIVE RESEARCH PLATFORM IN PERSONALIZED MEDICINE: DRUGS, DIAGNOSTICS AND NEW THERAPEUTIC APPROACHES)* is implementing all the public-private collaborative projects on this subject, consistent with the PE6 theme.

Key elements/strengths
a) patient registers: Italian (GITMO) and European Bone Marrow Transplantation (EBMT)
b) ongoing trials: a number of phase II, III, IV multicenter RCTs on cancer, blood, liver and fibrotic diseases, Center authorized for phase I studies. Pilot trial on lung cancer approved by Italian Ministry of Health.
c) active national and international research networks:
- Liver diseases: Testing Marker Utility in Steatohepatitis (LITMUS), ITA.LI.CA. database (Italian Liver Cancer), European Network for the Study of Cholangiocarcinoma (ENS-CCA); International PSC study group (IPSCSG)
- Blood diseases: GIMEMA, Fondazione Italiana Sindromi Mielodisplastiche (FISiM), Fondazione Italiana Linfomi (FIL), Gruppo Italiano trapianto Midollo Osseo (GITMO); European Hematology Association AML working group; European Mieloma Network; International Bone Marrow Adiposity Society (BMAS) COST Action EUROGRAFT-Integrated Network on Chronic Graft Versus Host Disease
- Fibrotic diseases: Gruppo Italiano Lotta alla Sclerodermia (GILS), European Scleroderma Trials and Research (EUSTAR), European Reference Networks (ERNs) RECONNET and LUNG;

d) patient associations: AIPASIM (Associazione Italiana Pazienti Sindromi Mielodisplastiche); AIPIT (Associazione Italiana Porpora Trombocitemica); ASIMAS (Associazione Italiana Mastocitosi ONLUS); AIL (Leukemya), GILS (Gruppo Italiano Lotta alla Sclerodermia)
e) biobanks:Marche BioBank, 150 m^2 aptly designed space located at walking distance between the UNIVPM Departments and Hospital, dedicated to the collection, characterization and distribution of samples and primary cell cultures and organoids obtained from patients with rare and chronic diseases, and with hematological-oncological malignancies. Marche BioBank is part of the BBMRI-Biobanking and Biomolecular Resources Research Infrastructure (https://www.bbmri.it/home).
f) Marche BioBank has a 150 m^2 associated laboratory endowed with single cell analysis facility (BD FACS Melody, Laser capture dissection microscope, digital PCR, Illumina NGS, Luminex, Mass spectrometer) for the most complete omics characterization of liquid and tissue biopsies of patients
f) public-private partnership between Marche BioBank and the 3 main biotech and pharmaceutical companies of Marche region (Angelini, Diatheva, Diatech) which are actively contributing to ongoing research projects on precision medicine
g) hybrid imaging (PET/CT, PET/MRI) facility for the morpho-functional-metabolic study of organs and systems + spectral imaging facility (dual energy CT) for perfusion studies of organ and tissues
h) mouse facility for disease models: humanized model of skin and lung fibrosis, models of liver carcinogenesis, models of mesothelioma and lung cancer
i) access to cell factory of AMU hematological consortium
l) Center authorized for cellular therapies and *CAR-T*
m) Hybrid room for cardiac imaging and electroanatomical voltage mapping (EAM)

UNIVPM and ISS will coordinate the Spoke 7 dedicated to the Prevention Strategies. UNIVPM and ISS have a longstanding collaboration and ability of managing and implement projects within the PE6 area.

National and international collaborations

National: Ministry of University and Research (MUR), Ministry of Health, ISS (Istituto Superiore di Sanità), AIRC (Fondazione Italiana per la Ricerca sul Cancro)

International: European League Against Rheumatism (EULAR), National Cancer Institutes.

UNIVERSITÀ DI PISA Hub founder and Spoke 8 Leader
Short Name: UNIPI

General information

The University of Pisa (UNIPI) is a public institution, nowadays representing a modern and prestigious facility of advanced teaching and research with 12,190 first year enrolled in bachelor and master's degree courses (2021), 20 departments, 17 libraries and 13 museums, besides to 26 centres to carry out the most solid research in all disciplinary areas. UNIPI offers 61 undergraduate courses (first cycle), 71 master's degree courses (second cycle) and 7 single-cycle degree courses, together with 36 PhD courses, 49 specialization courses and 67 one-year master's courses. In the QS World University Rankings 2022.

National and international project management skills

UNIPI ranked 7th at the Italian level and 388th worldwide and was confirmed as one of top 10 Italian Universities according to the Times Higher Education (THE) ranking 2021. According to the latest Academic Ranking of World Universities (ARWU, 2021), published by the Shanghai JaoTong University, UNIPI ranks amongst 151-200 academic institutions worldwide. Moreover, according to ARTU, the Aggregate Ranking of Top Universities, in 2021 UNIPI ranked the 280th place at an international level and confirming its ranking not only on a national level among the top 10 Italian universities but also the 1st in the Tuscany Region.

Technology transfer capacity - patents - spin-off

UNIPI is currently involved in **236 EU-funded R&I projects** under Horizon Europe, H2020 and other programmes (**EU contribution: €84.5 million**), 68 of them as coordinator (EU contribution: €38.9 million). With regards to **national and regional projects**, in the period 2017-2021 UNIPI has received around **€44 million** for a total of **380 projects** funded by Ministries, Tuscany Region, foundations. Since 2003, UNIPI has presented 121 Italian patent applications filed, of which **85 patents** granted and 115 foreign patent applications. Moreover, UNIPI created **34 spin-off companies**.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

UNIPI has a long-lasting experience (**40 years**) in the field of precision medicine. For this application UNIPI brings together a strong interdisciplinary academic team involving **25 researchers** from several different and complementary areas (Endocrinology, Oncology, Cardiology, Imaging and Radiotherapy, Pharmacology, Organic Chemistry, Pharmaceutical Chemistry, Genetics, Electronic Engineering, Materials Science and Technology, Electronic and Information Bioengineering, Experimental Physics). During the last 10 years, the researchers involved in Spoke 8 of PE6 have published **more than 900 papers** in this field and they have high *h*-index values **(up to 92),** with an **average of 43**

Key elements/strengths

To achieve the aims of PE6, UNIPI relies on the scientific skills and research experience of nine departments: i) **Clinical and Experimental Medicine**, ii) **Surgical, Medical and Molecular Pathology and Critical Care Medicine**, iii) **Translational Research and of New Surgical and Medical Technologies**, iv) **Biology**, v) **Pharmacy**, vi) **Chemistry and Industrial Chemistry**, vii) **Physics**, viii) **Information Engineering**, and ix) **Civil and Industrial Engineering**. All of them are very active in theoretical and applied research (including technology transfer and interdisciplinary initiatives) as well as in advanced clinical care within the local University Hospital **- Azienda Ospedaliero Universitaria Pisana** (AOUP). Registers containing information on hundreds of patients affected by solid tumors (thyroid, colorectal, upper gastrointestinal tract, lung, breast and prostate cancer), diabetes, obesity and specific cardiovascular diseases (heart failure, atrial fibrillation [ETNA-AF], valvulopathies) are available, as well as registers of patients who underwent liver, kidney and pancreas transplantation. For some of them biological samples have been/are being banked, which includes the use of the ISO 9001 2015 biobank of the UO Biobanche of the AOUP. In addition, two registers will serve as radiological images repository and will be interrogated. 41 academic clinical trials in the field of precision medicine are currently ongoing at UNIPI, enrolling patients affected by cancer or cardiometabolic diseases. Furthermore, UNIPI will trust in the **Centre of Instrument Sharing of UNIPI** (CISUP), an interdepartmental laboratory platform offering access to a wide range of analytical instrumentation for life science researchers, which will support the activities planned in PE6 (e.g. Field emission scanning electron microscope (SEM) FEI Quanta 450 FEG; High Resolution Field Emission Gun Transmission Electron Microscope (TEM) (HR FEG-TEM); and confocal laser scanning microscopy (Zeiss LSM 900/Airyscan 2). Since 2012 UNIPI has established a partnership with the Massachusetts Institute of Technology (MIT) and with the Joint Research Centre (JRC) and is member of several European networks (e.g. EUA, Science Business, UNIMED, Tour4EU). Furthermore, it is an active member of many of the most important EU initiatives acting in fields related to PE6, like the European Technology Platform Nanomedicine (ETPN). UNIPI also adheres to SLAGEN Consortium and to the

European Alzheimer's Disease Initiative Investigators. Moreover, UNIPI joins the international network Spark Global. Several collaborations with patients' associations are in place, including Rete Oncologica Pazienti Italia (ROPI), Lega Italiana Lotta ai Tumori (LILT), Associazione GIovani Diabetici (AGID), aBRCAdabra supporting patients bearing germ-line *BRCA* mutations and their families, Salute Donna Onlus. Two recent conferences were organised by UNIPI on the topic of precision medicine: 1) *"Technology supporting precision medicine – How the fusion of physical, digital and biological technologies impacts patient care"* (17/12/2021) and, 2) *"Medicina e sanità pubblica di precisione: nuove sfide per una salute efficace e sostenibile"* (27/02/2020).

Since 2003, UNIPI has presented 121 Italian patent applications filed, of which **85 patents** granted and 115 foreign patent applications, 31 of them in the life sciences sector and in the precision medicine area, e.g. "*Diagnostic probes to detect cells and tissues afflicted with a pathological or metabolic condition by means of infrared spectroscopy*" (International patent application number PCT/IB2021/061061 - 29/11/2021); "*Device for rehabilitation of tongue muscles*" (Italian registration number: 102019000006406, 2019); "*Method and apparatus for the acquisition of data for positron emission tomography in hadrontherapy with beam on*" (Italian n. 102018000000867 (a1) - 15.01.2018. International n. wo2019138384 (a1) 18/7/2019); "*New activators of sirt1 enzyme for the treatment of cardiovascular and cardiometabolic pathologies*" (WO2019/162911, 2019). Moreover, UNIPI created **34 spin-off companies**, some of them dealing with precision medicine (such as QUIPU S.r.l., specialized in medical imaging and innovative ultrasound technologies, and IVTech, an Italian biotech company that produces advanced cell culture chambers to refine in-vitro models.

UNIPI has obtained **22 European projects** (Horizon Europe, Horizon 2020, Third Health Programme, IMI2, PhotonicSensing - ERA-NET Cofund) focused on precision medicine, for a total of approximately **€10 million** in the last 10 years. Within the conceptual and research framework of Spoke 8, the research groups of this University have a strong expertise proved by the numerous research projects dealing with precision medicine, such as: Horizon Europe: **AInCP** – "*Clinical validation of Artificial Intelligence for providing a personalized motor clinical profile assessment and rehabilitation of upper limb in children with unilateral cerebral palsy*" (GA ID: 101057309 - UNIPI coordinator); **RESORB** – "*On-Demand Bioresorbable OptoElectronic System for In-Vivo and In-Situ Monitoring of Chemotherapeutic Drugs*" (GA ID: 101046946 - UNIPI coordinator). H2020: **T2DSystems** – "*Development of a systems biomedicine approach for risk identification, prevention and treatment of type 2 diabetes*" (GA ID: 667191 - UNIPI beneficiary); **PRIMAGE** – "*PRedictive In-silico Multiscale Analytics to support cancer personalized diaGnosis and prognosis, Empowered by imaging biomarkers*" (GA ID: 826494 - UNIPI beneficiary); **EuCanImage** – "*A European Cancer Image Platform Linked to Biological and Health Data for Next-Generation Artificial Intelligence and Precision Medicine in Oncology*" (GA ID: 952103 - UNIPI beneficiary); **ProCAncer-I** – "*An AI platform integrating imaging data and models, supporting precision care through prostate cancer's continuum*" (GA ID: 952159 - UNIPI beneficiary); **AffecTech** – "*Personal Technologies for Affective Health*" (GA ID: 722022 - UNIPI beneficiary). IMI2: **INNODIA HARVEST** – "*Translational approaches to disease modifying therapy of type 1 diabetes*" (GA ID: 945268 - UNIPI beneficiary). 3rd Health Programme: **INTEGRATE** – "*Integrating patients reported outcomes, clinical data and quality indicators to physician driven data in clinical management of chronic rheumatic diseases: the paradigm of Systemic Lupus Erythematosus"* (GA ID: 769736 - UNIPI coordinator). PRIN 2020: **FREE2D** – "*Filling the gap between Risk assessment and prEcision mEdicine in type 2 Diabetes complications*" (UNIPI coordinator). Bando Ricerca Salute 2018: **NAVIGATOR** – "*An Imaging Biobank to Precisely Prevent and Predict cancer, and facilitate the Participation of oncologic patients to Diagnosis and Treatment*" (UNIPI coordinator); **PREMED2** – "*Precision Medicine for Preventing Type 2 Diabetes: a Step Forward"* (UNIPI coordinator) and others (e.g. **IN BILICO**, **PROBIO**, **ADAPTA** with UNIPI coordinator).

Strategic national, European and other international scientific collaborations have been developed by participating in national and European projects (see above), thanks to the signed **286 International interuniversity agreements** worldwide to promote and carry out joint activities related to teaching, research and training. Among them, **197 collaboration agreements** were signed in the field of Medicine. International public and private institutions and SME/industries the UNIPI researches involved in this PE collaborate with, in the field of precision medicine, include: Université Libre de Bruxelles and Catholic University of Leuven, University of Oxford, King's College London and Imperial College London , Lunds Universitet (Sweden), INSERM Institut National de la Santé et de la Recherche Médicale (France), Université de Lausanne (Switzerland), Sanofi (Germany), Novo Nordisk (Denmark), JDRF (US), and others.

UNIVERSITÀ DI FOGGIA - Hub founder and Spoke's Affiliate
Short Name: UNIFG

General information

Organization skills

The University of Foggia (UNIFG), formally established as an autonomous and independent University on August 5, 1999, is a young university, which in a few years assumed an important role in the national and international scientific world. Many achievements have been made in research and training which made it a point of reference for the social, cultural and economic context of the Territory. Important results were obtained in the VQR 2004-10 and 2011-14, thanks to the quality of the scientific production expressed. UNIFG has accumulated in the last years wide experience in model development and biomarkers discovery in the field of precision medicine. The research staff and equipment are placed in a dedicated structure accessible to the whole research group with 8 core facilities, dedicated to laser spectroscopy, spectrophotofluorimetry, proteomics, flow cytometry, genomics, confocal microscopy, mass spectrometry and the laboratories of experimental biology

National and international project management skills

UNIFG manages several projects, such as "lattiero-caseario", "ofr.al.ser", "iscocem", "in.te.rra" "pl.a.s.s", "microtrigenpot", "safemeat", "log.in. (2012)", "ittico", "nutrafast", "(asparago)", "bivini", "h-bio", 7 prin 2010-2011, firb 2012, 2 mipaaf, PON "ricerca e competitività 2007-2013" regioni convergenza asse i - pon01, PON "ricerca e competitività 2007-2013" bando startup linea 2, cultura ad impatto aumentato, 3 prin 2012, futuro in ricerca, dm 29099 (codice cig n. 6018751e6f), domanda pon03pe_001,36 - progetto pon03pf.-00l36-1., 4 prin 2015, 7 a.i.m., 11 prin 2017, riu.s.a, fami 3438, percival, foster, or in the european context: star-agroenergy, quafety, sonetto, "tempting streets", isci, unifederlab, hera(partner), cost: oc-2013-2-16751, up-running, simra, skin, pro-infant, eu-jam rai, e-parks, fish and c.h.i.p.s, correct it!, cut-it short, innodevo, unveil, treasure, ern-apulia, ern-apulia2, ern-apulia3, pimento and corenet network.

Technology transfer capacity - patents - spin-off

In the three-year period of reference, UNIFG obtained 85 patents, together to the II PCT phase of the Gluten Friendly international patent application and entered into 5 research enhancement agreements. Finally, it should be noted that in the three-year period of reference, the University has an average of six spin-offs active per year, three competence centers and two technological districts

Experience in the field of "Diagnostica e terapie innovative nella medicina di precisione" PE 6

Our group includes competences, skills and technologies of different units, truly complementary, each being expert of a specific field of human diseases. The workgroup constitutes an ideal network able to tackle all the aspects of the human diseases, from onset to progression and therapy. Members are leading experts in human diseases; units collaborate with experienced bioinformatics researchers characterized by excellent skills in programming and deep experience in computational cancer biology to apply to NGS-analysis. The units are composed also by experienced pathologists, able in processing of samples for anatomopathological analysis and immunohistochemistry. The project follows the translational pathway, from identification of cellular alteration in carcinoma, to results application in clinical practice and developing of new therapies.

List of publication published on the institution's website is available at the following link https://fair.unifg.it/; on the top it is possible to perform a search by topic keywords or names to list the contribution to the field.

Projects

1) fondazione per la ricerca sulla fibrosi cistica (#ffc#6/2021); 2) fondazione cariplo/telethon joint call 2021. 3) pra: combining genetic variants and metabolic factors to improve risk prediction in metabolic associated fatty liver disease 4) effects of resveratrol and polydatin on mesenchymal stem cells from dental tissues and their possible application in bone formation. 5) PON ricerca e innovazione 2014-2020 asse i "investimenti in capitale umano"- azione i.2 "mobilità dei ricercatori" "resveratrol and polydatin effects on bone tissue metabolism: mscs osteogenic induction and regenerative potential".

Key elements/strengths

A) Strutture: Laboratori di Patologia Generale, di Patologia Orale, Centro di Ricerca "Emanuele Altomare", C) Network And Collaborations: University of Palermo, Bari, Trieste and La Sapienza. Memory of Understanding with the Anadolu University, Eskishir, Turkey. Collaboration with Cavalcanti-Adam, E.Ada, Group leader of the Institute for Physical Chemistry Department of Biophysical Chemistry, Heidelberg, Germany; CINBO (Consorzio Interuniversitario per la Bio-Oncologia), University of Melbourne, Mississipi, Tokushima, Hiroshima, Umea, Oulu

The participants have wide background experience in development of prognostic models based on several features of diseases, in particular head and neck squamous cell carcinoma. Previous developed model accounted of genetic features, mutations, mRNA expression and histological signatures, able to improve current standard TNM staging system stratification, highlighting their potential role in clinical managing of patients. The UNIFG researchers have background experiences in study of proteomics, genomics on several biological matrices, such as saliva, blood, urine.

The training offer 2019-20 is divided into three cycles: 21 three-year degree courses; 12 master's degree courses; 3 single-cycle master's degree courses. The university also offers further learning opportunities: 2 first level masters; 6 second level masters; 14 advanced courses and 16 schools of specialization in the medical area.

UNIFG researchers will bring their expertise in some spokes of the project, such as spoke 1, spoke 4, spoke 6, spoke 7. In spoke 4 - **TASK 2.1 Title:** Alternative matrix for biological monitoring of inorganic lead and cancer (Saliva as potential noninvasive alternative) Lo Muzio, Troiano e Ranieri will perform circulating extracellular vesicles (EV) profiling in solid cancer (breast, head and neck, GI) together scientists from UniMoRe, while Mori and Pannone will collaborate to the activities of WP4. In spoke 6 UNIFG will collaborate to the activities of WP 1- Task 1.2, WP2 - Task 2.1, WP 3 – Task 3.2, WP 4 – task 4.1, in spoke 7 UNIFG will collaborate to the activities of WP1 task 1.1.1 At the following link the list of awarded projects showing the ability of the Unit to manage and implement projects https://www.unifg.it/it/ricerca/finanziamenti-alla-ricerca/proposte-progettuali. With reference to the area of the partnership, several projects have been awarded such as 3rd Health Progamme, PRIN 2015, Bando 'Ricerca Finalizzata,2011-2012', Bando Aiuti a sostegno dei Cluster Tecnologici Regionali per l'Innovazione, A.I.M ATTRACTION AND INTERNATIONAL MOBILITY, PRIN 2017 ec

National and international collaborations in the area of PE6 specialisation

University of Palermo, Bari, Trieste and Roma"La Sapienza". Memory of Understanding with the Anadolu University, Eskishir, Turkey. Collaboration with Cavalcanti-Adam, E.Ada, DMD, MS, PhD, Group leader of the Institute for Physical Chemistry Department of Biophysical Chemistry, Cell Adhesion group, Heidelberg, Germany; CINBO (Consorzio Interuniversitario per la Bio-Oncologia), University of Melbourne, University of Mississipi, University of Tokushima, University of Hiroshima, University of Umea, University of Oulu; SIPMO (Società Italiana di Patologia e Medicina Orale)

UNIVERSITÀ DEGLI STUDI DI CATANIA - Hub founder and Spoke's Affiliate
Short Name: UNICT

General information

Founded in 1434, the University of Catania (UNiCT) is the oldest university in Sicily. Currently, it has more than 42.000 students, 1.034 professors, 216 researchers and 1.125 administrative staff. The UniCT educational system is run and overseen by 17 Departments, a Medical School and 2 additional educational units located in the cities of Ragusa and Syracuse. Another special unit is the "Scuola Superiore di Catania", an excellence education center founded in 1998 that offers a variety of studies including analysis, research and experimentation to selected brightest students. The *Scuola* has its own laboratories and invests in industrial research in collaboration with many firms of the "Etna Valley".

Organization skills

The University of Catania governance is made up of a Rector, an academic senate, a board of directors and auditors, an evaluation body and a director general. The Central Administration is made up of 12 Administrative Divisions, each of them dealing with a particular sphere of activity and internally split into various organizational units (sectors, services, offices) in charge of specific tasks. The Research Division is organized in order to provide professors and researchers with all the support that is necessary to carry out their scientific activities, including administrative, organizational and managerial assistance throughout the life cycle of research projects. The Research Division works closely also with all other administrative offices involved in the management of the research projects both at the central and departmental level. The University of Catania uses an economic-patrimonial accounting (or accrual accounting) that leads to obtaining clear view of all single and consolidated financial statements, budget and financial accounting reports, and a three-year economic financial plan to guarantee the sustainability of all the activities. The U.P.B. (*Unità Previsionali di Base*) are the main articulations into which the revenues and expenditures are divided. These units are identified so that each of them corresponds to a single administrative responsibility centre, which is entrusted with their management.

National and international project management skills

UniCT pays great attention to research and a remarkable part of its resources is allocated, every year, to fund research projects in all scientific fields. It also supports young researchers in all departments by providing, each year, about 200 research grants to young fellows. UniCT is strongly committed to implement EU policies for the development of scientific careers and the principles of the European Charter of Researchers and the Code of conduct for recruitment. To this end, its Research Division hosts one of the 18 Italian Mobility Centers participating to the EURAXESS network, created by the European Commission to support international mobility and careers' development. UniCT has also an intensive collaboration with research organizations and enterprises present on the territory, which has led to the implementation of many joint research projects and activities

Technology transfer capacity - patents - spin-off

Great attention is paid to the exploitation of research results through the management of its patents and the creation of "spin-offs". UniCT has a long experience of participation, both as coordinator and/or partner, to international, European and Italian projects. It has been the recipient of funds from EU framework Programs and other international and Italian programs since the end of 90's. UniCT is currently participating to many projects funded by Horizon 2020, Horizon Europe and many other Italian and European research and training programs related to all scientific fields (such as ERA-NET actions, INTERREG programmes, LIFE+, ITALIA-MALTA projects, ENI ITALIE-TUNISIE projects, ERASMUS+ initiative, etc.). In the last 5 years, about 300 projects have been funded, with a total financial contribution of almost € 47.000.000,00.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine

UniCT researchers involved in the Project have obtained several grants to support projects related to the field of diagnostics and innovative therapies for precision medicine. Notable examples are financed projects entitled: a) "Molecular oncology: genetic variations to evaluate the response to precision therapies" (€ 2.500.000,00); b) "New antimicrobials obtained from compounds of natural origin" (€ 1.219.120,47); c) "Association of biomolecules extracted from Sicilian blood oranges to reduce the side effects of anticancer therapy" (€ 600,000.00); d) "Dissecting and targeting a novel immune-metabolic checkpoint in multiple myeloma" (€ 583.220,00); e) Effects of Lactobacillus Rhamnosus GG * (ATCC 53103) in oncology" (€ 450.000,00); f) "Role of the immunological impairment in progression from MGUS to Multiple Myeloma" (€ 314.209,00); g)

"Evaluation of microvascular changes of psoriasis during treatment: from molecular basis to high resolution skin imaging" (€ 210.00,00); h) "Tackling biological barriers to antigen delivery by nanotechnological vaccines (NanoTechVax)" (€ 182.400,00); i) "Prevalence and profile of non-responders alopecia areata patients: a multicenter retrospective and prospective analysis from hospital-based aa clinics" (USD 152.359,00); j) "Analysis of circulating microRNAs in the early diagnosis of bladder cancer" (€ 127.500,00); k) "Reduction of breast cancer recurrence in women: lifestyle strategies and microRNA expression" (€ 68.000,00).

Key elements/strengths

With respect to the topic of diagnostics and innovative therapies in precision medicine, UniCT features several skills, infrastructures networks and services for the activities in support of the current partnership, including and not limited to: a) patient registers (e.g., Prader-Willi Syndrome Registry, Diasmoke Registry, Eur IPF, Eur ILD, and national Registries for NSCLC, hereditary breast and ovarian cancer, CML, Ph-negative myeloproliferative disorders); b) ongoing trials (e.g., on new treatments for diabetes, thyroid cancer, pulmonary disease, smoking cessation, multiple myeloma, Grawes' ophtalmopathy, and several other ongoing studies); c) active national and international research networks (e.g., Italian Alliance against Cancer, European Myeloma, International Microdeletion/Mutation, CoEHAR, Italian Network for Public Health Genomics, GENISAP, IC2PerMed, Birthcohorts.net, Cardio-pulmonary Institute, Lung Center Justus, Italian Society of anti-infective Therapy, Italian Society of Pulmonology, FIBRONET, ISILD, IPF, ILD, and more); d) patient associations (Regional Prader-Willi Syndrome Association, LIAF, FAND, Federazione Diabete Sicilia, Catania Section of the Associazione Italiana Linfomi e Leucemie, Respirare ONLUS for Rare Interstitial Lung Diseases, and more); e) biobanks (e.g., CoEHAR; Biobanking and Biomolecular Resources Research Infrastructure, Hematology biobank at Catania Policlinic; and f) facilities (e.g., Catania Policlinic [CTU], Bio-nanotech Research and Innovation Tower [BRIT])

Previous experiences, scientific and design skills of participants concerning the topic of diagnostics and innovative therapies in precision medicine in terms of innovation and technology transfer include, for example: a) expertise in targeting endocrine-related tumors with special regard to the relevance of the Insulin/IGF axis, insulin receptor isoforms and RAGE pathway; b) CoEHAR, a Center of Excellence for the Acceleration of Harm Reduction established in 2018 to accelerate efforts to study and reduce health impacts and deaths from smoking through use of approved pharmacological approaches as well as innovative technology, featuring a 5-year collaboration with a University spin-off, Eclat srl, for technological transfer.

Researchers from UniCT in support of the current partnership have *competences* on the following aspects: cancer (cell biology, genetics, histology, molecular and cellular mechanisms, non-invasive imaging, innovative diagnostic and prognostic tests and models, next-generation therapies, nanoparticles, robotic surgery, metabolomics, proteomics), metabolic disease (genetics, prevention), cardiovascular disease and respiratory disease (genotyping, phenotyping, prevention), rare genetic diseases (genotyping, phenotyping, management), behavioural and weight management, digital health, data management and machine learning; public health genomics, personalized nutrition, precision health and public health informatics.

Researchers from UniCT in support of the current partnership also have study *collaborations in place* with Departments and Institutions in the United States (e.g., University of California, University of Philadelphia, Huck Institutes of the Life Sciences, George Mason University, St. Jude Children Research Hospital, Duke University, Temple University, UCLA, University of Florida), Europe (e.g., Erasmus Medical Center, Amsterdam University Medical Center, University of Antwerp, University of Giessen, University of Heidelberg, Mossakowski Medical Research Centre Polish Academy of Sciences, University of Southampton, Institute for Tobacco Studies [ITS], St. Anne's University Hospital Czech Republic, University of Barcelona, University of Manchester, King's College London, Imperial College London, Royal Brompton Hospital London, INSERM, Paris) and Middle East (e.g., Sultan Qaboos University).

UNIVERSITÀ DEGLI STUDI DI CAGLIARI - Hub founder and Spoke's Affiliate
Short Name: UNICA

General information

Established in 1620, the UniCa is a public multidisciplinary research University, the largest Higher Education Institution in Sardinia. With more than 25,000 students, UniCa plays a strategic role in the development of knowledge across the regional ecosystem of Sardinia. With more than 1,000 researchers, 279 laboratories (equivalent to 38.845 sqm), 17 PhD programmes, and more than 100 visiting professors per year, UniCa encourages investigation across disciplinary boundaries. UniCa commitment to equality and sustainability are evidenced by the implementation of a gender equality plan since 2018, and by promoting interdisciplinary training and research projects on SDGs of the UN's 2030 Agenda and by its position as 392° in 2021 UI GreenMetric World University Rankings. At the national "AQ/AVA/ANVUR periodic accreditation of universities and their programmes", UniCa was awarded by MUR Decree n 135/2019: fully satisfactory-B. UniCa is organized in 15 research Departments: 4 at the forefront in life sciences research and integrated medicine, 6 actives in Physical Sciences and Engineering, and 5 dedicated to Social Sciences and Humanities. UniCa offers international PhD programmes in Molecular and Translational Medicine, in Life, Environmental and Drug Sciences, in Neuroscience, and in Chemical Science and Technology. The Depts of Medical Sciences and Public Health, of Biomedical Sciences, and of Surgical Sciences are integrated medical depts: strictly linked to the Azienda Ospedaliero-Universitaria of Cagliari (University Medical Hospital) which has as its objective the coordinated development of medical assistance, teaching and research. UniCa hosts residency training in medical genetics, diagnostic radiology, rheumatology, clinical pharmacology and toxicology, medical oncology, in hematology, gastroenterology, clinical pathology and biochemistry, and in clinical immunology and allergy. UniCa PE researcher team comprises of preclinical and clinical researchers: concerning the publication track record of the last 10 years, the team has an average H-index of 20.8, 2007

Key elements/strengths

UniCa research is supported by 10 service centers, two divisions for the financial and administrative support of research, and 35 research infrastructures (RI). Specifically, the most relevant to the PE topics:

CeSAR Service Centre for Researche stablished in 2017, is a multidisciplinary RI, that brings a range of essential research support services to UniCa researchers but also to external users; hosts equipment of the highest technological level for advanced analysis in the fields of chemistry, biomedicine (genomics, proteomics, metabolomics, flow cytometry, immunology, fluorescence/electron microscopy, cell culture) and physics (X-ray diffraction facility, spectroscopy, magnetometry, electron microscopy, NMR facility). It contributes to the creation of national/international networks, international projects, innovation and scientific-technological development at national and international level. CeSAR offers research training programmes to students, PhD students and users.

CREA Centre for Innovation and Entrepreneurship Activities: established in 2016, it supports an entrepreneurial culture, enhances inter-disciplinary activities and creates innovative business projects through the contamination among diverse areas of studies. Nominated in 2017 by MUR, national leader of Italian Clab Network.

CeSASt: established in 2018, manages all UniCa's animal care facilities, in order to standardize research protocols, optimizing the management of resources, as well as to ensure optimal conditions for carrying out experimental research in compliance with current regulations. It offers a wide range of equipment for preclinical studies.

Clinical Trials: 2019-present: NIVO-COLLECT-Nivolumab in Oncology Practice: Data collection on safety and effectiveness"; 2019-present: cBO 16348/HERA "A randomised three-arm multi-centre comparison of 1 year and 2 years of Herceptin® versus no Herceptin® in women with HER- positive primary breast cancer who have completed adjuvant chemotherapy; 2016 GIM15-NEPA "One day antiemetic prophylaxis of NEPA (netupitant plus palonosetron) and dexamethasone to prevent chemotherapy-induced nausea and vomiting (CINV) in breast cancer patients receiving a combination chemotherapy of doxorubicin or epirubicin with cyclophosphamide (AC-based regimen)";2016 "fRida" funded by Dompè; 2016 "CINC280B22011" funded by Novartis; 2015 -Trial "Asteroid-2 - 17541" funded by Bayer; 2012-2015 "LUX-Head & Neck 1-A randomised, open-label, phase III study efficacy and safety of oral afatinib (BIBW 2992) versus intravenous methotrexate in patients with recurrent and/or metastatic head and neck squamous cell carcinoma who have progressed after platinum-based therapy".

UniCa Industrial Liaison Office (ILO): since 2005, knowledge transfer activities are managed by ILO, which supports UniCa's exploitation of research results. ILO serves a wide range of industrial sectors, from

pharmaceuticals to cosmetics, from agribusiness to materials, from engineering to economics. From the skills of UniCa research groups, 24 new spin-offs are born; they bring latest scientific and technological discovers to the market. In particular, spin-offs cover various fields including healthcare and biomedical (Maga Orthodontics srl), and chemistry, physics, new materials and manufacturing processes (Bacfarm). The patent portfolio consists of 44 patent families, 52% of which are in health and biomedical; 25% in chemistry, physics, new materials and manufacturing processes; 16% in informatics, electronics and communication systems; 5% in energy and renewable sources; 2% in aerospace. Several patents are more related to PE 6: in the health and biomedical area (e.g. method for the diagnosis and/or prognosis of biliary tract cancer; in vitro diagnosis of Multiple Sclerosis; procedure and kit for the early in vitro diagnosis of Parkinson's disease; double-layer lipid vesicles containing adrenaline, for use in the treatment of cardiac emergencies; method for detecting and/or prognosis colorectal neoplasms), some in informatics, electronics and communication systems (e.g. sensorized garment; manual activity level monitoring system for hand and wrist and monitoring method; procedure for verifying the correct functioning of the implantable cardiac device; an organic transistor-based system for electrophysiological monitoring of cells and method for the monitoring of the cells; an apparatus, a system and a relating method for local or remote rehabilitation and functional evaluation of the hands).

UniCa is currently involved in 38 H2020 projects and 6 Horizon Europe. UniCa most relevant EU projects related to the PE area: BioMeld (HORIZON-CL4-2021-DIGITAL-EMERGING-01-2), Search and Rescue (H2020-SU-SEC-2018-2019-2020); ProDGNE (EJP RD - JP Rare Diseases JTC 2020), SUPERA (H2020-SwafS-2017-1), Dr VCoach (H2020-MSCA-IF-2020), DISCHARGE (FP7-HEALTH), HYMEC (FP7-NMP), i-FLEXIS (FP7-ICT), ND4BB TRANSLOCATION(IMI1 - Call 6), 3TR (JTI-IMI2-2018-14-two-stage), C4C (H2020-JTI-IMI2-2016-10-two-stage), RESET-ME (ERANET, JPIAMR), Capice (H2020-MSCA-ITN-2016). Other international funded projects relevant to PE topics: 1 National Institute of Health USA project (AI136799), MILCH (LIFE + 2018), PROSPECT (EuropeAid DG DEVCO 2018), TEAMS (MAECI Italia-Israele 2019). At national level, UniCa has 55 ongoing projects under the PRIN calls of the last 5 years for a budget of € 6.260.181; specifically related to the topics of the PE: PRIN 2020 GLUCOMFORT. Other relevant national projects: MIUR – PON RI 14-20"TEX-Style", ARS0100996, POC project ORGANOI3D, 2012 FIRB grant "New molecular and cellular mechanisms involved in colitis-associated colorectal cancer". Projects funded by Foundations and Companies: 2012 IG AIRC grant "T cell plasticity in colitis-associated colorectal cancer"; 2018 ASPIRE (Pfizer) research grant AVATAR; Research Project Genomic Parameters Related with the healing effect of the TMO" funded by CARIPLO; 3 projects funded by CSL-Behring Italy. Total budget of projects funded by Regional Government: € 2.887.971; total research funding by the RFO Sardinia Foundation: € 176.885.

Long-term international and national collaborations: the European Society of Medical Oncology (ESMO), Geffen School of Medicine at UCLA, Los Angeles, USA, IRCCS San Raffaele, Sanofi Aventis, Astrazeneca, Glaxo Smith, Basilea Pharmaceutica, Qpex Biopharma, biotech GenomeUp, CyberTech - Engineering group, Roche Italy, IBM, Menarini Group. UniCa is affiliated partner of the spoke Biophamacology, involved in the project's proposal ECS_00000038 "e.INS Ecosystem of Innovation for Next Generation Sardinia" which has passed the first selection step: it consists of the Leading Partner CyberTech – Engineering Group, and the affiliated Partners UniCa (CESAR), Menarini, GenomeUp.

UNIVERSITÀ DEGLI STUDI DI VERONA - Hub founder and Spoke's Affiliate
Short name-UNIVR

General information

The University of Verona (UNIVR) is a State institution with about 27,500 students and 1,500 teaching and administrative/technical staff. Founded in the 1950s, it was established as autonomous State University in 1982.

Organization skills

UNIVR is organized in 12 Departments and 3 Schools, offering a total of 69 Degree and 16 PhD programmes. UNIVR hosts an Euraxess Contact Point and a Europe Direct contact point and has recently adopted a Gender Budgeting and a Gender Equality Plan. All UNIVR gender policies and documents are available at https://www.univr.it/it/politiche-di-genere.

National and international project management skills

UNIVR has many projects supported by the major national programmes and funders, e.g. Ministry of Research and Universities, Ministry of Health, Regione of Veneto, the private funders AIRC, Intesa San Paolo, Fondazione Cariverona.UNIVR involvement in EU Framework Programs has constantly improved, as shown in the table below.

Updated on December 4, 2021	**FP7**	**H2020**
Net EU Funding	20.71 Million €	29.59 Million €
No. of projects	59 (35.6% as coordinator)	55 (52.7% as coordinator)
ERC PIs	5	9
MSCA Actions	15	23

Under these EU programmes, the first 10 collaborations are with: CNRS; Fraunhofer; Commissariat à l'Energie Atomique et aux Energies Alternatives; CNR; ETH Zürich; Karlsruher Institut fuer Technologie; Max-Planck-Gesellschaft; Danmarks Tekniske Universitet; Katholieke Universiteit Leuven; United Kingdom Research and Innovation. Additional international funding comes on programs from NIH-USA.

Technology transfer capacity - patents - spin-off

From 2005 to today, n. 31 spin offs have been established, and there are 27 patent families owned by the University of Verona. As for the enhancement of these patents, the Liaison Office periodically updates the data sheets of these technologies within the Knowledge Share website (https://www.knowledge-share.eu/), a platform dedicated to the enhancement of the results of the research generated by universities, IRCCS and EPR on the national territory, with the aim of making the contents of patents easily usable, to convey in a clear and simple way the advantages that technologies can bring within the sectors of reference and put in contact the world of business, investors and innovators with researchers.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

Key elements/strengths

Two Centres of UNIVR are of specific interest to the present PE6 proposal on Precision Medicine:

1- The ARC-Net (Applied Research on Cancer - network) Centre (www.arc-net.it) founded in 2008, designed for the identification and clinical validation of diagnostic/prognostic markers and therapeutic targets in oncology. The Centre boasts a biobanking facility with primary cancer/normal tissues with complete clinical pathological information and molecular characterization using targeted next generation sequencing. The biobank has also accrued a patient patient-derived cancer models, including over 250 xenografts and 42 organoids complete of genomic and transcriptomic characterization. It is a certified partner of BBMRI.it, the National Node of the European Research Infrastructure of Biobanks and Bio Molecular Resources (BBMRI-ERIC). It is also partner in the Biobank and Cohort Building Network (BCNet) created by the International Agency for Research on Cancer (IARC - WHO) (https://bcnet.iarc.fr). The centre has all equipment necessary for the execution of activities including (i) next-generation sequencing platforms (10X Genomics, Illumina, Ion Torrent) (ii) digital profiling platform (Nanostring ncouter); and (iii) Digital Image assisted Cell sorting DEPArray and FACS facility for cell sorting and immunofluorescence.

2- The Centro Ricerche Cliniche (CRC) (www.crc.vr.it) founded in 2005 with the mission to assist both commercial and non-profit research organizations in early clinical drug development and conduction of clinical trials, including Phase I and Proof-of-Concept trials. CRC is legally a state organization under Verona University and Teaching G.B. Rossi Hospital ownership. A full range of Phase I services can be provided (e.g. first time in human, drug-drug interaction, pharmacokinetics, food effects, bioequivalence) by means of an in-house Clinical Pharmacology Unit (CPU), equipped to a high standard expertise and operating in close connection with

specialists. The CPU has 20 beds with bedside monitors for ECG, BP, HR and SO2 saturation. These are arranged into 10 rooms with 2 beds. Also 10 armchairs are available in a separated room for day-hospital therapies. The majority of staff have more than 10-year experience managing trials with Heathy Volunteer subjects and Patients.
Since June 2012 CRC has been carrying out a total of 240 industry-sponsored or investigator-initiated clinical trials in oncology and other different therapeutic areas. The CRC is in full compliance with International Good Clinical Practice (GCP) standards and it has a UNI EN ISO 9001:2015 certification carried out by BSI S.r.l. Since October 2016 CRC meets the requirements according to the new Italian regulation for Phase 1 Units and it is included in the Phase 1 Unit list available on the Italian Regulatory Agency (AIFA) web site.

UNIVR has implemented projects in fundamental and applied research in the area of PE6 Innovative Diagnostics and Therapies in precision medicine. The following projects are worth mentioning for their relevance to PE6, see table below:

- "Accelerating precision medicine in pancreatic cancer through definition of novel classifications and mol Ref.: Scarpa Aldo; funded by AIRC; € 1,147,000; 02/01/2022 - 01/07/2024
- "ASTEROID- breASt Thyroid cancERs endOcrIne Disruptors - Gene/environment interactions in brea cancers: defining the biological role of and actioning endocrine disruptors (ED) and lifestyle to de therapeutic/preventive interventions."; Ref.: M. Milella, funded by MUR PRIN; € 168,381; 22/03/2022 - 22/
- "S.T.E.P.S. Shared Time Enhances People Solidarity"; Ref.: F. Schena; funded by EU UIA; € 376,680; 30/06/2023
- "Organoid Models Development Center for ATCC of Biliopancreatic, Gastric and Colorectal Cancer". Re funded by NCI-USA (grant HHSN26100008); $ 1,651,792; 15/06/2017-31/12/2022
- "ADAIR - Novel biomarkers for air pollution effects in Alzheimer's disease"; Ref.: R. Giugno; funded by 146,994; 01/01/2020 - 31/12/2022
- "Mechanistic basis for improving the efficacy of targeting TGFβ pathway in poor prognosis pancreatic ca Ref.: D. Melisi; funded by AIRC; € 1,025,915; 02/01/2020 - 01/01/2025
- "Emerging players shaping the responses of tumor inflammatory infiltrate"; Ref.: M. Cassatella; funded by 117,146; 25/09/2019 - 25/09/2022
- "TUBE - Transport derived Ultrafines and the Brain Effects"; Ref.: R. Giugno; funded by H2020 SC 01/05/2019 - 30/04/2023
- "BRCA and beyond: Dissecting BRCAness and overcoming therapeutic resistance"; Ref.: A. Scarpa; funde of Health; € 78,000; 10/06/2021 - 09/06/2024
- "Identifying the immunological role of the various neutrophil subpopulations in human tumors" Ref.: M. Cas by AIRC; € 757,900; 02/01/2018 - 01/01/2023
- "A Biomarker-driven Therapeutic Strategy for Esophageal Cancer"; Ref.: D. Melisi; funded by Ministry 450,000; 14/08/2019 - 13/12/2022
- "Italian Cancer Genome Project"; Ref. A. Scarpa; funded by FIRB; €8,000,000; 24/02/2011- 04/05/2018

In the specific field of Precision Medicine, main topic of the proposed PE6 initiative of the Heal Italia consortium, the University of Verona through the ARC-Net Research Center has over time acquired a particular know-how in the coordination and management of multicentre projects supported by competitive funding, being: i) Partner of the International Cancer Genome Consortium (ICGC) and leader of the Italian contribution to this project, funded by MIUR in 2010-2016; ii) Founder and part of the organizing committee of the application phase of the Consortium's activities, ICGC-ARGO (Research Acceleration in Genomic Oncology; www.icgc-argo.org) which aims to create a Precision Oncology aimed at the development of useful applications in clinical practice; iii) Coordinator of the seven-year multi-center project funded by AIRC (5x thousand n.12182) in 2013-2020 for the search for diagnostic and therapeutic markers in hepatobiliopancreatic tumors; iv) Coordinator of the Center of Excellence on Neuroendocrine Tumors certified by the scientific society ENETS (European NeuroEndocrine Tunour Society); v) Certified partner of the Italian node (BBMRI.IT) of the European infrastructure for biobanks and biomolecular research BBMRI (biobanking and biomolecular research infrastructure). In addition, in the last 10 years, UNIVR has invested more than 1.8 Million € to finance over 250 incoming mobilities for Visiting Professors and Visiting Researchers and over 180 outgoing mobilities for UNIVR academic staff.

ISTITUTO SUPERIORE DI SANITÀ - Spoke's Affiliate
Short Name: ISS

General information

ISS is the main Italian research institute in the biomedical and public health field, as well as the technical and scientific body of the Italian National Health Service (Servizio Sanitario Nazionale, SSN). At the ISS, 1800 people, including researchers, technicians and administrative staff, work daily with the aim of protecting the citizens' health. The research management at the ISS is structured with 6 Departments, 16 National Centres, 2 Reference Centres, 5 technical-scientific services and a Notified Body for the assessment of the suitability of medical devices.

Organization skills

ISS cooperates with the Ministry of Health, the Italian Regions and the whole SSN, to accomplish health policies on the basis of scientific evidence, in the field of prevention and health promotion to the fight against cancer and chronic and neurodegenerative diseases, from autism to rare diseases, from infectious diseases to pathological addictions.

ISS is the technical body for evaluation of research protocols involving animals, and ISS reseachers have unique expertise in the applicative and theoretical aspects of the implementation of the Directive 2010/63/EU. This includes the application of the 3Rs Principle to specific experimental protocols, with the related cost/benefit analysis, and more theoretical argumentation in the field of animal ethics.

ISS hosts the nationa nodes of two ESFRI-Research Infrastructures in Life Sciences, namely Ecrin and Eatris, devoted to clinical trials and traslational research, respectively, as well as the secretariat of BBMRI, the ESFRI-Research Infrastructure for Biobanking. ISS also participates in the European Research Infrastructure ELIXIR (European Life-science Infrastructure for Biological Information) which deals with infrastructures for high-intensity data analysis and in METROFOOD, a Research Infrastructure created to harmonize scientific research in the field of food quality and safety and promote the metrology for food and nutrition.

National and international project management skills

ISS has a longstanding experience in international and European research projects, as partner as well as leader in several of those. The Institution, in order to sustain the burden of administrative obligations, adopts an integrated financial and economic-equity plan of accounts, in compliance with the plan of accounts referred to in the decree of the Italian Minister of Economy and Finance of 25 January 2019 (in line with the European system). The financial system currently in use is SCI (integrated accounting system). The ISS, by using SCI, manages a separate accounting system for all transactions relating to an operation, make all documents available for possible inspection, provides information on the onset and completion of operations and all data necessary for the purposes of monitoring.

Technology transfer capacity - patents - spin-off

The ISS intellectual property portfolio, consists of 59 patent families, at national and international levels; some of these are jointly owned with other research bodies or companies, reflecting the intense scientific collaboration of the institution at an international level. In particular, with George Mason University (GMU), Maryland, USA, the ISS co-owns 24 patent families.

Many patent applications have been granted or are in the process of active internationalization in the most important international markets. In addition, the ISS has supported the creation of a spin-off called "Cardionics". Cardionica was born in September 2014 and won the "Best Social Innovation Project" category of the Lazio Innovator award.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

In the frame of Precision medicine, ISS has a long-lasting experience in developing multiple advanced approaches for individually tailored prevention, diagnosis, screening and drug delivery in relation to metabolic, cardiovascular, cancer and rare diseases. Main in vitro approaches include patient-derived 3D and 4D cell cultures, co-coltures of cross-talking tissues, microfluidic and organ-on-chip devices. As supporting tools, also organotypic slices from animal models have been implemented. In addition, experimental studies on personalized therapies are performed in the FaBio Cell GMP facility at ISS. Omics and computational data analysis integrating results from the different approaches are supported by the ISS Core Facilities. The experience in the field is proved by the several projects carried in the last years, which are listed in a section below.

Key elements/strengths

The ISS is endowed with several facilities, including animal facilities for conventional or SPF experimentation; a cell factory (FaBioCell), specialized in the production of cells for human clinical trials, with a major experience in immunotherapy. In addition, the ISS Core Facilities include: a state-of-the-art MRI for small animals; NMR spectroscopy for metabolomics; instruments for protein investigations (mass spectrometry, Biacore, Reverse Phase Protein Arrays); confocal and electron microscopy (transmission, scanning, and correlative); several multiparameter cytometers, including a mass cytometer, and cell sorters; Electron Paramagnetic Resonance for studying free radical and inflammation; an NGS area. In 2021, a new Level 3 Biosecurity Laboratory (BSL3) and a new animal house have been open. Finally, the Core Facilities include a Scientific Computing area, with competence in bioinformatics, biostatistics, and artificial intelligence. The ISS owns a population biobank based on the collection of biological specimens belonging to the general population, enrolled in epidemiological studies conducted between 1993 and 2019 (Progetto CUORE; https://www.cuore.iss.it/). The biobank contains more than 290,000 specimens collected in 40,000 donors of all ages, associated with the individual data collected at the time of enrolment (lifestyles, risk factors, conditions at risk, pathologies and environmental factors). All donors are followed over time for morbidity and mortality. A second important Biobank (the Italian Twin Register biobank) contains biological material (plasma, buffy coat, erythrocytes, serum, umbilical cords) from over 3000 newborns and mothers from the Piccolipiù cohort, for a total of over 70,000 aliquots. It also has DNA from saliva of about 2000 twins (children and adults), and other specimens (plasma, buffy coat, serum) of about 400 twins (adults). All individuals also have a deep phenotype characterization (health information, lifestyle factors and environmental exposure). ISS hosts several patient registries (https://www.iss.it/en/registri-e-sorveglianze).

The ISS researchers involved in the present proposal have a extended experience in the topic of the *Extended Partnership* PE6; such experience is proved by the several research projects, as follow:

AIRC - 20744 “Silent seeds at the root of cancer: targeting quiescent therapy-resistant stem cells in lung and colorectal tumors”; TRANSCAN-2(JTC 2016). “Early Detection of Prostate Cancer by Liquid Biopsies-(Prolipsy)”; MUR PON_ARS01_00492. “Liquid biopsy in solid tumor management (BiLiGeCT)”; Regione Lazio. “Terapia antitumorale del melanoma metastatico: sviluppo di nano-particelle immunocompetenti in grado di veicolare efficacemente il miR 126/126* nelle cellule bersaglio”; AMD1 to Memorandum of understanding on cooperation in cancer research between IARC and ISS “Mechanisms of carcinogenesis in colorectal cancer: the role of bacterial toxins”; NIH-NIDCR; R01DE029471. “Determining How A Werner Helicase (WRN) Tumor Suppressor Complex Regulates The Human Papillomavirus 16 Life Cycle”; NIH-NCI; R01CA232425. “The role of human RAD52 protein in genome stability”; AIRC- IG21428.” Novel functions of RAD52 in protection of perturbed DNA replication and implication for genome instability of cancer”; Italian Ministry of Health; RF-2016-02362022. “Investigating the molecular mechanism leading to genome instability in tumors: analysis of role of the replication stress response and its potential for early diagnosis and target therapy of cancer”; Italian Ministry of Health, RF2018-12366565. “Search of a prognostic biomarker of cardiovascular disease in the Italian population: focus on the potential role of circulating microRNA”; Italian Ministry of Health, RF 101018317. “Population Health Information Research Infrastructure H2020 IBA –INFRA-CORONA-2020"; AIRC-21366. “Exploring the antitumoral functions of IL-33-induced eosinophils for improving chemoimmunothrapy against melanoma”; Nando & Elsa Peretti Foundation.” Autophagy and mitochondria influence susceptibility of lymphoma cells to target-therapy"; CJD Foundation grant 2019. “Investigating the influence of CYP4X1 gene of age at onset of CJD as potential preventive therapeutic approach”; HORIZON-INFRA-2021-SERV-01 -#101058620 – 2022. “canSERV- Providing cutting edge cancer research services across Europe”; European Commission Erasmus+ ADVANCE “Educating the next generation of Advanced Therapy (ATMP) professionals and advancing ATMP development”; Fondazione Fibrosi Cistica Ricerca Onlus- FFC8 2021. “Theratyping Cystic Fibrosis”.

ISTITUTI FISIOTERAPICI OSPITALIERI – IRCCS - Hub founder and Spoke's Affiliate (Short Name: IFO-IRE) (

General information

IFOs are a public law entity established by R.D. of 04/08/1932, n. 1296, a center of excellence for cancer research, prevention and treatment, recognized as a Scientific Research and Treatment Institute since 1939 and reconfirmed with D.M. of the Ministry of Health of 8 May 2020 relating to the discipline of "oncology" as the Regina Elena National Cancer Institute IRCCS and "dermatology" as the Santa Maria and San Gallicano IRCCS Institute. It bases its mission on the development of new methods of cancer prevention, diagnosis and treatment through basic, preclinical, clinical and translational research.

According to the Strategic Research Plan (2020-2022) adopted with resolution 1281 of 11 December 2020, the main objective of the Institute is the care and translational management of cancer patients (PRESTO). With this focus, the purpose is on a better understanding of the distinct changes in patients' tumors, aimed at administering personalized therapies based on principles of medicine and precision surgery, to give effective answers to the health needs of the assisted population, through scientific and technological innovation.

Organization skills

The IRE Department of Research and Advanced Diagnostics (RIDAIT) and Clinical Department and Oncological Research, with their laboratories currently consists of over 220 units including senior executives, medical/biologist researchers, technicians, contract staff, fellows and PhD students The Institute's clinical research activity is supported by the expertise of translational research interest groups: Genomics, Non-Coding RNAs (NCR), Melanoma, Rare Tumors, Brain Tumors, Immunotherapy, Artificial Intelligence and Imaging. The Molecular Tumor Board (MTB) to date has treated over 150 patients, suffering from various cancers (lung, colorectal, breast carcinomas) or rare as sarcomas). The Institute's Immuno-oncology task force with resolution no. 475 of 9/4/2020 can officially count on seven Translational Interest Research Groups (TRIG). The Bioinformatics group uses different analysis systems through a complex combination of workflows in High Performance Computing. The Unit Porphyrias and Rare Diseases ISG (Clinical Dermatologic Department) is devoted to the diagnosis and management of dermatological rare diseases with non-invasive diagnostic techniques for skin, innovative treatments and molecular diagnosis and follow-up of rare skin conditions.

National and international project management skills

IFO-IRE is involved actively in high quality national and regional collaborative research programmes with over 80 projects. IFO has explored and increased its opportunities within the Horizon program, which is difficult to access for a clinical / care research reality such as IRCCS, traditionally linked to grants from the Ministry of Health and private sponsors. Actually, the Institute is the beneficiary of 5 European grants and 5 submissions, 4 of which on the Cancer Mission Calls.

Technology transfer capacity - patents - spin-off

IFOs have a Technology Transfer Office (TTO), a Patent Commission and a regulation on industrial property and spin-off creation, considered as the first example at a national level for IRCCS from Ministry of Health and Italian Office Patents and trademarks. It manages an active patent portfolio of 4 families of inventions and 20 patents obtained, and 39 MTA/MTDA in the context of research collaborations, framework agreements.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

Since 1939 of experience in carrying out preclinical and clinical research with important translational implications with several national and international publications of its researchers. Dedicated resources: facilities offering a wide range of instruments to investigate the experimental and clinical aspects of the proposed research and commitment of human resources to implement projects. In recent years, IRE, in terms of oncogenomics and personalized medicine, has become a regional and national reference point for NGS diagnostics and high-throughput sequencing. In addition to the equipment of a technologically advanced machine park for the execution of transcriptomics analysis, WES, single cell sequencing, genomics-epigenetics, a group of bioinformaticians has been set up for data analysis in support of the numerous projects that IRE conducts both internally and within the ACC network (Alliance Against Cancer). One of the main results of this investment is the establishment (resolution no.468 of 19/6/2018) of one of the first national Molecular Tumor Boards, for the identification of cutting-edge personalized therapeutic profiles for the treatment of cancer patients who have run out of standard therapies. At IFO robotics is used daily, thanks to the acquisition of

advanced technologies, in the field of Gynecological Surgery, Thoracic Surgery, Otorhinolaryngology Surgery and Hepatobiliopancreatic Surgery, thus moving the theme no longer on the effectiveness of the operation, but on the possible expansion of patients who can benefit from it.

Key elements/strengths

According to the latest institutional census, IRE can count on about 180 equipment/instruments of various sizes, dedicated to Traslational health Research. A preclinical enclosure is under construction at the Institute. In 2021, the IRE institute carried out 34.306 first visits. The institute has 383 active Clinical Studies involving 6370 patients. These studies are divided into 138 interventional studies with 370 patients and 245 observational studies with 6000 patients; 68 studios are for profit and 315 are non-profit; 53 studies are single-center with 2256 patients and 330 studies are multi-center with 4114 patients. BBIRE biobank oncology resolution no. 180 of 14/03/2014: present in the Institute since 2014 in support of medical-scientific research. BBIRE currently stores more than 60.262 samples of biological fluids (whole blood, serum, plasma, PBMC) from more than 2587 patients and more than 18690 tumor tissue samples (Formalin-fixed paraffin-embedded (FFPE), Snap Frozen, Optimal Cutting Temperature (OCT) and Fresh Tissue (96 cases for Organoids) from more than 1635 patients.
IFO is active as a partner on national and regional initiatives in the Life Science area, many of which in collaboration with universities, other IRCCS and local or national PMI, with a focus on precision medicine: Regional Strategic Projects with SMEs and Universities, Research Groups POR FESR, PON MUR, MAECI Projects international collaboration. Actually IRE is beneficiary of ongoing six grants in Horizon 2020 and Horizon Europe. Almost three Networks on Current Research (ACC, RIN, RAMS), to which to add numerous translational research projects on national grants as coordinator (AIRC, Finalizzate, INAIL, ISS, Private grants). Consolidated experience in single-center and multi-center clinical trials, managed with the support of the Clinical Trials Center with ISO 9001: 2015 accreditation, it has been authorized by AIFA since September 2018 to operate as a Center for Phase1-Early Phase Studies (CSEP), which combines experimental and clinical research and clinical research and direct assistance to the patient, carrying out all the phases of the clinical trial, 'early phase' (phases 0, I and II), also in agreement with the clinical departments of the universities.

IFO, in the laboratories of the RIDAIT Departments and in the Clinical area, has equipped itself with indispensable instrumentation for the research activity integrated in the clinic, with a modern and equipped infrastructure for the biobank that holds tissue and liquid samples from cancer patients, up to some of the most recent technologies for “omics” analysis (Nanostring, Hight Throughput Sequencing Machine with Library Generator for single-cell sequencing, fluorescence microscopy, machine for metabolomics) as well as for surgery, such as robotics, stereotaxic radiotherapy, advanced imaging. With these facilities and expertise IFO participates as Spoke in the thematic Spoke1, 3 and 4 with its expertise in molecular profiling and 3D prediction modelling. In the Spoke 8 IFO will play a decisive role clinical validation and implementation of precision medicine approaches in particular versus certain oncologic and metabolic diseases, for available treatments, daily clinical practice, with the patient database and to build new clustering methods.

At national level IFO-IRE is one of the founders of Alleanza Contro il Cancro (ACC), an association established in 2002 by the Ministry of Health of six high-level IRCCS cancer research and treatment centers. IFO-IRE participates in various projects of the "Italian Network of Biobanks for Oncology (RIBBO)" and in "BBMRI-IT" (Italian node of the European research infrastructure Biobanking and Biomolecular Resources Research Infrastructure). Internationally, IFO-IRE is the Referral Center of the World Health Organization (WHO) and a member of the European Organization for Research and Treatment of Cancer (E.O.R.T.C. - Early ClinicaI Trial Group), Digital Institute for Outcomes Cancer Research (DIGICORE), of the Union International Contre le Cancer (U.I.C.C.) and of the European Organization of Cancer Institutes (O.E.C.I.), from which it has received the prestigious accreditation as Comprehensive Cancer Center since 2016. Since 2017 IFO-IRE is the Reference Center of the European EURACAN project (EUropean RAre CANcers) and is currently accredited at the highest level as a Comprehensive Cancer Center (CCC).

ISTITUTO NEUROLOGICO MEDITERRANEO NEUROMED I.R.C.C.S. - Hub founder and Spoke's Affiliate
Short name NEUROMED

General information

INM Neuromed (www.neuromed.it), according to regional and national programming, carries out health care, biomedical, clinical and translational research in neuroscience, confirming itself as a center of national reference. The Institute is accredited with the National and Regional Health Service, for both activities of outpatient and hospitalization, with 41 clinical specialties. It is recognized by the Italian Ministry of Health as Scientific Institute for Hospitalization, Treatment and Research (IRCCS).

Organization skills

National and international project management skills

Technology transfer capacity - patents - spin-off

Research is carried out at the Research centre, and is essentially used to update knowledge and offer patients advanced care. Neuromed research is focused on the study of the molecular mechanisms involved in the anatomophysiology of cells and systems (neuro and related cardiovascular) and in the processes of neurodegeneration / neuroprotection and is aimed at identifying new "targets" for symptomatic and neuroprotective drugs that are potentially used in stroke and in the main chronic neurodegenerative diseases. Moreover, the aspects of the relationship between cardiologic and neurologic diseases is a key-focus of the Neuromed research. The research, in Neuromed, is conducted from animal models to clinical application, to epidemiology and public health. The wide series of scientific results described above is reached thanks to a unique technical and technological infrastructure, built in order to ensure the complete coverage of translational research. Below, the details of Labs at the Research Center-Biotechnology Park.

Imaging systems. A complete set of imaging technologies is available, including: Digital microPET/CT (RAYCAN E180), 7T (PHARMASCAN 70/16) High field microMRI, in-vivo optical imaging technologies, including 2-photon tomography (FMTPerking Elmer), 2-photon microscope (LSM 7 MP, ZEISS), a micro CT (SkyScan 1178) and micro US (Vevo 2100 VISUALSONICS). Available multiple stations for the measurement of vital parameters of small animals, including a System of pressure and volume measurement (ARIA1 MILLAR), Pressure measurement (BP 2000 M6), Radiotelemetric System (Data Science International), Laser Doppler (Periflux System).

Molecular and cellular biology/pathology facilities. Cell culture, Cell analysis lab, sequencing stations for genetical studies: Sonicator (Covaris), Sequencer Hightroughput HiScan, Sequencer for MiSeq diagnosis (ILLUMINA), Slice functional analysis (Slice scope pro 6000) with one Control Cube and one PatchPad-Metric, SilicaMate SM-4000 with 4 working stations. Radio-Chemistry lab equipped with g counter, b counter, preparation of samples with a and b radioactive sources.

Animal preparation and handling. Micro-surgery stations for small animals, with know-how for the development and evaluation of pathologies in animal models (SMZ 800 Nikon), PZMIII-BS (WPI), 304913-9901 (ZEISS). Stations for mice behavior analysis: Morris Water Maze, Ethovision XT (Noldus Information Technology). A fully equipped mice and rat housing facility (hosting approximately 7000 animals), with dedicated staff. Know-how in the generation of new animal models (knockout, transgenic, etc.).

Biobanking. The Neuromed Biobanking Center is devoted to collection and storage of biological materials, managed according to standardized operating procedures, established at national and international levels. Available medical and behavioral data related to each sample. The Biobank is member of the European Infrastructure of Biobanks and Biomolecular Resources (BBMRI-ERIC).

High Performance Computing and artificial intelligence. Available Big Data storage and interpretation with Artificial Intelligence techniques.

INM Neuromed researchers produced 424 scientific publications in 2020 and 403 in 2021 with a normalized impact factor of 1.848 in 2020 and 1.974 in 2021.

Research activities conducted at Neuromed led to the creation of a start-up **R4pso**, highly specialized in the the design and production of customized bionic orthotics and prosthetics to support the recovery of the limb after injury through the use of advanced prototyping technologies and innovative materials.

Key elements/strengths

The INM Neuromed participates in programs financed at national and international level in collaboration with Universities, organizations, research centers, IRCCS and companies. Neuromed leads and coordinates numerous national and international projects for a total of approximately € 40 million in the last five years.
The Department of Epidemiology and Prevention (DEP) has been working for many years on the impact of genes and environment and their interaction on cardiovascular risk and on the role of nutrigenomics in the development of metabolic diseases, cardio-cerebrovascular disease and cancer. At DEP there are extensive experience in large-scale genotyping analysis of complex diseases and functional polymorphisms studies in animals and in cell systems. Scientist at DEP coordinated and collaborated in EU and NIH founded studies on the role of genetic polymorphisms on the risk of coronary heart disease and metabolic disease namely IMMIDIET (QLK1-CT-2000-00100) and IDEFICS study (QLK1-6FP-016181) and NIH-RP-R01HLO-75389-01. More recently DEP has developed an innovative epidemiology model based on the connection between fundamental-translational (-omic) and clinical research data and "real-life" clinical data: the integration and analysis of large amounts of structured and unstructured big-data have the aim of developing holistic models of personalized prediction, through the application of artificial intelligence techniques (cohort of general population (Moli-sani project), cohorts of hospitalized patients (Platone project), cohorts of patients with specific pathologies (CORIST project), cohorts of elderly population in polyfamacotherapy (Epipol project).

Over the years, the Neuromed Institute has strengthened its ability to network with national and international research structures capable of giving added value to research, training and clinical performance activities by signing numerous agreements with bodies of excellence. In the last two years, the Institute was active in the participation in the ministerial work of building thematic networks between IRCCS (cardiological, aging, neuroscience and neurorehabilitation). Neuromed has a large number of national and international collaborations. In the Italian context, both public and private researcher centers, such as: University of Sapienza, of Insubria, Varese, of Siena, Brescia, Naples "Federico II", Catania, Padua, Trieste, Milan-Bicocca, the S. Anna School of Pisa, Palermo, the Catholic University, Florence, the Canavese Bioindustry Park; Area Science Park in Trieste, Human Technopole, Milano. Among the international collaborations: Huazhong University Of Science And Technology Whuan, CNRS University of Provence, Autonomous University of Barcelona- Spain, University of Amsterdam- Holland, Stanford University, USA and Cardiff University, University of Alberta and Western University. London, Canada, University Heart & Vascular Center Hamburg, Germany. The Department of Epidemiology and Prevention is involved in the Global Dietary Database (GDD; https://www.globaldietarydatabase.org/); The Global Burden of Disease (GBD) network (https://www.healthdata.org/gbd/2019); The Global Lung Function Initiative (GLI) Network (www.lungfunction.org); The NCD Risk Factor Collaboration (NCD-RisC) group (https://www.ncdrisc.org/); the European Infrastructure of Biobanks and Biomolecular Resources (BBMRI-ERIC; https://www.bbmri-eric.eu/).

IRCCS CENTRO DI RIFERIMENTO ONCOLOGICO DI AVIANO - Hub founder and Spoke's Affiliate
Short Name:- CRO-AVIANO-

General information

The Centro di Riferimento Oncologico (CRO) is an Institute for Research, Hospitalization and Health Care (IRCCS) of national importance, entirely dedicated to cancer research and care. It is devoted to patient-centered tumor treatment and research, encompassing all phases of the disease: Prevention, Diagnosis, Treatment and Rehabilitation. CRO's clinical services include innovative medical treatments (precision medicine, immunotherapy and autologous bone marrow transplantation), oncological surgery, cutting-edge radiotherapy approaches to cure and palliate cancer, and technology transfer.

Organization skills

The organization of CRO enables activities to advance scientific knowledge, disease prevention and treatment, and technology transfer in collaboration with the private sector. The organization of research lines is based on five lines, namely tumor genetics and biology, tumor epidemiology and prevention, hematologic neoplasms, solid tumors, tumors associated with infectious agents, and immunosuppression.

National and international project management skills

Over the past 10 years, 287 national competitive projects and 16 international competitive projects have been conducted. In addition, 65 non-competitive national projects and 1 non-competitive international project have been conducted in the last 10 years.

Technology transfer capacity - patents - spin-off

The Office of Technology Transfer (TTO https://www.cro.it/it/ricercatori/technology_transfer.html) benefits from a competitive advantage acquired in the 15 years of its activity. The capacities reported to the Ministero della Salute - according to the new performance indicators introduced in 2018 (MoH - Ricerca Corrente, Criterio E) - generated an economic value of €8,165 through licenses, €22,000 through in-kind contributions with industry and 13 joint developments. Spinoff activities are key to our vision of bringing technologies to the right stage of development to strengthen the economy. In fact, only 15 companies have been spin-off from Italy's 51 public and private research hospitals, 5 start-ups created by translating the results of CRO translational research into societal use.

The MoH / Direzione Generale della Ricerca e dell'Innovazione in Sanità promoted a dedicated working group coordinated by CRO TTO that developed the spin-off guidelines for IRCCS (February 2020), followed by joint spin-off regulations (forthcoming, May 2022).

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

Precision medicine, which refers to both therapy and diagnostics, has been the main objective of CRO since its foundation to promote a true translational research. Mainly clinical and translational research concerns 1) tumor genetics and biology, 2) epidemiology and prevention, 3) hematological tumors, 4) solid tumors, and 5) tumors associated with infectious agents.

The CRO was opened in 1984 and accredited by the Italian Minister of Health in 1990.

Information about the clinical and research activities of CRO is published in the CRO Clinic-Scientific Relation (available at https://www.cro.sanita.fvg.it/it/ricercatori/relazione_clinico_scientifica.html). Information on the research activities of CRO is also published by the Italian Ministry of Health (https://moh-it.pure.elsevier.com/en/organizations/centro-di-riferimento-oncologico). In the last decade, a total of 2348 articles have been published. In particular, in 2017-2019, a total of 812 peer-reviewed articles were published with an impact factor of 4351.

The CRO is supported by the Friuli-Venezia Giulia Region (FVG), the Ministry of Health, and a number of competitive grants, in particular the Associazione Italiana per la Ricerca sul Cancro (AIRC). 285 teaching sessions and 131 on-site training sessions were held. Collaboration with the Universities of Trieste, Udine and others allows the presence of an average of 30 residents in medical oncology and other relevant disciplines and 40 PhD students at CRO. Young researchers find hospitality at the nearby campus, which also hosts educational facilities.

Key elements/strengths

The Institute has technology platforms equipped with state-of-the-art equipment for clinical and research activities: Multispectral cytofluorimetry/cell sorting, next generation sequencing, gene/microRNA expression

profiling, epigenetics, microscopy and cell imaging, preclinical in vivo and in vitro models, pharmacogenomics, nanomedicine and spectroscopy. State-of-the-art equipment is also available for diagnostic imaging (CT-, 3T MR-, Pet-ct; and Spect-ct-scan) and radiotherapy (IMRT, VMAT, tomotherapy and intraoperative radiotherapy), resulting in improved treatment efficacy and reduced toxicity. The establishment of a proton therapy and cell factory (CAR -T) is also underway. CRO also operates the FVG population-based cancer registry and a biobank that includes approximately 131,000 frozen blood and/or tissue samples from about 8,000 patients and healthy individuals. CRO FVG library supports medical staff, patients and caregivers through the Patient Education & Empowerment project. CRO is included in Bbmri - Network of Italian Biobanks, and Iarc/Who. CRO strives for innovation, which includes experimenting with new organizational and care models. In 2017-2019, an average of 50 clinical trial studies (for-profit and not-for-profit) were launched, often in collaboration with other oncology institutions and the pharmaceutical and diagnostic industry, and over 2000 patients were recruited per year.

A strong synergy has been created at CRO between the research staff and the clinical staff (see institutional working groups). Projects are characterized by multidisciplinary support (physicians, biologists, engineers, physicists, etc.), also to promote technology transfer with impact on the region.
The CRO sponsors conferences, trainings, participates in university courses and also financially supports PhD schools.

The proposing Department of Experimental and Clinical Pharmacology of CRO in Aviano is actively involved in the development of CAR -T projects and nanomedicine projects. The director of the department Giuseppe Toffoli coordinated the AIRC 5 promille project "Application of Advanced Nanotechnology in the Development of Innovative Cancer Diagnostics Tools", where he was Principal Investigator (PI) coordinating the activity of more than 50 working groups with the participation of more than 300 researchers (college professors, directors of SOC, contractors and fellows). Dr Toffoli also participates in the Horizon 2020 project (DIACHEMO) as head of a merged division. In the field of cell therapies, he is the scientific leader of the Institute's CAR -T cell project and participates in the ACC - CAR -T - RCR-2019-23669115 Alliance Against Cancer project "Research project on CAR -T cells for malignant haematological diseases and for solid tumors ". Dr Dal Bo coordinated as PI an Italian MoH Young researcher project in the haematological field: “New genetic lesions characterizing marginal zone lymphomas clinical and functional implications”.

The CRO is one of the founding members of Alleanza Contro il Cancro (ACC). The CRO shares the mission and is part of the Organization of European Cancer Institutes (OECI). Since its inception, the CRO has been a member of the Union International Contre le Cancer (UICC), the leading international, non-profit, non-political and non-sectarian non-governmental organization dedicated to global cancer prevention. The C

IRCCS AZIENDA OSPEDALIERO-UNIVERSITARIA DI BOLOGNA UNIBO - Hub founder and Spoke's Affiliate
Short Name: S.ORSOLA

General information

The IRCCS Azienda Ospedaliero-Universitaria (University Hospital) di Bologna St. Orsola-Malpighi (IRCCS AOUBO) is the first hospital in Bologna, with over 400 years of history, and ranking 60th among the World's Best Hospitals 2022 and 3rd among the Italian's Best Hospital 2022. The IRCCS AOUBO hospital is the official venue of the School of Medicine and Surgery of the University of Bologna and represent a city campus in the heart of Bologna. Its hospital wards, distributed among 32 pavilions, is currently organized into thirteen Departments, including 87 Operative Units. Every year the IRCCS AOUBO admits approximately 53,000 patients and performs an estimated 3,700,000 outpatient visits.

Organization skills

IRCCS AOUBO hosts over 60 disease registries, and a large number of datasets for observational studies and clinical trials. Its r**esearch activities** encompass all phases of **translational research (from T1 to T4), in particular (but not limited to) in the two fields of oncology and of both solid organ and bone marrow transplants**. It hosts three units for Phase I studies in Oncology and Onco-hematology, and a **certified unit for CAR-T treatments and studies**. In research, IRCCS AOUBO is very active in all research domains, with more than 1300 publications per year (JIP > 8377.8) and outstanding results in attracting research competitive funding at both European and national level. At regional level, IRCCS AOUBO accounts for more than 52 funded projects in 2021 (12M€).

The hospital is currently working to centralize biobanks and collections into a Biological Resources Centre for a secured and quality assured management.

National and international project management skills

IRCCS AOUBO has obtained 13 European Projects within FP7, H2020 and Horizon Europe programmes, 5 as full Beneficiary and 8 projects as third party of the University.

Key elements/strengths

For the third consecutive year, the University Hospital IRCCS AOUBO is confirmed on the podium of Italian hospitals. In the "Best Hospital 2022" ranking, published by the historic American magazine "Newsweek", the Polyclinic is in fact ranked third among the best Italian facilities and 60th in the global ranking.

In support of the current Partnership, the IRCCS AOU Bologna/University of Bologna hosts more than 60 disease registries and numerous datasets for observational and clinical studies. The total number of **multicenter clinical trials** in the last five years is 346, with a total number of patients recruited in 2020 equal to 2.546.

TASKs on Spoke HEALTH DATA SCIENCE - Data management and development of advanced methods, algorithms, and machine learning approaches integrating health big data. In 2021, the IRCSS set up a new inter-disciplinary Platform dedicated to the analysis of data coming from omics approach. The Head of this Platform is Dr Tommaso Pippucci who has a great experience and expertise in the field. In particular in Bioinformatic analysis of Next Generation Sequencing data; Development of tools for analysis of Exome/Genome data; Identification of susceptibility genes in complex disorders; Identification of mutations and genes in Mendelian disorders.

TASK: Identifying predictors of benefit from available treatments. In particular, WP on predicting the effectiveness of immunotherapy through the deep molecular characterization of patients treated with anti-PD1 drugs and in developing CAR-T approach. In 2021, the IRCCS set up a new laboratory unit named Immunobiology of Transplant laboratory (IBT-Lab) with the aim of performing biologic and molecular studies on HSC transplantand CAR-T patients. Dr Francesca Bonifazi who is the Head of this Laboratory ha a great experience in the field. Starting 2017 she is the Director of the CIC240 haematopoietic stem cell (HSC) transplant program. The transplant expertise regards2164 years/patients, 1,300 allogeneic HSCtransplants, 300 autologous transplants, 300 induction/consolidation phases inacute leukemia, 300 chemotherapy phases in lymphomas and myelomas, 70 CAR-Ttherapyfor non Hodgkinlymphomas, 20 CAR-T for multiple myeloma.

The IRCCS Azienda Ospedaliero-Universitaria (University Hospital) di Bologna St. Orsola-Malpighi (IRCCS AOUBO) will manage and implement the project of the partnership PE6 with the following activities:

- In the projects PE6, the Head of new inter-disciplinary Platform dedicated to the analysis of data coming from omics approach, Dr. Tommaso Pippucci, will provide the Coordination and supervision of bioinformatic genomic data analysis.
- In the projects PE6, the Head of this Laboratory Immunobiology of Transplant laboratory (IBT-Lab), Dr. Francesca Bonifazi, will provide her expertise in the clinical management of CAR-T treated patients. She will also lead the Immunobiology of Transplant (IBT) team at IRCCS who is in charge to collect biologic samples of CAR-T patients and perform cellular and molecular analyses.

IRCCS AOUBO is part of the Italian network "Alliance Against Cancer (ACC)" and is member of 12 European Reference Networks (ERNs): Rare Adult Cancers -solid tumors - EURACAN, Rare Lung Diseases – ERN-LUNG, Rare Endocrine Conditions -Endo-ERN, Rare and Undiagnosed Skin Disorders - ERN-Skin, Rare Hepatological Diseases - ERN-RARE Liver, European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability – ITHACA, rare genetic tumour risk syndromes - ERN GENTURIS, rare urogenital diseases and complex conditions in both children and adults - ERN eUROGEN, rare kidney diseases – ERKNet, Rare Hepatological Diseases - ERN-RARE Liver, Rare Hematological Diseases - ERN-EuroBloodNet, Rare and Low Prevalence Complex Diseases of the Heart - ERN Guard Heart, Rare Hereditary Metabolic Disorders - MetabERN.

FONDAZIONE TOSCANA LIFE SCIENCES - Hub founder and Spoke's Affiliate
Short Name: TLS

General information

Fondazione Toscana Life Sciences (FTLS) is a private non-profit research organization founded by public (including all universities and schools for advanced studies) and private institutions in Tuscany. FTLS operates in the life sciences (LS) sector pursuing scientific research of particular social interest, especially for the prevention, diagnosis and treatment of human pathologies with particular attention to the development of personalised medicine. FTLS is also actively involved in supporting innovation processes and creation of new companies, leveraging on expertise acquired over the years in intellectual property strategies, business intelligence, technology transfer, licensing and funding opportunities, while at the same time providing laboratories, equipment and research services.

Organization skills

In 2007, when the foundation started its operations, the incubator housed less than 10 people. Today we are faced with a very lively ecosystem with 51 different research groups, start-ups and companies and more than 500 people, mainly scientists (40%), who have been able to cumulatively attract more than 50 million in research grants, published > 650 scientific articles and secured > 50 patents. FTLS employs 76 people, almost half of them involved in R&D activities, and hosts 12 young scientists, including PhD students and postdoctoral fellows. 60% of all employees are female and 56% have a PhD or a Master degree.

Although FTLS started its own research activities in 2016, the results so far are significant. A total of 88 manuscripts have been published, some of them in prestigious scientific journals, and 8 patent applications have been submitted to date (two of them already granted).

National and international project management skills

Projects granted in national and international competitive bids are also among FTLS main KPIs and a measure of its excellence for shareholders and stakeholders. Currently, FTLS carries out more than 10 R&D&I national, European and international projects, as well as scientific dissemination project and an investment project (42 Million € total investment). The overall number of R&D grants secured by FTLS since 2017 is impressive. Together the grants issued by EU, international charities (Wellcome Trust and Wellcome Leap), national and regional institutions, public-private partnerships (EU Malaria Fund) exceeds 19 million EUROs and involve more than 30 intramural researchers and several collaborators around the world.

Technology transfer capacity - patents - spin-off

At present, FTLS manages two bio-incubators equipped with laboratories and research facilities available to research groups and hosted companies.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

One of FTLS's main strategic projects is the Tuscany Center for Precision Medicine (CREMEP). CREMEP is a multicenter consortium aimed at favoring a multidisciplinary approach to biomedical and translational research. CREMEP represents an initiative, funded by Tuscany Region, in close synergy between the University of Siena (UNISI), FTLS and the Siena University Hospital (AOUS) and focusing on the specific interaction between research and care. The Centre will introduce a new approach to care, for the benefit of the health of citizens, who will be able to rely on a model based on personalized services for each patient, taking into account individual variations in the genetic heritage, environment and lifestyle, particularly in the oncology and metabolic area.

Such multidisciplinary approach favors consortia generation; noteworthy, the affiliated partners benefit from shared highly qualified technicians and biotechnologists, technology platforms and laboratories fully equipped to carry on molecular and cellular biology and immunological research.

Among granted R&D projects based on personalized medicine, it is to mention IDF SHARID, "Innovative devices for shaping the risks of diabetes" https://www.toscanalifesciences.org/en/projects/idf-sharid/ . This involved in fibrosis will be investigated by NGS deep sequencing and mass spectrometry, respectively).

Key elements/strengths

FTLS is coordinator of the EU funded project Regions4PerMed, whose main aim is to facilitate the implementation of personalised medicine within healthcare systems by leveraging the role regional authorities in Italy and Europe. FTLS also seats in the executive board of the International Consortium on Personalised Medicine (IC-PerMed) who is developing the next European Partnership on Personalised Medicine (which will invest 1 billion € on PM research and innovation between 2023 and 2032). Lastly FTLS is part of the ERA-NET co-fund on PM, which is funding transnational joint research projects on PM since 2018.

FTLS closely work with the European Beating Cancer Plan patient representative Bettina Ryll and has ongoing collaborations with Italian and European patient organisations: **Raknroll** (https://www.raknroll.pl/en/), **Being Dense** (http://www.beingdense.com; **Onda Foundation** (https://ondaosservatorio.it/en) **EVITA.**
Regarding the Biobank, FTLS cooperates with da Vinci European BioBank (https://www.davincieuropeanbiobank.org/it). The biobank was created as an infrastructure to support the activities of the Fiorgen Foundation (http://www.fiorgen.net/), a nonprofit organization that promotes research in the field of pharmacogenomics and personalized medicine. FTLS has also technically designed the regional reform for the reorganisation of clinical and research biobanks in Tuscany, which has been approved in 2021 by the regional government.
Thanks to CREMEP consortium, FTLS, UNISI and AOUS scientists have access to shared facilities. These facilities include a Core service Lab for innovative high throughput next generation sequencing, a Core Service for flow cytometry cell sorting and Single cell sorting, a Core service Lab for mass spectrometry specialized in the analytical characterisation of small metabolites, macromolecules and for advanced proteomics analyses, a Core Service for ultrastructural studies and microscopy imaging studies fully equipped with fluorescence confocal microscopy, vital confocal microscopy, a high content screening (HCS) and high-resolution imaging system (Opera Phenix), as well as for Laser Capture Microdissection. Of note, a state-of-the-art animal facility equipped for high-sensitivity bioluminescence, fluorescence, high-resolution x-ray, high frequency ultrasound for real-time in vivo microimaging.

The Proponent has the right IPR and Business Development expertise that will represent an added value for the overall development and successful outcome of the proposed research project.
In mid 2020 research activities of the FTLS Monoclonal Antibody Discovery Laboratory (MAD-Lab) led to the discovery of a promising mAb-based treatment to cure coronavirus disease 2019 (COVID-19). These studies have resulted in 9 manuscripts, many of which have been published in prestigious journals (Cell, Nature, Nature Medicine, Lancet), 5 patent applications and the execution of 2 clinical trials (EudraCT N.: 2020-005469-15 (Phase 1); EudraCT Number:2020-005532-29 (Phase 2/3)). The isolated antibody has been also the subject of three licensing deals for the development of diagnostic devices (PrimaLab; Diasorin, Diesse Diagnostica Senese).
As detailed in the previous sections, FTLS has been able to achieve important goals in terms of attracting talented young researchers, establishing solid R&D networks, securing major research grants and successfully managing complex research projects.

The scientists involved in this project have established several collaborations over the years. Among them: Dr. Danilo Licastro, Area Science Park biohub, Trieste, Italy (Transcriptomics and genomics); Dr. Andrea Armirotti, Istituto Italiano di Tecnologia, Genova, Italy, (Metabolomics); Dr. Paolo Ghia, Università Vita-Salute San Raffaele (CLL clinical samples) Dr. Malgorzata Firczuk, Department of Immunology, Medical University of Warsaw, Poland (Preclinical models of B cell tumours in vivo); Dr. PierPaolo DiFiore, University of Milan, IEO, Milan (*Characterization of novel cancer bio-markers)*.

UPMC ITALY SRL - Hub founder and Spoke's Affiliate
(Short Name: UPMC)

General information

UPMC Italy Srl (UPMCI) is the Italian branch of the University of Pittsburgh Medical Center (UPMC). Founded in 1997, it operates in the field of healthcare, biomedical research, telemedicine and in general is active in all development and consultancy activities IT, administrative-management, business planning in the sectors directly or indirectly connected to the institutional ones of medicine and research. Since June 2013, it has been wholly owned by UPMC Overseas (hereinafter also OVERSEAS), a non-profit company of UPMC which coordinates, organizes and provides a wide range of services and resources to other UPMC affiliated organizations that participate in projects on national and international scale with aims of social utility in the health sector. UPMCI is present at: IRCCS/ISMETT (transplant hospital, Palermo-Sicily, managed by UPMCI), SMIH (private general hospital, Rome, Lazio, wholly owned by UPMCI), UPMC Institute for Health (Chianciano Terme, Siena-Toscana, specialized in primary prevention of non-communicable diseases, NCD, local unit of UPMCI), UPMC San Pietro FBF, (radiotherapy center, Rome, Lazio, local unit of UPMCI) and UPMC Villa Maria (radiotherapy center, Avellino-Campania, local unit of UPMCI) that belong to the Hillman Cancer Center group. UPMCI has acquired considerable experience in different medical specialties, in Italy. As regards to oncology, with reference to stereotactic radiotherapy (performed with a True Beam™ STx linear accelerator), UPMCI operates in Rome, with UPMC San Pietro FBF Advanced Radiotherapy Center, since2013 and in Mirabella Eclano (Avellino, Campania), with UPMC VillaMaria since 2018. In much the same way, UPMCI has focused on NCD prevention and management, at the UPMC Institute for Health Chianciano Terme since 2014, a medical center for research and preventive medicine for hepatobiliopancreatic diseases, gastrointestinal diseases, cardiac diseases, metabolic syndrome, and diabetes, and offering personalized screening programs. In Palermo (Sicily) UPMCI manages the IRCCS ISMETT, since 1997, a transplant hospital which also provides specialized therapies for patients with high complex pathologies (also oncology). Finally, UPMCI manages Salvator Mundi International Hospital since 2018, a private general hospital, which provides, inter alia, high specialized orthopedic procedures and oncologic treatments. *Organization skills* At UPMCI, healthcare personnel carry out research activities directly related to and deriving from clinical activity, making UPMCI n intrinsic vocation for research and the organization of structures aimed at promoting research itself through dedicated infrastructures. UPMCI, researchers have workstations dedicated to their research activities and thanks to the high level of computerization, specific software resources for data/image analysis, for statistical processing (e.g. SPSS statistical software), for the management of clinical trials (RedCap), as well as access to world research platforms such as Scopus, Web of Science, Scientific IRCCS Network on Scival, EMBASE, which allows them to be continuously updated. In UPMCI, he IT staff has developed considerable experience in the management of clinical applications (CCE, LIS, RIS) also with regards to the extraction of data aimed at carrying out research activities (database query through data extraction, generation and population data research base, etc). Equally high is the presence of equipment specifically dedicated to research activities, such as the research laboratory in ISMETT. Furthermore, on the occasion of participation in research projects, the equipment is enhanced with respect to specific needs (purchase of centrifuges, freezers, and specific laboratory materials kits for research activities), as was the case, for example, of Chianciano Terme for the LifeStyle4Health project (see next sections), where gym equipment was specifically purchased. With reference to the staff, the offices in Lazio and Campania are specialized in radiotherapy treatments, with regard to head and neck, pancreas, central nervous system and liver metastases, while in Palermo the focus is on abdominal cancer surgery and Chianciano Terme in primary prevention. UPMCI has dedicated resourced for project management activities that are involved in identifying the tools and opportunities for research and development to support the Company Mission, to managing the entire Project Management cycle, starting from design, passing from implementation and monitoring to the physical and financial reporting of project activities. They work closely with UPMCI's Chief Operating Officer, as in fact once the research topics on which the company intends to focus are known, they identify the most suitable opportunities and take care of setting the correct approach to the design of the research project, with respect to the requirements of each specific call. The project management resources are responsible for supporting medical researchers in the realization of the project and in exploiting the potential of the research carried out with a funded project, in order to identify the ways in which the results of the research can be translated into concrete tools that can be used in the clinical routine

National and international project management skills

UPMCI will manage and monitor the project from an operational and financial progress viewpoint. The coordination and management of the project will be inspired by internationally recognized project management standards such as PMBOK and PRINCE II [PMBOK, PMI, 2017, A Guide to the Project Management Body of Knowledge: (PMBOK® Guide), sixth ed., Project Management Institute, Newtown Square, PA', PRINCE II,

'Office of Government Commerce, 2017, Managing Successful Projects with PRINCE2'], and will entrust on a project management team. Based on the Gantt of the project, the project team will periodically steer and monitor the progress of all activities and milestones, responding to any roadblocks in a timely manner. Each member of the team will regulaly report on all action items that support each milestone within their area ofexpertise. In addition, should any deviations be highlighted, the project management team will explore the reasons and implement timely corrective actions to align the performed tasks with the expectations. Once an activity is completed, a corresponding report will be finalized (if needed). In parallel with the operational progress of the activities, the financial progress will also be monitored to determine the expenses incurred (e.g. over spending).

Technology transfer capacity - patents - spin-of-Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

UPMCI operates in the field of the precision medicine from several years, as is shown by the research and development projects to which regularly participates (see below the description of the projects LifeStyle4Health, PROBIO, OngOIng)»Year of experience:9; UPMCI provides high specialized and personalized treatments in oncology starting from the 2013 in SanPietro FBF, where stereotactic radiotherapy treatments are available.

Key elements/strengths

UPMCI is active in the field as the research projects below mentioned show; Networks: UPMCI is partner of the network named "Lifestyle4Health" , whose main purpose is to collaborate to projects in the field of precision medicine for the primary and secondary prevention (see Lifestyle project); UPMCI is partner of the Bi-Rex consortium in which it collaborates in the field of LiveScience, whose main focus is the precision medicine (precision treatments, see PROBIO project). Thanks to its strong international presence UPMC has signed numerous collaboration agreements. In Italy are active, for example, about N.30 collaborations with Italian Universities and companies in the field of AI and HPC in Life Science and Healthcare. Upmc has numerous patents thanks to the activity carried out by the University of Pittsburgh in America and the Rimed Foundation in Italy. »The agreement between UPMCI and UPMC OVERSEAS leads that UPMCI has free access to the protocols and clinical research that are the result of the research and development carried out jointly within University of Pittsburgh Medical Center (UPMC). With reference to Research and Development, UPMCI has free access to the results achieved by the UPMC Group and has the right to use the protocols derived from them. In addition to the know-how of the UPMC group, in relation to the oncology field, it must also be said that the same group has undertaken collaborations aimed at achieving new expertise in the field of machine learning for precision medicine. For this purpose, the Pittsburgh Health Data Alliance (PHDA) was born, a collaboration between Carnegie Mellon University, the University of Pittsburgh and the UPMC, which integrates the experience of the University of Pittsburgh with the competences of Carnegie Mellon University, in computer science and machine learning,and the dataset that UPMC will be able to make available. The goal of the collaboration is to derive new knowledge starting from the information present in medical records, those deriving from diagnostic imaging, prescriptions, genomic profiles, insurancerecords and even data from wearable devices, with the aim of preventing the onset of the diseas and improving the diagnosis and the quality of cares.

UPMCI managed/is managing the following projects in the field of precision medicine: »LifeStyle4Health:(PON MISE progr. 2014-2020): The project aimed at studying the impact of the physical/rehabilitation activity on the health status of people. The project applied Machine Learning techniques to: 1)derive new knowledge about the role of specific biomarkers in mediating the positive effect of the physical activity in preventing/reducing the risk factors of CVD (such as, obesity, hypercolesterolemia, diabetes), 2) identify personalized physical activity programs able at achieving specific health related targets (also linked to cognitive improvement)(Start year: 2017, End year: 2021; UPMCI acted as leading partner); »PROBIO (MISE Bi-Rex consortium):The project aimed at developing a decision support system, in cloud, for helping physicians to the better characterization of brain metastases, in pre and post treatment phases, for the definition of response to radiation therapy treatment and personalization of thetreatments (Start year: 2020, End year: 2022; UPMCI acted as leading partner);»OngOing (1.1.5 measure, PO FESR Sicilia 2014-2020):The project aims to systematize a series of components already available on the market such that clinical information and, more specifically, genetic information, can be associated with standards of care, but also with off-label drugs and open trials. An informatic system will automate the search on the basis of validated medical knowledge and will summarize the information in organized reports, in order to make the obtained information effective and immediate for the clinician, thus powering and accelerating the medical knowledge and allowing the physicians to make personalized treatments in oncology (Start year: 2019, End year: 2022

ISTITUTO ONCOLOGICO DEL MEDITERRANEO SPA - Hub founder and Spoke's Affiliate (Short Name: IOM)

General information

Istituto Oncologico del Mediterraneo Spa (hereinafter "IOM") is one of the few highly specialized, level III, oncology departments across Sicily; together with IOM Ricerca Srl, and REM Radioterapia Srl it constitutes the "Polo Oncologico di Viagrande", which aims to create a cluster of scientific excellence to fulfill both the scientific and clinical needs in the oncology sector. Since its foundation, IOM operates both as research and healthcare oncology hub, joining together highly skilled clinicians and researchers, operating in an integrated manner, each of them playing a role in both clinical and research (basic, and molecular science) activities. By following a sustainable approach to diagnosis and treatment of cancer diseases, leveraging on both advanced diagnostic technologies and professionals from different specialties working as a multi-disciplinary team, IOM follow its patients through the entire journey from diagnosis to treatment that can guarantee better living conditions.

Organization skills

IOM was founded in 2003 as cancer center with the following operative units: Medical Oncology, Onco-hematology (comprised of a bone marrow transplantation unit), Palliative Care and Analgesic Therapy, Oncological Surgery, Dermato-oncological Surgery, Interventional Neurosurgery and Neuroradiology, Breast and Reconstructive Surgery, Oncological Gynecology and Urology. There are also the following outpatient clinics and services: Anesthesia and Intensive Care, Colonoscopy, Oncological Dermatology, Digestive, Bronchoscopic and Thoroscopic Endoscopic Diagnostics, Diagnostic Imaging with interventional section, Oncological Endocrinology, Pharmacy, Cardiovascular Pathophysiology, Analysis Laboratory, Nuclear Medicine, Neurosurgery and Spinal Intervention, Oncological Pneumology, Psychoncology, Pathological Anatomy, Clinical Chemistry, Microbiology and Molecular Diagnostics Laboratories.

Furthermore, by virtue of the strong propensity for research, the Oncology Center boasts a very modern research center equipped with cell biology laboratories, imaging laboratories, experimental radiotherapy laboratories, biorepository, biobank and cryogenic storage, technological platforms and a modern specialized animal facility (mice and rats) which has laboratories for preclinical experimentation and an internal OPBA for the evaluation of research projects and support for sending authorization requests to the Ministry of Health.

National and international project management skills Since several years, IOM participated in numerous funding programs managed by MIUR, MISE and the Sicilian Region for scientific research in the "life science" field, as a partner or know-how provider. The management and/or participation in scientific research projects allowed the Institute to develop significant expertise in the topics of molecular oncology, experimental radiotherapy, digital pathology and, in general, translational medicine. Project management is the responsibility of the "R&D Project Management" Office which carries out the planning, monitoring, auditing and scientific and administrative management of the projects, in line with the relevant legislation and according to quality procedures which provide for internal SOPs prepared for the management of R&D projects. In recent years, the main research topics of the IOM have been: the identification of biomarkers for diagnostic and therapeutic purposes, experimental radiotherapy, organoids, digital pathology, biobanking and technologies based on exosomes.

IOM aims to customize diagnosis and therapy and it is strongly dedicated to translational research: the Center was born from the idea of developing research paths particularly oriented towards clinical output and effectively transferring research results to the patient's bed. To this end, the IOM has created a qualified research laboratory equipped with advanced technical-scientific equipment: IOM Ricerca Srl, a spin-off of the Istituto Oncologico del Mediterraneo Spa. IOM Ricerca Srl has a molecular biology laboratory that has implemented an area of innovative diagnostic services; it offers a wide range of biotechnological and customized solutions to realize various research projects or to validate new scientific protocols; it has been identified by the Sicilian Region as a technological Campus that offers skills at the service of technological, strategic, organizational and commercial innovation of SMEs.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

To carry out research activities, IOM dedicates a substantial part of its medical, nursing, technical and research staff and makes use of external professionals with a high scientific profile, as evidenced by the numerous

publications in international journals and participation in important scientific societies. In recent years, in particular, the research activities of the IOM have focused on the increasingly topical issue of precision medicine, with the aim of identifying new and superior technological solutions and implementing synergistic systems to support the clinical decision that favors the personalization of the diagnosis also in a pro-therapeutic sense. In particular, the research activity conducted by IOM focused on experimental radiotherapy; digital pathology; liquid biopsy; exosomes. Below is a short list of IOM publications related to the topics/activities that the Institute will develop within the project. Furthermore, with reference to the issue of Digital Pathology, IOM has already developed several hypotheses of scientific articles relating to the results achieved, not yet disseminated as the intellectual outputs are currently being protected.

Key elements/strengths

The laboratories have been designed and equipped, following the highest technical and scientific standards and, together with the clinical activity, are constantly engaged in research and development. They are equipped with instruments dedicated to molecular investigations allowing efficiency and automation for pre-analytical (extraction and purification of nucleic acids through automated extractors), and analytical (NGS sequencing, realtime PCR High- throughput) processes. The pathological anatomy unit has invested considerable efforts in streamlining the flows of biological samples and it has extensive research experience in the areas of molecular diagnostics and biobanking and over the years has created an organized collection of biological materials, annotated with clinical information, both fresh and waxed. IOM biobank is part of the European network of biobanks BBMRI thanks to the high standardization of the procedures applied in the operational process of the structure. The clinical pathology unit is equipped with automated equipment for clinical chemistry and in particular has considerable experience in the development and conduct of assays through ELISA.

. In recent years, the research activities of IOM have focused on some main issues including: experimental radiotherapy and digital pathology. As part of the first research topic, the center that qualifies as one of the very few capable of carrying out experimental radiotherapy, in vitro and in vivo, continues to carry out various scientific research studies in the oncology field relating to the innovation of radiotherapeutic protocols personalized anticancer drugs. In particular, the IOM, in collaboration with other companies and entities of the IOM Oncology Center, has carried out a study entitled "*Development of innovative personalized radiotherapy treatments, based on the individual sensitivity of cancer stem cells*", created with the aim of creating a new process of personalized radiotherapy treatment in oncology, able to develop specific therapy techniques for Tumor Stem Cells (CSC) in order to ensure patients a very high rate of success in radiotherapy treatments, preventing relapses and therefore the need for subsequent treatments and increasing the quality of life and survival rates. This scientific research study, in addition to generating very interesting outputs for the scientific world, has led to a significant growth from the technological and procedural point of view and from the professional point of view of the personnel involved: the staff has acquired various expertise in the field of "in vitro" irradiation of CSC cultures, in the techniques of immobilization, centering and irradiation of small animals (mice), as well as in the evaluation of effectiveness of radiotherapy protocols developed “in vivo”. In addition, the results achieved within this project were used by the IOM researchers as a starting point for the launch of new research studies on this topic, also thanks to the instrumental equipment enjoyed by the IOM Oncology Center. The theme of Digital Pathology was also developed thanks to the management of a research project entitled "*DiOncoGen Innovative Diagnostics*" which, through the digitization of the activities of the Departments of Pathological Anatomies, aims to facilitate the diagnostic process for anatomopathologists, allowing them to use digital images and related information treated with the paradigms of Artificial Intelligence. Therefore, the development and validation of advanced all-in-one systems will be able to favor the personalization of the diagnosis also in a pro-therapeutic sense, through an "assisted interpretation" approach. The IOM boasts over ten years of significant experience in the management of research projects and verticalized observational and prospective studies in the oncology sector. With reference to research projects funded by third parties, the IOM has always fully achieved its scientific objectives which have made a contribution to the clinic with new diagnostic and/or therapeutic approaches and have become important starting points for the start of further research studies. In addition, the experience gained by the managers and professionals working in the administrative offices has made it possible to achieve excellent results also in the management and reporting of costs incurred. Therefore, the IOM has all the skills and professionalism, both scientific and administrative, to successfully manage research projects.

BI-REX - BIG DATA INNOVATION & RESEARCH EXCELLENCE - Hub founder and Spoke's Affiliate
(Short Name: BI-REX)

General information

The BI-REX Competence Center - Big Data Innovation & Research Excellence, is one of the eight highly specialized Competence Centers selected by the Ministry of the Economic Development (MISE), whose focus is the promotion of research projects of excellence in the field of Industry 4.0 technologies, with a focus on the Big Data area.

The BI-REX constitutive partnership includes activities in Emilia Romagna, Campania, Lazio, Lombardy, Marche, Piedmont, Tuscany, Sicily and Trentino Alto Adige. The Consortium is strongly committed to the implementation of industrial development projects for the experimental definition of important national and international sectors of intelligent specialization. **The BI-REX Life Sciences** Sector aims to facilitate the implementation of Research and Development projects functional to the transfer of innovation to the health service system and translational medicine, through Big Data Technologies and Artificial Intelligence applied to clinical research.

The Supply Chain promoted by Bi-REX and by consortium members with high expertise in the Life Science field, such as the Alma Mater Studiorum University, UPMC (University of Pittsburgh Medical Center) and the Rizzoli Orthopedic Institute, is characterized as a network composed of public and private entities, with an inter-regional value and as the main place of continuous comparison among networks of universities and research centers, industrial players, SMEs and a specialized network of clinical centers on a national and international basis. Through many consortium members (IBM, Siemens, ATOS, Leonardo, CINECA, INFN) Bi-Rex guarantees access to HPC facilities.

Organization skills

Bi-Rex has a proven track record of organization skills; in the first three years it has achieved the following KPIs:

- Opening of Pilot Plant, a smart factory I4.0
- Organization of more than 50 webinars, training courses and thematic workshops with more than 3000 participants from 1200 companies
- Creation of a syllabus containing more than 90 training courses on I4.0 technologies
- Activation of two e-learning platforms with synchronous and asynchronous training courses
- Launch of the Industry 4.0 Observatory
- Implementation of BI-REX for Life Science network
- Agreements and networking: + 50 collaborations between conventions and agreements
- + 600 Companies met
- Enrollment to the Regione Emilia-Romagna High Technology Network

National and international project management skills

BI-REX has open 3 competitive calls under MISE supervisions to stimulate the demand of innovation project delivering over 6M€ grants. BIREX coordinated the evaluation of over 200 proposals selecting 35 Small Consortia and triggering further 7M€ additional private investments. The 35 projects will deliver in BIREX over 50 use cases, demos and prototypes in which the advanced digital technologies integrate to generate business value for technology transfer. The 35 projects relate to the following 8 thematic areas: Big data for sustainability; Big data for manufacturing; ICT for machines and production lines; Advanced systems for the management of production processes; Security and Blockchain; Additive & Advanced Manufacturing; Collaborative robotics, warehousing and Automated Guided Vehicle, Sustainability and Social Responsibility. BI-REX consolidates the wide ecosystem of National Stakeholders, with a capillarity coverage in many Italian Regions, including other Competence Centers, Industry Associations, Universities, Research- and Training Centres, Financial Institutions as well as Corporations and partners of European impact as well as structured connections with international organizations all supporting the digital and twin transition of SMEs in the manufacturing sectors and with a focus on data valorization. Unique mix of advanced digital capabilities enable to foster the twin transition and the adoption of advanced technologies in the Manufacturing sectors.

Short list of European and regional projects managed:

- CASTIEL and EuroCC, Two projects, within the H2020 program, whose goal is to enhance European knowledge and opportunities in the HPC field, the Consortium plays the role of Cineca's linked Third Party
- Higher Training Project Services and innovation for industry 4.0 (SII40)
- Sanitize project: application of COBOT and MOBOT to sanitize workplaces

» BI-REX application as EDIH
» Application as Partner on Digital Europe design: first target draft of the Work Package of cluster 4 2021-2022
» 2 Start-up projects and an Emilia Romagna Region project ex Emilia Romagna regional Law 14

Technology transfer capacity - patents - spin-off

For its unique capability and specialization BI-REX is targeting to reach manufacturing SMEs widely on the whole Italian territory. The targeted multi-level networking and implemented mechanisms bring significant benefit in access to practices, technologies and impose high impact to the whole ecosystems in national and international level. The main outcomes of this activities correspond to the increased support for effective transition of SMEs to more digitized and sustainable business models and more resource-efficient and circular processes and infrastructures via tailored advisory services. To overcome the structural barriers in digital adoption, BI-REX contributes to fill internal skills gap that prevents managers and workers to identify the available and applicable digital solutions, and new digital business models and processes. To close this gap, we offer easy access to technology transfer, networking and training activities for start-ups, SMEs, and mid-caps to widen the understanding, introduce available technologies and solutions that meet the needs of company and supports the business ambitions.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"With respect to the topics of the research program, expose the previous experiences, scientific and design skills of each participant concerning the Specialization Area of the Extended Partnership PE6 "Diagnostica e terapie innovative nella medicina di precisione" (see art. 7 par. 2 Call for proposal "Partenariati estesi") *in terms of innovation and technology transfer. Illustrate the demonstrated ability of individual Spoke and affiliated entities to manage and implement projects in fundamental and/or applied research with reference to the area of the partnership* PE6 "Diagnostica e terapie innovative nella medicina di precisione" (see art. 7 par. 2 Call for proposal "Partenariati estesi").*Specify any existing national and international collaborations with other institutions and centers of high scientific quality, as regards the area of specialisation* "Diagnostica e terapie innovative nella medicina di precisione" PE 6 (see art. 7 par. 2 Call for proposal "Partenariati estesi").

Key elements/strengths

Bi-Rex, through the involvement of its consortium members, will support the project partners by providing the infrastructures and skills for the use of supercomputing (HPC) for the development of

- advanced algorithms and machine learning approaches integrating electronic health records (EHR) with imaging and pre-clinically validated high-throughput data,
- advanced prediction models for prognosis and therapeutic response based on comprehensive data treatment

in order to deliver new, cost-effective, and evidence-based predictive and non-invasive diagnostic pathways for faster, earlier, more precise, accessible, and affordable prediction, detection, and monitoring of monogenic (rare diseases), polygenic (cardiovascular and metabolic) disorders, and cancer, as well as to identify new effective therapeutic approaches and employing innovative and non-invasive technologies and solutions, based on the analysis of digitalised integrated diagnostics data.

Bi-Rex will also provide its 5G facilities as a test and demonstration environment to test the applicability of these technologies for the connection of infrastructures and machinery in a clinical and research environment and the establishment of networks of highly specialized laboratories and innovative services for digital transformation and Big Data management to support healthcare, pharmaceuticals, the world of research and innovation. Bi-Rex will also be involved in Technology Transfer activities to and from companies, the National Health System, and in public engagement activities.

ENGINEERING INGEGNERIA INFORMATICA S.P.A - Hub founder and Spoke's Affiliate (Short Name: ENGINEERING)

General information

Engineering Ingegneria Informatica S.p.A (ENG) is the head company of the ENGINEERING Group. ENG was founded in 1980 and the Group is now a Digital Transformation Company, leader in Italy and expanding its global footprint, with around 12,000 associates, with over 60 offices in 12 countries spread across Europe, the United States, and South America and global delivery. The Group has been supporting the continuous evolution of companies and organizations for more than 40 years, thanks to a deep understanding of business processes, fully leveraging on advanced digital technologies. The Group boasts a diversified portfolio built around proprietary solutions, best-of-breed market solutions, and managed services.

Organization skills

40+ years presence in all market segments (Finance, Healthcare, Agrifood, Utilities, Manufacturing, Retail, Public Administration, Transport, Security, Defence, Space) has allowed the company to build deep knowledge of business needs and anticipate them by exploring constantly the evolution of technologies, especially in the field of Cloud, Cybersecurity, Metaverse, Artificial Intelligence and Big Data.

With a strong and relentless focus on Innovation, through the R&I division that comprises over 450 researchers and data scientists (and a global innovation network of universities, startups, and research firms), the Group continues to invest in international R&D projects while exploring ground-breaking technologies and developing new business solutions. Specifically, ENG participates in the Heal Italia PE through its IT for Health (IT4H) R&D Labs. With over 50 researchers, IT4H holds different responsibilities within the international e-health community, including technical and overall coordination of large research projects and consortia, cross fertilisation of know-how at a European level and future exploitation of the project outcomes.

Key elements/strengths

ENG has matured deep experiences on: i) innovative monitoring, prevention and prediction services through the application of technologies such as AI, BD, mHealth, IoT, human-computer interaction, machine learning and data mining, ii) management of chronic disease, iii) patients' empowerment, iv) management, integration and enhancement of healthcare data, v) improvement of human-computer interaction and communication models, vi) integrated care, vii) innovative EHR, viii) architectures for the integration of complementary healthcare processes. The involved group has deep experience in data modelling, health data integration and exchange based on international standards (e.g., HL7/FHIR, CDA) and terminologies (e.g., ICD-10, LOINC, ATC).ENG has an IT & Management Academy in Ferentino, near Rome, with 500+ courses in catalogue and 2500+ training days for internal and external professionals, equipped with 16 state-of-the-art computerized classrooms, a lecture hall which can host up to 140 people, a library, a testing centre where professional certification exams are carried out and a corporate restaurant. Moreover, ENG has an integrated network of 3 Data Centers located in Pont-Saint-Martin (Aosta), Turin and Vicenza, with a service system and infrastructure that guarantee the best technological, qualitative standards and security, offering High Performance Computing solutions. ENG's strategic role within the **software research community** in Europe is evidenced by its active participation in international initiatives designed to stimulate and promote innovation in different areas: **Gaia-X** in which ENG is a member in the board of directors and participates to the following WGs: Federation Services, Architecture, Portfolio, X-Association, Provider; **EIT Digital** (European Institute of Innovation and Technology) where Engineering is a core partner; **BDVA (Big Data Value Association)**: Engineering is active participant of different working groups in domain committees; **FIWARE Foundation** (https://www.fiware.org/foundation/), as founding partner and active participant; ENG is co-founder of **International Data Space Association** (IDSA).Furthermore, the company is co-founder of the **Big Data Value cPPP**, co-founder of the European initiative **Future Internet PPP**, board member of the **European Organisation for Security** (EOS), board member of the **European Cyber Security Organisation** (ECSO), co-founder of the initiative **NESSI** (Networked European Software and Service Initiative) and supporter of **Living-in.eu** movement (to support the take up of technologies in local communities) and member of **Water Europe.**

ENG has matured deep experiences on modelling and integration of medical and non-medical health related data, as long as competences in widely used standards for medical data interoperability, like HL7/FHIR and OMOP, conveying the results of the modelling activities in a conceptual data model named UHM. Moreover, deep experience has been matured on architectural specification of IT systems and requirements elicitation, data storage management systems, EMR graphical interface design and implementation, machine learning enabling technologies, innovative patients monitoring through IoT platforms.ENG has

strengthened the business orientation of its research and assets, looking outwards to innovative markets, such as AI, BDA, Digital Therapeutics, Personalised and Precision Medicine and inwards to company vision and mission. Indeed, the internal process of technological & innovation transfer to the business structures is increasingly structured in order to produce cutting-edge innovations in line with needs and expectations of ENG customers. Among the others, ENG proposes to its customers products like Ellipse, an eHealth platform enabling the total digitalization of the "health system" specialized in the clinical and care dimensions, and DE4BIOS, a bio-surveillance platform which integrate and harmonize data from multiple sources to monitor COVID-19 pandemic evolution, geo-localize infected patients and identify clusters to be monitored.

The projects listed below could bring added value to the Spokes 2 – 3 - 4. Specifically, ENG's experience and activities in the following projects are: **CMP3VDA** (POR) aimed at accelerating the adoption of Precision Medicine in the current practice of healthcare delivery, by leveraging new generation genomics, bioinformatics, AI and big data technologies. It delivers a regional infrastructure as a reference genomic research center; an innovative holistic Electronic Health Record (EHR), integrating clinical and genomics data of patients; a big data infrastructure with HPC capabilities providing data intensive storage and processing. **CrowdHEALTH** (EU H2020) introduced the new paradigm of Holistic Health Records (HHRs) that include all health determinants (e.g., clinical, nutrition, lifestyle, demographical, social) and developed policy modeling techniques to facilitate the inclusion of Key Performance Indicators (KPIs) in health policies and the correlation of these KPIs with all health determinants captured in HHRs. **eMORFORAD** (POR Campania FESR 2014-2020) developed a software platform for integrated radiomic analysis for medical professionals specialized in head and neck cancers, involving patients suffering of such disease in new personalized medicine pathways. The solution integrates tissue biomarkers and radiomic imaging data and defines and develops predictive, diagnostic and prognostic algorithms using AI techniques, based on the defined holistic data model. **Amico** (PON) developed an "instrumented environment" consisting of both the home environment and of the person, both suitably equipped with sensors, a telemedicine service platform (IoT). This infrastructure offers both services oriented to the person in his home environment, and telemedicine services for cardiovascular patients. **SUMMIT** (PON 2014-2020) realized a dynamically configurable, adaptive and evolvable IoT infrastructure to enable the secure and dependable integration, as well as the runtime management and adaptation of smart objects (e.g., sensors, smart devices, smart IoT systems) available through heterogeneous IoT platforms. The infrastructure is validated in the area of smart health in robotic solution for ambient assisted living. **REHOME** (POR Piemonte 2014-2020) exploits innovative wearable and home sensors, for monitoring sleep and motor activities, and augmented/virtual reality, for cognitive (learning, memory, language, etc.) and motor (posture recovery, walking, upper limb functionality) rehabilitation, also offering social network services to facilitate the communication between patients and healthcare professionals, and big data analytics to follow the activities of patients at distance and to immediately adapt their therapy when needed. **PATHway** (EU H2020) developed an internet enabled, sensor-based home exercise platform that allows remote participation in cardiac rehabilitation programs, empowering patients to self-manage their cardiovascular disease using regular, socially inclusive exercise sessions as the basis upon which to provide a personalized, comprehensive lifestyle interventions. **TAS** (PON 2007-2013) developed a tele-monitoring system for wellbeing, primary and secondary prevention, which comprises sensor data management, interoperability among different e-health systems such as EHR and PHR, data sharing among healthcare workers facilitating the patient co-morbidity management, targeting pathologies like metabolic syndrome, chronic obstructive pulmonary disease (COPD), SLA and Alzheimer. **DMCoach** (EIT Digital 2018) developed a platform composed of mHealth APP (integrable with Hospital Information Systems) to unobtrusively monitor (activities, diet and anthropometrics), manage and coach (with personalized feedback and proposed intervention actions) citizens at risk of T2 diabetes and declared patients, so as to increase their awareness towards a healthier and appropriate lifestyle.

ENG has a long experience in participation to research and innovation projects in the health domain and is an important player in the health market. In his experience, ENG has entered into proficient long-lasting collaborations with relevant national and international scientific players, as hospitals, enterprises, university and research centres, consortia or other EU initiatives. Among many, there are ISMETT, BAYER, UPMC-I, Università Federico II, Fondazione Gemelli, CNI, Scuola Superiore Sant'Anna, Karolinska Institute, IIT, Fraunhofer, Università Politecnica di Madrid, EHTEL, HL7 Italia.

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" - Hub founder and Spoke's Affiliate
(Short Name: MARIO NEGRI)

General information

The Mario Negri Institute for Pharmacological Research (Istituto di Ricerche Farmacologiche "Mario Negri", *IRFMN*, www.marionegri.it) is a private, not-for-profit biomedical research organization. According to the Italian Legislation, effective January 2016, *IRFMN* is a "Istituto di Ricovero e Cura a Carattere Scientifico" (IRCCS). Currently, *IRFMN* consists of 3 different research headquarters which are located in Milano, Bergamo and Ranica. The 3 headquarters are part of the same organization and are run by a single Administrative Unit which operates inside the Institute in Milano (via Mario Negri 2, 20156 Milano, Italy). *Organization skills* The *IRFMN* scientific staff consists of approximately 700 researchers who are distributed in the three locations. About 50% of the scientists belong to the *IRFMN* permanent staff, while the remaining 50% consists of young individuals under training who are supported by pre-doctoral or post-doctoral fellowships which are generally issued by the Institute. *IRFMN* is endowed with the expertise, resources and equipment necessary to conduct experimental/pre-clinical and clinical studies involving pharmacological approaches in various areas of Biomedical Sciences. In particular, *IRFMN* focuses on Oncology, Neurosciences, Cardiovascular and Kidney Diseases including Rare Diseases, Toxic effect of Environmental contaminants, Food Safety, Mother and Children Health. Besides research, the Institute runs training courses for laboratory technicians , students and graduate researchers. It also organizes initiatives for the diffusion of scientific culture in biomedicine, in general and as specific backing for healthcare practice, and more rational use of drugs.

The expertise of the Institute in the specific field of Precision Medicine is supported by some of the most recent scientific papers published by IRFMN scientific staff' members, listed below:
-Cavalleri T et al. Alleanza contro il Cancro (ACC). Colorectal Cancer Working Group. Combined Low Densities of FoxP3+ and CD3+ Tumor-Infiltrating Lymphocytes Identify Stage II Colorectal Cancer at High Risk of Progression. Cancer Immunol Res. 2019 May;7(5):751-758. DOI: 10.1158/2326-6066.CIR-18-0661. - Novello S et al. International Tailored Chemotherapy Adjuvant (ITACA) trial, a phase III multicenter randomized trial comparing adjuvant pharmacogenomic-driven chemotherapy versus standard adjuvant chemotherapy in completely resected stage II-IIIA non small-cell lung cancer. Ann Oncol. 2022 Jan;33(1):57-66. Epub 2021 Oct 5. DOI: 10.1016/j.annonc.2021.09.017; - Vernieri C et al. Fasting-mimicking diet is safe and reshapes metabolism and antitumor immunity in cancer patients. Cancer Discov. 2021 Nov 17:candisc.0030.2021. Online ahead of print. DOI: 10.1158/2159-8290.CD-21-0030; -Nørskov AK et al. Assessment of assumptions of statistical analysis methods in randomised clinical trials: the what and how. BMJ Evid Based Med. 2021 Jun;26:121-12. DOI: 10.1136/bmjebm-2019-111268; - Caiola E et al. LKB1 Deficiency Renders NSCLC Cells Sensitive to ERK Inhibitors. J Thorac Oncol. 2020 Mar;15:360-370. DOI: 10.1016/j.jtho.2019.10.009; -Resovi A et al. Soluble stroma-related biomarkers of pancreatic cancer. Embo Molecular Medicine 2018,10, pii: e8741, DOI:10.15252/emmm.201708741; -Bizzaro F et al. VEGF pathway inhibition potentiates PARP inhibitor efficacy in ovarian cancer independent of BRCA status. Journal of Hematology & Oncology 2021, 14:186. DOI: 10.1186/s13045-021-01196-x; -Ghilardi C et al. PGC1α/β expression predicts therapeutic response to oxidative phosphorylation inhibition in ovarian cancer. Cancer Research 2022, 82:1423-34. DOI: 10.1158/0008-5472.CAN-21-1223. 35131872; -Bolis M et al. Network-guided modeling allows tumor-type independent prediction of sensitivity to all-trans-retinoic acid. Ann Oncol. 2017 Mar 1;28:611-621. DOI: 10.1093/annonc/mdw660; -Bolis M et al. Dynamic prostate cancer transcriptome analysis delineates the trajectory to disease progression. Nat. Comm. 2021 Dec;12:7033. DOI: 10.1038/s41467-021-26840-5.

Key elements/strengths

The IRFM has a thirty-year experience of planning activities, conducting analysis and monitoring of population epidemiological studies and clinical studies, with collection, management analysis of complex databases. This activity is conceived in collaboration with national and international research groups. The availability of doctors, computer scientists, research coordinators, statisticians, pharmacovigilance managers and quality assurance, certified monitors enable the coordination and conducting epidemiological research and clinical trials in various research fields. The possibility of management and use of in-house Biobanks in the field of oncology (for ovary cancer) and cardiovascular allows to expand the activity to studies of preclinical and translational research.

V.Torri, MD (S8). Input in methodological and statistical aspects of design, analysis and interpretation of results of preclinical, translational and clinical. E. Garattini, MD (S5). Molecular oncologist focusing on the nuclear receptor agonist, for the treatment of breast and stomach cancer. G. Taraboletti, Biologis (S5). Novel and repositioned antineoplastic compounds in the context of the tumor microenvironment; tumor molecular targets and cellular functions in multicellular *in vitro* systems recapitulating the organ-specific tumor microenvironments. G.Damia, MD (S 5). Generation of preclinical *in vitro* and *in vivo* models to identify molecular targets for new pharmacological intervention; screening of CRISPR and siRNA libraries. C.Ghilardi, Biotechnologist (S3). Molecular characterization of preclinical models (including PDX): "the case of ovarian cancer" for preclinical trials; metabolism reprograming to impair malignant progression. F.Ricci Biotechnologist (S3). Drug development in preclinical models focusing on drug resistance and cell metabolism (diets and pharmacological approaches) . All the senior participants have long experience in training and tutoring pre-doc student and post-doc scientist in the field of molecular oncology and pharmacology.

As for the experimental work, IRFMN contains all the structures and facilities necessary to carry out *in-vitro*, *ex-vivo* and *in-vivo* studies. In this last context, it must be stressed that IRFMN has one of the largest animal house facilities available in Italy, enclosed a dedicated area for immune-deficient mice and it is equipped with noninvasive imaging systems (optical-IVIS , MRI-tesla, and micro-CT). This setting is of fundamental importance for the conduction of the *in vivo* pre-clinical studies which are part of the present Research Proposal. Accessible institutional facilities include multiplex testing platform (BioRad Bio-PlexPlatform), flow cytometry, confocal microscopy, transmission and scanning electron microscopy, atomic force microscopy (Multimode 8-HR, Bruker), HPLC, mass spectrometry and MALDI imaging mass spectrometry (Mass spectrometry Centre for Health & Environment). Platform for high performance computing facilities for NGS , single-cell sequencing and omics analyses are available. Dedicated resources available in the Institute that will be used to implement various aspects of the current proposal include computational resources for the management and analysis of large clinical and pre-clinical data sets (Computational Oncology Unit). As for the clinical studies, *IRFMN* has a long-standing tradition in the organization and conduction of clinical trials via the established collaborations with numerous Clinical Centers throughout Italy and in various European countries.

The results obtained by researchers at the Institute are set out in more than 13,000 scientific publications in international journals. IRFMN has managed and coordinated numerous national and international projects, including the "GISSI-Italian Group" for the study myocardial infarction survival, which represented a milestone for cardiovascular diseases, or the "GISEN-Italian Group of Epidemiological Studies in Nephrology" within which was carried out the "REIN Study" whose results are still used by clinicians worldwide to slow the progression of chronic kidney diseases. Currently, IRFMN manages the ongoing networks of "GiViTi-Italian Group" for the Evaluation of the Interventions in Intensive Care Units involving more than 560 Intensive Care Units and the "Fenice-Italian Group" for Clinical Research in Emergency Medicine. At the EU level, IRFMN has been involved in more than 150 EU-funded grants (FP5, FP6, FP7, LIFE, H2020, Horizon Europe, etc.), 30 of them as Coordinator, and its researchers are still active in initiatives funded under the major EU funding schemes. Among the national and international collaborations of the Participants with other centers: the National Cancer Institute Milan, IT; The University of Chicago; Ulm University, DE; Faculty of Medicine, McGill University, Montreal, Canada; the National Cancer Centre Singapore; Metropolitan Hospital, Athens, GR; University of Wisconsin-Madison; University of Alabama at Birmingham; Department of Chemistry, UniPV, IT; Therapeutics Discovery Division, MD Anderson Cancer Center, Houston; Unit of Gynecological Oncology Research, European Institute of Oncology, Milan, IT; Institute of Biomedical Sciences Abel Salazar ,University of Porto, PT; Cancer Genomics, Fondazione Tempia Valente, Biella, IT. Furthermore, IRFMN has many years of experience in the design, organization, conduct and analysis of phase I, II and III controlled clinical studies. These studies are carried out through active collaborations with numerous national and international clinical centers.

OPELLA HEALTHCARE ITALY S.R.L. - Hub founder and Spoke's Affiliate

(Short Name: OPELLA)

General information

Opella Healthcare Italy S.r.l. belongs to the Sanofi Group as a recently created legal entity corresponding to the Consumer Healthcare division of Sanofi. Currently Opella is totally owned by Sanofi s.r.l., the Italian affiliate of the Sanofi Group.

Organization skills

(Sanofi Group) Sanofi is today an innovative global healthcare company, driven by one purpose: chase the miracles of science to improve people's lives. Around 100,000 people, across some 100 countries, are dedicated to transforming the practice of medicine by working to turn the impossible into the possible. Since September 2019, Sanofi worked to optimize its products portfolio and to leverage on all their 4 divisions to maximize results and growth opportunities: General Medicine, Specialty care, Vaccines and Consumer Healthcare.
Sanofi has prioritized its R&D resources:

- on potential first-in-class or best-in-class drugs. Today, 75% of R&D resources are allocated to potential first-in-class or best-in-class; - on the leading role of innovation. On a wide range of proprietary innovative technology platforms (mRNA and many others) acquired through a dozen strategic acquisitions over the last two years absolutely in line with its strategy, to expand its portfolio in oncology, immunology and rare diseases and to reinforce its growth axes;
- - on a process of continuous improvement and acceleration of clinical operations and the ability to process big data and real-world data to move forward as quickly as possible; - on the ability to rethink research and development activities in terms of simplifying governance, organizing and strengthening teams where necessary.

National and international project management skills

Technology transfer capacity - patents - spin-off

In Italy, Sanofi is an industrial reality of excellence, thanks to its factories, reference poles for the Group.
Sanofi Italy Headquarters and offices are in Milan and in Rome. 4 industrial sites: **Origgio (Va, Lombardy Region), belonging to Opella Healthcare Italy s.r.l**, in Anagni (Fr, Lazio Region), Scoppito (Aq, Abruzzo Region) and Brindisi (Puglia Region) owned by EuroApi Italy s.r.l.

Illustrate the demonstrated ability of individual Spoke and affiliated entities to manage and implement projects in fundamental and/or applied research with reference to the area of the partnership PE6 "Diagnostica e terapie innovative nella medicina di precisione" (see art. 7 par. 2 Call for proposal "Partenariati estesi").

Specify any existing national and international collaborations with other institutions and centers of high scientific quality, as regards the area of specialisation "Diagnostica e terapie innovative nella medicina di precisione" PE 6 (see art. 7 par. 2 Call for proposal "Partenariati estesi").

The Company boasts a diversified business that covers the entire drug value chain: clinical research and development, R&D in API and drugs production, medical information, and sales. Sanofi s.r.l. deals with the research, manufacture, processing, and packaging, on its own and on behalf of third parties of chemical, pharmaceutical products, including biological products (human vaccines, serums, diagnostics, processing of blood and its derivatives), of medicinal specialties, health aids, medical-surgical, medical devices, para pharmaceuticals, over-the-counter products but also probiotics, some supplements and more generally nutraceutical products. Sanofi employs in Italy more than 2,000 people including employees and collaborators: more than 1000 are employed in our industrial plants. Annual investments for 75 million euros for its industrial footprint and 11 million euros for Clinical development activities. In the last three years (2019-2021), Sanofi invested around 200 M€ in our industrial research apparatus, triggering a further share from the Regions and the Country. **Origgio** site is the **Sanofi Worldwide R&D&I center for Probiotics** with expertise in multiple delivery forms, manufacturing technologies, digital sustainability evolution, for international products covering more than 70 different markets worldwide & suppling key Brands, leaders in probiotics & digestives wellness. In 2019, an Innovation Agreement was approved by MISE (signed in 2022) for a research project creation of approximately € 20 million that will allow the manufacture of the innovative "Probiotikà" pilot Accelerator Center (around 500 sq m) focused on innovation and R&D. The activities of the Probiotikà Center are aimed at developing new innovative nutraceutical products to support the immune system and for the healthy aging area, through modulation of the **microbiome**.

Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

Microbiota area:

- human studies for the registration as drug of new formulation of our main probiotic;
- cooperation with several Universities (Pisa, Naples, Rome Catholic University, Piacenza Catholic University, Sacco Hospital – University of Milan) for the conduction of preclinical studies investigating the mechanism of action of Bacillus clausii strains;
- conduction of Observational Studies;
- scientific dissemination on the knowledge of microbiota and probiotics to Heath Care Providers
- advanced data analysis (i.e. in silico simulation; PBPK modelling, large data sets analysis)
- project management and team supervision
- innovation projects in the area of probiotics as food supplements;
- support to Origgio Industrial Site for the scientific aspects of the innovations projects they have been conducting in the last years;
- expertise in technology, formulations development and industrialization for consumer health care products;
- advanced analytical methods application to characterize the products;
- specific know-how in probiotics and oral dosage forms;
- process improvement projects in fermentation, preparation of suspensions, syrups, solutions, solid forms in different packaging presentations;
- application of innovation and lean methodologies to optimize products, process and flows;
- digital projects management, aligned to Industries 4.0

Key elements/strengths

Maria Chiara Uboldi and Paolo Pellegrino*:* human clinical studies for the registration as drug of new formulation of our main probiotic; cooperation with Universities for the conduction of preclinical studies; conduction of Observational Studies; scientific dissemination on the knowledge of microbiota and probiotics to Heath Care Providers; advanced data analysis (i.e. in silico simulation; PBPK modelling, large data sets analysis); project management and team supervision

innovation projects in the area of probiotics as food supplements;

Maria Cristina Trecciola*:* expert in technology, formulations development and industrialization for consumer health care products, with specific know-how in probiotics and oral dosage forms field (research in fermentation, preparation of suspensions, syrups, solutions, solid forms in different packaging presentations). Cooperation in several innovation projects with associated regional or national grants (from 2015 till now, with a total of 3 projects successfully completed, one project under completion and two new ones just submitted). Industrial scientific know how of products in the digestive wellness category, with special focus on the microbiota role in the treatment and prevention of human diseases and associated biotics benefits. Coordination of industrial technological tramsfer. Expert in continuous improvement, lean and manufacturing excellence methodology

S.I.T. – Sordina IORT Technologies S.P.A. - Hub founder and Spoke's Affiliate
(Short Name: SIT)

General information

S.I.T. - Sordina IORT Technologies S.p.A. (web: www.soiort.com) is an "Innovative SME" working in the designing, manufacturing, selling and maintenance of medical and research devices for oncological radiation therapy. The company is a leader in IORT technological solutions for the Intra-Operative Radiation Therapy treatment and over twenty years has sold its products in Italy, Europe and at international level (USA, East and Middle East countries) in hospitals and prestigious research centres. The company represents an excellence of the Italian technology, one of the few companies in the world capable of producing miniaturized mobile electron linear accelerators for radiation therapy. SIT is the first company in the world (and still unique) which has designed and manufactured a preclinical research accelerator (ElectronFlash, https://www.soiort.com/flash-rt-technology/) dedicated to Flash Radiotherapy, a methodology using electron beams and results to be iso-effective in the treatment of neoplastic cells significantly preserving healthy tissues unlike standard treatments. The Flash effect, discovered by the Institute Curie group in 2014, represents the most interesting perspective in radiation therapy, as confirmed by the last ESTRO Congresses in Madrid (August-September 2021) and Copenhagen (May 2022).

Organization skill National and international project management skills Technology transfer capacity - patents - spin-off Experience in the field of "Diagnostics and innovative therapies in Precision Medicine"

SIT has a wide national and international scientific cooperation network with Universities, Research Centres and Companies, reported in the section below. SIT intense scientific activities leads naturally both to scientific papers publications (more than 100 papers on peer reviewed journals) and to patents productions (more than 40 national and international patents, 7 of which dealing with FLASH related technologies). A quick reference to the main scientific publications can be found here: https://www.researchgate.net/profile/Giuseppe-Felici/research. SIT, being an 'Innovative SME', has in its own DNA the cooperation with research Institution; the development of ElectronFlash itself has been achieved thanks to a long-lasting cooperation with SBAI Department of La Sapienza University and many Flash research topics have been investigated together with Pisa University and Research Centres.

Key elements/strengths

SIT will share all its infrastructure needed for the development, testing and production of linear accelerators: vacuum laboratory, radiofrequency laboratory, assembling areas and two bunkers for final testing of the equipment within the Partnership. Furthermore, SIT will provide its expertise as Medical Device manufacturer and its expertise in Medical Technical Standards and in designing of Flash linear accelerators. Today, three ElectronFlash units have been successfully installed in Institute Curie, Paris, France (September 2020), UZA University, Antwerp, Belgium (December 2021) and University of Pisa, Pisa, Italy (June 2022).

SIT has a wide national and international scientific cooperation network with Universities, Research Centres and Companies, hereinafter detailed:

Research Centers and Universities:

- University of Pisa, Pisa;
- CNR, Institute of Neuroscience and Institute of Nanoscience, Pisa;
- INFN, National Institute of Nuclear Physics (Sections of Turin, Pisa, Rome, Catania);
- La Sapienza University of Rome, Department of Basic and Applied Sciences for Engineer (SBAI);
- Tor Vergata University of Rome, Department of Physics;
- Scuola Superiore S. Anna Pisa, Pisa.
- University of Perugia, Perugia;
- ENEA, INMRI.

Italian Hospitals and IRCCS:

- Policlinico Universitario Agostino Gemelli, Roma;
- IEO, European Institute of Oncology, Milan;
- AOUP - Pisa University Hospital;
- Maggiore della Carità University Hospital, Novara.

European Institutions:

- Curie Institut, France;
- GZA University, Antwerp, Belgium;
- University of Santiago de Compostela, Santiago de Compostela, Spain;
- University Clinic of Navarra, Madrid, Spain.

European Companies:

- Thales, France (https://www.thalesgroup.com/en/markets/market-specific-solutions/microwave-imaging-sub-systems/radio-frequency-microwave-sources-3) ;
- Bergoz, France (https://www.bergoz.com/);
- Advacam, Czech Republic (https://advacam.com/);
- STLab, Catania, Italy (https://www.stlab.eu/).

SECTION B

CHARACTERISTICS, FEASIBILITY AND CONTROL

B. CHARACTERISTICS, FEASIBILITY AND CONTROL

B.1 – Composition of the Consortium and Spoke's Decription

HEAL ITALIA is based on 8 thematic Spokes established at entities from the **academic sector** (red acronyms in the chart) where entities from both the **private** and **clinical research sectors** (black names/acronyms in the chart) are affiliated to realize the proposed research and innovation program.

Moreover, according to its acronym, the HEAL ITALIA network is distributed throughout the different regions of the Mediterranean peninsula and its major islands. The selection of spokes and affiliates was based on an expression of interest towards an innovative vision of the *Precision Medicine Paradigm* that aimed at developing best methods of precision medicine and extend their application from oncology to other classes of pathologies (e.g. cardiovascular, metabolic and rare disease), where patient-centred healthcare can be implemented. The process involved invitation to extended mailing list (Dec. 2021), competences, facilities, and skills mapping (Jan. 2022) and was followed by a shared vision of the proposal draft (Mar. 2022) and an open call for expression of interest (Apr. 2022) resulting in a partnership between public (16) and (9) private entities. Each Spoke has its own Scientific Advisory Board composed by international scientists that voluntarily contribute as supervisors of the quality of the program's implementation.

The Extended Partnership is organised in 8 thematic Spokes, each of them performing from 3 to 5 research Work Packages (WPs) divided into the relative tasks. Initial Spoke's composition (Leader and affiliates) is described in the table below without compromising the possibility of further affiliations in a Spoke of other entities already belonging to the partnership.

The themes of the spokes are based on the research flow of methodologies that contribute to the full implementation of precision medicine, from omics mapping (Spoke 1) to data treatment (Spoke 2), model development (Spoke 3), innovative diagnosis and therapeutic approaches (Spokes 4 and 5), tailored devices (Spoke 6), prevention strategies (Spoke 7) and clinical applications (Spoke 8).

HEAL ITALIA - Health Extended ALliance for Innovative Therapies, Advanced Lab-research, and Integraded Approaches of Precision Medicine							
Spoke 1 Holistic Nosology	**Spoke 2 Intelligent Health**	**Spoke 3 Prediction models**	**Spoke 4 *S4D Precision Diagnostics***	**Spoke 5 *Next-Gen Therapeutics***	**Spoke 6 *Healthy Toolbox***	**Spoke 7 *Prevention Strategies***	**Spoke 8 *Clinical Exploitation***
Spoke Leader TOR VERGATA	**Spoke Leader UNIBO**	**Spoke Leader UNIPA**	**Spoke Leader SAPIENZA**	**Spoke Leader UNIMIB**	**Spoke Leader UNIMORE**	**Spoke Leader UNIVPM**	**Spoke Leader UNIPI**
Affiliates ISS NEUROMED SAPIENZA TLS UNIBO UNICA UNIFG UNIMORE UNIVPM UNIVR	**Affiliates** BIREX ENGINEERING IFO-IRE ISS NEUROMED SAPIENZA TOR VERGATA UNICA UNICT UNIMIB UNIMORE UNIPI UNIVR UPMC	**Affiliates** BIREX IFO-IRE IOM ISS M. NEGRI SAPIENZA SIT TOR VERGATA UNIBO UNICA UNICT UNIFG UNIMIB UNIMORE UNIPI UNIVPM	**Affiliates** S. ORSOLA TOR VERGATA UNIBO UNICA UNICT UNIFG UNIMIB UNIMORE UNIPA UNIPI UNIVPM UNIVR	**Affiliates** CRO Aviano ISS M.NEGRI NEUROMED OPELLA SAPIENZA SIT TLS UNIBO UNICA UNICT UNIMORE UNIPA UNIPI UNIVR UPMC	**Affiliates** CRO Aviano UNIBO UNICA UNICT UNIFG UNIMIB UNIPA UNIPI UNIVR	**Affiliates** IFO-IRE ISS NEUROMED SAPIENZA UNIBO UNICA UNICT UNIFG UNIMORE UPMC	**Affiliates** IFO-IRE M. NEGRI S.ORSOLA SAPIENZA TLS TOR VERGATA UNIBO UNICA UNICT UNIMIB UNIMORE UNIPA UNIVR UPMC

Additionally, the following actions run through the entire HEAL ITALIA network and will be performed involving all of the Spokes and their affiliates:

- Technology transfer, dissemination and exploitation of HEAL ITALIA research results.
- Promoting self-entrepreneurship of HEAL ITALIA's young researchers fostering incubation and acceleration of research spin-offs.
- Cross-sectoral (businesses, clinics and universities) professional skills empowerment focused on KETs exploitation in precision medicine.
- Higher education and research training (PhD programs) in precision medicine

B.2 Critical mass and recruitment requirements

The project's critical mass satisfies the criteria of the call and consists of a total of 350 structured researchers, divided in 8 thematic spokes each one fulfilling the minimum critical mass criteria in terms of persons and months dedicated to the program. The selection of involved individuals has been based on competence in the specific field of precision medicine, high ranking in bibliometric indicators in each own field of research, capacity of attracting funding. Gender balance has been taken into account as well as the involvement of young researchers. Nevertheless, the starting percentage of involved young researchers holding a PhD awarded later than 2013 is just below 10% (33/350), while female researchers represent only the 35% (122/350) of the critical mass. These percentages will be potentially improved through dedicated recruitment of more than 200 young scientists including at least 100 short term researchers, with an allocated budget of at least 15 million €, and more than 100 PhD students.

			SPOKE LEADER						SPOKE'S AFFILIATE				SOUTHERN REGION							
			SPOKE 1		SPOKE 2		SPOKE 3		SPOKE 4		SPOKE 5		SPOKE 6		SPOKE 7		SPOKE 8		TOTAL	
	ENTITY	SECTOR	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M
1	TOR VERGATA	public	12	141	2	21	4	42	3	36							1	12	22	252
2	UNIBO	public	1	9	8	90	5	51	3	27	3	27	4	36	1	9	5	45	30	294
3	UNIPA	public					10	147	4	45	8	123	5	63			2	36	29	414
4	SAPIENZA	public	3	27	2	18	2	18	15	143	4	39			1	9	5	54	32	308
5	UNIMIB	public			2	18	4	36	4	45	6	54	3	27			1	9	20	189
6	UNIMORE	public	3	30	2	18	1	15	4	45	3	30	9	98	3	36	1	12	26	284
7	UNIVPM	public	2	18			1	9	1	9					12	108			16	144
8	UNIPI	public			1	12	4	36	1	12	2	18	4	45			13	132	25	255
9	UNIFG	public	4	51			1	12	5	69			6	87	1	12			17	231
10	UNICT	public			1	9	2	27	4	36	2	18	4	42	4	39	2	18	19	189
11	UNICA	public	2	21	3	45	1	9	6	72	2	24	2	18	1	9	2	21	19	219
12	UNIVR	public	7	63	3	27			2	18	2	18	1	9			2	18	17	153
13	ISS	public	1	12	2	18	4	48			1	15			12	120			20	213
14	IFO-IRE	public			3	27	5	45							2	18	7	63	17	153
15	NEUROMED	private	2	18	2	18					1	9			1	12			6	57
16	CRO AVIANO	public									1	24	1	12					2	36
17	SANT'ORSOLA	public							1	9							1	9	2	18
18	TLS	private	2	18							2	18					3	27	7	63
19	UPMC	private			1	9					1	9			1	9	1	9	4	36
20	IOM	private					3	27											3	27
21	BI-REX	private			2	18	2	18											4	36
22	ENGINEERING	private			3	27													3	27
23	MARIO NEGRI	private					1	9			2	18					1	9	4	36
24	OPELLA	private									3	27							3	27
25	SIT	private					1	9			2	18							3	27
	TOTAL		39	408	37	375	51	558	53	566	45	489	39	437	39	381	47	474	350	3688

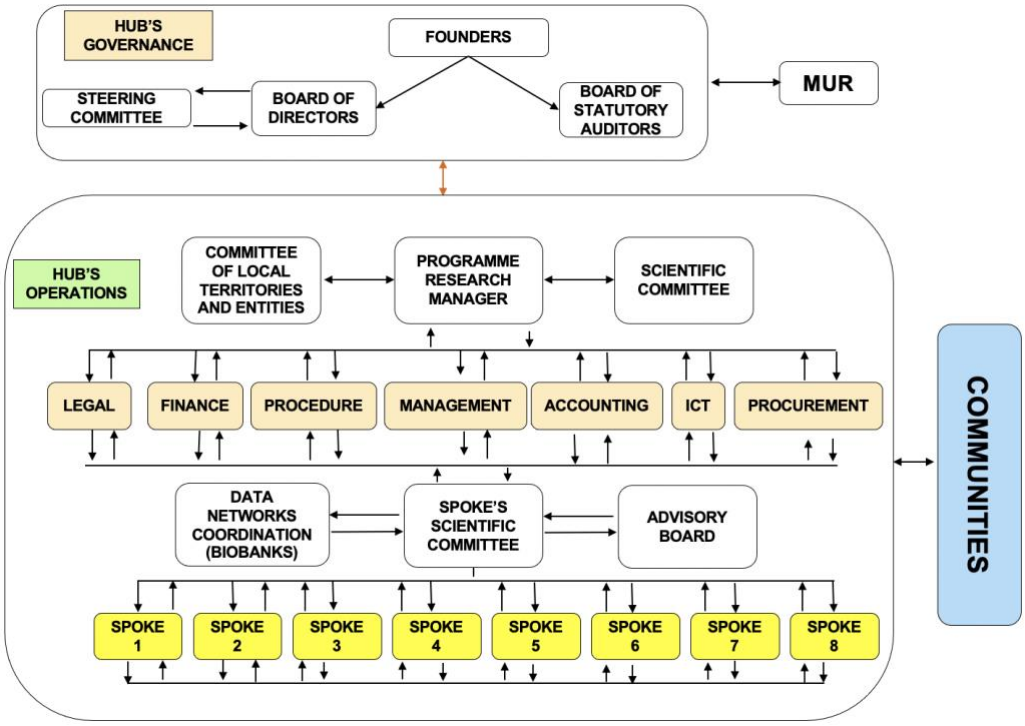
The intensive program of PhD recruitment will be developed in two phases, starting from already activated PhD courses in fields consistent with the precision medicine area within the partnership (Period Nov. 22 – Oct. 25), through the financing of PhD grants to entities external to the consortium and leading to the implementation of a coordinated national PhD program on Precision's Medicine (Period Nov. 23 – Oct. 26) that will be running over the period financed by the NRRP and will be co-financed by adhering institutions and private entities. Recruitment and selection procedures will respect the best practices of equal opportunities guidelines and the Gender Equality Plan (GEP) of each recruiting institution. In particular, for PhD grants, a minimum of 40 % of female recruitment can be respected by initially assigning the grants to females in the final rankings until the 40% rate is fulfilled, successively assigning the grant to the other candidates in order of rankings. As for fixed-term researchers, in case of more than one available position per scientific sector, the calls will be organized by reserving at least 40% of positions to female candidates in the rankings. With an envisaged 40 % rate of female recruitment, the HEAL ITALIA scientists population will increase its rate of female scientists to 37 % (202/550).

B.3 Description of the HUB – Management and administrative structure

Hub's legal form and governance

The parties involved will establish a not-for-profit Foundation, without prejudice to any definitive assessment, which will be shared and validated by the MUR in the course of the subsequent phase, always taking into account the regulation applicable to publicly held entities. All involved universities will be members of the Hub together with other public (S.ORSOLA, IFO-IRE, CRO Aviano) and private (NEUROMED, TLS, UPMC, IOM, BIREX, ENGINEERING, MARIO NEGRI, OPELLA, SIT) partners. The majority of the Hub's governance will be determined by public entities and the private entities will

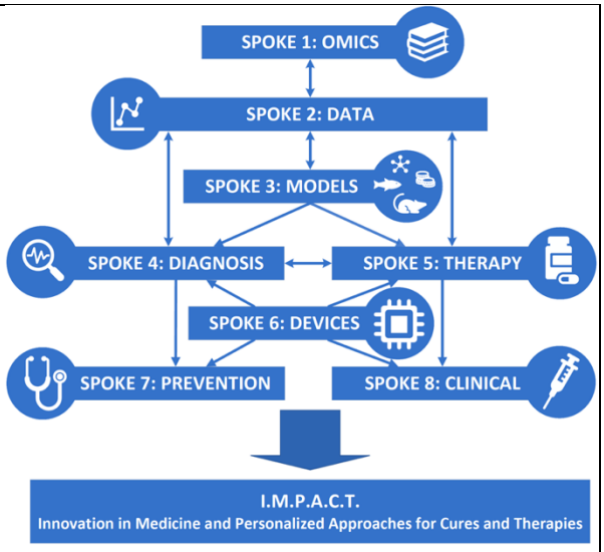
enter into the governance of the Hub in compliance with all regulations applicable to their specific case.



The Hub will have the following governance model: **a) the Founders ("*Membri Fondatori*")** will mainly have the function of appointing the members of the management and control bodies, defining the general Hub's strategic policies, which will be implemented at the level of the Hub's operational bodies (*e.g.* management body, programme research manager, etc.). For specific resolutions the Founders will require an absolute majority of the Founders for the approval of strategic decisions; **b) the Board of Directors ("*Consiglio di Amministrazione*")** will be formed by no. 5 to 11 members, with the majority designated by public entities, and will be entrusted with the task of guaranteeing an adequate organization of the Hub considering its functions within the Enlarged Partnership and the activities to be carried out by the Hub itself (*e.g.* medical research on precision medicine, training, technology transfer, spin-off, etc.). The main tasks is the establishment of a management structure at the Hub's level, to carry out the tasks necessary for the Enlarged Partnership operations (*e.g.* management functions, connection, coordination, reporting, etc.); **c) the internal control body ("*Organo di Controllo*")** will be formed by no. 5 members (no. 3 effectives and no. 2 alternates) and will carry out its own functions provided for by law, supervising the activities of the Hub as a whole; **d) the steering committee ("*Comitato Direttivo*")** will primarily have advisory functions to the management body in carrying out its Hub's management activities; **e)** two consulting committees, the **Scientific Committee** and the **Territorial Entities and Communities Committee** will be established by the Hub's by-laws to provide opinions and identify territorial macro-trends and to address liaison activities of the Enlarged Partnership; **f) the programme research manager ("*Responsabile dell'attività di progetto*")** will primarily have implementation, project execution functions, ordinary operation of the Hub's activities and overseeing the implementation of the various work performed within the HEAL ITALIA partnership and will coordinate the various areas in which the Hub's operations are divided (*e.g.* legal affairs, management control, finance, procedure, etc.); **g) the Data Networks Coordination (*i.e.* Biobanks)** will ensure the quality of biometric data management; **h) Spoke's scientific committee ("*Comitato Tecnico Scientifico degli Spokes*")** will be formed by a representative for each Spoke and will primarily have coordination functions, overseeing the implementation of each Spoke's activities according to the subject of the activities performed by each of them. The procedure for the appointment of the members of the various committees and bodies and the governance model of the Hub will ensure the respect for equal opportunities and gender equality. The Hub&Spoke's relationships will be governed on the basis of written agreements, which will describe the specific commitments that the Spokes will undertake taking into account the purposes of the Call and of the respective research project, with the aim of ensuring the coordination of the Hub and the reporting duties towards the MUR, in accordance with the requirements of the Call. The same model could also be used by the Spokes towards their affiliates. Along and after the 3-year program, HEAL ITALIA Hub will also rely on administrative personnel of the spokes and their affiliates in order to manage all the activities that will include further participation in other research programs, as well as the provision of research services based on the networked laboratories and competences.

B.4 Work Plan & Costing

The HEAL ITALIA work plan has a 36 months' timeframe, although the set-up network is planned to persist

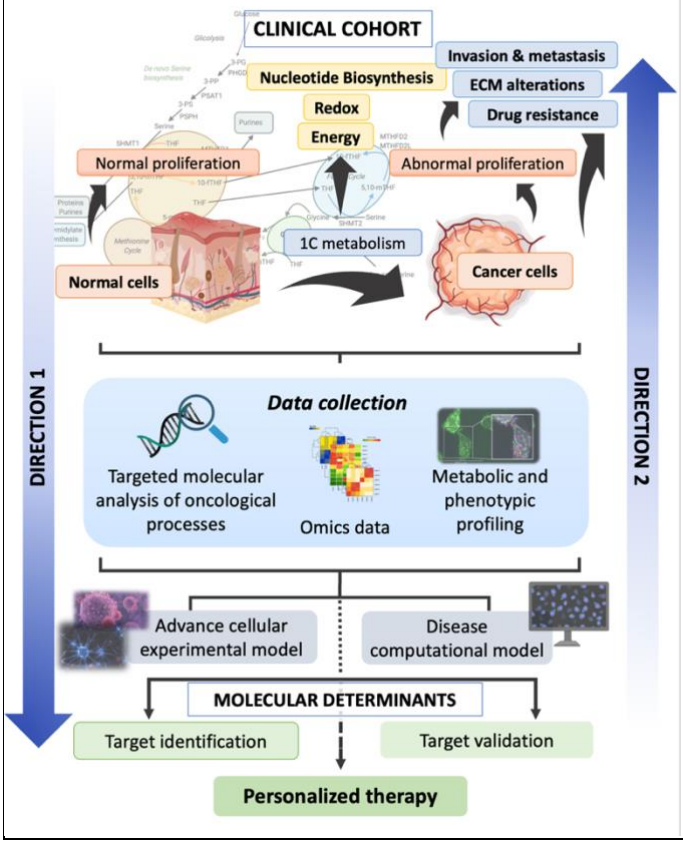


after the end of the NRRP investment period. For this reason, the entire network has been designed on the basis of 8 thematic interconnected spokes (see Pert diagram below), each one with its own focus along the research workflow, thus ensuring the **feasibility** and accountability of the planned activities within the timeframe of the project and beyond. The **scientific and technical feasibility** of the project relies on the high-level skills (see CVs) and international experiences of project partners and on their ability to successfully participate in multidisciplinary applied research activities involving enterprises. The duration of the project and the strong interaction with industry will ensure a successful implementation of the project in the long-term period. The **implementation capabilities** of the project will be ensured by sound project management methodologies applied at each Spoke level. Each partner will operate under the coordination of the Spoke's Leader to guarantee high scientific quality and transferability of the output. The achievement of the expected results will also be granted by the direct link with technology transfer actions foreseen in each Spoke. An overview of the activities of each Spoke is provided below and fully detailed in "*Annex B – Description of Spokes*" and "*Project Logic Board*" attachments where executors are indicated as the entities majorly responsible for undertaking the efforts to achieve each WP objectives without compromising the possibility of further involvement in the WP of other entities within the partnership.

SPOKE - 1

HOLISTIC NOSOLOGY

FROM PATIENTS TO MOLECULES & BACK: Mapping the omic landscape of clinical to molecular environment, to identify, classify, and refine the phenotypes of multifactorial diseases.



GENERAL OBJECTIVE: To identify, in a **normal healthy large population**, FACTORS controlling/protecting development or progression towards distinct diseases, to be used as preventive/prognostic biomarkers and potential drug target for personalized medicine intervention. OMICS and in vitro/vivo investigations will be essential, and connected to all other spokes and external biotech.

The spoke aims to investigate a **normal population** slowly progressing towards disease as well as disease models in order to define molecular markers predictors of adverse or protective events. The identified genes/molecules will be studied in vitro and in vivo (DIRECTION 1: from patients to molecular determinants). Conversely, current knowledge on metabolic and biochemical pathways, moving in the opposite direction (DIRECTION 2: from molecular determinants to patients) will be deepened. At the basis of this experimental design are 3 cohort of >24.000 (Moli-Sani), 4.000 (RoCAV), 200,000 (Genoma Sardo) subjects, testing the "common-soil" hypothesis. The longitudinal cohort which includes samples (blood, buffy coat, serum, urine), collected every 5 years during the last

15 years, medical records, exposome. Multi-omics and multidimensional epidemiological integration will identify novel precision diagnosis biomarkers. The opposite direction originates from genetically modified mouse models, that will be investigated in the clinical cohort to evaluate their role in a human context. Underlying molecular mechanisms with potential novel therapeutic targets and clusters of prognostic/prevention predictors will be shared with Spokes 2, 3, 8. The spoke has its own **Scientific Advisory Board** (Boris ZHIVOTOVSKY (Karolinska Institut, Sweden), George CALIN (MD Anderson, TX, USA), Michele CARBONE (Cancer Centre, HI, USA), Xin LU (Ludwig, Oxford, UK), **PhD Course** for scientific training and **Editorial Office** for training on publishing, Rome (www.nature.com/cdd) (impact factor 15.8). **Biotech** involvement is also Highly consolidated.

WP 1: Population mapping: DNA seq, Exome Mapping aiming at the identification of pathogenic genetic variants

Task 1.1: Precision Medicine: the common soil hypothesis and the Moli-sani/RoCAV studies. The *Moli-sani study* (www.moli-sani.org) (24,325 subjects, from 2005) investigates the **common soil hypothesis** to evaluate risk/protective factors (environmental, genetics, biomolecular) linked to chronic-degenerative diseases (cardiovascular, cancer, neurodegenerative) and their intermediate phenotypes (hypertension, diabetes, dyslipidemia, obesity, metabolic syndrome). The *RoCAV Study* (http://epimed.uninsubria.eu/) (3777 subjects) and the *Genoma Sardo Study* (in Sardinia from 2010) expand the resource with samples, clinical information, exposome to elaborate OMICS and generate correlations cluster of biomarkers. ***Deliverable***: follow-up mortality on chronic degenerative disease.

Task 1.2: Genomics, Phenomics and Biomarkers. Identifying phenotypically coherent groups of patients within the cohorts, including single cell multi-omics profiling of induced pluripotent stem cells-derived organoids, the Task investigates how genetics, environment, social factors and healthcare interact to identify the genetic determinants capable of modulating the phenotype. Data (GWAS/EWAS, Polygenic Risk Score, PRS) compared to international consortia (UK biobank; Nevada Health Genome) will generate powerfull biomarkers. ***Deliverable***: biobank of pluripotent stem cells; genomic/non-genomic biomarkers for predictive diagnostics/prognosis/pathophysiology/pharmacogenetics; translation/drug development.

Task 1.3: Metabolome mapping from mouse to Moli-sani sub-cohorts and development of new therapeutic targets. Metabolic profiling of selected Moli-sani, mouse models and organoids genes involved in mitochondrial metabolism will provide fine mapping and genotype/metabolite correlations. ***Deliverable***: Set up a nanostring prognostic response for 1C metabolism or mitochondrial regulators.

WP 2: Transcriptomics: refinement of "common-soil" hypothesis & investigation on chronic pathological conditions for personalized stratification for therapeutics

This WP will refine the molecular identity of "common-soil" features within the 3 population cohorts. It will also be expected to address unmet clinical needs of the above indicated chronic-degenerative diseases.

Task 2.1: Omics biomarkers in the stratification of obesity, epithelial and related metabolic and functional complications. Technological support for the production of transcriptomic (genomic and epigenomic) data to WP1 on obesity, epithelial homeostasis and degeneration, and nonalcoholic fatty liver disease. ***Deliverable***: omics for obesity, NAFLD, selective epithelial targets.

Task 2.2: Multi-omics approach for big killers: stratification of treatment response and tailored interventions. Transcriptomic (genomic/epigenomic) data on cancer cardio-vascular diseases (heart failure), Immune checkpoint inhibitors, circulating tumor cells, evolution/therapy resistance in lympho-myeloproliferative disorders. ***Deliverable***: define novel omics predictors of ICPIs, LMOD; optimization of guideline-directed medical therapy and physical, nutritional, cognitive status in HF.

Task 2.3: Serine metabolism and epigenetic regulation through ncRNAs. Key players (histone modifications, DNA methylation) in epigenetic control regulating chromatin remodeling complexes, and ncRNAs, will be investigated in normal and cancer cells under stress/drugs (HDACi, DNMTi, ROS, H2S). ***Deliverable***: molecular mechanisms of selected ncRNAs; molecular signatures in the Moli-sani cohort and selected mouse models.

WP 3: Proteomic and metabolic analysis: an exciting avenue to advance the knowledge of dynamic interactomes

Task 3.1: Microbial metabolites impact on disease: from translational models to bedside. Gut microbial diversity, lipid/glucose metabolism, low-grade inflammation preceding common multimorbidity will help

understanding individual patients' disease mechanisms, with potential for early intervention approaches. Organ-Chip, organoids, transgenic and diet-induced models of metabolic syndrome will correlate inflammatory diseases and metabolic disorders to understand immunometabolic diseases and to develop novel diagnostic and therapeutic opportunities. ***Deliverable***: Bioinformatic immunometabolic analysis; identification morbidity markers.

Task 3.2: Protein degradation in physiology and pathology. E3 ubiquitin (Ub) protein ligases and de-Ub regulate cell functions and fate via both proteolytic and non-proteolytic actions and affecting individual patient's response. ***Deliverable***: Identification of substrate targets and their role in cell physiology and correlation to the subjects in WP1.

Task 3.3: Autophagy, cell cycle regulation and diseases. Will investigate, in subjects from WP1 and in mouse models, defects of the autophagic machinery that is associated with dysfunction of multiple metabolic tissues including pancreatic β cells, liver, adipose tissue and muscle, and is implicated in metabolic disorders such as obesity, insulin resistance and cancer. ***Deliverable***: Validate AMBRA1, TG2, necroptosis, innate immunity as a prognostic biomarker for cancer; pipeline for testing autophagy-modulators.

WP 4 Metabolic alterations, metabolites and metabolome maps

Task 4.1: Long Chain fatty acids enzymes and lipid metabolism. Lipid metabolism, fatty acid synthesis and their enzymatic regulators (ELOVL) determines cell survival, cell death and function. ***Deliverable***: Test ELOV amd ZNF750 regulators in epithelial and cancer context.

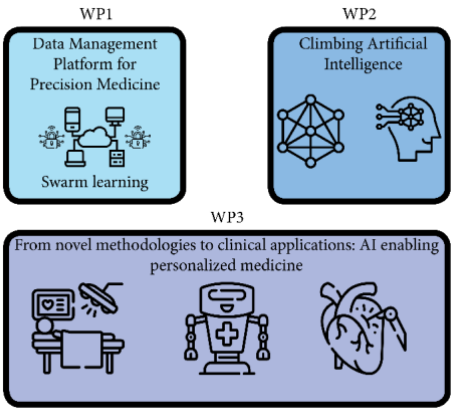
Task 4.2: Imaging & Ca2+ machinery as reporter of metabolic adaptations in physiology and disease. Cell imaging, organelles interactions and Ca2+ signaling represents one of the main mechanisms to fine-tune mitochondrial activity in response to energetic cellular demand. ***Deliverable***: ER-mitochondria Ca2+ homeostasis changes in selective disease.

Task 4.3: Genes versus environment, causing metabolic dysregulation leading to disease. The dysregulation of the molecular mechanisms underlying metabolic processes may cause cancer, as a consequence of cell-intrinsic/-extrinsic events. Cellular DNA can be damaged by spontaneous hydrolysis, reactive oxygen species or aberrant cellular metabolism. In fact, several environmental factors may damage the DNA, alter cellular metabolism or affect the ability of cells to interact with their microenvironment. Carriers of heterozygous germline mutations of the deubiquitylase BAP1 develop cancer (uveal melanoma, cholangiocarcinoma, mesothelioma, chronic myelomonocytic leukemia, pancreatic ductal adenocarcinoma). BAP1 mutant regulates type 3 inositol-1,4,5-trisphosphate receptor and modulates ER-to-mitochondria calcium-release and led to the same reduced mitochondrial respiration and increased aerobic glycolysis Warburg effect that is detected in malignancies. AIM is (i) to understand the gene versus environment interaction at the level of metabolic dysregulation leading to cancer development and relate that to specific epithelial cancers, (ii) their regulation with transcription and ncRNA. ***Deliverable***: Understanding metabolic dysregulation in environmental cancer development.

SPOKE – 2

INTELLIGENT HEALTH

HEALTH DATA SCIENCE: Data management and development of advanced methods, algorithms, and machine learning approaches integrating health big data for Precision Medicine



GENERAL OBJECTIVE: The general objective of spoke 2 is to establish a model of a collaborative data and analysis platform among the partners of the Consortium for Precision Medicine in full compliance with the Italian privacy legislation for enabling the processing of predictive computer models on large volumes of digital heterogeneous data, coherently with the Health Big Data (HBD) project, funded by the Italian Ministry of Health, which aims to enable the exchange of digital clinical data between all 52 Italian IRCCS. This data and analysis platform will also enable swarm learning – a decentralized machine learning approach to further accelerate the introduction of Precision Medicine in the clinic. Novel and innovative computational models, and multi-purpose AI frameworks – compatible with the data and analysis platform and integrating multilevel data (biological, imaging, and clinical data) for Precision Medicine will be developed to predict disease diagnosis,
personalized intervention, and precision treatments. Finally, novel AI methodologies will be developed and applied in real clinical settings enabling ready-to-use personalized medicine systems. The collaborative data and analysis platform and innovative AI frameworks will be shared with Spokes 3, and 4. The spoke 2 has its own **Scientific Advisory Board** (Riccardo Poli, Essex, UK and Pietro Liò, Cambridge, UK). **Birex** involvement is also highly consolidated.

WP 1: Integrate confidential clinical data with omic and imaging landscape map

WP1 will address the challenge of deploying a collaborative, clinical data and analysis platform for Precision Medicine, enabling feature extraction, harmonization, and analysis of heterogeneous health big data in full compliance with the Italian privacy legislation.

1.1 TASK: Development and deployment of a collaborative platform to integrate molecular, imaging, and clinical characteristics of individual patients. This task aims to develop and deploy a collaborative IT infrastructure for predictive computer models enabling: i) the processing of large volumes of digital clinical data in full compliance with the Italian privacy legislation; ii) the use and development of computational and data-driven models for clinical research applications (as provided by spoke 4) from risk prediction models to Precision Medicine. When all services, mechanisms (e.g., anonymous data access), and protocols will be implemented, the IT platform will be accessible among the partners of the Consortium via Virtual Machines that can be equipped with the required analysis stack for algorithm development and statistical analysis. A dedicated bioinformatics platform for research purposes will also be tested. When high-performance computing (HPC) resources are required, it will be possible to initialize a virtual machine on-demand in a local encrypted file system at the HPC facility. ***Deliverable***: final version of the clinical data infrastructure. Adopted technical, organizational, and legal solutions and outcomes of the technical validation.

Task 1.2: Swarm learning for integrated and decentralized biomedical data processing. This task aims to create a swarm learning framework simultaneously addressing the need for large medical datasets and highest-grade encryption and confidentiality requirements. A general swarm learning framework will be developed to support the training of models while completely removing the requirement of local, dedicated servers. This will allow users to share model parameters via the Swarm network and build the models independently on private data at the individual sites (swarm nodes). The framework will be developed in close collaboration with the platform created in Task 1.1, which will initially serve as a first use-case and benchmarking/development environment. Successively, nodes (i.e., hospitals and research institutions) will be added as swarm nodes, hence contributing large multimodal data volumes through safe decentralization. ***Deliverable***: Open access code available. Models trained in swarm mode with multiple contributors and nodes.

Task 1.3: Prototyping of software as a medical device application integrating molecular and clinical features to support tailored clinical characterization and novel therapeutics. This task aims to i) in collaboration with Spoke 4 WP2 create a software prototype able to collect and securely store molecular sequence data and clinical data, both for research and diagnostic purposes; ii) ensure that the prototype development will be carried out in compliance with all national and international regulatory standards as part of the CE-mark approval procedure. The software prototype will serve as a basis for the creation of a multi-action platform serving patients, caregivers, health professionals, and the community of potentially affected people to i) manage clinical studies in terms of patient enrolment and randomization; and ii) identify patterns for

supporting precise clinical characterization and diagnosis. ***Deliverable***: The software prototype and outcomes of the clinical validation

WP 2: Climbing Artificial Intelligence

WP2 will address the development of ground-breaking basic research frameworks leveraging AI methods to make a notable leap in personalized medicine

Task 2.1: Beyond supervised learning. This task will develop a breakthrough deep learning framework using self- and semi-supervised methods for a new generation of personalized risk-stratification models. Deep generative models (e.g., deep adversarial autoencoders combined with contrastive loss functions) will be employed and trained to faithfully reproduce 3D/4D multimodal images as well as non-imaging data through the generation of lower-dimensional embeddings. The models' internal representations and regularities discovered by these approaches will form a set of effective degrees of freedom for the disease continuum that will be fed into similarity-based clustering and dimensionality-reduction algorithms to derive new phenotypical categorizations hence uncovering the true degrees of freedom that govern a single patients' trajectory. ***Deliverable***: framework and open-source repository of the framework

Task 2.2: Integration and modeling of multi-omic data. This task aims to analyze and integrate multi-omic data (Next Generation Sequencing, Transcriptomics, Methylation, etc.) with bioinformatics, AI, and statistical learning methodologies such as automatized pipelines by using snakemake, complex network and graph neural networks to quantify, e.g., tumor heterogeneity and similarity after mapping of omics on protein-protein interaction network and laplacian diffusion. This task will ensure: i) dimensionality reduction of multi-omic measurements with methods based on manifold learning techniques and density-based clustering for characterizing multi-omic signatures, disease modules and genomic landscapes; ii) together with Spoke 4 WP3, feature extraction (morphological and texture) from digitalized histopathological images (Whole Slide Imaging) and prioritization; iii) generation of synthetic, disease, gender, and age-specific multi-omics data to circumvent the privacy problem to be used for transfer learning and data quality improvement. ***Deliverable***: deployment of open source software through a public platform.

Task 2.3: Digital twins for computational modeling and personalized intervention. This task will develop digital twin solutions for the prediction of disease diagnosis, personalized intervention, and precision treatments using computational modeling and simulations of complex physiological systems. This task will address some of the open methodological issues in the field (e.g., multiscale and multiphysics integration of computational models; reliable understanding and description of the genotype-phenotype relationships to fully exploit a personalized medicine approach) and will ultimately lead to the development of a Digital Twin for a specific target organ (e.g., heart) or a specific target pathology (e.g., cancer, cardiac arrhythmias). It will have as input patient-specific data, including omics data, biosignals, and images. Patient-specific simulations based on mechanistic models and machine learning algorithms will be applied to anatomical and functional patient information. ***Deliverable***: Full implementation of the Digital Twin for at least one target organ/pathology, including the automated procedure for its personalization based on patient-specific data.

WP 3: From novel methodologies to clinical applications: AI enabling personalized medicine

WP3 will develop and apply novel AI methodologies in real clinical settings enabling personalized medicine.

Task 3.1: Design of AI techniques for augmented reality in robotic surgery. This task aims to develop dedicated AI techniques for the automated registration of 3D virtual models during robotic surgery with augmented reality and to generate automatic surgical guidance. ***Deliverable***: Development of reliable and safe innovative AI AR robotic technologies for specific anatomical topographic regions

Task 3.2: Development of network analysis algorithms. This task will develop network analysis tools and algorithms to be used in Spoke 4, including i) tools for data preparation integrated with the collaborative platform; ii) AI-powered tools for patients' classification and stratification according to the disease stage; iii) network analysis algorithms analyzing data collected in the collaborative platform. ***Deliverable***: Data preparation tools for network analysis; release of the AI tool to be used by partners of Spoke 4; novel network medicine analysis to be used in Spoke 4 for developing a computational profile.

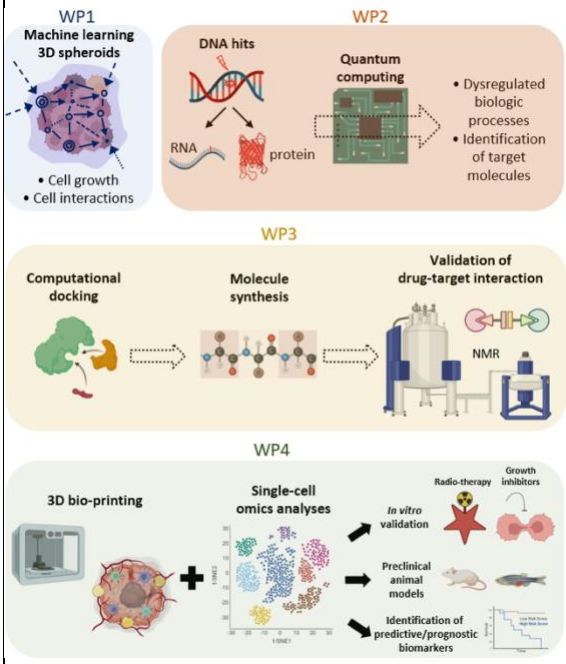
Task 3.3: Development of an AI-powered medical image system to support diagnostic and radiation protection in chest computed tomography (CT). This task will develop a Computer-Aided Detection (CAD) system for i) automatic detection of lung nodules and ii) segmentation and quantification of lung

areas affected by COVID-19 pneumonia based on a novel deep learning approach using radiomic features. The CAD system will be tailored to low-dose chest CT (LDCT), and CT reconstructed with different Adaptive Statistic Iterative Reconstruction (ASIR) blending levels. ***Deliverable***: Implementation of a CAD system for detection of lung nodules and segmentation and quantification of lung areas affected by COVID-19 pneumonia in LDCT chest CT

SPOKE – 3

PREDICTION MODELS

Development of advanced prediction models for prognosis and therapeutic response based on comprehensive data treatment



GENERAL OBJECTIVE: The realization of this activity will be facilitated by the already established protocol for the isolation and purification of organoids. On the bases of the knowledge acquired along the years through advanced computational approaches, a model recapitulating the complex evolution of a cell even following an action of an external agent (drug, radiation, etc.) will be created and experimentally verified through the use of 3D tissue models (multicellular organoids). In particular, organoids, fibroblasts, adipose, mesenchymal, endothelial and immune cells will be 3D bioprinted. This 3D structure, which will also be simulated through existing analytical tissue growth codes, will be investigated by evaluating the action of specific compounds selected by the consortium and radiotherapy. Its development following the external action will be monitored and the "physiopathology" will be analyzed and simulated with machine learning approaches capable of reproducing the complex mechanisms of cellular communication. Radiosensitivity of in vitro organoids and organoid zebrafish and mouse avatars has potential predictive value for individual response to radiotherapy, supporting clinical decisions for the management of affected by monogenic and polygenic diseases including those dependent from specific mutations, and cancer, thus avoiding radiation toxicity to resistant patients and reducing the treatment costs. The opposite direction originates from genetically modified mouse models, some not yet published, affecting metabolic pathways. The spoke 3 makes use of the **Scientific Advisory Board** with which has consolidated a collaboration for years: Ettore Appella, MD, National Cancer Institute (NIH), USA, Soldano Ferrone, MD, PhD, Harvard Medical School, Massachusetts General Hospital, Cristina Maccalli, PhD, Sidra Medicine, Doha, Qatar.

WP 1: Integrated experimental and computational models of 3D cultures of human cells with specific gene mutations or biogenesis alterations of RNA/Proteins

Task 1.1: 3D simulation of spheroid structures through machine learning. This task aims to develop Machine Learning (ML) models for cell growth prediction under the action of external agents on its DNA (e.g. drug, radiation, etc.). ML algorithms will be trained using biological and imaging features extracted from 3D tissue models (i.e. multicellular spheroids). In particular, a mixed cellular 3D model will be studied, with cellular spheroids of normal fibroblasts (3T3 cells) and "pathological" cells. Trained algorithms will predict the action of specific inhibitors of neoplastic growth processes and the "physiopathology" or the reproduction of the single cell of each of the individual tissues or of their whole. ***Deliverable:*** XAI-based ML models to predict the best 3D cell culture method for studying the proliferative and invasive capacity of cells.

Task 1.2: Regulatory molecular circuits of 3D cell growth affecting physio-pathological cell phenotypes. The goal of this Task is to establish organoid-relevant mechanisms and mutations affecting growth and the migration of cells in 3D. Particular attention will be given to ncRNA complexes, chromatin factors, DNA repair proteins and pathogenetic gene mutations in specific diseases such as cancer, neurodegeneration and immune deficiency. ***Deliverables:*** Engineered-organoid models with targeted CRISPR mutations; Knowledge of

pathogenetic gene mutations affecting 3D cell cultures.

WP 2: Simulation of mutated proteins and complex structures through quantum computing and AI

Task 2.1: Quantum computing techniques applied to biochemical systems, molecular biology and organic chemistry. This task will take advantage of hybrid quantum-classical algorithms to simulate protein folding, that is the molecular folding through which proteins obtain their three-dimensional structure. Due to the central role of proteins' structures in chemistry, biology and medicine applications, this subject has been intensively studied for over half a century. Although standard classical algorithms provide practical solutions for the sampling of the conformation space of small proteins, they cannot tackle the intrinsic NP-hard complexity of the problem. ***Deliverable:*** Simulation of protein folding in the presence of mutations.

Task 2.2: Integrative in silico assessment of the impact of mutations on protein structure, function, and interactions. This task aims to model the overall effect of mutations on proteins as well as estimating and ranking specific molecular alterations potentially affecting the phenotype. AI-based AlphaFold is currently the most useful tool to predict the structure of a single protein chain with a naturally occurring sequence. In the context of this task, an integrative predictive approach for in silico variant interpretation will be provided through the integration of different tools for evaluating the impact at different levels: *i)* contextualization of the mutation within protein functional and structural domains; *ii)* evaluation of the impact of the mutation on protein stability; *iii)* impairment of protein function and subcellular localization; *iv)* decrease/disruption of the protein binding affinity with other biological molecules. Based on recently developed non-dephosphorylatable phosphopeptidomimetic (NDP) inhibitors of SHP2 protein-protein interactions, targeting the N-SH2 domain (patent pending), the NDPs binding constant will be improved by in silico designing a novel NDPs targeted to the C-SH2 domain and enhancing sampling simulations of SHP2 allosteric transition. Existing machine learning-based computational tools, available at the consortium (Spoke 2), will be updated considering curated datasets of variations extracted from public data sources as well as recent advancements in the field of artificial intelligence. ***Deliverables:*** ***i)*** *In silico* analysis workflow for variant interpretation integrating different aspects of protein and protein variants; ***ii)*** A deeper understanding of the structure, function and regulation of the phosphatase and of its pathogenic mutations.

WP 3: Pharmacophoric dynamic docking simulations of genetic altered molecules

Task 3.1: Computation of molecule bearing genetic alterations able to bind to the target in the most effective way possible (docking) and with the greatest affinity (search for the best scoring). This task aims to develop and test novel computational methods tailored to compute accurate reaction and binding free energies for biomolecules and supramolecular systems (protein-drug interaction, protein-cofactor interaction, etc.) and to obtain a Hit molecule with the highest possible affinity for the targeting. Non-equilibrium thermodynamic integration (TI) methods will be used, coupled to molecular dynamics simulations and machine learning (ML) techniques, to develop high-throughput computational protocols to be used for the virtual screening of databases of small molecules (drug discovery projects) and proteins (prediction of thermodynamic property projects). ***Deliverable:*** High-throughput enhanced computational model for virtual screening.

Task 3.2: Molecular synthesis for the experimental validation of computation models. Scaffolds of hit-molecules will be synthesised with functional and steric groups in key points of the drug-target docking site in order to experimentally prove a different level of binding with respect to the drug-target match. The scope will not be to optimise the molecule's activity towards a given biomolecular target but rather to assess, experimentally, the differences in drug-target binding interactions and compare them to the computational dynamic docking at the atomistic level. The experimental validation of the simulated drug target interaction will be tested by high resolution NMR techniques in the presence of biomolecular target and its ligand. ***Deliverable:*** Simulation of drug-target interactions.

Task 3.3: Fight the enemy before you can see it: moving tools for early diagnosis from bench to bedside. To design, synthetize and clinically validate innovative molecular probes as precision diagnostic tools for cancer. ***Deliverable:*** Test of the bioimaging probes (gold-nano/fluorescent) that are selective for ADAM10 and ADAM17 in living cell models (also 3D) and in tissues isolated from patients; study of the effect of Anti-CD36-Ab conjugated with ADAM10 inhibitors in simple and organized 3D cell systems; Assay of the bioconjugated complexes in cultured cancer cells in order to measure their selective uptake by IR-microscopy or NMR techniques and, therefore, characterize the specific alterations occurring in the tested cancer cells; translate these assays to patient-derived tissues.

WP4: Preclinical models for precise therapeutic and diagnostic prevention strategies

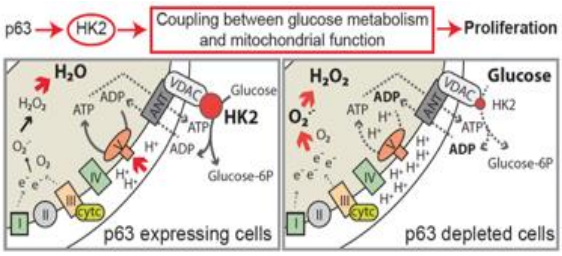
Task 4.1: Modeling of 3D approaches of multicellular spheroids structures for estimating the risk of disease initiation and progression. The main goal of this Task will be: 1) the prediction of the best 3D model to study the in vitro behaviour of cancer cells and to establish organoid-relevant mechanisms and mutations affecting the migration and growth of cells in 3D; 2) to understand the pathogenic mechanisms responsible for genotype/phenotype relationship in rare neurodegenerative diseases centered on mitochondrial dysfunction. These are tackled by somatic cells reprogramming to iPSCs, in turn differentiated into diseased cell types, including neuronal cells, glia and 3D tissue organoids. 3) self-assembling human heart organoids for the modeling of heart failure and genetic cardiomyopathies. ***Deliverables:*** *1)* Generation of new diagnostic and prognostic tools; *2)* Identification of new diagnostic, predictive and prognostic biomarkers; *3)* Generation of a platform for the identification of candidate drugs efficacious against cancer initiating cells and its stromal components; *4)* CRISPR/Cas9 screening and therapy testing and validation; *5)* the identification of specific druggable pathways implicated in mitochondrial disease mechanisms with the proof of therapeutic efficacy; *6)* Novel drug discovery and precision drugs for cardiovascular diseases using human cardiac organoids as valid and reliable preclinical models.

Task 4.2: Development of a powerful in vitro model for the response to radiotherapy and local hyperthermia (HT) in order to make clinical decisions more appropriate for treatment options. Radiation therapy is one of the technology-driven modalities in cancer management. Despite major advances in radiotherapy, some cancer patients do not benefit from treatment, which results in primary resistance or relapse after apparent eradication of the disease. The aim of this Task is to generate an in vitro model based on patient-derived bioprinted cancer organoids to be subjected to flash radiotherapy for the prediction of treatment efficacy to support clinical decisions, thus avoiding radiation toxicity to resistant patients and reducing the treatment costs. The planned model may result in identifying, in a clinically acceptable time frame, refractory patients who can be spared from side effects of ineffective radiotherapy. Similarly, the mechanism of action of local HT, and its use with radiotherapy, will be studied. ***Deliverable:*** Generation of in vitro tools to predict cancer patient's response to flash radiotherapy treatment.

Task 4.3: Validation at single-cell level. This will provide information on the best method to recapitulate tumor cellular heterogeneity *in vitro*. In particular, tissue specimens will be characterised by spatial single-cell RNAseq and proteomic analysis, in order to have a clear snapshot of the spatial distribution and percentage of each microenvironmental cell component to perform in vitro functional preclinical studies. In this context, 3D bioprinting will be used to recapitulate the complex TME cellular network, using different tridimensional plating geometries combined with microfluidic devices (to regulated concentration of nutrients, cytokines, growth factors, oxygen), to validate its ability to recapitulate the *in vivo* conditions in terms of spatial transcriptomic/proteomic profile. ***Deliverable:*** Development and fine-tuning of quantitative methods to *in vitro* study the dynamic evolution of patient-derived organoids, including the cell-cell and cell-matrix interactome and the response to curative and preventive treatments.

Task 4.4: Generation and optimization of preclinical animal models based on the use of organoids. The aim is to validate in vivo the data obtained *in vitro*, regarding the recapitulation of tridimensional TME dynamics. To this end, different optimised animal models (immunocompromised mice and zebrafish) will be exploited by labelling the cells with fluorescent tags or transducing them with luciferase. ***Deliverable:*** 1) Generation of key proof-of-concept data for precise diagnosis, prognosis and therapy in established models of preclinical efficacy; 2) Identification of molecular and metabolic adaptive responses underlying the heterogeneity and chemoresistance in epithelial cancers.

Task 4.5: Mouse models of mitochondrial metabolism.



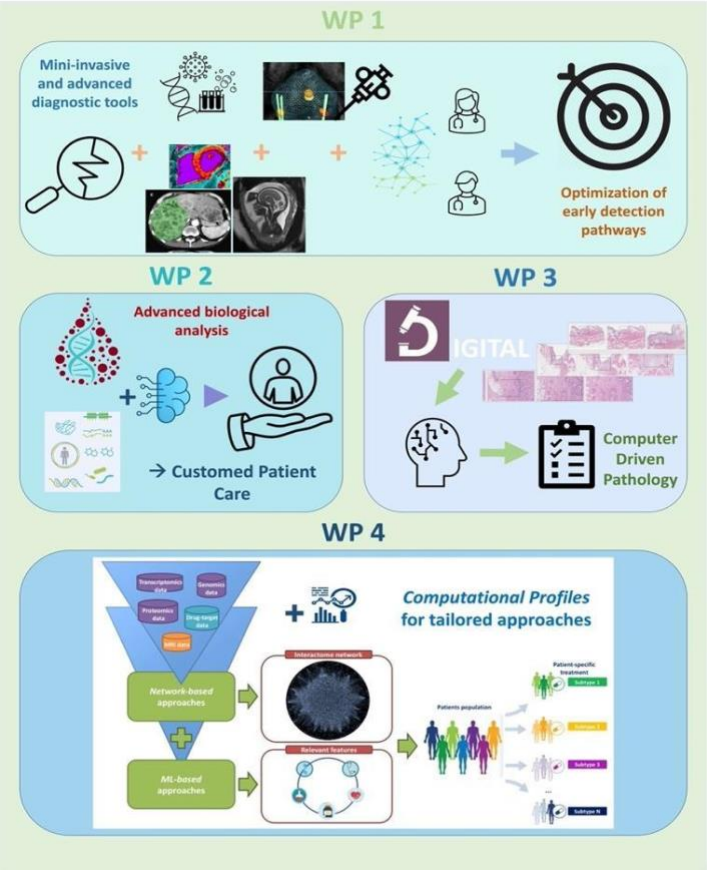
The molecular mechanisms involved in the cellular production of energy starting from glucose, fatty acids or glutamine, play a central role for mitochondria with their molecular dynamics. Mutations in The TCA enzymes show different rate of mutations, that result in the production of abnormal "oncometabolites", whose steady state levels interferes with the redox status and gene transcription. A clear example of this mechanism is represented by mutations in isocitrate dehydrogenase (IDH1 or IDH2), pathologically relevant in gliomas, cholangiocarcinoma or acute myelogenous leukaemia (AML). The aim of this Task will be to define novel therapeutic targets acting on metabolism, by developing or characterising mouse models to evaluate metabolic regulators. The GENERAL AIM is to define p63-related common and system-specific traits as well as cancer-type specific pathways and to identify novel prognostic and therapeutic targets. This will be achieved using in vivo and in vivo experimental models. The metabolic consequences of the loss of p63 isoforms and p63 overexpression will be studied using previous and novel genetically engineered mouse models and in vitro models. Particular attention will be given to address the functional differences of the C-terminal splicing forms of p63, not yet defined. To establish the in vivo role of specific p73 variants in cellular metabolism, metabolic rates of mice will be monitored by using metabolic chambers allowing continuous recording of food and water intake, energy expenditure via indirect calorimetry, oxygen consumption, and physical activity. ***Deliverable***: Generation of preclinical model to define novel therapeutic targets acting on metabolism.

Task 4.6: Innovative system for electron FLASH radiotherapy from in silico modelling to preclinical validation in organoids and animal models for deep seated tumours. To study the scattering effect of nanoparticles inside the tumour, a mouse model of the tumour will be created by orthotopically inoculating a certain number of cancer stem cells while recreating a human tumour. The advantages of nanoparticles in combination with the latest radiotherapy techniques, both conventional and hadronic, will be studied to possibly obtain an excellent definition of the dose in the area to be irradiated by carrying out highly accurate dosimetric treatments. The additional objectives of this task are: *1)* development of a treatment planning software (TPS) for high intensity and high energy electron beams. An extended use of AI methods such as Montecarlo simulation and machine learning based optimization is foreseen, in view of the clinical implementation. The goals of this task are to perform dedicated radiobiological experiments varying the electron beams parameters like average dose rate, dose per pulse, instantaneous dose per pulse and dose. ***Deliverables:*** 1) Clinical TPS for FLASH electron beams treating real in silico case of deep-seated tumours with complete beam parameters optimization; 2) Development of the dosimetric formalism for pencil beam delivery and development of a clinical protocol for low energy UHDP electron beam.

SPOKE – 4

4D PRECISION DIAGNOSTICS

Precision medicine integrating clinical and imaging biomarkers for a “precise in space and time” diagnosis



GENERAL OBJECTIVE: Technological innovations including multidisciplinary convergences enabling integrated diagnostics, and computational medicine to integrate health data can transform patient care. Still, these innovations are investigated and applied separately and unevenly throughout the national territory, with citizens and professionals having limited access and differences in the standards of care, reflecting regional inequalities. Spoke 4 will deliver novel, cost-effective, evidence-based, non-invasive predictive risk-adapted diagnostic pathways for faster, earlier, more precise, accessible, and affordable detection and screening of mono- and polygenic diseases and cancer, to achieve optimal clinical outcomes. This objective will be supported by the integrated analysis of digital data including bioimaging, omics and data derived from medical devices, performed using computational tools developed in Spoke 2; essential for indolent and aggressive pathologies (i.e. lung, prostate, breast, colorectal, hepato-biliary & head-neck cancer), and recurrent, long-term complex and chronic pathologies that burden the National Health System - SSN - (i.e. cardiovascular, metabolic and rare diseases).

Spoke 4 will address these issues by bringing together 13 Partners. Specific objectives span from application of high-performance computing, clinical validation of innovative technologies and methods (incl. AI-empowered tools and network medicine analysis), to health technology assessment analysis (further performed in Spoke 8). Spoke 4 as part of the HEAL ITALIA network will, for the first time, provide effective, game-changing computational-based solutions and non-invasive medical devices for the promotion of precision diagnostics, based on the analysis of digitized integrated diagnostics data across the different geography areas, that will lead to millions of euros saving for the Italian National Health System. Spoke 4 will contribute to implement the PNRR MISSION 4 "Education and Research" component to strengthen the digital and the technical-scientific skills, the research and technology transfer within the Italian territory. Also, Spoke 4 will contribute to the EC's 'Europe's Beating Cancer Plan' flagship initiatives. Spoke 4 will moreover explore intersections with European programmes, such as the Innovative Health Initiatives, the EU4Health and the Digital Europe programmes, or the European Universities Initiative, and seek synergies with projects funded under these programmes. Spoke's **Scientific Advisory Board**: Rami Aqeilan (Jerusalem Univ, Israel), Nichlas Bazan (LSU University, Luisiana, New Orleans, USA), Thomi Brunner (Univ. Konstanza, DE).

WP 1: Optimization of early detection pathways using mini-invasive and advanced diagnostic tools (M1-M30)

Task 1.1: **Integrated bioimaging for early diagnosis of polygenic diseases (e.g., cardiovascular and metabolic diseases) and cancer.** This Task aims: *i)* to provide data obtained with pilot studies, in order to create anonymized shared databases, based on algorithms developed by Spoke 2; *ii)* to validate new diagnostic pathways using non-invasive and advanced imaging tools (i.e. ultrasound, confocal in vivo, MRI, PET, and radiomics), integrated with innovative clinical data acquisition (chronobiology, remote monitoring, biomarkers repurposing), based on imaging as biomarkers, post-processed from the data shared through the platform; HTA affordability analysis will be performed; iii) features extraction (radiomics) from PET/CT images for prognostic purposes and bio-simulation including an appropriate cellular kinetic model, the morphological rules for tumor expansion and shrinkage, the cell killing and the hypoxia model; *iv)* to apply artificial intelligence algorithms to reduce the variability of the diagnostic performance of imaging tools and integrated biomarkers. ***Deliverables:*** *1)* Analysis of stored patients' data previously enrolled in clinical studies for early diagnosis of each polygenic disease and cancer; *2)* Definition of new imaging biomarkers and computational profiles, validation of identified

novel diagnostic pathways and relative standard cost-effectiveness model (HTA); *3)* A set of features for optimizing both specificity and sensitivity of PET/CT for predictive purpose; *4)* New artificial intelligence-based algorithms for organ-specific segmentation, index lesion detection, pre-biopsy modeling and clinical condition.

Task 1.2: **Assessing the applicability of innovative "intelligent" software for decision-making and biopsy planning and with implementation of robotic systems for organ-targeted biopsies.** This task aims: *i)* To improve planning of biopsy procedures; ii) to validate the diagnostic accuracy of AI-based software incorporated into targeted biopsy workstations for the detection of target lesions; *iii)* to test the inter-user reproducibility of AI-empowered solutions. ***Deliverables:*** *1)* Report on biopsy procedure planning; *2)* New artificial intelligence-based algorithms for biopsy planning; *3)* Final databases for AI solutions inter-rater reproducibility testing.

Task 1.3: **Development of novel techniques for precision diagnostics: from low energy beta tracers and detectors to nonlinear multimode fibers for endoscopy and real-time optical biopsy.** This task will develop: *i)* and test new radiotracers marked with beta emitters and corresponding detectors to enlarge the range of tutors for which margin delineation and absorbed dose measurement in RT treatments can be performed; *ii)* an endoscope based on an evolution of the complex propagation of light in multimodal optical fibers for real-time optical biopsy; *iii)* remotely guided micro-robots for on-site biopsies. ***Deliverables:*** *1)* Identification of at least 1 new beta minus emitting tracer with a PK profile in mice suitable for testing in (pre)-clinical tumor models; *2)* one software (simulation) and/or hardware (detectors) tool for beta radiation detection for nuclear medicine; *3)* Real-time diagnosis capability of the endoscopic system device on 3D-bioprinted cancerous tissues.

WP 2: Advanced biological analysis for diagnosis and monitoring of mono-polygenic diseases and cancer (M4-M34)

Overall aim: to produce molecular information, generating genomic, proteomic, metabolomic, transcriptomic, miRNAs and immune profiling data, using innovative platforms allowing molecular characterization of complex and polygenic diseases.

Task 2.1: Alternative matrix for biological monitoring of inorganic lead and cancer (Saliva as potential noninvasive alternative). This task aims to establish circulating extracellular vesicles (EV) profiling in solid cancer (breast, head and neck, GI). ***Deliverables:*** *1)* creation of EV-based predictive profile in solid cancer treated by anti-EGF receptors; *2)* creation of an EV-based mirnome prognostic profile in BRCA carriers; *3)* creation of an algorithm to combine radiomic features with the EV-based profiles.

Task 2.2: Molecular profiling of circulating nucleic acids by liquid biopsy for a more in-depth characterization, classification and stratification of polygenic disease, cancer and tumor aggressiveness and assessment of response to therapy. In this task the Partners will: *i)* validate new tissue and circulating biomarkers, providing a set of new tissue and circulating biomarkers for personalized approach that will allow a better stratification of oncologic patients; *ii)* validate new tissue and circulating biomarkers, providing a set of new tissue and circulating biomarkers for personalized approach that will allow a better stratification of polygenic diseases (such as cardiovascular, endo-metabolic, immunological, etc); *iii)* design and validate a genomic workflow based on a multidisciplinary approach directed to offer the best diagnostic pathway; *iv*) to perform systematic evaluation of accuracy of using liquid biopsy for cancer. ***Deliverables:*** *1)* Database on genetic and molecular on both tissue and circulating cells in oncologic patients (liquid biopsies); *2)* Database on genetic and molecular on both tissue and circulating cells in polygenic diseases (liquid biopsies); *3)* Selection of CTCs and their precise characterization through the analysis of surface markers and molecular genetic analysis obtained by techniques of qRT-PCR/NGS; *4)* A novel genomic workflow for disease diagnosis.

Task 2.3: Multi-omics approach in monogenic diseases, complex disorders and rare diseases and cancer, supported by multilevel AI tools developed by Spoke 2. This task aims to: *i)* set-up a diffuse Italian genomic center to speed up the diagnostic pathway, therapy and monitoring relapse and response to treatment, for monogenic diseases, complex disorders and rare diseases and cancer; *ii)* define the combination and sequence of omics approaches that most efficiently allows patients stratification and early diagnosis in selected disorders, exploiting multi-omic signatures developed using machine learning methods (Spoke 2); *iii)* identify and evaluate health policy, economic and ethical issues for the introduction of these approaches in the clinical practice offering flowcharts and guidelines for guiding the use of novel technologies in the diagnostic setting. ***Deliverables:*** *1)* Assessment of the efficacy of a workflow based on an integrated genome scan in the diagnosis of monogenic diseases, complex disorders and rare tumors; *2)* Analysis of the sustainability of the application of these genomic tools in clinical practice; *3)* Application of multilevel AI tools for data integration and interpretation; *4)* Design of recommendations for the implementation of multi-OMICs approach in diagnostics;

5) Software release for prediction of patients trajectories using the Markov models and reinforcement learning and final report.

WP 3: Digital pathology: standardization of acquisition and analysis of digital images for AI-based solutions (M4-M32)

Task 3.1: Pathology sample collection for digital acquisition using dedicated scanners. This task will: *i)* create the technical requirements for a centralized digital imaging facility; *ii)* use of storage system developed by Spoke 2 to house the physical slides to ensure their safe storage and traceability and to facilitate the immediate use of the digital images for the evaluation of a fit for blocks for downstream omics selection; *iii)* create standard operating procedures for preparation and acquisition of the digital slides to ensure quality control and guarantee downstream comparison of different sets; *iv)* develop and validation of workflow for sample collection and transport of slides to the centralized digital imaging facility. ***Deliverables:*** *1*) Creation of digital imaging facility; *2)* Employment of the automated storage system; *3)* Standardized protocols handbook; *4)* Validation of workflow for collection and transport among the participating centers.

Task 3.2: Definition of specific histopathologic panels for the diagnosis and prognostic stratification of complex polygenic diseases and cancer. This task aims: *i)* to define the histopathological panels for diagnosis and prognosis stratification of diseases included in the project, comparing them to the feature (morphological and texture) extracted from histopathological images by Spoke 2 (Task 2.2), with employment of the repository system of digital histopathological images developed by Spoke 2; *ii)* to design of standard operating procedure for histopathologic panels; *iii)* to analyze in details the cancer immune environment and identify immune signatures predictive of response to immune checkpoint inhibitors and activation of the immune system in response to chemotherapy; *iv*) to evaluate stromal cell-type repertoire and specific gene expression signatures in prostate and breast cancer as prognostic markers. ***Deliverables:*** *1)* Validation of histopathological panels; *2)* Standard operating procedure handbook for histopathologic panels; *3)* Repository of integrated digital histopathological and immuno-phenotypical images; *4)* Creation of ad hoc ad hoc in vitro models able to recapitulate specific molecular events of interest.

Task 3.3: Computer Driven Pathology Assessment. This task sought to: *i)* exploit the expert opinion database (developed by Spoke 2) to feed the digital repository in consensus; *ii)* use machine learning algorithms (developed in Spoke 2); *iii)* validate a computer driven AI pathology expert system in a clinical context. ***Deliverables:*** *1)* Achievement of standardization for acquisition and interpretation of digital formats, application and test of AI-based algorithms for quality assurance and control of biomolecular, clinical, digital pathology, and diagnostic imaging data; *2)* results on the use of machine learning algorithms, developed in Spoke 2; *3)* provide a clinically validated computer driven AI pathology expert system.

WP 4: Application, test and validation of computational profiles based on the network medicine approach, for a personalized management of polygenic diseases and cancer (M10 -36)

Overall aim: To apply network medicine tools developed in SPOKE 2 for analyzing and integrating omics data (genomics, transcriptomics, epigenomics, metabolomics, proteomics), imaging, laboratory, and clinical data of the patient (signs/symptoms, medical history, as well as relevant demographic data, such as age, ethnicity, and sex) to understand the heterogeneity of human diseases and thus pave the way for precision medicine care.

Task 4.1: Validation of network-based tools developed by Spoke 2, for analyzing and integrating omics, imaging, and clinical data to understand the heterogeneity of human diseases for precision medicine. This task will: *i)* interpret the collected health care data on platform (developed by Spoke 2); *ii)* build phenotypic networks applied to oncological diseases; *iii)* Build phenotypic networks applied to polygenic diseases. ***Deliverables:*** *1)* use of AI-powered tool (ML) developed in SPOKE 2, for patients' classification and stratification according to disease stage; *2)* Database for phenotypic networks validation on large cohorts of oncologic patients; *3)* Database for phenotypic networks validation on large cohorts of patients with polygenic diseases.

Task 4.2: **Test of benchmark analytic tools based on interpretable available network analytic models for risk stratification, prediction of relapse and complications rate in cancer and polygenic diseases, considering the possible impact of early-life stressors, analyzed by Spoke 7.** This task will: *i)* apply a ML model developed in SPOKE 2 to specific precision medicine analysis objectives for Network Analysis; *ii)* apply and test a combined application of Artificial Intelligence, Machine Learning and Semantic Technologies for cancer and polygenic diseases; *iii)* generate personalized risk prediction models based on early-life stressors (implemented in SPOKE 2). ***Deliverables:*** *1)* ML model applied to cancer and polygenic diseases; *2)* Release of network analysis tools for data analytics in precision medicine; *3)* Reports on the relationships of early-life

exposures with health trajectories and NCDs risk from birth to adulthood and Recommendations for the early, stratified and targeted prevention of adverse pregnancy outcomes and NCDs.

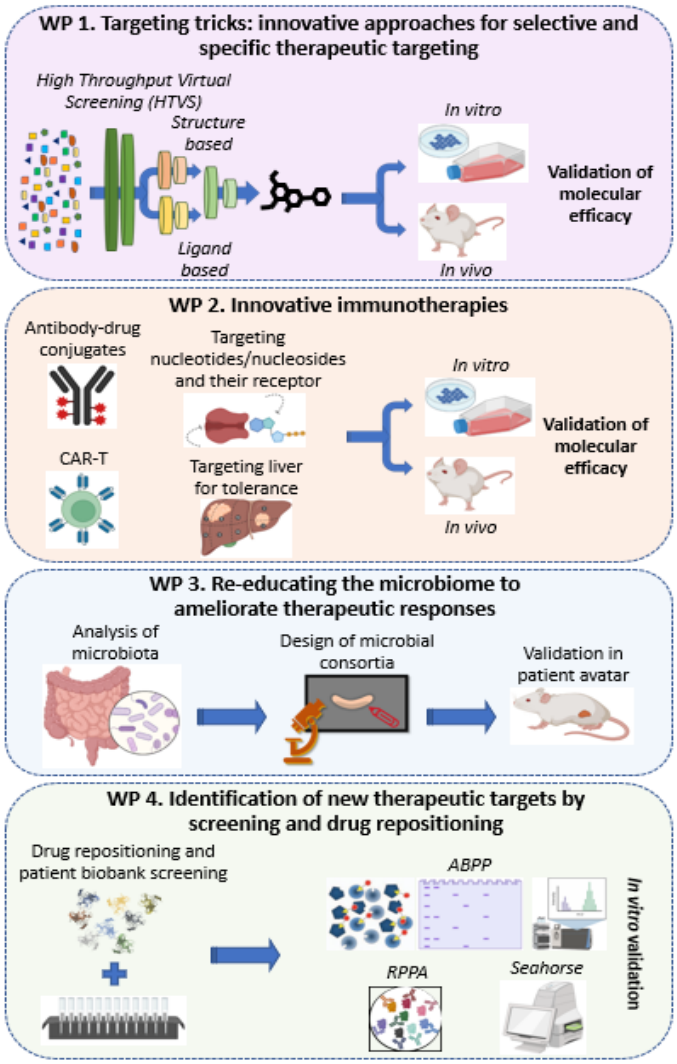
Task 4.3: Application of the network analysis-based algorithms for the definition of new early diagnosis and screening pathways of polygenic and cancer. This task will define: *i)* analysis of global data for the implementation of precision medicine, to identify cluster of patients at risk, suitable for promoting automated systems of early diagnosis, when the disease has already developed, as well as screening and surveillance programs; *ii)* validation of the new computational algorithms based on network science approach, with the definition of new biomarkers and computational profiles to implement precision medicine in oncology and polygenic diseases; *iii)* Clinical application of the identified new algorithm for the definition of new primary and secondary screening pathways to fight against the incidence of the oncological and polygenic diseases. ***Deliverables:*** *1)* Datasets on integrated diagnostic data for training of network analysis algorithms for identification of population at risk, of genetic driver mutations and immune checkpoints for response to therapy, of predictive factors of adverse drug reactions, and of prognostic factors of survival; *2)* Release of the validated network medicine algorithms; *3)* Clinical study protocols for multicenter randomized trials to test the clinical applicability on the validated algorithms.

SPOKE - 5

NEXT GEN THERAPEUTICS

"From silico to bedside" design and validation of innovative tailored and personalized therapeutic strategies

GENERAL OBJECTIVE: This spoke aims at developing innovative and comprehensive drug-screening and validation platforms allowing to overcome the limits of currently available systems and accelerate the identification of next-generation effective drugs in the field of precision medicine for oncology, cardiology, rare diseases, microbiota alterations etc. Platforms available to HEAL ITALIA's consortium are based upon the definition and implementation of a workflow composed by a series of intertwined *in silico*, *in vitro* and *in vivo* assays, tentatively grouped into successive stages of selection, progressively more refined and specific. A highly qualifying element of this approach is the use of unique human *in vitro* models, high throughput screenings, nanotechnologies and animal models available thanks to the expertise gathered together in the present network. The spoke is conceived in order to achieve the following: *i)* validating new targets and therapeutic effectors; ii) assessing the *in vitro* efficacy of compounds libraries; iii) determining the cellular and molecular targets on which the candidate drugs act; *iv)* validating candidate drugs; *v)* defining the key mechanisms of action that underlie the therapeutic efficacy of the drug; *vi)* completing the analysis of their effects in pre-clinical settings with unprecedented accuracy. This approach will enable the simultaneous definition of drug candidate and drug action mechanisms at the cellular and molecular level with an unprecedented level of predictivity. This, therefore, will allow filing dossiers for clinical trials in a more simple and reliable manner, incorporating experimental evidence.



The experimental setting and the complementary expertise of HEAL ITALIA's consortium make it possible to establish reproducible and quick pipelines for the preclinical validation of innovative therapies. The spoke 5 makes use of the **Scientific Advisory Board** that will be shared with Spoke 3: Ettore Appella, MD, National Cancer Institute (NIH), USA and Soldano Ferrone, MD, PhD, Harvard Medical School, Massachusetts General Hospital. Annalisa Scopinaro, UNIAMO (Italian Federation of Rare Diseases) President; Antonio Bertoletti PhD, Program in emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

WP 1: Targeting tricks: innovative approaches for selective and specific therapeutic targeting

Task 1.1: TRIDS that make the Tricks – Design, synthesis and validation of Translational Readthrough Inducing Drugs (TRIDS) to overcome nonsense mutations. This task will be pursued by identifying biologically active molecules capable of rescuing the expression of full and functional proteins in cellular and animal models. *In silico* analysis through High Throughput Virtual screening (HTVS) will be performed using ligand-based and structure-based strategies to identify pharmacophores to be used for the screening of chemical libraries. Best candidates selected by HTVS will be synthetized and validated *in vitro* and *in vivo* for efficacy and specificity**.** ***Deliverable:*** In vitro and in vivo validation of the activity of selected TRIDs on different genetic diseases (e.g. Retinitis Pigmentosa, Fabry disease, Primary Immunodeficiencies, Duchenne Muscular Dystrophy, Choroideremia).

Task 1.2: Identification of correctors of misfolding and post-transcriptional functional defects of CFTR protein in Cystic Fibrosis. New correctors of mutant CFTR will be designed via *in silico* analysis using ligand-based and structure-based strategies: docking studies will be performed to identify the putative binding site of selected correctors. We will then be synthesized chemical libraries and select lead candidates as correctors of mutation *del*F508 able to produce synergistic effects with other CFTR modulators and validate them in vitro. ***Deliverable:*** In vitro validation of a library of potential correctors of misfolding for CFTR.

WP2: Innovative immunotherapies

Task 2.1: Synthesis of antibody-drug conjugates (ADCs) for the treatment of selected tumors. This task aims to: *1)* Synthesize ADCs by conjugation of anti-GPC1, anti-GPC3, anti-CD138 mAbs with the cytotoxic drug emtansine; *2)* Chemical-physical characterize the synthesized ADCs; *3)* Evaluate ADC in-vitro killing capacity in tumor associated antigens (TAA) expressing cancer-derived cell line cells; *4)* Evaluation of in-vivo biodistribution and cell killing capacity of ADCs in TAA-expressing xenograft mouse models obtained by the injection of the TAA expressing cancer-derived cell line cells from Glioblastoma multiforme (GBM): Pancreatic ductal adenocarcinoma (PDAC); hepatocellular carcinoma (HCC); multiple myeloma (MM). ***Deliverables:*** new therapeutic anti-GPC1, anti-GPC3, anti-CD138 ADCs targeting GBM, PDAC, HCC, MM.

Task 2.2: Targeting nucleotides/nucleosides and their receptors and extracellular catabolism of nucleotide substrates to affect immunotherapy responses against solid tumors. Validation of purinergic check-points as viable targets in cancer therapy, in particular development of potent and selective P2X7 and A2A antagonists; pharmacological inhibition of the ectoenzymatic activities of CD38, CD39, CD73 and CD203a, with the goal of i) blocking or reducing the generation of immunosuppressive adenosine in the TME from the catabolism of

ATP, NAD and cGAMP; ii) boosting extracellular ATP and cGAMP stability to enhance their immunostimulatory functions. ***Deliverable:*** Novel immunotherapeutic approaches for cancer therapy.

Task 2.3: Generation and characterization of CAR-modified cells for the treatment of solid tumors and fibrosis. Generation of CARs (i.e CIK, T) anti fibroblast activation protein (FAP) and anti-TAA (GPC1, GPC3, GD2 and CD138 to target GBM, PDAC; HCC and MM). CAR cells will be tested in vitro, and in vivo in appropriated mouse models. Studies of safety and efficacy will be conducted, and clinical protocols will be preliminary defined. ***Deliverable***: planning and management of a phase I clinical protocol of the viral and non-viral CAR-FAP and CAR-TAA manufacturing platform.

Task 2.4 Development of a new generation immunotherapy to address unmet needs in cancer, infection and autoimmune disease. Development of TCR bi-specific T-cell engaging receptors (TCERs) to offer a new approach for the treatment of tumors, chronic infections by redirecting T cells to kill *(i)* tumor cells of both hematopoietic and non-hematopoietic origin; *(ii)* cells infected by intracellular microrganisms (viruses, mycobacteria, etc.); *(iii)* to induce organ-specific immune suppression in patients with autoimmune diseases. ***Deliverables:*** TCR bi-specific TECR in vitro and in pre-clinical mouse models in vivo.

Task 2.5 Delivering autoantigens (autoimmune disease relevant peptides) in the liver to induce tolerance and treat autoimmune diseases. This task will: *1)* generate and purify nanoparticles (NP), pMHC-NP, disease relevant peptide-NP and peptide microparticles (MP); *2)* evaluate liver targeting and the expansion of T regulatory cells in vivo after peptide-NP conjugate administration; *3)* evaluate in vivo disease-specific bystander immunoregulation in appropriate mouse models of autoimmune diseases. ***Deliverable:*** Development of a quick, robust, and scalable process suitable for large-scale synthesis of in vivo effective and efficacious conjugates.

WP3: Re-educating the microbiome to ameliorate therapeutic responses

Task 3.1: Design of patient-, disease-and tissue-specific microbiota-targeting intervention. This task will provide a disease-specific microbial profile of patients: *i)* identifying and implement tissue- and biomass-specific effective sequencing methods; *ii)* creating a disease-specific database comprising microbial and clinical data of patients; creating prediction models of disease risk and/or therapy responses. ***Deliverable:*** provide risk and /or therapy response prediction models, according to disease and microbial signature.

Task 3.2: design of microbiota-targeting intervention by personalized microbial consortia and next gen microorganism. This task will design personalized microbiota targeted therapeutic interventions based on patient's microbial signature and intend to: *i)* rationally design in silico microbial consortia complementing patients' microbial alterations; *ii)* create personalized living microbial consortia to be administered; *iii)* to enhance therapeutic activities of probiotics (ie, SCFA production, anti-or pro-inflammatory enzymatic activities according to the patient need, specific tissue delivery) by targeted bioengineering in specific microorganisms (NextGen microorganisms) or modulate their GUS content for drug bioavailability. ***Deliverable:*** create probiotics with enhanced therapeutic activity by targeted bioengineering.

Task 3.3: in vivo validation of therapeutic activity of personalized microbial consortia in patients' microbiota avatars. This task will validate in vivo in patient's avatars the therapeutic activity of microbial consortia and Nextgen probiotics: *i)* creating patients' microbiota avatars via transplantation of fecal patients' microbiota murine models; *ii)* therapeutically administer the personalized, in silico rationally designed microbial consortia or the next-gen probiotics in mice at different timepoints and concomitantly or not to the disease-specific therapeutic interventions. ***Deliverable:*** establish patients' microbiota avatars as screening platforms for microbiota-targeted therapies.

WP 4: Identification of new therapeutic targets by screening and drug repositioning

Task 4.1: Definition of putative therapeutic targets via computational tools, i.e. virtual screening of drug libraries. This task will in silico define lead compounds via virtual screening of drug libraries containing EMA, FDA and AIFA approved small will be performed. The aim is to identify a shortlist of compounds having already a favorable profile for use in the clinic. ***Deliverable:*** Definition of a list of candidate drugs.

Task 4.2: In vitro validation of selected drug efficacy and selectivity. This task will: *1)* in vitro validate the efficacy of the compounds selected in task 4.1 and in other platforms of the consortium (Spoke 1, 2, 3) on disease-relevant cellular models; *2)* Off-targets characterize using of high-throughput proteomics and metabolomics platforms [Activity-Based Protein Profiling (ABPP), Reverse-Phase Phosphoprotein microArrays (RPPA) and Seahorse) to circumscribe the molecular targets of the identified drugs and evaluate their effect on cellular homeostasis (e.g., signal transduction and energy metabolism). ***Deliverable:*** identification of new repurposed drugs and characterization of their activity

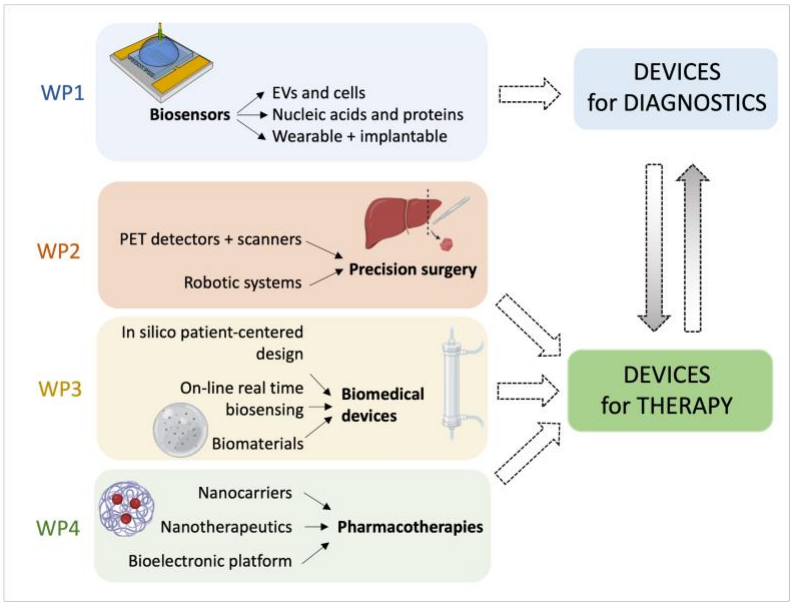
Task 4.3: Drug repositioning through large patient biobank screening, the case of NAFLD/NASH GDR Study. This task will determine whether medications prior to diagnosis of NAFLD/NASH have a positive effect by modifying the rate of deaths for any cause, the occurrence of gastrointestinal medications, and the occurrence of cardiovascular ischemic complications compared to not taking the medications. ***Deliverable**:* Identification of drugs or combination of drugs influencing the identified clinical outcomes by statistical analyses. All subjects who will be enrolled in the study will be included in the full analysis set irrespective of their adherence and continued participation in the study. The primary variable is the time to first event included in the primary composite endpoint. The primary analysis will be based on prior medications' using principle.

Task 4.4: Development and validation of new targeted radionuclides and Precision Flash Radiotherapy procedures for precision medicine. This task will develop new therapeutic radiopharmaceuticals with improved efficacy and reduced toxicity in order to build a platform to design and validate new targeted radionuclide-based therapeutic approaches selected on tumor specific biological features. The efficacy target selectivity, kinetics of bio-distribution in target and non-target organs will be validated. ***Deliverables:* *i)*** Define molecular mechanisms associated with radionuclide efficacy; ***ii)*** Kinetics of in vivo bio-distribution and dosimetry of novel radiopharmaceuticals for imaging and therapy.

SPOKE – 6

HEALTHY TOOLBOX

Development of innovative devices for precision diagnosis and personalized therapy



GENERAL OBJECTIVE: This spoke is a technological toolbox of devices for applications both in precision diagnostics and therapy, closely interacting with Spoke (S)1 for omics investigations, with S2 for AI implementation, with S4 for the identification of biomarkers to be quantified by innovative biosensing platforms. WPs are divided into two subgroups: WP1 is devoted to devices for diagnostics; WPs 2 to 4 aim at developing devices for therapeutic interventions. The broad definition of "devices" in the Heal Italia project encompasses molecular systems to miniaturizable diagnostic assays and assistive tools for surgery to hardware solutions. **WP1** develops a portfolio of biosensors for early diagnosis, for monitoring the efficacy of a therapy during patient follow-up, and for wellness tracking. Different transduction mechanisms will be explored to mitigate the risks and to identify the best-performing biosensors, in terms of selectivity and sensitivity for a given target. WP1 sensors will be validated for selected target pathologies and adapted within the platforms developed in S6 or in others. Robotics supporting surgeons in their actions is developed in **WP2**, starting from imaging to micro-robotic technologies together with AI tools developed in collaboration with S2, for example to assist in detection of tumor margins and in minimally invasive operation sparing healthy tissues within a novel personalized surgery. **WP3** will develop and validate devices and materials for precision medicine, covering different device-based therapeutic strategies other than pharmacological treatments, identifying kidney disease as target pathologies. The introduction of novel materials and devices for optimized tissue regeneration approaches is included in the WP, as one of the most promising precision tools for unmet medical needs. **WP4** designs, synthesizes and screens novel nanomolecular systems as carriers for drug delivery and as stimuli-responsive therapeutic agents and to develop electronic devices to monitor response of cellular and small animal models to innovative therapies. WP4 also relies on inputs from S5 that will identify promising/repurposed drugs, for which ad hoc targeting nanosystems should be developed. The spoke has its own **Scientific Advisory Board**: Wolfgang Knoll (Austrian Institute of Technology, AT), Raymond Schiffelers (University Medical Center

Utrecht, NL), Giorgio Mari (Modena, IT), Nick Barlev (CAS, St Petersburg, RU), Pier Paolo D'Avino (Univ Cambridge, UK), Qiang Sun (CAS, Beijing, China)

WP 1: Sensing devices for precision diagnostics and remote health monitoring

Task 1.1: Biosensing platforms for the detection of extracellular vesicles and cells in biological fluids. Sensors will be developed for the ultra-sensitive, rapid and consistent detection of biological entities ranging in size from tens-hundreds of nanometers (exosomes, extracellular vesicles -EV) to the micrometer scale of whole cells. Novel assays will be designed for detection of EVs, with a focus on identification of exosomes produced by a specific pathological tissue (i.e tumor), both with organic electronic devices and with optical methods. In parallel, the demonstration of microfluidic-based biosensors will be achieved, taking identification of fetal cells in maternal biological samples for prenatal diagnostic genetic testing as a paradigmatic application. ***Deliverable:*** Report on detection of EV and fetal cells in biological samples by novel biosensing platforms.

Task 1.2: Detection of nucleic acids and protein biomarkers: liquid biopsies with optical biosensors. This task will develop devices for the PCR-free detection and quantification of nanometer-sized biomarkers, i.e. nucleic acids and proteins, based on optical biosensing. A novel (electrochemi)luminescent diagnostic platform will be developed for point of care testing based on signal amplification, by nanoparticles and phages, that will be applied first to the quali-quantitative characterization of nucleic acids without the PCR amplification step, and further adapted to small molecules and protein biomarkers. ***Deliverable:*** Prototype of a portable platform for rapid (< 2 h), sensitive (as PCR) detection of nucleic acids and other tumor biomarkers.

Task 1.3: Detection of nucleic acids and protein biomarkers: label-free electronic biosensors. This task explores novel strategies for detection of nanoscale biomarkers based on electronic, rather than optical, response. Electronic devices are amenable to miniaturization and future implementation in portable platforms deployable in the field. Bioelectronic sensors will be developed towards tumor biomarkers both established and identified by other spokes within the project, featuring silicon (Ultra-High Frequency Capacitance Spectroscopy (UHF-CS) biochip architecture) as active material. ***Deliverable:*** Report on fully electronic recognition and dynamic imaging of the tumor biomarker with UHF-CS platform.

Task 1.4: Wearable and implantable electronic biosensors. As a last set of biomarkers, this task will address monitoring of levels of small molecules (namely cytotoxic drugs) and of electrophysiological signals. Novel materials and device architectures will be developed for wearable monitoring systems to be used as sensors for physical and chemical parameters of medical interest, on flexible substrates, for the unobtrusive monitoring of the human body. In parallel, implantable and biodegradable biosensors will be developed and validated to measure the concentration of cytotoxic drugs in human tissues. ***Deliverable:*** Report on the characterization and assessment of wearable biosensors against golden standard systems. In vitro and clinical validation of developed implantable biosensors.

WP 2: Assistive tools for precision surgery

Task 2.1: Expanding the potential of PET: enhancing sensitivity and fabrication of intraoperative scanner for surgical margin assessment. The twofold aim is: i) to further improve PET sensitivity through new scintillating heterostructures to control the system coincidence time resolution (CTR) and therefore the PET sensitivity. A CTR of 10 ps would allow real-time molecular imaging for cancer diagnosis/therapy; ii) to develop a compact, high sensitivity, high resolution PET scanner integrated with an optical 3D scanner, producing 3D representations of the tracer uptake in excised tissue samples and to provide the surgeon feedback in real time. ***Deliverable:*** Device demonstrators of fast Time-of-flight-PET detectors; Development and validation of the developed intraoperative PET scanner on phantoms and tissues samples.

Task 2.2: Toolbox to Minimally Invasive Robotic Surgery. Aim is to develop and test innovative precision devices leading to the application of new surgical procedures by implementation of a novel robot-assisted mini-invasive surgery, focusing on liver diseases and colorectal cancer as model pathologies and combining surgical expertise, artificial intelligence, augmented reality, 3D printed models and robotic systems. We will also explore and test advanced and experimental surgery procedures on the cadaver, starting from morphological and molecular data to create an interdisciplinary platform. ***Deliverable:*** Software for Robotic-assisted, real time navigation during resection as an extension of the current robotic platform.

WP3: Innovative tools for precision therapeutics

Task 3.1: Integrating patient-centered device design and real-time sensing with biomedical technology. We aim to implement a multidisciplinary approach to personalized treatment, combining state-of-the-art medical equipment with frontier research that enables patient-tailored in silico design of a medical device and ad hoc developed solutions for real time monitoring of biomarkers, taking chronic kidney diseases as target pathology. We will combine in silico and in vitro approaches, integrated with clinical images, to design and characterize novel hemodiafiltration (HDF) devices at a patient-specific level, compare two alternative dializers for HDF in a non-randomized open-label observational study and integrate them with ad-hoc developed IT and sensing technologies for collection and analysis of patient's data. ***Deliverable:*** Reports on newly designed devices for HDF. Integration of new diagnostic tools and methodologies based on advanced IT techniques for personalized hemodialysis treatments.

Task 3.2: Novel biomaterials and devices in regenerative medicine. Aim is to develop a platform of devices based on new biomaterials, implants and nanostructures for tissue regeneration. Integration with molecular data of each single subject will allow personalization of the therapy according to the patient's specific needs. Specific case studies will be new hydrogels and scaffolds ad-hoc engineered to progressively release anti-biofilm and anti-microbial agents in potential infection sites (wound bed, bone fracture, prosthesis implantation site) and development of new biomaterials for oral surgery and maxillofacial applications. ***Deliverable:*** Design and characterization of novel biomaterials and antibiotic-free therapeutic approaches to tackle and manage infections.

WP4: Precision micro- and nanotools for innovative pharmacotherapies

Task 4.1: Development of smart drug-delivery systems. Aim is the development of nanoplatforms for the transport of poorly bioavailable and/or highly toxic therapeutic agents to selectively direct a drug towards a specific organ or tissue or cellular system, and to achieve the release/maintenance of the therapeutic agent at the target site, eventually also by external stimuli, for an extended period. The main target pathology will be cancer but metabolic syndrome, cystic fibrosis, rare diseases such as retinitis pigmentosa and degenerative diseases such as osteoarthritis will also be considered. ***Deliverable:*** Selection of the most effective formulations based on preclinical studies in advanced cellular tissue models.

Task 4.2 Development of nanotherapeutic agents. Aim is the preparation of nanotherapeutic agents whose function can also be activated by external stimuli such as radiation or hyperthermia, including "theranostic" systems that combine therapeutics and diagnostics components in one multifunctional device. Cancer will be the main target pathology. We will develop photo- or thermo-activable molecular pro-drugs or nanoparticles as well as therapeutics innovative systems all-in-one including in one single structure components for diagnosis, monitoring, and mini-invasive treatment of solid tumors. ***Deliverable:*** Nanotherapeutic and theranostic nanosystems characterized, tested and optimized.

Task 4.3: Organic Bioelectronic platform to monitor response to therapy of in vitro cell models and in vivo in small animal models. Aim is to develop innovative systems for 2D and 3D in vitro cell monitoring based on sensor arrays that combine soft mechanical behavior with highly sensitive electrical transduction properties to be confirmed by small animal models with radiological read-outs. A recently demonstrated organic-semiconductor based technology will be applied with the aim of deriving complex information (e.g. electrical and metabolic) on the cellular behavior as a consequence of the interaction between the nanodrug and the biological target. ***Deliverable:*** Demonstration of multichannel recording with platform from 3D cell cultures (in-vitro) and in small animal models.

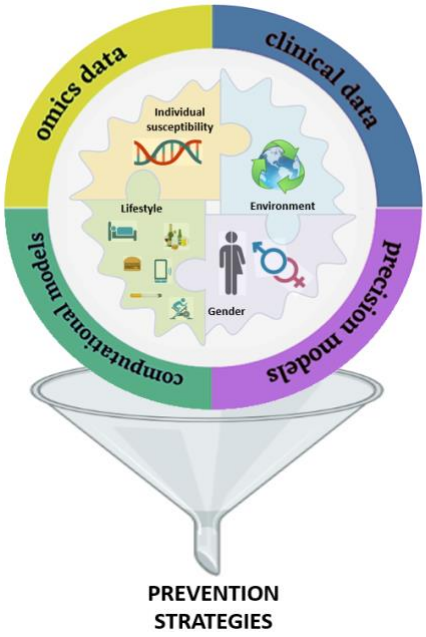
SPOKE – 7

PREVENTION STRATEGIES

Integrated and gender medicine approaches for prevention strategies based on environmental, lifestyle and clinical biometric data

GENERAL OBJECTIVE: Prevention is a pillar of public health policies aimed at reducing the burden of

diseases. The level of implementation of preventive strategies is influenced by, but is also a determinant of, the socio-economical conditions of a Country. Conventional prevention strategies do not consider individual susceptibility to develop diseases but find application on large groups or an entire population, and interventions that could obtain relevant results in selected cases might not show efficacy when adopted on a large scale. Individual genetic features or biologic conditions in concurrence with environmental agents and life habits determine the risk of a disease, and translation of knowledge in targeted interventions would maximize their cost-effectiveness.



In the framework of a precision health approach – which includes disease prevention and health promotion activities for "precision public health" – the spoke's aim is to identify, in a gender medicine approach, determinants of the individual risk to develop diseases with a high impact on the health status of a population, such as cardiovascular, cancer, or endocrine-metabolic diseases. The identification of individual risk determinants can be the base of innovative new strategies for prevention. The spoke has its own **Scientific Advisory Board**: Anthony Letai (Harvard, MA, USA), Haining Yang (Hawaii Cancer Center, HI, USA), Daniele Bano (DZNE, Bonn, DE).

WP 1: Translation of precision medicine tools into novel strategies for cancer prevention

Task 1.1: Predicting cancer development in chronic inflammatory conditions of the digestive system: molecular profiling, individual risk prediction and potential prevention strategies

1.1.1: Specific animal models of chronic liver diseases and in vitro facilities will be used to identify markers of liver injury progression and cancer development. Data will be compared with clinical prognostic features of disease progression from local, national and international databases. Specific environmental factors and lifestyle habits will be evaluated as non-transmissible aetiological parameters able to modify morbidity and mortality. A platform for multigenic characterization will be developed, together with tools in support of clinical decision-making. ***Deliverable:*** molecular profile of patients at risk of developing primary liver cancers. **1.1.2:** Studying the role of bacterial toxins in colorectal tumor- and carcino-genesis, to define genetic profiles associated with increased risk and actionable mechanisms, with the final goal to explore preventive strategies based on dietary of microbioma-modificating interventions. ***Deliverable:*** Identification of immune, metabolic, epigenetic mechanisms modulated by bacterial toxins.

Task 1.2: Weighting the exposome: a model for disease prevention

A BioBank for biological samples of former asbestos exposed (ex-EXP) subjects, patients with malignant pleural mesothelioma (MPM) and healthy subjects has been set up. Circulating biomarkers of different origin linked to asbestos exposure, such as asbestos-related miRNAs, autophagic biomarkers of mesothelial transformation ATG5 and HMGB1, and soluble mesothelin related proteins (SMRPs) will be investigated for MPM prediction and follow-up. ***Deliverables:*** i) an algorithm for prevention and early detection of the asbestos-related cancer diseases; ii) molecular signatures for asbestos-related cancer diseases and follow-up mortality of MPM.

Task 1.3: Accelerating the development of innovative therapies for solid tumors by advanced preclinical evidence and 3D systems

Microfluidic devices (immune-on-a-chip) generated with the patient's primary cells to analyze the interactions of specific immune cell populations with the host's pathological tissues, and to test the activity of immunotherapies or the effect of conventional therapies on the immune system, in a patient-specific way, facilitating the pathways of personalized medicine. ***Deliverable:*** Identification of key immune signals driving the effectiveness of cancer therapies.

Task 1.4: Head and neck oncological predictive medicine by artificial intelligence omic analysis

Identifying genomic and molecular landscape that underlies the phenotypic diversity in HNSCC liquid biopsy using single-cell transcriptomic analysis and next generation sequencing and cells, correlating these findings to specific phenotypes by Artificial Intelligence approaches. ***Deliverables:*** genomic, transcriptomic, and microbiome biomarkers for predictive diagnostics, prognosis, and drug development in HNSCC patients.

Task 1.5: Cancer risk prediction in the Italian population
Implementing a novel and comprehensive method for cancer risk prediction in the Italian population, through the outline of comprehensive cancer gene panels (by NGS) as well as the selection of common SNPs to be genotyped for the definition of polygenic risk scores (PRSs) for most frequent cancer types. Algorithms for the evaluation of cancer specific PRSs and tools for the integrative analysis of high/moderate/ low penetrance genetics and environmental exposure will be developed in collaboration with external companies. ***Deliverable:*** To provide effective models for the stratification of the Italian population for cancer risk estimates

WP 2: Novel personalized strategies for prevention of cardiovascular diseases
Task 2.1: Prevention of Arrhythmogenic Cardiomyopathy (ACM)
Arrhythmogenic Cardiomyopathy (ACM) is a rare, genetic, inherited heart muscle disease that is a main cause of sudden cardiac death (SCD), especially in young adults and athletes and that is frequently misdiagnosed. With laboratory test, ECG recordings, cardiac MRI, an accurate electroanatomical voltage mapping, an endomyocardial biopsy, and genetics, it is hypothesized to reduce the portion of misdiagnosis and to better identify phenocopies. ***Deliverable:*** a diagnostic pathway capable of recognizing ACM patients from pathological phenocopies and a multiparametric score that allows the identification of patients with high arrhythmic risk, using clinical, imaging, electrophysiological, histological and genetic data.
Task 2.2: Risk factors assessment for chronic heart failure
To develop and validate a suitable model for monitoring the density of the ß1AR as a possible predictive marker of heart failure (HF) development using monocytes of the peripheral blood isolated from male and female healthy subjects. ***Deliverable:*** identification of possible predictive markers of heart HF.
Task 2.3: A novel female-specific risk score predictive of atherosclerosis in women for personalized prevention strategies
Cardiovascular (CV) injury is associated with a significant proportion of deaths in women. Women are exposed to specific sex related CV risk factors including menopause and depression and also the traditional risk factors act differently in women compared to men. Three different cohorts will be studied from pre- to postmenopause. Machine Learning methods to integrate molecular, phenotypic and lifestyle data will be used generate a novel female-specific risk score predictive of atherosclerosis in women. ***Deliverables:*** sex-specific cardiovascular risk score ready and validated
Task 2.4: Novel early predictors of infections of cardiovascular implantable electronic devices
The use of cardiovascular implantable electronic devices (CIEDs) is widespread and has been shown to improve patients' quality of life and long-term survival. A deep characterization of patients with suspect CIED infection is proposed in order i) to identify the risk factors for systemic vs. local infection; ii) to identify the risk factors for CIED infection relapse or adverse outcomes; iii) to provide novel early predictors of CIED infection. ***Deliverables:*** a novel stratification tool to predict the risk of CIED re-infection after hardware removal and create a novel algorithm for management of CIED patients after hardware removal.

WP 3: Integrating old risk factors and novel predictive models for the prevention of Metabolic and Endocrine Diseases
Task 3.1: Risk factors, lifestyle and new biomarkers in obesity and related diseases
To study the mechanisms and specific effects of nutrients and metabolic disrupting compounds (MDCs) on adipose tissue and human iPSC-derived organoids in human cohorts taking into account sex, age, health status, dietary intake and cross-talk with other organs, including gut and brain. Validated murine models will be used to verify gut-brain-axis signaling and inflammatory processes involved in metabolic diseases regulation and mood disorders. ***Deliverables:*** Characterization of specific at-risk groups for metabolic diseases onset related to nutrients and/or MDC effects
Task 3.2: Diasmoke - Randomised controlled trial evaluating changes in cardiovascular risk in type 2 diabetic patients who switch to combustion-free nicotine delivery systems.
Large randomized switching trial investigating changes in CV risk factors and metabolic parameters in diabetic smokers who switch to combustion free nicotine delivery systems - CF NDS (e.g. e-cigarettes, heated tobacco products) compared to continuing smoking. ***Deliverables:*** identification of CV risk factors changes in diabetic patients
Task 3.3: Targeting reproductive functions for novel prevention and prediction strategies
Identifying novel prevention and early treatment strategies for reproduction-related diseases, to ensure sexual and reproductive health. The methodological approach will include generation, collection and analysis of large sets of in vivo and in vitro data, covering environmental, molecular, endocrine, pharmacogenetic, genotoxic and

epigenetic data around reproductive functions. Epigenetics, clinical and exposure data will be used, especially to endocrine disruptors, with a particular focus on gonadotropins. Investigations on infertility prevention will be combined with research on infertility prediction with the identifications of genetic, biochemical and clinical markers associated with infertility. ***Deliverables:*** Predictive algorithms for infertility and human reproductive prevention strategies combined with novel biomarkers identification.

WP 4: Case studies for personalized prevention strategies

Task 4.1: Risk factors assessment for Cardio-Vascular Diseases (CVD)

Using data from Italian population-based studies of the Progetto CUORE (Health Examination Surveys (OEC 1998-202, OEC/HES 2028-2012, HES 2018-19) and MATISS cohort, the improvement of the risk assessment personalization elaborating sex specific or age-group specific CVD risk functions will be quantified; study the utility of different analysis methods for elaborating individual CVD risk assessment functions (Cox-proportional hazard models, Artificial Intelligence techniques, such as Machine Learning). ***Deliverables:*** updated functions and tools for the individual CVD risk assessment, possibly specific fo sex- and age-groups.

Task 4.2: Effect Modifiers, epigenetics and gene-environment interaction: comparison among different cohorts in a gender perspective

Evaluation of how environmental exposures impact on later-life health/disease consequences for the child via epigenetic mechanisms will be assessed to predict the risk associated to early-life stressors and to understand whether dietary factors could potentially counteract environmental pollution-induced epigenomic defects, using data and samples from distinct Italian cohorts of women of childbearing age and of mother-child pairs. ***Deliverables:*** predictive models for the risk associated to early-life stressors.

Task 4.3: Multi-omics and AI approach in rare diseases: implementing an innovative diagnostic pathway and precision medicine tool for fibrotic diseases

Fibrosis is the late stage of many chronic, rare diseases characterized by immune-mediated inflammation such as Systemic Sclerosis (SSc), Idiopathic Pulmonary Fibrosis (IPF), chronic Graft Versus Host Disease (cGHVD). Predicting fibrosis onset and progression is an unmet medical need.

i) Liquid (blood, bronchoalveolar lavage) and tissue (skin, lung, heart, liver) biopsies from patients of the multicenter network will be transferred to the biobank connected to a single cell analysis facility (www.marchebiobank.it) for extraction of single cell omics information; ii) Novel single cell data, conventional laboratory data, imaging data and clinical data from each patient will be transferred to the AI facility (Unibo, spoke 2, task 2.2) for integration into new algorithm models enabling stratification of risk of developing fibrosis and progression to severe forms of fibrosis. ***Deliverables:*** a novel biobank of fibrotic diseases' samples; a novel algorithm enabling stratification of patients (at very early or early disease steps) based on the risk of developing progressive fibrosis.

Task 4.4: Omics Research in Epidemiology and Preventive Medicine

To provide in-depth understanding of the relationship of multilevel omics biomarkers measured in available cohorts. Combining detailed data on established life-styles, risk factors and metabolic features, exposome and "omics" (metabolomic, epigenomic and genomic) data, (newly generated and made available by large international consortia as UK biobank, Morgam/BioMarCARE and Nevada Health Genome, precision medicine algorithms will be defined to predict the risk of the major chronic disease. Machine learning methods will be employed for feature (variable) selection, estimation of effect sizes and of conditional predictive impact to construct a super learner. ***Deliverables:*** Identification of predisposing or protecting markers, personalized algorithms for chronic disease prediction.

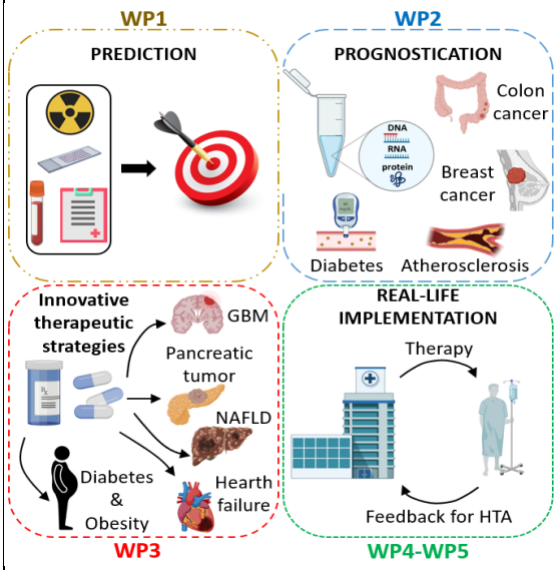
SPOKE - 8

CLINICAL EXPLOITATION

Clinical validation and implementation of innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches, based on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols

GENERAL OBJECTIVE: This part of the project aspires to validate and implement, in the clinical setting, innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches founded on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols. Specific

aims include:
1) To provide proof-of-concept evidence for the clinical usefulness of new strategies supported by sound preclinical backgrounds; **2)** To improve the ability of prognostication and risk stratification to offer early intervention when needed, while avoiding useless treatments; **3)** To better use available resources to prevent treatment toxicity while expanding the magnitude of expected benefit in properly selected patients; **4)** To make the current state-of-art of precision medicine available for patients and caregivers by implementing networks for the management of molecular and clinical information; **5)** To assess the value of applying precision medicine strategies by Health Technology Assessment (HPA) evaluations.



According to the call, this will be mainly (but not only) applied to cancer, metabolic diseases (METAB) and cardiovascular diseases (CVDs), and each WP and its related tasks may address issues concerning more than one single disease. The achievement of the general objective of this Spoke will be facilitated by: i) the already ongoing collaborations between several of the partners of the Spoke, particularly in the field of certain cancer types, type 2 diabetes and major cardiovascular ailments, which will be further implemented to develop new combined efforts; and ii) the continuous exchanges of information with the other Spokes, in particular for the WPs specifically involved in the study of various omics, A.I. approaches, new devices and preclinical evaluations. The Spoke takes advantage of its own **Scientific Advisory Board**: Laszlo Fesus (Debrecen Univ, Hungary), Inna Lavrik (Univ Magdeburg, DE), Wei Jia (Univ. Hong Kong).

WP 1 Optimizing the arrows into the quiver: from prediction markers to targeted treatments

Task 1.1: Catching the clinical heterogeneity and dynamic evolution of diseases. This Task will capture the heterogenous clinical trajectories of certain malignancies (including lung, gastric, colorectal, hepatocellular and pancreatic cancer) and type 2 diabetes (T2D) to identify and apply personalized strategies for disease prevention, follow-up, prognosis and effective therapies. ***Deliverables***: Prospective validation of radiomic signatures in gastrointestinal cancers and correlation with ongoing treatments; Classification of lung adenocarcinoma based on tumor immune microenvironment and its impact on the use of immune checkpoint inhibitors (ICIs); Identification of biomarkers associated with the prognosis and the response to therapy in hepatocellular cancer; Risk stratification for T2D and impact of mHealth on its prevention.

Task 1.2: Identifying predictors of benefit from available treatments. In this Task, omics and functional data from cancer tissue and patients will be used for assessing responses to treatment, in order to optimize personalized treatment benefits in definite tumours and CVDs. ***Deliverables***: Correlation of epigenetic, single cell transcriptomic, metabolomic, and immunophenotypic data with clinical data in patients on ICIs or CAR-T cell therapy; Identification and validation of targeted drugs in dedifferentiated/anaplastic/medullary thyroid cancers; Personalized therapy of heart failure through marker-related phenotyping that predicts response to treatments.

Task 1.3: Predicting treatment-related toxicity. This Task aims to contribute to improve the safety of cancer therapies by personalized approaches. Time-to-event models to quantitate the effects of mixed factors will be built, and biodegradable sensors for measuring the concentration of cytotoxic drugs will be used to enhance patients' protection. ***Deliverables***: Validation and implementation in patients of the TTE models and the used sensors, in order to provide prediction and prevention of drug tolerability.

Task 1.4: Predicting all cause mortality in patients with diabetes. In this Task the aim is to validate the association between novel metabolites, miRNA and all-cause death in two independent cohorts with adult-onset diabetes (AOD) from the "Sapienza Mortality and Morbidity Event Rate (SUMMER) study in diabetes" (n=5,000) and the aggregate "Gargano Mortality Study" (A-GMS) (n=2,000). Both studies are part of established networks, comprising several academic/research centers in Italy (L'Aquila, Pisa, Foggia, Tor

Vergata-Rome and abroad (Boston, Munich). The role of such markers in improving discrimination and reclassification of the ENFORCE and RECODe algorithms, two gold standards for predicting mortality in AOD, will be investigated. Finally, this task will investigate whether the markers associated with all-cause death are also associated to coronary heart disease (CHD) and/or chronic kidney disease (CKD) (mediation analysis). ***Deliverables***: New pathogenic pathways of CHD and CKD involving the newly identified markers; Novel risk score for prediction of mortality in adults with diabetes; Novel risk score for prediction of diabetes outcomes in different types of AOD; Novel insights about the role of CHD and CKD as mediators of all-cause mortality in diabetes; Novel diagnostic biomarkers of interest for industries.

WP 2: Beyond the crystal ball: improving prognostication in cancer and cardiometabolic diseases

Task 2.1: Circulating markers of minimal residual disease: exploiting liquid biopsies to predict disease relapse through the analysis of circulating tumor DNA. In this Task, the clinical application of novel technologies to detect minimal residual disease will be assessed, in order to personalize the adjuvant treatment of cancer patients. The main focus will be on the post-operative treatment of radically resected colorectal cancer patients to enhance their chance to be cured, and the identification of new personalized treatments for breast cancer patients by exploiting hormone-mediated mechanisms for cancer metastatic spread and immunoescape associated with obesity and hyperinsulinemia. ***Deliverables***: Demonstration of the clinical efficacy of targeted treatment strategies in the above mentioned clinical scenarios.

Task 2.2: **Clinical, genomic and epigenetic markers of outcome in malignancies and cardiometabolic diseases.** In this Task, comprehensive models to catch the multifactorial complexity of cancer and CVDs will be investigated with a focus on acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), myelodysplastic syndromes (MSD and atherosclerosis. In particular, the contribution of molecular markers to the prognosis of affected patients will be evaluated. ***Deliverables***: Recognition of precise disease mutational patterns in the spectrum of MDS/AML, to predict clinical outcome and improve prognostic stratification; Appreciation of the clinical impact of chemo-free combinations in CLL; Conduction of a prospective trial on atherosclerosis moving from two ongoing retrospective experiences.

Task 2.3: Recognising colo-rectal cancer (CRC) behaviour, aggressiveness, and prognosis through radiogenomic signature. This task will characterize biological processes in a voxel-wise high-spatial resolution approach, extracting first, second and third order radiomics features from CT datasets of patients with CRC. Radiomic features will be subsequently computed by AI and integrated with clinical data to eventually generate a radiogenomic signature for personalised management of patients with CRC. By an artificial intelligence-based algorithm of radiomic features combined with clinical factors, biochemical biomarkers and genomic data the tumor behaviour, aggressiveness, and prognosis, will be investigated identifying a radiogenomic signature of the tumor. ***Deliverables***: Generation of segmentation AI model for colon cancer; Generation of radiogenomic AI model for colon cancer signature.

WP 3: Reverse translation: from the patient to the lab and back

Task 3.1: Finding the niche for drug clinical repurposing. This Task intends to contribute to the repositioning of certain drugs for the treatment of definite cancer types (such as glioblastoma multiforme and pancreatic cancer) and T2D (targeting the pancreatic beta cells). ***Deliverables***: Repositioning of chlorpromazine for the therapy of glioblastoma multiforme; Repositioning of decitabine in refractory pancreas cancer; identification and validation of beta cell targeted treatments in T2D.

Task 3.2 Identifying mechanisms of acquired resistance to available treatment options to select candidate drugs for clinical investigation. In this Task, efforts will focus on the detection and validation of markers to predict and prevent atherosclerotic vascular disease, heart failure and mortality in patients with cardiovascular diseases and/or myocardial infarction. ***Deliverables***: Confirmation of the role of the selected biomarkers on both atherosclerotic vascular disease and heart failure progression in the populations prospectively enrolled in Task 1.2 and 2.2, and in the retrospective populations of the ongoing CAMP and SMARTool studies; Identification of arrhythmic structures and mortality risk factors within specific heart rate variability (HRV) patterns.

Task 3.3 As a matter of fat. This Task will address the clinical impact of adipose tissue when it accumulates in the liver (NAFLD) of genotyped people, and its role in the prognosis of graft and patient survival after organ transplantation; it will also identify patient-specific biomarkers to be correlated with adipose stromal cell therapy

in skin complication of systemic sclerosis (SSc). ***Deliverables***: Identification of the appropriate treatment in genotyped NAFLD patients; Identification of role of body weight and cardiometabolic risk factors in transplanted patients, and respective treatments; Establishment of a personalized adipose cell therapy strategy for SSc.

Task 3.4 Molecular, mutational, radiomic and histo-morphologic profile of HBP cancers: assigning the right treatment to the right patient at the right time. Thanks to shareable data from the international network (ENSCA) that includes the largest cohort (approx. 3,000 cases) of fully characterized (clinic, histopathologic, morphologic and radiologic data) HBP tumors, this task will: 1) provide the first in-depth characterization of HBP tumors based on clinical, histomorphological and molecular profiling in tissues and liquid biopsy; 2) identify, with the help of a multidisciplinary platform (matching clinical data, histomorphology, molecular profiling in tissues, molecular data from liquid biopsy and data from patient-derived organoids) individualized prognosis and therapy for patients with HPB tumors. ***Deliverables***: Comparative genomic-radiomic-histomorphologic profile of liver and pancreas cancer; Clinical outcomes of specific target therapies in a clinical trial; Identification of a transcriptomic fingerprint predicting the therapeutic response to FGFR2-, HDH1-target therapies; Validation of an innovative contrast enhanced MRI cholangiography as a tool for early diagnosis of HPB cancer in at risk patients (PSC, chronic pancreatitis, etc.).

WP 4: Make it happen in the real-life: precision medicine is ready for prime time

Task 4.1 Building networks for precision medicine implementation in daily clinical practice. The experience of local molecular tumor boards (MTBs) has just started in many institutions, but a relevant expertise is needed to fully appreciate the contribution of the large amount of clinical and molecular information, so that building a network of MTBs at national level would be a relevant added value to let precision medicine enter the daily practice. ***Deliverables***: Creation of MTBs at national level and periodical review of achieved results also through the implementation of AI technologies.

Task 4.2 Assessing the cost-effectiveness of available techniques to "pick the winner" in the perspective of sustainability. The availability of many different technologies able to provide different levels of information requires to clearly identify sustainable flows for the analysis of tissue samples with the aim of offering an adequate molecular characterization to all cancer patients. Point of care tools may provide useful information to monitor patients in their environment. ***Deliverables***: Development of cost-effective diagnostic algorithms; clinical validation of biosensors for patients' monitoring

Task 4.3 From extended trailers to clinical proofs of concept. Innovative personalized strategies to reduce the cardiometabolic/inflammatory atherosclerotic risk, to lose weight and to treat alkaptonuria will be evaluated in prospective trials. ***Deliverables***: Results from above mentioned clinical trials.

WP5: From bench to bedside: the evaluation and implementation of innovative precision medicine technologies in clinical care

Task 5.1 Identification of the HTA evaluation process core components and key aspects regarding innovative clinical precision medicine technologies. This Task aims to systematically review the existing HTA frameworks used for the evaluation of innovative technologies, such as omics biomarkers and AI-based tools. ***Deliverables***: Systematic review of HTA frameworks used for the evaluation of innovative technologies and the pertinent HTA reports; Identification of the core components and key features of the HTA evaluation process regarding innovative precision medicine technologies.

Task 5.2 Identification of the existing methodologies and development of a standardized approach to generate evidence on the clinical validity, utility, and technical aspects of innovative precision medicine technologies, and provide a tailored HTA framework to evaluate personalized procedures and promote their implementation in clinical care. In this Task the existing approaches and methodologies used to generate evidence regarding innovative precision medicine technologies, and the gaps and challenges of the processes will be identified, to develop evidence-based standardized and tailored tools. ***Deliverables***: Development of an evidence-based methodology to generate evidence for innovative precision medicine technologies; Development of a new HTA framework to be used for the evaluation of innovative technologies.

[***]

Costs breakdown

The following tables provide an overview of HEAL ITALIA's costs broken down for each organisation (public/private; Spoke/Affiliated to the Spokes). In terms of territory allocation, the entire budget has been distributed among the regions of Central-Northern and Southern Italy, ensuring that the requirement of 40% of resources allocated to the South is met. In particular, the Cascade calls dedicated to the research activities of the Spokes will contain a requirement of allocating at least 75% of the calls' budget to Southern regions. In this way, the overall economic impact of HEAL ITALIA's program towards the **Southern regions** reaches a total of **47.2 Mln euros (41% of the total approved contribution of 114.7 Mln €),** calculated as follows: 75% of the cascade calls dedicated to research activities independently from the territorial location of the entity issuing the call; 100% of the Hub's costs since the Hub is located in Sicily; 100 % of the budget allocated to Southern region's entities within the HEAL ITALIA's participants (UNIPA, UNICT, UNIFG, UNICA, NEUROMED, IOM, UPMC). **Cascade calls are totaling 11.47 Mln euros** (**10 % of the total approved contribution)**, thus falling within the range allowed by the call.

The costs breakdown per entity and per type of activity are summarized in the table below and demonstrate consistency with article 7 paragraph 2 of the call fulfilling the 20 % of activities in the *field 022* (23%) and 77 % of activities in the *field 006*.

	Costs by Type of Activity expressed in Euros								
ENTITY	**Fundamental Research**	**Industrial Research**	**Experimental Development**	**Innovation Hub**	**Education and Training**	**Total Costs**	**Field of Intervention 022**	**Field of Intervention 006**	**Contribution**
TOR VERGATA	4.432.000	1.351.000	611.000		921.000	7.315.000	1.445.000	5.870.000	7.315.000
UNIBO	5.760.000	1.875.000	810.000		720.000	9.165.000	1.705.000	7.460.000	9.165.000
UNIPA	5.988.000	2.141.000	882.000		1.504.000	10.515.000	2.223.000	8.292.000	10.515.000
SAPIENZA	6.055.000	1.978.000	816.000		306.000	9.155.000	1.445.000	7.710.000	9.155.000
UNIMIB	4.529.000	1.575.000	611.000		600.000	7.315.000	1.445.000	5.870.000	7.315.000
UNIMORE	4.067.000	1.637.000	611.000		1.000.000	7.315.000	1.445.000	5.870.000	7.315.000
UNIVPM	3.812.000	1.310.000	570.000		533.000	6.225.000	1.445.000	4.780.000	6.225.000
UNIPI	4.149.000	1.639.000	631.000		1.086.000	7.505.000	1.445.000	6.060.000	7.505.000
UNIFG	3.700.000	1.086.000	697.000		767.000	6.250.000	1.299.000	4.951.000	6.250.000
UNICT	3.330.000	1.783.000	738.000		749.000	6.600.000	1.329.000	5.271.000	6.600.000
UNICA	3.700.000	1.074.000	697.000		779.000	6.250.000	1.299.000	4.951.000	6.250.000
UNIVR	2.565.000	727.000	492.000		656.000	4.440.000	902.000	3.538.000	4.440.000
ISS	878.000	1.435.000			287.000	2.600.000	345.000	2.255.000	2.600.000
IFO-IRE	898.000	1.412.000				2.310.000	291.000	2.019.000	2.310.000
NEUROMED	1.169.500	1.770.000			60.000	2.999.500	588.000	2.411.500	2.350.000
CRO AVIANO	502.000	833.000				1.335.000	202.000	1.133.000	1.335.000
SANT'ORSOLA	437.000	738.000				1.175.000	148.000	1.027.000	1.175.000
TLS	954.000	984.000	492.000			2.430.000	550.000	1.880.000	2.430.000
UPMC		3.100.000				3.100.000	3.100.000		2.015.000
IOM	246.000	2.392.000			100.000	2.738.000	614.000	2.124.000	2.100.000
BI-REX	350.500	1.030.000	335.000		35.000	1.750.500	521.000	1.229.500	1.400.000
ENGINEERING		1.750.000				1.750.000	1.750.000		1.137.500
MARIO NEGRI	360.000	785.000			195.000	1.340.000	194.000	1.146.000	1.340.000
OPELLA	572.950	637.000	270.000		30.000	1.509.950	301.000	1.208.950	1.110.000
SIT	400.000	734.000	434.000		32.000	1.600.000	326.000	1.274.000	1.270.000
HUB				4.077.500		4.077.500	953.500	3.124.000	4.077.500
Total	**58.854.950**	**35.776.000**	**9.697.000**	**4.077.500**	**10.360.000**	**118.765.450**	**27.310.500**	**91.454.950**	**114.700.000**

The timeline and costs breakdown per "*Project Milestone"* (different from Scientific Milestones described later on) are summarized in the table below together with their timeline (envisaged *starting* and *ending* date) and costs/approved grant.

				Costs by Type of Activity expressed in Euros					
Project Milestone	*Description*	*Start Month*	*End Month*	**Fundamental Research**	**Industrial Research**	**Experimental Development**	**Innovation Hub**	**Education and Training**	*TOTAL*
M1	Hub Establishment, Agreements &Open Kick-off	1	2	-	-	-	246.000,00	-	**246.000,00**
M2	Manager selection, HUB & facilities management	1	36	-	-	-	1.163.500,00	-	**1.163.500,00**
M3	Hub governance and planning activities (IPR and other management plans)	1	36	-	-	-	328.000,00	-	**328.000,00**
M4	Scientific Meetings, Public Engagement and OpenDays	7	36	-	-	-	319.000,00	-	**319.000,00**
M5	Web services & FAIR Open Data Platform	1	36	-	-	-	552.000,00	-	**552.000,00**
M6	Networking Interoperable Platforms	7	36	-	-	-	361.000,00	-	**361.000,00**
M7	IP&TT support services (Brokering/patenting)	13	36	-	-	-	310.000,00	-	**310.000,00**
M8	MOOC platform & courses management	13	36	-	-	-	146.000,00	-	**146.000,00**
M9	Spin-off/Start up incubation service and support	19	36	-	-	-	202.000,00	-	**202.000,00**
M10	Administrative and Management activities	1	36	-	-	-	450.000,00	-	**450.000,00**
M11	RTD/TTD recruitment & research activities within the spokes and their affiliates	1	36	14.110.000,00	5.161.000,00	-		1.903.000,00	**21.174.000,00**
M12.1	PhD selection&research @ spokes 1-8	1	36	-	-	-		3.500.000,00	**3.500.000,00**
M12.2	PhD national selection&research@spokes 1-8	7	36	-	-	-		3.750.000,00	**3.750.000,00**
M13.1	1st Round Professional Training/Masters	7	24	-	-	-		567.000,00	**567.000,00**
M13.2	2nd Round Professional Training/Masters	19	36	-	-	-		640.000,00	**640.000,00**
M14.1	1st round Spoke&affiliate's R&I (Low TRL 1-3)	1	6	15.545.000,00	1.281.000,00	-		-	**16.826.000,00**
M14.2	2nd round R&I Spokes 1-8 (Low TRL 1-3)	7	12	11.102.450,00	1.400.000,00	-		-	**12.502.450,00**
M14.3	3rd round R&I Spokes 1-8 (Low TRL 1-3)	13	18	9.577.000,00	1.950.000,00	-		-	**11.527.000,00**
M14.4	4th round R&I Spokes 1-8 (Low TRL 1-3)	19	24	8.520.500,00	2.330.000,00	-		-	**10.850.500,00**
M15.1	1st round R&I Spokes 1-8 (Mid TRL 3-5)	7	12	-	4.300.000,00	470.000,00		-	**4.770.000,00**
M15.2	2nd round R&I Spokes 1-8 (Mid TRL 3-5)	13	18	-	3.550.000,00	638.000,00		-	**4.188.000,00**
M15.3	3rd round R&I Spokes 1-8 (Mid TRL 3-5)	19	24	-	3.050.000,00	860.000,00		-	**3.910.000,00**
M15.4	4th round R&I Spokes 1-8 (Mid TRL 3-5)	25	30	-	2.284.000,00	1.060.000,00		-	**3.344.000,00**
M16	Early products/demonstrators/services in Spokes 1-8	25	36	-	-	969.000,00		-	**969.000,00**
M17.1	1st-TechTransfer&Dissemination in Spokes 1-8	13	24	-	1.400.000,00	600.000,00		-	**2.000.000,00**
M17.2	2nd-TechTransfer&Dissemination in Spokes 1-8	25	36	-	1.800.000,00	900.000,00		-	**2.700.000,00**
M18.1	1st Round Open Call - Industrial Research	1	24	-	2.770.000,00	-		-	**2.770.000,00**
M18.2	2nd Round Open Call - Industrial Research and Experimental Development	7	36	-	3.500.000,00	1.800.000,00		-	**5.300.000,00**
M18.3	3rd Round Open Call - Industrial Research and Experimental Development	13	36	-	1.000.000,00	2.400.000,00		-	**3.400.000,00**

The costs breakdown and project milestone timeline will be updated periodically, in agreement with the financing body (MUR), on the basis of project monitoring activities.

Roles and responsibilities

The following table illustrates the roles and responsibilities for each subject involved in the project, distinguishing between institutions from the Southern Regions (light orange), Spoke leaders (light blue), WP leaders (red), contributors/spokes affiliates (yellow).

Color Codes:	Spoke Leader		Spoke Affiliates/ Contributors												Southern Regions					WP Leader					
	TOR VERGATA	UNIBO	UNIPA	SAPIENZA	UNIMIB	UNIMORE	UNIVPM	UNIPI	UNIFG	UNICT	UNICA	UNIVR	ISS	IFO-IRE	NEUROMED	CRO AVIANO	S. ORSOLA	TLS	UPMC	IOM	BIREX	ENGINEERING	MARIO NEGRI	OPELLA	SIT
S1																									
WP1																									
WP2																									
WP3																									
WP4																									
S2																									
WP1																									
WP2																									
WP3																									
S3																									
WP1																									
WP2																									
WP3																									
WP4																									
S4																									
WP1																									
WP2																									
WP3																									
WP4																									
S5																									
WP1																									
WP2																									
WP3																									
WP4																									
S6																									
WP1																									
WP2																									
WP3																									
WP4																									
S7																									
WP1																									
WP2																									
WP3																									
WP4																									
S8																									
WP1																									
WP2																									
WP3																									
WP4																									
WP5																									

B.5 Time schedule and monitoring indicators

The activities of the Extended Partnership will last 36 months. Each Spoke and WP will have its own detailed time schedule, including specific activities and deliverables. The following table summarizes the timeline of *Scientific Milestones* (which differ from *Project Milestones* above) and *Deliverables*, described in annex B for all Spokes, to enable effective monitoring of the progress status of the activities.

Key monitoring indicators are summarized in the Gantt chart below with the indication of the bimester where intermediate scientific milestones and final deliverables are achieved. The timeline will be updated periodically on the basis of project monitoring activities.

			MONTHS																		Indicators of Work Package monitoring (in itinere & ex post)
		Task	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	
SPOKE 1	WP1	1.1						M1.1							D.1.1						rate of population mapping (%)
		1.2												M1.2						D.1.2	
		1.3												M1.3						D.1.3	
	WP2	2.1											M 2.1							D.2.1	number of novel omics predictors of therapy resistance
		2.2									M2.2							D.2.2			
		2.3								M.2.3								D2.3			
	WP3	3.1									M 3.1									D.3.1	number of new identifid interaction
		3.2									M 3.2									D.3.2	
		3.3								M 3.2								D3.2			
	WP4	4.1							M.4.1								D 4.1				percentage of realization of matabolic maps (%)
		4.2									M.4.2							D 4.2			
		4.3											M.4.3							D 4.3	
SPOKE 2	WP1	1.1								M.1.1								D 1.1			percentage of realization of framework and open-source repository of the framework (%)
		1.2									M.1.2							D 1.2			
		1.3								M.1.3								D 1.3			
	WP2	2.1								M.2.1								D 2.1			percentage of realization of realizazion of Digital twins for computational modeling and personalized intervention (%)
		2.2									M.2.2							D 2.2			
		2.3								M.2.3								D 2.3			
	WP3	3.1								M.3.1								D.3.1			number of AI tool developed
		3.2								M.3.2								D.3.2			
		3.3								M.3.3								D.3.3			
SPOKE 3	WP1	1.1							M.1.1						D.1.1						percentage of realization of integrated experimental and computational models of 3D (%)
		1.2									M.1.2							D.1.2			
	WP2	2.1									M.2.1							D.2.1			number of models of mutated proteins
		2.2												M.2.2						D.2.2	
	WP3	3.1							M.3.1						D.3.1						number of validate test
		3.2									M.3.2							D.32			
		3.3												M.3.3						D.3.3	
	WP4	4.1								M.4.1								D.4.1			number of Preclinical models for precise therapeutic and diagnostic prevention strategies
		4.2								M.4.2								D.4.2			
		4.3										M.4.3								D.4.3	
		4.4									M.4.4							D.4.4			
		4.5										M.4.5								D.4.5	
		4.6										M.4.6								D.4.6	
Spoke 4	WP1	1.1						M1.1							D.1.1						percentage of realization of advanced diagnostic tools (%)
		1.2							M1.2								D.1.2				
		1.3										M.1.3						D.1.3			
	WP 2	2.1									M2.1								D.2.1		percentage of realization of a workflow based on an integrated genome scan in the diagnosis of monogenic diseases, complex disorders and rare tumors (%)
		2.2										M2.2							D.2.2		
		2.3											M2.3							D.2.3	
	WP3	3.1								M3.1							D.3.1				number of standardized imaging acquisition process
		3.2										M3.2						D.3.2			
		3.3											M3.3						D.3.3		
	WP4	4.1											M4.1							D.4.1	number of Application, test and validation of computational profiles
		4.2												M4.2						D.4.2	
		4.3												M4.3						D.4.3	
SPOKE 5	WP1	1.1								M.1.1								D.1.1			number In vitro validated hit compounds
		1.2								M.1.2							D.2.2				
	WP2	2.1								M.2.1								D.2.1			percentage of realization of a quick, robust, and scalable process suitable for large-scale synthesis of in vivo effective and efficacious conjugates (%)
		2.2										M.2.2								D.2.2	
		2.3									M.2.3									D.2.3	
		2.4								M.2.4								D.2.4			
		2.5										M.2.5								D.2.5	
	WP 3	3.1					M.3.1					D.3.1									number of patients avatar
		3.2										M.3.2						D.3.2			
		3.3											M.3.3							D.3.3	
	WP 4	4.1					M.4.1					D.4.1									number of alternative targets discoverd
		4.2											M.4.2							D.4.2	
		4.3									M.4.3									D.4.3	
		4.4									M.4.4									D.4.4	
Spoke 6	WP 1	1.1								M.1.1									D.1.1		percentage of realization of of Sensing devices for precision diagnostics and remote health monitoring (%)
		1.2								M.1.2									D.1.2		
		1.3										M.1.3								D.1.3	
		1.4										M.1.4								D.1.4	
	WP2	2.1									M.2.1								D.2.1.		percentage of realization of assistive tools for precision surgery (%)
		2.2									M.2.2									D.2.2	
	WP3	3.1								M.3.1									D.3.1		
		3.2									M.3.2									D.3.2	
	WP4	4.1								M.4.1									D.4.1		number of nanotools reaching the proof of concept
		4.2									M.4.2									D.4.2	
		4.3									M.4.3									D.4.3	
SPOKE 7	WP1	1.1									M1.1									D.1.1	percentage of realization of effective models for the stratification of the Italian population for cancer risk (%)
		1.2									M1.2									D.1.2	
		1.3									M1.3									D.1.3	
		1.4									M1.4									D.1.4	
		1.5									M.1.5									D.1.5	
	WP2	2.1									M.2.1									D.2.1	number of novel personalized strategies for prevention of cardiovascular diseases
		2.2									M.2.2									D.2.2	
		2.3									M.2.3									D.2.3	
		2.4									M.2.4									D.2.4	
	WP3	3.1									M.3.1									D.3.1	percentage of realization of integrating old risk factors and novel predictive models for the prevention of Metabolic and Endocrine Diseases (%)
		3.2									M.3.2									D.3.2	
		3.3									M.3.3									D.3.3	
	WP4	4.1									M.4.1									D.4.1	number of case studies for personalized prevention strategies
		4.2									M.4.2									D.4.2	
		4.3									M.4.3									D.4.3	
		4.4									M.4.4									D.4.4	
SPOKE 8	WP1	1.1									M.1.1									D.1.1	number of case studies for personalized prevention strategies
		1.2									M.1.2									D.1.2	
		1.3									M.1.3									D.1.3	
	WP2	2.1									M.2.1									D.2.1	number of prognostic indicators
		2.2									M.2.2									D.2.2	
		2.3									M.2.3									D.2.3	
	WP3	3.1									M.3.1									D.3.1	number of characterized cohorts
		3.2									M.3.2									D.3.2	
		3.3									M.3.3									D.3.3	
		3.4									M.3.4									D.3.4	
	WP4	4.1									M.4.1									D.4.1	percentage of realization of the proof of concept (%)
		4.2									M.4.2									D.4.2	
		4.3									M.4.3									D.4.3	
	WP 5	5.1									M.5.1									D.5.1	percentage of realization in terms of evaluation and implementation of innovative precision medicine technologies in clinical care (%)
		5.2									M.5.2									D.5.2	

B.6 Promotion of gender equal opportunities and stakeholders' engagement

Gender equal opportunities (see also par. B2)

Moreover, in accordance with the transversal strategy on gender equality promoted by the PNRR itself, the project Team of **Heal Italia** has defined the proposal and intends to develop it over the next 3 years consistent with the following principles:

- ensure fair, open, inclusive and gender equal access and career paths in research and consider intersectional perspectives on gender inequalities
- facilitate mutual learning opportunities on these issues among actors of the ecosystem
- employ existing and newly developed Gender Equality Plans, to facilitate systemic institutional and cultural change
- support active monitoring and evaluation to ensure continuous improvement.

In line with legal constraints of the call "Enlarged Partnership" at least 40% of staff hired on fixed-term contracts must be female and at least 40% of doctoral fellowships must be awarded to female researchers.
It is worth mentioning that team members have been already selected, based on both their scientific relevance and representativeness of male and female genders. As it can be seen in this proposal, the research and innovation program involves a whole of n. 350 components, of which n. 122 female (35 %).

Young Researchers (see also par. B2)

Regarding young researchers, **Heal Italia** intends to take in order to involve scholars who have achieved their PhD since no more than 10 years (excluding maternity and parental leave) and to attract young talents from other countries (EU and non-EU), using the following tools:

- recruitment and selection procedures will be based on main diversity management and inclusion principles, in order to favor the application of women, foreigners, and people with special needs.
- the scientists with a PhD obtained no more than 10 years before, will be chosen on the basis of international standards of transparency and of the curricula that best fit spokes activities, on the scientific assessment of the previous career of the candidate, also in the light of the most widely used bibliometric indicators of scientific production (suitably revised to take parental or health leave periods into account)
- standard parameters will be established that a doctorate must respect to be able to automatically recognize the title
- the scientific experience of candidates will be considered as well and will be privileged those with a participation in competitive European, national and regional projects and a better scientific production adhering to the themes of the Program Research **Heal Italia**
- when people will present the same evaluation, women and younger people will be favored. Gender and age will also be taken into account in the assessment.

In summary, besides scientific goals of the partnership, a crucial impact of HEAL ITALIA will be that to recruit and foster next generation of scientists in the field of precision medicine. Therefore a solid plan of both training (through PhD programs) and recruitment of researchers and technologists who obtained their PhD no more than 10 years ago will be implemented from the start of HEAL ITALIA's activities. With a minimum of 100 newly recruited researchers current percentage (33/350) of young scientists involved will be at least tripled to 133/450. HEAL ITALIA will offer to recruited researchers both the support of experienced scientists in a multidisciplinary environment and the access to networked facilities, thus boosting their career opportunities and accelerating their path to scientific independency. The "HR Excellence in Research" seal awarded to several institutions within the HEAL ITALIA network and the solid engagement of industrial partners will guarantee success of both the training and recruiting program. Recruitment will be performed according to gender equality plans (GEPs) of hosting institutions including quality of scientific background as selection criteria; besides publishing recruiting calls in English, mobility indemnities are envisaged for successful candidates from abroad, in analogy with MSCA actions. Through a *Young-to-Young-Training* (YtYT) program, recruited researchers will have a key role also in developing HEAL ITALIA higher educational agenda (Innovative PhD, Master and professional training/reskilling/upskilling) centered in scientific and technological fields related to precision medicine. Additionally, both recruited researchers and PhDs will be involved in a secondment program with co-supervisors from both the academic and non-academic sectors and requiring periods spent at other research entities and enterprises, with respect to the recruiting one, within the network and abroad.

Engagement of large companies, SMEs established less than 5 years ago, innovative start-ups and research spin-offs (including in terms of co- funding)

Large and mid-sized companies such as Engineering, SIT and Opella, have been involved in the consortium both as participant in the research and innovation program and as founders of the Hub, thus contributing in both kind and cash to the sustainability of the HEAL ITALIA program. Part of the cascade calls will be dedicated to the incubation and acceleration program towards start-up and research spin-offs (see Section C).

Besides taking advantage of HEAL ITALIA partners experience in technology transfer in life science, the organization structure of the hub foresees the creation of a system of services to support:

i) incubation of new science-based start-ups/spin-offs benefitting from the experience of business incubators of the partners and from the supervision of SMEs that would act as *Business-to-Business* trainers.

ii) acceleration of existing science-based start-ups/spin-offs that would benefit from the experience of large enterprises that would act as *Business-to-Business* trainers with a particular focus on business acceleration and scaling-up issues.

Intellectual Property Right (IPR) management plan will be defined at the beginning of the HEAL ITALIA program which includes external matching meetings, and the creation of a repository of HI network patents boosting the brokerage opportunities (for patent licensing and research valorization), improving the matchmaking between "*knowledge producers*" and "*innovation demanders*" fulfilling the main scope of Mission 4 Component 2 of the NRRP "*from research to business*".

In this context, HEAL ITALIA's open calls/cascade fundings will be issued with a focus on industrial research and experimental development and will promote (through rewarding selection criteria) the participation of existing or to-be-established start-up/spin-off alone or in partnership with research entities not belonging to current HEAL ITALIA members. Particular attention will be paid to business centered on key enabling technologies able to be integrated with the fundamental research offered by HI partners fully exploiting the open science and open innovation paradigms.

Al least 75% of such cascade calls will be required to have impact in Southern regions including main islands thus contributing to reduce the economic North-South divide.

SECTION C

IMPACT OF THE PROGRAMME

C. PROGRAMME IMPACT

C.1.1 Economic, social and cultural system impact analysis

Heal Italia is supported by an **impact assessment framework** which is a strategic tool for supporting the implementation of the Programme and to be accountable also with respect to the gap identified by the NRRP which recommends strengthening the digital and technical-scientific skills, research and technology transfer, with particular regard to the regions of southern Italy. **Heal Italia** involves the following subjects based in regions of southern Italy:

- University of Catania, Sicily.
- University of Palermo, Sicily.
- University of Cagliari, Sardinia.
- University of Foggia, Puglia.
- IRCCS Neurological Mediterranean Institute -Neuromed, Molise.
- IOM Istituto Oncologico del Mediterraneo based in Catania, Sicily
- University Of Pittsburgh Medical Center Italy based in Palermo, Sicily.

Heal Italia intends to contribute to the gap between the southern and northern regions by pursuing a double objective:

» (1) on the one hand, it supports the recruitment of young researchers (<10 years of experience) and female researchers in the southern regions involved in the research program. The programme will put great attention to the gender gap issue, promoting female participation in research and innovation activities, supporting woman aimed at satisfying their ambition to work in innovation and industrial research, thus enhancing human resources and their potential and contributing to overcoming stereotypes that they would like woman less inclined to study STEM scientific disciplines (Science, Technology, Engineering, Mathematics). Furthermore, through the proposed activities it is intended to support PhD students and researchers' early stage, on topics of strategic importance for the research program, giving them a great opportunity for professional and scientific growth. Through this involvement of researchers' early stage and doctoral students it will be possible to raise the level of participation in the national and international dimension of the Universities, Public Research organization, Enterprises affiliate to **Heal Italia** . Researchers will be able to play an increasingly decisive role as "facilitators" of national and international relationships with other innovation ecosystem players: that's why it is so important for the Program involving scholars who have obtained their PhDs for no more than 10 years and attraction from other EU and non-EU countries, based on the quality of their scientific curriculum. It is important to point out how the research period abroad experienced by the young researchers will also stimulate in the near future (after the implementation of the research program) the attraction of talents from abroad and at mean time will promote a better strategic positioning in the world of our research system.

» (2) on the other side, the research program focuses on territorial gaps with reference to the mortality rate from cancer and chronic diseases: it is known indeed that in Italy - according to recent epidemiological data provided by the Istituto Superiore di Sanità (ISS) - people die less from cancers and chronic diseases but only where prevention works, that is mainly in the northern regions. In the South, however, the situation is the opposite: the death rate from these diseases is in fact higher than the northern regions.

These two factors/objectives will make it possible to increase the prestige of the international image of all the partners involved, also regarding the research system of the southern regions involved in the research program.
Here some of the most relevant key aspect.

Given the network of highly qualified clinical research units, rapid transfer of results to the clinical practice is expected. Research groups will share the obtained results with other researchers. The most significant results will be disseminated by the scientific community from qualified scientific programs.
In addition, at the end of the Research Program a meeting will be organized for researchers and SSN operators. Particular attention will be given to the innovative prevention strategies and diagnostic and therapeutic approaches that will be obtained from the scientific experimentation of the research program. The development of bio-molecular research projects points to early diagnosis, treatment and follow up of complex diseases, including monogenic (rare diseases), polygenic pathologies (cardiovascular and metabolic disease), and

cancer.

The deployment of the Digital Platform will facilitate the analysis and evaluation of health data, starting from imaging data, for both healthy subjects (population-based) and/or patients suffering from complex pathologies (disease-oriented), (i) to develop faster, more precise, accessible and affordable early diagnosis and screening methods for the citizens, (ii) to apply precision medicine by developing risk-based stratification algorithm, and (iii) to provide scientific open-access evidence to health policy makers.

Models of diagnosis, continuous monitoring and treatment of complex pathology will be provided, allowing breaking down, among other things, the long waiting lists for all the services that revolve around the patient. In particular: (i) the early diagnosis will avoid the patient to undergo unnecessary and expensive diagnostic tests; (ii) the achievement of targeted treatment plans will avoid the patient to undergo ineffective procedures/treatments; (iii) the change of therapeutic strategy during monitoring through advanced evaluation criteria will determine an improvement in the management of the pathology itself increasing the Quality of Life and survival/outcome of the patient.

Moreover, the integration of datasets of complex diseases, including monogenic (rare diseases), polygenic pathologies (cardiovascular and metabolic disease), and cancer may pave the way for greater understanding the disease and may have consequences in future prognostic studies as well as in the definition of new therapeutic approaches. The World Health Organization is predicting, in 2023, an increase of 50% of tumors, 20% of metabolic and cardiovascular diseases and 3% of rare disease outbreaks.

The high incidence of rare, cardiovascular, metabolic and oncological diseases are the largest pharmaceutical and socio-economic cost worldwide. The efforts of scientific and industry research are aimed to identify, at the molecular level, biological markers responsible for the cascade of events that determine the onset of the disease. This will result in the development of biomarker platforms and diagnostic tests that will allow detection and identification at a very early stage of multiple forms of cancer with consequent and significant benefits in terms of survival and health costs. On the therapeutic side, the identification of biological markers and target specifics will increase the marketing of new therapeutic classes, more specific and effective for tumor care. At the same time, it is necessary to create Biobanks to have reliable preclinical models that recapitulate the patient's tumors to develop innovative therapies. The information obtained from both strategies will lead to the use of databases accessible for all Italian laboratories to optimize the insertion of patients with similar mutational profiles into specific experimental treatment protocols.

As previously mentioned, molecular oncology aims to identify mutations that may have different implications in oncological clinical practice. Thus, one of the "side effects" associated with the implementation of this process could be the introduction of molecular diagnostics in southern regions of Italy, with the potential to reduce pharmaceutical costs through effective selection of eligible patients for a specific therapy. Therefore, the implementation of the project will enable the creation of a new working group, coordinated by reference experts, which will interface with the national collaborative groups in order to program clinical research and optimize the use of targeted therapies for each individual specific patient affected by cardiovascular, cancer, rare and metabolic disease.

The innovation of molecular diagnostics and the creation of up-to-date databases will favor the contribution of capital to the profit and non-profit research of Southern, Central and Northern Italy. This will help develop new diagnostic procedures and new therapeutic molecules. The partnerships that have been signed in recent years among some large multinationals of the drug and some biotechnology start-up operating in the field of the targeted diseases, studied in the project, show the interest of this business in the pharmaceutical market. Interest due to the size of the business turnover that the drug market has today, but above all for what it will be able to generate from here in the coming years. Over the next decade, significant advances in pharmacotherapy will come from improvements in the traditional classes of drugs and immunotherapy, and, above all, by the identification of biomarkers, such as early predictive indexes, and therapies innovative features, due to their growing selectivity on the target, greater therapeutic effectiveness and lower toxicity.
The growth of the market and the consortium will provide the basis for a strengthening of the specialized staff employed in research and employees in the industrial area (production and marketing) with positive effects on the economic situation in the convergence regions.

C.1.2 The impact assessment framework

The impact assessment framework develops along all the duration of the Programme and is organized in **three stages.** The first stage is the **ex-ante evaluation** which supports the definition of the objectives addressing

each spoke and consists in the design and identification of the goals (objectives, tasks, milestones, deliverables) and related indicators (KPI). The second stage is the ***in-itinere*** **evaluation** which develops during the implementation of the Programme, it serves to monitor the implementation of the results. The third stage is **the *ex-post* evaluation**, namely the final evaluation which is carried out after the conclusion of the Programme and measures and reports on the achieved results but also on the medium-long term impact on the economic, social and cultural system.

The three-stage evaluation process follow a **virtuous circle of programming, implementation, measurement and evaluation, learning, and confirmation/review**. This virtuous circle requires a continuous dialogue among all project partners (Spoke and Affiliate), in fact the strategic monitoring assumes a fundamental role for assessing the state of implementation of the Programme of each Spoke and putting in place appropriate adjustments. In order to mitigate the risk that not all the goals can be met, the assessment framework prescribe the monitoring of early stages, with particular regards to the project management and scientific milestones and deliverables. Then the final impact assessment examines the causal links between the actions and the effects produced which can inform the design of subsequent policies.

A dedicated **impact team/taskforce** (Hub and the Leader of each Spoke) will preside the impact assessment framework during all the stages and finalize the indicators with respect to the three stages. In fact, the evaluation process is not a top-down exercise, rather it is a participatory process among the stakeholders, especially because the Programme is built along a shared agenda which requires several adjustments in progress.

The Program's impact assessment framework - in terms of scientific tasks- is also in line with the Horizon Europe Framework. The Cancer Mission (see Annex 2) should mobilize R&I for the achievement of Europe's Beating Cancer Plan to improve the lives of more than 3 million people by 2030 through prevention, cure and solutions to live longer and better. It`s important to note that health emergencies such as COVID-19 pose a global risk and have shown the critical need for preparedness. The pandemic provides a watershed moment for health emergency preparedness and for investment in critical 21st century public services. Prevention is the key factor for a global health security.

While enabling economic goals, it also drives social and cultural achievements **in line with the UN Agenda 2030 for Sustainable Development**.especially in line with the Goal 3 SDG's: *Ensure healthy lives and promote well-being for all at all ages.* **Heal Italia** intends to create a strong *health extended alliance for innovative therapies, advanced lab-research, and integrated approaches of precision medicine* that is expected to help eradicate a wide range of diseases and address many different persistent and emerging health issues. This will allow for a positive impact on economic, social system, including also scientific and cultural in the regions where the program will be implemented.

More precisely, **the Programme's scientific impact** relates to the creation and diffusion of high-quality new knowledge, technologies, skills strengthening the human capital, solutions supporting the health system. Scientific achievements - as illustrated in the activities of each Spoke – will contribute to gaining an international scope (publications and research lines) and strengthening the national and international reputation and image of the subjects involved. Also, the Programme includes activities for the diffusion of knowledge (academic and non-academic dissemination activities) and talent attraction. The indicators include, but are not limited to, scientific and technical publications, patents in *innovative diagnostics and therapies in the medicine of precision* and scientific and technical conferences/conferences, pilots for innovative product/process.
Some relevant aspects about the Programme's scientific impact are summarized below.

The results of the activities related to the study of rare diseases will allow to deepen the knowledge on the role of the genetic component in susceptibility to various rare diseases and to identify new genetic markers associated with the response to treatments. It will therefore be possible to identify the subjects who can respond to a given therapy obtaining the best efficacy and the reduction of adverse reactions. This will result in greater appropriateness of treatments with consequent reduction of costs for the National Health System. Furthermore, the possibility of expanding the banked biological material will allow institutions to have greater attractiveness for European funding requests. In particular (in line with the objectives of the National Research Plan 2021/2027), studies will be conducted on the identification of the determinants responsible for the pathogenesis of currently incurable diseases and the variability in the individual response to drugs to:

a) reveal the factors influencing individual pharmacological responses;
b) identify non-invasive prognostic / diagnostic disease biomarkers (including RNA, cfDNA, proteins and other molecular entities that can also be measured through liquid biopsy and analysis of the content of

extracellular microvesicles.

Heal Italia will generate positive impacts, contributing to:

a) guarantee higher levels of therapeutic performance by making performance more homogeneous between regions;
b) encourage the development of emerging and innovative biotherapies responding to the needs of health services at local and national level;
c) integrate specific therapies and interventions in the oncology field with a system of supportive therapies;
d) implement the constantly evolving and commonly used computational platform that integrates genomic and molecular analyzes with clinical ones, to define the precision treatment strategy for each cancer patient;
e) develop the Research and Health system with a view to greater and better usability of the products and services offered by the Hub;
f) accelerate the development of new and effective therapies both for diseases widely spread in the population and for rare diseases will allow the optimization of personalized therapeutic solutions;
g) guarantee higher and greater therapeutic performance between regions, determining concrete support for overcoming the North-South gap and ensuring equality in accessibility to the best treatments even in the South;
h) create a constantly evolving computational platform of common use that integrates genomic and molecular analyzes with clinical ones, to define the precision treatment strategy for each cancer patient.

The **Programme's economic impact** emerges from the connection between the developed innovations and the business community involved which can grasp innovation applications for the national and international markets. Market deployment of innovative solutions has a positive effect on growth (thanks to new products and innovative processes), economic activities (in terms of increasing the transactions and new innovative enterprises), investments in R&D, investments' attraction, but also in terms of jobs quality, internalization of young researchers (<10 years of experience), new enterprises (spin-off/start-up).

The **Programme's societal and cultural impact** relates to the uptake of innovative solutions in terms of new products, processes and services in the society providing local responses to address the health system. Societal impacts of R&I relate also to the development and strengthening the Southern welfare system. Other social impacts involve thestrengthening of values and rights and fostering social participation of the subjects of the quadruple helix (science, policy, industry, and society), thus avoiding unfair exclusion of specific groups not only from participation in the research process and/or access to research results but also in benefit from the innovation and being part of the research Program. Program's impact relates also to the cultural dimension in the form of supporting and promoting the scientific culture to disseminate the basic elements of diagnostics and innovative therapies in precision medicine to citizens, patients and target subjects and also the results of the project. Moreover, the inclusion of principles and actions for women empowerment and gender equality will ensure gender equality both in academic and business careers and in the research/innovation content.

The following section define the **high-level time schedule** and the **main dimensions of the expected impact** for each SPOKE/WP. However, the impact dimensions and indicator will be further finalized in the first stage of the Program's implementation, also with regards to **Indicators of evaluation** (see **par. C 1.3.3**) .

C.1.3 How to measure and monitor the results and activities of the Research Program "Heal Italia"

C.1.3.1 High-level time schedule

The activities of the Program Research **Heal Italia** will last 36 months. The activities of HUB and each Spoke will be marked by the following high-level schedule and key deliverables:

Months from 1 to 6	» Definition, publication and awarding of first round of cascade calls » Publication of calls for personnel recruitment, including RTD, PhDs, TD
Months from 1 to 36 (Every 2 months)	» Progress Report (technical and financial) on project status, including status of WPs and Task activities, deliverables, and KPIs (evaluation on recruitment, SMEs and companies involved, gender balance, publications, and patents, etc) and financial reporting » Technical and Financial Report (one per Spoke)

Month 18	» Interim Report on status of the Program Research & cascade calls
Month 30	» Final Report on status cascade funding calls including status of demonstrators development
Month 36	» Final Report on progress achievements, including status of WPs and Task activities, deliverables, and KPIs (evaluation on recruitment, SMEs and companies involved, gender balance, publications and patents, etc) and financial reporting » Final Technical and Financial Report (one per Spoke) » Report on costs, returns and industrial transferability » Report on impact

C.1.3.2 Main dimensions of the expected impact for each SPOKE

Each Spoke and WP will have its own detailed time schedule, including specific activities and deliverables focused on scientific tasks. The following table summarizes indicators based on KPI of all spokes to enable effective monitoring of the progress status of the activities.

- **Spoke 1 - Holistic Nosology**

Output	Target	Impact Indicator
Identification of disease associated omic signatures	Molecular signatures for each studied disease	Number of markers improving early diagnosis, prognosis, and therapeutic targets

- **Spoke 2 - Intelligent Health**

Output	Target	Impact Indicator
Development of a collaborative platform of integrated dataset	A platform for each type of dataset	Number of protypes for managing the outcomes of clinical studies

- **Spoke 3- Prediction models**

Output	Target	Impact Indicator
Development of advanced prediction models	Preclinical models for precise therapeutic, diagnostic and prevention strategies	Number of validated biomarkers & effective therapeutic approaches

- **Spoke 4- 4D Precision Diagnostics**

Output	Target	Impact Indicator
High- Performance of computational tools for precision diagnosis	Success rate of correct early diagnosis	Optimization of *timeliness* of diagnosis

- **Spoke 5- Next-Gen Therapeutics**

Output	Target	Impact Indicator
Preclinically validated therapies	New therapeutic approaches	Increase of quality of life & survival rate

- **Spoke 6-Healthy Toolbox**

Output	Target	Impact Indicator
Design of precision medicine devices	Realization of new tools for precision medicine	Number of tools developed

- **Spoke 7- Prevention Strategies**

Output	Target	Impact Indicator
Holistic prevention approaches	Person-oriented screening campaigns	Percentage of population involved

- **Spoke 8- Clinical Exploitation**

Output	Target	Impact Indicator
Application of a new approach/new solution to face the prevention, diagnosis, prognosis and therapy	Validated applications	Percentage of successfulness of new approaches/new solutions

C.1.3.3 Indicators of evaluation (impact)

The following table identify the indicators that will be used to evaluate the performance of the entire Research Program "**Heal Italia**".

Key Performance Indicators for evaluation
People and research excellence » Number of hired people (personnel - including RTD, PhDs, TD and researchers)* » Rate of involving scholars who have obtained their PhDs for no more than 10 years. » Rate of attraction of researchers from other EU and non-EU countries. » Rate of involving female scholars/researchers. » Number of publications. » Number of scientific articles or submissions in international scientific journals. » Number of tests and benchmarks. » Number of prototypes. » Number of applicants to the cascade calls.* » Number of Research Networks established. **Gender, age (under 36) will also be considered*

Key Performance Indicators for evaluation
Technology Transfer and Innovation - with reference to the Spokes that develop products/services with a high technological maturity » Number of participants, companies and solutions created in SME challenge-based initiatives. » Rate of hiring after PhDs fellowships hosted in companies. » Rate of Startup/Spin off born as a result of the research program Heal Italia. » Number of prototypes, solutions or apparatus* as a result of the research program Heal Italia. » Number of published or submitted patents as a result of the research program Heal Italia. **Dissemination of the results of Heal Italia and societal and cultural impact** » Number of engaged enterprises** and other organizations (such as associations, public administrations, citizens, etc) e in the research program Heal Italia activities. » Number of participants and rate of satisfaction at the community events. » Number of public engagements activities. » Number of information and dissemination events. » Number of people taking part to the events. » Number of investors involved within the feasibility plan programmes and acceleration programmes. (*) For the evaluation of prototypes, solutions or apparatus: if the prototype, solution or apparatus was made and if it is functioning; if applicable, their performances will be evaluated on the basis of specific KPIs (**) Enterprises, including a detailed monitoring of SMEs

C.2 Synergies with other research programs

The **Heal Italia** program was developed taking into consideration the potential synergies with the NRRP M4C2 measures to maximize the impact of the expected results. Sharing strategies and trajectories with other NRRP centers, consortia or projects isa goal of this Research Program proposal. **Heal Italia** is thus expected to offer enabling technologies, projects, and expertise also to several other programmes of the NRRP. Possible joint activities will be considered according to NRRP rules and guidelines.

The link with the other measures of the NRRP will be ensured by the participation of the universities and research centers leader and affiliate to each SPOKE, but also by the affiliated laboratories and innovation centers involved in the implementation of the programme. The project proposal enables a full synergy with Mission 4 Component 2 of the "National Recovery and Resilience Plan" (NRRP), in particular:

- 1.1- Fund for the National Research Programme (NRP) and Research Projects of Significant National Interest (PRIN).
- 1.4- Strengthening research structures and creating R&D "national champions" on specific key enabling technologies.
- 3.1-Fund to create an integrated system of research and innovation infrastructures
- 3.2- Start-up financing
- 3.3- Introduction of innovative doctorates responding to the innovation needs of companies and

promotion of recruiting researchers from businesses.

» Innovation agreements.

The table shows the measures that intercept those envisaged by the **Heal Italia** research program for each single Spoke.

Methodology: The different color gradation within the corresponding boxes is determined by the thematic coherence between the measures of Mission 4 component 2 selected and the thematic specializations of SPOKEs, by the participation of SPOKE leaders and affiliates in the ongoing planning on the other measures identified for NRRP M4C2 .

M4 C2 NRRP INVESTMENTS	SPOKE 1	SPOKE 2	SPOKE 3	SPOKE 4	SPOKE 5	SPOKE 6	SPOKE 7	SPOKE 8
1.1- Fund for the National Research Programme (NRP) and Research Projects of Significant National Interest (PRIN)								
1.4-Strengthening research structures and creating R&D "national champions" (NC) on specific key enabling technologies	See below							
1. *High performance simulations, computation and data analysis*								
2. *Agricultural Technologies (Agritech)*								
3. *Development of gene therapy and drugs with RNAtechnology*								
4. *Sustainable mobility*								
5. *Bio-diversity*								
2.3- Strengthening and subject-specific and territorial extension of technology transfer centres for industrial segments								
3.1-Fund to create an integrated systemof research and innovation infrastructures								
3.2- Start-up financing								
3.3- Introduction of innovative doctorates responding to the innovation needs of companies and promotion of recruiting researchers from businesses								
Innovation agreements								
Legend			Low		Medium		High	

There will be great attention to the convergence of objectives, actions and resources with the Investment **1.4** *Strengthening research structures and creating R&D "national champions" (NC)* and specifically with "*Development of gene therapy and drugs with RNA technology*" and "*High performance simulations, computation and data analysis.* The **Investment 1.4** is about specific key enabling technologies, which integrate from a point of thematic view and in terms objectives with the provisions of the project proposal.

In fact, if the national centers have to reach a priority capacity for innovation and research through collaboration

with other centers, universities, companies, for **Heal Italia** it will be possible to maximize the impact and put together the research activities carried out on which to graft other related activities training, reducing the mismatch between demand and supply of skills, technology transferring local businesses, support for startups and the involvement of the local community on sustainability and innovation issues.

Furthermore, it is relevant in terms of thematic coherence the **Investiment 3.1 - Fund to create an integrated system of research and innovation infrastructures** and **3.3 Introduction of innovative doctorates responding to the innovation needs of companies and promotion of recruiting researchers from businesses** for the above reasons set out.

Lastly the **Investiment 3.2** Start-up financing has a strong coherence with the activities/WP of the Spoke 2,4 6 & 5 that involve the development of products, services and solutions with a high technological maturity (Spoke 2; 4; 6: TRL 1-6| Spoke 5: TRL 1-7)

Regarding other national and regional programmatic frameworks**,** it is important to analyze how the research activities of **Heal Italia** are completely consistent with national and regional programme framework (NRP).

The table shows which area of research and innovation dealt with in the NRP 2021-27 relate to the specializations of the SPOKE. Those linked to domain "Health" find complete compliance with the thematic focus of the proposal.

The following table summarizes the most representative connections between the research and innovation areas and the areas of intervention of the NRP 2021-27 and the specializations of the SPOKEs.

NRP 2021-27 R&IFIELDS	AREAS OF INTERVENTION	SPOKE 1	SPOKE 2	SPOKE 3	SPOKE 4	SPOKE 5	SPOKE 6	SPOKE 7	SPOKE 8
HEALTH	General themes								
	Pharmaceutical and pharmacologicaltechnologies								
	Biotechnology								
	Technologies for health								
HUMANISTIC CULTURE, CREATIVITY, SOCIAL TRANSFORMATIO N, SOCIETY OF INCLUSION	Cultural heritage								
	Historical, literary and artistic disciplines								
	Antiquistics								
	Creativity, design and Made in Italy								
	Social transformations and society of inclusion								
SECURITY FOR SOCIAL SYSTEMS	Security of structures, infrastructures and networks								
	Natural systems security								
	Cybersecurity								
DIGITAL, INDUSTRY, AEROSPACE	Digital transition - i4.0								
	High performance computing and big data								
	Artificial intelligence								
	Robotics								
	Quantum technologies Innovation for the manufacturingindustry								
	Aerospace								
CLIMATE, ENERGY, SUSTAINABLE MOBILITY	Sustainable mobility								
	Climate change, mitigation and adaptation								
	Industrial energy								
	Environmental energy								
FOOD PRODUCTS, BIOECONOMICS, NATURAL RESOURCES, AGRICULTURE, ENVIRO\NMENT	Green technologies								
	Food science and technology								
	Bioindustry for the bioeconomy								
	Knowledge and sustainable management of agricultural and forestry systems								
	Knowledge, technological innovationand sustainable management of marine ecosystems								
Legend				Low		Medium		High	

Moreover, it is worth to notice that national "Fondo per le Scienze applicate" established with the 2022 budget law (legge di bilancio) which allocated 50 million euros for 2022 and has forecast growth over time to reach 250 million starting from 2025, aims to promote the competitiveness of the national production system through enhancement of industrial research and experimental development, and joins the Italian Science Fund dedicated, instead, to fundamental research.

In general, the provision intends to enhance the most relevant innovative ideas proposed by individuals (Principal Investigator-PI) belonging to the national research system, public and private, and to put them in the condition, having the guarantee of a broad decision-making capacity, to develop innovative project proposals, at the home institution or at a different host institution.

Heal Italia will be able to take advantage of the fund or in any case create synergies with the planned investments financed that demonstrate a thematic coherence with the activities of the Spokes (1-8).

Regarding the *National Plan for Complementary Investments to the NRRP,* it is worth to mention the following two initiatives:

» Initiative A: Research initiatives for innovative technologies and pathways in the health and assistance sector (Ministero dell 'Università e della Ricerca/Ministry of University and Research- MUR).

» Initiative B: Innovative health ecosystem (Ministero della Salute/Ministry of Health)

The measure Research initiatives for health, under the responsibility of the MUR, provides for the financing, with 500 million euros, of research programs with the aim of putting into an innovative system the strengthening of research on enabling technologies in the health sector to improve the diagnosis, monitoring, assistance and rehabilitation care of certain reference communities. It will be implemented through four major initiatives - for which funding between 75 and 150 million euros each is expected - based, for example, on robotics and digital tools, remote monitoring, process re-engineering, data mining.

The *Innovative Health Ecosystem*, under the responsibility of the Ministry of Health with a funding of 80 million euros to be divided between four initiatives, aims to create clinical-transnational networks of excellence to enhance national biomedical research, able to pool the existing skills.

These two measures are strictly coherent with all the activities of the Heal Italia research program and show a multiplicity of synergy in terms of collaborations with the various projects that will be financed, of virtuous exchange between the research results.

Therefore, an absolute priority is to build research programs capable of producing usable knowledge to maintain good health for as long as possible to prevent, treat and improve the management of both high epidemiological and clinical and rare pathologies. Chronicity above all is constantly increasing in Italy and in countries with advanced economic development and the management of chronicity represents an important challenge for the Italian research system and for the sustainability of the National Health Service. The increase in the prevalence of chronically ill patients is a multifactorial phenomenon, which depends on the demographic trend of aging of the population, correlated with the increase in survival, the improvement of economic and social conditions and the availability of new therapies. In particular, in line with the *National and Regional Health Plan* in force, with the *National Chronicity Plan 2016* and with the National Prevention Plan 2021-2025, the program of activities to be carried out aims to contribute to the strengthening of a system that connection: pathology networks (rare diseases, oncology, cardiovascular diseases, etc.) networks for research and health services.

Heal Italia intends to offer further answers to the need for health in the field of: Oncology, Cardiovascular, Rare & Metabolic Diseases. In this context, the project intends to develop therapeutic strategies for the diagnosis and treatment of neoplasms currently lacking in resolving therapies or with poor efficacy and to promote technology transfer in the clinical setting with entrepreneurial repercussions for the various territories involved.

C.3 HEAL ITALIA's Technology Readiness Levels

The **Heal Italia** research program has used the TRL metric since the processing of the activities of each Spoke or WP. This allowed the project to develop a framework of WP, tasks and deliverables consistent with the planned results. As illustrated, the following table crosses the starting and final TRLs for each Spoke of Heal Italia. Beside Spoke 1, that will contribute to the observation of basic principles, thus triggering further research in the field of precision medicine, spokes 2-6 will take advantage of key enabling technologies to advance knowledge and innovate products/services at the point that they will be available through spokes 7 and 8 for validation in relevant environment for prevention strategies or as clinical solutions.

The expected impact of Spoke 7 "PREVENTION STRATEGIES" is to improve existing disease prevention programs, thereby reducing morbidity and mortality related to the disease areas covered by the research project. In detail, the combination and integration of multi-level information coming from advanced omics analysis, clinical data and disease models (as proposed in this project) into composite tools able of predicting with higher accuracy the risk of disease onset or disease progression, will provide novel instruments for daily medical practice. It is conceivable that the novel screening tools and prevention programs developed through the 3-year program will exert their impact in terms of morbidity/mortality/costs reduction over the following years, well after the end of the project, as expected for a precision medicine project aiming at deep innovation and long standing amelioration of social and health system. With reference to TRL/SRL advancement, Spokes 7 activities can be better classified with a Starting TRL of 3 aiming at reaching a final TRL of 6 during the program development.

S = Starting TRL E = TRL reached at the end of the program	Spoke 1	Spoke 2	Spoke 3	Spoke 4	Spoke 5	Spoke 6	Spoke 7	Spoke 8
	Prototypes, solutions or apparatus* as a result of the research program							
		AI Platform	Preclinical Models	Diagnostic tools	Innovative Therapies	Devices	Prevention Strategies	Clinical Exploitation
TRL 1 Basic principles observed	Spoke 1 contributes to implement ation of TRL 1 Basic principles observed	S	S	S	S	S		
TRL 2 Technology concept formulated								
TRL 3 Experimental proof of concept							S	S
TRL 4 Technology validated in lab			E					
TRL 5 Technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies)								
TRL 6 Technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies)		E		E		E	E	
TRL 7 System prototype demonstration in operational environment					E			E
TRL 8 System complete and qualified							Spoke 7 & Spoke 8 contribute to validation of activities related to Spoke 2-6	
TRL 9 Actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space								

Spoke 8 "CLINICAL EXPLOITATION" will be the final step of our research programme to bring innovation "**from the bench to the bedside…**" and the beginning step of the **"…and back"** phase providing the new clinical data that will be considered a new starting point of the iterative research workflow.
The expected impact of the WPs of Spoke 8 is to demonstrate and validate the usefulness of innovative tools for prediction, prevention, prognostication and treatment strategies in the relevant clinical setting for people at risk of or affected by certain complex, multifactorial diseases. During the development of the project, research outputs coming from other Spokes as well as Spoke's 8 WPs will feed the planned activities, thus ensuring a high level of dynamic and timely novelty.
Some of Spoke 8 milestone are dedicated to clinical trials for drug repurposing thus accelerating path to cures. Other trials will lead to an improvement of specific clinical parameters such as Progression Free Survival or Overall Survival. Phase 2 studies will pave the way to new studies towards the approval of new therapeutic approaches. The creation of Molecular Tumor Board foreseen as scientific milestone 4.1 in Annex B will generate a significant amount of data with an impact at the national level in terms of survival periods in response to therapies tailored to the molecular framework of disease alterations.
A special focus will be directed to the application of precision medicine in the real-life scenario and to the path of Health Technology Assessment (HTA) with particular attention to consequent regulatory activity, of which patients will be the ultimate beneficiaries, and the mission to offer the best precision medicine opportunities in the frame of the Italian National Health System. With reference to TRL/SRL advancement, Spokes 8 activities can be better classified with a Starting TRL of 3 aiming at reaching a final TRL of 7 during the program development.

As already mentioned, **Heal Italia** intend to establish a synergy between the scientific community and the industrialand social system, to foster a reuse of research results and to improve the productivity of companies, especially SMEs, and the social capacity to create common value.

As already explained in Section B, in addition to the technological transfer of the results obtained during the first phases of the research and innovation activities, **Heal Italia** intends to actively contribute:

- to enhance the research results, and technology transfer,
- to develop the creation of a network of collaboration between parties,
- to promote the birth and growth of entrepreneurial initiatives with a higher technological content (innovative start-ups and spin-offs from research),

- to raise the technical and scientific skills and the attraction of highly qualified human capital,
- to contrast to the migration phenomena of qualified personnel.

Moreover, **Heal Italia** intend to disseminate knowledge and innovative technologies from universities into the market by fostering the creation of start-ups and Research Spin-Offs (RSOs): the Research Program foresees the economic exploitation of knowledge with the aim of promoting economic growth by transforming the knowledge produced by research into knowledge that can be used for production purposes.

In this regard, **Heal Italia** intends to ensure:

- definition, publication and awarding of cascade calls aimed not only at recruiting staff but also at selecting *start-ups* /RSOs to involve in the activities.
- promotion of self-entrepreneurship tools that will foster the creation of start-ups and spin-offs by the young researchers involved in the Research activities.

To achieve these objectives, the Research Program intend to develop an *Incubation & Acceleration Programme* (IAP) to enhance the research results of universities and for transferring them to the industrial world, through the creation of a new entrepreneurial activity, of an innovative nature, based on a research background developed in the academic field. IAP will work in the business idea generation stage, as part of the activities of the following spokes:

- **Spoke 2** concerning **Intelligent Health –** TRL 1-6
- **Spoke 4** concerning **4D Precision Diagnostics**. – TRL 1-6
- **Spoke 5** concerning **Next-Gen Therapeutics**. TRL 1-7
- **Spoke 6** concerning **Health Toolbox.** TRL 1-6

IAP will be organized and promoted by all project partners and open to potential investors and foresees the involvement of students, PhD students, researchers in feasibility plan programmes aimed at enhancing the innovative and deep-tech ideas through the transfer of the most important results of **Heal Italia** to the market.
The expected impact can be identified at the end of the Research Program considering the following dimensions: *quality and scalability of ideas, business plans developed and finalized; SMEs/Corporates involved in the programmes, public and private funds attracted*.

Moreover, the IAP is focused on the improvement and the sharing of methodologies, tools and services to exploit the potential of the research-based RSOs as key actors for the territory's economic competitiveness and innovation, with a clear scale-up potential and ready access to international markets. This goal is devoted to fill the gap identified by the NRRP related to the low number of research- based enterprises able to grow and become innovation providers, leaders on a global scale.
Some key elements of the research program are illustrated below in order to bring out more clearly the area in which the results of the research will be exploited.
The activities planned for the construction of the Hub foresee the networking of the services and structures made available by the beneficiary entities. Specifically, the following will be put online:

a. The IT structures for the management and analysis of big data. This platform will support all the activities of the spokes and will allow the management and storage of omics, imaging, clinical and laboratory data.

b. Biobanks and collections of biological samples. This will make it possible to have biological material collected, stored and distributed with standardized criteria in order to guarantee the quality of health services provided and accelerate translational research.

c. Pre-clinical trial services. This will also allow to accelerate the proof-of-concept process and subsequent pre-clinical validation of new drugs and innovative therapies.

d. The facilities to produce advanced therapies (Cell Factories / GMP Facilities). The presence of Cell Factories will allow the possibility of producing according to criteria of Good Manufacturing Practice Medicinal Products of Advanced Therapy, one of the new frontiers of medicine especially in the southern regions where there is a shortage.

e. Services for clinical trials. The presence in the partnership of IRCCS and hospitals and their services

dedicated to clinical trials (Clinical trial Center etc.) will allow to cover all phases of clinical research.

f. The infrastructures made available by the proposing subjects will make it possible to cover the entire path of research, validation, and subsequent production of innovative therapies.

In this way, the HEAL ITALIA program aims at creating a diffuse network of competences, facilities, and services focused on advancement in precision medicine approaches. The achievement of this goal is intrinsically connected to strong collaborations, stable networking activities, reliable data treatment and sharing. The collaborative IT infrastructure for predictive computer models will be developed to maximize the use of open-source software in full compliance with Italian privacy legislation. Indeed, the ability to examine the code will facilitate public trust in the software and more rapid innovation as it encourages the sharing of resources and information that, in turn, supports more technical development.

The Hub can count on the presence of:

- » Laboratories equipped with standard and latest generation equipment: *Biomaterial Preparation and Analysis Laboratories; Laboratories of Mechanics of materials and biomaterials, Laboratories of Standard and Advanced Microscopy and Spectroscopy, Laboratories of Bioimaging; Atomic Molecular Structure Characterization Laboratories; Surfaces, Thin Films and Devices Laboratories; Mass Spectrometry Laboratories; Cell biology laboratories; Biochemistry laboratories; Proteomics and Genomics Laboratories; Molecular Biology Laboratories, Immunology Laboratories; Microbiology laboratories*.
- » Nurseries for pre-clinical experimentation for generation and characterization of advanced in vivo pathology models and in vivo validation of new therapies
- » Biobanks for the standardized collection and storage of biological samples
- » Pharmaceutical workshops for the production of Advanced Therapy Medicinal Products
- » Radio-pharmacy services for the production of radiopharmaceuticals

To support the infrastructures for research and production, the Hub has services for the management of pre-clinical and clinical trials. The range of services offered by the Hub and for the Hub are completed by services for the training of technical, scientific and managerial personnel and services for the promotion of businesses and start-ups.

The set of all the tools and services of the Hub allows to cover all the phases of research and development of new products / therapies / technologies and will allow to support and accelerate the production process through:

- » *Development of animal models that constitute the proof of concept of the therapeutic efficacy of the products developed.*
- » *Development of production procedures in compliance with GMP standards and definition of the criteria for the identification, safety and efficacy of products.*
- » *Development of phase 1 or phase 1/2 clinical trials for therapeutic products and medical devices.*
- » *Development of production scale-up procedures to be proposed to biotechnological or pharmaceutical companies for industrial production purposes.*
- » *Development of decision-making algorithms for personalized medicine.*
- » *Development of PDTA (therapeutic diagnostic pathways) for oncological pathologies and rare pathologies*
- » *Development of randomized multicentre trials for the validation of experimental results*.

Enhancement of human capital and raising of technical and scientific skills, attraction of highly qualified human capital, contrast to the migration phenomena of qualified personnel.

As part of the outlined purposes, **Heal Italia** intends to enhance human capital, pursuing the following objectives:

- » recruitment of young people and attraction of highly qualified human capital (see Section B - Young People);
- » effective incentive and ability to attract scientific excellence and development of professional growth opportunities for young researchers, also through the promotion of self-entrepreneurship tools;
- » improvement and enhancement of the quality of research training (Innovative PhDs programmes and professional training), focusing on the internationalization, interdisciplinary and intersectoral aspects of research in the topics covered by **Heal Italia**;
- » effective enhancement of knowledge transfer skills by researchers to support the development of innovative spin-offs and start-ups, Open Innovation ecosystems, proof of concept, etc. (as part of Spoke 2,4,5,6).

Finanziato dall'Unione europea NextGenerationEU

Ministero dell'Università e della Ricerca

Call for tender for the presentation of intervention proposals for the Creation of Enlarged Partnerships extended to Universities, Research Centres, Enterprises and funding basic research projects to be funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 funded from the European Union - NextGenerationEU.

Annex B – Spokes Description

(Article 10, paragraph 3 and Article 12 of the Call)

(This attachment must be completed and digitally signed by the legal representative of the proposing entity)

HEAL ITALIA

H*ealth* ***E****xtended* ***AL****liance*

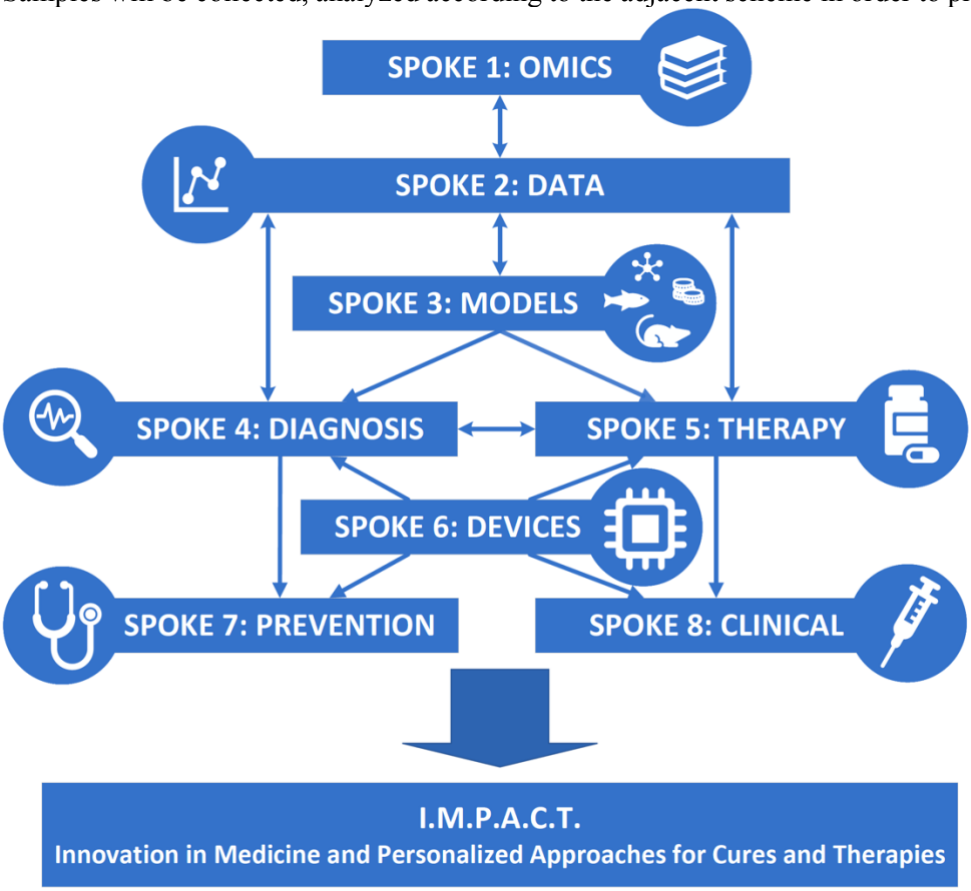
for

I*nnovative* ***T****herapies,* ***A****dvanced* ***L****ab-research, and* ***I****ntegrated* ***A****pproaches*

of Precision Medicine

The overall objective of the project is to deliver new, cost-effective, and evidence-based predictive and non-invasive diagnostic pathways for faster, earlier, more precise, accessible, and affordable prediction, detection, and monitoring of monogenic (rare diseases), polygenic (cardiovascular and metabolic) disorders, and cancer, as well as to identify innovative and effective therapeutic approaches. The project will allow to apply precision medicine approaches by developing *risk-based stratification algorithms*, and to provide scientific open-access evidence to health policy makers. The completion of this proposal intends to *overcome the concept of "one gene, one disease, one drug"*.

The accomplishment will take advantage of two existing population cohorts started since 2005, expanded by a third cohort, to follow the progression of normal healthy people towards distinct diseases. Here, it will be applied a multi-omics screening strategy and analysis to identify factors relevant for disease progression. Samples will be collected, analyzed according to the adjacent scheme in order to produce compelling data that will allow the development of modern competitive Precision Medicine. The specific arms of the project are articulated in distinct spokes highly inter-related between each other both at technical and translational level. In brief:



1. **Holistic Nosology**. *From patients to molecules and back:* mapping the omic landscape of clinical to molecular environment, to identify, classify, and refine the phenotypes of multifactorial diseases;
2. **Intelligent Health.** *Health Data Science:* Data management and development of advanced methods, algorithms, and machine learning approaches integrating health big data;
3. **Prediction models**: Advanced prediction models for prognosis and therapeutic response based on comprehensive data treatment;
4. **4D Precision Diagnostics.** Precision medicine integrating clinical and imaging biomarkers for a "*precise in space and time*" diagnosis;
5. **Next-Gen Therapeutics.** *From silico to bedside:* design and validation of innovative tailored and personalized therapeutic strategies;
6. **Healthy Toolbox:** Development of innovative devices for precision diagnosis and personalized therapy;
7. **Prevention Strategies:** Integrated and gender medicine approaches for prevention strategies based on environmental, lifestyle and clinical biometric data;
8. **Clinical Exploitation:** Clinical validation and implementation of innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches, based on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols.

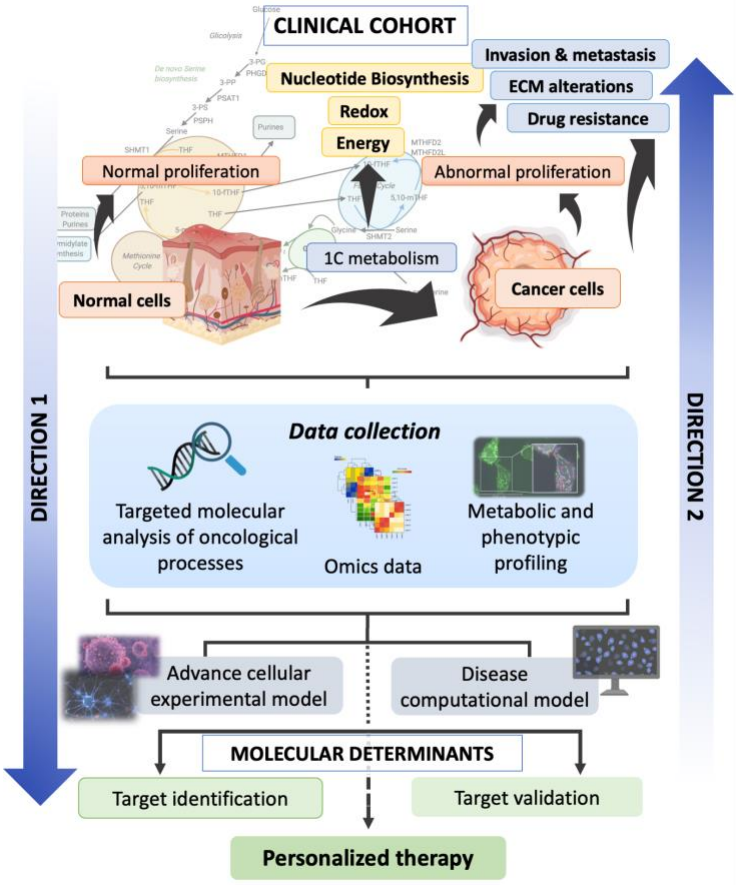
SPOKE 1: Holistic Nosology

FROM PATIENTS TO MOLECULES & BACK: Mapping the omics landscape of clinical to molecular environment, to identify, classify, and refine the phenotypes of multifactorial diseases.

Description of the Spoke Activities

SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
TorVergata	ISS/Neuromed/Sapienza/UniBO/UniCA UniFG/TLS/TorVergata/UniMORE UnivPM/UniVR	1	36	36

1. Context description:



To identify, in a **normal healthy large population**, FACTORS controlling/protecting development or progression towards distinct diseases, to be used as preventive/prognostic biomarkers and potential drug target for personalized medicine intervention. The normal population is based on 3 distinct cohorts: Moli-sani (>24.000 subjects since 2005), RoCAV (4,000 since 2010), Genoma Sardo. OMICS and in vitro/vivo investigations will be essential, and connected to all other spokes and external biotech. This spoke has huge synergy with spokes 2,3,7,8 as well as other national (NRP) programs (ageing, infectious diseases, Ministry Health).

2. General objective:

This spoke aims to investigate a **<u>normal population</u>** slowly progressing towards disease as well as disease models in order to define molecular markers predictors of adverse or protective events. The identified genes/molecules will be studied in vitro and in vivo (DIRECTION 1: from patients to molecular determinants). Conversely, it will be deepen the current knowledge on metabolic and biochemical pathways, moving in the opposite direction (DIRECTION 2: from molecular determinants to patients).

At the basis of this experimental design is a biobank available at the IRCCS Neuromed named the Moli-Sani "common-soil" Program. It consists of >24.000 volunteered individuals from the Molise Region (and the RoCAV study with nearly 4.000 subjects). It is a longitudinal cohort which

includes: *i)* samples (blood, buffy coat, serum, urine), collected every 5 years during the last 15 years; *ii)* individual medical records; *iii)* professional exposures; *iv)* pollution records at single areas; *v)* diseases: all cases/multipathologies or single case: cancer, cardiovascular diseases, degenerative neurological diseases, i.e. cognitive-Alzheimer/vascular, Parkinson. The Moli-Sani biobank, which is still recruiting, is useful for multi-omics and multidimensional characterization. It is functional to integration of epidemiology, molecular-cellular medicine and technologies applied to "precision diagnosis", "precision therapeutics-discovery of drugs-better appropriate use of existing drugs", "precision technologies-imaging" and whole "precision medicine".

The opposite direction originates from genetically modified mouse models, some not yet published, affecting metabolic pathways. These genes will be investigated in the clinical cohort to evaluate their role in a human context. The definition of the underlying molecular mechanisms will offer potential novel therapeutic targets and clusters of prognostic predictors.

The spoke has its own **Scientific Advisory Board** (Boris ZHIVOTOVSKY (Karolinska Institut, Sweden), George CALIN (MD Anderson, TX, USA), Michele CARBONE (Cancer Centre, HI, USA), Xin LU (Ludwig, Oxford, UK), **PhD Course** for scientific training and **Editorial Office** for training on publishing, Rome (www.nature.com/cdd) (impact factor 15.8). **Biotech** involvement is also highly consolidated.

3. Project WPs structure

WP 1: Population mapping: DNA seq, Exome Mapping aiming at identification of pathogenic genetic variants **(Leader: Neuromed)**

Task 1.1: Precision Medicine: the common soil hypothesis and the Moli-sani/RoCAV studies

Executors	Starting month	Ending month	Duration (months)
Neuromed/UniCA/UniFG/UnivPM/UniVR	1	24	24

The border between health and disease is set by a complex equilibrium between genetics, metabolism and environmental exposure. Evidence accumulated on the hypothesis of a "common soil" for chronic degenerative diseases. The **Moli-sani study** (www.moli-sani.org) is an epidemiological study aimed at investigating the **common soil hypothesis**. The Moli-sani Study is a cohort study aimed at evaluating the risk/protective factors (environmental, genetics, biomolecular) linked to chronic-degenerative disease, especially cardiovascular disease, cancer, neurodegenerative disease and their intermediate phenotypes such as hypertension, diabetes, dyslipidemia, obesity and metabolic syndrome. Indeed, its unique design allows to simultaneously investigate risk factors and molecular pathways related to several chronic degenerative diseases with both a longitudinal or a nested case-cohort design. The study recruited 24,325 participants (70% participation rate), aged ≥ 35 from the general population by a multistage sampling, from 2005-2010. Biometric and clinical data (electrocardiogram and spirometric test, anthropometric and blood pressure measures), samples (plasma, serum, cell pellet, DNA; biobank-stored), exposome (geolocalization by living-addresses and link with the regional air pollution map), life styles (dietary habits, physical activity, smoke habits, socioeconomic status) psycho-emotional variables have been collected. A follow-up registry based on hospital records, pharmacological records and mortality regional registry was performed on December 2018 and has been updated every two years. Genome wide epigenomic and polymorphism analysis have been performed in random subsamples of the Moli-sani population (n=4000). Radiomic features from neuroimaging are available for subsamples of the Moli-sani population (n=3600).

Table 1. Summary of data points collected in the Moli-sani study

Questionnaires	Quality of life (SF36); depressive status, stress response (resiliency) and attitude to suicide
	Lifestyle: One year-FFQ (EPIC); physical activity, smoking habits
	Demographics: education, work, income, present social status and at childhood.
	Prevalent diseases and pharmacological therapy
Serum/plasma screening	Fasting: Lipid biomarkers (Apo A, Apo B, HDL, LDL, TG, Lipoprotein(a), glucose, C-peptide, Insulin), AST, ALT, N-pro B-NTP, hsTroponin I, Testosterone, Vitamin D, D-Dimers, thrombin generation, fibrinogen, anti-thrombin III, uric acid, albumin, creatinine, cystatin C, microalbuminuria, eGFR, hs-CRP, INFLAscore, RBC, Ht, Hb, MCV, MCH, MCHC, RDW, WBC, mon, gran, lymph, eos, platelet count, MPW
Genotyping	Candidate genes SNP, GWS and EpigGWS on going
Clinical test	BMI, waist and hip circumferences, BP, ECG, spirometric test (pulmonary diffusion capacity, gas diffusion and pulmonary volumes through plethysmography).
Imaging	3600 brain RMN and 700 brain PET
Follow-up (10 yrs)	Deaths (with specific causes of death), fatal or Non-fatal AMI, cardiac revascularization, fatal and non-fatal stroke events, heart failure, atrial fibrillation, DVT, PE, diabetes and cancer, neurodegenerative disease, pharmacological therapy. First hospitalizations for all and specific causes, including cardiovascular disease, stroke, ischemic heart disease, type 2 diabetes and cancer.
Cohort Re-call (on-going)	Cognitive function (MOCA test), sleep (quality and frequency), frailty, bone densitometry, examination of the sense organ function (taste, smell, hearing), behavioral questionnaires
Biobank (liquid nitrogen)	Serum, citrate plasma, EDTA plasma, buffy coats, DNA 3hr morning spot urines (-80°C)

The **RoCAV Study** (http://epimed.uninsubria.eu/) is a cohort study aiming at identifying new risk biomarkers and algorithms for abdominal aorta aneurism and cardiovascular disease risk stratification and determinants associated with the occurrence of diseases with high impact in public health. **3777 subjects** were recruited between 2013-2016. Biometric, clinical and biobanking samples are available for omics assessment.

Table 2. Summary of data points collected in the RoCAV study

Questionnaires	Lifestyle: One year-FFQ (EPIC); physical activity, smoking habits, sleep habits
	Family history
	Occupational history

	Demographics: education, work, income, present social status and at childhood.
	Prevalent diseases and pharmacological therapy
Serum/plasma screening	Fasting: Lipid biomarkers (HDL, LDL, TG), glucose-homeostasis biomarkers (glucose, Insulin), AST, ALT, creatinine, RBC, Ht, Hb, MCV, MCH, MCHC, RDW, WBC, mon, gran, lymph, eos, platelet count, MPV, FGF-19, bile acids, and, only at T1 (5 years), hsTroponin T and hs-CRP
Clinical test	BMI, waist and hip circumferences, plicometry (8 sites), BP, ECG, spirometry (expiratory flow), abdominal aortic diameters (4 sites, antero-posterior and latero-lateral diameters), ABI and PWV.
Follow-up (10 yrs)	Deaths (with specific causes of death), hospitalizations, pharmacological therapy.
Biobank (liquid nitrogen)	Serum, citrate plasma, EDTA plasma, buffy coats, red blood cells

The **Genoma Sardo Study** (in Sardinia over last 10 years) will expand the robust research resource comprehending information provided by participants and data collected by health professionals, including environmental, physiological and health data and biological samples. *Sub-Tasks 1.1.1* Establishment of the centralized and harmonized Project Database. *Sub-Tasks 1.1.2* Follow-up update of the involved cohort. *Sub-Tasks 1.1.3* Identification of specific case-studies and set-up of study designs. *Sub-Tasks 1.1.4* DNA, plasma, urine samples retrieval for "omics" analyses.

Milestone: DNA seq of all subjects, exposome database availability, aliquots of biological samples for epigenomics and metabolomics and analysis.

Deliverable: follow-up update for mortality and chronic degenerative disease.

Task 1.2: Genomics, Phenomics and Biomarkers

Executors	Starting month	Ending month	Duration (months)
TorVergata/Sapienza	12	36	24

This task aims at identifying phenotypically coherent groups of patients within the cohorts for in vitro experimentation through extensive genomic and functional analysis, including single cell multi-omics profiling of induced pluripotent stem cells (iPSC)-derived organoids. The Task intends to better understand how genetics, environment, social factors and healthcare interact to identify the genetic determinants capable of modulating the phenotype. This task will also characterize genomic and non-genomic biomarkers useful for predictive diagnostics, prognosis, pathophysiology, and pharmacogenetics for translational benefits and drug development. Data involves analysis and re-analysis of GWAS / EWAS data on common pathologies, with meta-analyzes; Polygenic Risk Score (PRS) produced by large international consortia (UK biobank and Nevada Health Genome) for various complex diseases (e.g. breast and other solid cancer, atrial fibrillation, coronary heart disease, type 2 diabetes, kidney diseases, hypercholesterolemia and other susceptibility endophenotypes) in order to validate the Italian data obtained and their translation of the international PRS. *Sub-Tasks*

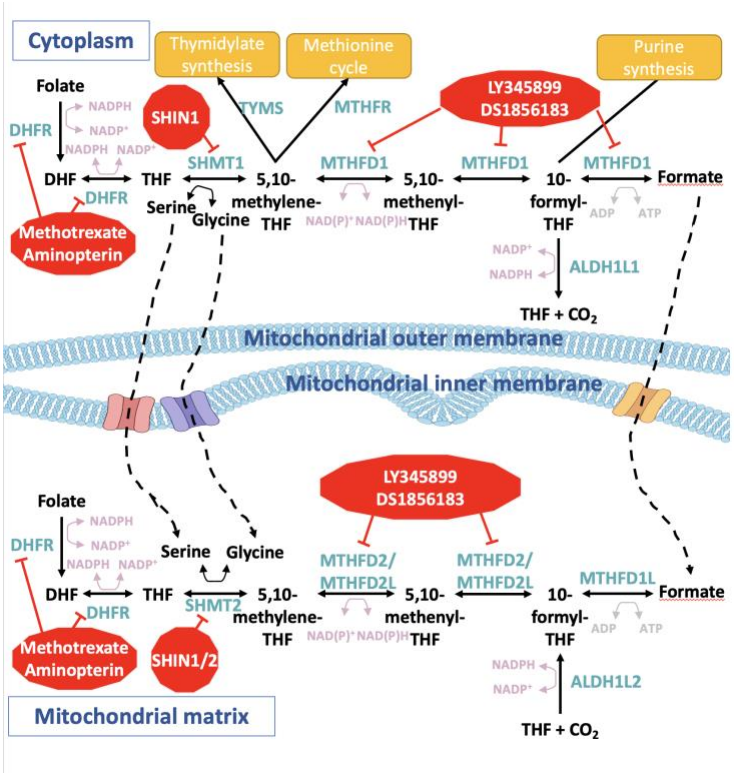
1.2.1. Re-contact participants on a disease risk-stratified basis (i.e. recall-by incident neurodegenerative disease, cancer or cardiovascular disease) over time specifically to enable secondary studies. *Sub-Tasks* 1.2.2. Isolate pluripotent stem cells from biological samples to be used for organoids formation.

Milestone: sub-cohorts of disease risk stratified subjects.

Deliverable: biobank of pluripotent stem cells, characterization of genomic and non-genomic biomarkers useful for predictive diagnostics, prognosis, pathophysiology, and pharmacogenetics for translational benefits, and drug development

Task 1.3: Metabolome mapping: from mouse to Moli-sani sub-cohorts and development of new therapeutic targets

Executors	Starting month	Ending month	Duration (months)
TorVergata/Sapienza/UniCA	12	36	24



Metabolic profiling of selected sub-cohorts of the Moli-sani as well as mouse models of genes involved in mitochondrial metabolism regulation will provide fine mapping and genotype/metabolite correlations. This will be expanded on organoids for both sources to be followed from a more refined mapping and correlations. Organoids would provide also fine testing of selective traditional or innovative pharmacological testing. Analyzing the expression profiles of metabolic regulatory genes will provide information on (i) the metabolic programs of malignant and non-malignant cells; (ii) the different contributors of genetic and metabolic heterogeneity for different cell populations; (iii) the metabolic characteristics of infiltrating and circulating cell subpopulations; (iv) novel use of old drugs. This task will contribute to the broader characterization performed on the metastatic disease by using state-of-the-art technologies, which allow studying individual cells of the tumor, its microenvironment, and the circulating peripheral blood mononuclear cells. Together with the transcriptional profile, bioenergetics (basal and maximal respiration, proton leak and spare respiratory capacity) and mitochondrial functions will be assessed. Data obtained from these single-

cell studies will be used for computational analyses to investigate metabolic programs of individual cells.

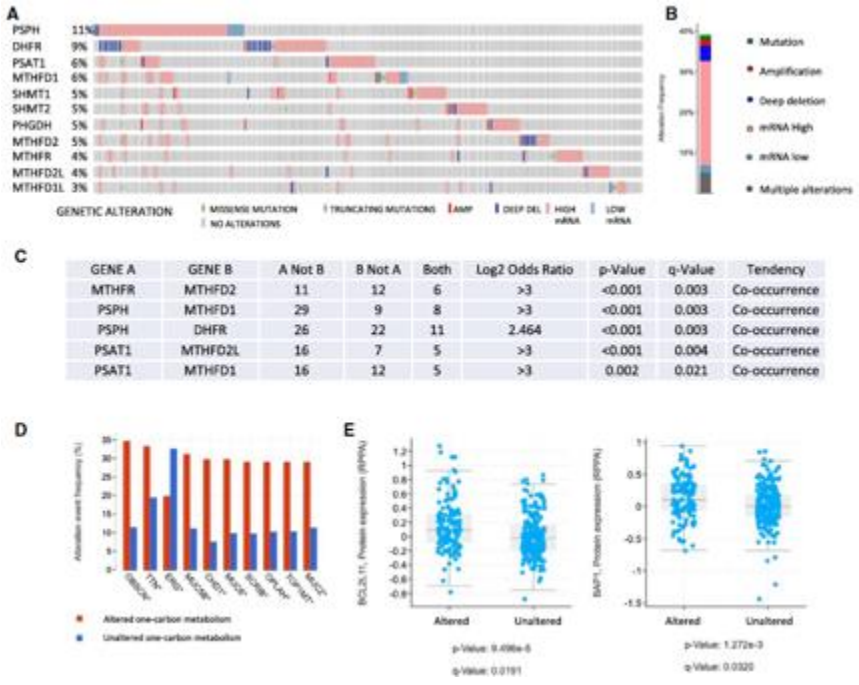


Figure One-carbon metabolism enzymes in prostate cancer overview. **A** OncoPrint profiling of genetic or expression alteration in genes involved in the one-carbon metabolism in a cohort of 498 patients from TCGA (AMP, amplification, DEL, deletion); **B** cumulative alteration frequency of the one-carbon enzymes analysed; **C** co-occurrence analysis of the alterations of transcription the one-carbon enzymes genes, p-values are derived from one-sided Fisher Exact Test, q-values are derived from Benjamini–Hochberg FDR correction procedure; **D** genomic alteration frequency of known prostate cancer driver genes in one-carbon metabolism genes altered group vs control; **E** log2 scale protein expression of BCL2L11 and BAP1 in one-carbon metabolism genes altered group vs control, p-values are calculated from Student's T-test, q-values are calculated from Benjamini–Hochberg procedure

The aim of this task would be of linking omics knowledge between population genetics, disease susceptibility/protection and molecular mechanisms. The final result would be to design innovative nanostring devices to predict therapeutic response acting on one-carbon metabolism or mitochondrial regulators. The end result would be a clinical service open at national level.

Milestone: Definition of metabolic pathways form Moli-sani, mouse models and related organoids.

Deliverable: Set up a nanostring prognostic response for one-carbon metabolism or mitochondrial regulators.

WP 2: Transcriptomics: refinement of "common-soil" hypothesis & investigation on chronic pathological conditions for personalized stratification for therapeutics **(Leader: UniBO)**

In synergy with WP1, WP2 will refine the molecular identity of "common-soil" features within the 3 population cohorts by testing subsets of individuals characterized by unique risk profiles for the development of chronic-degenerative disorders (cardiovascular disease, cancer and intermediate phenotypes, like obesity and metabolic syndrome). By using transcriptomics approaches, WP2 is also expected to address unmet clinical needs of the above indicated chronic-degenerative diseases, as described in the Tasks that follow.

Executors	Starting month	Ending month	Duration (months)
UniMORE/UniFG	6	36	30

Task 2.1: Omics biomarkers in the stratification of obesity, epithelial and related metabolic and functional complications

This task will produce transcriptomic, genomic and epigenomic profile of patients' samples selected in WP1. *Sub-Task 2.1.1.* Obesity can produce adiposity, a condition sufficient to impair human health, by inducing low-grade inflammation and oxidative stress, and, in turn, cardiovascular disease, diabetes and increased risk of cancer. Based on the production of transcriptomic as well as genomic data, the aim of this task is to apply omics approaches to recognize biomarkers towards precision therapeutic strategies in obesity. Samples (blood, fecal, adipose tissue) collected through the HealITALIA consortium and from WP1, will be investigated for GWAS, RNAseq, gut microbiome (metagenomics NGS). *Sub-Task 2.1.2.* Nonalcoholic fatty liver disease (NAFLD) complicates dysmetabolism and leads to liver fibrosis. It will be developed Omics-based score for NAFLD at risk of fibrosis and screen strategy targeting advanced fibrosis in high-risk patients by multi-omics biomarkers in liver and, by non-invasive approaches, in blood to monitor disease severity for more personalized clinical follow-up and treatments. *Sub-Task 2.1.3.* Similarly, epithelial samples and generate data from skin pathologies (psoriasis, genodermatoses and related cancers) will be collected. Data will be shared with other Spokes and investigated with artificial intelligence approaches developed at **Spoke 2**.

Milestone: recognition of key points for the development of targeted drugs for the treatment of obesity and functional adiposity and identification of a panel of biomarkers involved in NAFLD progression.

Deliverable: multi-omics data for a molecular understanding of obesity etiology and patho-mechanisms, toward personalized strategies for the prevention and treatment of obesity and establishment of a clinical/omics-based score system for PM in high-risk NAFLD; identification potential selective skin therapeutic targets.

Task 2.2: Multi-omics approach for big killers: stratification of treatment response and tailored interventions

Executors	Starting month	Ending month	Duration (months)
Neuromed/UniBO/UniMORE/UniVR /ISS	6	30	24

Sub-Task-2.2.1. Immune checkpoint inhibitors (ICPIs) have revolutionized therapeutic approaches in several types of human cancer in the last few years. However, available biomarkers are not consistently able to predict the actual individual response. Thus, by genome-wide transcriptomic and genomic analyses, isolation and characterization of circulating tumor cells (CTCs), and development of novel TME-eATP probes or functional tests of A2A-A2B axis, AIM of the Sub-Task is to generate multiple precision-medicine molecular tools useful for predictive response to ICPIs and new tools for the application of novel and more effective anti-cancer drugs. *Sub-Task-2.2.2*. Characterization of the clonal dynamics associated with evolution/resistance in lympho-myeloproliferative disorders (LMOD) has the potential to identify transformed clones and their tumor microenvironments molecular and metabolic drivers. Single cell omics technologies next to bulk analysis will identify rare events driving transformation and resistance for a better stratification of high-risk patients. *Sub-Task-2.2.3.* In heart failure (HF) patients, cardiologists make decisions based on clinical acumen

(signs and symptoms), imaging (from electrocardiogram to RMN), and conventional blood work-up. Integration of omics studies with clinical variables will be implemented to identify patients at risk of adverse outcomes. Data will be shared with other Spokes and investigated with artificial intelligence approaches developed at Spoke 2.

Milestone: design of innovative diagnostic and interventional omics strategies.

Deliverable: define novel omics predictors of therapy resistance.

Task 2.3: Serine metabolism and epigenetic regulation through ncRNAs

Executors	Starting month	Ending month	Duration (months)
TorVergata	1	30	30

Key players in epigenetic control are histone modifications and DNA methylation which establish chromatin structure and its transcriptional activity. miRNAs and long non-coding RNAs (lncRNAs) can act to control activity of such players. Metabolites such as methionine, S-Adenosylmethionine (SAM), aKG, acetyl-CoA, ketone bodies all potentially regulate the function of chromatin remodelers and the expression of miRNAs and lncRNAs. Recent data show that the tumor suppressor p53 preserves genomic integrity by empowering adequate levels of the methyl-donor, SAM. In p53 deficient cells, stress perturbing the epigenome promotes instability of silent heterochromatin. Moreover, stress/drugs (i.e. HDACi, DNMTi) induced the cryptic transcription of many non-annotated transcripts epigenetically by perturbing the epigenome repressed in normal cells. The aim of this Task is to (i) identify keys ncRNAs (miRNAs and lncRNAs) associated to metabolic stress; (ii) investigate the underlying molecular mechanisms and (iii) the expression of identified pathways/genes in selected Moli-sani cohort.

Milestone: Identification of ncRNA determinants associated with metabolic stress in normal human cells (serine/glycine, methionine deprivation and HDACi, DNMTi).

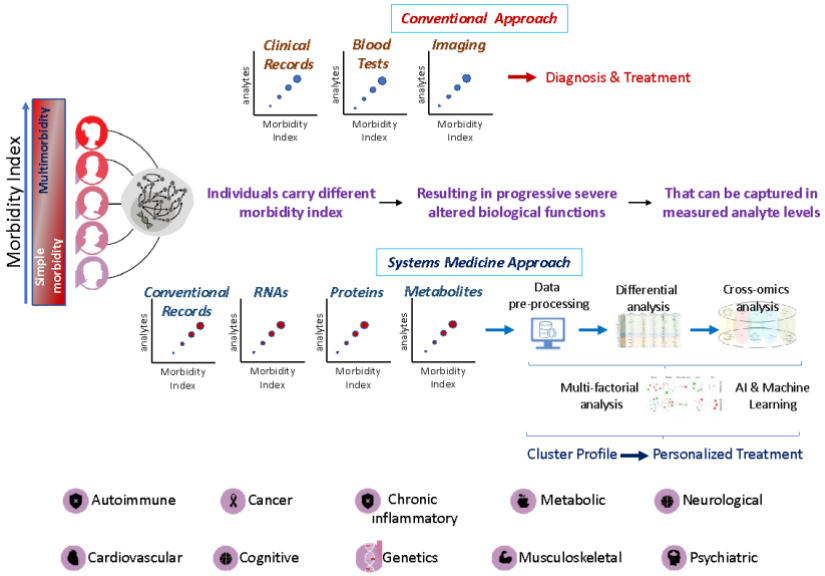
Deliverable: Assess if the identified molecular signatures are relevant for prediction/diagnosis decisions using selected sub-groups in the Moli-sani cohort.

WP 3: Proteomic and metabolic analysis: an exciting avenue to advance the knowledge of dynamic interactomes **(Leader: TorVergata)**

Task 3.1: Microbial metabolites impact on disease: from translational models to bedside

Executors	Starting month	Ending month	Duration (months)
TLS/TorVergata/UniVR	1	36	36

The simultaneous presence of multiple chronic conditions is an increasing health problem. Research into its determinants is of high priority. In this context gut microbial diversity, lipid and glucose metabolism, low-grade inflammation precedes common multimorbidity with potential for early intervention approaches. Current understanding of the contribution of gut microbiota to disease is incomplete, because of heterogeneity of microbial community, interindividual differences in disease evolution and incomplete understanding of the mechanisms that integrate microbiota-derived signals into host signaling pathways. This task will integrate analyses of multi-omics data, including metagenomics, gut and blood proteomics and metabolomics along with measurements of host response (clinical phenome) with the aim to identify microbiome derived biomarkers for inflammatory and metabolic disorders.



More detailed AIMS are:
1) validate microbial metabolites in translational models testing whether already identified metabolites (microbial derived Branched Chain Amino Acids (BCAAs), PhenylAcetic Acid (PAA) and Tryptophan derivatives and metabolites that will be identified, may have a direct eumetabolic or anti-metabolic effect using: 1) Organ-Chip models (from Emulate Bio) including brain, kidney, liver, intestinal and lung chips; 2) brain, kidney, liver, intestinal and lung organoids (from StemCell Technologies); 3) transgenic and diet-induced models of metabolic syndrome
2) test whether the relevant molecular signatures derived from the interomics approach are relevant for clinical decision making i.e. precision prediction of major outcomes or precision use of expensive treatments in high-risk subjects.

Milestone: collection of a large cohort of subjects with multimorbidity.

Deliverable: identify commonalities between inflammatory diseases such as IBD and metabolic disorders such as type 2 diabetes mellitus (T2DM) or combined immunometabolic diseases such as atherosclerosis, which will be translated to clinical practice to improve diagnostic and therapeutic opportunities.

Task 3.2: Protein degradation in physiology and pathology

Executors	Starting month	Ending month	Duration (months)

TorVergata	1	36	36

Due to its major role in many cellular pathways, the ubiquitin proteasome system (UPS) has been extensively reported to be deregulated in a variety of pathological events, including cancer. This task aims to explore how the improper functioning of the UPS is functionally connected with tumor cell growth, energy metabolism and ROS/H2S production, and how this functional connection may be targeted in the development of novel therapeutic strategies. *Sub-Task 3.2.1.* i) Set up a CRISPR/Cas9-based screening for E3/de-Ub involved in glucose addiction of tumor cells; ii) biochemical characterization of relevant E3/de-Ub and (iii) evaluation of their clinical relevance. *Sub-Task 3.3.2*. This sub-task aims to dissect the involvement of the WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) in tumor redox homeostasis. Based on the impairment of the antioxidant defences observed in WWP1 depleted cells, it will be studied the Thioredoxin-dependent peroxide reductase (Prdx3), a mitochondrial Thiol-specific *peroxidase* playing a role in cell protection against oxidative stress as a relevant protein target of WWP1.

Milestone: Generate reagents, validate in vitro models for ITCH, WWP1, E3 screening studies.

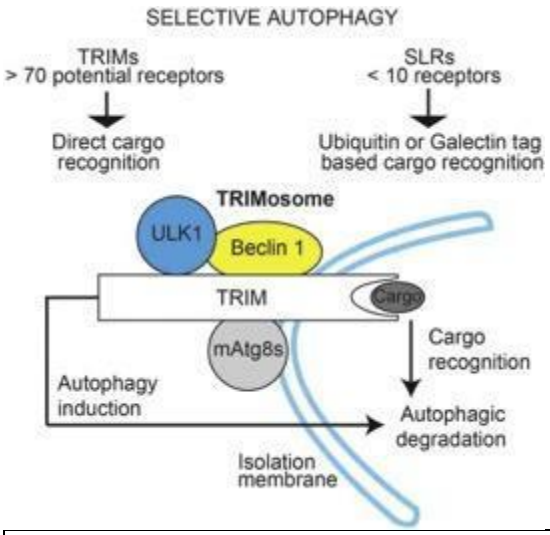
***Deliverable*:** Identification of substrate targets and their role in cell metabolism and redox homeostasis.

Task 3.3: Autophagy, cell cycle regulation and diseases

Executors	Starting month	Ending month	Duration (months)
TorVergata	1	30	30

The increasing prevalence of metabolic disorders is a threat to human health. Genetic features, environmental aspects and lifestyle changes are major risk factors determining metabolic dysfunction in the body. Autophagy is a housekeeping stress-induced lysosomal degradation pathway, which recycles macromolecules and metabolites for new protein synthesis and energy production and regulates cellular homeostasis by clearance of damaged protein or organelles. An increasing number of studies have shown that defects of the autophagic machinery is associated with dysfunction of multiple metabolic tissues including pancreatic β cells, liver, adipose tissue and muscle, and is implicated in metabolic disorders such as obesity, insulin resistance and cancer. Autophagy plays a dual role in carcinogenesis, the "paradox of autophagy": it can inhibit the development of cancer or can protect cancer cells. More and more research indicate that autophagy plays an important role in

the development of drug resistance by protecting cancer cells. The aim of this task is to investigate autophagy mechanisms in the Moli-Sani cohort for AMBRA1 and Transglutaminase manipulation. Type D cyclins are central regulators of the cell division cycle and are among the most frequently deregulated therapeutic targets in human cancer. Autophagy and in particular AMBRA1, have been identified as the main regulators of cyclin D as they mediate the ubiquitination and degradation of cyclin D as substrate receptor for the E3 ligase complex of cullin 4. Then the analysis of the underlying molecular mechanisms of regulation by Ambra1 in cancer is of fundamental importance in the definition of new therapeutic approaches for the treatment of tumors. Accumulating evidence indicates that autophagy operates as a critical quality control mechanism for the maintenance of cellular homeostasis. The aim of future studies is to clarify the role of AMBRA1 and its interplay with TRIMs in the regulation of autophagy under pathological conditions such as cancer. In particular, considering that mammalian cells harness autophagy to eliminate physiological by products of metabolism and to cope with microenvironmental perturbations. It will be investigated how the autophagy regulation by AMBRA1 links transformed and non-transformed components of the tumour microenvironment and how the autophagic network is important for cancer initiation, progression and response to therapy.



Milestone: Define the role of TG2 in hepatocarcinogenesis; Identify major interactors of AMBRA1 in the regulation of different cancer type progression and their mechanism of action; Identify novel autophagy factors involved in cell stem potential in medulloblastoma.

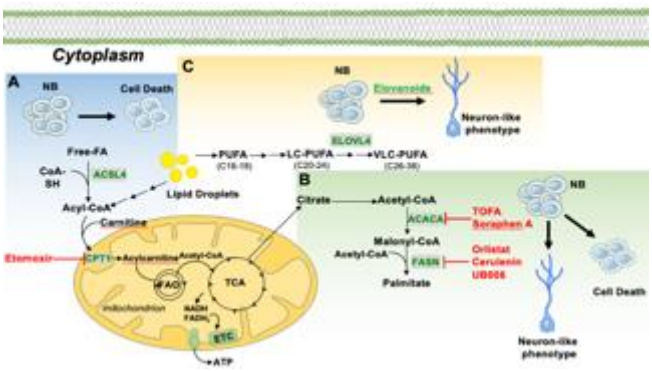
Deliverable: Validate the use of AMBRA1 as a prognostic biomarker for melanoma and medulloblastoma development; Generate a pipeline for testing the effects of autophagy-specific molecule modulation on stemness and differentiation of cancer cells; Define the role of TG2-dependent regulation of TBK1 and innate immunity in cancer development; Dissect out the role of TG2 in necroptosis in cancer.

WP 4: Metabolic alterations, metabolites and metabolome maps **(Leader: TorVergata)**

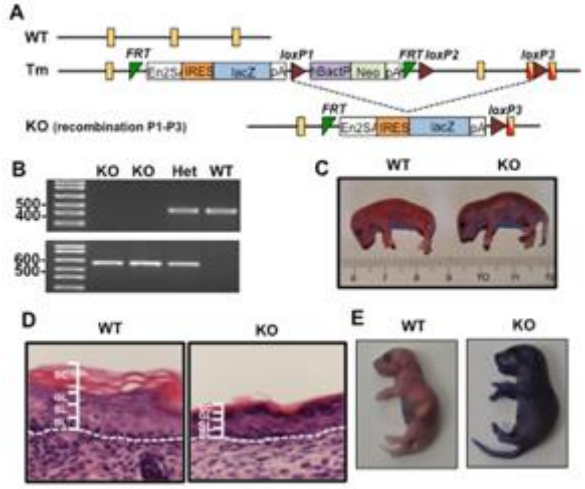
Task 4.1: Long Chain fatty acids enzymes and lipid metabolism

Executors	Starting month	Ending month	Duration (months)
TorVergata	1	28	28

A common feature of tumors is the reactivation of fatty acid synthesis (FAS) to support growth, as cells need lipids both as membrane components and as signaling molecules involved in cell survival, cell death and metastasis. FAS, which take place in cytoplasm is mainly regulated by two enzymes, acetyl-CoA carboxylase (ACACA) and fatty acid synthase (FASN). Small molecule inhibitor of these two enzymes including TOFA and Soraphen A, target ACACA, and Cerulenin, Orlistat, and UB006,

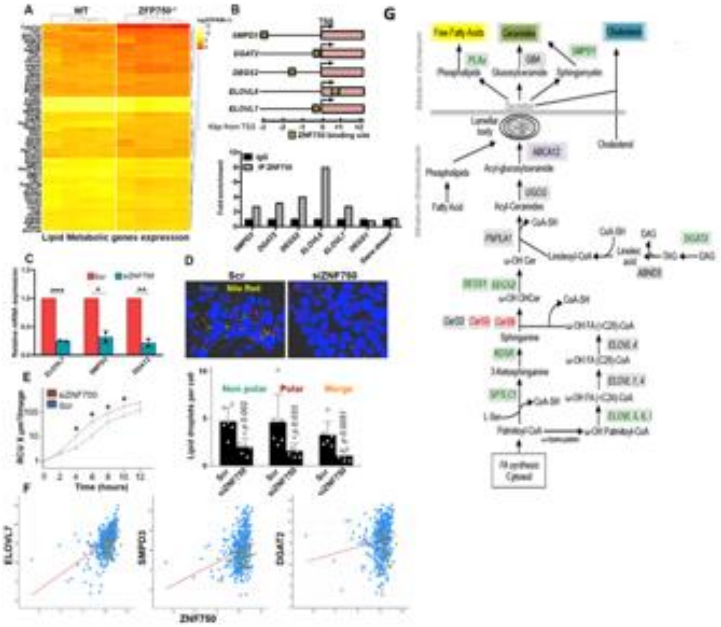


target FASN are available and largely used in preclinical experiments where have shown promising antitumor activity. The AIM of this Task is to characterize the impact of the eicosapentaenoic acid (EPA)-derived lipid mediators, called elovanoids, in the regulation of cell fate. Regulation of elovanoids synthesis by the ZNF750 dependent regulation of elongase ELOVL4 as well as generation of selective inhibitors will be studied in physiological and pathological context.



Preliminary unpublished data 1: **ZNF750**. In vivo function of ZNF750. Schematic representation of the strategy employed for generating the KO mouse. **B)** PCR genotyping of Wild-Type (WT), Het and Knock-out (KO) mice. **C)** Gross appearance of newborn WT and KO. KO mice show shiny skin and lack of eyelid. **D)** Histology of dorsal skin sections from newborn WT and KO mice stained with hematoxylin and eosin. KO mice show reduced thickeness and tightly packed stratum corneum (SC). **E)** Skin permeability assay. Mice of the indicated genotype were stained with Toluidine blue. BL, basal layer; SL, spinous layer; GL, granular layer; SC, stratum corneum.

Preliminary unpublished data 2: **ZNF750**. Genetic deletion of ZFP750 deregulates genes involved in lipid metabolism **A)** Heat map of the 55 genes involved in lipid metabolism deregulated in the epidermis of ZFP750$^{-/-}$ mice. **B)** ChIP-qPCR analysis showing the binding of endogenous ZNF750 to the promoter region of the indicated genes. The ZNF750 binding site position is shown. Gene desert was used as a negative control region. **C)** Inhibition of human ZNF750 expression by siRNA in Fadu cancer cell line deregulates the expression of human SMPD3, DGAT2 and ELOVL7. **D and E)** ZNF750 knockdown in Fadu resulted in the reduction of intracellular lipid droplets content and deregulation of ceramide metabolism. **F)** ZNF750 positive correlates with ELOVL7, SMPD3 and DGAT2. **G)** Metabolic scheme of the lipid affected pathways.



***Milestone*:** Generate ELOVL4 experimental models; generate regulators (siRNA & chemical synthesis); Generate ZNF750 full knockout in pure genetic background and characterize it.
***Deliverable*:** Test ELOVL4 and ZNF750 regulators in epithelial and cancer context.

Task 4.2: Ca2+ machinery as reporter of metabolic adaptations in physiology and disease

Executors	Starting month	Ending month	Duration (months)
TBD/UniVR	6	30	24

Ca2+ signaling represents one of the main mechanisms to fine-tune mitochondrial activity in response to energetic cellular demand. Indeed, Ca2+ signaling is involved in several of the mitochondrial activities, including ATP generation, NADH formation, ROS production, and the regulation of the activity of key metabolic enzymes. The physical interaction between mitochondria and the endoplasmic reticulum (ER), provided by specialized membrane domains at the ER-mitochondria, the mitochondrial-associated membranes (MAMs), profoundly impacts the correct mitochondrial Ca2+ signal. Alterations at MAMs levels can deregulate phospholipid metabolism, mitochondrial morphology, dynamics and activities leading to pathological conditions. The aim of this Task is to gain mechanistic insights into the possibility that mitochondrial energy metabolism, essential for cell life, could be modulated by alterations of ER-mitochondria contacts during pathological conditions. Importantly, instrumental for the project will be the possibility to visualize and quantify organelle contact sites in living cells and translate this concept in preclinical murine models. These technologies collaborating with other Tasks of all the other spokes in selected subsets of the Moli-Sani cohort and in disease models will be applied.

Milestone: Role of ER-mitochondria Ca2+ transfer in cell cycle regulation, cell proliferation, metabolism, and cell death.

Deliverable: ER-mitochondria Ca2+ homeostasis changes are selective disease.

Task 4.3: Gene versus environment, causing metabolic dysregulation leading to disease

Executors	Starting month	Ending month	Duration (months)
TBD	6	36	30

The dysregulation of the molecular mechanisms underlying metabolic processes may cause cancer, as a consequence of cell-intrinsic/-extrinsic events. Cellular DNA can be damaged by spontaneous hydrolysis, reactive oxygen species or aberrant cellular metabolism. In fact, several environmental factors may damage the DNA, alter cellular metabolism or affect the ability of cells to interact with their microenvironment. Carriers of heterozygous germline mutations of the deubiquitylase BAP1 develop cancer (uveal melanoma, cholangiocarcinoma, mesothelioma, chronic myelomonocytic leukemia, pancreatic ductal adenocarcinoma). BAP1 mutant regulates type 3 inositol-1,4,5-trisphosphate receptor and modulates ER-to-mitochondria calcium-release and led to the same reduced mitochondrial respiration and increased aerobic glycolysis Warburg effect that is detected in malignancies. AIM is (i) to understand the gene versus environment interaction at the level of metabolic dysregulation leading to cancer development and relate that to specific epithelial cancers, (ii) their regulation with transcription and ncRNA.

Milestone: Models of mitochondria, Ca2+ metabolism and malignant transformation.
Deliverable: Understanding metabolic dysregulation in environmental cancer development.

SPOKE 2: Intelligent Health

HEALTH DATA SCIENCE: Data management and development of advanced methods, algorithms, and machine learning approaches integrating health big data

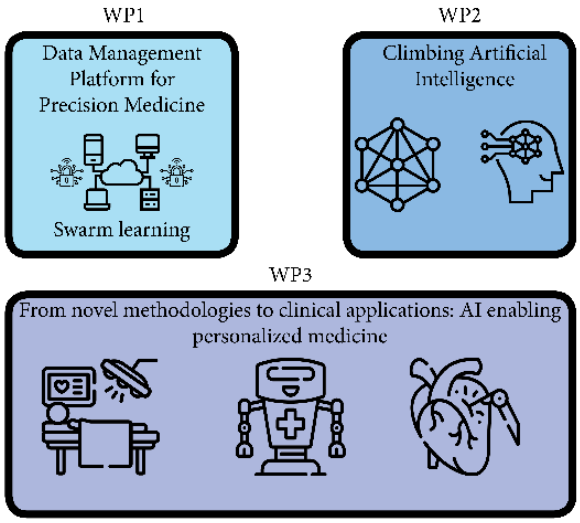
Description of the Spoke Activities

SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
UniBO	Birex/UniBO/Engineering/IFO/TorVergata/ UniCA/UniMORE/Neuromed/ISS/Sapienza/ UniCT/UniPI/UniVR/UniMiB/UPMC	1	36	36

1. Context description:

Intelligent health is based on the use of large-scale data for promoting precision medicine in diagnostics, intervention, person-centered support, and improving healthcare services through joint efforts of public and private sectors. Integrating EHR data with imaging and high-throughput data is essential for decomposing heterogeneity across diseases, improving aetiological understanding, and identifying dimensional biomarkers. This goal directly invokes artificial intelligence (AI) techniques which are gradually changing the landscape of biomedical research and healthcare. AI solutions rely intrinsically on large training datasets and need reliable, fast, secure, confidentiality- and privacy-preserving algorithms to be developed. In particular, they must address ethics and data protection appropriately, ensuring data safety and privacy, according to existing European and international standards and legislation (EU GDPR, General Data Protection Regulation (EU Regulation 679/2016 GDPR), balancing data access and privacy to advance healthcare by applying a dedicated and secure technical procedure. These solutions will improve public health by equipping healthcare providers with appropriate infrastructures to systematically store and analyze big health data. This efficient management and analysis can change the game by opening new avenues for modern healthcare and revolutionize personalized medicine.

General objective:



The general objective of spoke 2 is to establish a model of a collaborative data and analysis platform among the partners of the HealITALIA consortium for Precision Medicine in full compliance with the Italian privacy legislation for enabling the processing of predictive computer models on large volumes of digital heterogeneous data, coherently with the Health Big Data (HBD) project, funded by the Italian Ministry of Health, which aims to enable the exchange of digital clinical data between all 52 Italian IRCCS. This data and analysis platform will also enable swarm learning – a decentralized machine learning approach to further accelerate the introduction of Precision Medicine in the clinic. Novel and

innovative computational models, and multi-purpose AI frameworks – compatible with the data and analysis platform and integrating multilevel data (biological, imaging, and clinical data) for Precision Medicine will be developed to predict disease diagnosis, personalized intervention, and precision treatments. Finally, novel AI methodologies will be developed and applied in real clinical settings enabling ready-to-use personalized medicine systems. The collaborative data and analysis platform and innovative AI frameworks will be shared with Spokes 3, and 4. The spoke 2 has its own Scientific Advisory Board (Riccardo Poli, Essex UK and Pietro Liò, Cambridge, UK). Birex involvement is also highly consolidated.

2. Project WPs structure

WP 1: Integrate confidential clinical data with omic and imaging landscape map **(Leader: UniBO)**

WP1 will address the challenge of deploying a collaborative, clinical data and analysis platform for Precision Medicine, enabling feature extraction, harmonization, and analysis of heterogeneous health big data in full compliance with the Italian privacy legislation.

Task 1.1: Development and deployment of a collaborative platform to integrate molecular, imaging, and clinical characteristics of individual patients

Executors	Starting month	Ending month	Duration (months)
Birex/Engineering/IFO/UniBO/UniCA	1	30	30

This task aims to develop and deploy a collaborative IT infrastructure for predictive computer models processing large volumes of digital clinical data in full compliance with the Italian privacy legislation and to acquire and deploy a collaborative IT infrastructure enabling: *i)* the processing of large volumes of digital clinical data in full compliance with the Italian privacy legislation; ii) the use and development of computational and data-driven models for clinical research applications (as provided by spoke 4) from risk prediction models to Precision Medicine. The infrastructure will be developed using as reference two ongoing implementations: the AlmaHealthDB project, which is developing an infrastructure that links UNIBO to three National Research Hospitals (Istituti di Ricovero e Cura a Carattere Scientifico, IRCCS) located in the city of Bologna; and the Health Big Data (HBD) project, funded by the Italian Ministry of Health, which aims to enable the exchange of digital clinical data between all 52 Italian IRCCS. AlmaHealthDB is part of HBD as a local node provider for the three Bologna-based IRCCS. These are long-term efforts: AlmaHealthDB will run until 2025, while HBD will be completed in 2029. This project operates in a much shorter time frame, so it will require its own infrastructure, more limited in scope but aligned with the general specifications of these two projects. By M06 all specifications for an exemplary pilot will be available; these include the type of clinical data to be transferred, the legal treatment consent framework within which the data transfer takes place, and the location of the sending hospital and the receiving data processing facility. Ideally, the pilot should require molecular, imaging, and clinical data, or at least two of the three data types. The IT platform that will host the stack dedicated to data anonymization, its management, storage, and accessibility by authorized users will be subjected to a preventive cybersecurity analysis. The most appropriate rules for data anonymization and obfuscation will be selected, depending on the type of data and the management phase within the data lifecycle, from a wide list of possibilities: Attribute Suppression, Record Suppression, Character Masking, Pseudo-anonymisation, Generalisation, Swapping, Data Perturbation, Synthetic Data, Data Aggregation, Data Hashing, Salting and K-Anonymity Index and Risk-Measure, together with L-Diversity and T-Closeness. Governing bodies and DPOs of institutions involved will define and approve the Standard Operating

Procedures, and the Data Management Plan, and a board will oversee the management of the infrastructure and SOP application. Input data sources undergo an assessment process to establish their origin, permitted uses, presence of valid consents and authorizations, and conditions for processing. Outcomes of this assessment process are coded into a set of standardized metadata. AlmaHealthDB has a FAIR-by design approach spanning the entire data and software lifecycle. Input data sources are curated, standardized, and harmonized using internationally recognized standards such as the Observational Medical Outcomes Partnership (OMOP) Common Data Model and vocabularies such as ICD and LOINC. A general and project-specific data quality plan verifies the completeness of subject records, the accuracy of manual entries, and the adherence to the protocols and standard operating procedures (SOP); it also minimizes the risk of sensitive data being retained within the infrastructure. In addition to the rule-based mechanisms, more advanced data quality mechanisms, e.g., AI-based, can be developed and employed by the partners (as provided by spoke 4). By M12 the complete technical specifications should be ready, which will allow the submission to the hospital ethical committee, which needs to authorize the research treatment of these sensitive data. A detailed report on the clinical data Infrastructure and its use for the exemplary pilots (from spoke 4) connecting a limited number of partners will be produced by M18 (MS1.1). When all services, mechanisms (e.g., anonymous data access), and protocols will be implemented, the IT platform will be accessible among the partners of the HealITALIA consortium via Virtual Machines that can be equipped with the required analysis stack for algorithm development and statistical analysis. A dedicated bioinformatics platform for research purposes will be tested too. The functionalities of the bioinformatics platform will be monitored and technically validated to ensure the correctness of the results. While computational capabilities of the IT platform hosting the infrastructure are adequate in many cases, when HPC resources are required, it will be possible to initialize a virtual machine on-demand in a local encrypted file system at the HPC facility. A dedicated gateway will support the data transfer encryption and ensure interoperability between the two nodes; the same gateway receives back the output of the data processing, and the HPC facility terminates the virtual machine and permanently deletes the data. The final implementation of the Clinical Data Infrastructure and its deployment in all participating hospitals will be reported by M36 (D1.1).

Milestone: A detailed report on the Clinical Data Infrastructure and its use for the exemplary pilot is available. It will include the description of the architecture, the adopted anonymizations mechanisms, the Standard Operating Procedures and the Data Management Plan.

Deliverable: Report on the final version of the clinical data infrastructure as deployed in participating hospitals. Adopted technical, organizational, and legal solutions and outcomes of the technical validation.

Task 1.2: Swarm learning for integrated and decentralized biomedical data processing

Executors	Starting month	Ending month	Duration (months)
UniBO/UniVR	6	30	24

To create a swarm learning framework that will incorporate the platform developed in Task 1.1, simultaneously addressing the need for large medical datasets and highest-grade encryption and confidentiality requirements. As medicine is inherently decentral, the volume of local data is often insufficient to train reliable classifiers, but centralization poses critical concerns on privacy and

confidentiality. General swarm learning framework able to support the training of models while completely removing the requirement of local, dedicated servers will be developed. This will allow to share model parameters via the Swarm network, as well as to build the models independently on private data at the individual sites (swarm nodes). The framework will be developed in close collaboration with the platform created in Task 1.1, which will initially serve as a first use-case and benchmarking/development environment. Successively, nodes (i.e., hospitals and research institutions) will be added as swarm nodes, hence contributing large multimodal data volumes through safe decentralization. All new nodes will enroll via a blockchain smart contract, obtain the model, and perform local model training until defined conditions for synchronization are met. Model parameters will be exchanged via a dedicated Application Programming Interface (API) which will be developed within this task and hosted in the cloud and merged to create an updated model with updated parameter settings before starting a new training round. Top-level security measures based on private permissioned blockchain technology will ensure data sovereignty, security, and confidentiality. Once nodes are enrolled, the swarm learning framework will allow the decentralized training of models using large volumes of data without confidentiality and privacy constraints.

Milestone: First release of the API. First model trained in swarm mode with single-platform data.

Deliverable: Final release of the API. Open access code available. Models trained in swarm mode with multiple contributors and nodes.

Task 1.3: Prototyping of software as a medical device application integrating molecular and clinical features to support tailored clinical characterization and novel therapeutics

Executors	Starting month	Ending month	Duration (months)
Engineering/IFO/TorVergata/UniCA/UniMORE	1	30	30

This task aims to *i)* in collaboration with Spoke 4 WP2 create a software prototype able to collect and securely store molecular sequence data and clinical data, both for research and diagnostic purposes; *ii)* ensure that the prototype development will be carried out in compliance with all national and international regulatory standards as part of the CE-mark approval procedure. " The software prototype will serve as a basis for the creation of a multi-action platform serving patients, caregivers, health professionals, and the community of potentially affected people to *i)* manage clinical studies in terms of patient enrolment and randomization; *ii)* identify patterns for supporting precise clinical characterization and diagnosis; and *iii)* establish data-driven methods for dynamically manage the corresponding clinical pathways."

For both retrospective datasets and prospective data collection, data will be collected via eCRF in REDCap, either by manual imputation or with direct file upload. REDCap also supports patients' randomization and online data quality control mechanisms; it will be installed and integrated with the IT platform developed in Task 1.1, and Partners will log in with a two-factor authentication. The transfer of very large files and/or large volumes of data will be managed through dedicated, secure point-to-point connections. As part of Task 1.1., appropriate anonymization or pseudo anonymization methods will be applied for data collection and processing. Collected data within the IT infrastructure will be processed by means of the available bioinformatics platform for advanced feature extraction and statistical analysis. The identification of specific intended uses and regulatory requirements will guide the implementation of the mathematical and data-driven methods required for the development and validation of pathology-specific predictive models. Software and algorithms development will

be carried out according to the standard ISO 13485:2016 in order to prepare the technical file that will be required, together with the evidence on clinical validity, for obtaining the medical CE-mark approval after the end of the project.

Milestone: Retrospective datasets and prospective data are available within the IT platform for statistical analysis, algorithms development, and testing.

Deliverable: The software prototype and outcomes of the clinical validation.

WP 2: Climbing Artificial Intelligence **(Leader: UniBO)**

WP2 will address the development of ground-breaking basic research frameworks leveraging AI methods to make a notable leap in personalized medicine

Task 2.1: Beyond supervised learning

Executors	Starting month	Ending month	Duration (months)
UniBO/UniCT/TorVergata	1	30	30

This task will develop a breakthrough deep learning framework using self- and semi-supervised methods for a new generation of personalized risk-stratification models. It will be employed using deep generative models (e.g., deep adversarial autoencoders combined with contrastive loss functions) which will be trained to faithfully reproduce 3D/4D multimodal images as well as non-imaging data through the generation of lower-dimensional embeddings (reconstruction fidelity will be included in the loss function). The embeddings will be conditioned to iteratively separate into patient subgroups according to the latent data structure. Multiple-timepoint features will be embedded in the learning to augment the separation ability of the architecture in the latent space, hence taking into account longitudinal patient trajectories. The embeddings created by the model along with all other patient data, will be used as inputs for a set of evolutionary machine-discovery and model-building algorithms that will build predictive models of the evolution of the disease through a wrapper approach. The models' internal representations and regularities discovered by these approaches will form a set of effective degrees of freedom for the disease continuum that will be fed (possibly after further coarse-graining) into similarity-based clustering and dimensionality-reduction algorithms to derive new phenotypical categorizations, i.e., candidate new phenotypic versions of the clinical spectrum represented in the data, hence uncovering the true degrees of freedom that govern a single patients' trajectory.

Milestone: First release of the framework and report on the developed framework.

Deliverable: Final release of the framework and open-source repository of the framework.

Task 2.2: Integration and modeling of multi-omic data

Executors	Starting month	Ending month	Duration (months)
ISS/Neuromed/IFO/Sapienza/TorVergata/UniBO/UniVR	3	30	28

This task aims to analyze and integrate multi-omic data (Next Generation Sequencing, Transcriptomics, Methylation, etc) with bioinformatics, AI and statistical learning methodologies such as automatized pipelines (Mutect, Mutect2 and GATK) by using snakemake, complex network and graph neural networks to quantify, e.g., tumour heterogeneity and similarity after mapping of omics on protein-protein interaction network and laplacian diffusion. It will be ensured: *i)* dimensionality reduction of multi-omic measurements with methods based on manifold learning techniques and density based clustering for characterizing multi-omic signatures, disease modules and genomic landscapes; *ii)* together with Spoke 4 WP3, feature extraction (morphological and texture) from digitalized histopathological images (Whole Slide Imaging) in and prioritization in order to identify the most important ones; *iii)* generation of synthetic, disease, gender and age-specific multi-omics data to circumvent the privacy problem and to be used for transfer learning (data augmentation) and for data quality improvement (imputation of missing data).

Milestone: Intermediate report and communication event of obtained results.

Deliverable: Deployment of open source software through a public platform.

Task 2.3: Digital twins for computational modeling and personalized intervention

Executors	Starting month	Ending month	Duration (months)
Engineering/ISS/UniBO/UniMIB	1	30	30

This task will develop digital twin solutions for the prediction of disease diagnosis, personalized intervention, and precision treatments using computational modeling and simulations of complex physiological systems. The proposed task will address some of the open methodological issues in the field (multiscale and multiphysics integration of computational models; algorithm optimization to reach real-time or close to real-time simulations; reliable understanding and description of the genotype-phenotype relationships to fully exploit a personalized medicine approach; integration of mechanistic, biophysical, statistical and population models; uncertainty quantification) and will ultimately lead to the development of a Digital Twin for a specific target organ (e.g., heart) or a specific target pathology (e.g., cancer, cardiac arrhythmias). It will have as input patient-specific data, including omics data, biosignals, as well as images. Patient-specific simulations based on mechanistic models and machine learning algorithms will be applied to anatomical and functional patient information. Simulation output will possibly support: *i)* diagnosis; *ii)* drug safety and efficacy assessment; *iii)* therapy planning; and *iv*) risk stratification.

Milestone: Implementation of the computational model of at least one target organ/pathology. The code will be uploaded on an open access web platform. ***Deliverable:*** Full implementation of the Digital Twin at least one target organ/pathology, including the automated procedure for its personalization based on acquired patient-specific data.

WP 3: From novel methodologies to clinical applications: AI enabling personalized medicine **(Leader: UniCA)**

In WP3, it will be developed and applied novel AI methodologies in real clinical settings enabling personalized medicine

Task 3.1: Design of AI techniques for augmented reality in robotic surgery

Executors	Starting month	Ending month	Duration (months)
UniBO/UniCA	1	30	30

In this task, it will be overcame the use of conventional 3D-guided robotic surgery in which the 3D virtual model is overlaid manually into the surgical field during robotic surgery. Indeed, the virtual 3D model is not automatically aligned with the real organ during its mobilization and traction in surgery, and this non-automated AR may be affected by gross errors due to the manual alignment of the 3D model over the surgical target anatomy leading to a potential inaccurate dissection. Thus, this task aims to develop dedicated AI techniques for the automated registration of 3D virtual models during robotic surgery with augmented reality (AR) and generate automatic surgical guidance.

Milestone: Evaluation of specific surgical endpoints to be developed in AI AR on human cadavers through AI techniques for AR in robotic surgery; assessment of specific innovative robotic surgical procedures in different anatomical regions
Deliverable: Development of reliable and safe innovative AI AR robotic technologies for specific anatomical topographic regions

Task 3.2: Development of network analysis algorithms

Executors	Starting month	Ending month	Duration (months)
Sapienza/UniMIB/UniVR	1	30	30

This task will develop network analysis tools and algorithms to be used in Spoke 4, including i) tools for data preparation integrated with the collaborative platform; *ii)* AI-powered tools for patients' classification and stratification according to the disease stage; *iii)* network analysis algorithms analyzing data collected in the collaborative platform.

Milestones: Protocols on data preparation for network analysis; AI tool architecture; network analysis algorithms.
Deliverables: Data preparation tools for network analysis; release of the AI tool to be used by partners of Spoke 4; novel network medicine analysis to be used in Spoke 4 for developing computational profile.

Task 3.3: Development of an AI-powered medical image system to support diagnostic and radiation protection in chest computed tomography (CT)

Executors	Starting month	Ending month	Duration (months)
Neuromed/UniPI/UPMC	1	30	30

This task will develop a Computer-Aided Detection (CAD) system for i) automatic detection of lung nodules and ii) segmentation and quantification of lung areas affected by COVID-19 pneumonia based on a novel deep learning approach using radiomic features. The CAD system will be tailored to low-dose chest CT (LDCT), and CT reconstructed with different Adaptive Statistic Iterative Reconstruction (ASIR) blending levels.

Milestones: Optimization and validation of radiomics and deep learning systems of standard- and low-dose chest CT using public datasets and evaluation of imaging properties of chest CT reconstructed with different Adaptive Statistic Iterative Reconstruction blending levels. ***Deliverables:*** Implementation of a CAD system for detection of lung nodules and segmentation and quantification of lung areas affected by COVID-19 pneumonia in LDCT chest CT.

SPOKE 3: Prediction Models

Development of advanced prediction models for prognosis and therapeutic response based on comprehensive data treatment

Description of the Spoke Activities

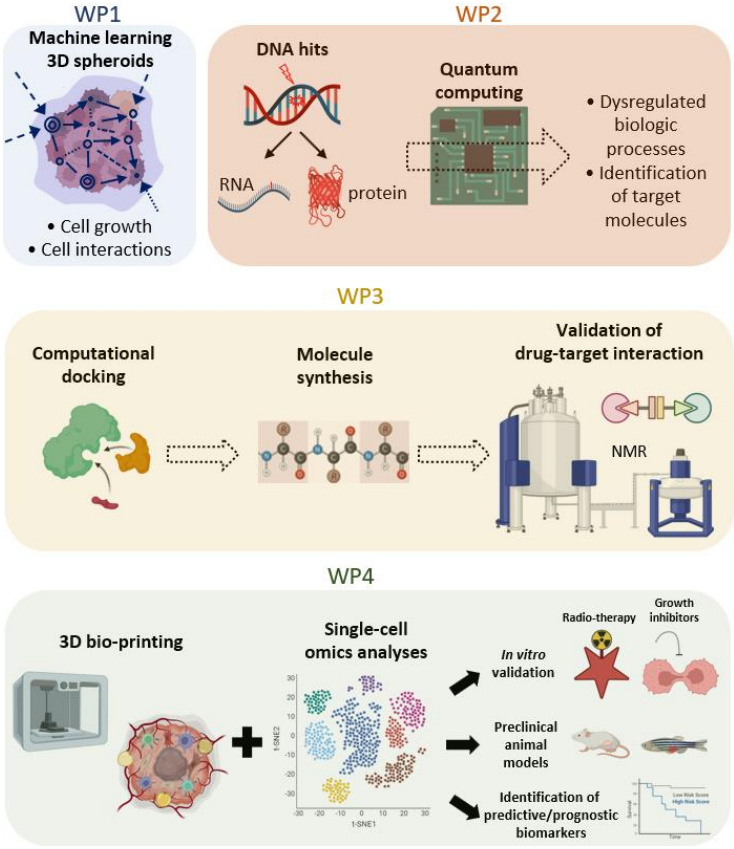
SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
UniPA	Birex/IOM/ISS/IFO/M.NEGRI/ Sapienza/SIT TorVergata/UniBO/UniCA/UniCT/UniFG/ UniMIB/UniMORE/UniPA/UniPI/UnivPM	1	36	36

1. Context description:

The longstanding acceptance that disease evolution can only be interpreted through the selfish gene theory has emerged to be insufficient to elucidate the complexity of heterogeneity. The latter is better explained by an intricate interplay of genomic instability, epigenetic regulations and micro-environmental factors leading to different phenotypic features. Although some consequences of distributional heterogeneity in diseases arise as a result of continuing mutagenesis, it is likely that this peculiarity also derives from the aberrant differentiation status of cells. For instance, in neoplasia, two current theories attempt to describe the establishment and maintenance of tumor heterogeneity: the clonal evolution model and the cancer stem cell hypothesis. According to the clonal evolution model, each cell within the tumor has an equal capacity to proliferate and thereby sustain the tumor growth. Differently from the tumorigenesis paradigm, the cancer stem cell hypothesis states that irrespective of the cell-of-origin, many cancers may be hierarchically organized in much the same manner as normal tissues. In this model a small population, called “cancer stem cell” (CSC), is univocally able to drive tumor growth much like normal stem cells (SCs) gain proliferation and differentiation in normal tissue. Their self-renewal and differentiation lead to the production of all cell types of a tumor, thereby generating tumor heterogeneity. In adult mammals, tissue homeostasis and repair of injured organs depends on small reservoirs of tissue-specific stem cells able to self-renew and differentiate. Indeed, the hierarchical organization of adult tissue has been accomplished to slow aging, help the organism recover from injury, and finally protect cells from accumulating damage that would ultimately lead to cancer. Although adult stem cells and their immediate progeny, the progenitor cells with limited lifespan escape the risk of accumulating mutations, could potentially be transformed either by acquiring mutations inducing self-renewal, or by inheriting existing mutations from stem cells. Rapidly self-renewing tissues, that harbor actively cycling stem cell populations, offer the opportunity to study adult stem cells, as well as their relationship with cancer initiation and progression. Major advances in adult stem/progenitor cell biology have allowed researchers to study specific physiological functions of these immature cells and their early progeny, endowed with self-renewal and multi-lineage differentiation potential. On the other hand, the growing body of experimental evidence has revealed that an accumulation of genetic abnormalities in tissue-resident stem cells or in their more committed progenies, concomitant with niche epigenetic alterations, DNA damage caused by mechanical forces and the dynamic ecosystems that evolve in response to selection pressures from non-genetic contributors among which cell plasticity and microenvironment, may result in rare and metabolic disorders, cardiovascular and cancers. This observation mostly reflects the need to better understand the events which occur in tissue-resident stem cells in order to explain the transition from non-malignant hyper-proliferative lesions to well established diseases and translate the identified alterations into new therapies. Of note, advancements

in 3D in vitro culture have allowed for the development of tissue models, which accurately recapitulate the microenvironment and can inform about the genetic changes that lead to disease initiation and the evolution of drug resistance, understanding of the evolutionary dynamics which govern the initiation and progression of the diseases. Once identified biomarkers or pharmacological agents endowed with potential pathogenetic or therapeutic activity, respectively their efficacy will be tested in zebrafish or mouse models.

2. General objective:



The realization of this activity will be facilitated by the already established protocol for the isolation and purification of organoids. On the bases of the knowledge acquired along the years through advanced computational approaches, a model recapitulating the complex evolution of a cell even following an action of an external agent (drug, radiation, etc.) will be created and experimentally verified through the use of 3D tissue models (multicellular organoids). In particular, organoids, fibroblasts, adipose, mesenchymal, endothelial and immune cells will be 3D bioprinted. This 3D structure, which will also be simulated through existing analytical tissue growth codes, will be investigated by evaluating the action of specific compounds selected by the other spokes and affiliated involved in the HealITALIA consortium and radiotherapy. Its development following the external action will be monitored and the "physiopathology" will be analyzed and simulated with machine learning approaches capable of reproducing the complex mechanisms of cellular communication. Radiosensitivity of *in vitro* organoids and organoid zebrafish and mouse avatars has potential predictive value for individual response to radiotherapy, supporting clinical decisions for the management of affected by monogenic and polygenic diseases including those dependent from specific mutations, and cancer, thus avoiding radiation toxicity to resistant patients and reducing the treatment costs. The opposite direction originates from genetically modified mouse models, some not yet published, affecting metabolic pathways. The spoke 3 makes use of the **Scientific Advisory Board** with which has consolidated a collaboration for years: Ettore Appella, MD, National Cancer Institute (NIH), USA, Soldano Ferrone, MD, PhD, Harvard Medical School, Massachusetts General Hospital, Cristina Maccalli, PhD, Sidra Medicine, Doha, Qatar. Distinct interactions and benefits are, in addition to other spokes of the same programs, also with other national research programs from the Ministry of University and Ministry of Health.

3. Project WPs structure

WP 1: Integrated experimental and computational models of 3D cultures of human cells with specific gene mutations or biogenesis alterations of RNA/Proteins **(Leader: UniPA)**

Task 1.1: 3D simulation of spheroid structures through machine learning

Executors	Starting month	Ending month	Duration (months)
UniPA/Birex	1	24	24

This task aims to develop Machine Learning (ML) models for cell growth prediction under the action of external agents on its DNA (e.g. drug, radiation, etc.). ML algorithms will be trained using biological and imaging features extracted from 3D tissue models (i.e. multicellular spheroids). In particular, a mixed cellular 3D model will be studied, with cellular spheroids of normal fibroblasts (3T3 cells) and "pathological" cells. Trained algorithms will predict the action of specific inhibitors of neoplastic growth processes and the "physiopathology" or the reproduction of the single cell of each of the individual tissues or of their whole. Explainable AI (XAI) algorithms will also be used to understand the complex relationships between the input biomarkers discovered by the trained algorithms and the external agent response. A study on the features inhibiting cell growth will also be performed. XAI enhanced models, able to make correct predictions about a therapy efficacy, will also explain the main factors affecting the prediction.

Milestone: Most cell growth influencing biomarkers identification through predictive ML models using Explainable AI algorithms.

Deliverable: XAI-based ML models to predict the best 3D cell culture method for studying the proliferative and invasive capacity of cells.

Task 1.2: Regulatory molecular circuits of 3D cell growth affecting physio-pathological cell phenotypes

Executors	Starting month	Ending month	Duration (months)
UniPA/UniBO	6	30	24

Regulation of gene expression (DNA and RNA epigenetics) defines cell phenotypes upon interactions with micro-environmental conditions. Altered environment-gene interactions affecting expression profiles thus connect to cardiovascular, metabolic, neurodegenerative, developmental and cancer diseases. Key molecular players in gene expression regulation are chromatin remodeling enzymes, modified histones, transcription factors and non-coding (nc)RNAs. Mechanical forces constitute environment-dependent factors, which modulate the shape of cells and organelle, such as nuclei and mitochondria, during migration and adaptation to space constrictions in a tissue. Mechanical forces are known to affect gene expression and genome integrity. DNA damage is increased during cell migration and DNA damage response activates several molecular pathways regulating cell functions. Several diseases are caused by gene mutations affecting DNA repair mechanisms, which are regulated by many proteins and ncRNAs. The functional interaction networks of ncRNAs and chromatin factors in an organoid context are still poorly characterized. In particular, their interplay under 3D growth conditions in relation to DNA damage/repair and expression profiles need to be fully defined. Various therapeutic strategies fail at different stages of clinical trials because of lack of efficacy. To overcome this limitation, there is a need to identify the specific pathways and gene mutations determining cell phenotypes in their natural micro-environment. Thus, the goal of this Task is to establish organoid-relevant mechanisms and mutations affecting growth and the migration of cells in 3D. Particular

attention will be given to ncRNA complexes, chromatin factors, DNA repair proteins and pathogenetic gene mutations in specific diseases such as cancer, neurodegeneration and immune deficiency.

Milestone: Transcriptome-wide characterization of ncRNAs, chromatin factors and DNA repair genes in 3D cell culture.

Deliverables: Engineered-organoid models with targeted CRISPR mutations; Knowledge of pathogenetic gene mutations affecting 3D cell cultures.

WP 2: Simulation of mutated proteins and complex structures through quantum computing and AI **(Leader: UniBO)**

Task 2.1: Quantum computing techniques applied to biochemical systems, molecular biology and organic chemistry

Executors	Starting month	Ending month	Duration (months)
UniPA	6	30	24

Chemistry is considered as one of the more promising applications to science of near - term quantum computing. In the current noisy intermediate-scale quantum (NISQ) regime, the leading quantum processors contain about 50 to a few hundred qubits, but are not advanced enough to reach fault-tolerance nor large enough to profit sustainably from quantum supremacy. In such a scenario the most effective strategy in quantum chemistry simulations is to split the work over classical and quantum computers. Such an approach has proved effective in computational speedup and in the decrease of the degree of complexity of the problem. This project will use such hybrid quantum-classical algorithms to simulate protein folding, that is the molecular folding through which proteins obtain their three-dimensional structure. Due to the central role of proteins' structures in chemistry, biology and medicine applications, this subject has been intensively studied for over half a century. Although standard classical algorithms provide practical solutions for the sampling of the conformation space of small proteins, they cannot tackle the intrinsic NP-hard complexity of the problem.

Milestone: Development of variational quantum algorithms specifically adapted to classical cost functions and evolutionary strategies to simulate the folding of polipeptides.

Deliverable: Simulation of protein folding in the presence of mutations.

Task 2.2: Integrative in silico assessment of the impact of mutations on protein structure, function, and interactions

Executors	Starting month	Ending month	Duration (months)
TorVergata/UniBO	12	36	24

This task aims to model the overall effect of mutations on proteins as well as estimating and ranking specific molecular alterations (protein destabilization, functional impairment, disrupted macromolecular binding, ablation of post translational modification sites) potentially affecting the phenotype. AI-based AlphaFold is currently the most useful tool to predict the structure of a single protein chain with a naturally occurring sequence. However, several structure features cannot be defined by the tools. For instance, AlphaFold does not predict multi-chain complexes effectively, even if some AlphaFold versions have been developed for complex prediction. Relevant to this project, AlphaFold cannot predict the effect of mutations. In particular, it does not produce an unfolded structure given a sequence containing a destabilizing point mutation. Mutations in proteins can induce a large variety of effects on protein structure, stability, function, and interactions. The extent of those effects is strongly dependent on the type of variation, its position in the context of the protein structure, and on the role of proteins in complex biological networks. In the context of this task, an integrative predictive approach for in silico variant interpretation will be provided through the integration of different tools for evaluating the impact at different levels: *i)* contextualization of the mutation within protein functional and structural domains; *ii)* evaluation of the impact of the mutation on protein stability; *iii)* impairment of protein function and subcellular localization e.g. through disruption of active sites and/or localization signals; *iv)* decrease/disruption of the protein binding affinity with other biological molecules (proteins, DNA/RNA, ligands). Existing machine learning-based computational tools, available at the HealITALIA consortium, will be updated considering curated datasets of variations extracted from public data sources as well as recent advancements in the field of artificial intelligence. Novel approaches, based on machine-learning algorithms, will also be implemented. In addition, SHP2 is required for survival of RTK-driven cancer cells, contributes to resistance to anti-cancer drugs and modulates immune checkpoints. Molecules targeting the catalytic site are poorly selective; allosteric drugs, stabilizing an autoinhibited conformation, are more promising, but they are ineffective on PTPN11 mutants destabilizing the closed state of the protein. It was recently developed non-dephosphorylatable phosphopeptidomimetic (NDP) inhibitors of SHP2 protein-protein interactions, targeting the N-SH2 domain (patent pending). NDPs can be improved in their binding constant in silico designing a novel NDPs targeted to the C-SH2 domain and enhancing sampling simulations of SHP2 allosteric transition.

Milestone: Updated tools for evaluating/modeling the impact of mutations on protein stability, function, and interaction.

Deliverables: ***i)*** *In silico* analysis workflow for variant interpretation integrating different aspects of protein and protein variants; ***ii)*** A deeper understanding of the structure, function and regulation of the phosphatase and of its pathogenic mutations.

WP 3: Pharmacophoric dynamic docking simulations of genetic altered molecules **(Leader: UniMIB)**

Task 3.1: Computation of molecule bearing genetic alterations able to bind to the target in the most effective way possible (docking) and with the greatest affinity (search for the best scoring

Executors	Starting month	Ending month	Duration (months)
Birex/UniPA/UniMIB	1	24	24

This task aims to develop and test novel computational methods tailored to compute accurate reaction and binding free energies for biomolecules and supramolecular systems (protein-drug interaction, protein-cofactor interaction, etc.) and to obtain a Hit molecule with the highest possible affinity for the targeting. It will be planned to use non-equilibrium thermodynamic integration (TI) methods, coupled to molecular dynamics simulations and machine learning (ML) techniques, to develop high-throughput computational protocols to be used for the virtual screening of databases of small molecules (drug discovery projects) and proteins (prediction of thermodynamic property projects).

Milestone: Optimization of hit molecules with high affinity for the targeting.

Deliverable: High-throughput enhanced computational model for virtual screening.

Task 3.2: Molecular synthesis for the experimental validation of computation models

Executors	Starting month	Ending month	Duration (months)
UniPA	6	30	24

Differently from classical computer-driven drug design (CDDD) where the computational tools are used to design molecular scaffolds for potentially bioactive molecules, this task is devoted to the synthesis of molecules with the scope of validating the simulation results in complex systems of drug-target interactions. Scaffolds of hit-molecules will be synthesized with functional and steric groups in key points of the drug-target docking site in order to experimentally prove a different level of binding with respect to the drug-target match. The scope will not be to optimize the molecule's activity towards a given biomolecular target but rather to assess, experimentally, the differences in drug-target binding interactions and compare them to the computational dynamic docking at the atomistic level. The experimental validation of the simulated drug target interaction will be tested by high resolution NMR techniques in the presence of biomolecular target and its ligand.

Milestone: Optimization of differences in drug-target binding interactions.

Deliverable: Simulation of drug-target interactions.

Task 3.3: Fight the enemy before you can see it: moving tools for early diagnosis from bench to bedside

Executors	Starting month	Ending month	Duration (months)
UniPI	12	36	24

To design, synthetize and clinically validate innovative molecular probes as precision diagnostic tools for cancer.

Milestone: Development of selective bioimaging probes for ADAM10 and ADAM17 sheddases as gold multivalent nanoprobes (GMNPs) suitable through electron microscopy (EM) and/or bioactive fluorescent probes, and of anti-CD36-Ab conjugated with inhibitors of ADAM10; Synthesis and characterization of bioconjugate probes, where metal complexes absorbing in the IR "cell transparency window" (Cp-rhenium-tricarbonyls) or being NMR-active (lantanides, such as europium or terbium), are linked to carbohydrate units and/or peptide portions.

Deliverable: Test of the bioimaging probes (gold-nano/fluorescent) that are selective for ADAM10 and ADAM17 in living cell models (also 3D) and in tissues isolated from patients; study of the effect of Anti-CD36-Ab conjugated with ADAM10 inhibitors in simple and organized 3D cell systems; Assay of the bioconjugated complexes in cultured cancer cells in order to measure their selective uptake by IR-microscopy or NMR techniques and, therefore, characterize the specific alterations occurring in the tested cancer cells; translate these assays to patient-derived tissues.

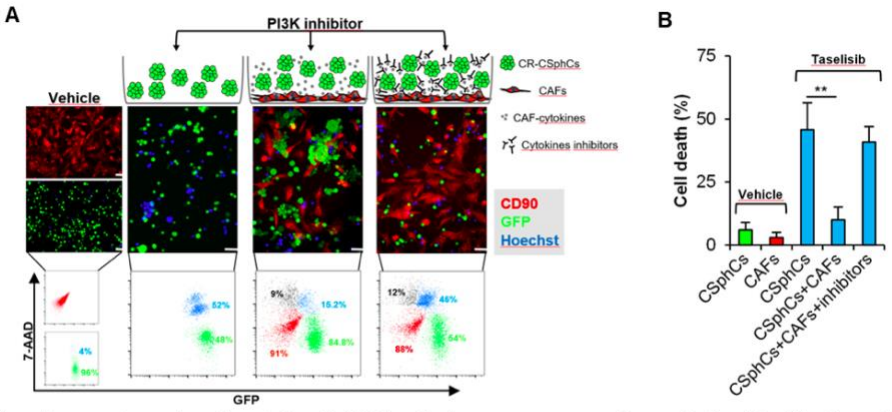
WP4: Preclinical models for precise therapeutic and diagnostic prevention strategies **(Leader: UniPA)**

Task 4.1: Modeling of 3D approaches of multicellular spheroids structures for estimating the risk of disease initiation and progression.

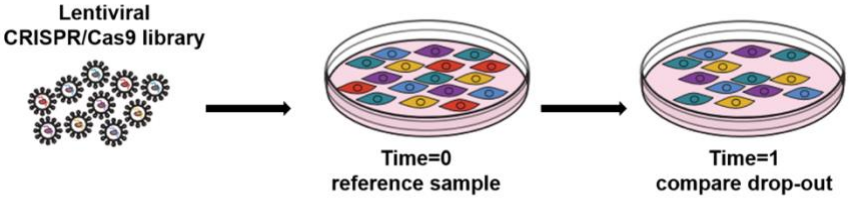
Executors	Starting month	Ending month	Duration (months)
UniPA/UniMORE/IFO/UniPI/ISS/UniMIB/TorVergata	1	30	30

The main goal of this Task will be: 1) the prediction of the best 3D model to study the in vitro behavior of cancer cells and to establish organoid-relevant mechanisms and mutations affecting the migration and growth of cells in 3D; 2) to understand the pathogenic mechanisms responsible for genotype/phenotype relationship in rare neurodegenerative diseases centered on mitochondrial dysfunction. These are tackled by somatic cells reprogramming to iPSCs, in turn differentiated into diseased cell types, including neuronal cells, glia and 3D tissue organoids. 3) self-assembling human heart organoids for the modeling of heart failure and genetic cardiomyopathies. For the first aim, attention will be given to ncRNA complexes, chromatin factors and DNA repair proteins, mutated in specific diseases such as cancer, neurodegeneration and immune deficiency. It is expected to define the influence of ncRNAs, mutations and chromatin factors on 3D growth, and on the ability of cells to migrate and to form tissue-type structures, such as blood vessels and cell layer organization. To this aim, primary cells and the pre-existing collection of cancer initiating cells and enriched with new samples provided by pathologists involved in this proposal will be printed in 96-well non-adhesive U-bottom cell culture plates at different confluence, in cell culture medium solution containing low concentration of the selected matrix, and spheroid formation will be monitored for 2-5 days after plating. This time frame will lead to the evaluation of cell proliferative potential. Preliminary data show that the co-culture of cancer cells with TME cells, and non-cellular component, allow the study of crucial molecular mechanisms involved in tumor progression, such as cancer cell proliferation and chemoresistance. Thus, model will be optimized through the use of a drop-on-drop 3D bioprinting technique, in which cells will be embedded in a solution with higher concentration of matrix, in order to evaluate the invasive capacity of cells. This experimental setting will allow us to develop a deep learning-based method for monitoring cell proliferative and invasive potential and correlate these functional parameters to the available clinical information of parental patients (i.e., staging, grading, histotype, mutational background), in order to predict which are the best tridimensional culture conditions optimized for each cell line. For the second aim, patient-derived iPSCs from a group of

rare monogenic phenotypes will be differentiated into neuronal precursor cells. These will be instrumental to standard functional and metabolic characterization, including single cell analysis with multi-omics. Neuronal and organoid differentiation will be used to deepen the mechanistic understanding of the pathogenic mechanisms. Then, this task will identify phenotypic suppressors by CRISPR/Cas9-based library screening to be targeted as druggable pathways. For the third aim it will be generated cardiac disease relevant human heart organoids by self-assembly using cardiac sample-derived human pluripotent stem cells, pre-differentiated into cardiac muscle cells, endothelial cells, and smooth muscle cells together with patient-specific cardiac fibroblasts. Clinical trials could be indeed performed using patient-derived organoids as a tool for personalized medical decisions to predict patients' responses to therapeutic regimens and potentially improve treatment outcomes. Living organoid biobanks encompassing several cardiovascular types are currently being established, providing a representative collection of well-characterized models that will facilitate drug development.



Co-culture systems for the study of TME role in cancer progression and the identification of predictive/prognostic biomarkers. **A**, Cell death (blue colour) evaluated by immunofluorescence (upper panels) and flow cytometry (lower panels) in sphere cells transduced with GFP (green colour) cocultured with CAFs CD90 positive (red colour) and treated with a PI3K inhibitor (taselisib) for 72 hours in the presence or absence of hepatocyte growth factor (HGF), stromal cell-derived factor-1 (SDF-1) and osteopontin (OPN) blocking antibodies (inhibitors). Scale bars, 40 μm. **B**, Percentage of cell death in cells as in (A). Data are mean±SD of three independent experiments using Ras/Braf-wt, Braf-mutant and Kras-mutant sphere cell lines.



Schematic overview of set-up of lentiviral CRISPR-based drop-out screen.

Milestones: 1) Establishment of bioengineered 3D bioreactored /bioprinted organoid models; 2) Development of indices for analysis of tumor spheroid proliferative and invasive capacity, to set a deep learning-based standardized and quantitative method, based on bright-field images. 3) Validation of the functional properties of the identified molecules and their impact in mimicked therapeutic protocols; 4) Identification of differentially expressed biomarkers in vitro mechanisms of action; 5) iPSCs/neuronal precursors generation and characterization, with single cell analysis by multi-omic approaches; 6) 3D bioprinting to generate advanced human cardiac organoids to closely mimic the maturity of adult myocardial tissue.

Deliverables: 1) Generation of new diagnostic and prognostic tools; 2) Identification of new diagnostic, predictive and prognostic biomarkers; 3) Generation of a platform for the identification of candidate drugs efficacious against cancer initiating cells and its stromal components; 4) CRISPR/Cas9 screening and therapy testing and validation; 5) the identification of specific druggable pathways implicated in mitochondrial disease mechanisms with the proof of therapeutic efficacy; 6) Novel drug discovery and precision drugs for cardiovascular diseases using human cardiac organoids as valid and reliable preclinical models.

Task 4.2: Development of a powerful in vitro model for the response to radiotherapy in order to make clinical decisions more appropriate for treatment options

Executors	Starting month	Ending month	Duration (months)
UniPA/IOM/UniCA	1	30	30

Radiation therapy is one of the technology-driven modalities in cancer management. Despite major advances in radiotherapy, some cancer patients do not benefit from treatment, which results in primary resistance or relapse after apparent eradication of the disease. The aim of this Task is to generate an in vitro model based on patient-derived bioprinted cancer organoids to be subjected to flash radiotherapy for the prediction of treatment efficacy to support clinical decisions, thus avoiding radiation toxicity to resistant patients and reducing the treatment costs. The planned model may result in identifying, in a clinically acceptable time frame, refractory patients who can be spared from side effects of ineffective radiotherapy. The same approach will be applied to study the efficacy and cellular mechanism of action of local hyperthermia (HT). Indeed, according to the Dutch Deep Hyperthermia Trial, HT showed enhanced effects when used with radiotherapy.

Milestone: Assessment of the in vitro radiotherapy based model.

Deliverable: Generation of tools to predict cancer patient's response to flash radiotherapy treatment.

Task 4.3: Validation at single-cell level

Executors	Starting month	Ending month	Duration (months)
UniPA/UniCT/UniBO/UniMIB	6	36	30

Normal/immune cell interactions are critical for several pathogenetic mechanisms. The full lists of genomic loci, mutations and epigenetic alterations affecting immunotherapies are not yet known. Immunotherapy is highly effective in immunological hot tumors; however new personalized chemo-immune combinations must be developed to treat unresponsive tumors. Based on multi-omic data of tumors samples, including TCGA and European datasets (ELIXIR-IIB infrastructures, https://www.elixir-europe.org/, http://elixir-italy.org/), focused on DNA repair, metabolic, epigenetic, immunity-related, adhesion protein genes and driver oncogenes, novel gene interactions with compounds (DNA repair and metabolic inhibitors, DNA/RNA interactors and epigenetic agents) will be established using bioprinted organoids to assess their evolution at single-cell levels. This task aims at validating at single-cell level the previously identified mixed profile associated with disease progression and response to therapies, thus giving a proteomic tag, to design a pathologist-based classifier prognostic tool for a better stratification of patients who could benefit from the use of adjuvant therapy for the prevention of progression disease. This Task will provide information about the best method to recapitulate tumor cellular heterogeneity *in vitro*. In particular, tissue specimens will be characterized by spatial single-cell RNAseq and proteomic analysis, in order to have a clear snapshot of the spatial distribution and percentage of each microenvironmental cell component to perform in vitro functional preclinical studies. In this context, 3D bioprinting will be used to recapitulate the complex TME cellular network, using different tridimensional plating geometries combined with microfluidic devices (to regulated concentration of nutrients, cytokines, growth factors, oxygen), to validate its ability to recapitulate the *in vivo* conditions in terms of spatial transcriptomic/proteomic profile.

Milestone: Set-up of 3D in vitro tools that recapitulate cellular heterogeneity and complexity to study molecular and biochemical mechanisms regulating disease progression.

Deliverable: Development and fine-tuning of quantitative methods to *in vitro* study the dynamic evolution of patient-derived organoids, including the cell-cell and cell-matrix interactome and the response to curative and preventive treatments.

Task 4.4: Generation and optimization of preclinical animal models based on the use of organoids

Executors	Starting month	Ending month	Duration (months)
UniPA/UniBO/IFO/IOM/M. NEGRI	3	30	27

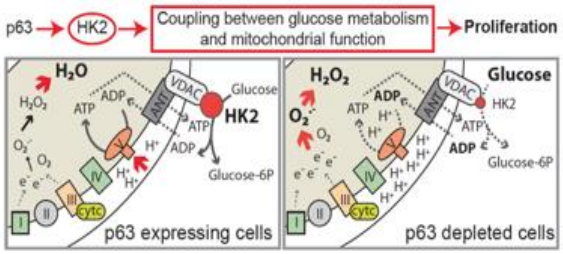
This Task aims to validate in vivo the data obtained *in vitro*, regarding the recapitulation of tridimensional TME dynamics. To this end, different optimized animal models (immunocompromised mice and zebrafish) will be exploited by labeling the cells with fluorescent tags or transducing them with luciferase. Luciferase transduced bioprinted organoid cells will be implanted into animals and monitored by an in vivo imaging detector. This experimental approach will provide crucial information about the molecular and metabolic mechanisms underlying disease initiation/progression. Further, the analysis at single cell level will be suitable to dissect the heterogeneity of the disease lesions and to identify the cell subclones able to drive initiation, promotion and progression. Overall, the generation and use of organoids will be an added value for the identification of prognostic biomarkers that can be potentially druggable with innovative single- or combinatorial-therapies. This tool can be exploited in epithelial carcinoma, rare, and polygenic diseases.

Milestones: 1) Optimized diagnostic preclinical validation at early-stage; 2) Correlation of clinical parameters with candidate biomarkers responsible for disease progression; 3) Identification of prototype therapeutic derivations.

Deliverable: 1) Generation of key proof-of-concept data for precise diagnosis, prognosis and therapy in established models of preclinical efficacy; 2) Identification of molecular and metabolic adaptive responses underlying the heterogeneity and chemoresistance in epithelial cancers.

Task 4.5: Mouse models of mitochondrial metabolism

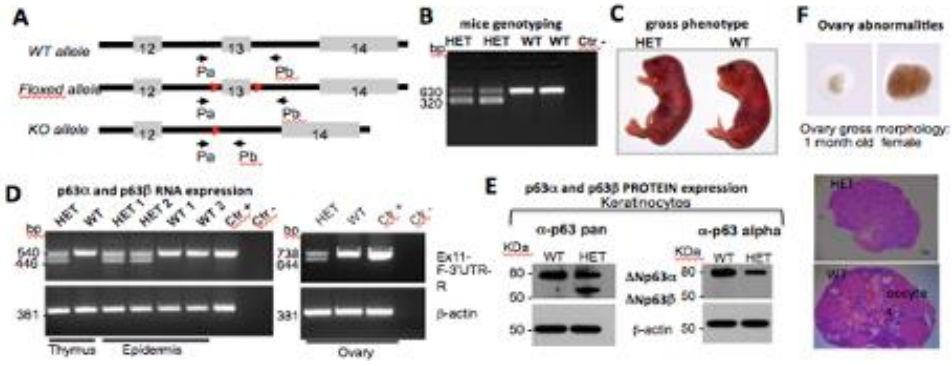
Executors	Starting month	Ending month	Duration (months)
TorVergata/UniFG/UniPI/UnivPM	6	36	30



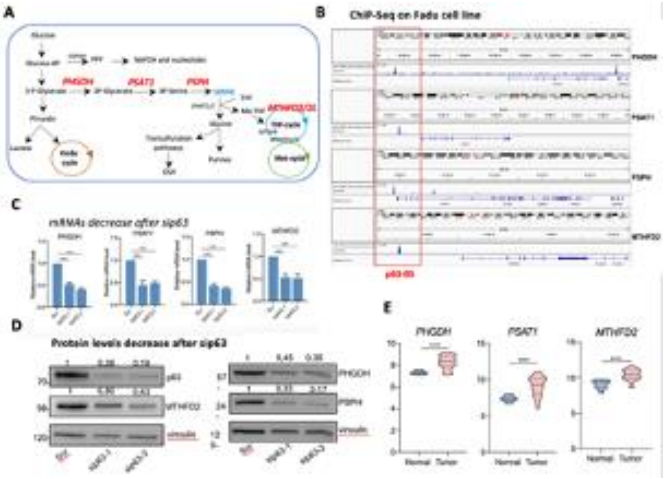
The molecular mechanisms involved in the cellular production of energy starting from glucose, fatty acids or glutamine, play a central role for mitochondria with their molecular dynamics. Mutations in The TCA enzymes show different rates of mutations that result in the production of abnormal "oncometabolites", whose steady state levels interferes with the redox status and gene transcription. A clear example of this mechanism is represented by mutations in isocitrate dehydrogenase (IDH1 or IDH2), pathologically relevant in gliomas, cholangiocarcinoma or acute myelogenous leukemia (AML). The aim of this Task will be to define novel therapeutic targets acting on metabolism, by developing or characterizing mouse models to evaluate metabolic regulators. In conjunction with Spokes 1, and 8, the GENERAL AIM is to define p63-related common and system-specific traits as well as cancer-type specific pathways and to identify novel prognostic and therapeutic targets. This will be achieved using in vivo and in vivo experimental models. Additionally, it was sought to study the metabolic consequences of the loss of p63 isoforms and p63 overexpression, using previous and novel genetically engineered mouse models and in vitro models. Particular attention will be given to address the functional differences of the C-terminal splicing forms of p63, not yet defined. To establish the in vivo role of specific p73 variants in cellular metabolism, metabolic rates of mice will be monitored by using metabolic chambers allowing continuous recording of food and water intake, energy expenditure via indirect calorimetry, oxygen consumption, and physical activity.

The novel models (unpublished, shown here as preliminary data) were done to address the role of the C-terminus p63 variants in vivo. By adopting the CRE recombinase-dependent deletion of exon 13 of the mouse TP63 gene, it was recently generated a constitutive p63α defective mouse (p63aKO mice), in which the p63α isoform is genetically ablated expressing instead the p63a isoform (Figure 4). Unfortunately, the heterozygous females expressing in the oocyte TAp63a are not fertile (Figure 4) due to massive apoptosis triggered by the active TAp63a isoform. To overcome this issue, by crossing the floxed mice with Keratin14-driven CRE ricombinase mice, active in embryonic skin epithelial stem cells it was generated a mouse model lacking the p63a isoform in K14 expressing cells (K14-p63D13/D13 mice). The mice are born with 20-30 weight reduction, that it is maintained during the mice life span. The K14-p63D13/D13 mice have a reduction in the survival rate. Metabolic chambers revealed a reduction of the mice activity, while detect an increase of the energy expenditure, possibly due to an increase of brown fat thermogenic activity. Brown adipose tissue looks abnormal

as indicated by the small lipid droplets and smaller brown adipocytes. Recently, it has been demonstrated that mitochondrial cristae biogenesis protein optic atrophy 1 (Opa1) facilitates cell-autonomous adipocyte browning (Bean et al. 2021), and the Opa1 trangenic mice show a similar metabolic profile of the K14-p63D13/D13 mice). Having identified p63 binding site closeby the Opa1 promoter, it will be investigated the functional link between p63b and the mitochondrial protein OPA1. Preliminary data also indicate that the transcription factor p63 is could be involved in controlling serine biosynthesis (PHGDH, PSAT1, PSPH) and one carbon metabolism enzyme MTHFD2. ChIP sequencing experiment performed in a SCC cell line (Fadu) indicate the presence of p63 binding site in their promoters' proximity. In addition, p63 silencing by RNA interference reduced both PHGDH, PSAT1, PSPH, MTHFD2 mRNA and protein levels. A bioinformatic analysis of these enzymes in a cohort of HNSCC from TCGA confirmed an increased expression of these enzymes in human tumours (GEO datasets GSE12452).



PRELIMINARY DATA. Strategy to obtains p63aKO. A) Primers for genotyping are indicated. B) Genotypes of the mice obtained. C) WT and heterozygous (.HET) mice did not show gross morphology differences at 1 day post-natal and at 1 month of age (not shown). D) RT-PCR showing the expression of the mutated allele, that encode for the p63b isoform, in total mRNA extracted by thymus, epidermis and ovary. E) Western blot showing p63a and p63b expression in keratinocytes. F) Gross-morphology of the ovary. HET females have about 1/10 small ovary. EO staining confirms that in 1 month old female there are no oocytes. This phenotype explains HET female infertility.



PRELIMINARY DATA. p63 controls the expression of serine biosynthesis and one-carbon metabolism enzyme in HNSCCs. A) Metabolic scheme. B) ChIP sequencing for p63 binding site in FaDu cell line in proximity of the indicated enzymes. C) PHGDH, PSAT1, PSPH and MTHFD2 mRNAs expression in FaDu cell line after sip63, ***p<0.001. D) PHGDH, PSAT1, PSPH and MTHFD2 protein levels in FaDu cell line after sip63. E) The expression of serine biosynthesis enzymes and MTHFD2 enzymes is upregulated in Head and Neck Squamous Cell Carcinoma dataset (GSE12452), ****p<0.001.

Milestone: 1) define the metabolic regulation of current knockout mouse models generated by the group, namely p73, p63, p53; 2) generate new knockout mouse models affecting metabolism (e.g. ZNF750); 3) investigate alteration of these genes in the Moli-Sani cohort.

Deliverable: Generation of preclinical models to define novel therapeutic targets acting on metabolism.

Executors	Starting month	Ending month	Duration (months)
Sapienza/IOM/SIT	6	36	36

The main aim of clinicians-pharmacologists is to overcome resistance to conventional therapies in the treatment of advanced diseases. For instance, the optimization of an experimental model of highly aggressive tumors would give the whole partnership the opportunity to learn about the molecular mechanisms underlying resistance to standard therapies including neo-adjuvant radiotherapy. Neoadjuvant chemoradiotherapy is the reference treatment for advanced stage of colorectal cancer that uses the use of 5 fluorouracil or capecitabine for a few months associated with radiotherapy. The combination of the two is not free from side effects to the patient who is already in the presence of an advanced stage tumor or to the possible presence of comorbidities that could make neoadjuvant chemoradiotherapy contraindicated. Neoadjuvant radiotherapy alone is used to reduce the size of the tumor before surgery or after surgery to eliminate residues of tumor cells or lymph nodes, but it is often unsatisfactory as physiological damage to the cells of healthy tissues must be considered. The adverse effects of neoadjuvant flash radiotherapy could be overcome by the use of magnetic nanoparticles that conveyed inside the tumor or any distant metastases can be considered radio sensitizers, i.e., substances that increase the predisposition of tumor tissues to suffer damage. The nanoparticles of iron oxide or cobalt ferrite or magnetite or graphite have been used for this purpose as they are the only ones that are not toxic to the body and when administered in smaller quantities, they have the power to heat. By means of external magnets, the nano particles that have been inoculated into the bloodstream will be conveyed inside the tumor and following localized radiotherapy these will increase the scattering allowing the administration of low doses of Gy. Once their task is completed, the nano particles are transported to the liver and destroyed without causing damage. Furthermore, the use of magnetic nanoparticles has the advantage of being able to be exploited as a contrast agent for magnetic resonance imaging by reducing T1 and T2 by improving image contrast. To study the scattering effect of nanoparticles inside the tumor, a mouse model of the tumor will be created by orthotopically inoculating a certain number of cancer stem cells while recreating a human tumor. This task aims to study the advantages of nanoparticles in combination with the latest radiotherapy techniques, both conventional and hadronic, it is possible to obtain an excellent definition of the dose in the area to be irradiated by carrying out highly accurate dosimetric treatments.

The additional objectives of this task are: *1)* development of a treatment planning software (TPS) for high intensity and high energy electron beams. An extended use of AI methods such as Montecarlo simulation and machine learning based optimization is foreseen, in view of the clinical implementation. The goals of this task are to perform dedicated radiobiological experiments varying the electron beam parameters like average dose rate, dose per pulse, instantaneous dose per pulse and dose.

Milestones: 1) Establishment of CR-CSC mouse avatars to preclinically validate flash radiotherapy options; 2) Simplified (fixed beam energy) version TPS for FLASH electron beams treating real in silico case of deep-seated tumors; 3) Full realization of multiparametric radiobiological experiments with FLASH Radiation.

Deliverables: 1) Clinical TPS for FLASH electron beams treating real in silico case of deep-seated tumors with complete beam parameters optimization; 2) Development of the dosimetric formalism for pencil beam delivery and development of a clinical protocol for low energy UHDP electron beam.

SPOKE 4: 4D Precision Diagnostics

Precision medicine integrating clinical and imaging biomarkers for a "precise in space and time" diagnosis

Description of the Spoke Activities

SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
Sapienza	Sant'Orsola/Sapienza/TorVergata/UniBO/ UniCA/UniCT/UniFG/UniMIB/UniMORE /UniPA/UniPI/UnivPM/UniVR	1	36	36

1. Context description:

The stratification of the Italian population based on disease-specific risk is an unmet need and a big challenge to address in order to guide prevention and treatment in line with the cornerstones of PRECISION MEDICINE. New models for risk assessment at national level are needed, integrating extensive genomic screenings (for high/moderate penetrance risk alleles and low penetrance/polygenic risk alleles, specifically mapped for the Italian population) with exposure to environmental and metabolic factors as well as with bioimaging and omics data. Technological innovations to be acknowledged and exploited include multidisciplinary convergences, leading the way to integrated diagnostics and computational medicine as methods to integrate data inhomogeneous access and substantial differences in the standards of care, reflecting regional inequalities. In this context, the need arises for an all-inclusive digital infrastructure (ecosystem) to analyze healthcare data pertaining integrated diagnostics - molecular, genetic, clinical and imaging biomarkers with uptake of the developed solutions in SSN (Sistema Nazionale Sanitario, National Healthcare System), supported by the application of artificial intelligence (AI)-empowered solutions and non-invasive medical devices for the promotion of precision diagnostics. The complexity, the etiopathogenetic and prognostic heterogeneity of mono- and polygenic diseases, and cancer makes the application of precision medicine imperative, addressing individualized pathways for diagnosis, risk stratification and treatment, to achieve optimal clinical outcomes. This is essential for indolent, recurrent and long-term complex and chronic pathologies that burden the SSN and for aggressive diseases that often present in an advanced stage. The optimization of resources and the return of health to the population, with a relatively good quality of life, represent the basic methods of savings in terms of reduction of health care and welfare costs.

Specifications on the state of the art (SoA) and issues on monogenic rare diseases, polygenic diseases (cardiovascular and metabolic) and cancer management are detailed below:

Rare diseases

SoA: Recent advances in genomics and their implementation in clinical practice are widely recognized as diagnostic milestones. More than 8,000 disorders with proven or suspected Mendelian basis have been reported to date. Among these, a large fraction refers to rare diseases. The application of whole exome sequencing (WES) has notably improved the diagnostic yield. Nevertheless, a large fraction (about 50%) of patients remain undiagnosed. Moreover, the diagnosis is the starting point for proper clinical management and care. Of note, UNIRM1 has a longstanding expertise in the field

and offers integrated clinical, molecular, and functional approaches. Several national and international research projects as well as the profitable interactions with industrial partners makes feasible the proposed plan.
Issues: Subjects affected by rare diseases suffer diagnostic delay, uncertainty in genetic counselling, and lack of proper clinical management. Indeed, an early diagnosis is required to optimize patient care and escape avoidable diagnostic assessment. This critical issue could be overcome by implementing more effective public health policies directed to improve the diagnostic process based on genomics and bioinformatics applications which, in turn, can improve patient-specific therapies.

Cardio-vascular diseases: heart failure
SoA: Cardiovascular diseases represent the main reason of death in industrialized countries. Among them, heart failure, aortic aneurysmal pathology and cerebral vasculopathy represent a significant healthcare and social problem, affecting the growing elderly population. The current evidence supports a range of strategies to improve cardiovascular health, including the following: individual-focused approaches, which target lifestyle and treatments at the individual level; healthcare systems approaches, which encourage, facilitate, and reward efforts by providers and patients to improve health behaviors and health factors, and population approaches, which target lifestyle and treatments in schools or workplaces, local communities, and states, as well as throughout the nation. Although all of these measures have brought effective improvement in controlling vascular pathologies, still accurate diagnostic tools are missing for predictive purposes. Heart failure (HF) with preserved ejection fraction (HFpEF) is a highly morbid phenotypically heterogeneous syndrome, associated with a survival at 1 (78%), 3 (58%) and 5 (43%) years from the diagnosis. The main pathophysiological substrate still needs to be defined; therefore, a specific treatment is currently not available. Aging-related metabolic, renal, and hypertensive derangements; myocardial inflammation; and coronary microvascular dysfunction play a pivotal role, leading to myocardial stiffness, diastolic dysfunction and lung congestion. Considering the complexity of this syndrome, an integrative translational approach is mandatory to clarify the key molecular events predisposing to its development of HFpEF to find new therapeutic targets. For diagnostic precision medicine, a multidisciplinary approach starting from nosology through a TNM-like classification to multi-diagnostic assessment will allow better understanding of this complex syndrome.
Issues: Today, HFpEF is managed with a one-size-fits-all approach. A major challenge is to identify pathways and biomarkers which drive specific solutions. in this spoke it is proposed multidisciplinary studies: i) in validated pre-clinical animal models of HFpEF to investigate the emerging molecular mechanisms to be translated to the clinical setting in human; ii) in consecutive HFpEF patients, by integrating clinical, imaging, functional, tissue, genetic data, and artificial intelligence, aimed to find pathophysiologic pathways. This approach will start from bedside going to bench and back to bedside. Concerning vascular pathologies, although medical imaging methods have been developed for the measurement and visualization of the condition of the vessel wall, such as Ecocardiography, Computed Tomography, Magnetic Resonance Imaging, none of them has a real predictive and quantitative nature. Such a limitation of current medical instrumentation impairs the possibility of understanding the modifications of the vessel wall and the reduction of plaques in response to a therapy. Autophagy, for instance, is one of the potential therapeutic targets to prevent and cure the cerebrovascular damage caused by aortic aneurysmal pathology and cerebral vasculopathy. However, it remains to be clarified how the vascular autophagic state is influenced by cardiovascular risk factors, drug therapy and how autophagy is associated with structural and functional alterations of the aortic wall and with endothelial dysfunction in humans, particularly in subjects. suffering from pathologies of the aorta and cerebral vessels. In one word, it remains therefore the problem to early asset the risk of aortic rupture and vascular stroke. Positron Emission Tomography (PET) has the potential to visualize non-invasively and dynamically the changes in the aortic wall, induced by therapy and its role should be further evaluated.

Metabolic diseases

SoA: Metabolic comorbidities results from accumulation of damage caused by accelerated aging, disease-related impairment of the repair network and inappropriate drug prescriptions. Assessing cumulative dysfunction in different systems (hormonal, metabolic, immune, cardiovascular, and skeletal) is crucial as their relationship with outcome is nonlinear. Patients with multiple endocrine and metabolic comorbidities are at high risk for inappropriate prescriptions. Endocrine and metabolic comorbidities often coexist in patients requiring intensive medical care. Concomitant multiple medications aggravate frailty by increasing the risk of metabolic interactions, adverse effects and reducing efficacy. The pathophysiology of metabolic frailty is still poorly understood. Omics have broadened the diagnostic approach; however, they still require deeper integration into multi-omics to achieve clinical meaning. The interaction of predisposing genetic/epigenetic factors with acquired metabolic determinants is key to predict the outcome trajectories of frailty.

Issues: Understanding the interaction of multi-system endocrine-metabolic interactions will reduce the need for the multi-drug prescriptions in the fragile population.

Omics must be integrated into a multi-omics platform, specifically metabolomics and genomics should be more integrated in clinical settings. Too many metabolic patients are off target, despite the availability of new treatments. HealthCare data are not exploited enough to identify inappropriate prescription patterns. Earlier recognition of complex endocrine disorders, liver failure, sarcopenia and fractures are needed to program, in advance, the best long-term management, through de-prescription and re-calibration of drug use.

Cancer

SoA: Cancer represents one of the most urgent and complex issues of current biomedical research and unequivocally deserves to be included in a national network for the optimization of its management. The complexity, and the etiopathogenetic and prognostic heterogeneity of oncological disease makes the application of precision medicine imperative, which can address individualized pathways for diagnosis and treatment of patients with cancer, to achieve an optimal clinical outcome. This is essential for indolent/recurrent/long-term neoplasms that burden the NHS (breast, prostate, colorectal) and for aggressive neoplasms that often present in an advanced stage (head and neck, lung, pancreas, hepato-biliary). The proper management of the oncological patient is multidisciplinary and must be integrated between the various specialist professionals and caregivers. In addition, with the recent introduction of immunotherapy, that has improved life expectancy in cancer patients' new ways for stratifying patients to develop personalized immunotherapies are needed (i.e. "circulome" (e.g., tumor DNA, immune cells, soluble factors and free or extracellular-associated non-coding RNAs represents a major step forward toward the prediction of response to immunotherapy).

The scenario of the fight against cancer is rapidly changing. The new personalized therapies, based on molecular profiling and on the identification of alterations responsible for tumor growth, allow physicians to evaluate more therapeutic options for the patient, but that often have high costs posing a challenge to the health system in terms of sustainability. Abrupt change in clinical practice must be accompanied using digital tools to address the challenges that this change provides.

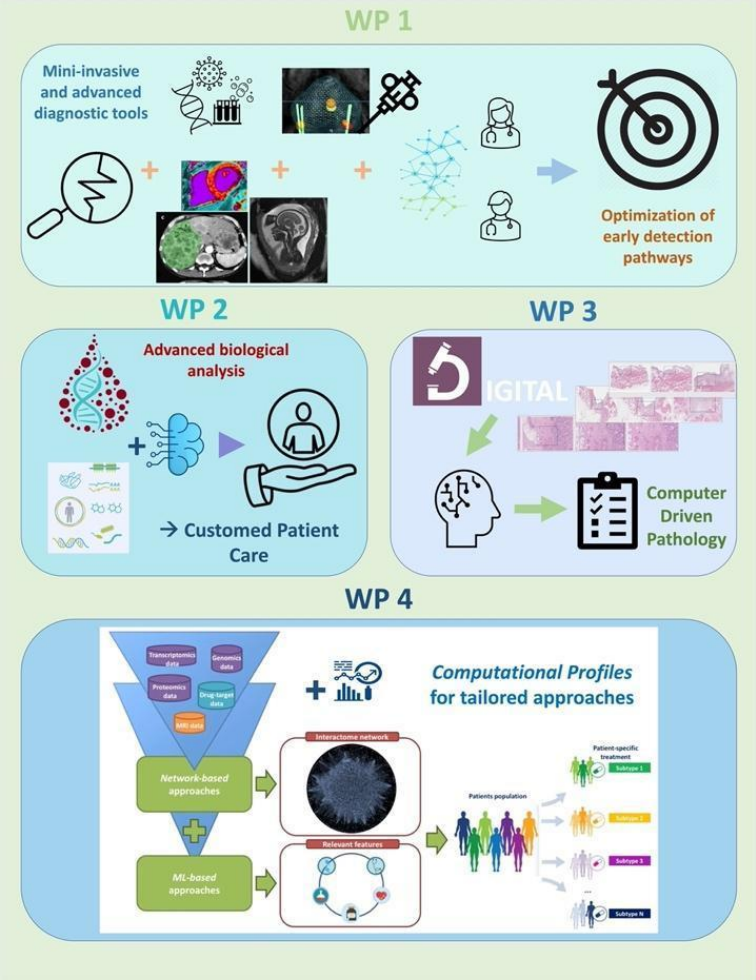
Issues: Cancer is a complex disease that demands an integrative and dynamic approach. To address this issue a set of cutting-edge technologies (i.e. genomics, proteomics, radiomics) along with AI approaches will be used as a strategy to implement hi-precision oncological diagnostics. For example, immunotherapy efficacy must be assessed considering a correct homing of effector leukocytes, the activation of their effector functions and the possibility to predict response; also, the so-called "circulome" should be further evaluated (e.g., tumor DNA, immune cells, soluble factors and free or extracellular-associated non-coding RNAs represents a major step forward toward the prediction of response to immunotherapy),

Finally, integration of molecular data with advanced quantitative imaging and other healthcare data will offer a tool for improved precision diagnostics and staging, which may allow personalized therapy.

These issues will all be addressed in WPs included in Spoke 4.

2. General objective:

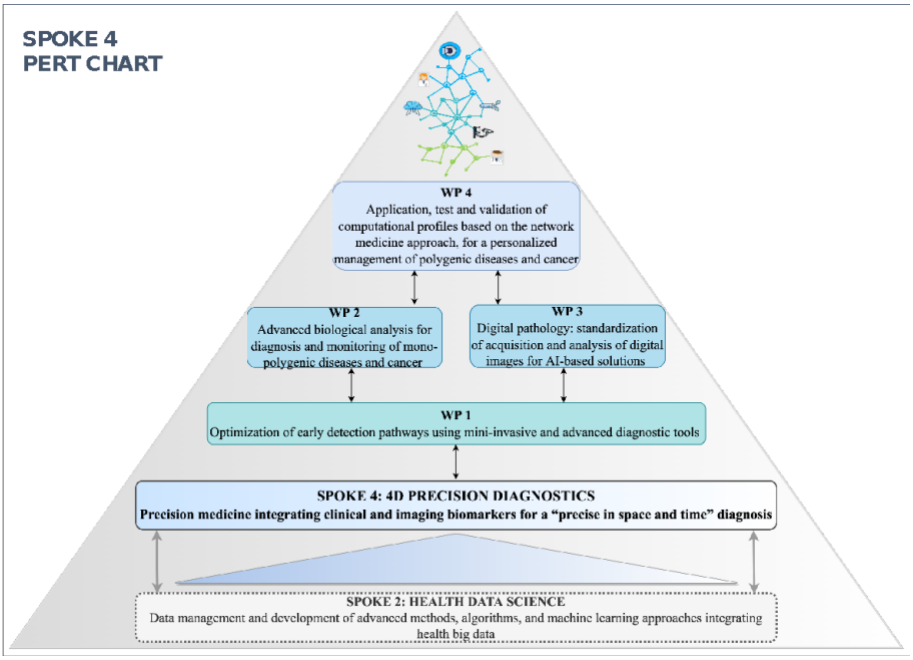
The overall objective of Spoke 4 is to deliver new, cost-effective, evidence-based, predictive risk-based and non-invasive diagnostic pathways for faster, earlier, more precise, accessible, and affordable early detection and screening of mono- and polygenic diseases and cancer. This proposal represents an opportunity to define early pathways for precision medicine focused on complex and chronic diseases care, supported by the integrated analysis of digital data including bioimaging, omics and data derived from medical devices, performed using computational tools (AI and network medicine). The main deliverables of the Spoke 4 will be the use and implementation of the computational tools developed in Spoke 2 in the clinical practice. Indeed, during the clinical studies in Spoke 4, large amounts of healthcare data (i.e. electronic medical records including clinical/environmental data and laboratory testing, diagnostic imaging, digital pathology, genetics and molecular phenotyping and omics data) will be collected and analyzed to define disease-specific stratification biomarkers and personalized diagnostic and prognostic pathways. Spoke 4 will significantly go beyond the current state of the art and develop innovative pathways for complex diseases, precision diagnostic pathways, exploiting digital technologies and computational methodologies. For the development of innovative pathways, technological innovations will be supported by standardized clinical methods to integrate high quality data (from acquisition to interpretation) and transform patient care. Spoke 4 will address these goals by bringing together 11 Partners and developing a network of research and clinical care, combining the skills of highly experienced centers. Spoke 4 is also intended to demonstrate new benefits in different fields of biomedical research, impacting multiple vertical markets. Again, the challenge for the advancement of the new "standard of care" and biomedical scientific research is the application of advanced technologies to health data, to achieve a high and equal level of specialization for complex disease management. Indeed, to develop effective preventive interventions and optimize diagnostic actions, the development of tools to interpret healthcare data derived from multicenter clinical validation studies is required on an unprecedented scale, requiring continuous and collaborative initiatives that advance discovery and transform the delivery of patient-centric healthcare based on precision medicine. The strength of the proposal is based on its interconnected multidisciplinarity that would provide a matrix in which each technological objective converges with clinical once and vice versa. The pathways proposed will be supported by a research infrastructure aimed at the cooperative design of innovative methodologies and their testing in the clinical practice, through clinical trials producing high-level open-access evidence for the scientific communities and policy makers. The proposed solutions will also aim to be ready usable by Italian health professionals. Spoke 4 will contribute to implement the



NRP MISSION 4 "Education and Research" component to strengthen the digital and the technical-scientific skills, the research and technology transfer within the Italian territory. Also, Spoke 4 will contribute to the EC's 'Europe's Beating Cancer Plan' flagship initiatives. Spoke 4 will moreover explore intersections with European programmes, such as the Innovative Health Initiatives, the EU4Health and the Digital Europe programmes, or the European Universities Initiative, and seek synergies with projects funded under these programmes. Spoke's **Scientific Advisory Board**: Rami Aqeilan (Jerusalem Univ, Israel), Nichlas Bazan (LSU University, Luisiana, New Orleans, USA), Thomi Brunner (Univ. Konstanza, DE).

3. Project WPs structure

Spoke 4 will develop on a pyramidal scaffolding with Spoke 2 as the pre-requisite for its implementation. At the base of the pyramid WP 1 will set the standard for the definition of new precision diagnostic pathways for mono- and polygenic diseases and cancer. WP 2 and WP 3 will promote the development of advanced biological analysis and digital pathology, as mid-term goals, using the healthcare data collected in WP 1 and the technological tools developed in Spoke 2. Finally, at the apex of the pyramid advanced analysis of data will be performed using the network analysis approach for defining tailored approaches and disease specific computational profiles.



WP 1: Optimization of early detection pathways using mini-invasive and advanced diagnostic tools **(Leader: Sapienza)**

Proposed research: The proposal represents an opportunity to define risk-based early detection pathways for precision medicine, supported by the integrated analysis of bioimaging, omics and all the health data available in digital format (integrated diagnostics, including IVD tests), through the deployment of the technological innovation developed in Spoke 2. The innovation of the project translates into readily available new ways of non-invasive early detection, using advanced technologies. In this WP, in addition to integration of bioimaging for early diagnosis of polygenic diseases (i.e. cardiovascular, and metabolic diseases) and cancer, innovative "intelligent" software for biopsy planning and of robotic systems will be implemented. Innovative biopsy methods are essential for the classification of oncological pathologies and for their effective diagnosis. UNIRM1 has already witnessed a remarkable success in the past with the use of innovative software/hardware for precise biopsy and pre-operative planning (i.e Mammotome for stereotactic breast biopsy; elastic fusion of ultrasound and MRI images – TRUS/MRI fusion biopsy - and MRI in-bore biopsy, for prostate and gynecological tumors). These tools have allowed a significantly higher diagnostic accuracy compared to older methods. In addition, since the chance of a successful of surgical treatments is highly dependent on improved pre-operative histological characterization and diseased tissue identification during the surgical treatment, it is essential to implement endoscopic, micro-

robotic and optical approaches and develop a real-time in situ detection to delimit the tumor margins, improving the prevention of cancer local recurrence and limiting the burden of resections. Furthermore, the precise evaluation of dose in all the organs of a radiotherapy patient is a requirement of the European Directive 59/2013, and for RMT, this is an unmet need, and the community lacks detectors to measure the biodistribution of β- tracers in pre-clinical tests. That is why this WP aims also at developing innovative techniques such as: new radiotracers for β-RGS to be able to target a larger number of tumors (previous experience demonstrated successful outcomes with marked MIBG with Y90 for neuroendocrine tumors); low energy detectors for radio-guided surgery, dosimetry in RMT, and evaluate biodistribution in animal tests of β- radiotracers (previous experience: developed probes based on p-terphenyl crystals read out by SiPMs); the new scintillators patented by UNIRM1 or devices directly based on solid-state devices. (β-RGS-det in the following); endoscope based on multiple methods of identifying the constitution and properties of tissues, thus allowing us to perform real-time optical biopsy (prev. experience: propagation of light in multimodal optical fibers, allowing for imaging based on linear and nonlinear fluorescence processes); and remotely guided micro-robots for on-site biopsies.

Objectives: To define and validate innovative pathways for complex diseases' early diagnosis (integration of demographic data, imaging, tumor margin delineation, molecular data and liquid biopsy biomarkers) to impact citizens' health, using key enabling technologies (highly performing computational tools).

Task 1.1: Integrated bioimaging for early diagnosis of polygenic diseases (e.g., cardiovascular and metabolic diseases) and cancer

Excutors	Starting month	Ending month	Duration (months)
Sapienza/TorVergata/UniMIB/UnivPM/UniMORE	1	24	24

Task aims: *i)* to provide data obtained with pilot studies, in order to create anonymized shared databases, based on algorithms developed by Spoke 2; *ii)* to validate new diagnostic pathways using non-invasive and advanced imaging tools (i.e. ultrasound, in vivo confocal, MRI, PET, and radiomics), integrated with innovative clinical data acquisition (chronobiology, remote monitoring, biomarkers repurposing), based on imaging as biomarkers, post-processed from the data shared through the platform; HTA affordability analysis will be performed; iii) features extraction (radiomics) from PET/CT images for prognostic purposes and bio-simulation including an appropriate cellular kinetic model, the morphological rules for tumor expansion and shrinkage, the cell killing and the hypoxia model; *iv)* to apply artificial intelligence algorithms to reduce the variability of the diagnostic performance of imaging tools and integrated biomarkers.

Milestones: ***MS1.1.1.*** 50% of patients recruited; ***MS1.1.2*** Definition of novel integrated imaging and clinical biomarkers to deliver novel cost-effective diagnostic pathways; ***MS1.1.3*** Number of PET scans acquired; ***MS1.1.4*** AI-algorithms training and validations sets available.
Deliverables: ***D1.1.1***. Analysis of stored patients' data previously enrolled in clinical studies for early diagnosis of each polygenic disease and cancer. ***D1.1.2*** Definition of new imaging biomarkers and computational profiles, validation of identified novel diagnostic pathways and relative standard cost-effectiveness model. (HTA); D1.1.3 A set of features for optimizing both specificity and sensitivity of PET/CT for predictive purpose; ***D1.1.4*** New artificial intelligence-based algorithms for organ-specific segmentation, index lesion detection, pre-biopsy modelling and clinical condition.

Task 1.2: Assessing the applicability of innovative "intelligent" software for decision-making and biopsy planning and with implementation of robotic systems for organ-targeted biopsies.

Excutors	Starting month	Ending month	Duration (months)
Sapienza	1	28	28

This task aims: *i)* To improve planning of biopsy procedures; ii) to validate the diagnostic accuracy of AI-based software incorporated into targeted biopsy workstations for the detection of target lesions; *iii)* to test the inter-user reproducibility of AI-empowered solutions.

Milestones: ***MS1.2.1.*** Multidisciplinary meetings to draft optimization protocols*;* ***MS1.2.2.*** AI-algorithms training and validations sets available; ***MS1.2.3.*** 50% of data needed for the reproducibility testing collected.
Deliverables: ***D1.2.1.*** Report on biopsy procedure planning. ***D1.2.2.*** New artificial intelligence-based algorithms for biopsy planning; ***D1.2.3.*** Final databases for AI solutions inter-rater reproducibility testing.

Task 1.3: Development of novel techniques for precision diagnostics: from low energy beta tracers and detectors to nonlinear multimode fibers for endoscopy and real-time optical biopsy.

Excutors	Starting month	Ending month	Duration (months)
Sapienza	10	30	20

This task will develop: *i)* and test new radiotracers marked with beta emitters and corresponding detectors to enlarge the range of tutors for which can be performed margin delineation and absorbed dose measurement in RT treatments; *ii)* an endoscope based on an evolution of the complex propagation of light in multimodal optical fibers for real-time optical biopsy; *iii)* remotely guided micro-robots for on-site biopsies.

Milestones: ***MS1.3.1*** Preparation and validation in cells of 2-3 new beta emitting radiotracers suitable for biodistribution studies in mice; ***MS1.3.2*** Design of one software (simulation) and/or hardware (detectors) tool for beta radiation detection for nuclear medicine; ***MS1.3.3*** performance validation and initial testing evaluation of the multimodal optical fiber endoscope.
Deliverables: ***D1.3.1*** Identification of at least 1 new beta minus emitting tracer with a PK profile in mice suitable for testing in (pre)-clinical tumor models; ***D1.3.2*** one software (simulation) and/or hardware (detectors) tool for beta radiation detection for nuclear medicine; ***D1.3.3*** Real-time diagnosis capability of the endoscopic system device on 3D-bioprinted cancerous tissues.

WP 2: Advanced biological analysis for diagnosis and monitoring of mono-polygenic diseases and cancer **(Leader: Sapienza)**

Proposed research: The identification of novel biological markers, including markers of tumor cell metabolism, and quantitative assessment of mono- and polygenic diseases and cancer represent the major challenges for hi-precision diagnostics and would be a critical need to design a tailored therapy adapted to type- and stage-specific patients. This is relevant for either hematologic (i.e. myeloid and lymphoid leukemias) and solid tumours (i.e. colon, pancreas, hepao-biliary, breast and lung cancers, head and neck neoplasms, melanoma, medulloblastoma), but also for cardiovascular and metabolic diseases, for which several studies have provided evidence that specific tumour-associated molecular signatures can be used for more precise patient's stratification. High-throughput and innovative approaches combined with artificial intelligence (AI) and machine learning algorithms offer the opportunity to obtain new data from tissue and biological fluids samples, integrate and translate them into actionable information allowing earlier diagnosis and precise treatment options.

The Spoke will produce molecular information that will be integrated, using AI algorithms, with quantitative imaging and other features of patients with mono-polygenic diseases and cancer. The Teamwork can rely on infrastructures, platforms and biobanked samples (tissue and liquid biopsies) while new samples will be prospectively collected. Genomic, proteomic, metabolomic, transcriptomic, miRNAs and immune profiling data will be generated using innovative platforms (e.i. GeoMx DSP) allows a molecular characterization at single-cell level, in tissue samples. Liquid biopsy samples obtained at diagnosis, prior to any treatment, and during follow-up will be evaluated for cfDNA, ctDNA, small non-coding RNAs (free and linked to exosomes), and immune biomarkers. Data will be validated in independent cohorts and integrated with radiomic data and clinico-pathological features, using an AI approach to obtain a quantitative assessment of tumour and to identify novel and powerful non-invasive biomarkers usable in the clinical practice

In addition, this Spoke proposes to design and validate a genomic workflow based on a multidisciplinary approach directed to offer the best diagnostic path. The program will benefit from an already instituted network of 9 partners coordinated by the UNIRM1 well integrated into national and international initiatives (e.g., national rare diseases networks, ERNs). The enrolled cohorts will be stratified based on clinical presentation, genetic/genomic data and availability of biological specimens. The state-of-the-art technologies will be used to obtain a personalised genomic card for each individual and FAIR data. This approach is expected to improve the diagnostic yield for rare diseases in the SSN. This task will anticipate the identification of novel disease genes/novel nosology entities. The relevant genomic variants identified in the project will be clinically and functionally validated (2D and 3D patient-specific cellular models including brain organoids).

Objective: To produce molecular information, generating genomic, proteomic, metabolomic, transcriptomic, miRNAs and immune profiling data, using innovative platforms allowing molecular characterization of complex and polygenic diseases.

Task 2.1: Alternative matrix for biological monitoring of inorganic lead and cancer (Saliva as potential noninvasive alternative)

Executors	Starting month	Ending month	Duration (months)
Sapienza/UniCA/UniFG/UniMORE	4	32	28

This task aims to establish a circulating extracellular vesicles (EV) profiling in solid cancer (breast, head and neck, GI)

Milestones: ***MS2.1.1*** to identify anti-HER2/EGFR agents predictive biomarkers (protein, miRNA) in EV correlating clinical parameters in solid cancer (breast, head and neck); ***MS2.1.2*** to assess EV miRNAs profiles among mutation carriers (BRCA et other genes) affected by solid cancers and mutation carriers without solid cancers; ***MS2.1.3*** to correlate the identified biomarkers with radiomic features of response evaluating the presence of significant differences in the different solid cancers settings, also considering BRCA (for breast only) mutated and no mutated patients.
Deliverable: **D2.1.1** creation of a EV-based predictive profile in solid cancer treated by anti-EGF receptors; **D2.1.2** creation of a EV-based mirnome prognostic profile in BRCA carriers; **D2.1.3** creation of an algorithm to combine radiomic features with the EV-based profiles.

Task 2.2: Molecular profiling of circulating nucleic acids by liquid biopsy for a more in-depth characterization, classification and stratification of polygenic disease, cancer and tumor aggressiveness and assessment of response to therapy

Excutors	Starting month	Ending month	Duration (months)
Sapienza/UniBO/UniCT/UniPA	6	33	27

In this task the Partners will: *i)* validate of the new tissue and circulating biomarkers, providing a set of new tissue and circulating biomarkers for personalized approach that will allow a better stratification of oncologic patients; *ii)* validate new tissue and circulating biomarkers, providing a set of new tissue and circulating biomarkers for personalized approach that will allow a better stratification of polygenic diseases (such as cardiovascular, endo-metabolic, immunological, etc); *iii)* design and validate a genomic workflow based on a multidisciplinary approach directed to offer the best diagnostic pathway; *iv*) to perform systematic evaluation of accuracy of using liquid biopsy for cancer.

Milestones: ***MS2.2.1.*** Specific panels of genetic and molecular data of oncologic patients; ***MS2.2.2***. Specific panels of genetic and molecular data of polygenic diseases*;* ***MS2.2.3*** isolation, vitality test and cell culture of viable cells from new LB platforms, evaluated and compared to cell search for cancer diagnosis; ***MS2.2.4*** Multidisciplinary meetings to draft optimization protocols.
Deliverables: ***D2.2.1.*** Database on genetic and molecular on both tissue and circulating cells in oncologic patients (liquid biopsies); ***D2.2.2.*** Database on genetic and molecular on both tissue and circulating cells in polygenic diseases (liquid biopsies); ***D2.2.3*** selection of CTCs and their precise characterization through the analysis of surface markers and molecular genetic analysis obtained by techniques of qRT-PCR/NGS; ***D2.2.4*** A novel genomic workflow for disease diagnosis.

Task 2.3: Multi-omics approach in monogenic diseases, complex disorders and rare diseases and cancer, supported by multilevel AI tools developed by Spoke 2

Excutors	Starting month	Ending month	Duration (months)
Sant'Orsola/Sapienza/UniBO/UniCT	10	34	24

This task aims to: *i)* To set-up a diffuse Italian genomic center to speed up the diagnostic pathway, therapy and monitoring relapse and response to treatment, for monogenic diseases, complex disorders and rare diseases and cancer; *ii)* to define the combination and sequence of omics approaches that most efficiently allows patients stratification and early diagnosis in selected disorders, exploiting multi-omic signatures developed using machine learning methods (Spoke 2); *iii)* to identify and evaluate health policy, economic and ethical issues for the introduction of these approaches in the clinical practice offering flowcharts and guidelines for guiding the use of novel technologies in the diagnostic setting.

Milestone: ***MS2.3.1*** Cohorts' collection and stratification; ***MS2.3.2*** Multi-OMICs approach completed for 125 patients; ***MS2.3.3*** Generation and validation of a dedicated platform for clinical and multi-OMICS data; ***MS2.3.4*** Application of AI tools integrating and intersecting multilevel multiple levels data (biological, clinical, pathological and imaging); ***MS2.3.5*** Design and dissemination of a survey dedicated to evaluate the suitability and sustainability of the proposed approach; ***MS2.3.6*** Two open-source software release for multi-omic signatures and for outcome prediction.
Deliverable: ***D2.3.1*** Assessment of the efficacy of a workflow based on an integrated genome scan in the diagnosis of monogenic diseases, complex disorders and rare tumors; ***D2.3.2*** Analysis of the sustainability of the application of these genomic tools in clinical practice; ***D2.3.3*** Application of multilevel AI tools for data integration and interpretation; ***D2.3.4*** Design of recommendations for the implementation of multi-OMICs approach in diagnostics; ***D2.3.5*** Software release for prediction of patients trajectories using the Markov models and reinforcement learning and final report.

WP 3: Digital pathology: standardization of acquisition and analysis of digital images for AI-based solutions **(Leader: UniVR)**

Proposed research: In this spoke a Computer Driven Pathology Assessment will be implemented. The use of digital pathology and virtual microscopy will provide a rapid access to archived digital images, accelerated access to samples, and faster response delivery, especially for complex cases. Histological specimens will be collected for digital acquisition using special scanners. Clinicians will have access to data and images through an online server for shared consultation. The pathologist will examine the digital images of the tumor sections and will note the morphological characteristics necessary for the diagnosis and the stratification of the risk of aggression. The set of histopathological characteristics required will be defined according to the most updated diagnostic criteria for the specific type of neoplasm. A training cohort will be used to use artificial intelligence models for the automatic detection of specific characteristics. Developed in Spoke 2. The performance of the models will then be evaluated to determine if they accurately identify the morphological characteristics in the clinical setting. The standardized review will allow the identification of new histopathological and immunophenotypic variables that may be useful for prognosis stratification. To achieve the proposed objectives, AI-based standardization algorithms of digital formats and quality standards control of biomolecular, clinical, histopathological data (digital pathology), and diagnostic imaging will be developed by Spoke 2.
Objective: To use the dedicated inter-operable digital framework *developed by Spoke 2* for both data and knowledge sharing and analysis of digitalized integrated diagnostics data by AI-based standardization algorithms of digital formats and quality control. The main technological deliverable of the project will be a digital image acquisition framework to permit the evaluation and diagnosis of integrated digital image pathology diagnostic data for computer driven pathology assessment and.

These will also provide large datasets for novel research and further integration with molecular omics data using machine learning and artificial intelligence computational analysis.

Task 3.1: Pathology sample collection for digital acquisition using dedicated scanners

Excutors	Starting month	Ending month	Duration (months)
Sapienza/UniVR	4	28	24

This task aims to: *i)* create the technical requirements for a centralized digital imaging facility; *ii)* Use of storage system developed by Spoke 2 to house the physical slides to ensure their safe storage and traceability and to facilitate the immediate use of the digital images for the evaluation of a fit for blocks for downstream omics selection; *iii)* Creation of standard operating procedures for preparation and acquisition of the digital slides to ensure quality control and guarantee downstream comparison of different sets; *iv)* Development and validation of workflow for sample collection and transport of slides to the centralized digital imaging facility.

Milestones: ***MS3.1.1*** Creation of technical requirements for digital imaging facility**;** ***MS3.1.2*** Employment of the automated storage system; ***MS3.1.3*** Standardized protocols; ***MS3.1.4*** Creation of workflow for collection and transport among the participating centers.
Deliverables: ***D3.1.1*** Creation of digital imaging facility**;** ***D3.1.2*** Employment of the automated storage system; ***D3.1.3*** Standardized protocols handbook; ***D3.1.4*** Validation of workflow for collection and transport among the participating centers.

Task 3.2: Definition of specific histopathologic panels for the diagnosis and prognostic stratification of complex polygenic diseases and cancer

Excutors	Starting month	Ending month	Duration (months)
Sapienza/UniPA/UniPI/ UniVR	10	30	20

This task aims: *i)* to define the histopathological panels for diagnosis and prognosis stratification of diseases included in the project, comparing them to the feature (morphological and texture) extracted from histopathological images by Spoke 2 (Task 2.2), with employment of the repository system of digital histopathological images developed by Spoke 2; *ii)* to design of standard operating procedure for histopathologic panels; *iii)* to analyze in details the cancer immune environment and identify immune signatures predictive of response to immune checkpoint inhibitors and activation of the immune system in response to chemotherapy; *iv*) to evaluate stromal cell-type repertoire and specific gene expression signatures in prostate and breast cancer as prognostic markers.

Milestones: ***MS3.2.1*** Definition of histopathological panels; ***MS3.2.2*** Design of standard operating procedure for histopathologic panels; ***MS3.2.3*** Employment of the repository system of digital histopathological images, developed by Spoke 2; ***MS3.2.4:*** stroma-enriched FFPE tissue samples TMAs generated and multiplex immunofluorescence performed, and slides digitised.
Deliverables: ***D3.2.1*** Validation of histopathological panels; ***D3.2.2*** Standard operating procedure

handbook for histopathologic panels; ***D3.2.3*** Repository of integrated digital histopathological and immuno-phenotypical images; ***D3.2.4*** Creation of ad hoc ad hoc in vitro models able to recapitulate specific molecular events of interest

Task 3.3: Computer Driven Pathology Assessment

Excutors	Starting month	Ending month	Duration (months)
Sapienza/ UniMIB/UniMORE/UniVR	12	32	20

This task sought to: *i)* exploit the expert opinion database (developed by Spoke 2) to feed the digital repository in consensus; *ii) u*se machine learning algorithms (developed in Spoke 2); iii) validate a computer driven AI pathology expert system in a clinical context.

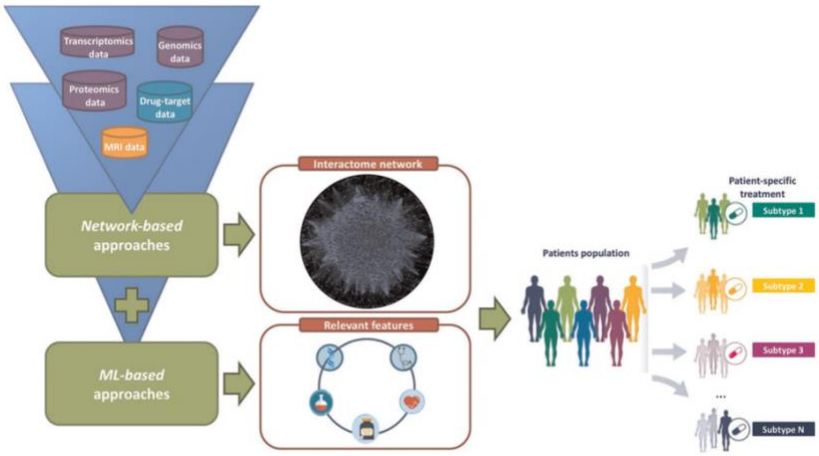
Milestones: ***MS3.3.1*** Use of an expert opinion database to feed the digital repository; ***MS3.3.2*** Qualitative and quantitative evaluation with extraction of morphological computational features for selection of patho-phenotypic features**;** ***MS3.3.3*** Evaluate market available computer driven AI pathology expert systems based on cancer types.
Deliverables: ***D3.3.1*** Achievement of standardization for acquisition and interpretation of digital formats, application and test of AI-based algorithms for quality assurance and control of biomolecular, clinical, digital pathology, and diagnostic imaging data; ***D3.3.2*** results on the use of machine learning algorithms, developed in Spoke 2; ***D3.3.3*** Provide a clinically validated computer driven AI pathology expert system.

WP 4: Application, test and validation of computational profiles based on the network medicine approach, for a personalized management of polygenic diseases and cancer **(Leader: Sapienza)**

Proposed research: This WP will be based on a case-study-driven approach, to ensure the feasibility in the allocated project timeframe and to pave the way for a strong and long-lasting impact of the project outcomes. Data derived from healthcare sources will be analysed and integrated with network analytical methods to identify new and unbiased relationships between health status and risk factors for a given disease. This way, individual clustering will be performed to customize environmental exposure, promote behavioural changes, predict the disease, identify biomarkers of the disease, and optimize drug administration. Computational approaches based on the theory of networks build on the network of interactions between individual cellular components (i.e., the human interactome), represent a valuable tool and will be developed, among others, for efficient disease screening/diagnosing, but also for determining potential new indications for approved drugs with well-established pharmacokinetic and pharmacodynamic (drug repositioning), safety and tolerability profiles or previously unidentified adverse events. UNIRM1 has a long-standing experience in network medicine approaches being part of a worldwide alliance and strong collaboration with Harvard medical School.

Objective: To apply network medicine tools developed in SPOKE 2 for analyzing and integrating omics data (genomics, transcriptomics, epigenomics, metabolomics, proteomics), imaging, laboratory, and clinical data of the patient (signs/symptoms, medical history, as well as relevant

demographic data, such as age, ethnicity, and sex) to understand the heterogeneity of human diseases and thus pave the way for precision medicine care.



Schematic representation of the integrated computational approach for precision medicine applicable to different pathologies. The cylindrical shapes represent different data types given as input of the computational model: drug-target therapeutic associations data (drug-target data), genomic, transcriptomics, and proteomics data, and bioimaging data. The rectangular shapes represent the innovative parts that will be ad-hoc developed: (i) network-based approaches to identify disease genes and disease pathways; and (ii) application of ML approaches to identify the more relevant morphological features related to the pathology for tailored approaches and definition of computational profiles.

Task 4.1: Validation of network-based tools developed by Spoke 2, for analyzing and integrating omics, imaging, and clinical data to understand the heterogeneity of human diseases for precision medicine.

Excutors	Starting month	Ending month	Duration (months)
Sapienza/UniVR	10	34	24

This task will: *i)* interpretate the collected health care data on platform (developed by Spoke 2); *ii)* build phenotypic networks applied to oncological diseases; *iii)* Build phenotypic networks applied to polygenic diseases.

Milestones: ***MS4.1.1*** 50% of patients recruited.
Deliverable: ***D4.1.1*** use of AI-powered tool (ML) developed in SPOKE 2, for patients' classification and stratification according to disease stage; ***D4.1.2*** Database for phenotypic networks validation on large cohorts of oncologic patients; ***D4.1.3*** Database for phenotypic networks validation on large cohorts of patients with polygenic diseases.

Task 4.2: Test of benchmark analytic tools based on interpretable available network analytic models for risk stratification, prediction of relapse and complications rate in cancer and polygenic diseases, considering the possible impact of early-life stressors, analyzed by Spoke 7

Excutors	Starting month	Ending month	Duration (months)
Sapienza/UniFG	12	36	24

This task will: *i)* apply a ML model developed in SPOKE 2 to specific precision medicine analysis objectives for Network Analysis; *ii)* apply and test a combined application of Artificial Intelligence, Machine Learning and Semantic Technologies for cancer and polygenic diseases; *iii)* generate personalized risk prediction models based on early-life stressors (implemented in SPOKE 2).

Milestones: ***MS4.2.1.*** AI-algorithms (ML) training and validations sets available; ***MS4.2.2*** Testing tools and protocols for data preparation, data ingestion and data analytics for Network Analysis; ***MS4.2.3.*** Best metrics for evaluation of Network Analysis methods and tools.
Deliverables: ***D4.2.1.*** ML model applied to cancer and polygenic diseases; ***D4.2.2.*** Release of network analysis tools for data analytics in precision medicine; ***D4.2.3*** Reports on the relationships of early-life exposures with health trajectories and NCDs risk from birth to adulthood and Recommendations for the early, stratified and targeted prevention of adverse pregnancy outcomes and NCDs.

Task 4.3: Application of the network analysis-based algorithms for the definition of new early diagnosis and screening pathways of polygenic and oncological diseases

Excutors	Starting month	Ending month	Duration (months)
Sapienza/UniCA	14	36	22

This task will define: *i)* analysis of global data for the implementation of precision medicine, to identify cluster of patients at risk, suitable for promoting automated systems of early diagnosis, when the disease has already developed, as well as screening and surveillance programs; *ii)* validation of the new computational algorithms based on network science approach, with the definition of new biomarkers and computational profiles to implement precision medicine in oncology and polygenic diseases; *iii)* Clinical application of the identified new algorithm for the definition of new primary and secondary screening pathways to fight against the incidence of the oncological and polygenic diseases.

Milestones: ***MS4.3.1*** 50% of data needed for the reproducibility testing collected; ***MS4.3.2*** Validation of network medicine algorithms on large cohorts of integrated diagnostic data for identification of population at risk; ***MS4.3.3*** Ethics approvals complete**.**
Deliverables: ***D4.3.1*** Datasets on integrated diagnostic data for training of network analysis algorithms for identification of population at risk, of genetic driver mutations and immune checkpoints for response to therapy, of predictive factors of adverse drug reactions, and of prognostic factors of survival; ***D4.3.2*** Release of the validated network medicine algorithms**;** ***D4.3.3*** Clinical study protocols for multicenter randomized trials to test the clinical applicability on the validated algorithms.

SPOKE 5: Next Gen Therapeutics

"From silico to bedside" design and validation of innovative tailored and personalized therapeutic strategies

Description of the Spoke Activities

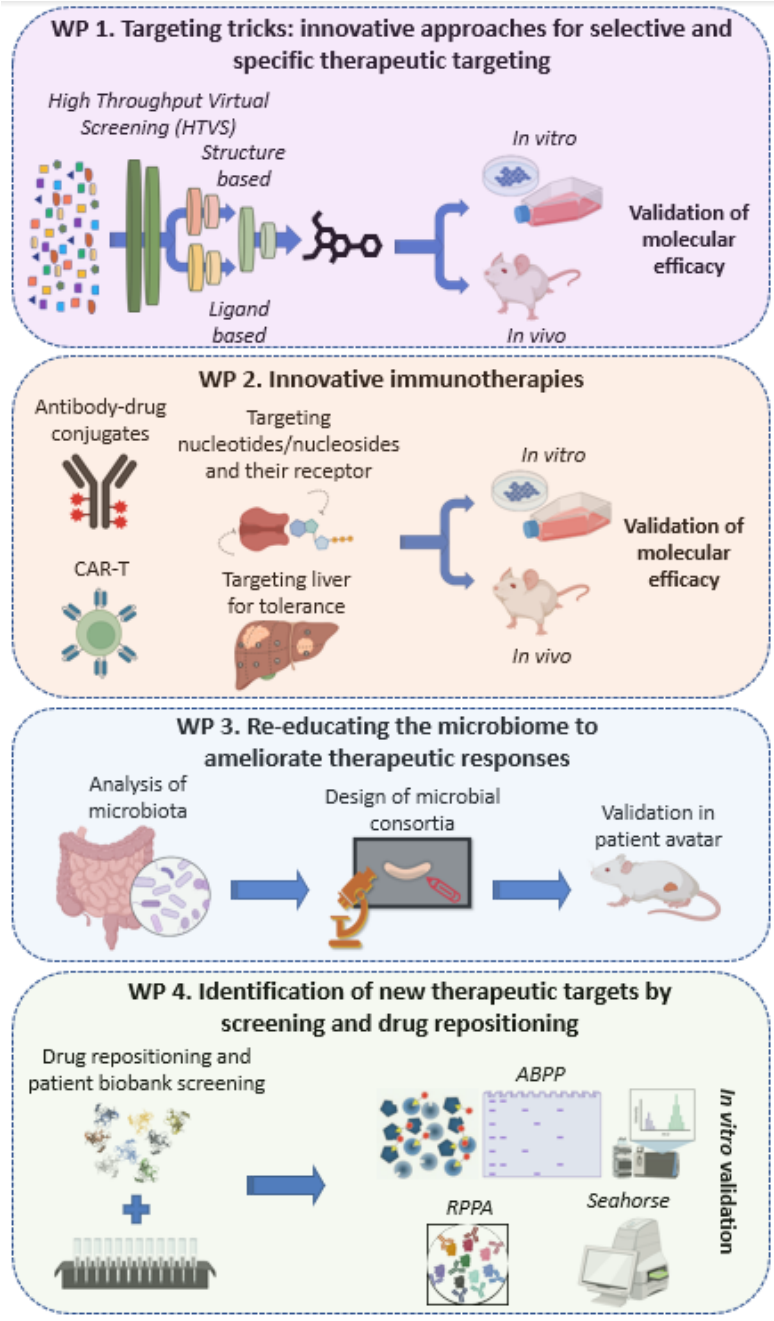
SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
UniMIB	CRO/ISS/M.Negri/Neuromed/Opella/ Sapienza/SIT/TLS/UniBO/UniCA/UniCT/ UniMIB/UniMORE/UniPA/UniPI/UniVR/ UPMC	1	36	36

1. Context description:

The costs and time needed to identify new lead compounds and taking them to the Investigational New Drug phase level is one of the limiting factors in the area of new therapeutics development. A bottle-neck element that needs to be addressed to accelerate the process and define cogent therapeutic targets is the identification of high-throughput pre-clinical screening systems, allowing to test drug efficacy with high reproducibility and predictability. This can be achieved only through the use of state-of-the-art human *in vitro* models, upon which compounds' effects can be compared. It is crucial to establish systems which efficiently verify drug specificity in view of personalized medicine approaches. Therefore, the model shall mimic the original disease in an extremely accurate way, should remain stable and reproducible in the behavior over time, also after genetic modifications are introduced to develop specific or customized assays.

2. General objective:

This spoke aims at developing innovative and comprehensive drug-screening and validation platforms allowing to overcome the limits of currently available systems and accelerate the identification of next-generation effective drugs in the field of precision medicine for oncology, cardiology, rare diseases, microbiota alterations etc. Platforms of this spoke are based upon the definition and implementation of a workflow composed by a series of intertwined *in silico*, *in vitro* and *in vivo* assays, tentatively grouped into successive stages of selection, progressively more refined and specific. A highly qualifying element of the spoke's approach is the use of unique human *in vitro* models, high throughput screenings, nanotechnologies and animal models available thanks to the expertise gathered together in the present network.



The spoke is conceived in order to achieve the following:
i) validating new targets and therapeutic effectors;
ii) assessing the *in vitro* efficacy of compounds libraries;
iii) determining the cellular and molecular targets on which the candidate drugs act;
iv) validating candidate drugs;
v) defining the key mechanisms of action that underlie the therapeutic efficacy of the drug;
vi) completing the analysis of their effects in pre-clinical settings with unprecedented accuracy. These approaches will enable the simultaneous definition of drug candidate and drug action mechanisms at the cellular and molecular level with an unprecedented level of predictivity. This, therefore, will allow filing dossiers for clinical trials in a more simple and reliable manner, incorporating experimental evidence. The experimental setting and the complementary expertise of the HealITALIA consortium make it possible to establish reproducible and quick pipelines for the preclinical validation of innovative therapies. The spoke 5 makes use of the **Scientific Advisory Board** that will be shared with Spoke 3: Ettore Appella, MD, National Cancer Institute (NIH), USA and Soldano Ferrone, MD, PhD, Harvard Medical School, Massachusetts General Hospital. Annalisa Scopinaro, UNIAMO (Italian Federation of Rare Diseases) President; Antonio Bertoletti PhD, Program in emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

Project WPs structure:

WP 1: Targeting tricks: innovative approaches for selective and specific therapeutic targeting **(Leader: Unipa)**

To develop successful therapeutic strategies for genetic diseases due to non-sense mutations or mutations causing functional defects.

Task 1.1: TRIDS that make the Tricks – Design, synthesis and validation of Translational Readthrough Inducing Drugs (TRIDS) to overcome nonsense mutations

Executors	Starting month	Ending month	Duration (months)
UniPA	1	30	30

This task will be pursued by identifying biologically active molecules capable of rescuing the expression of full and functional proteins in cellular and animal models. *In silico* analysis will be performed through High Throughput Virtual Screening (HTVS) using ligand-based and structure-based strategies to identify pharmacophores to be used for the screening of chemical libraries. Best candidates selected by HTVS will be synthesised and validated *in vitro* and *in vivo* for efficacy and specificity**.**

Task 1.1.1: In silico analysis through High Throughput Virtual Screening (HTVS) using ligand-based and structure-based strategies allowing to generate a pharmacophore to be used for the screening of chemical libraries to select new readthrough active molecules;
-Synthesis of the best candidates selected by HTVS;

Task 1.1.2: In vitro validation of the readthrough activity of selected TRIDs on different genetic diseases (e.g. Retinitis Pigmentosa, Fabry disease, Primary Immunodeficiency, Duchenne Muscular Dystrophy, Choroideremia).
-Selection of appropriate in vitro cell model systems to be used in order to validate the molecules activity;
-Study of protein expression and functionality after treatment with new TRIDs molecules
-Study of toxicity in vitro

Task 1.1.3: In vivo validation of the readthrough activity of selected TRIDs in specific murine models related to specific nonsense associated with genetic disease.
-Study of the safety profile in vivo.
- Study of the rescue of protein expression and functionality after molecules administration

Milestone: in silico Identification and synthesis of potential TRIDs.

Deliverable: In vitro and in vivo validation of the activity of selected TRIDs on different genetic diseases (e.g. Retinitis Pigmentosa, Fabry disease, Primary Immunodeficiencies, Duchenne Muscular Dystrophy, Choroideremia).

Task 1.2: Identification of correctors of misfolding and post-transcriptional functional defects of CFTR protein in Cystic Fibrosis

Executors	Starting month	Ending month	Duration (months)
UniPA	1	28	28

New correctors of mutant CFTR via *in silico* analysis using ligand-based and structure-based strategies will be rationally designed: docking studies will be performed to identify the putative

binding site of selected correctors. Chemical libraries will be sensitized and selected candidates as correctors of mutation *del*F508 able to produce synergistic effects with other CFTR modulators and validate them in vitro.

Task 1.2.1: Design of new correctors of mutant CFTR.
- In silico analysis using ligand-based and structure-based strategies.
- Docking studies to identify the putative binding site of selected correctors.

Task 1.2.2: Synthesis of new correctors of mutant CFTR able to produce synergistic effects with other CFTR modulators.
- Production of chemical libraries as suggested by in silico screening, to select new active molecules.
-Identification of the best candidates as correctors of mutation F508del.

Task 1.2.3: In vitro validation of the rescue of CFTR protein after correctors treatment in CF cell model systems
-Study of the activity of CFTR in CF cell model systems
-Study of toxicity in vitro

Milestone: Identification and synthesis of a set of potential correctors.
Deliverable: In vitro validation of a library of potential correctors of misfolding for CFTR.

WP2: Innovative immunotherapies **(Leader: UniMIB)**

To generate tools for the selective killing or control of pathogenic cells with the purpose to obtain a high treatment efficacy, contain side effects and reduce the time of therapy administration

Task 2.1: Synthesis of antibody-drug conjugates (ADCs) for the treatment of selected tumors

Executors	Starting month	Ending month	Duration (months)
CRO	1	30	30

This task aims to: *1)* Synthesize ADCs by conjugation of anti-GPC1, anti-GPC3, anti-CD138 mAbs with the cytotoxic drug emtansine; *2)* Chemical-physical characterize the synthesized ADCs; *3)* Evaluate ADC in-vitro killing capacity in tumor associated antigens (TAA) expressing cancer-derived cell line cells; *4)* Evaluation of in-vivo biodistribution and cell killing capacity of ADCs in TAA-expressing xenograft mouse models obtained by the injection of the TAA expressing cancer-derived cell line cells from Glioblastoma multiforme (GBM): Pancreatic ductal adenocarcinoma (PDAC); hepatocellular carcinoma (HCC); multiple myeloma (MM).
It will be defined:
1) the in-vitro capability to kill TAA expressing cancer-derived cell line cells for the custom-made produced anti-TAA mAbs; the viability values of cancer cell line cells upon mAbs treatment in-vitro; *2)* the capability of the custom-made produced anti-TAA mAbs to reach TAA-expressing cells in-vivo; the mAbs accumulation levels in the tumor masses and mouse organs by in-vivo and ex-vivo imaging in xenograft mouse models; *3)* The accumulation of mAbs-dependent and activity of immune system-related cell populations (*e.g.* macrophages, NK cells).

Milestones: ***i)*** chemical-physical characterization of the synthesized ADCs; ***ii)*** viability values of cancer cell line cells upon ADC treatment in-vitro.

Deliverable: new therapeutic anti-GPC1, anti-GPC3, anti-CD138 ADCs targeting GBM, PDAC, HCC, MM.

Task 2.2: Targeting nucleotides/nucleosides and their receptors and extracellular catabolism of nucleotide substrates to affect immunotherapy responses against solid tumors

Executors	Starting month	Ending month	Duration (months)
Sapienza/UniMORE	6	36	30

Validation of purinergic check-points as viable targets in cancer therapy, in particular development of potent and selective P2X7 and A2A antagonists; pharmacological inhibition of the ectoenzymatic activities of CD38, CD39, CD73 and CD203a, with the goal of i) blocking or reducing the generation of immunosuppressive adenosine in the TME from the catabolism of ATP, NAD and cGAMP; ii) boosting extracellular ATP and cGAMP stability to enhance their immunostimulatory functions.

Milestone: In vitro and in vivo validation of clemastine and polymyxin B as potent and selective positive allosteric modulators of the P2X7 receptor; elucidation of role and functionality of A2A and A2B adenosine receptor subtypes on cancer cells; identification of metabolic pathways contributing to adenosine generation and cGAMP catabolism.

Deliverable: Novel immunotherapeutic approaches for cancer therapy.

Task 2.3: Generation and characterization of CAR-modified cells for the treatment of solid tumors and fibrosis.

Executors	Starting month	Ending month	Duration (months)
CRO/UniMIB/UniPA/UniVR/UniMORE	1	36	36

Generation of CARs (i.e CIK, T) anti fibroblast activation protein (FAP) and anti-TAA (GPC1, GPC3, GD2 and CD138 to target GBM, PDAC; HCC and MM). CAR cells will be tested in vitro, and in vivo in appropriated mouse models. Studies of safety and efficacy will be conducted, and clinical protocols will be preliminary defined.

Task 2.3.1: generation and in vitro characterization of CAR cells
Objectives: to generate anti-FAP or anti-TAA CAR cells
Cloning and generation of CARs anti fibroblast activation protein (FAP) or anti-TAA in Sleeping Beauty (SB) transposon; viral and non-viral genetic engineering of cytokine-induced killer (CIK) cells to express anti-FAPor anti-TAA CARs and *in vitro* characterization.

Task 2.3.2: In vivo studies of safety and efficacy

Objectives: Assess the safety and efficacy of the anti-FAP and anti-TAACAR-CIK cells in relevant diseased animal models

Characterization of the effect of anti-FAP or anti -TAA CAR cells in a mouse model of hypertensive heart disease or bearing solid tumors. Reduced fibrosis in heart and rescued of both systolic and diastolic function or reduced tumor volume will be evaluated.

Task 2.3.2: Toward the development of a clinical protocol

Objectives: Set-up of a clinical protocol for anti-FAP or anti-TAA CAR-T cells for cardiac fibrosis in humans

Set-up a multicentric clinical study to assess the safety and feasibility of infusing CAR anti-FAP or anti-TAA in patients with cardiac fibrosis or bearing solid tumors.

Planning and management of a phase I clinical protocol of the viral and non-viral CAR cells anti-FAO or anti-TAA manufacturing platform.

The proof of concept of using CAR T cells to target cardiac fibrosis will pave the way for targeting other forms of human fibrotic disorders.

Milestone: viral and non-viral engineered CAR cells expressing anti-FAP and anti-TAA receptors.

Deliverable: planning and management of a phase I clinical protocol of the viral and non-viral CAR-FAP and CAR-TAA manufacturing platform.

Task 2.4 Development of a new generation immunotherapy to address unmet needs in cancer, infection and autoimmune disease

Executors	Starting month	Ending month	Duration (months)
Sapienza/TLS/UniPA/UniVR	1	30	30

Bispecific T-cell engagers (BiTE) are bispecific antibody constructs with a unique function, simultaneously binding a target antigen and a surface molecule (typically CD3) on T cells designed to redirect the immune system to recognize and kill target cells. Bispecific T-cell engaging receptors (TCERs) are off-the-shelf biologics that leverage the body's immune system by redirecting and activating T cells towards target cells, regardless of the T-cells' intrinsic specificity. γδ T cells have unique attributes that make them especially well-suited to be used for immunotherapy. They express an invariant TCR that recognizes antigens broadly expressed by metabolically-stressed cells, and kill them (while sparing normal cells) in a MHC-independent manner.

Aim: Development of TCR bi-specific T-cell engaging receptors (TCERs) to offer a new approach for the treatment of tumors, chronic infections by redirecting T cells to kill *(i)* tumor cells of both hematopoietic and non-hematopoietic origin; *(ii)* cells infected by intracellular microrganisms (viruses, mycobacteria, etc.); *(iii)* to induce organ-specific immune suppression in patients with autoimmune diseases.

Milestone: To design and synthetize TCR bi-specific TECRs.

Deliverable: TCR bi-specific TECR in vitro and in pre-clinical mouse models in vivo.

Task 2.5 Delivering autoantigens (autoimmune disease relevant peptides) in the liver to induce tolerance and treat autoimmune diseases

Executors	Starting month	Ending month	Duration (months)
Sapienza/UniCA/UniMIB	3	36	33

This task will: *1)* generate and purify nanoparticles (NP), pMHC-NP, disease relevant peptide-NP and peptide microparticles (MP); *2)* evaluate liver targeting and the expansion of T regulatory cells in vivo after peptide-NP conjugate administration; *3)* evaluate in vivo disease-specific bystander immunoregulation in appropriate mouse models of autoimmune diseases.

Task 2.5.1: To generate and purify nanoparticles (NP), pMHC-NP, peptide-NP and peptide microparticles (MP). A quick, robust, and scalable process suitable for large-scale synthesis of the compounds will be generated. Quality control will be performed by transmission electron microscopy, dynamic light scattering, and native and denaturing gel electrophoresis. pMHC or peptide content measurement will be performed by Bradford assay, denaturing SDS–PAGE, amino acid analysis, dot-ELISA.

Task 2.5.2 Evaluation of the expansion of T regulatory cells in vivo. Evaluation of the actual reprogramming of cognate antigen-experienced CD4+ T cells into FOXP3-CD25- T regulatory type 1-like (TR1-like) cells in both healthy and diseased mice, by FACS analyses and functional assays.

Task 2.5.3: Assess the safety and efficacy of the NPs in relevant diseased animal models. Molecular characterization of the effect on the inflammatory/immunological pathways involved in the target organ (joints, liver and bile ducts) and proximal lymphoid tissues. Autoantigen-loaded antigen-presenting cells (APCs) across different treatment arms will be evaluated for the production of regulatory cytokines (IL-10, TGF-beta and IL-21. *A* safety report based on evaluation of systemic cellular and humoral immunity will be generated.

Milestone: production of endotoxin-free nanoparticles conjugates.

Deliverable: Development of a quick, robust, and scalable process suitable for large-scale synthesis of in vivo effective and efficacious conjugates.

WP3: Re-educating the microbiome to ameliorate therapeutic responses **(Leader: UniMIB)**

Alterations in the composition and function of the microbiota have been linked to the initiation and progression of several diseases. Moreover, the presence of specific microbial signatures has been shown to be predictive of response or failure of therapies. Thus, interventions aimed at restoring an

eubiotic microbiota are key to promote long-lasting therapeutic success. This WP aims at providing innovative microbiota modulating therapies tailored on the individual patient's microbial dysbiosis.

Task 3.1: Design of patient-, disease-and tissue-specific microbiota-targeting intervention

Executors	Starting month	Ending month	Duration (months)
Neuromed/OPELLA/UniBO/UniCT/UniMIB/UniMORE	1	18	18

This task will provide a disease-specific microbial profile of patients: *i)* identifying and implement tissue- and biomass-specific effective sequencing methods; *ii)* creating a disease-specific database comprising microbial and clinical data of patients; creating prediction models of disease risk and/or therapy responses.
Objective: Provide a disease-specific microbial profile of patients
- to identify and implement the most effective sequencing method (ie, 16S rRNA seq or whole Genome Shotgun or whole RNA Shotgun) according to the tissue origin of the biomass
- to implement the most suitable metabolomics approach for microbiota-derived molecules according to the tissue origin of the biomass
- to create a disease-specific database comprising microbial multi-omics data and clinical data of patients
- to create prediction models of disease risk and/or therapy responses
-to isolate microorganisms and/or microbial consortia for further biological/mechanistic investigations (through culturomics)

Milestone: generate tissue- and disease-specific microbial signatures of patients.

Deliverable: provide risk and /or therapy response prediction models, according to disease and microbial signature.

Task 3.2: design of microbiota-targeting intervention by personalized microbial consortia and next gen microorganism

Executors	Starting month	Ending month	Duration (months)
OPELLA/UniCT/UniMORE	12	30	18

This task will design personalized microbiota targeted therapeutic interventions based on patient's microbial signature and intend to: *i)* rationally design in silico microbial consortia complementing patients' microbial alterations; *ii)* create personalized living microbial consortia to be administered; *iii)* to enhance therapeutic activities of probiotics (ie, SCFA production, anti-or pro-inflammatory enzymatic activities according to the patient need, specific tissue delivery) by targeted bioengineering in specific microorganisms (NextGen microorganisms) or modulate their GUS content for drug bioavailability.

Milestones: ***i)*** in silico generation of personalised microbial consortia; ***ii)*** Next gen defined microorganisms' preparation.

Deliverable: create probiotics with enhanced therapeutic activity by targeted bioengineering.

Task 3.3: in vivo validation of therapeutic activity of personalized microbial consortia in patients' microbiota avatars

Executors	Starting month	Ending month	Duration (months)
OPELLA/UniMIB	12	36	24

This task will validate in vivo in patient's avatars the therapeutic activity of microbial consortia and Nextgen probiotics: *i)* creating patients' microbiota avatars via transplantation of fecal patients' microbiota murine models; *ii)* therapeutically administer the personalized, in silico rationally designed microbial consortia or the next-gen probiotics in mice at different timepoints and concomitantly or not to the disease-specific therapeutic interventions.

Milestone: generation of patients' avatars.

Deliverable: establish patients' microbiota avatars as screening platforms for microbiota-targeted therapies.

WP 4: Identification of new therapeutic targets by screening and drug repositioning **(Leader: Sapienza)**

Current rules for drug registration are producing a high increase in cost and time of development. Multi-target, "polypharmacological" drug candidates have become increasingly important as an alternative to single-target drugs and/or drug combinations. Drug repurposing can cut research expenditure and the overall development time required to bring an effective drug to the clinic. Repurposing is also considered safer, due to known parameters regarding drug toxicity and dosage; but the question is how to identify the right drug for specific purposes. The objective of this WP is the optimization of a pipeline for the identification of novel candidate drugs and testing of available drugs for human diseases. Modeling tools and AI to guide the selection of new candidates with already favourable safety profiles will be exploited.

Task 4.1: Definition of putative therapeutic targets via computational tools, i.e. virtual screening of drug libraries

Executors	Starting month	Ending month	Duration (months)
UniPA/UniMORE	1	18	18

This task will in silico define lead compounds via virtual screening of drug libraries containing EMA, FDA and AIFA approved small will be performed. The aim is to identify a shortlist of compounds having already a favorable profile for use in the clinic.

Milestone: Optimization of in silico screening methods. ***Deliverable:*** Definition of a list of candidate drugs.

Task 4.2: In vitro validation of selected drug efficacy and selectivity

Executors	Starting month	Ending month	Duration (months)
ISS/M.Negri/UniBO	12	36	24

This task will: *1)* in vitro validate the efficacy of the compounds selected in task 4.1 and in other platforms of the HealITALIA consortium (Spoke 1, 2, 3) on disease-relevant cellular models; *2)* Off-targets characterize using of high-throughput proteomics and metabolomics platforms [Activity-Based Protein Profiling (ABPP), Reverse-Phase Phosphoprotein microArrays (RPPA) and Seahorse) to circumscribe the molecular targets of the identified drugs and evaluate their effect on cellular homeostasis (e.g., signal transduction and energy metabolism).

Milestone: in vitro validation of drug activity in relevant cellular models. ***Deliverable:*** identification of new repurposed drugs and characterization of their activity.

Task 4.3: Drug repositioning through large patient biobank screening, the case of NAFLD/NASH GDR Study

Executors	Starting month	Ending month	Duration (months)
Sapienza	1	36	36

This task will determine whether medications prior to diagnosis of NAFLD/NASH have a positive effect by modifying the rate of deaths for any cause, the occurrence of gastrointestinal medications, and the occurrence of cardiovascular ischemic complications compared to not taking the medications. **Rationale:** Non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) may represent pathological conditions in which drug repurposing can have a useful area of application. Indeed, NAFLD/NASH have emerged as the most prevalent liver diseases and are linked to an increased risk of gastroenterological (hepatocellular carcinoma and cirrhosis) and cardiovascular ischemic complications. Moreover, there is no currently approved treatment for NAFLD/NASH. Anti-obesity drugs, anti-diabetic drugs, anti-inflammatory drugs, lipid-lowering drugs (namely statins), antioxidant vitamins, vitamin D, antibiotics, bile acids have been reported by anectodical observations as well by small clinical trial as potentially effective therapeutic measures to reduce liver fat content, indices of liver injury or degree of fibrosis. However, none of these interventions have been evaluated for their ability to reduce the risk of gastrointestinal or cardiovascular NAFLD/NASH

-driven complications. Therefore, a study has been designed to assess global drug repurposing in NAFLD/NASH (The NAFLD/NASH GDR Study). The general hypothesis for this study is that some current medications may modify the clinical course of subjects with NASH/NAFLD compared to those who are not taking the same medications. The strength of this proposal in the context of a research network is the ready access to subjects' information and to computational expertise as well as to bioinformatic platforms and tools to carry BigData analyses.

Task 4.3.1: To determine whether medications prior to diagnosis of NAFLD/NASH modify the rate of deaths for any cause compared to not taking the medications. To this purpose, a large cohort of patients (about 50.000 subjects) with NASH / NAFLD will be enrolled and collection of demographic and clinical information will be generated (in particular, prior history of drug uses and gastroenterological and cardiovascular complications). This collection will be performed using a large national and international database (UKBiobank). This database is already available to the proposers and access to it does not require any specific approval other than the submission of the protocol with amendments.

Task 4.3.2: To determine whether medications prior to diagnosis of NAFLD/NASH modify the occurrence of gastrointestinal complications (liver cirrhosis and hepatocarcinoma) compared to not taking the medications. Drugs or combination of drugs influencing the identified clinical outcomes will be identified by statistical analyses. All subjects who will be enrolled in the study will be included in the full analysis set irrespective of their adherence and continued participation in the study. The primary variable is the time to the first event included in the primary composite endpoint. The primary analysis will be based on prior medications' using principle.

Task 4.3.2: To determine whether medications prior to diagnosis of NAFLD/NASH modify the rate of occurrence of cardiovascular ischemic complications compared to not taking the medications. The analysis will be performed as in task 4.3.2.

Milestone: Enrolment of a large cohort of patients (about 50.000 subjects) with NASH / NAFLD and collection of demographic and clinical information. This collection will be performed using a large national and international database (UKBiobank). *This database is already available to the proposers and access to it does not require any specific approval other than the submission of the protocol with amendments.*

Deliverable*:* Identification of drugs or combination of drugs influencing the identified clinical outcomes by statistical analyses. All subjects who will be enrolled in the study will be included in the full analysis set irrespective of their adherence and continued participation in the study. The primary variable is the time to the first event included in the primary composite endpoint. The primary analysis will be based on prior medications' using principle.

Task 4.4: Development and validation of new targeted radionuclides and Precision Flash Radiotherapy procedures for precision medicine.

Executors	Starting month	Ending month	Duration (months)
Sapienza/SIT/UniMIB/UniPI/UPMC	1	36	36

This task will develop new therapeutic radiopharmaceuticals with improved efficacy and reduced toxicity in order to build a platform to design and validate new targeted radionuclide-based therapeutic approaches selected on tumor specific biological features. The efficacy target selectivity, kinetics of bio-distribution in target and non-target organs will be validated.

Task 4.4.1: In vitro evaluation of the effect of beta minus, alpha and Auger electron emitters on patients derived cancer cells. Dose and rays dependent effects of radionuclides capable of inhibiting cancer cell growth will be identified as well as the molecular mechanism associated with radionuclide efficacy.

Task 4.4.2: Evaluation of the biological effects of different doses of radionuclides in vivo using well-established targeted radiopharmaceuticals labeled with different radionuclides in tumor mouse models showing different dimensions and heterogeneity of target expression. Reduction of tumor volume will be evaluated in appropriate mouse models as well as the in vivo molecular mechanism of tumor lesion associated with the radionuclide.

Task 4.4.3: Development of novel radiopharmaceuticals based on the use of target specific radioactivity carrier structures (NP, antibodies fragment or peptides). The radiolabelling process and the evaluation of the kinetic properties in vivo of radiopharmaceuticals will be evaluated in preclinical models of cancer in order to evaluate the selectivity for the tumor, the dosimetric parameter and calculate the dose required for efficacy studies. Kinetics of in vivo bio-distribution and dosimetry of novel radiopharmaceuticals for imaging and therapy will be determined.

Milestones: ***i)*** dose and rays dependent effects of radionuclides on cancer cell growth in vitro and in vivo; ***ii)*** radiolabeling for imaging and therapy of novel radiopharmaceuticals.

Deliverables: ***i)*** Define molecular mechanisms associated with radionuclide efficacy; ***ii)*** Kinetics of in vivo bio-distribution and dosimetry of novel radiopharmaceuticals for imaging and therapy.

SPOKE 6: Healthy Toolbox

Development of innovative devices for precision diagnosis and personalized therapy

Description of the Spoke Activities

SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
UniMORE	CRO/UniBO/UniCA/UniCT/UniFG/ UniMIB/UniMORE/UniPA/UniPI/UniVR	1	36	36

1. Context description:

Personalized medicine relies on the ability to design tailored therapies to patients, promptly adjustable over time following frequent monitoring of patient conditions. Consequently, new technologies and devices, in principle operating in a highly interconnected way, must be in parallel developed to consolidate two of the main pillars on which personalized medicine is based: precision diagnostics and precision therapeutics, the latter combining medicine, surgery (i.e robotics) and medical devices for health-care.

Precision diagnostics can be achieved by a variety of different approaches, requiring profiling of biomarkers to prognose and monitor the disease progression and predict response to therapy. In this context, precision diagnostics shall rely on devices with ultra-sensitive (for early diagnosis) and cost-effective (for repeated testing) technologies (i.e biosensors) that can be also operated at the Point-of-care with high versatility for diverse targets (DNA/RNA, proteins, metabolites, extracellular vesicles). In addition, precision devices shall be capable of supporting the implementation of personalized medical interventions by non-invasive monitoring of physical and chemical parameters of medical interest by portable/wearable devices, also linked with real-time precise diagnostics, to achieve a comprehensive assessment of the patient's health status for the applicable medical decision.

As for precision therapy, three main areas of intervention in terms of development of innovative devices, encompassing different time- and length- scales were identified: i) **precision surgery**, ii) biomedical **instrumentation for precision therapies** (including development of novel **biomaterials**) and iii) **micro- and nano-tools for precision drug delivery and nanotherapeutics**.

Aiming to precision diagnostics and remote health monitoring, it will be developed a portfolio of biosensors, encompassing electrochemical, electronic and optical devices, also integrated with microfluidics, for early diagnosis, for monitoring the efficacy of a therapy during patient follow-up, and for wellness tracking. This allows the detection of the different biomarkers also accounting for the distinct biological matrix. The advent of these technologies will allow higher sensitivity, frequent monitoring using low volume of biological specimens and unprecedented adaptability in different contexts/devices, for a next generation of diagnostic tools.

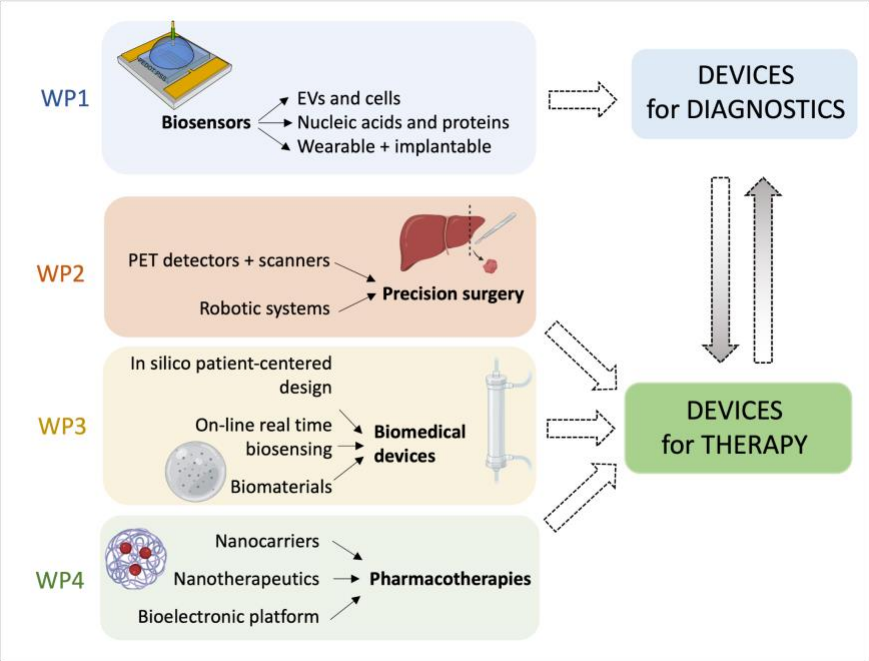
Surgery is the cornerstone in the treatment of many solid and localized tumors. In particular, robotic surgery represents a strong therapeutic opportunity in the context of precision surgery, also by the support of AI; ultimately this will generate personalized approaches conjugating procedural safety with a radical intent within a variety of indications.

Medical devices for precision therapies encompass a variety of technologies, spanning from high precision radiotherapy, to novel models of biomedical instrumentation that may integrate on-line sensors, arriving to game changing tools for regenerative medicine approaches.

As for nanoscale devices, the design and fabrication of strategies to selectively direct a drug towards a specific organ or tissue or cellular system, and to obtain the release/maintenance of the therapeutic

agent at the target site for an extended period, is key in precision medicine. In this context, the role of engineered nanocarriers designed to overcome issues on drug solubility, stability, toxicity, low bioavailability and lack of selectivity, along with the development of nanotherapeutic agents for minimally invasive treatment, is crucial.

2. General objective:



This spoke develops a technological toolbox of devices for applications both in precision diagnostics and therapy, closely interacting with Spoke (S)1 for omics investigations, with S2 for AI implementation, with S4 for the identification of biomarkers to be quantified by innovative biosensing platforms. To this end, WPs are divided into two subgroups: WP1 is devoted to devices for diagnostics, while WPs 2, 3 and 4 aim at developing devices for therapeutic interventions. The broad definition of "devices" in the Heal Italia project encompasses from molecular systems to miniaturizable diagnostic assays and from robotic systems to hardware solutions. The spoke has its own Scientific Advisory Board: Wolfgang Knoll Wolfgang Knoll (Austrian Institute of Technology, AT), Raymond Schiffelers (University Medical Center Utrecht, NL), Giorgio Mari (Modena, IT), Nick Barlev (CAS, St Petersburg, RU), Pier Paolo D'Avino (Univ Cambridge, UK), Qiang Sun (CAS, Beijing, China).

3. Project WPs structure:

WP 1: Sensing devices for precision diagnostics and remote health monitoring **(Leader: UniMORE)**

To develop a portfolio of biosensors, encompassing electrochemical, electronic and optical devices, also integrated with microfluidics, for early diagnosis, for monitoring the efficacy of a therapy during patient follow-up, and for wellness tracking. Detection of biological entities poses different challenges depending on the nature and size of the analyte, as well as on the biofluid that contains it and on the kind of sought information. In WP, it will be developed biosensors for detection of biomarkers that can be divided into three groups: 1) membranous vesicles such as exosomes and whole cells; 2) nucleic acids and proteins; 3) small molecules and electrophysiological signals. The

work of this WP will be organized on the basis of target analyte and further classify the sensors based on the transduction mechanism. Exploring different transduction mechanisms allows us to mitigate the risks and to identify the best-performing biosensors, in terms of selectivity and sensitivity, for a given target. Still, a common feature sought in the developed biosensors will be the ultra-high sensitivity (enabled by innovative amplification strategies), rapid response and the possibility to deploy the sensing platforms at the point of care, as they all share high miniaturizability. The WP1 sensors will be validated for selected target pathologies but will be designed to be a proper platform, i.e. readily readjusted to detect different biomarkers, which can be identified as outcome of the work carried out by Spoke 4.

Task 1.1: Biosensing platforms for the detection of extracellular vesicles and cells in biological fluids

Executors	Starting month	Ending month	Duration (months)
UniCT/UniMORE/UniPA	1	32	32

This task aims at the development of a set of sensors for the detection of biological entities ranging in size from tens-hundreds of nanometers (exosomes and, more broadly, extracellular vesicles) to the micrometer scale of whole cells. EVs are heterogeneous populations of membranous vesicles of different diameters and cellular origin which are emerging as potential biomarkers for a number of pathologies (with a major focus on cancer), since they can be detected in various kinds of bodily fluids and can provide minimally invasive snapshots of disease status. While a large number of optical and electronic biosensors have been developed for "traditional" biomarkers such as proteins and nucleic acids, EVs pose a new challenge to the biosensing field. A major aim of Task 1.1 is therefore to develop novel assays for the ultra-sensitive, rapid and consistent detection of EVs, with a focus on identification of exosomes produced by a specific pathological tissue (i.e tumor), both with organic electronic devices and with optical methods. In parallel, this task aims at the demonstration of microfluidic-based biosensors to detect whole cells. Microfluidic systems have emerged as a key ingredient of innovative diagnostic tools for precision medicine. Besides integration into optical or electronic biosensors, they can be operated as standalone platforms to achieve efficient separation of biological entities, as is the paradigmatic case of rare cell capture devices. Microfluidic biosensors taking identification of fetal cells in maternal biological samples for prenatal diagnostic genetic testing as a paradigmatic application will be developed. Specific objectives are:
- Development of a label-free organic electronic biosensors based on the Electrolyte Gated Organic Transistor (EGOT) architecture for the detection of EVs using the EV-containing biological fluid as electrolyte for the device operation;
- Fabrication and testing of optical biosensors for isolation of exosomes by luminescent nanostructures to conduct a liquid biopsy, including assessment of the biotoxicity of the biosensor;
- Development of microfluidic devices to detect fetal cells in maternal biological samples, based on micro-structured polymeric membranes with purposely engineered pore/channel structure surface composition using nanofibrous mat electrospinning and/or hydrogel 3D printing. The devices will be functionalized with antibodies/aptamers to combine size-based sorting with immunocapture techniques;
- Lab-scale validation and optimization of the above-described assays for operation with real samples.

Milestone: Electronic, optical and microfluidic devices for EV detection designed, including identification of functionalization strategies for selective EV recognition.

Deliverable: Report on detection of extracellular vesicles in real samples with the biosensing platforms.

Task 1.2: Detection of nucleic acids and protein biomarkers: liquid biopsies with optical biosensors

Executors	Starting month	Ending month	Duration (months)
UniBO/UniFG	1	32	32

This task aims at the development of devices for the PCR-free detection and quantification of nanometer-sized biomarkers, i.e. nucleic acids and proteins, based on optical biosensing. An innovative, (electrochemi)luminescent diagnostic platform for point of care testing (POCT) based on signal amplification, by nanoparticles and phages, that will be applied first to the qualitative and quantitative characterization of nucleic acids without the PCR amplification step, and further adapted to small molecules and protein biomarkers will be designed and developed. Specific objectives are:
- Development of efficient recognition elements for analyte recognition with (electrochemi)luminescence platform and of bio-nanotechnological structures for high signal amplification;
- Development of a portable (electrochemi)luminescence-based platform for POCT for the qualitative (and possibly quantitative) detection of nucleic acids without the aid of PCR amplification and for detection of cardiovascular, oncological, neurodegenerative diseases biomarkers.
-Validation by clinical end-users.

Milestone: Bio-nanotechnological structures for signal amplification allowing the sensitivity required for PCR-free analysis of nucleic acids developed.

Deliverable: Prototype of a portable platform for rapid (< 2 h), sensitive (as PCR) PCR-free detection of nucleic acids and other oncological biomarkers.

Task 1.3: Detection of nucleic acids and protein biomarkers: label-free electronic biosensors

Executors	Starting month	Ending month	Duration (months)
UniMORE	5	36	32

This task further explores novel strategies for detection of nanoscale biomarkers such as DNA, RNA and proteins, but based on electronic, rather than optical, response. Electronic devices are amenable to miniaturization and future implementation in portable platforms deployable in the field. Bioelectronic sensors, featuring silicon or organic thin films as active materials, will be developed towards tumor biomarkers both established and identified by other spokes within the Heal Italia project. Specific objectives are:

- -Setup of a newly designed and automated wide band platform integrated with microfluidics to operate a Ultra-High Frequency Capacitance Spectroscopy (UHF-CS) biochip, for detection of

nucleic acids and proteins. The platform will be based on electrical transduction of biorecognition processes at large arrays of nanoscale electrodes, obtaining wide-band capacitance fingerprints of selected analytes by experiments and simulations.

Milestone: Models and simulation tools to predict biomarker capacitance spectra of the biomarker with UHF-CS platform calibrated and verified.

Deliverable: Report on fully electronic recognition and dynamic imaging of the tumor biomarker with UHF-CS platform.

Task 1.4: Wearable organic electronic biosensors

Executors	Starting month	Ending month	Duration (months)
UniCA/UniPI	5	36	32

As a last set of biomarkers, monitoring of levels of small molecules (namely cytotoxic drugs) and of electrophysiological signals will be addressed. The aim is the demonstration of novel materials and device architectures to be integrated into wearable, implantable or biodegradable electronic biosensors. The task includes validation even through rational assessment of the potential of the technology. Specific objectives are:
- Development of wearable monitoring systems based on the use of organic semiconductors allowing devices and sensors for different parameters of medical interest, either physical or chemical. Organic technologies can be exploited for the development of such devices on flexible substrates, from smart textiles to nanofilms for epidermal electronics, which can be adopted for the unobtrusive monitoring of the human body.
- Production of a full Health Technology Assessment (HTA) for wearable devices and benchmarking against commercial devices.
- Development and validation of implantable and biodegradable biosensors to measure the concentration of cytotoxic drugs in human tissues.

Milestones: ***i)*** Set of prototypes including textiles and epidermal systems endowed with different functionalities (from passive electrodes to active transistor-based chemosensors); ***ii)*** Development and in-vitro validation of implantable and biodegradable biosensors for cytotoxic drugs.

Deliverable: ***i)*** Report on the characterization and assessment of wearable biosensors against golden standard systems; ***ii)*** Clinical validation of developed implantable biosensors.

WP 2: Assistive tools for precision surgery **(Leader: UniVR)**

The main goal of precision surgery is to obtain the maximum efficacy against the unhealthy tissues or organs, while preserving as much as possible healthy ones. This goal may be achieved through a variety of possible strategies. Robotic surgery is one of them and, though extensively employed in a variety of operative fields, still is the object of an intense research effort addressing on one hand new fields of application and, on the other hand, the reproduction of the complete set of feedback normally experienced in traditional operation by the surgeon. A set of tools supporting surgeons in their actions are developed in this WP, starting from imaging (i.e Positron Emission Tomography, PET) and micro-

robotic technologies together with AI tools developed in collaboration with Spoke 2, for example to assist in detection of tumor margins and in minimally invasive operation sparing healthy tissues within a novel personalized surgery.

Task 2.1: Expanding the potential of PET: enhancing sensitivity and fabrication of intraoperative scanner for surgical margin assessment

Executors	Starting month	Ending month	Duration (months)
UniFG/UniPI/UniMIB	1	33	33

Among the approaches tested to improve margin detection in cancer surgery, PET has captured attention because of its high sensitivity. Pushing the timing performance of detectors is a straightforward method to improve the PET sensitivity. The time of flight TOF-PET accuracy is ultimately given by the instrument coincidence time resolution (CTR). A CTR of 10 ps would allow a real-time monitoring of radiopharmaceuticals activity distribution, therefore the possibility of real-time molecular imaging for cancer diagnosis/therapy and other medical applications. Realizing new scintillators is a decisive synthetic challenge to control the system CTR and therefore the PET sensitivity. As for PET application in precision surgery, the effectiveness of surgery depends on the size of resection margins, as a compromise between complete tumor removal and preservation of healthy tissues. Within this task, it will be pursued a twofold aim: to further improve PET sensitivity through materials strategy and to develop a prototype PET/optical imager for tumor margin detection in precision surgery. Specific goals are:
-to realize a scintillating heterostructure employing a polymeric nanocomposite embedding scintillating nanocrystals, as fast emitter, coupled to an industrially processable dense crystalline scintillator. Thanks to the excellent time response of the nanocomposite recently demonstrated, this architecture will optimize the outcoupling of the fast scintillation thus realizing a prototype device with a CTR below 50 ps that will be tested in operative conditions;
-to develop a compact, high sensitivity, high resolution PET scanner integrated with an optical 3D scanner, able to produce 3D representations of the tracer uptake in excised tissue samples and to provide the surgeon feedback in real time.

Milestone: Tailored fast scintillating composites with CTR 50ps; Construction of the PET scanner with a geometry tailored for intraoperative imaging and integration with the optical 3D scanner.

Deliverable: Device demonstrators of fast ToF-PET detectors; Development and validation of the developed intraoperative PET scanner on phantoms and tissues samples.

Task 2.2: Toolbox to Minimally Invasive Robotic Surgery

Executors	Starting month	Ending month	Duration (months)
UniMORE/UniCT/UniBO/UniVR	4	36	33

The main goal is to develop and test innovative precision devices leading to the application of new surgical procedures by implementation of a novel robot-assisted mini-invasive surgery. Based on the PIs expertise and to optimize resources, the task will be focused on liver diseases and colorectal cancer as model pathologies. Along this line, it will be also explore explored and test advanced and experimental surgery procedures on the cadaver, developing new surgical tools, starting from the morphological and molecular data of the cadavers to create an open and interdisciplinary platform, by developing and testing robotic surgery tools and specific minimally invasive techniques in different anatomical areas. By combining surgical expertise, artificial intelligence, augmented reality, 3D printed models and robotic systems this task aims to the following specific aims:

-assist the surgeon in surgical minimal invasive treatments for accurate planning of the intervention;
- reduce the steep learning curve of the interventions warranting precision and accuracy when executing the intervention;
- create a platform with surgical robot for tutoring and training in advanced cancer surgery;
- establish models and tools to support the organisation of precision robotic surgery activities.

With respect to liver diseases, along this task they will be identified positive predictors of liver regeneration in cases of small future liver remnant at risk of liver decompensation, thus reducing post-surgical complication, reduce the risk of cancer recurrence thanks to increased precision and accuracy of the resection, and collectively generate a more performing clinical outcome.

Milestone: Optimization of augmented reality with 3D reconstruction from CT scan with 40 consecutive cases. Elaboration of preoperative studies to be used during the perioperative management. Cadaver testing of image segmentation, 3D printed models and surgery procedures.

Deliverable: Software for Robotic-assisted, real time navigation during resection as an extension of the current robotic platform.

WP 3: Innovative tools for precision therapeutics **(Leader: UniMORE)**

The main goal is the development and validation of devices and materials for precision medicine, covering different device-based therapeutic strategies other than pharmacological treatments. Paradigmatic target pathologies, such as cancer and kidney diseases, have been identified. This WP will be focused on the integration of state-of-the-art methods in on-line hemodiafiltration (HDF) with hardware and software for big data analysis and patient-centered, in silico and in vitro approach to the design of medical devices. In addition, the introduction of novel materials and devices for optimized tissue regeneration approaches is included in the WP, as one of the most promising precision tools for still unmet medical needs.

Task 3.1: Integrating patient-centered device design and real-time sensing with biomedical technology.

Executors	Starting month	Ending month	Duration (months)
UniFG/UniMORE	1	32	32

The combination of state-of-the-art medical equipment with frontier research that enables patient-tailored design of a medical device and real time monitoring of biomarkers, enabling a complete

knowledge of the patient's status, can create an ecosystem of services and technologies capable of increasing safety, effectiveness, and efficiency of a given treatment. In this task, it will be applied such a highly multifaceted, interdisciplinary and patient-centered approach to device design, taking chronic kidney diseases (CKD) as a target pathology. In particular, it will be first used a combination of in silico and in vitro approaches, integrated with clinical images, to design and characterize novel hemodiafiltration (HDF) devices at a patient-specific level. It will be also compared two alternative dializers for HDF in a non-randomized open-label observational study and integrate them with ad-hoc developed IT and sensing technologies for real time collection and analysis of patient's data.
Specific objectives are:
- Application of in silico approaches (spanning from atomistic to continuous scale) to simulation of biotransport phenomena and blood recirculation for HDF device design;
- Simulation of personalized cases of interaction between the device and the patient, based on integration of multiscale/multiphysics simulations (empowered by machine learning) with clinical imaging;
- In vitro testing and characterization of the HDF devices, with particular attention to their effectiveness in terms of fluidics, transport of biochemicals, mechanical and fatigue behavior;
-Evaluation of the efficacy of two dialysis filters, based on High-flux polysulfone and cellulose triacetate, respectively, in terms of removal of uremic toxins and modulation of inflammation by monitoring a large set of parameters in CKD patients;
-Development of new measurement systems capable of online and real-time monitoring of biomarkers of HDF efficiency (e.g. uremic complexes, beta2microglobulin, vitamin B12);
-Development of new software solutions for merging clinical data of the patient with outputs from sensors for real-time-updated knowledge of the patient's status.

Milestone: In silico platforms for the multiscale and multiphysics design, characterization and optimization of devices for dialysis, including identification of functionalization strategies for personalized devices. In vitro platforms for the characterization and optimization of devices for HDF. Software and hardware for collection, integration and analysis of data from patients designed.

Deliverable: Reports on newly designed devices for HDF. Integration of new diagnostic tools and methodologies based on advanced IT techniques for personalized hemodialysis treatments.

Task 3.2: Novel biomaterials and devices in regenerative medicine

Executors	Starting month	Ending month	Duration (months)
UniBO/UniMORE/UniPA	4	36	33

The main goal of the task is to develop a platform of devices based on new biomaterials, implants and nanostructures for tissue regeneration. It will be develop a wide portfolio of formulations, whose multi-component nature might eventually be exploited, by integration with molecular data of each single subject, to personalize the therapy according to the patient's specific needs.
To establish favorable environmental conditions for tissue repair and regeneration as well as for implant integration, key targets are represented by infection tackling and control. Biofilm formation is another issue associated with infection treatment, requiring therapeutic approaches able to infiltrate and eradicate it while preventing its re-assembly and growth. Hence, the development of antibiotic-

free therapeutic approaches bearing anti-biofilm, anti-microbial, and pro-healing properties represents an urgent need in the biomedical field. It will be therefore developed new hydrogels and scaffolds ad-hoc engineered to progressively release in the infection site (e.g., wound bed, bone fracture, prosthesis implantation site) anti-biofilm and anti-microbial agents of natural origin.
As a paradigmatic model case, based on the expertise of the PIs involved in the task, it will be then focused the attention on the development of new biomaterials for oral surgery and maxillofacial applications. By integration with genetic analysis, liquid biopsy and NGS data of the patient, it will be engineered a platform of materials adaptable to the patient's needs.
Specific objectives are:
- Design of hydrogels and scaffolds using custom-made polymers of the poly(urethane) family coupled with biomaterials of natural origin (e.g., collagen, hyaluronic acid, gelatin) defining a cell-friendly environment to favor tissue repair and regeneration as well as implant integration in the host tissue;
- Encapsulation into micro- and nano-carriers (e.g., micelles, nanoparticles, mesoporous particles) to protect the therapeutic agents and provide an additional control over their release kinetics;
- Test of the hydrogels as injectable formulations and biomaterial inks for the 3D bioprinting of patient-specific patches;
- Fabrication of porous scaffolds in the form of sponges through conventional techniques and multi-layered structures with precise geometrical features for personalized medicine applications;
- As a model application, test of the effect of different concentrations of newly developed polymers on dentinal enzymatic activity by means of gelatin and in situ zymography, to reduce the instability of resin-dentin bonds;
-Physico-chemical and biological characterization of all the developed biomaterials and therapeutic formulations, including analysis of tissue interactions with biomimetic surfaces to evaluate the influence of new biomaterials on surface characteristics (e.g. morphology, roughness, elemental composition).

Milestone: Portfolio of new biomaterial formulations (synthetic and bioartificial biomaterials) designed. Procedures for samples collection and analysis standardized. Shared database to correlate clinical and pathological variables, while monitoring the effect of the new therapeutic tools, created.

Deliverable: Design and characterization of novel biomaterials and antibiotic-free therapeutic approaches to tackle and manage infections. Identification of molecular risk factors, design of supervised machine learning algorithms to develop prediction models to monitor the effect of the devices on the patient.

WP 4: Precision micro- and nanotools for innovative pharmacotherapies **(Leader: UniCT)**

To design, synthesize and screen novel molecular systems as precise carriers for drug delivery and as therapeutic, stimuli-responsive agents and to develop electronic devices to monitor response of cellular models to innovative therapies. The research activity of this WP will contribute to the progress of the field by developing a novel nanotechnology platform to achieve an optimized drug delivery, improve their efficacy and minimize their undesired side effects. WP5 will also rely on inputs from Spoke 5 that will identify promising/repurposed drugs, for which ad hoc targeting nanosystems should be developed.

Task 4.1: Development of smart drug-delivery systems

Executors	Starting month	Ending month	Duration (months)
CRO/UniCT/UniFG/UniMIB/ UniMORE/UniPA/UniPI/UniVR	1	32	32

The aim is the development of drug delivery systems for the transport of poorly bioavailable and/or highly toxic therapeutic agents to selectively direct a drug towards a specific organ or tissue or cellular system, and to achieve the release/maintenance of the therapeutic agent at the target site for an extended period. The main target pathology will be cancer (with a particular focus on CR, glioblastoma, pancreatic ductal adenocarcinoma, hepatocellular carcinoma and multiple myeloma, skin cancer) but metabolic syndrome, cystic fibrosis, rare diseases such as retinitis pigmentosa and degenerative diseases such as osteoarthritis will also be considered. Specific goals are:
-Structure-based drug discovery by atomistic computational modeling;
-Development of targeting strategies for the synthesis of bioactive entities able to selectively recognize specific biomarkers, including biodegradable polymeric drug nanocarriers;
- Development of smart biomaterials for the production of injectable or printable drug delivery systems with "programmed" and "on-demand" release, able to convert an external remote stimulus (visible light or NIR laser irradiation) into physicochemical perturbation of the bulk material that will be exploited to tailor or enable the release of active molecules or to activate a physiological cascade of events that favors the restoration of physiological functions;
-In vitro screening of the nanoplatform in terms of target selectivity, delivery efficiency and nanodrug safety/biocompatibility. In vitro tests will be conducted in 2D and 3D models to assess the nanocarrier toxicity, uptake, drug delivery and selective effect on targeted cells.
-Selection of most promising formulations to be tested in vivo. Establishment of appropriate preclinical models and evaluation of pharmacological effects of the nanodrugs after the administration. In addition, possible impacts in off-target organs will be assessed by means of biodistribution and toxicity studies.

Milestone: Identification of the most appropriate formulations for innovative controlled drug release platforms and their technological characterization.

Deliverable: Selection of the most effective formulations based on preclinical studies in advanced cellular tissue models

Task 4.2: Development of nanotherapeutic agents

Executors	Starting month	Ending month	Duration (months)
UniCT/UniMORE/UniPA/UniPI	4	36	33

The aim is the preparation of nanotherapeutic agents whose function can also be activated by external stimuli such as radiation or hyperthermia, including "theranostic" systems that combine therapeutics and diagnostics components in one multifunctional device. Cancer will be the main target pathology. Specific goals are:

-Development of photo- or thermo-activable molecular pro-drugs or nanoparticles. Examples include light-activable systems devoted to the release of non-conventional therapeutic species (nitric oxide (NO), singlet oxygen (1O2) and heat) and plasmonic nanomaterials for photothermal therapy of tumors.
-Development of therapeutics innovative systems all-in-one including in one single structure components for diagnosis, monitoring, and mini-invasive treatment of solid tumors. Two paradigmatic cases will be considered:
1) carbon dots nanotheranostics (CD-NT) with surface functional groups responsive to tumor microenvironment (TME) and able, in response to light and magnetic stimuli, to act as chemo- and photo-thermic agents, able to release locally and on demand drugs and heat. CD-NTs also act as thermo-sensible contrast agents in Magnetic Resonance (MR) or in Fluorescence (FL) for the monitoring of local hyperthermia, as nanotools for measuring the efficiency of therapeutic treatment monitoring TME variationsand as radiosensitizing agents;
2) Production of a radio-labeled engineered Fn3 Mesothelin Binding Protein Variant for early detection and treatment of mesothelin-overexpressing cancers;
-In vitro (and eventually in vivo) validation of the nanotherapy agents in 2D and 3D models

Milestone: Optimization of the protocol of production of nanotherapeutic and theranostic nanosystems for diagnosis, monitoring and treatment of solid tumours. Physico-chemical characterization of obtained nanosystems.
Deliverable: Nanotheranostic devices tunable for diagnosis, monitoring and thermic precision ablation of solid tumor; one nanodevice for the monitoring of parameters of the MTE such as pH, temperature and reducing properties.

Task 4.3: Organic Bioelectronic platform to monitor response to therapy of in vitro cell models and in vivo in small animal models

Executors	Starting month	Ending month	Duration (months)
UniBO/UniCA/UniMORE	6	36	31

Along with state-of-the-art methodologies for assessing the effect of nanodrugs on targeted cells (see Task 4.1), it will be developed innovative systems for 2D and 3D in vitro cell monitoring based on sensor arrays that combine soft mechanical behavior with highly sensitive electrical transduction properties to be confirmed by small animal models with radiological read-outs. A recently demonstrated organic-semiconductor based technology will be applied with the aim of deriving complex information (e.g. electrical and metabolic) on the cellular behavior as a consequence of the interaction between the nanodrug and the biological target.
Specific objectives are:
-Design of multisensing platforms aimed at detecting electrical and physiological parameters of several kinds of cell cultures (2D and 3D);
-Development of microfabrication protocols for multichannel recording sites based on both passive and active devices (field effect and electrochemical transistor based), eventually including 3D elements as pillars or meandering connectors;
-Microfabrication of sensor arrays with multichannel recordings sites. Low-impedance coatings will be developed based on organic ionic-electronic conductors. Supporting substrate is polymer thin film adaptable to complex tissue or device conformation;
- Lab validation of the platforms, including the development of the readout electronics;

- Optimization of the above-described platforms for operation with real samples and validation in drug-screening tasks.

Milestone: Demonstration of multichannel recording with platform from 3D cell cultures (in-vitro) of electrogenic cells.

Deliverable: Demonstration of multichannel recording in small animal models.

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

SPOKE 7: Prevention Strategies

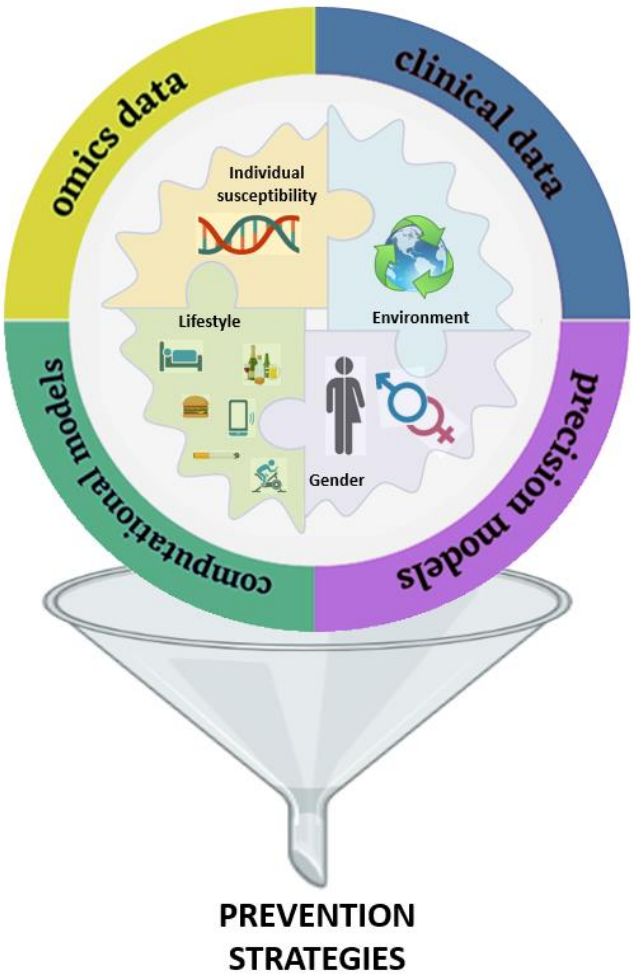
Integrated and gender medicine approaches for prevention strategies based on environmental, lifestyle and clinical biometric data

Description of the Spoke Activities

SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
UnivPM	ISS/Neuromed/IFO/Sapienza/UniBO/UniCA/ UniCT/UniFG/UniMORE/UnivPM/UPMC	1	36	36

1. Context description

Prevention is a pillar of public health policies aimed at reducing the burden of diseases in any country. The level of implementation of preventive strategies is influenced by, but is also, a determinant of the socio-economic condition of a country. Conventional prevention strategies do not consider individual susceptibility to develop diseases but find application on large groups or on an entire population; thus interventions that could obtain relevant results in selected cases are likely to miss efficacy when adopted on a large scale. Individual genetic features or biologic conditions in concurrence with environmental agents and life habits determine the risk of a disease and translation of knowledge in targeted interventions would maximize their cost-effectiveness. According to the Precision Medicine Initiative, precision medicine is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person". In the framework of a broader precision health approach – which includes disease prevention and health promotion activities for "precision public health" - the studies proposed in Spoke 7 aim at predicting more accurately which prevention strategies/interventions for a particular disease will work in which groups of people and will assist in establishing public health recommendations for specific subgroups based on phenotype, genotype and the exposome. This approach is in contrast to a "one-size-fits-all approach", in which disease treatment and prevention strategies are developed for the average person, with less consideration for the differences between individuals. The proposed research aims at identifying determinants of the individual risk to develop diseases with a high impact on the health status of a population, such as cardiovascular, cancer, or endocrine-metabolic diseases. A "gender approach" will also be adopted. The identification of such individual risk determinants will be at the base of innovative new strategies of prevention. This spoke has direct interactions and benefits with other national (PNRR) projects of Ministry University and Ministry Health.



2. General objective:

The final aim will be to develop tools applicable to the population for prevention (primary / secondary).
To this aim, WPs 1, 2 and 3, through a holistic approach that integrate genetic, epigenetic and environmental factors including diet, lifestyle, will be devoted to the identification of personalized clinical or biological factors, the identification of risk factors and new biomarkers and the integration of database for individual risk prediction.
Finally, WP4 will present specific case studies to provide evidence, by taking advantage of existing well characterized cohorts, for the development and implementation of prevention strategies based on precision medicine approaches for complex diseases, including monogenic (rare diseases) and polygenic pathologies (cardiovascular, metabolic, fibrotic diseases). Novel AI methodologies will be used in the framework of a broader precision health approach, including disease prevention and health promotion activities for "precision public health" which will include clinical applications and prevention strategies enabling ready-to-use personalized medicine systems. The spoke has its own **Scientific Advisory Board**: Anthony Letai (Harvard, MA, USA), Haining Yang (Hawaii Cancer Center, HI, USA), Daniele Bano (DZNE, Bonn, DE).

1. Project WPs structure:

WP 1: Translation of precision medicine tools into novel strategies for cancer prevention **(Leader: UnivPM)**

Cancer is one of the main causes of disability and mortality in the world, WHO foresees a significant increase in cancer incidence in the next two decades mainly affecting developing countries but also industrialized countries. Prevention of cancer is presently based on modification of life habits and avoiding exposure to environmental agents but also vaccination has a role in primary prevention *e.g.* anti-EPV to prevent cervical cancer or anti-HBV for liver cancer. Genetic alterations which increase the risk of developing different type of cancers can have a hereditary transmission and their detection is used for therapeutic decisions or for primary and secondary prevention in relatives of cancer patient's carriers of the alteration (e.g. BRCA 1/2). The aim of the present WP is the identification of individual risk profiles to develop cancers linked to endogenous genetic profile, reactivity and/or exogenous agents such as microbiome and its products or environmental agents.

Task 1.1: Predicting cancer development in chronic inflammatory conditions of the digestive system: molecular profiling, individual risk prediction and potential prevention strategies

Executors	Starting month	Ending month	Duration (months)
Neuromed/UniCA/UnivPM	1	36	36

Sub-task 1.1.1: Chronic liver diseases represent an outstanding health problem since they determine 15.000 deaths/year due to the complication of cirrhosis or the occurrence of primary liver cancer such as hepatocellular carcinoma or cholangiocarcinoma. During the last years the aetiology of chronic

liver diseases is dramatically changing. Due to the availability of direct antiviral agents, the previously most frequent aetiology of chronic liver diseases, viral hepatitis, has now a minor role. The most frequent etiologies are now represented by chronic liver diseases induced by excess alcohol consumption (alcoholic fatty liver disease, AFLD) and/or metabolic syndrome (nonalcoholic fatty liver disease, NAFLD) associated with obesity and type 2 diabetes. Other less frequent conditions are represented by autoimmune diseases such as autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). All these conditions are associated with an incomplete knowledge of the pathogenetic mechanisms and thus to the lack of well-defined therapeutic strategies.

The main aims of the project will be the identification of molecular mechanisms related to the progression of non-transmissible chronic liver disease and the definition of clinical pathways for the early diagnosis of disease progression and primary liver cancer development.

To address these aims, specific animal models of chronic liver diseases and in vitro facilities will be used to identify markers of liver injury progression and cancer development. In vivo and in vitro data will be compared with clinical prognostic features of disease progression from local, national and international databases. By these comparisons, clinical pathways based on clinical risk stratification will be derived. Specifical environmental factors and lifestyle habits will be evaluated as non-transmissible aetiological parameters able to modify morbidity and mortality. This will lead to the development of new experimental platforms to identify molecular pathways involved in the progression of chronic liver diseases and in primary liver cancer development. Identification of clinical pathways based on risk stratification for prevention and/or early diagnosis of cirrhosis complications will be obtained. Another aspect that will be taken into account will be the creation of a specifically designed Molecular Tumor Board, for the interpretation and analysis of data, in particular those obtained by Next Generation Sequencing. The Molecular Tumor Board will support the interpretation of the patient molecular profile, to support and implement clinical strategies.

Milestones:
1. Sample collection and arrangement of clinic-pathological variables in a shared database;
2. Genomics, transcriptomics, and microbiomics analysis;
3. Data and bioinformatics analysis and interpretation using Artificial Intelligence;
4. Biomarker selection.

Deliverables:
1. Translational workflow;
2. Unravel new prognostic and therapeutic biomarkers with the aim to develop quick-use decision kits;
3. Highlighting genetic, molecular, and microbiological differences;
4. Highlighting new molecular pathways in different in vitro models to find new druggable targets;
5. Public database of genetic, molecular, and microbiological characteristics and data.

Sub-task 1.1.2: Colorectal cancer (CRC) is the 3rd most common cancer and the 2nd cause of cancer-related mortality worldwide (Globocan 2020). CNF1 toxin produced by toxigenic *E. coli* strains producing toxins in concert with chronic inflammation is associated with epithelial to mesenchymal transition of both normal and transformed intestinal cells. This can play a role in the development or progression of CRC and may open the way to novel preventive strategies against CRC. In fact, the current endoscopic examinations could be implemented with a multi-modal analysis of bacterial factors to redefine risk categories for CRC development. In addition, the results of this project may open the way to the study of the efficacy of antibiotics and of prebiotics in the treatment of pre-cancerous lesions to prevent progression to cancer stages. The aim of this project is to study the role of bacterial toxins in colorectal tumor- and carcino-genesis, to define genetic profiles associated with

increased risk and actionable mechanisms, with the final goal to explore preventive strategies based on dietary of microbioma-modificating interventions.

Milestone: Assessment of pro-carcinogenic and pro-metastasizing effects of bacterial toxins in vivo.
Deliverable:
1. Identification of immune, metabolic, epigenetic mechanisms modulated by bacterial toxins in vivo;
2. Definition of epigenetic modifications induced by bacterial toxins and inflammatory stimuli in advanced 3D cultures.

Task 1.2: Weighting the exposome: a model for disease prevention

Executors	Starting month	Ending month	Duration (months)
ISS/UnivPM	1	36	36

The global impact of asbestos-related diseases is around 231,000 cases / year (doi: 10.1016/S0140-6736(18)32225-6), where the main source of exposure always remains in the workplace, even if the effect of environmental exposure is not negligible: it is estimated in fact, that about 5-20% of mesotheliomas in the general population are of environmental origin (doi: 10.1136/oemed-2014-102297).

There is much evidence that exposure to asbestos causes malignant pleural mesothelioma (MPM), lung, larynx, and ovarian cancers. Although some progress in therapy has been made, the prognosis of asbestos-related cancers is still dismal.

Prevention, early diagnosis and therapy all contribute to reducing the mortality of asbestos-related diseases.

miRNA microarray analysis performed in patients affected by MPM and asbestos-related lung cancer identified a 4-miRNA panel able to detect the malignancy at an early stage (doi: 10.1158/1055-9965.EPI-18-0453). Although the miRNA showed high sensibility for cancer detection, they exhibited low specificity (doi: 10.1016/j.lungcan.2015.09.0219. Recently, two autophagic biomarkers (ATG5 and HMGB1) have been involved in the development of asbestos-related diseases (doi: 10.1073/pnas.2007622117). One strategy for improving biomarker performance is to combine molecules of different origins.

Despite several advantages associated with miRNA for early detection and cancer therapy there are some critical issues to be addressed. MiRNA for early diagnosis: 1) the serum levels of miRNAs changed not only in the presence of the tumor, but also following other pathologies such as metabolic and cardiac diseases, 2) not all MPMs secrete miRNAs in the serum. Therefore, other biomarkers are needed to predict the development of asbestos-related tumors.

The study aims to evaluate the pre-diagnostic role of deregulated miRNA and autophagic biomarkers in sera from a court of subjects previously exposed to asbestos (ex-EXP) and patients with MPM that will be compared with healthy control subjects (CTRL).

A retrospective and prospective study will be carried out. A BioBank for biological samples (serum and tumour biopsies) of ex-EXP subjects, patients with MMP and CTRL subjects has been set up.

In the period 2005-2020, former EXP subjects were recruited as part of the health surveillance project. The subjects were checked for lung function by spirometry, and if a more in-depth analysis was required, a chest CT scan was performed.

In the same period, patients with MMP were recruited at the Occupational Medicine Clinic as part of the specialist medical examination for the reporting of occupational disease.
The healthy subjects were obtained from workers who presented themselves at the Occupational Medicine Clinic for the annual medical checkup.
The subjects after demographic and work anamnestic analysis, and after having signed the informed consent, proceeded to give a blood sample.
Serum samples from the enrolled population stored in the BioBank and those obtained from new recruits will be analyzed for the miRNA-panel and two autophagy markers ATG5 and HMGB1. The blood levels of autophagy biomarkers will be related to the previously evaluated levels of miRNA and serum mesothelin expression. Autophagic biomarkers will also be evaluated in pre- and post-diagnosis serum samples in ex-EXP subjects who developed asbestos-related cancer during follow-up.

Milestone: Detection of miRNA-panel and autophagic biomarkers (ATG5 and HMGB1) in serum samples of the enrolled population.

Deliverable: Development of an algorithm for prevention and early detection of the asbestos-related cancer diseases

Task 1.3: Accelerating the development of innovative therapies for solid tumors by advanced preclinical evidence and 3D systems

Executors	Starting month	Ending month	Duration (months)
ISS	1	36	36

It has been known for a long time that the immune system actively participates in the control of transformed cells' growth and that host immune defects or immune escape strategies adopted by cancer cells foster tumor growth. In recent years the introduction in the therapy of monoclonal antibodies blocking immune-modulatory interactions, immune checkpoint inhibitors, has changed the clinical practice for several cancers. However, only a fraction of cancer patients shows a prolonged response to treatment with immune checkpoint inhibitors even when a prediction marker can be identified, e.g. PD-L1 expression on tumor cells and/or on immune cells. These inhibitors are usually better tolerated than traditional chemotherapy but they have a specific toxicological profile and recognizing patients with a low probability of response would be important to avoid unnecessary adverse events. In vitro models to dissect the interactions of immune cells with cancer cells and tumor microenvironment can contribute to design strategies for a more precise indication to therapy with immune checkpoint inhibitors.
The project involves the design of a microfluidic device for in vitro reconstruction of key sites of the immune system at the interface with a host tissue, affected by a pathology, such as a cancerous process, a viral infection or chronic inflammation. This "Immune-on-a-chip" represents an advanced microfluidic device capable of reproducing certain degrees of complexity of the pathological condition, such as the endothelial vessels, which the immune cells have to pass through in order to reach the site of interest, and a flow within the same that mimics the peripheral system of man. Immune-on-a-chip is generated with the patient's primary cells and allows the analysis of the interactions of specific immune cell populations with the host's pathological tissues, offering the possibility to study the immune response in a patient-specific way and to test the activity of

immunotherapies or the effect of conventional therapies on the immune system, facilitating the pathways of personalized medicine.

Milestone: Providing novel kinematic and morphometric data, which could be a valid support to better define the effect of drugs to cancer cells, to evaluate the migratory behavior of immune cell towards cancer cells within an organ-on-chip system, to recapitulate complex tumor-immunity interactions in presence of drugs with organ-on-chip platforms.

Deliverable: Identification of key immune signals driving the effectiveness of cancer therapies.

Task 1.4: Head and neck oncological predictive medicine by artificial intelligence omic analysis

Executors	Starting month	Ending month	Duration (months)
UnivPM/UniFG	1	36	36

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer worldwide, accounting for 5% of all cancers, and it is associated with high recurrence and mortality rates. The prognosis of HNSCC is still poor and often unpredictable, showing a 5-year survival rate of 50%, with negative consequences on the National Health System. Clinical outcome of HNSCC patients is currently established by AJCC Staging System; however, some patients show distinctive outcomes, with very aggressive tumours at low stages. Although immunohistochemistry and other techniques are well-established methods for identifying new prognostic markers, the variable results reported in literature currently hinder their usefulness in clinical practice. The reason is probably due to the fact that studies rely on procedures that analyze cancer tissue as a unique bulk, limiting the understanding of intra-tumoral biology. Indeed, tumour bulk puts together cancer, normal, and stromal cells, characterized by different genetic and molecular features. Therefore, recent improvements in "Omics" approaches, such as single-cell transcriptomic analysis (STA) and next generation sequencing (NGS), can help address the heterogeneity issue, leading to change in existing clinical algorithms.

The first goal of the project is to define the transcriptomic profile by STA and NGS to measure the genomic landscape and the molecular activity that underlies the phenotypic diversity in HNSCC cells and to correlate to specific histological phenotypes. Based on these results, the most promising biomarkers will be subsequently sorted and investigated both in cell-line models and tissue samples through different approaches, such as biochemistry, Raman scattering, Fourier-Transform Infrared Spectroscopy (FTIR), immunohistochemistry, to unravel their role in different pathways. Another goal of the project is to define head and neck microbiome features of HNSCC patients. Non-invasive methods of collecting reliable specimens (i.e., liquid biopsy) will be used, followed by NGS analysis. The information obtained will be combined with the clinic-pathologic features, also with the use of Artificial Intelligence-based approaches.

The first aim is to build a combined prognostic algorithm that will challenge the accuracy of current clinical and pathologic standards for risk classification of HNSCC patients. The second aim of the project is to define significant "Omics" changes among healthy subjects, patients affected by potentially malignant disorders and patients affected by HNSCC, as an alert regarding head and neck-related health disorders that might lead to cancer. The third aim is to define and characterize molecular and genetic targets for the development of new targeted therapies.

Milestones:
1. Sample collection and arrangement of clinic-pathological variables in a shared database;
2. Genomics, transcriptomics, and microbiomics analysis;
3. Data and bioinformatics analysis and interpretation using Artificial Intelligence;
4. Biomarker selection.

Deliverables:
1. prognostic algorithm for risk classification of HNSCC patients

Task 1.5 Cancer risk prediction in the Italian population

Executors	Starting month	Ending month	Duration (months)
Sapienza	1	36	36

This proposal aims at defining a novel and comprehensive method for cancer risk prediction in the Italian population, through which more correct approaches for personalized prevention can be implemented.
Data already available to the proponents by virtue of their own work[1], or through the participation to national or international consortia[2], will be used to define comprehensive cancer gene panels to be analyzed by Next Generation Sequencing (NGS), as well as the selection of common SNPs to be genotyped for the definition of polygenic risk scores (PRSs) for most frequent cancer types.
The large cohort of Italian population series collected from "Moli-sani" study[3], characterized in SPOKE 1 and analyzed and integrated with clinical data in SPOKE 2, will be used for the validation of the cancer panels and PRSs. This cohort will be also analyzed as a training set, to evaluate both genetic risk factors and gene-environment interactions associated with cancer risk.
Algorithms for the evaluation of cancer specific PRSs and tools for the integrative analysis of high/moderate/ low penetrance genetics and environmental exposure will be developed in collaboration with the company "Allelica". This integrated system analysis for precision-directed cancer prediction will be validated on further series, and eventually implemented in the clinical practice.
The Italian Thyroid Cancer Observatory (ITCO) web-based database will be used as a model to process genetic and clinical-pathological data from cancer patients to instruct machine learning approaches for the identification of reliable predictors of risk of disease recurrence and outcome.

Milestones:
1. To define the comprehensive cancer gene panels suitable for the representation of the genetic landscape of cancer susceptibility.
2. To evaluate and customize at national level the recently developed PRS models.
3. To instruct machine learning approaches for the identification of reliable predictors of risk of disease recurrence and outcome.

Deliverables:
1. To provide effective models for the stratification of the Italian population for cancer risk estimates based on the integrative analysis of genetic factors and gene-environment interactions.
2. To develop models for the identification of reliable predictors of risk of disease recurrence and outcome to treatment.

WP 2: Novel personalized strategies for prevention of cardiovascular diseases **(Leader: UnivPM)**

Cardiovascular diseases (CVD) are still the principal cause of death in the population of the economically developed world. The identification of risk factors of CVD has led to the application of primary and secondary prevention politics promoting positive life habits to substitute negative ones. In this WP, personalized prevention strategies for several specific pathological conditions will be studied.

Task 2.1: Prevention of Arrhythmogenic Cardiomyopathy (ACM)

Executors	Starting month	Ending month	Duration (months)
UnivPM	1	36	36

Arrhythmogenic Cardiomyopathy (ACM) is a rare (1:1000 to 5000), genetic, inherited heart muscle disease that is a main cause of sudden cardiac death (SCD), especially in young adults and athletes. Data from literature indicate that 11-22% of cases of SCD in young athletes (<35 years) occurred during physical exercise are due to the undiagnosed presence of ACM.
Frequent misdiagnoses are because ACM diagnosis does not rely on a single gold standard test but is achieved using a scoring system, which encompasses familial and genetic factors, ECG abnormalities, arrhythmias, and structural/functional ventricular alterations.
The major challenge is to distinguish ACM from a normal heart with physiological adaptation to hemodynamic overload, such as occurs in athletes, and from so-called disease phenocopies.
The most important objective of clinical treatment is prevention of SCD. Current therapeutic options include lifestyle changes, antiarrhythmic drugs, catheter ablation, implantable cardiac device (ICD), and heart transplantation.
Despite several improvements for ACM detection and therapy, there are still some critical issues. 1) High percentage of misdiagnosis. Most of the information on ACM comes from studies of the usual form of the disease with predominant right ventricle involvement. However, no diagnostic criteria are yet defined for the pattern characterized by early and predominant left ventricle involvement. 2) only recently a SCD arrhythmic risk score has been introduced but it is not validated in all ACM forms. 3) there is currently no treatment to prevent arrhythmic events. In individuals considered at risk, an ICD is the only proposed therapeutic measure.
With an extensive work-up, including: laboratory test, ECG recordings, cardiac MRI, an accurate electro anatomical voltage mapping, an endomyocardial biopsy, and genetics, it will be hypothesized

to reduce the portion of misdiagnosis and to better identify phenocopies, in particular physiological adaptation to hemodynamic overload, such as occurs in athletes.
Rare genetic diseases are due to a heterogeneous group of variants consisting mainly of single nucleotide variants (SNV/INDEL) or copy number Variation (CNV). The genetic origins of ACM are partly clarified and the genes that, mutated, cause cardiomyopathies are many tens. Analysis by massive parallel sequencing (MPS) often allows to highlight the causative genetic variant but in more than 40% of cases a variant is discovered whose meaning cannot be defined and remains undetermined (so-called VUS, variants of uncertain significance). To dispel doubt about the impact of these mutations, molecular (functional studies) and bioinformatic tools can be performed. It will be hypothesized to establish an analytical pipeline that allows us to define the clinical significance of a VUS. These innovative bioinformatics models could be the basis of new software for the interpretation of genetic data, providing added value for understanding the genetic causes of cardiomyopathies and also helping to optimize health diagnostic pathways.
Moreover, this diagnostic extensive work-up could improve the correct identification of biventricular and left dominant ACM cases that have a higher proportion of misdiagnosis.
It will be also hypothesized that the variable genetic expressivity, in particular the susceptibility to develop life-threatening ventricular arrhythmias, can be explained by the involvement of pathogenic cofactors, both of genetic and environmental origin.
Moreover, it will be investigated whether arrhythmia treatments, particularly catheter ablation procedure, could improve the cardiovascular mortality and arrhythmia free-survival in the acute and long-term follow-up.

Milestones:
1. Data collection and arrangement of clinic-pathological variables in a shared database;
2. Clinical, Imaging, Electrophysiological, Histopathological and Genomics analysis;
3. Data analysis and interpretation using Artificial Intelligence;
4. Diagnostic indicators and arrhythmic risk predictors selection.

Deliverables:
1. The primary objective of this research is to develop a diagnostic iter capable of recognizing ACM patients from pathological phenocopies and, in particular, from the so-called athlete's heart.
2. Furthermore, it is expected to better characterize the left dominant and biventricular presentation which, to date, are underdiagnosed and underdescribed.
3. A further goal is to develop a multiparametric score that allows the identification of patients with high arrhythmic risk, using clinical, imaging, electrophysiological, histological and genetic data analyzed by AI. Furthermore, it is anticipated that, in such patients, catheter ablation of ventricular arrhythmias could improve cardiovascular mortality and arrhythmia-free survival in acute and long-term follow-up.

Task 2.2: Risk factors assessment for chronic heart failure

Executors	Starting month	Ending month	Duration (months)
ISS	1	36	36

Patients with heart failure (HF) have a devastating 5-year mortality rate and the median survival rate after first hospitalization is very low. Risk factors for HF are ischemic heart disease, hypertension,

diabetes mellitus, obesity. Iatrogenic forms of HF also occur in patients undergoing anticancer therapy. Patients with HF can be divided into those with a reduced ejection fraction (HFrEF) and those with a preserved EF (HFpEF). Epidemiological data indicate that the incidence of HFrEF is particularly low in women whereas patients with preserved HFpEF are older and more likely to be female. Although the molecular hallmark in chronic HF is cardiac beta-adrenergic receptors (B-AR) desensitization and downregulation, particularly ß1-AR, unfortunately HFpEF do not respond to ß blocker therapy. There are currently no predictive biomarkers for the development of HF. Since ß1AR is also implicated in the evolution of HF, there is considerable interest in the mechanisms that regulate the density of this receptor on the cell surface, in the development of pharmacological treatments able to restore the cell surface ß1-AR density, as well as in understanding whether monitoring the density of this receptor can provide predictive information on the development of HF. Peripheral blood monocytes have been proposed as useful biomarkers in some pathological cardiac conditions. In light of this, it will be verified if the human monocytes of the peripheral blood isolated from male and female healthy subjects could represent a useful model for studying the modulation of the cell surface density of ßARs thus representing useful biomarkers to implement preventive therapies of the development of HF. Starting from the hypothesis that the decrease in cell density of the ß1AR on cardiac tissue may represent an early event in the development of HF, the principal aim of this project is to develop and validate a suitable model for monitoring the density of this receptor as a possible predictive marker of HF development.
Considering the gender differences observed in the incidence of HFpEF, another important question will be to establish whether and how estrogen signaling is involved in the regulation of the human ß1AR density.

Milestone: Standardize: the method for isolating monocytes from peripheral blood samples, the quantification of ß1-2AR on the plasma membrane of monocytes and on cardiac tissue; the time it takes for heart failure to develop in selected mouse models.
Deliverable: Determine whether: catecholamines downregulate ß1AR; estrogen signaling is involved in the regulation of β1AR density; PBR is involved in the regulation of ß1AR expression both in vitro and in vivo; PBR ligands can rescue cardiac function in several SC mouse models.

Task 2.3: A novel female-specific risk score predictive of atherosclerosis in women for personalized prevention strategies

Executors	Starting month	Ending month	Duration (months)
UniMORE	1	36	36

Cardiovascular (CV) injury is associated with a significant proportion of deaths in women. Women are exposed to specific sex related CV risk factors including menopause and depression and also the traditional risk factors (i.e.dyslipidemia) acts differently in women compared to men. However, women were underdiagnosed and under-treated in relation to CV disease. It is needed to change this approach in order to provide better health care to women at any age. Although women's individual risk might be significant, they are less likely to receive guideline-indicated imaging screening or lifestyle recommendations. It will be studied three different cohorts from pre- to postmenopause. Using cutting-edge noninvasive imaging technology, it will be detected early signs of injury in the heart, vascular system, and brain. It will be used state-of-the-art noninvasive imaging techniques to phenotype atherosclerosis in women and define its impact on the cardiovascular system. Functional, anatomical, and metabolic aspects will be assessed, including vascular mechanical properties and

plaque characterization. Machine Learning methods to integrate molecular, phenotypic and lifestyle data will be used to generate a novel female-specific risk score predictive of atherosclerosis in women.

Milestones:
1) Non-invasive imaging data set ready;
2) Lifestyle data set ready;
3) results from clinical studies collected;
4) big data platform ready to be populated (settled);
5) data mining and text and web/mining.
Verifiable/measurable indicators
At least 80% of the enrolled patients underwent all the tests.

Deliverables:
1)Machine learning;
2) predictive algorithm ready and validated;
3) optimization and simulation;
4) sex-specific cardiovascular risk scores ready and validated.

Task 2.4: Novel early predictors of infections of cardiovascular implantable electronic devices

Executors	Starting month	Ending month	Duration (months)
UniBO	1	36	36

In the last years the use of cardiovascular implantable electronic devices (CIEDs) have largely spread. All the available devices, including permanent pacemakers and implantable cardioverter-defibrillators, have been shown to improve patients' quality of life and long-term survival. The clear beneficial effect of CIEDs implantations is unfortunately burdened by a severe and dangerous adverse event, the occurrence of CIEDs infections. Several factors contribute to the development and evolution of CIEDs: comorbidities, clinical characteristics, surgical procedure, device type. A personalized approach of patients with suspect or overt CIEDs infection starting from early diagnosis to planning of the specific management is therefore urgently needed. This task aims to propose a deep characterization of patients with CIED infections from the early diagnosis to medium-term follow-up. It will be prospectively enrolled a cohort of patients with suspected CIED infection who will undergo a complete evaluation during all the process. The patient will undergo baseline and post-extraction: transesophageal echocardiography, Nuclear imaging, serial culturing (swabs, blood and hardware after explantation) and biochemical characterization of the inflammatory response (at presentation and after CIED removal). These data will be the basis to develop a database of deeply characterized patients to enable the identification of the specific role of the different variables promoting CIED infection and the best approach for its management. The objectives of this task are 1) to identify the risk factors for systemic vs. local infection; 2) to identify the risk factors for CIED infection relapse or adverse outcomes; 3) to provide novel early predictors of CIED infection.

Milestone:
Characterization of different patterns of CIED infections according to microbiological agent and patient characteristics.

Deliverables:

1) Identification of mechanisms and risk factors underlying the preferential development of systemic vs. local CIED infections; 2) to develop a novel stratification tool to predict the risk of CIED re-infection after hardware removal; 3) to create a novel algorithm for management of CIED patients after hardware removal

WP 3: Integrating old risk factors and novel predictive models for the prevention of Metabolic and Endocrine Diseases **(Leader: UniCT)**

Metabolic and endocrine diseases have a multifactorial pathogenesis. The observed increased incidence in the last decades may be ascribed to progressively changing dietary and life habits as well as to exposure to environmental stressors, which may exert different outcomes according to individual genetic background and gender. WP3 aims to assess and integrate the contribution of all these components to define precise risk profiles and prevention strategy adoption for these pathologies with a high impact on the national health system.

Task 3.1: Risk factors, lifestyle and new biomarkers in obesity and related diseases

Executors	Starting month	Ending month	Duration (months)
ISS/UPMC	1	36	36

Obesity (OB) is a main risk factor for the occurrence of many life-threatening metabolic diseases (MD). Being a complex condition, it reflects the interactions among genetic, epigenetic and environmental factors including diet, lifestyle, exposure to metabolic disrupting compounds (MDCs) as well as to stressful life events. Diet is one of the most important lifestyle behaviors which influences the development of many pathological conditions. In particular, consumption of some dietary components (red-meat, dairy products, and saturated fatty acids) may influence the inflammatory process promoting MD while others (monounsaturated fatty acids, fiber, fruits, vegetables, fish, seeds, nuts) may have protective effects, also towards exposure to MDCs. Among MDCs there are several chemical contaminants present in everyday life products such as plastics, textiles, furniture, as well as in the food chain. They represent risk factors for metabolic diseases as they affect adipocyte development with a consequent alteration of autocrine and paracrine endocrine signaling. In this context, gender and age-specific differences may greatly influence nutrients and MDCs effects on disease susceptibility and hazard identification. Further, metabolism is different in the two sexes and the same diet and/or exposure to MDCs may determine different effects in men and women.
The research aims at studying the mechanisms and specific effects of nutrients and MDCs on: the phenotype of adipose tissue (AT) and human iPSC-derived organoids taking into account sex, age and health status as well as the cross-talk with other organs, including the gut and brain, which are sexually dimorphic and have an endocrine dialogue with AT, controlling energy balance and food intake messages. The completion of this task will take advantage of already available human cohorts characterized for dietary intake, body-mass index (BMI), age and gender. Cohort-derived samples such as blood and faces will be profiled to firstly define specific at-risk individuals. AT samples from different population groups (BMI, age and gender) will be then implied in vitro to define specific responses to nutrients and/or MDCs exposure. This approach will be paralleled by well-validated murine models and a main focus will be given to the gut-brain-axis and the inflammatory processes affecting the nervous circuits involved in the regulation of MD and mood disorders. These skills are instrumental in the development of specific in vitro models capable of reproducing the variability observable in complex systems such as the whole organism.

Milestones:
1) End of patients' recruitment and set up a tissue biobank of human tissues deriving from a cohort of OB male and female subjects;
2) development of 3D models from AT;
3) development of 3D models (organoids) and "organs-on-a-chip" systems from iPSCs from fibroblasts of healthy and OB patients.

Deliverables:
1) Set up of *in vitro* co-coltures to implement the Cross-talk characterization between central and peripheral tissues with AT for assessing diet/MDCs effect;
2) High throughput screening of potential agents working against MDCs in 3D and organs-on-a-chip" systems;
3) Identification of specific biomarkers to be used for a personalized hazard identification through the impact of metabolic stress and MDCs on selected mouse models;
4) Characterization of the specific effects of selected promising disease modifying MDCs/nutrients on brain organotypic cultures, established from mouse models.

Task 3.2: Diasmoke - Randomised controlled trial evaluating changes in cardiovascular risk in type 2 diabetic patients who switch to combustion-free nicotine delivery systems

Executors	Starting month	Ending month	Duration (months)
UniCT	1	36	36

This large randomized switching trial investigates changes in CV risk factors and metabolic parameters in diabetic smokers who switch to combustion free nicotine delivery systems - CF NDS (e.g. e-cigarettes, heated tobacco products) compared to continuing smoking. First study to address the challenge of smoking cessation and harm reduction in diabetes, with an expected clinical impact on reduction of cardiovascular mortality and morbidity in diabetic patients who quit/switch.
Objective of this research is to show reduction of cardiovascular risk factors and improvements of functional/metabolic parameters in smokers with diabetes who abstain from smoking by switching to CF NDS vs. patients who continue to smoke.

Milestones:
1) activation of initial administrative procedures; 2) establish research coordination; 3) partner selection; 4) draft protocol and SOPs; 5) CRO selection; 6) submission to ERBs; 7) Registration to ClinicalTRial.gov; 8) electronic CRF development and testing; 9) App tracker development and testing; 10) ERBs approvals; 11) protocol publication; 12) research agreements and contracts; 13) website launch; 14) kick-off meeting; 15) patients recruitment; 16) last patient in; 17) last patient out; 18) database lock; 19) data analysis; 20) study report; 21) final publication and dissemination.

Deliverables: 1) signed contracts; 2) registration to ClinicalTRial.gov; 3) electronic CRF live; 4) App tracker live; 5) ERBs approvals; 6) protocol publication in a peer review journal; 7) study website; 8) kick off meeting report; 9) database lock document; 10) final report of the study; 11) final publication in a peer review journal; 12) closing meeting report.

Task 3.3: Targeting reproductive functions for novel prevention and prediction strategies

Executors	Starting month	Ending month	Duration (months)
UniMORE	1	36	36

Sexual and reproductive health belong to the Sustainable Developmental Goals of WHO and, besides concerning infertility treatment, contraception and family planning, include the "healthy" transmission of genetic and epigenetic information to the next generation, as the basis for a healthy life. Drugs, diseases and environmental pollutants affect reproductive function of the parents and the health of the offspring. Epigenetic modifications affect susceptibility to cancer, cardiovascular, metabolic and various other diseases. This task aims to identify potential predictive algorithms able to detect connection between human reproductive function and environmental exposure to produce adequate biomarkers and prevention strategies. Reproduction is under endocrine control: gametogenesis depends on hormones (FSH, LH, hCG, steroids) and hormonal drugs are commonly used in humans and farm animals for reproductive/contraceptive technologies, contributing to environmental harm (e.g. steroids released in the waters). Acting through specific G protein-coupled receptor, gonadotropins induce the synthesis of sex steroids via specific pathways susceptible to environmental pollutants, such as endocrine disruptors (ED). With an increasing human population, ED represents a major concern for human health and the global ecosystem since they interfere with hormone action and may have epigenetic effects. It was already deepened the action of gonadotropins and its relationship with the environment by in vitro and clinical studies, big data analysis and machine learning. However, the mechanism of action of gonadotropins and reproductive hormones in general are still largely unknown, and reliable predictive models allowing the identification of novel biomarkers are completely missing.

This activity will be complemented by research on infertility as a common problem in modern society with 15% of men and women remaining childless. Contrary to common perception, IVF does not guarantee success; between 38 and 49% of couples who start IVF will remain childless, even after undergoing up to six IVF cycles. Therefore, the availability, consequences, and costs of IVF continue to stimulate discussion and disagreement, as does the effectiveness of treatment. The probability of achieving a pregnancy in different couples is strongly dependent on basal characteristics of patients and on the aetiology of infertility and may vary from zero to almost normal range. It is therefore important that infertile couples are well informed about their chances of success with IVF. Based on their specific probability of success, the couple can decide whether the risks of the treatment along with the emotional and, in many cases, financial burden can be justified. An accurate prediction of the success for individual couples would permit not only to optimize counselling for the couples on their chances of live birth after IVF but also to possibly improve the definition of eligibility and reimbursement criteria to plan and guide state funding for IVF. These objectives can be achieved through a comprehensive project including clinical studies for the definition of the role of individual prognostic factors and analysis studies based on Machine learning (ML). Such algorithms with strong data processing ability have become a promising methodology for clinical decision making and medicine study, including clinical outcomes prediction, with the development of prediction models being its main purpose.

Milestones:
1. Environmental data over the last two decades collected to detect epigenetic effects of pollutants in experimental models characterized;
2. *In vitro* data sets on endocrine control of gametogenesis ready;
3. Results from clinical studies collated to populate big data platform;
4. Genetic, biochemical and clinical markers associated to infertility identified;
5. Development of prediction models with the machine learning methodology.

Deliverables:
1. Predictive algorithms ready and tested;
2. Novel biomarkers identified.

WP 4: Case studies for personalized prevention strategies **(Leader: ISS)**

Data from existing and well characterized cohorts will be analyzed to provide evidence in support of the feasibility to develop and implement prevention strategies based on precision medicine approaches for complex diseases. This task will evaluate the known risk factors, metabolomics, epigenetic and genetic factors and their interaction with environmental agents in different phases of the life to provide, also with the aid of AI, an evaluation of the individual risk of deterioration of his/her health status or of developing chronic degenerative or immune-mediated diseases.

4.1 Risk factors assessment for CVD

Executors	Starting month	Ending month	Duration (months)
ISS	1	36	36

The identification of individual CVD risk is one of the main targets of primary prevention and the first step to reduce modifiable risk factors, from lifestyle changes to pharmacological treatments. Risk charts and risk scores, based on global absolute risk, are key tools for CVD risk assessment and determine the likelihood of developing the disease over the following years, provided that the value of several risk factors is known. In Europe, systematic coronary risk evaluation (SCORE) risk charts are recommended in clinical practice as a valid tool to estimate CVD risk. They were built using data from the SCORE study, a pooling project of several European longitudinal studies, with base-line examination conducted from 1967 to 1991. In Italy, the CUORE project, funded by the Italian Ministry of Health, elaborated the individual CVD risk score, based on the longitudinal studies started in the 1980s and 1990s in the Italian adult population. CVD risk scores differ according to different population characteristics (mean risk factors, risk coefficients, and survival).
Using data from Italian population-based studies of the Progetto CUORE (Health Examination Surveys (OEC 1998-202, OEC/HES 2028-2012, HES 2018-19), in addition to the MATISS cohort, with the associated biological samples, this task intends to: enlarge baseline data test by including more recent population-based cohorts; update the follow-up for cause specific mortality and possibly for non fatal coronary and cerebrovascular major events; test the use of classic risk factors in a different modality (i.e. continuous glycemia instead of dicotomic diabetes); test the introduction in statistical models of treatments widely adopted and strictly related to CVD risk factors (i.e. statins and lipid-lowering agents); test the improvement of the risk assessment personalization elaborating sex specific or age-group specific CVD risk functions; study the utility of different analysis methods

for elaborating individual CVD risk assessment functions (Cox- proportional hazard models, Artificial Intelligence techniques, such as Machine Learning); elaborate simple tools for the individual CVD risk assessment for the Italian population usable by health professionals; improve capacity building of health professionals on CVD risk assessment in the clinical practice for primary prevention, by training courses.

Milestones:
1. To include more recent population-based cohorts;
2. To update the follow-up for cause specific mortality;
3. To evaluate the utility of implementing laboratory activities to assay new biomarkers from the biological samples;
4. To implement preliminary statistical analysis using different methodologies to elaborate risk prediction models.

Deliverables:
1. To elaborate updated functions for the individual CVD risk assessment, eventually specific for both sexes and for age-groups;
2. To elaborate tools for the individual CVD risk assessment in primary prevention;
3. To elaborate a training course, targeted to health professionals, on the use of individual CVD risk assessment in primary prevention.

Task 4.2: Effect Modifiers, epigenetics and gene-environment interaction: comparison among different cohorts in a gender perspective

Executors	Starting month	Ending month	Duration (months)
ISS/UniCT	1	36	36

The present task will be conducted in the framework of the "precision health" vision that integrates biological, genomics, lifestyle, social and environmental information and/or big data to assess health status and provide interventions for maintaining or restoring health. Precision health takes a lifespan perspective in health monitoring, identifying actionable risks and intervening early through personalized strategies.
The first 1,000 days of life - the time spanning roughly between conception and one's second birthday - is an important window of opportunity to improve health throughout life. Epidemiological studies suggest that prenatal exposure to stressors (e.g., environmental exposures, unhealthy behaviours, social disadvantage, etc.) can induce persistent changes in the fetus leading to different susceptibility to various adverse outcomes at delivery (e.g., preterm birth, low birth weight) and chronic diseases (e.g., obesity, cardiovascular, diabetes, and even cancer) in later life. Birth cohorts provide unique opportunities to study early-life exposures in association with child development and health, as well as, with longer follow-up, the early life origin of adult diseases. The general aim of this task is to evaluate how environmental exposures impacts on later-life health/disease consequences for the child via epigenetic mechanisms, to predict the risk associated to early-life stressors and to understand whether dietary factors could potentially counteract environmental pollution-induced epigenomic defects, using data and samples from distinct Italian cohorts of women of childbearing age and of mother-child pairs; the specific objectives are to: i) investigate epigenetic signatures including DNA methylation in relation to environmental exposure; ii) identify novel markers of early-life stressors, also using AI methods and models applied to birth-cohorts data, in order to predict their evolution towards damage and the onset of diseases throughout life; iii) analyze the effect of genetic variants

as effect modifiers of the relationship between environmental exposure and DNA methylation and adverse pregnancy/neonatal outcomes; iv) study the role of gene-environment interaction in predicting adverse pregnancy/neonatal outcomes; v) evaluate if dietary factors and/or the adherence to specific dietary patterns and other lifestyles could counteract the detrimental effect of environmental exposure; and vi) examine real world data to generate personalized risk prediction models based on early-life stressors and their molecular markers.

Milestones:
1. Identification of a panel of biomarkers of early-life stressors;
2. Identification of genetic variants that modify the relationship between exposure, identified biomarkers and adverse pregnancy/neonatal outcomes;
3. Development of predictive models for the risk associated with early-life stressors.

Deliverables:
1. Recommendations for prevention interventions for specific subgroups of individuals based on their phenotype, genotype and exposome.

Task 4.3: Multi-omics and AI approach in rare diseases: implementing an innovative diagnostic pathway and precision medicine tool for fibrotic diseases

Executors	Starting month	Ending month	Duration (months)
UnivPM/UniCT/UniMORE	1	36	36

Fibrosis is the late stage of many chronic, rare diseases characterized by immune-mediated inflammation such as Systemic Sclerosis (SSc), Idiopathic Pulmonary Fibrosis (IPF), Chronic Graft Versus Host Disease (cGHVD). Predicting fibrosis onset and progression is an unmet medical need, which makes the current clinical management of fibrotic diseases suboptimal. In particular, studies on lung tissue aiming at identifying predominant pathogenic mechanisms and molecular alterations are currently lacking and no targeted therapies have not been available yet. This is partly due to historically limited access to lung tissue for biological studies, as the standard sampling procedure, surgical lung biopsy, is characterized by an unfavorable risk-benefit profile. In the last years, the adoption of cryobiopsy, an innovative less invasive tool to obtain lung tissue, has changed the landscape of diagnostic work-up, offering also clinicians the chance to directly identify and characterize pathogenetic processes as wells as the leading biomarkers involved, leading to "endotype" each single patient.
Resolving the heterogeneity of fibrotic disorders not only at early disease stages but also at later disease stages, through stratification of patients, could be achieved by multi-omics approach and artificial intelligence (AI) algorithms enabling integration of multi-level information (clinical, imaging, laboratory, omics) coming from a multitude of single patients.
Aim 1) to develop a novel network of centers inside the national health system dedicated to fibrotic diseases, based on multi-OMICs technologies and bioinformatic/AI tools. The centers of this network are highly qualified and certified by their inclusion into renowned worldwide, European and Italian existing reference networks for rare diseases. Every single patient affected by rare diseases potentially evolving into fibrotic diseases will be studied according to guidelines, but the biological samples, clinical and imaging data will be processed in a new fashion:
a) Liquid (blood, bronchoalveolar lavage) and tissue (skin, lung, heart, liver) biopsies will be transferred to the biobank connected to a single cell analysis facility (www.marchebiobank.it) for extraction of single cell information both at DNA/RNA and protein level (relevant cell types and

molecular pathways involved in fibrosis will be investigated by NGS deep sequencing and mass spectrometry, respectively).
b) Novel single cell data, conventional laboratory data, imaging data and clinical data from each patient will be transferred to the AI facility (Unibo, spoke 2, task 2.2) for integration into new algorithm models enabling identification of new subsets of affected individuals across different diseases, stratified for risk of developing fibrosis, risk of progression to severe forms of fibrosis and possible response to new and existing targeted therapies.
Aim 2) to identify the new best algorithm model for stratification of fibrotic risk in patients affected by rare diseases.

Milestones:
1. Creation of a biobank of primary cell cultures and organoids and liquid and tissue biopsies obtained from subjects affected by fibrotic disorders;
2. Identification of a panel of biomarkers associated to fibrosis in different patients (markers of senescence, programmed death ligand-1/programmed cell death 1 [PD-L1/PD-1] axis, PDGFR and other TKRs pathways, HLA molecules);
3. Identification of the role of mesenchymal stromal cells (MSCs) in the onset and progression of fibrosis across different ILDs

Deliverables:
1. Correlation of clinical parameters with candidate biomarkers responsible for fibrosis progression;
2. Identification of criteria for discrimination of stable vs progressive phenotypes of patients affected by fibrotic diseases;
3. Novel algorithm enabling stratification of patients (at very early or early disease steps) based on the risk of developing progressive fibrosis, combining endotypic and phenotypic features (molecular and immunohistochemical characterization with radiological semiquantitative data and AI)

Task 4.4: Omics Research in Epidemiology and Preventive Medicine

Executors	Starting month	Ending month	Duration (months)
UniCT/IFO	1	36	36

This task aims to provide an in-depth understanding of the relationship of multilevel omics biomarkers measured in the available cohorts. Combining detailed data on established life-styles risk factors and metabolic features, exposome and "omics" (metabolomic, epigenomic and genomic) data, the following tasks aim to define precision medicine algorithms to predict the risk of the major chronic disease with the approach of the "common soil" hypothesis.
Sub-Tasks:
4.4.1 Metabolomic analysis of available cohorts
4.4.2 Epigenomic analysis of available cohorts
4.4.3 Integration with available "omics" data, made available by large international consortia (UK biobank, Morgam/BioMarCARE and Nevada Health Genome) for various complex diseases (https://kclhi.org/phenoflow/phenotype/all/phenotype.id/), in order to validate the Italian data obtained and their translation of the international PRS.

4.2.4 Joint multidimensional analyses of the omics-exposome interplay with chronic diseases. A model based on this interplay will be developed and internally validated by utilizing machine learning approaches. Several machine learning methods will be employed for feature (variable) selection, estimation of effect sizes and the estimation of conditional predictive impact. It is the aim to construct a super learner from the different machine learning approaches.

Milestones: exposome database availability, aliquots of biological samples for epigenomics and metabolomics and analysis, expansion of the database, data-base of "omics" measurements in the available cohort.

Deliverable: follow-up update for mortality and chronic degenerative disease. Identification of predisposing or protecting markers, personalized algorithms for chronic disease prediction.

SPOKE 8: Clinical Exploitation

Clinical validation and implementation of innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches, based on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols

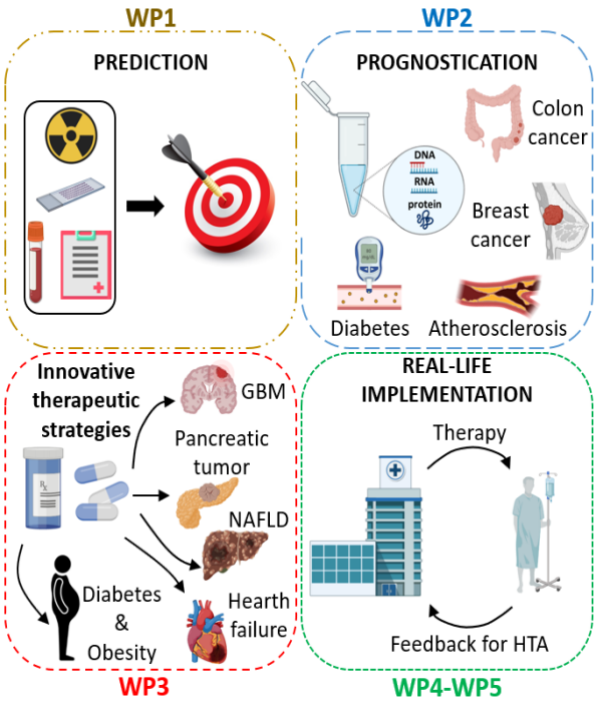
Description of the Spoke Activities

SPOKE Leader	Executors	Starting month	Ending month	Duration (months)
UniPI	M.Negri/IFO/Sant'Orsola/Sapienza/TLS/ TorVergata/UniBO/UniCA/UniCT/UniMIB/ UniMORE/UniPA/UniPI/UniVR/UPMC	1	36	36

1. Context description:

Precision medicine is expected to improve the practice of medicine by taking into account the individual variability in genetic and other molecular characteristics, the interaction with the environment and the impact of lifestyle of each subject. Over the recent years, there has been an outstanding increase, at multiple levels, of capability to generate, store, analyze and interpret data, which is greatly contributing to advance the knowledge in this field. However, the implications of precision medicine in the clinical setting remain challenging, due to the need of generating more molecular data at greater granularity and in larger cohorts, developing better and safer integrated analysis systems, applying reliable validation models, and providing evidence in patients. This spoke will be focused on the clinical validation and implementation of precision medicine approaches, mainly based on data currently available in the participating centers or being generated by the applicants in this Spoke, and with readiness to exchange and apply emerging knowledge by interactions with all other Spokes of this application.

2. General objective:



This part of the project aspires to validate and implement, in the clinical setting, innovative predictive, preventive, diagnostic and therapeutic precision medicine approaches founded on established or emerging molecular and clinical phenotyping and AI-driven decision-making protocols. Specific aims include:

1) To provide proof-of-concept evidence for the clinical usefulness of new strategies supported by sound preclinical backgrounds;

2) To improve the ability of prognostication and risk stratification to offer early intervention when needed, while avoiding useless treatments;

3) To better use available resources to prevent treatment toxicity while expanding the magnitude of expected benefit in properly selected patients;

4) To make the current state-of-art of precision medicine available for patients and caregivers by implementing networks for the management of molecular and clinical information.

According to the call, this will be mainly (but not only) applied to cancer, metabolic diseases and cardiovascular diseases, and each WP and its related tasks may address issues related to more than one single disease. The achievement of the general objective of this Spoke will be facilitated by: *i)* the already ongoing collaborations between several of the partners, particularly in the field of certain cancer types, type 2 diabetes and major cardiovascular ailments, as well as the development of new combined efforts, which is planned for most of the tasks; and *ii)* the continuous exchanges of information with the other Spokes, in particular for the WPs specifically involved in the study of various omics, A.I. approaches and preclinical evaluations. The spoke 8 makes use of the **Scientific Advisory Board** with which has consolidated a collaboration for years: Dominik Modest, Charité - Universitätsmedizin Berlin; Alessandro Doria, Joslin Diabetes, Harvard Medical School, Boston; Steel Dalby Kristensen, Aarhus University, Aarhus, Denmark.

3. Project WPs structure:

WP 1: Optimizing the arrows in quiver: from prediction markers to targeted treatments **(Leader: UniPI)**

This WP aims at capturing the heterogeneous clinical trajectories of certain oncologic and metabolic diseases in order to identify predictors of treatment-associated benefit and toxicity.

Task 1.1: Catching the clinical heterogeneity and dynamic evolution of diseases

Executors	Starting month	Ending month	Duration (months)
IFO/TLS/UniBO/UniPA/UniPI	1	36	36

-To exploit radiomic features to follow the evolution of gastrointestinal malignancies across subsequent lines of treatment.

Milestone: Analysis by means of radiomic software of CT scans of patients affected by colorectal, gastric and pancreatic cancer, and correlation of radiomic changes with ongoing treatments.
Deliverable: Prospective validation in independent cohorts of preliminary radiomic signatures.

-To build an evolutionary clustering method for lung adenocarcinoma able to inform a new classification based on tumor immune microenvironment with potential predictive ability with regard to immunotherapy.

Milestone: Building of a new classification for lung adenocarcinoma based on microenvironmental features.
Deliverable: Evaluation of the predictive role of the new classifier with regard to the use of checkpoint inhibitors.

-To perform risk stratification of type 2 diabetic patients by clusters for personalized prevention strategies.

Milestone: Recruitment of approximately 800 subjects, evaluation of the risk of type 2 diabetes progression and development of a program of mobile health in clusters of subjects with risk ≥ 20%.
Deliverable*:* Verification of the mobile health approach in comparison with traditional recommendations on indexes of glucose metabolism.

-To identify a panel of circulating and tissue biomarkers of prognosis and response to therapy in decompensated cirrhosis and NAFLD-associated hepatocellular carcinoma (HCC).

Milestone: Definition of a preliminary panel of biomarkers derived from an exploratory series of well annotated cirrhotic patients and of subjects with NAFLD-associated hepatocellular carcinoma.
Deliverable*:* Identification of biomarkers associated with the prognosis and the response to therapy in cirrhosis and NAFLD-associated HCC.

Task 1.2: Identifying predictors of benefit from available treatments

Executors	Starting month	Ending month	Duration (months)
IFO/Sant'Orsola/UniBO/UniCT/UniMIB/UniPI	1	36	36

-To predict the effectiveness of immunotherapy through the deep molecular characterization of patients treated with immune checkpoint inhibitors (ICIs) and CAR-T cell therapy approaches.

Milestone: Tumor genomic profiling and circulating immune cells profiling of samples from patients treated with immunotherapies and epigenetic, trascriptomic, metabolomics, single-cell transcriptomic, immunophenotypic analysis of patients treated with CAR-T cell therapy, and clinical data collection.
Deliverable: Correlation of epigenetic, transcriptomic, metabolomics, single-cell transcriptomic, immunophenotypic data with clinical data.

-To individualize treatment options for thyroid cancers.

Milestone: Comprehensive Genomic Profiling of a wide series of biobanked thyroid cancers (around 600 already available) to disclose potentially targetable alterations.
Deliverable: Prospective demonstration of the efficacy of the targeted-driven treatment approach for medullary, anaplastic and dedifferentiated thyroid cancer patient candidate to systemic therapy.

-To assess the predictive role of miRNA signatures in advanced lung cancer and melanoma patients receiving immunotherapy.

***Milestone**:* Identification and validation of the miRNA signature in samples already biobanked, and assay engineering.
Deliverable: Further validation of the miRNA signature in an independent cohort of patients prospectively enrolled.

-To perform personalized therapy of heart failure through marker-related phenotyping that predicts response to treatments.

***Milestone**:* Completion of a retrospective study on the available data of the ongoing GREEN-VASS Study to establish different marker-related phenotypes of heart failure patients; Set-up of the prospective randomized study and complete prospective enrollment of 50% of patients in both arms with full multi-sonographic/radiological and molecular characterization to personalize treatment for heart failure
Deliverable: Prospective enrollment of the remaining 50% of patients; Completion of the follow-up phase of the prospective study on the entire enrolled population; Final assessment of at least 90% of patients; Completion of the prospective study data analyses.

Task 1.3: Predicting treatment-related toxicity

Executors	Starting month	Ending month	Duration (months)
UniPI/UniVR	1	36	36

-To setup and validate time-to-event (TTE) models to quantitate the effects of mixed factors, including pharmacokinetics and pharmacogenetics, to provide a predictive tool for drug tolerability.

Milestone: In-house datasets including pharmacokinetic, pharmacogenetic, environmental, clinical and quality-of-life data will be interrogated to build a TTE model predictive of tolerability of different treatment strategies.
Deliverable: To validate results from TTE models in independent series.

Task 1.4: Predicting all-cause mortality in patients with diabetes

Executors	Starting month	Ending month	Duration (months)
Sapienza	1	36	36

To validate the association between novel metabolites, miRNA and all-cause death in two independent cohorts with adult-onset diabetes (AOD) from the “Sapienza Mortality and Morbidity Event Rate (SUMMER) study in diabetes” (n=5,000) and the aggregate “Gargano Mortality Study” (A-GMS) (n=2,000). Both studies are part of established networks, comprising several

academic/research centers in Italy (L'Aquila, Pisa, Foggia, Tor Vergata-Rome and abroad (Boston, Munich). This task will also investigate the role of such markers in improving discrimination and reclassification of the ENFORCE and RECODe algorithms, two gold standards for predicting mortality in AOD. Finally, it will be investigated whether the markers associated with all-cause death are also associated with coronary heart disease (CHD) and/or chronic kidney disease (CKD) (mediation analysis). The analyses will be conducted in the whole cohort and stratifying participants in those with T2D and with Autoimmune Diabetes (AID), based on the measurement of pancreatic autoantibodies and on AID genetic risk score. In vitro studies on cultured human aortic endothelial cells (HAEC) and immortalized human podocytes (iHP) will be conducted to unveil novel pathogenic pathways specific for the newly identified markers.

Milestones: Evaluation of the added value of the newly identified markers in improving discrimination (C-statistics and % integrated discrimination improvement) and reclassification (net reclassification improvement) of the ENFORCE and RECODe algorithms; Evaluation of differences in risk factors for mortality, CHD, and CKD between people with T2D and AID; - Evaluation of the biological activity of the newly identified markers within pathogenic pathways of CHD and of CKD; Validation of the association between the markers identified in the preliminary study and all-cause death; Validation of the association between the markers as above and CHD and/or CKD; Differential association of the newly identified metabolites and diabetes outcomes (mortality, CHD, CKD) among people with T2D and people with AID.
Deliverables: New pathogenic pathways of CHD and CKD involving the newly identified markers; Novel risk score for prediction of mortality in adults with diabetes; Novel risk score for prediction of diabetes outcomes in different types of AOD; Development of artificial intelligence algorithms for precision diagnostic in diabetes which will be able to predict hard outcomes in diabetes; Novel insights about the role of CHD and CKD as mediators of all-cause mortality in diabetes; Novel diagnostic biomarkers of interest for industries.

WP 2: Beyond the crystal ball: improving prognostication in cancer and cardiometabolic diseases **(Leader: UniPI)**

This WP aims at exploiting innovative technologies for the early detection of certain oncological and cardiometabolic diseases and for the improvement of their prognosis, based on clinical and molecular markers.

Task 2.1: Circulating markers of minimal residual disease: exploiting liquid biopsies to predict disease relapse through the analysis of circulating tumor DNA

Executors	Starting month	Ending month	Duration (months)
UniPI	1	36	36

-To personalize the adjuvant therapy of colorectal cancer patients through the quantitative and qualitative analysis of ctDNA.

Milestone: To take advantage of an already existing platform for liquid biopsy analysis for the detection of the minimal residual disease to design a targeted-oriented arm to treat patients bearing

specific molecular alterations and with post-operative ctDNA-positive with matched drugs. Trial start-up and EC approval.
Deliverable: To evaluate targeted treatment results by analyzing the ctDNA clearance at the end of the adjuvant therapy. Completion of enrollment and primary data analysis.

-To assess new hormone-mediated mechanisms for cancer metastatic spread and immunoescape in obese and hyperinsulinemic patients, with the ultimate goal of identifying novel personalized therapeutic approaches.

Milestone: To establish appropriate cell models to investigate the role of signaling networks involving the Insulin/IGF/DDR1 axis and RAGE pathway in driving therapy resistance, metastasis and immune evasion in invasive cancers of the breast and the thyroid gland; to assess the specific role of Insulin/IGF/DDR1 signaling and RAGE pathway as crucial determinant for dissemination and escape in breast and thyroid cancer.
Deliverable: Design and validate new combination target therapies to overcome obesity-driven mechanisms of BC metastatic dissemination and immunoescape.

Task 2.2: Clinical, genomic and epigenetic markers of outcome in malignancies and cardiometabolic diseases

Executors	Starting month	Ending month	Duration (months)
TLS/UniPI/UniCT/TorVergata	1	36	36

-To perform multiparametric assessment of critical site atherosclerosis.

Milestone: Completion of a retrospective study on the available dataset of the ongoing CAMP and SMARTool Studies; Set-up of the prospective study and completion of prospective enrollment of at least 60% of patients with full multi-imaging and molecular characterization to assess critical site atherosclerosis.
Deliverable: Prospective enrollment of the remaining 40% of patients; Completion of the follow-up phase of the prospective study on the entire enrolled population; Final assessment of at least 90% of patients with full multi-imaging and molecular characterization; Completion of the prospective study data analyses.

-To personalize the treatment approach to chronic lymphocytic and myeloid leukemia.

In this Task, comprehensive models to catch the multifactorial complexity of cancer and CVD will be investigated with a focus on acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), myelodysplastic syndromes (MSD and atherosclerosis. In particular, the contribution of molecular markers to the prognosis of affected patients will be evaluated.

Milestone: DNA sequencing will be assessed in CLL and AML patients from diagnosis to several follow-ups. First, it will evaluate the correlation between specific mutations and clinical manifestation. It will be necessary to define a multigene panel (25 genes), with selected genes expressed with certainty related, associated, or predictive of evolution in CLL e AML. It will analyse the pathways involved in apoptosis, DNA damage, NOTCH signaling, NFkB pathway, inflammation, and RNA processing. To focus on temporal patterns of driver acquisition – based on the distinction between clonal versus subclonal alterations in a cross-sectional analysis – defining a temporal map for the evolutionary history of CLL.
Deliverable: Over an extended follow-up to evaluate the impact of targeted therapies (i.e., BTKi, anti-bcl2) and future combinations, totally chemo-free, on CLL patients with specific mutations with a prospective analysis (clonal evolution). Recognition of precise disease mutational patterns in the spectrum of MDS/AML, to predict clinical outcome and improve prognostic stratification; appreciation of the clinical impact of chemo-free combinations in CLL; Conduction of a prospective trial on atherosclerosis moving from two ongoing retrospective experiences.

Task 2.3: Recognising colo-rectal cancer (CRC) behaviour, aggressiveness, and prognosis through radiogenomic signature

Executors	Starting month	Ending month	Duration (months)
Sapienza/UniCA	1	36	36

- The AIM is to characterize biological processes in a voxel-wise high-spatial resolution approach, extracting first, second and third order radiomics features from CT datasets of patients with CRC. Radiomic features will be subsequently computed by AI and integrated with clinical data to eventually generate a radiogenomic signature for personalised management of patients with CRC. By an artificial intelligence-based algorithm of radiomic features combined with clinical factors, biochemical biomarkers and genomic data it will be recognised the tumor behaviour, aggressiveness, and prognosis, identifying a radiogenomic signature of the tumor. Development of a radiogenomic signature of colon tumor, will have a great impact in oncology. A strong impact will be found at different levels, from research to patient care, thus involving not only high-end oncologic research centres, but also oncologic multidisciplinary teams working in community hospitals.This AI-based model approach will be based on the extraction of radiomic quantitative parameters from Computed Tomography images of colon tumors (Stage II-III) combined with pathological, genomic information. This model will allow for a more accurate and precise understanding of tumor behavior, aggressiveness, and prognosis, ultimately impacting the therapeutic strategies.The development of a radiogenomic signature will overcome the main limitation of conventional biopsies, incomplete tissue sampling, which might lead to a misinterpretation of real tumor characteristics.

Milestones: i) Inclusion of 300 retrospective cases; Definition of cancer hallmarks (radiology, pathology, genomic); Radiomic features calculations; Creation of the ATTRACT AI-model v1; ***ii)*** Recruitment of 210 prospective patients; Training of the ATTRACT AI-model v1; Validation of the ATTRACT AI-model v1 Patient follow-up; ***iii)*** Networking Dissemination and exploitation.
Deliverable: Generation of segmentation AI model for colon cancer; Generation of radiogenomic AI model for colon cancer signature; Attract AI-model v2.

WP 3: Reverse translation: from the patient to the lab and back **(Leader: Sapienza)**

This WP aims at providing proof-of-concept evidence of the clinical efficacy of a precision medicine-driven approach to the management of specific clinical conditions. The opportunity of drug re-purposing, the cross-talk between metabolism and cancer, and the possibility to build a biomarker-informed *continuum of care* for patients affected by cardiometabolic diseases and solid malignancies will be explored.

Task 3.1: Finding the niche for drug clinical repurposing

Executors	Starting month	Ending month	Duration (months)
IFO/UniPI/UniVR	1	36	36

-To reposition chlorpromazine for the treatment of glioblastoma multiforme
-To run the ORIENTATE (TailOred dRug repurposIng of dEcitabine in KRAS-dependeNt refracTory pAncreaTic cancEr) trial

Milestone: 75% of expected accrual for both trials (the protocol of these Phase II trials have already been approved by competent authorities)
Deliverable: Primary analysis of primary endpoint results (6 months-Progression Free Rate and Objective Response Rate, respectively).

-To exploit tumor dependency on aerobic glycolysis for therapeutic purposes: a phase I/Ib study with the LDH inhibitor BIT23836.

Milestone: Trial start	up and first patient in.
Deliverable: Accrual completion and set	up of the phase Ib expansion cohort.

-To identify pancreatic beta cell targeted therapy for type 2 diabetes.

Milestone: to generate/collect multiomics data from pancreatic islets of > 600 organ donors to be correlated with beta cell function in non-diabetic subjects and patients with type 2 diabetes.
Deliverable: Identification of at least 3 chemical compounds/known drugs to be repurposed for the use in type 2 diabetes therapy.

Task 3.2: Identifying mechanisms of acquired resistance to available treatment options to select candidate drugs for clinical investigation

Executors	Starting month	Ending month	Duration (months)
UniPI/UniBO	1	36	36

-To detect and validate markers and predictors (also through AI) of atherosclerotic vascular disease and heart failure progression.

Milestone: Collection of all validated markers assessed at 18 months of Task 1.2 and Task 2.3 on atherosclerotic vascular disease and heart failure progression; Set up of two deep learning libraries to run AI analysis for atherosclerotic vascular disease and heart failure progression
Deliverable: Confirmation of the role of the selected biomarkers on both atherosclerotic vascular disease and heart failure progression in the populations prospectively enrolled in Task 1.2 and Task 2.3 and in the retrospective populations of the ongoing CAMP and SMARTool studies.

- To detect and validate heart rate variability (HRV) patterns behaving as markers and predictors for heart failure, arrhythmia and mortality in cardiovascular diseases or myocardial infarction.

Milestone: Collection of validated HRV signatures with cardiovascular diseases and/or myocardial infarction on the onset and progression of heart failure, arrhythmias and mortality in cardiovascular diseases;
Deliverable: Confirmation of the role of the selected HRV signatures as predictor markers for the onset and progression of heart failure, arrhythmias and mortality in cardiovascular diseases

Task 3.3: As a matter of fat

Executors	Starting month	Ending month	Duration (months)
UniPI/UniPA/UniMORE/UPMC	1	36	36

-To predict and treat Non-Alcoholic Fatty Liver Disease (NAFLD) in genotyped patients.

Milestone: Implementation and analysis of a large retrospective cohort of patients with NAFLD, with a median follow-up of 60 months
Deliverable: Identification of genetic and clinical markers associated with progression of disease and hepatic as well as extra-hepatic morbidity and mortality.

-To determine the role of weight gain and other cardiometabolic risk factors in transplanted patients.

Milestone: Evaluation of anthropometric characteristics (AC) and cardiometabolic risk factors CRF) in patients with kidney (> 1,000), liver (> 2,000) and pancreas (> 400)

Deliverable: Identification of AC and CRF associated associated with organ/recipient survival

- To identify patient-specific biomarkers to be correlated with adipose stromal cells therapy outcome targeting immune dysregulation and impacting on highly invalidating skin complications in systemic sclerosis (SSc).

Milestone: To correlate circulating and tissue biomarkers in SSc with the therapeutic outcome of adipose cell transplantation.
Deliverable: To establish a personalized adipose cell therapy strategy for SSc.

Task 3.4: Molecular, mutational, radiomic and histo-morphologic profile of HBP cancers: assigning the right treatment to the right patient at the right time.

Executors	Starting month	Ending month	Duration (months)
Sapienza	1	36	36

- Thank to shareable data from the international network (ENSCA) and the activities that will be performed by the SPOKE 4 in the WP4, Task 4 that include the largest cohort (approx. 3000 cases) of fully characterized (clinic, histopathologic, morphologic and radiologic data) HBP tumors, this task aims to: 1) provide the first in-depth characterization of HBP tumors based on clinical, histomorphological and molecular profiling in tissues and liquid biopsy; 2) identify, with the help of a multidisciplinary platform (matching clinical data, histomorphology, molecular profiling in tissues, molecular data from liquid biopsy and data from patient-derived organoids) individualized prognosis and therapy for patients with HPB tumors.

Milestones: Identification of molecular and mutational profile of HBP cancers by machine learning on histomorphological features; Molecular and genetic profiles will be correlated to histological subtypes of HBP tumors;Morpho-genomic classification: integration of clinical, morphological and radiological parameters using AI-guided neural networks; Evaluation of ctDNA as a useful tool to monitor tumor progression and recurrence in HBP cancer patients; Profiling a personalized medicine approach in CCA patient-derived molecularly defined organoids; to test Pemigatinib on FGFR2-wt and FGFR2 mutated PDOs and correlate in vitro drug response with transcriptomic profile to identify a transcriptomic fingerprint predicting therapeutic responsiveness; obtain IDH1-wild type and aberrant IDH1 PDOs by using CCA samples and to perform transcriptome analysis; To test Ivosidenib on IDH1-wt and IDH1 mutated PDOs and correlate in vitro drug response with transcriptomic profile to identify a transcriptomic fingerprint predicting therapeutic responsiveness; identify the most promising target compounds as IDH1 inhibitors available in an in house library available at Sapienza University, based on the available X-ray crystal structure of IDH1 mutants and computational studies, mostly carried out at the structure-based level.; To assess NPs selected by in silico screening against IDH1-mutated PDOs. identify qualitative and quantitative MRI parameters as potential markers for bile duct fibrosis, inflammation and dysplasia/cancer in subjects with disease at risk for CCA; analyze transcriptomic and proteomic profile in tissue and liquid biopsy in primary sclerosing cholangitis or IgG-4 related cholangitis and in CCA emerging in theses chronic diseases to unveil predictive biomarkers for CCA diagnosis and

screening; evaluate in a group of HCC patients initially out of the conventional transplantability criteria the down staging efficacy of the total tumor burden and the transplant outcomes (survival) using a multimodal approach comprehending the immune checkpoint inhibitors; Molecular profiling (transcriptomic, genetic) of HBP tumors at spatial level by Nanostring® in order to characterize innovative biomarkers for targetable pathways: i) IDH1/2 expression; ii) FGFR alterations; iii) checkpoint inhibitors (PD1/PDL1 and CTLA-4 pathways, DNA mismatch repair pathway by immunohistochemistry); iv) VEGF pathways; v) IL-6 pathways; vi) HER2, EGFR, mTOR, MEK1/2 pathways.
Deliverables: comparative genomic-radiomic-histomorphologic profile of liver and pancreas cancer; clinical outcomes of specific target therapies in a clinical trial; proteomic fingerprint predicting the emerging of molecular findings from liquid biopsy; platform of HBP patient-derived molecular defined organoids; Identification of potential therapeutic agents in HBP patient-derived molecular defined organoids; Identification of a transcriptomic fingerprint predicting the therapeutic response to FGFR2-, HDH1-target therapies; validation of an innovative contrast enhanced MRI cholangiography as a tool for early diagnosis of HPB cancer in at risk patients (PSC, chronic pancreatitis, etc..

WP 4: Make it happen in the real-life: precision medicine is ready for prime time (**(Leader: IFO)**

This WP aims at translating promises of precision medicine into the real-life of affected patients on a large scale. Strategies to implement findings from the lab in the clinic through large dedicated networks and to make cutting-edge technologies available for the majority of patients, feasible and sustainable, will be investigated.

Task 4.1: Building networks for precision medicine implementation in the daily clinical practice

Executors	Starting month	Ending month	Duration (months)
UniPI/UniBO/IFO/UniCA	1	36	36

-To create "tumor boards" at national level through hub and spoke networks to apply knowledge to clinical practice and clinical trials' enrollment.

Milestone: Creation of "tumor boards" at national level through hub and spoke network involving professionals with different skills (clinical, biological, molecular and bioinformatics) aimed at developing collaborations and shared knowledge on the subject; promotion and carrying out of translational research studies aimed at developing and validating AI tools or pipeline and, with these ones, at identifying molecular markers by means of a multilevel technological approach;
Deliverable: Promotion of clinical research studies aimed at the development and application of new drugs and treatment strategies incorporating the AI tools and information; Promotion of AI technology in medicine and precision oncology in the field of the health culture through the dissemination of scientific data, discussion of guidelines, and training events; Progressive introduction of AI approaches in the clinical governance of health care system.

-To create a network for knowledge sharing and trial development in rare tumors.

Milestone: Creating a collaborative networking on rare tumors integrated with national rare tumors network (RTRN) to develop cancer registries, epidemiological studies, translational research studies on molecular biology and immunology with advanced approach and technologies, derived patients' preclinical models, structured clinical and multi -omic databases for efficient data management, AI tools, exploratory clinical studies, ethical working group.
Deliverable: Networking on rare tumors including Health Care Institutes, professionals, regulatory agencies and advocacy participation; collaboration with ERN and pre-existing national networks; Epidemiological studies on the cancer tumors population; Design of translational studies for tumor molecular characterization by high throughput technologies; Development of new algorithms and novel bioinformatics pipelines for the analysis of generated data; Development of derived-single patient preclinical models to assess innovative therapeutic targets and strategies; Ethical and legal discussion and preparation of position papers and specific requests to regulatory agencies.

-To Profile OrphaN Tumors for therapy sElection: extensive molecular profiling (WES/WGS and/or Trascriptomics) and MTB-based case management of "untargettable" tumors.

Milestone: Identification/validation of the most cost/effective molecular testing strategy for selected orphan tumors.
Deliverable: Development of a functional case management process, to guide patients from molecular diagnosis to clinical treatment; Creation of a public database, mapped per histotype and actionability; Analysis of the impact of the proposed strategy (including detailed description of the actionable cancer vulnerabilities identified and health economics assessment) on the outcome.

Task 4.2: Assessing the cost-effectiveness of available techniques to "pick the winner" in the perspective of sustainability

Executors	Starting month	Ending month	Duration (months)
UniPI	1	36	36

-To compare step-by-step versus one-shot analyses for molecular characterization of malignancies in the real-life.

Milestone: Analysis of tissue and liquid biopsies of patients diagnosed with colorectal and NSCLC will be analyzed with a multi-omics approach, via both solid and liquid biopsies. The proportion of patients with uncommon actionable molecular alterations will be determined.
Deliverable: The cost-effectiveness of a deep molecular characterization of tumors, as defined above, will be defined in relation to tumor type and analysis level. The most appropriate diagnostic algorithms will be developed accordingly.

-To develop point of care tools based on biosensors and telemedicine for monitoring individuals in their daily environment.

Milestone: development of innovative biosensors to be included in a portable point of care device (POC) capable of remote communication for the rapid identification of pathogens in fragile subjects.
Deliverable: final set-up and clinical validation of the developed biosensor.

-To validate data sanitization algorithms for privacy-aware dissemination.

Milestone: Formal modeling of real cases of dissemination of biomedical data with specific regard to privacy requirements, and development of related algorithms of data sanitization.
Deliverable: Application of algorithms previously identified to real-case scenarios and optimization in a perspective of scalability to big data.

Task 4.3: From extended trailers to clinical proofs of concept

Executors	Starting month	Ending month	Duration (months)
M. Negri/TLS/UniPI	1	36	36

-To tackle residual cardiometabolic/inflammatory atherosclerotic risk through a comprehensive lifestyle intervention on top of optimal medical treatment in a randomized controlled trial.

Milestone: Set up of the randomized controlled trial with comprehensive lifestyle intervention on top of optimal medical treatment (including dietary restriction); Enrollment of at least 70% of patients in both arms
Deliverable: Prospective enrollment of the remaining 36% of patients; Completion of the follow-up phase of the prospective study on the entire enrolled population; Final assessment of at least 90% of patients; Completion of the prospective study data analyses.

-To personalize weight loss therapies.

Milestone: Set-up of a "metabolic" (respiratory) chamber and recruitment of 40 non-diabetic, obese subjects, clinically phenotypes.
Deliverable: Completion of a study to compare a GLP-1 receptor agonist and a centrally acting drug on body composition, 24h energy expenditure and RQ, to correlate phenotype and pharmacological effectiveness.

-The case of alkaptonuria.

Milestone: Implementation of an already established digital platform for patients with alkaptonuria, to include genetic, biochemical, histopathological, clinical and quality of life data.
Deliverable: Identification of novel therapeutical approaches for the treatment of alkaptonuria and its comorbidities.

WP 5: From bench to bedside: the evaluation and implementation of innovative precision medicine technologies in clinical care **(Leader: Sapienza)**

This WP aims at developing an evidence-based strategy to assess the value of personalized medicine-based technologies and promote their implementation in clinical care.

Task 5.1: Identification of the HTA evaluation process core components and key aspects regarding innovative clinical precision medicine technologies

Executors	Starting month	Ending month	Duration (months)
UniPI/Sapienza	1	36	36

-To systematically review the existing HTA frameworks used for the evaluation of innovative technologies, such as genomics, proteomics and metabolomics biomarkers and artificial intelligence-based tools.
-To identify and summarize the core components and key features of the HTA evaluation process regarding innovative precision medicine technologies.

Milestone: Systematic review of HTA frameworks used for the evaluation of innovative technologies and of HTA reports conducted on innovative technologies.
Deliverable: Identification of the core components and key features of the HTA evaluation process regarding innovative precision medicine technologies.

Task 5.2: Identification of the existing methodologies and development of a standardized approach to generate evidence on the clinical validity, utility, and technical aspects of innovative precision medicine technologies, and provide a tailored HTA framework to evaluate personalized procedures and promote their implementation in clinical care

Executors	Starting month	Ending month	Duration (months)
UniPI/Sapienza	1	36	36

-To identify the existing approaches and methodologies used to generate evidence regarding innovative precision medicine technologies and identify gaps and challenges of the processes.

-To develop standardized and tailored approaches to generate evidence, suitable for evaluation frameworks, and promote their adoption.

Milestone: Systematic review of existing approaches and methodologies used to generate evidence regarding innovative precision medicine technologies; Analysis of the gaps in evidence generated regarding innovative technologies.
Deliverable: Development of an evidence-based methodology to generate evidence for innovative precision medicine technologies; Development of a new HTA framework to be used for the evaluation of innovative technologies.