

Index

RESEARCH STRATEGIC PLAN (2020-2022) OF IRCCS REGINA ELENA NATIONAL CANCER INSTITUTE	2
INTRODUCTION.....	3
1. ADVANCED DIAGNOSTIC / PROGNOSTIC RESEARCH (ARAD) (in Orange).....	5
<i>1.1 Biobank.....</i>	<i>6</i>
<i>1.2 Omics.....</i>	<i>10</i>
<i>1.3 Bioinformatics, Machine Learning, Biomarkers.....</i>	<i>16</i>
2. AREA OF PRECISION SURGERY (APC) (highlighted in purple)	19
3. AREA OF PRECISION MEDICINE (AMP) (in GREEN)	24
<i>3.1 Targeted Therapy.....</i>	<i>24</i>
<i>3.2 Immunotherapy.....</i>	<i>31</i>
<i>3.3 Stereotactic Radiation Therapy.....</i>	<i>35</i>
4. ADVANCED CLINICAL RESEARCH AREA (ARCA) (Area in Blue)	38
<i>4.1 Clinical Trial Center.....</i>	<i>39</i>
<i>4.2 Phase I Study Centre.....</i>	<i>43</i>
<i>4.3 Molecular Tumor Board.....</i>	<i>47</i>
<i>4.4 Centre for Rare Cancers.....</i>	<i>52</i>
FINANCIAL RESOURCES.....	57
CONCLUSIONS.....	58

RESEARCH STRATEGIC PLAN (2020-2022) OF IRCCS REGINA ELENA NATIONAL CANCER INSTITUTE

IRE's main objective is:

**TRASLATIONAL RESEARCH TO MANAGE CANCER PATIENTS
 (PRESa in carico Traslazionale del paziente Oncologico - PRESTO)**

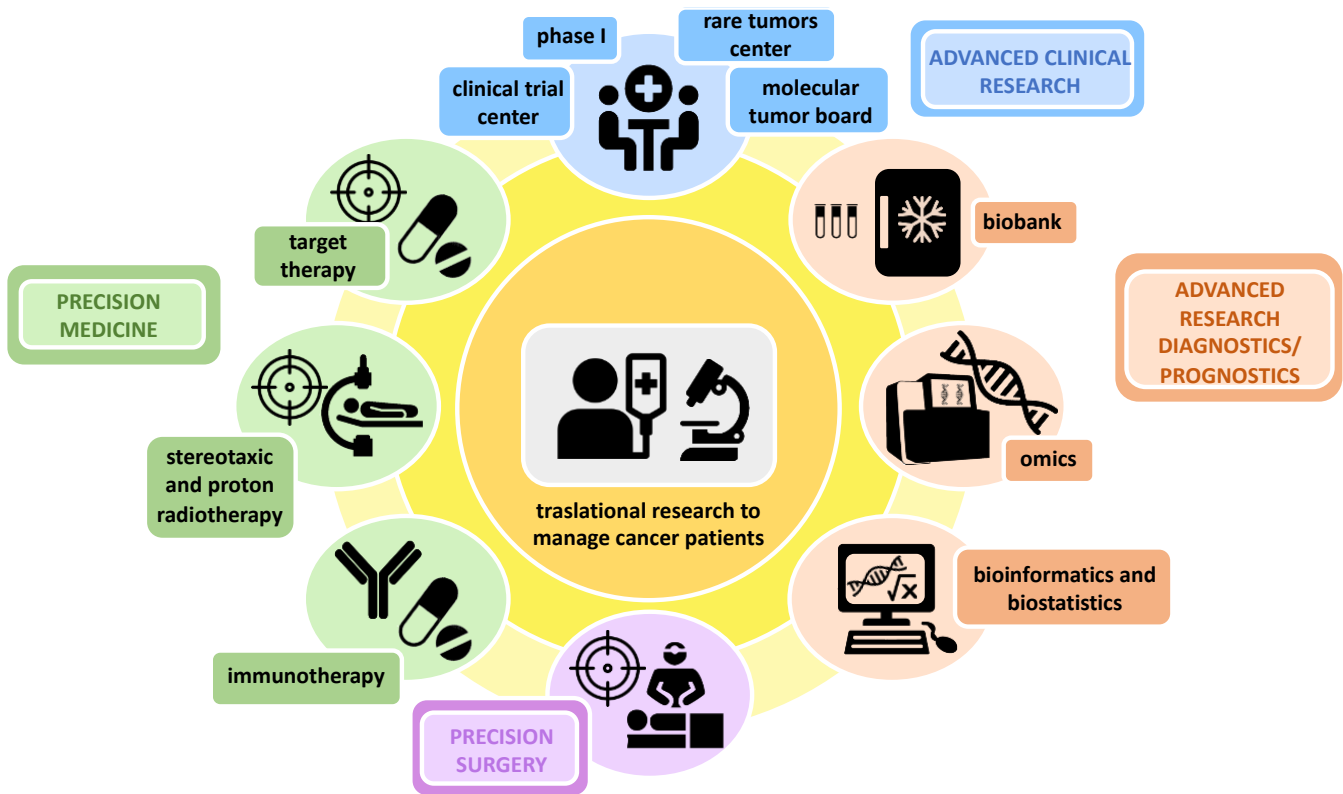


Figure 1

INTRODUCTION

The main research goal of the IRCCS Regina Elena National Cancer Institute is the translational management of cancer patients (PRESa in carico Traslazionale del paziente Oncologico - PRESTO). What does this mean? It means that the cancer patient is always placed at the center of our research objectives and with this focus, we aim to understand the distinct alterations of patients' tumors within the perspective of personalized and precision medicine; and then to transfer our research results to the patients' bedside in order to provide rapid answers to their health needs through scientific and technological innovation.

Today, providing care to cancer patients is essentially based on having several vital contributing facilities. IRE has therefore armed itself with a series of indispensable tools, from a modern and equipped biobank storing tissue and liquid samples deriving from cancer patients up to a number of the most recent technologies for "omics" analysis as well as for surgery such as robotic surgery, stereotactic radiotherapy, imaging and so on. Our Institute is the only facility in Lazio where these facilities combined with the necessary expertise (know how) are all physically concentrated in the same logistic area and dedicated to cancer patients' care. This allows us to apply all the standards of the state-of-the-art modern medicine to implement a more personalized approach towards the patient, not only considering their clinical characteristics, but also the expression of molecular determinants which are increasingly fundamental in improving diagnostic, prognostic, predictive and therapeutic performance.

It is now crucial to manage cancer patients by evaluating the expression of molecular biomarkers with cutting-edge "omics" technologies that allow to stratify patients that are able to respond or not to certain types of therapies. Even if it appears to be more challenging at first, obtaining knowledge, understanding and clinical management of these parameters provides us with an unquestionable advantage and together with the results generated can drastically improve the clinical picture (survival and quality of life) of the patient. By applying this modern approach towards cancer patient care it is also essential to increase the number of early diagnoses, which are now often possible due to the very high resolution in diagnostic imaging equipment provides, thus avoiding falling into making frequent errors in over-diagnosis and over-treatment.

The future of cancer care and patient medical records will therefore have to contain, in addition to the results of routine investigation, also a series of metadata (genomics, transcriptomics, metabolomics, proteomics, microbiomics, radiomics, etc.) that concern the molecular aspects of single neoplasms which provide the basis for an advanced prognostic / predictive / therapeutic approach. The objective is to obtain a personalized clinical report capable of highlighting the biological and clinical relevance of each identified molecular alteration. In fact, these methodologies allow identifying therapies and clinical studies that include potentially effective drugs (targeted therapy, immunotherapies and experimental therapies) that are best for each individual patient.

It is therefore important that the data provided by the most modern technologies are managed by a multidisciplinary team that includes not only oncologists, but also biotechnologists, molecular biologists, geneticists, pathologists, biostatistics, bioinformatics, etc.

PRESTO is summarized in the diagram above (Fig1). It is divided into four macro-areas which are highlighted by distinct colors: advanced diagnostic / prognostic research (in Orange), precision surgery (in Purple), precision medicine (in Green) and advanced clinical research (in Blue).

In the following sections the features of each macro-area will be described as well as short and long-term objectives. All together these sections constitute the elements of the research strategy, represent the research footprint of the institute and characterize its specificity in the context our national clinical cancer research.

It has to be pointed out that Regina Elena National Cancer Institute works in close collaboration within national and international networks, in first instance is one of the founders of the largest organization of clinical research cancer centers in Italy called Alliance Against Cancer (Alleanza Contro il Cancro -ACC). Furthermore, it is member of OECI, the Organization of European Cancer Institute where it has been given the highest level of accreditation as comprehensive cancer center, and finally has established strategic collaborations with prestigious research centers outside Italy such as the Weizmann Institute in Israel. All these network

collaborations help the realization of the strategic plan but we have decided not to specifically mention them in the following sections

1. ADVANCED DIAGNOSTIC / PROGNOSTIC RESEARCH (ARAD) (in Orange)

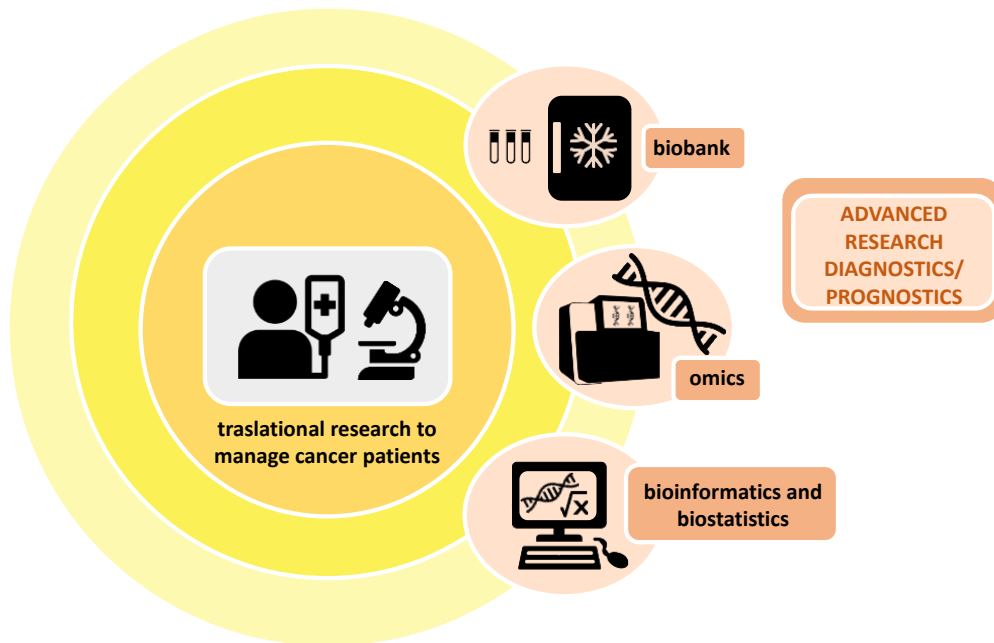


Figure 2

1.1 Biobank

Our main objective is to become the largest unit of storing tumor tissue and biological fluid samples from cancer patients in Lazio for the use of scientific research and for the benefit of the patient

- **Certified ISO 9001/2015 in June 2018**
- **Was inserted in the BBMRI network**
- **Quality Manual**
- **No. Liquid Samples > 60.000**
- **No. Tissue Samples > 13.000**

Background / state of the art:

Successful research in identifying the molecular causes behind complex diseases, such as cancer and of the possible therapeutic applications, including the development of new and specific drugs, makes use of the possibility of having a series of vast biological samples of people who are affected by cancer and are undergoing controlled/randomized therapeutic protocols or are carriers of molecular alterations predisposing them to specific cancers. The availability of biological samples associated with the relevant clinical and epidemiological data is essential in identifying the association between genetic and environmental factors that induce disease while, at the same time modify their severity and outcome, in order to identify new therapeutic targets and reduce the time required for the discovery and development and use of new therapies.

Numerous biological materials and information have been collected over the years as well as the number of different types of materials, methods of collection and conservation, and management of clinical-pathological data emerging. These collections have progressively transformed into increasingly better organized complex units, to which, the term "biobanks" was coined in the second half of the 1990s. The recent definition of international standards relating to the organization and management of biobanks (UNI ISO 20387), as well as the guidelines and documents produced by different Societies and Scientific Organizations require an adjustment of the modus operandi in order to make the biobanking procedures homogeneous with the prospect of being able to have homogeneous and qualitative biological samples.

The "Oncological biobanks for research purposes" Guidelines created in 2013 by the AIOM and SIAPEC-IAP Working Groups have defined a "research biobank" as: a service unit, with no direct profit, which is organized according to quality criteria, order and destination and aims at collecting, preserving and distributing human biological material and the data pertaining to it for scientific research purposes, guaranteeing and protecting the rights of the subjects involved. Biobanks offer to collect and conserve cells, tissues and biological liquids, including all the molecular fractions (proteins, RNA, DNA, etc.) that can be derived from them, as well as from healthy volunteers and patients. The type of research that can be carried out on these materials has not been defined a priori. The samples must be so versatile that they can be used in research not yet in progress and with techniques that have not yet been developed. The biological materials and related clinical data, in addition to being of high quality, must meet the ethical-legal requirements. That is, they must have been collected from subjects who were willing, conscious and with participatory consent and treated in compliance with the national and international standards, which relate to the protection of citizens' privacy and their right to self-determination. The biobank plays a public role, a functional service as well as a third party function, guaranteeing biobanking processes to all the players involved even towards the company. Sharing the biobanking services for results is what the research biobank is based on. Biobanks also represent a fundamental

tool for research in the field of rare diseases. There are now numerous cases in which the search for "disease genes" has been accelerated thanks to the existence of biobanks, leading to a greater understanding of pathogenetic mechanisms, the development of new diagnostic tools and the design of therapeutic strategies. In Italy, there are about 90 Biobanks, mainly disease-oriented (oncological, genetic, multi-specialist), which are organized in regional networks and national and international theme orientated networks.

The Regina Elena Biobank

The IRE (BBIRE) Bio-Oncological Research Biobank, formalized with Decree No. 180 dated 03/14/2014, is one of the Institute's strategic assets that aims to collect, store and distribute human biological material, tissues and biological liquids, and the data associated with it in order to implement basic, clinical and translational cancer research. In the spirit of strengthening and providing an accurate definition of its activities, a Quality / Regulation Manual was drafted and resolved (Decree no. 431 dated 13 June 2017) together with the necessary definition of the two operating bodies, the Steering Committee and the Operating Group. The Steering Committee (SC) has the task of assisting the Scientific Direction in evaluating and selecting scientific projects, presented by experimental and / or clinical Researchers, who are interested in using the Biobank both to provide and request human biological samples for their research. The SC is composed of the Scientific Director (SD), the Heads of Department, the Head of UOC Pathological Anatomy, the Head of UOSD Clinical Pathology, the Secretariat of the GRO and internal or external institutional figures of the Regina Elena Institute, and is convened by the SD according to the competence and transparency criteria for the selection of specific research projects at the OdG.

The Operating Group (GRO) is composed of the Head of the Pathological Anatomy UOC, the Head of the Clinical Pathology UOSD, the Quality Managers of BBIRE-T and BBIRE-LB executing administrative and management tasks. The GRO, which meets about every two months, in line with its functional and nominative organization chart, coordinates the two specific areas pertaining to Material and Biological Liquids and has the task of developing and managing the activities of the Biobank as well as implementing the Certification processes / Quality / Accreditation and defining the operational procedures and the quality control methods of the samples. It also manages, in agreement with the Technical and Clinical Engineering Unit, the various phases of construction, implementation and maintenance of the BBIRE in appointed spaces as well as all projects geared towards improvement and technological innovation.

The BBIRE has been collecting human biological samples systematically from patients under our care since 2015 in accordance with the quality standards and the ISO 9001 2015 certification.

Material and data collected are made available to all the Universities, Research Institutes, Scientific Institutes or Societies that collaborate with IRE to carry out research activities. BBIRE is a member of the European Research Infrastructure Network of Biobanks and Biomolecular Resources (BBMRI-ERIC) and participates together with European groups (BBMRI ERIC, EORTC) in realizing large-scale multi-center projects (HORIZON2020) and in the drafting guidelines. The BBIRE also belongs to the network of Biobanks of the Lazio Region in the framework of the regional project "Lazio Network for Translational Medicine and Tumor Biotherapies Development", coordinated by the Istituto Superiore di Sanità. This network aims to be an instrument and a driver in innovative medicine, focusing particularly on the development of bio-therapeutic and diagnostic products regarding health care, specially, in the field of anticancer biotherapies. BBIRE uses innovative management systems, in particular with regard to: traceability systems, the quality of the sample path (timing, compliance with the cold chain, etc.), storage with a dedicated room equipped with wireless monitoring systems and input protected by fingerprint identification. The entire biobanking process is periodically subjected to the Ethics Committee's guidance who approves the organization and makes any changes to informed consents that need to be regularly updated with the ethical-legal and European directives. The organization of the BBIRE is integrated both in the clinical and scientific setting, allowing to follow patients throughout their therapeutic pathway (diagnosis, therapies and follow up), thus contributing greatly

on a translational level. The BBIRE is an integral part of the workflow of the Advanced Diagnostic/Prognostic Research Area (Omics, Liquid Biopsy) and of the Advanced Clinical Research Area (Clinical Trial Center and Molecular Tumor Board).



Figure 3

To date, the BBIRE stores more than 60,000 samples of biological fluids (whole blood, serum, plasma, PBMC) from more than 2,500 patients and about 13,000 tumor tissue samples from more than 1,100 patients (numbers updated at October 2020). The main strength of the BBIRE is its availability in obtaining biological material from each patient that is essential for the correct diagnosis, prognosis and treatment based on the careful study of changes in molecular expression of the tumor which is crucial for achieving higher personalised or precision medicine. Recently, the collection of biological fluid samples from healthy subjects under the care of the Institute's Immunohematology and Transfusion Medicine Service has set up a control group to perform quality checks on stored biological samples.

BBIRE also has to be ready to develop specific subsections in case of sudden medical and epidemiological crisis such as what we are currently experiencing with the SARS-Cov-2 pandemic. Although the IFO Institute is considered a COVID free hospital and we don't hospitalize COVID-19 patients which are referred to COVID hospitals in the Lazio region, the BBIRE Biobank has started collecting and storing serum samples from employees and from patients (mainly cancer patients) for epidemiological surveys of seropositivity.

1 year Objectives

- Grow as a single repository unit of bioptic material and biological fluids deriving from cancer patients under the care of both IRE-ISG (ISG is San Gallicano Dermatological Institute also part of IFO)
 - BBMRI.it WG ELSI "Access and sharing" → Access policy MTA EU Biobank Week 2020 Matrix
 - COVID-19 Biobank IRE
-
- Implement collection, processing and storage according to quality and reproducibility criteria of tumor cell lines isolated from patients with different neoplasms
 - Start collecting stool samples from patients with solid tumors for research on the role of the "intestinal microbiome" in the development and neoplastic progression the response to immunotherapy.

3 years Objectives

- To become the largest repository recognized by the Regione Lazio for collecting tumor tissue samples and biological fluids from cancer patients that can assist scientific research and patient treatment.
- Strengthen the collection of stool samples to support multiple research projects on the link between gut microbiota and drug response.
- Start the systematic collection and biobanking of human tumors grown in PDX mouse models and their use for pharmacological purposes together with the formation of the Institute's Animal facility.
- To become known Internationally and to establish a series of external collaborations

1.2 Omics

The main objective is to become a Regional Reference Center for "OMICS" analyses on tissues and liquids (see Liquid Biopsy) that allow the refinement the appropriateness of oncological therapies

- Wide technological park available
- Next Generation Sequencing - NGS routinely applied to approximately 1000 cancer patients per year
- Definition of mutational signatures for assigning targeted target therapies
- Standardized reporting
- Liquid biopsy applied to patients with advanced disease
- Exome sequencing, epigenomics, RNAseq

Background and state of the art

Precision medicine (also known as personalised medicine) is a medical model that proposes the "personalization" of healthcare regarding medical decisions, practices and specific therapies targeted for the individual patient. It is based on the principle of heterogeneity of the individual and their disease, especially in diseases of genetic origin such as cancer. The treatment of this disease is rapidly transforming from broad-spectrum treatment to therapeutic strategies developed on the basis of the identification of the genetic and epigenetic singularities of an individual's tumor in a process increasingly focused on patient-centered care.

In oncology, precision medicine is currently based on identifying mutations that are potentially associated with transforming healthy cells into cancer and on the timely understanding of the evolutionary process of the tumor, thus generating the best choice of therapy for each specific neoplasm. The Next Generation Sequencing (NGS) methodology has characterized the last decade of oncological research, allowing sequencing of the tumor genome. From the applications resulting from this methodology, it was possible to generate specific large-scale molecular screening programs that enabled us to identify main driver genes, the progression of various types of tumors over time and achieve a classification with a molecular basis. These results led to creating numerous molecular panels that, through NGS technology, evaluate the presence of specific drive gene mutations in each particular tumor.

Omics at the Regina Elena Institute

A definite novelty taking place at our Institute, involving the complete spectrum of expertise on NGS technologies and "omics" analysis, is the proposal of a diagnostic system based integration of three levels of "omics" -genome / transcriptome / epigenome – to specifically define the molecular alterations capable of identifying new diagnostic markers and therapeutic predictions. This innovation is the result of an important technological investment made in recent years which has allowed, through a series of capital loans, to purchase three NGS Illumina sequencers (a Next-seq and two Mi-seq), a NGS S5 Thermo Fisher sequencer, a nCounter platform from Nanostring, a Thermo Fisher digital PCR, an Agilent analyzer for microRNA analysis, and more recently a DEPArray device, to analyze homogeneous populations of tumor cells from paraffin samples of solid neoplasms.

Molecular diagnostics with NGS is now routine for diagnostics the Regina Elena National Cancer Institute and has become increasingly important in the diagnostic activities of the Pathological Anatomy UOC. In this moment, the Unit performs integrated molecular diagnostics which in addition to the conventional methods of analysis (automated immuno-histochemistry, in situ hybridization, direct sequencing, real time PCR), uses a NGS S5 Thermo Fisher sequencer associated with an automatic library and template preparer (Ion Chef). The increasing availability of biological pharmaceuticals, already authorized by AIFA, and the validation in clinical trials of numerous molecules that will soon enter into current clinical practice, have led to a significant increase in the demand of oncologists for higher grade molecular tests of quantitative (number of genes to be analyzed) and qualitative complexities (type of genomic alterations to be identified). Therefore, in addition to the use of CE-IVD kits for current diagnostics, the Institute is increasingly resorting to the use of multigene panels that allow to quickly and accurately detect point mutations, small insertions / deletions, number variations of gene copies and specific gene rearrangements. The Regina Elena Institute possesses the skills and professionalism necessary to carry out all the molecular analyses, making use of dedicated software, interpreting the results and translating them into clinically relevant information. In particular, through the use of the "OncoPrint Knowledgebase Reporter" software, the service is able to provide the oncologist with a telematically available report in which the results of the genomic analyses are accompanied by the indication of potential therapeutic options, including the clinical trials currently underway on a national and international level.

The results of all these analyses allow to not only identify patients who can benefit from specific treatments already approved by the regulatory authorities, but also to 1) identify specific biological characteristics for diagnostic and prognostic purposes; 2) monitor the response to molecular target therapies and the onset of therapeutic resistance; 3) identify the molecular alterations necessary for enrolling patients in clinical trials; 5) carry out, for selected and particularly complex clinical cases, molecular analyses that may suggest personalised treatments that are not yet codified in guidelines (essentially to support the Molecular Tumor Board activities). At the same time, the Institute has developed several custom panels in the research field to carry out translational research in search of specific mutational signatures, predictive of drug response or a more or less favorable prognosis.

Thanks to over 4 years of experience gained in NGS analysis (with a total volume greater than 3000 analyzed

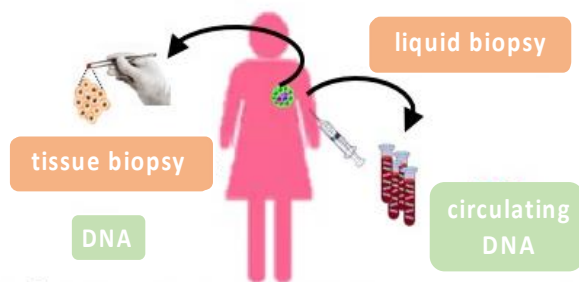


Figure 4. Liquid biopsy reveals tumor DNA in blood.

Genetic tumor fingerprinting reveals tumor vulnerabilities. Not only can DNA be obtained from surgically removed tissues, but also from blood, a far less invasive procedure that can be repeated at will (liquid biopsy). DNA ‘detaches’ from the tumor and is released into blood, drastically reducing inconvenience for the patient and cost to the National Health system. Liquid biopsy provides a continuous update of the tumor genomic profile. It is like taking the fingerprints of a serial thief at break-in and in each of the crime scenes. Knowing in advance theft strategies helps to anticipate the next moves. This is exactly what happens with liquid biopsy: we may learn in advance whether therapy is working, or the time has come to switch over to another drug. Quite often circulating vulnerabilities hint at new targets (new thieves) and identify the specific drug to be given next.

cases), the Institute coordinates a work group, within the SIAPEC-PMMP (Italian Society of Pathological Anatomy and Cytodiagnosics - Molecular Pathology and Predictive Medicine), for drafting recommendations concerning NGS reporting.

However, the application of precision medicine to oncology requires a dynamic understanding of the evolutionary process of cancer diversification along with the appearance of new mutations responsible for drug resistance. This issue is central if one wants to devise new therapeutic strategies. To this end, the Institute has already implemented specific solutions, including liquid biopsy (LB) (Fig 4), e.g. the analysing the circulating DNA released from the tumor (ctDNA). This is one of the most studied bio-markers of early-stage cancer, and it may be applied to virtually all body fluids including plasma, urine, saliva

and even cerebrospinal fluid. IRE has offered BL for diagnostic use since 2017 (Cobas EGFR mutation test

v2), i.e. ever since its clinical efficacy and value has been demonstrated in CE-IVD, fully ISO-certified formats. However, the variety of neoplastic diseases seen at IRE, in the context of exploratory and translational research mission, offers unique (at regional level) and exceptional (at national level) opportunities to establish a leadership within collaboration networks. A number of 'consensus' LB variants may then be deployed. One may foresee specific LB assays dedicated to specific nosological conditions, and one-size-fits all general multigene NGS panels. Potential applications range from big killers to rare tumors, and LB (for its peculiar positioning within patient management) provides unsurpassed potential for flexibility that makes it apt to both general 'surveillance and follow-up' protocols. In one word, medical necessity ultimately determines application. Recently, the ability to reveal minimal disease and relapse/progression in high-risk patients (e.g. sarcomas), as well as the combination of LB and radiomics, have been put in place at IRE. Possible development areas are the innovative follow-up of cancer patients in the colorectal cancer post-surgical period and in some rare neoplasms, areas in which much remains to be done. Ongoing studies document the existence of circulating traits of resistance and vulnerability during therapy, and tools have been developed to assign additional lines of therapy upon progression during advanced lines of treatment, particularly in breast cancer of the positive HER2 subtype. This unmet clinical need has fueled our interest in customized LB procedures that can actually be associated with clinical studies, are rapidly transferable to the clinical setting, and may accompany the patient throughout the course of the disease evolution. From a biotechnological perspective, IRE has long been active in a prestigious European Consortium (ULTRAPLACAD; www.ultraplacad.eu) and Regional Consortia (TURNOFF) with the aim of creating new industrial prototypes based on nanophotonic (PCR-free) technology. This represents a sophisticated, and yet simple, LB procedure that holds much promise of integration into a digital health framework. Although ctDNA is certainly an important chapter in the liquid biopsy book, IRE studies go much beyond the 'genomic layer'. In particular, miRNA signatures predictive of resistance and epigenetically defined sensitivity to treatments have been identified and are actively studied in numerous of neoplastic diseases.

Alongside daily routine activities, the IRE intensely pursues activities in the development and validation of NGS technologies in exploratory research, as well as in creating specific data analysis software of the data generated. In light of the scientific knowledge acquired, it has become clear that the molecular analysis of the genome is not sufficient to improve diagnosis and therapy alone, since the response of a neoplasm to therapeutic treatment does not depend solely on the characteristics of the genome, but also of the transcriptome and epigenome. RNA analyses provide important information on the transcriptional changes associated with the tumor phenotype, identifying aberrations in the regulation of gene expression and also in pathogenic gene fusions. The RNA analyses that exploit NGS technology have been carried out for several years at our Institute, using a specific pipeline developed in "house" for their interpretation. It is of utmost importance to note that by combining these analyses with exome sequencing, it is possible to identify specific neoantigens present in tumor cells as well as fusion proteins generated in the neoplasm. Furthermore, RNA-seq analyses are performed to analyse the composition of immune infiltrates in tumors, while the exomic analysis is often used to evaluate the mutational load of the single neoplasm. More recently, NGS methodology has also been used to analyse variations in miRNAs expression and long non-coding RNAs in different types of tumours.

Unlike the genome, the epigenome is not a static entity: an epigenetic variation can precede or proceed the formation of disease, an environmental exposure, or even reflect specific factors and lifestyles. This makes the epigenome an attractive field of investigation for identifying and transferring biomarkers of various types of diseases and states predisposing specific diseases such as tumors to the clinical setting. For several years IRE has carried out numerous epigenetic studies, through adopting developed and validated methods including ChIP-seq, ATAC-seq and more recently HiC-seq. Through these analyses, it is possible to determine how changes in chromatin conformation influence neoplastic transformation, altering access to specific DNA regions by transcription factors or RNA polymerases.

1 year Objectives:

PATHOLOGY:

- Implementing molecular diagnostics by using more complex panels capable of detecting all the main therapeutic targets (already consolidated and emerging ones):
 - OncoPrint Tumor Mutation Load Assay (select patients for immunotherapy)
 - Custom panels (specific pathology) and Archer panels (study of castings)
 - OncoPrint Comprehensive cancer panel (409 genes) (also supporting the activities of the MTB IRE)
- Improving the time between making a referral and taking the analysis (turnaround time from 10 to 5 working days).
- Carrying out an international multicenter study (sponsored by ThermoFisher) for the validation / introduction of the OncoPrint Comprehensive Assay Plus panel (+500 genes) in the diagnostic routine by means of NGS analysis on the Ion GeneStudio S5 Prime platform.
- Launching a feasibility study for the joint genomic diagnosis of genetic risks and sensitivity to new drugs in breast, ovarian and colon cancer through the ACC GerSom Panel (somatic and germline variants ~ 500 genes).
- Launching a multicenter study for the best possible detection of NTRK1,2,3 fusions in thyroid cancer (funded by Bayer)

SAFU:

- Boosting/reinforcing the offer of the following NGS omics technologies for translational research projects in order to identify and validate new biomarkers for prognosis and response to therapy, as well as the activities of the Molecular Tumor Board.
 - Transcriptome (mRNA, lncRNA, small RNA, scRNA-seq): (annual throughput approximately 100).
 - Targeted DNA (Exome and dedicated panels): (annual throughput around 200).
 - Epigenome: epigenetic analyzes such as ChIP-Seq, ATAC-Seq, HiC-Seq, DRIP-Seq, ChIRP-Seq (annual throughput about 300).
 - NanoString PanCancer IO 360™ Panel: (annual throughput approximately 60).
- Launching pilot projects (through external collaboration) for Single cell RNA-Seq (scRNA-seq) and epigenetic analyses on the heterogeneity of the tumor population and lymphocyte infiltrate (annual throughput about 10).

LIQUID BIOPSY:

- Improved sensitivity and testing breadth. Combined use of NGS panels, digital PCR (dPCR) and other technologies to detecting ctDNA and index mutations in cancer patients.
- Routine use of these technologies in the context of MTB activities. Demonstration of clinical usefulness: specific examples therapy assignment based on circulating vulnerabilities, especially if not detectable in neoplastic tissues.
- At least one, possibly multi-center, prospective clinical trial empowering LB as a major readout of clinical response and pharmacological resistance. LB as a tool to counteract tumor escape from therapeutic pressure.

RADIOMICS:

- Conclude the following studies:
 - MRI-based Radiomic protocols for parotid lesions.

- CT-based Radiomic protocols for lung lesions subject to lobectomy.
- Morphological and functional MRIs to identify imaging biomarker expression of the tumor microenvironment in head and neck neoplasms

3 year Objectives

ANATOMICAL PATHOLOGY:

- Centralize all molecular testing for cancer via MPS in our laboratory: our goal is to become a hub for molecular diagnostics in the Regione Lazio.
- Define the guidelines for NGS reports at a national level within the SIAPEC-PMMP (Italian Society of Pathological Anatomy and Cytodiagnosics - Molecular Pathology and Predictive Medicine)
- Promote quality controls for sequencing using MPS at a regional and national level (currently the IRE molecular diagnostics laboratory coordinates the "Molecular Pathology and Predictive Medicine Group" of the Regione Lazio - SIAPEC-IAP Regional Steering Group).
- Promote national multicenter studies in order to implement standards and guidelines in the annotation, interpretation and reporting of results obtained through MPS sequencing.
- Adopt a new integrated analysis system (IVDR labeled) that will allow you to combine fully automatically as well as a single workflow the following: nucleic acid extraction and purification, library preparation, sequencing and analysis reports:
 - Using a single MPS test (IVDR labeled) for profiling hundreds of genes simultaneously, capable of detecting all types of variants, key biomarkers for immuno-oncology research, the HRD gene family, TMB, MSI and the loss of heterozygosity.

SAFU:

- Making NGS technology accessible to all research laboratories following the purchase of the NovaSeq
 - Introduction of the clinical exome and expansion of its use for research but also for MTB.
 - Whole genome sequencing for translational research projects.
- Implementation of single cell sequencing (scRNA-Seq, scATAC-Seq, scChIP-seq, CNV, Immunoprofiling).
- Spatial characterization of proteins and mRNAs in the specific morphological context of the tissue using tools such as the GeoMX Digital Spatial Profiler or the Visium Spatial Gene Expression method.

LIQUID BIOPSY:

- Building on 2020 achievements, defining a robust and highly sensitive workflow to identify ctDNAs in patients developing drug resistance (e.g. breast or lung cancer).
- Participating in one or more multicenter trials for validating the use of ctDNA in assigning molecular target therapies. Overcoming the regulatory barrier that presently allows the prescription of targeted therapy only when alterations are seen in tissue DNA (tDNA).
- Participating in at least one multicentre trial to (a) validate ctDNA as a tool to estimate minimal residual disease, and (b) envisages interventional LB use, for instance therapeutic intensification in the neoadjuvant or adjuvant setting.
- Measurable objective: 1000 samples/year tested, and publish results in at least 4 high-level scientific journals.

RADIOMICS:

- Increase the use of radiomics in further diagnostic research activities and publish these in at least 3 scientific articles.
- Integrate radiomics data with other "omics" data (for example mutational state, study of the microenvironment by digital microscopy, etc...) in order to derive new predictive algorithms and decision-making tools (in collaboration with other units/departments: e.g. Bioinformatics and Pathological Anatomy)

1.3 Bioinformatics, Machine Learning, Biomarkers

The main aim is to explore and develop ways in which Big Data can be interpreted from omics analyses of cancer patients

- **Big Data generation and analysis**
- **Identification of predictive biomarkers for drug response**
- **Support for precision immunotherapy**

Background and the state-of -the -art

Extracting robust information from Big Data produced by the various omics platforms that is useful for patients and translational research is one of the main challenges on which many international Precision Medicine projects are based. To pursue these objectives, it is necessary to be equipped with the skills and know-how on how to deduce and derive the alleged information from the latest generation biotechnological platforms and validate the data with a statistical significance that can guarantee their reproducibility. The two fields of science that deal with these two tasks are broadly defined as Bioinformatics and Biostatistics. Bioinformatics has now become not only a simple tool, but a central and fundamental part of all the research processes involved in the world of Genomics. Similarly, Biostatistics forms a critical component of any clinical trial design. In recent years, there has been a great demand for experts to fill these professional roles which has led to establishing new study courses, in Europe and across the world, of three-year degree courses as well as specialized courses in order to make up for the lack of specialized personnel.

Bioinformatics, Machine Learning, Biomarkers at the Regina Elena Institute

The Regina Elena Institute has a Bioinformatics and Biostatistics unit. In the context of a regional IRCSS, it is unique in its kind. It is made up of 4 Biostatisticians, 7 Bioinformaticians and 1 IT Computer Programmer. It focuses on the study and analysis of cancer patient derived data.

The Bioinformatics group specializes in the analysis and integration of genomic and transcriptomic data, that is, data deriving from massive parallel sequencing of DNA or RNA.

With regard to bioinformatics analyses already applicable in clinical practice, for example as in the context of the Molecular Tumor Board (vv. Macroarea of advanced clinical research, Molecular Tumor Board), there are efforts placed toward the study and annotation of genomic variants of small and large size from experiments like the whole exome sequencing (Whole Exome Sequencing), which are not yet applicable due to costs and routine feasibility but are already in use for the study of rare tumors and difficult classification / treatment. In working closely with the Genomics Facility, it has worked towards the development of analytical and visualization techniques for innovative epigenetic experiments in order to study the molecular interactions external to DNA for translational research (for example, plasticity of zone enhancers through ChIP-seq and ATAC-seq and reconstruction of non-coding RNA modulations). This effort was inspired by the lack of understanding the many events underlying the pathological progression of tumors, firstly, the transition from primary tumor to metastasis. The classic paradigm of accumulation of somatic mutations does not explain this puzzling phenomenon in most of the cases. This is possibly due to the chain of epigenetic events that are not outlined in the current Guidelines of Precision Medicine.

The Bioinformatics group uses automated machine learning techniques and Artificial Intelligence (Automatic Learning or Machine Learning) to find interactions and correlations in large datasets, where tens of thousands of molecular characteristics are associated with each patient. Furthermore, the group develops analytical

algorithms and pipelines (workflows) that are available to the international scientific community on open-source platforms. From a technological perspective, the Bioinformatics group is equipped with internal servers, workstations, and storage space for studying and validating processes, and also collaborates with Italian supercomputing consortia for the exploitation of High Performance Computing. In the context of these projects, the unit is committed to creating training and internship projects, following 2-4 students a year for their thesis in various university degrees, from Biomedical Engineering to Pure Bioinformatics.

The Bioinformatics group collaborates closely with the Biostatistics Unit whose analyses take on a more correlated approach with patient clinical history that aims to build prognostic / predictive models based on omics. Creating models involves following well-defined rules that lead to the identification of molecular signatures starting with dimension reduction of specific clinical endpoints. The development and validation of molecular signatures is a very rigorous course when wanting to achieve reproducibility of claimed results from independent case studies and implementing these on a larger scale.

Further research activities include the field of predictive biomarkers in Immune-Oncology (Macroarea Precision Medicine, Immunotherapy), an area of great interest given the lack of robust biomarkers for drugs such as Immune-Checkpoint inhibitors (e.g. PD1). Scientific literature has been inundated with proposals regarding predictive characteristics of response to these drugs in different tumors and it is in fact the direction we are taking. On the one hand, we are working on the validation of different molecular signatures in datasets of clinical studies containing also omics data such as RNA-seq on tumor tissue, and on the other hand on the combination of different biomarkers in an attempt to improve its predictive performance. These markers are not just single genes or lists: in some cases, they are complex algorithms for calculating and separating patients. In line with this, we are also working on creating an immunoinformatics framework, a field that uses data sequencing (NGS) to calculate additional details for immunological problems, such as the HLA type and repertoire of T cells. One type of information that can be extracted by integrating DNA and RNA sequences is the list of tumor neo-antigens, or the list of mutations expressed by the tumor, for which experimental protocols for anti-tumor vaccination in more advanced phases exist.

1 year Objectives

- Add 1 researcher per unit specializing in Machine Learning and Artificial Intelligence.
- Add 1 master student per unit full time /totally committed to core-facility projects with Univ. Tor Vergata in 2020.
- Expand on non-HPC Computing Resources: +32 CPU - +256 GB RAM (local workstation: investing ~ 10,000 euros).

3 year Objectives

BIOINFORMATICS:

- Create 4 working groups consisting of 2-3 people, each coordinated by a senior bioinformatician (with > 2 years of experience) on macro-topics:
 - Epigenetics and Computational Molecular Biology
 - Variants Annotation, Precision Medicine, Medical Informatics
 - Machine Learning and Artificial Intelligence
 - Immuno-Computing and Deconvolution
- + 1 person dedicated to the "bioinformatics and IT infrastructure" of research, add 10 Units to the Medical Informatics group (+ 4 more units compared to the end of 2020)

BIostatistics:

- Create a manual for procedures on approaching large matrices to be shared with the Institute (e.g. Radiomics, Genomics)
- Invest in training on programming languages (e.g. R, 100 hours)
- Institutional training courses for researchers, biologists and doctors

2. AREA OF PRECISION SURGERY (APC) (highlighted in purple)

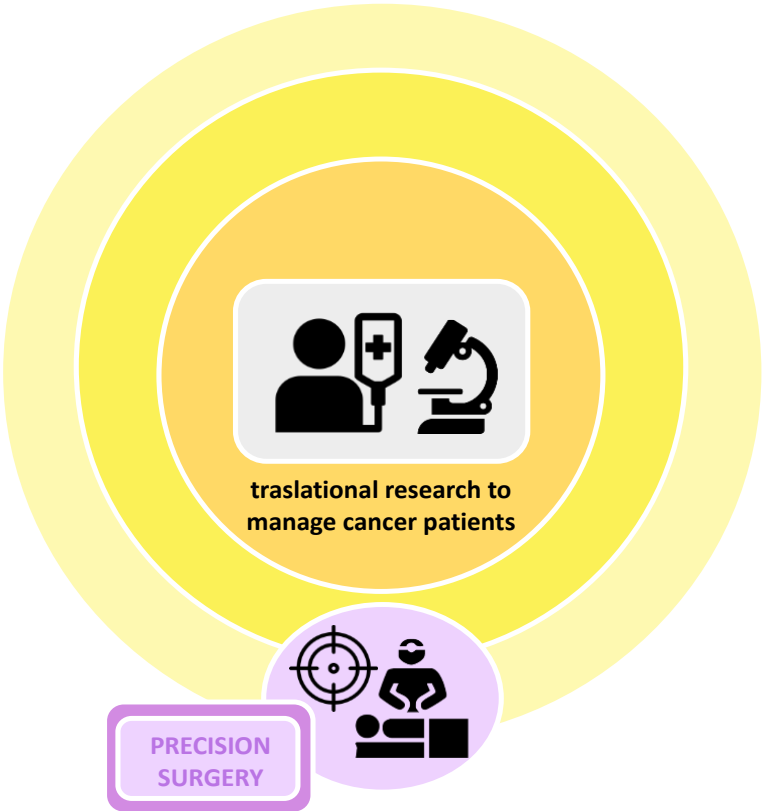


Figure 5

Background and state-of-the art

Surgery remains crucial for the treatment of most cancers with more than 80% of patients requiring one or more operations during their illness. Patients with advanced disease may also need surgical intervention. For example, in the case of hepatic metastases of colorectal carcinoma, 44.2% of those who underwent liver resection have a survival rate of 5 years after diagnosis compared to 18.4% of those treated only with systemic chemotherapy.

Within an IRCCS, surgeons also play a primary role in identifying cases of translational research specifically aimed at: (a) identifying biomarkers of molecular stratification of cancer patients; (b) finding cancer and non cancer tissues that faithfully represent the primary tumour site and its spread. The diagram below shows these aspects.

As a part of the multidisciplinary approach towards cancer treatment, one of the several distinguishing features of our Institute, the departments of surgery as well as medical oncology, radiotherapy, radiology and molecular surgery all play an equally major role. The development of intervention approaches that enhance multidisciplinary team work is undoubtedly one of the main objectives of modern oncology.

Is it better to use the term more accurate surgery or precision surgery?

Needless to say, the Surgery Departments have been purchasing more and more technological, clinical and scientific tools to improve its performance and to provide answers to questions that still need to be answered. With the introduction of laparoscopic instruments in the 1990s, it has enabled us to obtain functional and oncological results equal to or greater than those obtainable with conventional laparotomies, with less aesthetic damage, less pain and faster recovery after surgery. At our Institute 10 years ago, a decision was made to adopt the use of robotic surgery to develop minimally invasive surgery. Initially, the Urological community exclusively adopted the use of robotic Surgery but today, robotic surgery has also been embraced in the daily use by the Gynecological, Thoracic, Otorhinolaryngology and Epatobiliopancreatic Surgery communities, thus moving on from the effectiveness of the operation, and onto the possible expansion of patients who can benefit from it.

Today scientific research and the future of "precision surgery" have been substantially divided into three fields:

- 1) Precision surgery guided by biomolecular assessments of an individual patient;
- 2) Defining the operating technique and the extension of the intervention based on a patient's situation;
- 3) The possibility of extracting appropriate tissue samples to be analyzed in order to answer questions regarding cancer development and the underlying mechanisms of metastasis.

These are the fields in which surgical research at the Regina Elena National Cancer Institute will be progressively focusing on as well as increasingly developing the interdisciplinary collaboration with the Departments of Translational Research, Anatomical Pathology, Clinical Pathology and Radiology and Radiotherapy. The main priorities for each of the surgical units are summarized below in the following paragraphs.

Objectives over the next 1 to 3 years

Division of Liver, Pancreas and Biliary Tract Surgery

Liver metastases of colorectal cancer are treated in the field where the integration between surgery and providing biomolecular tumor specificity is an important frontier in the application of precision surgery. Therefore, the primary objective will be defining the individual patient's gene profile with one or more hepatic metastases from colorectal cancer, in order to plan a personalized treatment strategy. Guidelines can be implemented to define the most appropriate therapeutic pathway and determine whether the surgery patients must undergo is limited or unlimited to a certain site or establish whether chemotherapy is imminently necessary or not or decide what type of combination therapy should be adopted. This experimental approach may represent the basis of prospective clinical trials which can also be applied to other types of neoplasms.

This is the true prospect for applying the translational approach in the field of pancreatic hepatic biliary surgery in oncology.

Division of Gynecological surgery

In view of an increasingly multidisciplinary and integrated diagnostic and therapeutic approach, the evolution towards precision surgery is a major challenge in Oncologic Gynecology. The international guidelines have already started to design new clinical pathways bearing these aspects in mind.

The main aim will be to define the genetic/biomolecular indicators that help personalise the integration of both Surgery/Chemotherapy, particularly in advanced forms of disease, leading to a patient selection criterion which determines which patients need to undergo ultra-radical surgery or peritonectomy + HIPEC versus surgery after neoadjuvant CT. In the early stages of ovarian cancer, endoscopic surgery should be evaluated with the use of the sentinel lymph node technique versus extensive lomboartiac lymphadenectomy.

Studies aimed at evaluating the role of modifications in the qualitative and quantitative levels of cfDNA which are associated with the inflammatory state such as early biomarkers in aggressive types of cancers, system complications and potential metastases to better select patients who are candidates for autologous ovarian tissue transplant will be activated.

Division of Peritoneal Surgery

Precision surgery in peritoneal carcinosis is one of the major aims of the Institute. Since 2009, a pharmacogenetic project has personalized HIPEC treatment (intra-operative chemotherapy), defining the amount of drug to use in an individual patient. Today, ever more than before, personalized therapies and protocols have been recommended based on an evaluation of factors that identify prognosis and treatment.

In this context, the main research lines of peritoneum surgery will be: 1) defining the indications for treating colon carcinomas in mutated k-ras with cytoreduction and HIPEC. 2) very strict limits to PCI requirements (Peritoneal carcinoma index) in carcinomas due to gastric neoplasia, which always requires extremely precise evaluation and accurate decision making regarding the type of neoadjuvant CHT as well as timing of treatment, 3) evaluating the stroma/mucus ratio in carcinomas of the digestive tract of mucinous origin, 4) differentiating between low and high grade tumours with different treatment procedures.

A new and important method emerging is the use of molecular data for better treatment and peritoneal mesotheliomas targeted therapy, which has barely been studied even if the most significant data represents pleural ones, which behave rather differently.

Division of Orthopedic Surgery

Musculoskeletal oncology surgery is characterised by its marked use in cutting edge technology and the continuous evolution of surgical equipment and tools to improve surgical accuracy. The Computer Assisted Orthopedic Surgery (CAOS), through the use of navigation systems, aims to bring the surgeon and their instruments into a virtual space; offering a perspective of the operating field informing the operator about the accuracy of their gestures in real time. When 3D printing technology is associated with navigation, surgical solutions are transposed from the three-dimensional computerized model to reality, this is done through the creation of real building.

In light of these considerations, the primary objectives are; a) the development / improvement of navigation systems in performing complex surgical procedures on three-dimensionally complex anatomical sites such as the spine and pelvis; b) the realization of ad-hoc surgical instruments for achieving minimum invasiveness and maximum surgical accuracy; c) the development of customized composite prosthetic implants, based on bio-engineered structures, in order to achieve anatomically accurate reconstruction and maximum biomechanical and biological compatibility; d) the production of molds for custom-made cemented spacers to be used in revision surgery.

Division of Neurosurgery

The development of neurosurgery towards precision surgery aims to achieve the following main objectives:

- a) the implementation of surgical treatments for resection of primitive and secondary brain tumors with both morphological and functional imaging, integrating Neuronavigator (MRI with T1 sequences with contrast medium, Flair, Diffusion, Tractography, functional MRI), and Intraoperative and / or CT Ultrasound intraoperative, as soon as available. This combination makes it possible to update data on tumor removal directly in the operating room in real time, with safer, more precise and minimally invasive interventions. Sampling on pathological tissues will allow to perform the necessary comparisons for radiomic studies;
- b) the implementation of fluorescence-guided retrieval systems in favoring selective microsurgical removal of high-grade glial lesions and defining the target in the course of stereotactic or "open sky" surgical procedures for malignant brain tumors such as gliomas in a highly precise manner or lymphomas. The aim is to provide highly representative samples of the lesion to be treated with complementary therapies at the same time to promote the creation of reliable tissue banks for molecular tests;
- c) the implementation and standardization of neurophysiological monitoring techniques for the removal of brain lesions in critical sites, precisely defining the possible involvement of encephalic areas associated with superior motor, language and cognitive functions, also with "wide-awake surgery" techniques. Development of neurocognitive studies and tests that can better define the objectives and results of these surgical procedures;
- d) the implementation of the quality of endoscopic (4D endoscopy, esoscopy) and stereotaxic instruments, for the removal of tumor lesions of the sellar region, the ventricles and the brain in deep areas with greater accuracy and mini-invasiveness, favoring the diagnosis and the study of rare cancers.

Division of Breast Surgery

The aim of precision surgeon in the field of breast cancer is to offer the best treatment from a multidisciplinary perspective, providing the best result with fewer side effects. The modern breast cancer oncologist must have a broad understanding of the natural history of the disease, interacting and stimulating innovative preventive therapeutic aspects with the various professional figures, placing it at the center of the multidisciplinary disease management team. In light of these considerations, the main objectives will be: a) identify the molecular cancer risk signatures to be evaluated retrospectively on intramural and regional case studies for subsequent prospective validation; b) define new therapeutic standards for the treatment of non-palpable lesions; c) standardize innovative conservative surgical techniques.

Division of Urological Surgery

Urological malignancy surgery has changed over the last 20 years. On the one hand, increasingly better diagnostic and prognostic tools have enabled more accurate patient selection and more reliable surgical programming. The implementation of minimally invasive techniques and technological innovations has played an essential role in reducing surgery-related morbidity. These advances have fostered a personalized approach in patient management, entering the era of "precision surgery" combines surgical techniques of maximum treatment efficacy and minimal impact on patient functions and quality of life.

Thanks to the enhancements in diagnostic imaging techniques, such as multiparametric prostate MRI and PET-PSMA (prostate specific membrane antigen), our main objective is to standardize robotic surgery even in oligometastatic patients (understood as the presence of 5 or less extrapelvic secondary lesions), a much debated topic, applicable only in clinical studies and in reference centers.

By obtaining the definitive results of ongoing prospective randomized studies concerning the comparison of radical robotic and open sky cystectomy, we will be able to establish whether there are advantages of robotic surgery in the treatment of muscle-invasive neoplasia of the bladder.

In the near future, we intend to standardize the use of the purely off-clamp approach together with that of Firefly technology, in carrying out robotic partial nephrectomy, especially in more technically complex cases, maximizing the precision of the surgical procedure and its short and long term results both oncologically and inherently in terms of the preservation of post-operative renal function.

Division of Ear, Nose & Throat Surgery

Advances in head / neck cancer treatment over the past two decades have not improved the survival of operated patients. Furthermore, patients with the same clinical stage of disease do not have the same history as the disease. The molecular characterization of head / neck tumors could significantly improve the choice of treatment and therefore potentially contribute to increased survival.

Therefore the main objectives will be: a) identifying the molecular signatures (non-coding and coding) to be evaluated in retrospective cases to evaluate the risk of disease relapse; b) defining new therapeutic standards for the treatment of disease relapses; c) standardizing innovative conservative surgical techniques by following the molecular stratification of peri-tumor tissues and surgical resection margins

Division of Thoracic Surgery

Lung cancer still represents the leading cause of cancer deaths. Robotic and videothoroscopic techniques have widened the number of lung cancer patients undergoing surgery, including "fragile" subjects in regards to age, clinical conditions and stage of disease. Alongside the purely surgical procedures, the highly technological invasive diagnostic techniques have taken an everincreasing role: in particular the Transesophageal Echo Endoscopy (EUS) and the EcoBronchoscopy (EBUS) aimed at transesophageal and transbronchial mediastinal and / or pulmonary biopsies.

Therefore, the main goals are:

- 1) to collaborate with translational researchers and agree upon the selection criteria in order to widen the number of patients eligible for surgery that increasingly include peritumor molecular, genomic and microenvironmental data;
- 2) To conduct analysis of invasive diagnostic procedures (EUS and EBUS) aimed at the validating these combined techniques in terms of accuracy and yield of the material useful for molecular analyses
- 3) retrospective and prospective analysis of the outcomes of robotic and videothoroscopic techniques with particular reference to the extension and accuracy of lymphadenectomy compared to traditional techniques and its impact on prognosis
- 4) participate to an international project for defining the new TNM classification for lung cancer.

1 year Overall Objectives:

- Define clinical databases based on research:
 - verify the possibility of using computerized medical records for research and scientific purposes
- Identify possible data managers.
- Identify possible research "facilitators".
- Define and structure the relationship channels with the Institute's researchers:
 - dedicated meetings, Facilitators, Research projects oriented to pathology v.s. biological mechanisms

3 Year Overall Objectives:

- Define surgical procedures dedicated to patients with particular molecular characteristics
 - "Surgical" tumor boards
- Implement diagnostic pathways
 - Radiomics
- Further improvements of minimally invasive surgical techniques, with special focus on robotics
 - Identifying better surgical indications for each type of disease
 - Integrated vision methods

3. AREA OF PRECISION MEDICINE (AMP) (in GREEN)

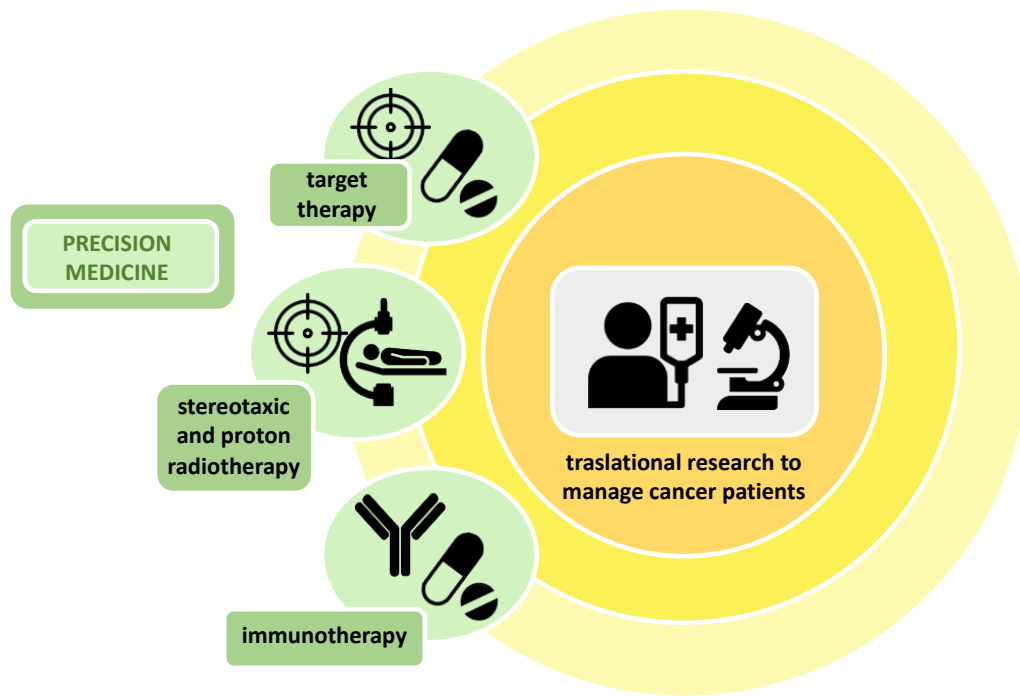


Figure 6

3.1 Targeted Therapy

The main objective is to identify new combination therapies as well as pharmacological repositioning to optimize treatment and genomic analysis

- **Rational use of Molecular Targeted Drugs**
- **Identification and validation of new therapeutic targets**
- **Transfer of predictive biomarkers to the clinic for response to drugs**
- **Drug Repositioning**

Background and state-of-the art

The enormous efforts made by cancer research in recent decades have allowed us to identify numerous biomolecular alterations in cancer cells that are the basis of carcinogenesis. In fact, studies that have been carried out since the late 1970s have identified those lesions that are responsible for the onset of tumors, attributable to two well-defined groups of genes, proto-oncogenes and tumor suppressor genes. Under physiological conditions, the proteins encoded by these genes guarantee the normal control of cell proliferation in response to the body's stimuli. Proto-oncogene products are responsible for transmitting proliferation stimuli from the cell surface to its nucleus, inducing DNA replication and subsequent cell division. The products of tumor suppressor genes, as opposed to the previous ones, instead are responsible for inhibitory signals that slow down cell proliferation. In neoplastic cells, the genetic lesions found in the protooncogenes are mutations that involve increased or uncontrolled activity of the protein product, i.e. the activation of the oncogene, or both of these characteristics. As a result, cell proliferation is hyperactivated and independent of extracellular signals. In contrast, alterations of tumor suppressor genes are inactivating mutations that cause loss of control of cell division

The principles of Targeted Therapy (Targeted Therapy)

The principle of molecular targeted therapy is to specifically block the mechanisms responsible for tumor growth. In fact, one of the major limitations of conventional chemotherapy is its lack of specificity. These therapies inhibit the reproduction of both neoplastic and normal cells, generating significant side effects that limit their use and consequently their effectiveness. Molecular targeted therapy in contrast to classical chemotherapy specifically directs its action against a target present only, or to a greater extent, in cancer cells than in normal ones. The specific action of these drugs against cancer cells is not only much more effective, but it allows to limit the side effects compared to traditional chemotherapy, consequently improving the quality of life of the cancer patient.

Molecular drugs are usually composed of small molecules capable of inhibiting the activity of some aberrantly activated enzymes in the tumor cell (e.g. protein kinase). In some cases, such as the epidermal growth factor receptor (EGFR), these enzymes are activated by specific mutations or their expression is abnormally increased due to gene amplification. In other cases, however, as with the anaplastic lymphoma tyrosine kinase receptor (ALK), there is an alteration at chromosomal levels that leads to the fusion of the ALK gene with another gene called EML4. The fusion of these two genes involves generating the aberrant fusion protein called EML4-ALK. Another class of drugs instead is composed of humanized monoclonal antibodies, which, by binding to specific receptors on the surface of neoplastic cells present in the tumor or in the bloodstream, are able to

interfere with the chain of proliferative signals or angiogenic stimuli that support the growing tumor vasculature, for instance trastuzumab, a molecule that is able to counteract the growth of tumor cells that show elevated levels of the HER2 / neu protein, or the drug Bevacizumab, inhibitor of endothelial cell growth factor (VEGF). Other monoclonal antibodies such as Nivolumab, are instead used to selectively stimulate the immune system so that it can recognize cancer cells as a foreign agent and subsequently neutralize them (immunotherapy).

Precision medicine, therefore, is an approach that uses molecular diagnostics to identify the most vulnerable changes in each tumor in order to identify the most effective "targeted" drug combination for each patient. This approach is increasingly feasible to carry out in clinical practice due to the significant advances made available in terms of targeted drugs and sophisticated molecular diagnostic techniques.

Molecular targeted therapies are not applicable in all cancer patients, however they are effective only for certain tumour subgroups that present specific molecular alterations. Therefore, in order to prescribe drugs with a molecular target an accurate diagnosis, including both the histology and the genetic-molecular profile of the tumor are required. Only by precisely identifying the molecular characteristics of the neoplasm, through appropriate diagnostic tests, is it possible to select patients whose tumor presents the molecular alteration against the drug it targets and can therefore benefit from specific treatment.

Diagnostic systems as well as the field of drugs have taken giant steps forward in recent: these improvements include molecular tests used to identify specific **predictive response biomarkers: whose molecules, whether present or activated, are capable of indicating sensitivity of a given targeted therapy.**

Some molecular-target compounds that have entered clinical practice for the treatment of some solid tumors, including colon and lung cancer, include the so-called "**anti-EGFR**" drugs for several years. These types of drugs can block the epidermal growth factor receptor (EGFR) with different mechanisms. These molecular diagnostic methods are best used for lung carcinoma, in which the EGFR gene mutations and ALK and ROS1 gene rearrangements represent alterations against commonly used drugs now available in clinical practice. Even in the case of colon cancer, the EGFR receptor represents a molecular target, which can be attacked by injecting monoclonal antibodies. In this case, however, mutations of the EGFR gene are very rare, as opposed to lung cancer, while alterations in the RAS gene are responsible for numerous processes of tumor proliferation and metastasis. In the presence of these mutations, anti-EGFR antibodies are not effective and it is therefore essential to obtain this information to avoid administering an ineffective therapy.

Molecular alterations capable of guiding clinical decision (defined "actionable" in English) have now been identified in almost all forms of cancers. Often these are alterations whose clinical use was initially established in a specific type of disease but was then extended across other diseases in subsequent studies (the so-called "drug repurposing"). For example, mutations of the BRAF gene are routinely searched in order to select the best therapeutic strategy in patients with metastatic melanoma, who may benefit, in the presence of V600E mutation, from treatment with specific BRAF inhibitors. However, subsequent studies have established how many other cancers, such as lung cancer, brain tumors and some rare forms of leukemia, can benefit exactly from the same drugs, when the same mutation is present.

Beside single gene alterations, of which only a few have been mentioned above, the importance of the so-called mutational "signatures", or multiple alterations of the genetic heritage associated with a specific neoplastic transformation, is on the rise. Multigene molecular analyses allow to achieve the mutational profile of a panel of genes in order to identify the most shared mutations in the neoplastic population, which are frequently extremely heterogeneous due to the presence of numerous clonal subpopulations, in order to formulate combined treatments with molecular targeted drugs or chemotherapy whose efficacy towards the type of tumor has already been documented. Characterizing multiple mutations is mostly done by adopting molecular tests based on sequencing other DNA processivity, i.e. the so-called Next Generation Sequencing, otherwise called NGS (see the Omics section above).

Genomic instability is a characteristic of most cancers and originates due to different stress conditions, such as errors that may occur during the DNA replication process, or deficiencies in the repair mechanisms of DNA damage. Homologous recombination (HR) is known as one of the main repair mechanisms of DNA damage. In this view, protein mutations involved in this process, such as BRCA1 and BRCA2, are more inclined to developing a neoplasm, especially in the breast and ovary. Furthermore, alterations of other genes involved in HR are present in a wide range of tumors. Tumors with this genotype are called "BRCAness" (all clinical cases where deficiencies present in HR do not involve germline mutations in the BRCA1 or BRCA2 genes) are particularly frequent in tumors such as high grade ovarian carcinoma (HSG-OvCa), triple negative breast tumors (TNBC), and pancreatic adenocarcinoma (PDAC). One important element to consider is that while on the one hand the deficiency in HR favors genomic instability, on the other it can represent the Achilles heel that can be used as a therapeutic target. In fact, defective tumors for the HR mechanism are sensitive to chemotherapy (Cisplatin and Trabectedin) or even to PARP inhibitors (Olaparib or Talazoparib). Identifying gene mutations associated with BRCAness serves as an essential mechanism for defining prognosis and choice of treatment.

Targeted Therapy at the Regina Elena Institute

In recent years, the Regina Elena National Cancer Institute has carried out numerous studies aimed at characterizing the functions of specific genetic alterations in different tumors, with the aim of ultimately identifying some vulnerabilities in cancer that can be exploited therapeutically. Below are some examples of the most relevant translational studies that are schematically represented in Fig 7.

- The use of PI3K inhibitors in head and neck tumors bearing TP53 mutations. The study of the transcriptomes associated with the presence of TP53 gene mutations, present in most head/neck tumors, led to identifying a molecular signature of transcripts highly expressed in these tumors predisposing the response to therapies with inhibitors of the PI3K pathway. In particular, the presence of high levels of this molecular signature is associated with a poor prognosis, mostly related to the onset of local recurrences and metastases. The study showed that the mutated p53 proteins are able to cooperate in the activation of the molecular signature and that the treatment with inhibitors of the PI3K pathway blocks the mutated p53 activity and determines a decrease in the expression of the molecular signature. Furthermore, this treatment causes sensitivity to conventional therapies (chemo- and radio-therapy). This body of evidence underline the importance of TP53 mutations as indicators of sensitivity to treatment with PI3K inhibitors, even in tumors that do not show mutations in the PIK3CA gene.

- Genomic instability as a therapeutic target. Over the past 20 years, considerable progress has been made in understanding the biological functions that regulate BRCA1 / 2 genes, whose mutations have an inherited predisposition to the onset of tumors. These studies have allowed the development of new therapeutic approaches that target those tumors in which BRCA1 / 2 mutations inactivate its function, such as PARP inhibitors (PARPi). There are also cancers that share the molecular characteristics with mutated BRCA1 / 2 tumors (BRCAness) and can respond to similar therapeutic approaches. Conversely, several mechanisms of resistance to this type of therapy have been identified, which compromise the effectiveness of these drugs. Thus, the development of effective alternative therapies for this class of patients is imperative. To this end, the Institute is working on several fronts:
 - i) Identifying BRCAness tumors: The identification of tumors with BRCAness phenotype by sequence analysis (NGS) of genes involved in DNA repair will allow to be able to extend the PARPi also to tumors that do not show germline mutations in BRCA1 / 2 genes;
 - ii) Drug repositioning: The repositioning of the "old" drugs for new therapeutic uses is emerging as a new drug development strategy. This promising line of research in the field of translational medical science has led to the identification of Chlorambucil as a drug to counteract PARPi resistance in BRCA-1/2 defective tumors.

iii) Ligands of G-quadruplex: striking the quadruple helix structure of DNA in order to interfere with telomere replication. The results of this study contributed to the initiation of a clinical phase I / II study in defective tumors in BRCA1 / 2 genes (Canadian trial, NCT02719977, opened May 2016).

- Identifying new vulnerabilities of ovarian cancer and new therapeutic strategies to overcome the onset of drug-resistance. This program focuses on high grade serous ovarian carcinoma (HGSOC), the most common and aggressive form of ovarian carcinoma (OC) associated with a particularly poor prognosis. HGSOC is characterized by a high heterogeneity, a high mutation frequency of TP53 (95%) and the acquisition of drug resistance. Signal transduction of ET-1 receptors (ET-1R) is critical for tumor growth, metastatic progression and for the acquisition of chemoresistance, indicating that ET-1Rs represent critical tumor vulnerabilities. Our data have highlighted a new mechanism by which the ET-1R / β -arrestin 1 axis activates the oncogenic cross-talk between mutated p53 and YAP, amplifying the YAP activity in HGSOC aberrantly. The objective of the study is to exploit the control node ETAR/ β -arr1/YAP/mutp53 as a target to inhibit the aggressiveness of this tumor form and increase its sensitivity to conventional treatments. In this context, the identification of the two ET-1 receptor subtypes as therapeutic targets expressed in HGSOC cells, as in the case of ETAR, and expressed in tumor microenvironment (TME) cells, and of ETBR, allowed the evaluation of Macitentan, an antagonist capable of blocking both ET-1 receptors, in monotherapy and in therapies combined with chemotherapy or PARP inhibitors in preclinical models derived from HGSOC patients. Repositioning in oncology of macitentan, approved by regulatory agencies for non-oncological indications (pulmonary arterial hypertension), in blocking ET-1R receptors and downstream effector β -arr1 / YAP / mutp53 could be an important therapeutic option for HGSOC, which to date is an orphan cancer of treatments penalized by a high percentage of recurrences. The evaluation of the therapeutic efficacy of the ET-1R antagonist, such as macitentan, or new ET-1R antagonists, such as aprocitentan, the active metabolite of macitentan (Idorsia), in combination with chemotherapy, is being evaluated or with PARP inhibitors, to overcome drug resistance and block metastatic progression. These studies are currently conducted in preclinical models of HGSOC derived from patients (primary cultures, co-cultures of HGSOC cells and fibroblasts or endothelial cells, and tumoroids) and in murine models (patient-derived xenograft; PDX) that summarize the phenotypic characteristics and molecular features of the neoplasm which they derive from.

- Use of PARP inhibitors for the treatment of ovarian cancer and mammary tumors. Historically, the use of PARP inhibitors (e.g. Olaparib) required the presence of mutations in the BRCA1 and BRCA2 genes. More recently, mutations of other factors responsible for DNA repair such as ATM have been identified as possible targets for this type of targeted therapy. ATM is considered an intermediate risk gene for breast cancers, but of the 4,000 variants identified, only 3% were classified as pathogenetic while 48% (> 1800) of these variants is still of unknown nature, the so-called VUS. To date, patients with VUS are excluded from molecular treatments. In our institute a diagnostic test was developed (p53-MCL, Prodosmo et al., JCI 2013) to identify, by means of a functional assay performed on peripheral blood lymphocytes, the pathogenetic variants of the ATM gene. We have adopted this test to classify VUS, in detecting pathogenetic ones, i.e. those that increase susceptibility to the development of tumors and / or that determine sensitivity to targeted therapy with PARP inhibitors. This will allow to extend the use of PARP inhibitors to patients presenting pathogenetic variants of the ATM gene even in the absence of BRCA1 / 2 mutations.

- Inhibition of HIPK2 as a sensitizing tool to TKI inhibitors in colorectal tumors bearing K-RAS mutations. The progression of colorectal tumors (CRC) is very often due to the activation of the RAS pathway or through alterations of membrane receptors such as EGF (EGFR) or due to downstream mutations. Targeted therapy with EGFR inhibitors is ineffective in the presence of mutations in the K-RAS gene (K-RAS-mut) which is found in about 40% of cases. The need therefore remains to find new therapies that can restore sensitivity to the aforementioned drugs. A retrospective study conducted on CRC patients of our Institute showed the association between the presence of KRAS-mut and a high expression of HIPK2, a kinase involved in most of

the deregulated signaling pathways in CRCs. The characterization at the molecular level has shown a bidirectional functional relationship between HIPK2 and the K-RAS pathway, demonstrating not only that the activation of the K-RAS pathway positively modulates HIPK2 but also at the same time that the presence of HIPK2 is necessary for the complete activation of the K-RAS pathway. In particular, we observed that silencing HIPK2 by genetic editing inhibits the activity of KRAS-mut. This "normalization" of the pathway in the presence of K-RAS mutations suggests the possibility, through the use of HIPK2 kinase inhibitors, to restore sensitivity to membrane receptor inhibitors, therefore greatly extend the effectiveness of this type of targeted therapy.

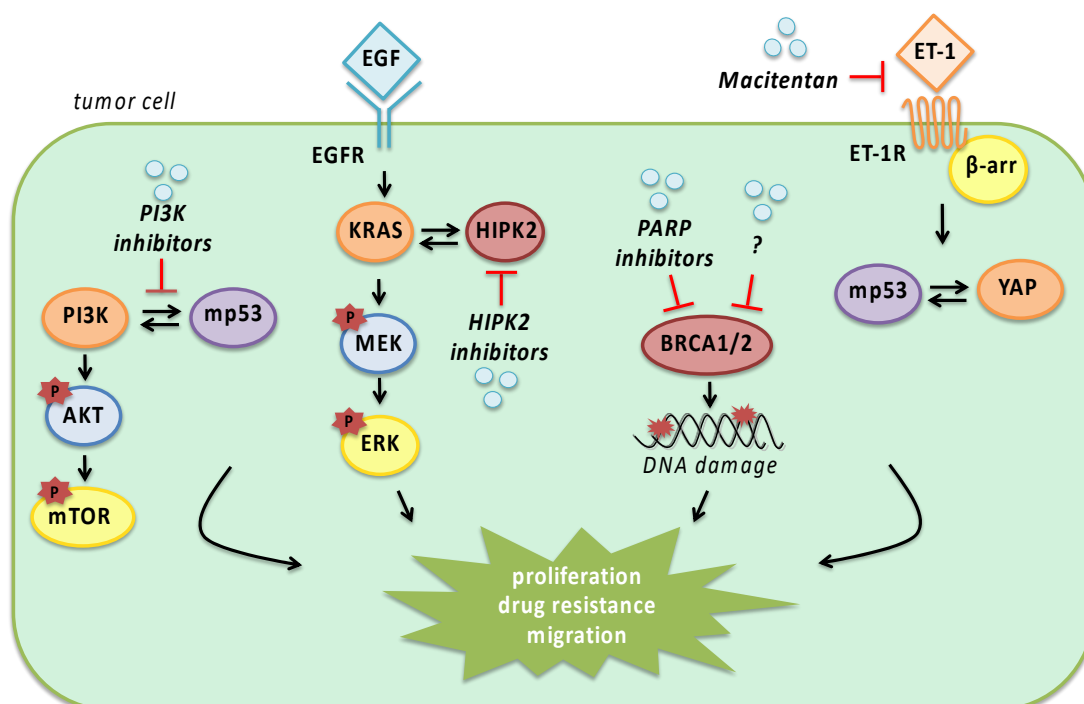


Figure 7 Target therapy

- Models of Intrahepatic cholangiocarcinoma driven by oncogenic FGFR2. Intrahepatic cholangiocarcinoma (iCCA) is a rare tumor of bile ducts. iCCA is most often diagnosed at a locally invasive or metastatic stage. Available therapeutic options provide for only a grim 5-10% 5-year survival rate in inoperable iCCA patients. Oncogenic FGFR2 fusions generated by chromosomal rearrangements are detected in about 15% of iCCA. FGFR tyrosine kinase inhibitors (F-TKIs) have been shown to provide clinical benefit in FF+ iCCA patients, although their efficacy is limited by therapeutic resistance. Three different F-TKIs are currently undergoing phase 3 clinical evaluation as first line single agent in FF+ inoperable iCCA patients and are expected to be approved by 2022. Because FF+ iCCA is a rare disease, clinical research aimed at amelioration of FF therapeutic targeting faces considerable challenges.

We have generated a model of FF-driven iCCA that exploits allotransplantation of genetically modified mouse liver organoids in NOD SCID mice. Our results indicate that FFs are sufficient to drive oncogenic conversion of Tp53 null liver bipotent precursors towards iCCA. We are pursuing further modeling in Bap1 or Cdkn2a null backgrounds, in order to obtain a collection of mouse iCCAs models capable of recapitulating the genetic makeup of the large majority of human iCCAs. We are trying to identify key molecular mechanisms

responsible for FF-mediated oncogenic conversion of bipotent liver precursors. 2D and 3D cellular models derived from FF+ mouse iCCAs are being used to identify collateral druggable vulnerabilities, using genetic and pharmacological in vitro screenings. We are generating in vitro and in vivo models of F-TKI resistance and aim to use these models to identify the underlying molecular mechanisms. We still have a limited knowledge of the extent to which our mouse models recapitulate the human disease. Work towards this direction is hampered by limited availability of genomic and transcriptomic profiling of FF+ iCCA in publicly available databases.

Because our institute has not been involved in F-TKI trials in iCCA, we have no access to blood and tissue samples from F-TKI resistant patients. It is difficult, if not impossible, to obtain this clinical material/info from pharms. Thus, it will be difficult to timely validate in the clinic our laboratory studies on F-TKI resistance. We are trying to circumvent this issue by creating an ad hoc consortium of European investigators.

1 Year Objectives

- Identify at least 3 molecular signatures that respond to the treatment of various types of tumors with specific molecular targeted drugs.
- Characterize genomic profiles of cell populations of metastatic and recurrent advanced tumors by single cell sequencing analysis in order to implement specific treatments of metastatic lesions and local relapses.
- Develop at least 3 pre-clinical in vitro and in vivo assays (crisp-Cas9, PDX, organoids and 3D cultures) in order to characterize molecular targets for therapeutic purposes.
- Establish an intramural Translational Group (Innovative Trial Group) coordinated by the head of the UOSD Phase I to transfer preclinical data into the activation of spontaneous Phase I and II clinical trials.
- Set up and promote private collaborative relationships aimed at implementing at least 2-3 patent applications for biomarker development.

3 Year Objectives

- Identify at least 10 molecular signatures that respond to the treatment of various types of tumors with specific molecular target drugs.
- Develop at least 6-7 pre-clinical in vitro and in vivo assays for the characterization of molecular targets for therapeutic purposes.
- Set up and promote private collaborative relationships aimed at implementing at least 5 patent applications for biomarker development.
- Integrate the Institute's electronic medical record system with ad hoc sections for entering patients' molecular data who are under treatment at IRE.
- Activate 5/10 "biomarker based" studies for validating biomarkers identified by genomic approaches by the Institute's research laboratories.
- Activate 2-3 Phase I or II interventional studies based on "drug repurposing" identified by the Institute's pre-clinical research

3.2 Immunotherapy

Main objectives:

- *Develop innovative ways to improve the efficacy and appropriateness of current immunotherapies for immune checkpoints*
- *Define innovative approaches aimed at increasing the efficacy of CAR-T for solid tumors*

- **Immune monitoring in periphery and in the tumor to define prognostic and theranostic biomarkers and novel combined radio-immunotherapy strategies**
- **Preclinical models for combining oncolytic virotherapy and CAR-T**

Background and state-of-the art

It is now a widely accepted concept that the host immune system controls neoplastic disease and is involved in the response to treatments such as chemo, radio and targeted therapy. Immunotherapy with antibodies to checkpoint inhibitors (ICB) of the immune system has recently transformed the clinical practice of several solid tumors, bearing in mind the efficacy and durability of response. It is however evident that a percentage of patients do not respond (innate resistance) or acquire resistance during the immunotherapy treatment (acquired resistance). Hence, the need to a) identify highly predictive biomarkers of response, in order to avoid toxicity to non-responsive patients and reduce the huge costs on the SSR / SSN b) identify resistance mechanisms to define new protocols for combined therapies and convert non-responsive patients into responsive ones.

Considering the complexity of the tumor microenvironment and the host immune system, it has not been hypothesized that a single marker can predict the response to therapy, as demonstrated by the poor predictive efficacy of PD-L1 expression levels in tumors.

ICB resistance is mediated by the convergence of intrinsic and extrinsic factors to the tumor that can generate mesenchymal traits, which include the rigidity of the extracellular matrix (ECM), the enrichment of tumor-associated fibroblasts (CAF) with activation of immunosuppressive pathways including those of TGF β and AXL consequently excluding the immune cell infiltrate. On the other hand, the immune infiltrate in the tumor microenvironment is considered a prognostic factor in several solid tumors. Important systematic studies available in the literature have demonstrated the relevance of the immunosensor as a prognostic factor and recently the tumor formation of lymph-like structures called tertiary lymphoid structures (TLS) has been indicated not only as a positive prognostic factor in several solid tumors, but also a positive response indicator to ICB. Hence, a schematic classification of "hot" or "cold" tumors and the need to decipher the different components of the tumor microenvironment (TME) to define resistance signatures and new selection criteria for patients to be treated with ICB. To this end, "Digital spatial profiler" platforms have recently been developed to simultaneously define the morphological context and the complexity of gene and protein expression of a single tissue section. These platforms, that are still not available in Italian centers, will contribute to the rapid development of new models of precision medicine.

In parallel, the possibility of multiparametric cytofluorimetric analyses in 12 colors and beyond allows to identify immune cell subpopulations and their longitudinal modulation diagnosis to therapy. The integration of data derived from the deconvolution of the tumor microenvironment with those obtained from the

multiparametric analysis of peripheral blood represent an innovative methodological approach for identifying biomarkers in Immuno Oncology (IO).

It is necessary to develop experimental preclinical 3D models that recapitulate the TME to identify the physical and biochemical characteristics responsible for excluding lymphocyte infiltrate from tumor nests and to define new effective immune-mediated therapeutic combinations.

Recently, in addition to sophisticated omic methodologies, clinical imaging has proven to be an important tool for defining the heterogeneity of different TMEs. Imaging techniques such as CT, MRI or PET scans and a texture analysis of diagnostic images based on diffusion and perfusion (radiomics), allow to obtain information on the microstructure, organization, composition and histological aspects of the tumor, providing a non-invasive tool, important guide to monitoring changes in the tumor microenvironment and the lymphocyte infiltrate during therapies, in particular those that include immunomodulatory treatments.

Radio immunotherapy protocols are proving to be among the most promising combinations with ICB, and are developing at a very rapid pace in clinical practice. Preclinical and clinical studies have demonstrated the immunomodulatory properties of RT which, at defined doses, can induce the production of pro-inflammatory cytokines capable of converting an immunosuppressive microenvironment into a more immunogenic one.

In the new Immuno-Oncology realm therapies are expanding and are involving the use of T cells isolated from patients and reinfused after the insertion of a chimeric-antigen receptor, CAR-T (Chimeric Antigen Receptor), which serves to activate and direct T lymphocytes to the tumor site. So far, their clinical efficacy has been demonstrated only in hematologic malignancies, and the real challenge is to be able to make these strategies more accessible to a large number of patients (expanding the number of accredited facilities to produce CAR-T) and to transfer the clinical experience with solid tumors. One of the main limitations owing to the inability of CAR-T cells to migrate to tumor sites, is due to the chemical-physical characteristics of the tumor microenvironment which presents a composition of the extracellular matrix that acts as a barrier to T cell infiltration. From here on, there is an urgent need to identify the mechanisms that limit the clinical efficacy of CAR-T cells in the treatment of solid tumors and to design new-generation CAR-Ts that favor the recruitment of these cells to the tumor site. The combination of oncolytic virotherapy and cell therapy can represent a valid strategy to hit solid tumors since the tumor cell infection by oncolytic viruses can convert an immunosuppressive TME into an immunostimulator, permitting the entry and activation of T lymphocytes.

Immunotherapy is a new reality that is continuously expanding, consequently requiring significant investments and the need to create national and international networks to increase clinical effectiveness in an endlessly growing number of patients.

Immunotherapy at the Regina Elena Institute.

In this new reality of translational research and innovative clinical approaches, which aim to 're-educate' the patient's immune system in recognizing and eliminating cancer cells, IRE has created an internal task force. This team is composed of highly skilled professionals across various disciplines dealing with the diagnostic therapeutic pathways and translational research with the aim to define criteria for precision medicine in relation to immune-profiling of the patient and the granular composition of TME.

Considering the central role that the patient immune system plays in tumour formation and in the therapeutic response not only in Immunotherapy but in all anti-cancer treatments, IRE has created a technological platform that has been implemented for multiparametric immunological monitoring in cancer patients before and during immunotherapy and radiotherapy treatment in order to identify predictive immunological parameters of response.

In this context, the Institute has created a working group that interacts with national and international networks to identify prognostic and theranostic and “actionable target” biomarkers, that aim to promote the activation of clinical studies (profit and non-profit) and offer cancer patients new treatments based on the paradigm of precision medicine. Clinical researchers from the Department of Anatomy Pathology, Facility NGS, Unit of Immunology and Immunotherapy, Radiology, Nuclear Medicine, Oncology and Radiotherapy, Medical

Physics, Bioinformatics and Biostatistics all work closely with the aim to understand the complexity of the tumor microenvironment (TME), analyzing a) the heterogeneity of tumor cells, b) the composition of the extracellular matrix and the c) spatial composition and distribution of stromal and immune cells, in order to define subclasses of TME.

Metastatic disease continues to be one of the major challenges in clinical practice and where the tumor microenvironment and the infiltrate of cells of the immune system all influence the progression and the process of metastasis. The Institute, thanks to the bio-banking of primitive and metastatic lesions, has identified, through analysis of transcriptomics and immunohistochemistry supported by digital pathology, immune-related signatures in melanomas and primary lung tumors that have developed pulmonary oligometastases to predict the metastatic potential of a tumor primitive and its relationship to the immunosuppressive microenvironment. These models can be transferred not only to distant metastatic processes, but also to locoregional metastatic processes, common in ovarian and head-neck tumors.

The Institute has financed internal advanced diagnostic imaging projects of and, thanks to the expertise of radiologists and medical physicists, radiomics projects have been activated in relation to CT and magnetic resonance perfusion imaging showing tumour characteristics and its microenvironment. This appears to be a great novelty for the near future because it will allow to identify perfusion parameters that define morphological aspects of the tumor to correlate with the composition and rigidity of the extracellular matrix evaluated in experimental models of co-cultures in 3D bioprinting of tumor cells, tumor-associated fibroblasts (CAF) and extracellular matrices. These models that are currently being developed at our Institute are an important study platform in defining mechanisms of resistance mediated by biochemical and physical characteristics of TME.

The Institute also has excellent skills in the isolation, phenotypic, molecular and functional study of T lymphocytes isolated from peripheral blood and tumor tissue and the possibility of transmitting them to the tumor using bispecific monoclonal antibodies. The Immunology and Immunotherapy Unit has isolated, cloned and characterized T lymphocytes from patients over the years and has also sequenced and identified specific T cell receptors (TCR) also for tumor antigens for which specific TCRs, such as antigens, were not known in melanoma associated with gp100. This expertise could be used towards activating projects in creating CAR-T in solid tumors.

An agreement has been signed with IRCCS-Bambino Gesù Children's Hospital in Rome to cooperate for launching phase I clinical trial protocols of CAR-T therapies in solid tumors.

1 Year Objectives

- Identify prognostic signatures generated by the interaction between cancer cells and components of the microenvironment in non-small cell lung carcinomas (NSCLC). The role of the hMENA cytoskeleton protein.
- Immunoscore as a prognostic factor in primary NSCLC N0 lung cancers.
- Identify immune-related signatures involved in the oligometastatic process of lung, melanoma, kidney and lung cancers.
- Identify immunological checkpoints to be inhibited in combination with RT in prostate cancer (one tumor resistant to the inhibition of immunological checkpoints) by longitudinal immunological monitoring of patients undergoing radiotherapy.
- Develop an experimental 3D preclinical model that recapitulates TME to define new effective combinations between CAR-T therapy and oncolytic virotherapy in order to convert an immunosuppressive microenvironment into immunogenic

3 Year Objectives

- ImmunoPortal: an Italian repository for more effective immunotherapy. Creation of a multicentre repository / portal including clinical and biological data of 2000 patients treated with ICB in accordance with current guidelines (i.e. Lung, Melanoma, Kidney)
- Define patient IBAP (immune-based actionable precision medicine) as an important innovation for precision medicine in Immuno-Oncology
- Develop digital spatial profiling and its use in experimental protocols for stratifying cancer patients and identifying new possible prognostic and theranostic markers
- Immunoscore as a predictor of response to immunological checkpoint inhibitors in primary lung tumors NSCLC
- Identify TIME subclasses (tumor immune microenvironment) in primary and oligometastatic tumors in the lung for the development of new combined therapies in oligometastatic disease
- Identify target-related subtypes of the tumor microenvironment that do not permit lymphocyte infiltration for the development of innovative strategies based on CAR-T
- Evaluate at least one of the pre-clinical models for combined CAR-T strategy therapy and immunostimulatory treatment with oncolytic virotherapy in order to increase the therapeutic efficacy of CAR-T therapies
- Implement the agreement made between IRCCS-Bambino Gesù Children's Hospital in Rome involving launching phase I clinical trial protocols of CAR-T therapies for solid tumors
- Start at least one new radio-immunotherapy clinical study

3.3 Stereotactic Radiation Therapy

The main objective is to be the Reference Center of the Lazio Region for stereotactic and proton therapy aimed at optimizing the therapeutic index

- **Precision radiotherapy**
- **Deep-seated Tumours**
- **Cyberknife**
- **Reduce Side Effects**

Background / State of the art

Stereotactic radiotherapy (SBRT) allows the administration of biologically important doses of ionizing radiation to the cancer tissue over a few sessions (generally <8). Stereotactic radiotherapy executes highly cytoreductive treatment rapidly avoiding surrounding tissues. The radio oncologist adopts this tool for the following purposes: 1. to treat small neoplasms (generally < 3 cm) that otherwise difficult to eradicate with conventional techniques; 2. to contribute, together with other pharmacological facilities, to the cytoriduction of metastatic (oligo) disease. The latter is defined by the presence of a limited number of distant metastases, generally <5.

Stereotactic radiotherapy treatment at the Regina Elena Cancer Institute.

The Institute is equipped with the Cyberknife, which delivers cranial and extracranial stereotactic treatments to a high degree of precision able to track the respiratory cycle, which is fundamental for some affected sites, such as the upper abdomen and the chest. Prospective innovative research studies are underway which aim to provide radical treatment in only three treatment sessions (against the usual 30 or more sessions) for laryngeal and prostate cancers. As part of the (oligo) metastatic disease (in response to systemic therapy) the goal is to consolidate the response and prevent / delay further dissemination of disease. In this regard, a prospective study aimed at assessing the impact of SBRT on oligometastatic breast cancer is being finalized.

Furthermore, SBRT can be associated with immunotherapy that aims to obtain an increase in the host's antineoplastic immune effect against the tumor in synergy. It has been hypothesized that SBRT may represent an in situ 'vaccine' for the patient, as substantial amounts of neo-antigens are released from the "immunogenic cell death", potentially capable of eliciting an immune response. Immunizing the host can help fight neoplasia even at a distance from the irradiated area, which has been defined as the 'abscopal' effect, i.e. at a distance from radiation. In IRE's Experimental Research Area, there are studies being carried out which aim to identify the molecular determinants underlying the 'abscopal' effect. Unfortunately, this effect has only been described anecdotally during conventional radiotherapy, but preclinical and clinical data suggest that its frequency may be significantly increased in association with immunostimulant drugs such as "checkpoint inhibitors". At the Institute, an ongoing phase II study aims to evaluate the association between SBRT and anti-PD-L1 in head and neck tumors.

Proton therapy situation in the world and in Italy and the prospect of a proton therapy center in the Lazio

There are currently 63 operating proton centers in the world. More than 30 of them are under construction. Of these, 24 centers are located in the USA, where there are also 11 centers currently being built.

From 1954 to 2014, around 120,000 patients were treated with proton therapy worldwide.

The first center was Loma Linda (CA) and was inaugurated in 1992. The center had 3 gantry / irradiation rooms and treated up to 160 patients a day in 2011. There are 20 operating centers across Europe including: Czech Republic (1); France (3); Germany (7); UK (2); Sweden (1); Russia (3); Italy (2).

In Italy, the clinical centers are located in Pavia (CNAO) and Trento. Occasionally, an accelerator made for high-energy experiments located in Catania is occasionally used for proton therapy. It uses a 62 MeV proton beam from a superconductor cyclotron of the National Southern Laboratories of Catania (INFN) to penetrate the tissues up to 3 cm in depth, typical of ocular pathologies. Obviously, this last accelerator is not able to treat tumors greater than 3 cm in depth.

Therefore, at the moment, patients with deep seated-neoplasms (almost all patients in CATEGORIES A and B) undergo irradiation treatment in the centers of Pavia and Trento, instead other patients must get treated abroad. The current ones installed in Italy are not in fact big enough to guarantee the necessary treatments for the Italian population, neither for those concerning diseases in Category A nor for Category B. For this reason, there is a consequent wave of patients setting off to foreign countries.

From this perspective, the goal is to increase the number of equally distributed centers across in Italy, in order to fully exploit the potential of these particles. The ideal geographical location to establish a proton therapy center for patients in Central and Southern Italy is Lazio.

Below are estimates demonstrating the need for both conventional radiotherapy and proton therapy limited to the Lazio Region. The population residing in Lazio in 1/1/2013 is 5,557,276, and in Rome, 2,638,842 (source <http://www.regione.lazio.it/statistica/areeTematiche>).

Every year, there are about 34,000 new cases of cancer are diagnosed in Lazio ("Together Against Cancer" Foundation). Currently, 50% of patients with solid tumors must undergo radiation therapy during their therapeutic course. In Lazio, about 17,000 patients undergo radiotherapy with conventional photon machines every year. This figure is compatible with the rate of radiotherapy services used, which is reflected in the services used in each ASL (subjects treated per 1000 residents), standardized by age and sex compared to the regional population. We use a value of 2.4 per 1000 residents, or 0.24% as a reference point. **In accordance with this value, the number of new cases expected for conventional radiotherapy is around 13500 in Lazio and 6300 in Rome.** It is recognized that at least 10% of patients treated with conventional therapy benefit from proton treatment, **only in Lazio does the number of patients / year reach about 1500, of which approximately half from in Rome.** To reach an estimated of 2500 treatable patients / year per center, the populations from Molise (314,000 inhabitants), Abruzzo (1,326,000 inhabitants), Umbria (895,000 inhabitants) and Marche (1,551,000 inhabitants) should be added. In other words, a proton therapy center in Lazio would reach 2,500 patients a year, covering some of the neighboring regions (Umbria, Abruzzo, Molise and Marche).

We need to add patients of Southern Italy (Campania, Basilicata, Puglia, Calabria and Sicily) to this estimate considering that the center of Catania, as already shown above, manages to treat only ocular and non-deep seated-tumors as that the accelerator for proton therapy treatments is available only one week every two months (for the remaining time the same is dedicated to basic physics research activities).

Last but not least, the IFO is a cancer IRCCS that provides assistance to patients living in Center-Southern Italy covering large area. It has already drawn interest from clinicians at the Bambino Gesù Children's Hospital and of the IRCCS Pascale di Napoli.

IFO is the perfect candidate as it:

- operates as a **strategic element** of the Lazio Region within the regional cancer network, which pursues both health care goals and translational research;
- Is the only IRCCS in the Lazio Region dedicated to the treatment of oncologic diseases and that is able to offer the most modern therapies to patients of Lazio and central-southern Italy;
- is already involved in the development of the TOP IMPLART project of the Lazio Region. This project could optimize the investment made by the Lazio Region in research. It aims to create an innovative accelerator for protons (TOP IMPLART project) otherwise limited to the industry, setting it up within a clinical setting, thus promoting translational research pathways through the direct application and implementation of innovative therapies to cancer patients;
- already possesses functional for realizing a Proton Therapy Center both for clinical and experimental purposes. These areas are adjacent and perfectly integrated with the main building of the Institute and the technology therein;
- has developed diagnostic therapeutic patient care pathways (PDTA) and is the center of attraction for the diagnosis and treatment of all the citizens of Lazio and the Center-South of Italy;
- has the medical, engineering and physical expertise necessary for implementing a proton therapy center.

Over the years the Department of Radiotherapy has proven to have the expertise and know how on adequately exploiting the installed equipment. Currently, there is an average of 96 treatments per day scheduled for conventional external beam radiotherapy. A fourth machine, the Cyberknife, which has also been installed, carries out treatments with precision for an additional 5-10 patients a day. The Department of Radiotherapy at IFO is one of the two public bodies in all of Lazio that treats the most patients per day on par with LINACs; An eventual proton therapy center would be able to sustain the care services that the Department of Radiotherapy delivers facilitating the workload at IFO. In fact, the department is undersized compared to its necessary demands. If it is estimated that 50% of patients with solid tumors need radiotherapy during their therapeutic course and given that not fewer than 5,000 new patients are seen per year at IFO, the department should be upsized so it is able to treat more than 2500 pcs, instead of the current 1200 or so. Furthermore, it has been shown that not only the qualitative but also the quantity requirements of therapy machines have increased by 3.5-4% per year (Slotman & Vos, Radiother Oncol 2013, 106: 266-270), and how the localities at IFO have been distributed has also in part changed in the last 15 years. 50% of IFO's Unit of Medical Physics Expert Systems staff is trained in proton therapy. It participates directly in developing the ENEA-ISS-IFO experimental proton accelerator and has designed the Trento center by finalizing the authorization procedure for the Ministry. Furthermore, the Unit is also currently carrying out studies in collaboration with the Trento proton therapy center on developing advanced methods for the generation of treatment plans, dosimetric tests and studies of automatic systems for prioritizing proton treatment, which make up the basis for launching a Proton Centre in Rome

1-3 years Objectives

Over the next three years we expect to achieve the following:

- Collect immuno-monitoring data after conventional and stereotaxic radiotherapy treatment in prostate cancer.
- Carry out the research line regarding head and neck cancer in a patient suffering from squamous carcinoma which aims to evaluate and quantify the existence of an abscopal effect in vivo after neoadjuvant treatment with PD-L1 and SBRT at low doses only on T.
- Implement research protocols in oligometastatic patients suffering from breast cancer, non-microcytoma lung and melanoma that aims to evaluate the effect of SBRT alone or in combination with immunotherapy in prolonging progression-free survival.
- Determine and conclude the executive project regarding the proton therapy center of the Regione Lazio at IFO.

4. ADVANCED CLINICAL RESEARCH AREA (ARCA) (Area in Blue)

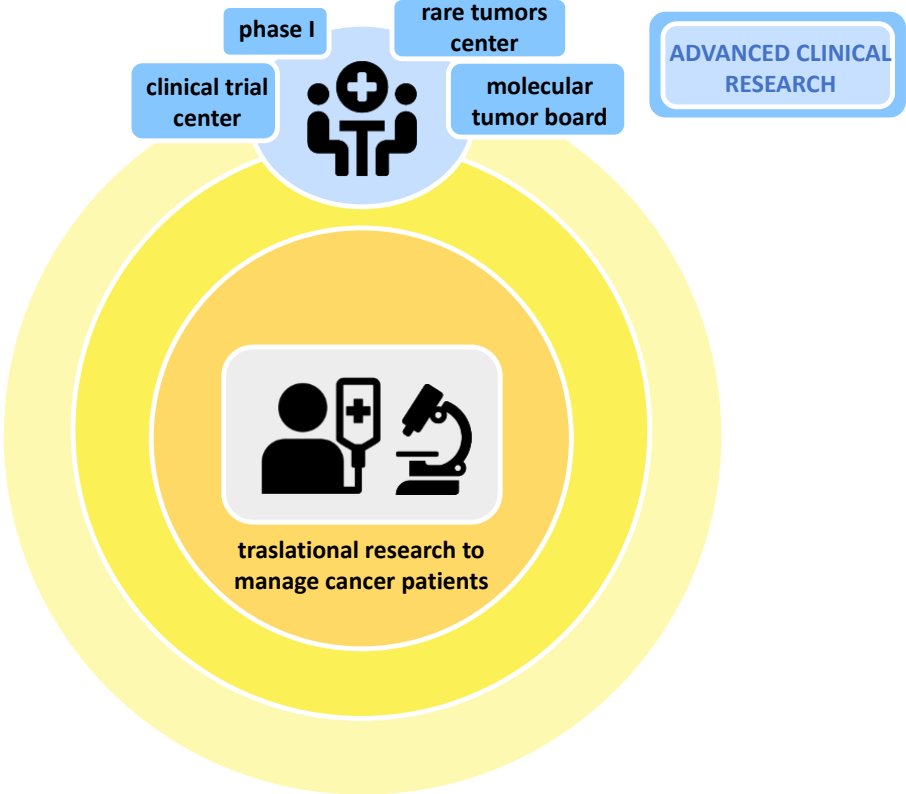


Figure 8

4.1 Clinical Trial Center

The main objective is to ensure coordinating and delivering high quality clinical trials in Lazio

- **Centralized coordination of clinical studies**
- **Training Data Manager and Nurses in Research**
- **Quality Assurance**
- **NON-Profit Research Support**

Background and state-of-the art

The objectives of clinical research in oncology include the following:

- Accelerate the development of new anticancer drugs in all solid and hematologic cancers (including biological agents and cell therapies) that can improve the quality of life and survival of cancer patients
- Develop therapies for specific subpopulations defined by certain biomarkers aiming to offer medical therapies tailored to each individual patient ("druggable" mutations)
- Making the approach towards patient care more innovative: designing clinical studies, sustainable sources of financing, better and more profitable use of resources, data collection and analysis
- Support, coordinate and collaborate with the laboratories in basic research that are involved in preclinical and translational trials in order to facilitate the discovery of new drugs, the identification of their targets, the mechanisms of action and resistance and promote subsequent experimental trials on humans.

The Clinical Trial Center (CTC) is an organizational body that centralizes and unifies the coordination and monitoring of clinical trials with the aim of providing management and methodological services to researchers as well as fostering collaboration between them. It also constitutes an incentive for private investments, favoring Institutes that have a unit or body capable of supporting, across the internal operating units, the entire process of clinical experimentation (phase I, II, III studies, IV)

IFOs CTC

The CTC was established by Decree No. 308 dated 24 April 2018 and subsequently amended with Decree No. 602 dated 06 August 2018. The components of both the CTC and External Units are shown in Figure 8. The CTC performs the following functions:

- Coordinates and monitors the functional activities regarding the management of clinical trials within the IFO, acting as a qualified reference point;
- guarantees greater control of the clinical trials to the Scientific Directions of Regina Elena and San Gallicano Institutes and IFOs Medical Office;
- particular supports in the spontaneous non-profit research;

- interacts with the Departments involved in experimental research activities, coordinating the activities of the experiments aimed at:

- providing administrative, managerial, methodological and statistical services to researchers for the conception, design, planning, start-up phase, conduction, analysis and reporting of clinical studies so that these activities are carried out in compliance with the GoodClinicalPractice (GCP) and the protocols;
- supporting the management of authorization procedures as well as the conduction and financial reports of clinical studies;
- promoting, in profit and non-profit research, the professional development of all participating researchers in terms of compliance with GCPs and regulatory aspects;
- guaranteeing quality control of studies (experimental and observational studies) with profit and non-profit study promoters;
- supporting monitoring of information regarding the feasibility of studies in terms of potentially enrolled patients;
- increasing the synergistic collaboration between researchers involved in the studies;
- evaluating the experiments proposed by researchers at IFO, for which IFO takes on the role of Promoter, and monitors the progress of the approved studies;
- identifying areas of great strategic interest for the Institute and propose initiatives necessary for promoting clinical trial projects in these areas.

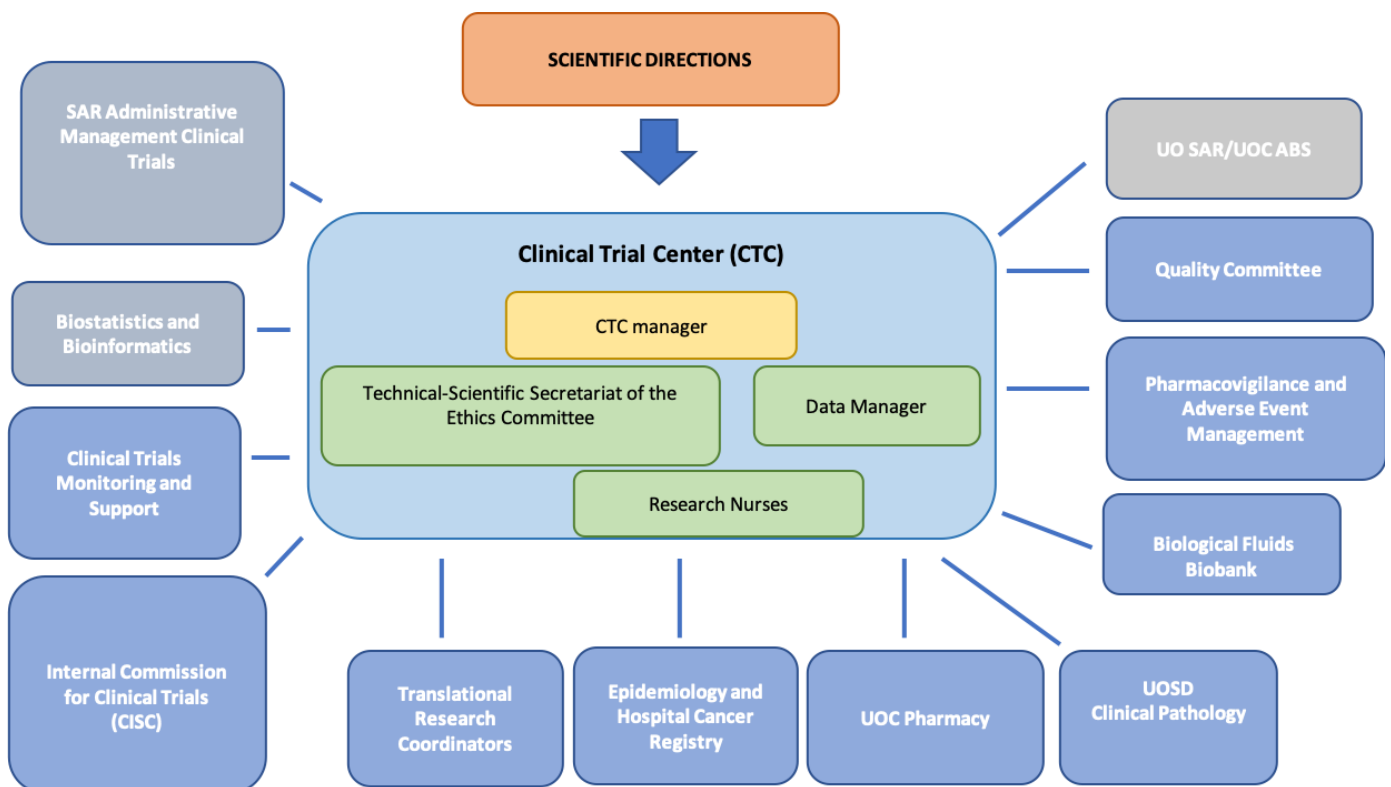


Figure 9

The CTC currently relies on the Technical-Scientific Secretariat of the Ethics Committee that charts the whole authorization process of the studies, and count on a consolidated group of data Manager experts who have been working continuously for more than a decade as well as a relatively younger group of Research Nurses. In September 2018, the CTC is located in a suitable location within the Institute, the so-called 'Open Space' which hosts 24 workstations, a meeting room to allow meetings and monitoring visits and several built-in wardrobes. 6 Data managers and 2 research nurses are based in this location and currently follow around 65 studies.

In 2018, the EC Technical Scientific Secretariat dealt with 105 studies for the Regna Elena Institute, 23 for San Gallicano and 19 for the Bietti Foundation, where it defined and negotiated the authorization procedures and contracts.

Together with the 'satellite' structures, the CTC can carry out its coordinating activities, with the help from the Biostatistics Unit, by guaranteeing statistical support for the study design in accordance with the most innovative types of studies such as n-of-1 trials, basket and umbrella studies, adaptive studies, observational studies to be analyzed with propensity score techniques and can carry out systematic reviews and meta-analyzes to contextualize the studies themselves.

It has made arrangements with: the Clinical Pathology Unit regarding the correct manipulation, preparation and shipment of biological samples, instead with the Pharmacy it agreed to identify the necessary harmonic features in the management of drugs and medical devices in the experimental field. With the Diagnostic Imaging Unit, it established procedures for accessing and scheduling examinations for patients enrolled in the clinical trials.

After several years not seeing IFO play the role as Promoter, except for one ISG study, 4 phase 2 and phase 3 clinical interventional trials started in 2019. It will demand great levels of commitment from the CTC and Monitoring and support section of the clinical trials. In this context, it was also necessary to clarify the relationship with the Pharmacovigilance Unit which has seen a significant increase in its activity. In collaboration with the Clinical Studies Monitoring and Support section and with the Quarc (Quality Assurance and clinical risk), an audit was carried out at the Institute's facilities in order to promote the quality of experiments and adherence to the current GCPs and regulations in force.

1 Year Objectives

- To establish and obtain certification of the CTQT (Clinical Trial Quality Team) in order to ensure and guarantee the quality of the management of the following:
 - non-profit clinical trials
 - studies for which IFO has the role of promoter
 - phase 1 studies with a non-profit promoter
- Implement internal auditing procedures at the institute and network monitor training to manage external studies
- Centralise all the Data Managers and increase the number of the Research Nurses present
- Optimize authorization times for any type of clinical study, bearing all the limitations set by the Single Opinion of the Coordinating Center and the AIFA authorization

3 Years Objectives

- Establish the CTC as a reference centre, both internally and for non-profit promoters in general, including external ones, in order to achieve multicentre studies covering the entire path from the researcher's idea to the drafting up of the protocol through to the completion of the authorization process, create web-based platforms with password-controlled access for the management of clinical and molecular data. The reference model and procedures will be those of a CRO.

To achieve this, several qualified personnel is required:

- A medical graduate with relevant specialist training: at least 1 unit
- An administrative assistant for managing the authorization process: 1 unit
- Monitor: 2 units
- Computer scientists: 2 units
- Clinicians: 1-2 units
- Web based space for data management in accordance with regulation

4.2 Phase I Study Centre

The main objective is to ensure coordinating and delivering high quality clinical trials in Lazio

- **AIFA Accredited infrastructure**
- **Phase 1 Clinical Trials**
- **Public / private collaborations**
- **New Patient Care Options**

Background and state-of-the art

Over last twenty years, pharmacological research has taken on a leading role in the field of Medical Oncology. However, while the phase that goes from the 'screening' the list of chemical compounds to realizing the 'first-in-human' studies of a new drug has been efficiently carried out by the pharmaceutical industry (as demonstrated by the crowded 'pipelines' of major international companies), the clinical / translational development of new drugs, in particular of molecular target agents, remains inefficient. In particular, in oncology the success rate in clinical registration studies is 15-45% (against 70% of infectious diseases and 80% of cardiology). This inequality between preclinical development and the clinical application of new drugs derives from multiple factors including:

- the poor integration between the laboratory and the clinic, with almost total absence of professional medical staff between experimental and clinical research (the so-called "physicians / scientists" present, albeit insufficiently, in the US system);
- the slow progress of clinical methodology towards more modern models able to integrate the evaluation of molecular and clinical "endpoints" in studies designed ad hoc;
- the growing number of molecular-targeted drugs produced by industry and often competing for specific market sectors;
- the lack of integration between industry and academia in the clinical / translational development of new drugs; the tendency towards adopting clinical development paradigms that contract the early clinical phase study (I and II) essentially for a preliminary assessment of safety in order to proceed as quickly as possible to the registration phase.

In this scenario, innovative Phase I clinical trials are increasingly important as they allow to increasingly obtain, through the use of biomarkers and the possible extension of the patients, useful information not only regarding the safety and tolerability of drug, but also the effectiveness and biomolecular identification of the subpopulations of responsive patients.

With the Decree n. 809/2015 ("Determines the minimum prerequisites necessary for health care facilities, needed to perform phase I experiments pursuant to Article 11 of the Presidential Decree dated 21 September 2001, No. 439 and referred to in Article 31, paragraph 3 of the Legislative Decree dated November 6, 2007, No. 200 ", Official Journal No. 158 of July 10, 2015), signed by the Director General, AIFA defined the minimum requirements necessary for running health care facilities that perform phase I clinical trials involving drugs. In compliance with the DM 19 March 1998, "Recognising adequate facilities/centers for performing clinical trials involving drugs", in general the Decree identifies the clinical centers / units / departments where it is possible to conduct phase I clinical trials on patients and / or on healthy volunteers. It also established procedures on self-certification outlining identification and description prerequisites, while self-certification modalities will be defined in a subsequent decree by AIFA. The text also covers phase I experiments that are carried out abroad and the experiments performed in non-compliant facilities/centres. In addition to the general aspects, Annex 1 of the Decree mentions the requirements of the Clinical Units, the laboratories and outlines the list of standard operating procedures (SOP). "It is a complex and detailed document that aims above all to guarantee the safety and well-being of the subjects participating in phase I trials (patients and / or healthy volunteers) as well as the correct conduct of the study according to the GCP / ICH (Good Clinical Practice / International Conference on Harmonization) the quality standards. It is from the latter perspective that the SOPs embraces the complete management of a clinical study including the simple physical / environmental organization of the structure, the skills and expertise of the health care personnel, the appropriate collection and reporting of clinical and laboratory data". According to clinicaltrial.gov, there are 264 phase I studies currently active in Italy, while in England there are 547. According to the latest AIFA report in regards to the year 2017, the percentage of applications for authorising phase I studies is equal to 14% of all clinical trial studies. Prior to this period, this value was undoubtedly lower, respectively 10.3% in 2015 and 11.2% in 2016. **This means that the Decree certainly contributed to the increase in phase I studies in Italy.**

The Phase 1 Study Center at IFO

In this context, and consistent with our Institute's mission in regards to research and patient care, the creation of the Phase 1 Study Center will: a) on the one hand, respond to the patient needs by providing excellent patient care, mainly through the possibility of gaining privileged and early access to new clinical trial drugs; b) on the other hand, it creates an absolutely innovative model for the collaboration between academia and industry, by gaining scientific and operational independence in managing early phase clinical / translational studies, will guarantee greater power in the innovation and fairness in evaluating development strategies in subsequent experimental study phases.

The Phase 1 Study Centre at IFO is dedicated to the early phase of clinical trials, including phase 1 studies and first-in-human studies. The Clinical Center for Phase 1 Studies, now known as the Department of Clinical Experiments: Phase 1 and Precision Medicine (Phase 1 and MP), based in the Department of Clinical and Oncological Research, and was established by Decree No. 538 dated 10 July 2018.

The Phase 1 Study Center (the only Phase 1 center in Lazio) contains the following features:

- Two locations: the first one is located on the eighth floor of Palace B for patients who do not require hospitalization. On the 8th floor there is a day room with two beds, a room with three armchairs for DH, a clinic for a medical visit, a nursing center, a storeroom, a meeting room and support services; the second location is room 208 located on the second floor within the Department of Medical Oncology 1 COC, for patients who require hospitalization or for those who need to under observation longer after 12 hours and / or for urgent needs,
- Monitoring and surveillance technologies of patients in sub-intensive therapy
- Dedicated staff
- A UUOO support network and Professionals for each type of study were prepared in accordance with AIFA Decree no. 809/2015

- In compliance with the AIFA 809/2015, Appendix 1, par. 4, the Phase I Clinical Center is composed of the following basic personnel, as outlined in the organization chart: Medical Director, Nurse Coordinator, Pharmacist (or with documented experience in the field), Medical Staff with at least 5 year degree, Head of Experimental Product Management (degree in CTF or pharmacy), Head of reception and treatment of Biological Samples in the Laboratory of Clinical Pathology (Degree in Biology), Manager for collection and dispatch of Biological Samples (Nurse), Nursing Staff, Study Coordinator / Data Manager, Quality Manager, Quality Assurance (according to DM 15.11.2011), Auditor (according to DM 15.11.2011), Archiver



Room 208



Room for Day hospital



Data Manager Office



Doctors Meeting Room

Figure 10

1 Year Objectives

- Complete self-certification for Phase 1 non-profit
- Complete the medical and nursing staff of the Phase 1 Clinical Center. The final goal is 1 Medical Director, 3 doctors, a coordinator, 5 nurses, a QA, an archivist, 1 full-time data manager / study coordinator.
- Relocate two beds h24 and 7/7 from the 2nd floor to the 8th floor.
- Evaluate at least one non-profit study with IFO promoter or in co-sponsorship.
- Publish an article reporting the experience of the first year and the difficulties of organizing and maintaining an efficient and competent Phase 1 center

3 Year Objectives

- Make the Phase 1 Clinical Center and all related units/departments more "appealing" for pharmaceutical companies and for national and international Experimental Centers in order to be selected and activate at least 3 new profit studies per year and at least 2 non-profit organizations with IFO Promoter or co-sponsor, including at least one on CAR-T.
- Apply for trials involving healthy volunteers (e.g. vaccines).
- Activate the Training Center on Phase 1 Trials and on Quality Assurance in Phase 1.
- Become a site for carrying out internships for students graduating in medicine-surgery, nursing, medical specializations, and post-doc research.

4.3 Molecular Tumor Board

The main objective is to develop a new patient care model based on the use of multidisciplinary expertise for the rational use of out-of-indication drugs in patients with advanced staged disease

- **Multidisciplinary table**
- **Recommendations for rational use of out-of-indication drugs for patients in an advanced state of disease**
- **Centralized database**
- **Ethical-Scientific Approach to the Patient**

Introduction and state-of-the art

In recent years, all the major cancer centers in the world, including the IRCCS National Cancer Regina Elena Institute, have established Molecular Tumor Boards (MTB). MTBs are created to scientifically and ethically regulate the access to drugs for uses that, although "beyond indication", are however supported by strong, and sometimes compelling, biological and/or clinical evidence. Accordingly, MTBs focus their attention exclusively on those cancer patients who have failed all previously approved lines of therapy and for whom no other viable options are available. It is widely agreed that patients referred to the MTB must be in good general conditions, e.g. they qualify to potentially benefit from non-conventional targeted treatment. At the same time, acceptable physical fitness is the pre-condition that allows healthcare professionals to evaluate drug efficacy and therapeutic appropriateness. These elements are crucial to determine possible future extension and applicability of the drug itself in indication. Indeed, this very sensitive ethical and deontological area requires tools and procedures that are absolutely immune to any possible censorship, both from a scientific-diagnostic point of view and from the good clinical practice and managerial perspectives.

To put it simply, MTBs were set up to explore the 'middle-earth' between 'classical' clinical studies that recruit pre-determined numerous cohorts and have pre-defined objectives, and the vast area of potentially actionable alterations. These diverse vulnerabilities make each tumor unique and define it as potentially susceptible to one or more molecular target drugs. This middle earth (in constant and continuous expansion) emerged when precision oncology began to make regular use of Next Generation Sequencing panels (massive sequencing, NGS) that identify in parallel a large number of potential vulnerabilities (mutations and other genomic aberrations). Vulnerabilities that cannot always be validated within a classical study, and in many cases require an "agnostic" approach to the tumor histotype. The general purpose of MTBs in this modified clinical-pathologic scenario goes beyond the application of therapy to the individual patient, and involves expanding the potential applicability to other tumor histotypes of drugs that may already be in use but with different indications. This delicate procedure, that intertwines with drug repositioning, must take place in a 'neutral' environment, in which potential conflicts of interest can freely emerge and be carefully considered. The IRCCS Regina Elena National Cancer Institute wants to contribute to this very sensitive topic, and guide the alignment between the interests of the NHS and the health needs of cancer patients, taking into account the role of all legitimate stakeholders, including pharmaceutical companies and biomedical technology providers.

Molecular Tumor Board at the Regina Elena Institute

We believe that the procedures that MTBs will adopt in the near future may build the foundation for general guidelines to be followed when EMA-AIFA therapeutic indications and recommendations are not available for that specific case/area. Our recent activities have all been performed with this final goal in mind. Our

priority is to start working on a competitive and innovative project that will establish a leadership in city of Rome and the greater Rome area (Regione Lazio). It was clear from the beginning that our experience would set the stage to establish state-of-art MTB procedures not only locally, but nation-wide. Our priority was to be a pilot center, a sort of catalyst for experiences meant to be expanded from a local, then regional, and finally national context. We are aware that one of the main limitations of these multidisciplinary bodies are the low number and/or peculiar features of the patients that come to MTB attention. Therefore, since September 2018 (the month in which the MTB was established with an Official Decree) we were ready to move forward. First we took action in Italy, with the intent to create much more than a 'treatment recommendation factory'. Our aim was to create a real methodological incubator, and generate an 'exportable' MTB workflow. In line with these initial intentions, our ultimate goal is presently to create a harmonized MTB national network. This will be organized on a national level, laying the grounds for sharing experiences, data and approaches. Such a network is intended to progressively acquire an active coordinating role in the context of future initiatives on the national level.

The milestones of our experience include: (1) establishing a constant and direct line of communication between the MTB and our Ethics Committee; (2) drafting internal guidelines which, although still are in the initial stages, may in the future become regulatory. At the core of these guidelines is the principle that any action within the mTB must be tracked; (3) To this end, we will support the adoption of a querable online platform for data sharing. The salient parts of this preliminary and paradigmatic work have been outlined, at least from an operational perspective, in the flow chart below.

The whole MTB workflow has so far been applied to all the 75 patients who were referred to our Institute is shown in Figure 11.

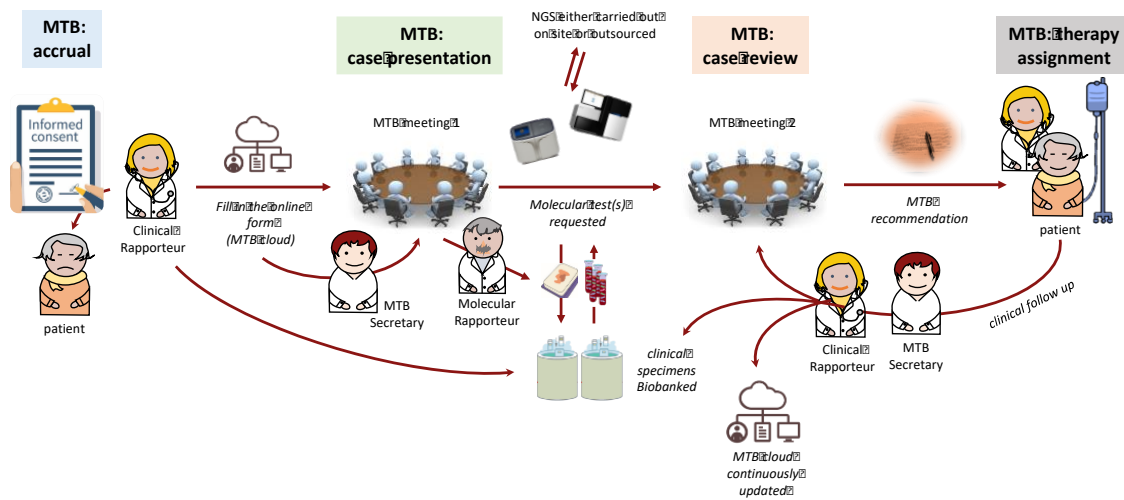


Figure 11. The IRE MTB workflow

The MTB adopts a special workflow that is centered on the clinical molecular rapporteurs, logistically connected to the other MTB members by the MTB Secretary. A specially designed MTB cloud (data repository) provides semi-automated data annotation, a session recording system, a number of case retrieval tools, and a connection with the institutional tissue/liquid biopsy BioBank. In summary, the patient signs an informed consent in which it is explicitly stated whether or not he/she opts to be informed of off-the-beaten-road therapeutic chances. Then, the Clinical Rapporteur files a new patient form that briefly describes the case and the reasons for MTB referral. Cases are discussed in multiple rounds (the MTB meets bi-weekly). In the first session/meeting the MTB analyzes the available clinical and molecular evidence, and requests additional molecular tests (e.g. NGS –WGS or targeted panels, liquid biopsy, genetic videat etc). Results are discussed in meeting 2, and also annotated in the cloud. This helps the Secretary to file an MTB report (in progress) that is electronically assembled using standard ‘blocks’ of information retrieved from the specifically designed cloud sections. The MTB report (recommendations) is electronically signed and returned to the Clinical Rapporteur who decides whether or not the patient may be treated as suggested. The system is continuously updated and there is an accessible (to the MTB members) follow-up pane to keep track of clinical outcome for future actions. The MTB cloud is unique in that it is dynamic. It describes patients on a timeline.

2018 today: cases and recommendations

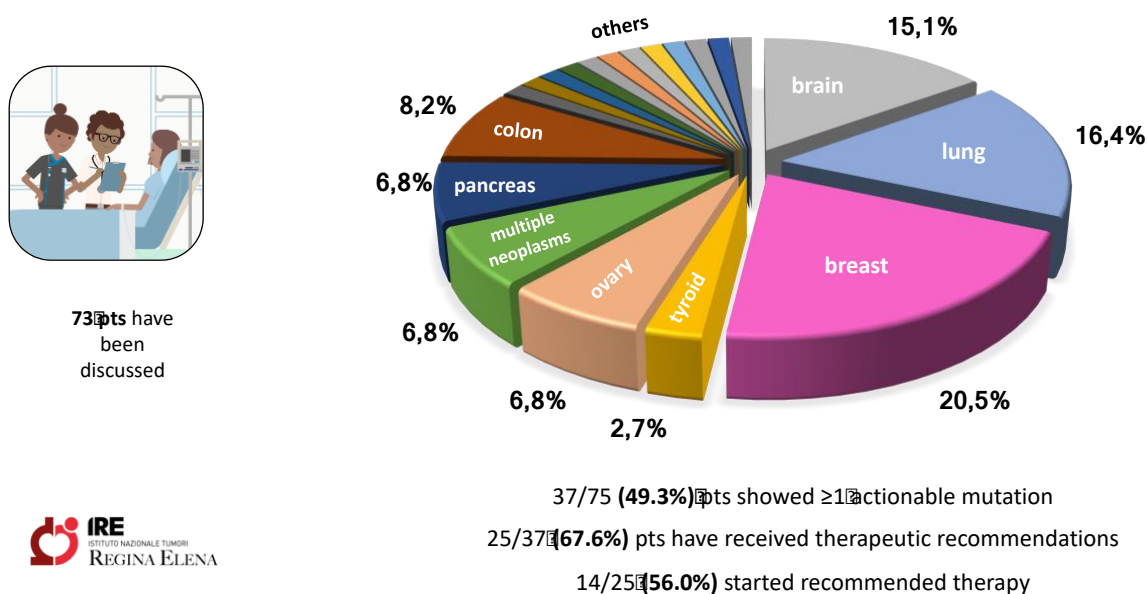


Figure 12

The pie chart on the side (Fig 12) represents at glance the variety and peculiarities (e.g. patients with multiple tumors) of the cases seen at our MTB. The 73 patients brought so far to the MTB attention have been broken down into cancer types, which illustrates at the same time disease variety and inclusion of rare tumors despite the apparently small number. Patients and recommended therapies are highlighted. The potential of liquid biopsy in the context of multidisciplinary MTB assessments deserves special mention. For about 10 years until now and throughout the post-genomic era, medical oncologists have treated their patients for the vulnerabilities they had at the time of histopathological and molecular diagnosis (often assessed years before) and not for their present vulnerabilities. Even innovative clinical trials, such as "basket" trials, i.e. those clinical studies in which patients are enrolled not on the basis of their pathology, but on the basis of their genomic lesions, fail to provide updated access to genomic tumor aberrations in real time. Liquid biopsy now offers the missing tool of updating the mutational status throughout disease evolution and therapy changes. Compared to the DNA of archival neoplastic tissues, aberrations in blood are a more accurate way to assign patients to "basket" trials and new lines of therapy. We believe disappointing results in some studies may be in part explained by treatment for vulnerabilities long gone at the time of standard treatment administration or trial enrolment.

1 Year Objectives

- Dedicated IT (intranet) platform for intramural MTB use: formalized, annotated, continuously updatable, and querable. The platform (GDPR-compliant) will be accessible to MTB Members only. It will monitor the efficacy of therapeutic recommendations, and will provide continuous clinical follow-up
- Use of the MTB cloud platform (1) to issue Molecular Reports including therapy recommendations. Semi-automated electronic fill-in (extensive use of drop-down menus) of defined fields. MTB Report on IRE letterhead, with a list of MTB Members, Clinical and Molecular Rapporteurs, and Coordinator. Electronic management, export of the document as PDF, and remote electronic signatures.
- Use of the MTB report for outpatients and physicians asking support from other oncological centers.
- Creation of a dedicated, public office (URL) on the IFO website primarily for the collection, filtering and management of external requests to access MTB IRE services.

- Establishment of the MTB ACC network (creation of a 'super-MTB'): defining electronic interoperability methods between centers, strengthen national leadership, write down a guide for adopting workflows, methods and annotation strategies based on those adopted by the IRE MTB.

3 Year Objectives

- Use an IT platform developed on the basis of the IRE intranet MTB cloud (but agreed and shared at a national level through ACC - see short-term objectives) for the multicentric management of the 'super-MTB' activities.
- Launch a multicentre Master Trial (ACC) for assigning targeted therapies beyond approved indications (but based on molecular indications), preferably according to the 'DRUP' scheme, derived from the Dutch model
- Alternatively, promote an equivalent master trial, possibly supported by the local Health Authorities (Regione Lazio), National Bodies (AIFA/Ministry of Health), or other public or private non-profit bodies. Envisage IFO/ACC institutional funds for co-financing this effort.
- Linked to (1) and (2), Launch a Master BL Trial for assigning therapy on the basis of molecular alterations and tumor vulnerabilities only found in blood (Liquid Biopsy) but absent (or minimally present) in neoplastic tissue, e.g. developing *in vivo* during previous multiple therapeutic lines.

4.4 Centre for Rare Cancers

The main objective is to become the regional hub for the study and therapy of rare tumors / rare genetic variants of adults thanks to the work carried out within the EURACAN network

- **Orphan Diseases**
- **Rare Cancer Network**
- **EURACAN – European Referring Center**

State-of –the art

Rare tumors (TRs), which by international definition are identified in tumors of various anatomical sites that have an incidence of less than 6 cases per 100,000 inhabitants per year, together represent around 25% of all newly diagnosed neoplasms, covering about 5 million patients in Europe. According to the 2015 AIRTUM report, it is estimated that the incidence of TRs in Italy is 89,000 new cases / year with approximately 900.00 who live with a TR. It should also be taken into account that 72% of them are considered very rare with an incidence <0.5 cases per 100,000 inhabitants / year. Moreover, thanks to advances in the field of genomics, a variety of histological subtypes and molecular subtypes are also emerging in the area of common cancers potentially increasing the percentage of TRs themselves. The TRs are notoriously an "unmet clinical need" due to various problems including: (a) epidemiological, given the difficult systemization and classification that prevents the annotation of all cases in a well standardized national register; (b) frequent errors and diagnostic delays particularly outside qualified centers; (c) therapeutic inappropriateness; (d) difficulty in producing guidelines based on recommendations with high levels of evidence due to the low number of patients (d) poor molecular knowledge that makes innovative treatments and target therapies less available to TRs than for any other neoplasia ; (e) delicate "ethics", seen as a vital in bringing high-level science and patient care to areas deemed unprofitable by pharmaceutical companies. This series of clinical difficulties unfortunately translates into a substantially worse prognosis compared to patients with common tumors, TRs therefore require special management because they are "orphan" diseases from many perspectives.

Rare Tumors in Adults at the IFO

With about 900 new cases accessing first time visits and about 3,000 patients followed up every year, the Regina Elena and San Gallicano Institutes (IFOs) together represent two crucial recruitment and assistance arms for the entire National Health Care Service. For over 10 years, the Regina Elena Institute has been a center for the Rare Tumors Network (RTR) within an Alliance Against Cancer project. It has actively participated in registering cases and in the virtual discussion of complex cases (mainly sarcomas of the soft tissue). Since 2016, after obtaining an endorsement granted by the Ministry of Health, IFO became one of the 67 centers of the European network EURACAN (EUropean network for RAre adult solid CANcer) (ERN n.2 of the European Union) and was accredited for 8 out of the 10 adult solid TR groups (soft tissue and bone sarcomas, rare neoplasia of the male genitourinary tract, neuroendocrine tumors, rare tumors of the gastrointestinal tract, rare endocrine tumors, rare chest tumors, rare skin tumors and ocular melanoma, rare brain tumors). Each group of TRs has a Domain Leader from IFO that helps to maintain relations with the European network within one's domain. During the first few years of EURACAN, a European platform for exchanging information between centers was created, the Clinical Patient Management System (CPMS), a platform for sharing and virtual discussions take place regarding difficult RT cases on a European level. In 2018, IFO was also involved in the elaboration and publication of international guidelines in collaboration with the European Society of Medical Oncology (ESMO) in order to homogenize the diagnostic and therapeutic approach of TRs at European level. To date, guidelines for soft tissue sarcomas, GISTs and bone sarcomas have been published and the Regina Elena Institute has taken part in the consensus board (Soft tissue and visceral sarcomas: ESMO-

EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018; Gastrointestinal stromal tumors: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up Ann Oncol 2018; Bone sarcomas: ESMO-PaedCan-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow up. Ann Oncol. 2018). In view of making a profitable contribution to the European network as well as systematically collecting all the cases that have access to the Institute, from 1st January 2018 a prospective collection of all new RT cases (Rare Tumor Clinical Registry - IFO) in a domestic platform called EURACAN platform is underway. It is for this purpose that a pool of data managers searches for all the TRs that access the Institute through the IFO computer systems (electronic folders, PACS, WEB CC, etc...) for which they have personal passwords. In order to standardize the data collection method, an operational protocol has been prepared describing the methodology used for searching and entering data in the IFOs internal platform. From January 2018 to date, 969 new cases of patients with RT have been prospectively recorded.

From the clinical point of view, RT cases are discussed in meetings held by multidisciplinary teams (DMT-Disease Management Team) which are scheduled on a weekly or bi-weekly basis (Fig. 13). DMTs are dedicated to managing various RT groups, in order to ensure adequate clinical, radiological and pathological assessments leading to the correct diagnosis and appropriate treatment within or outside national or international experimental trials. For some RT classes (for example sarcomas and CNS tumors) a tissue and serum Biobanking process is also currently active which will be extended to all RTs.

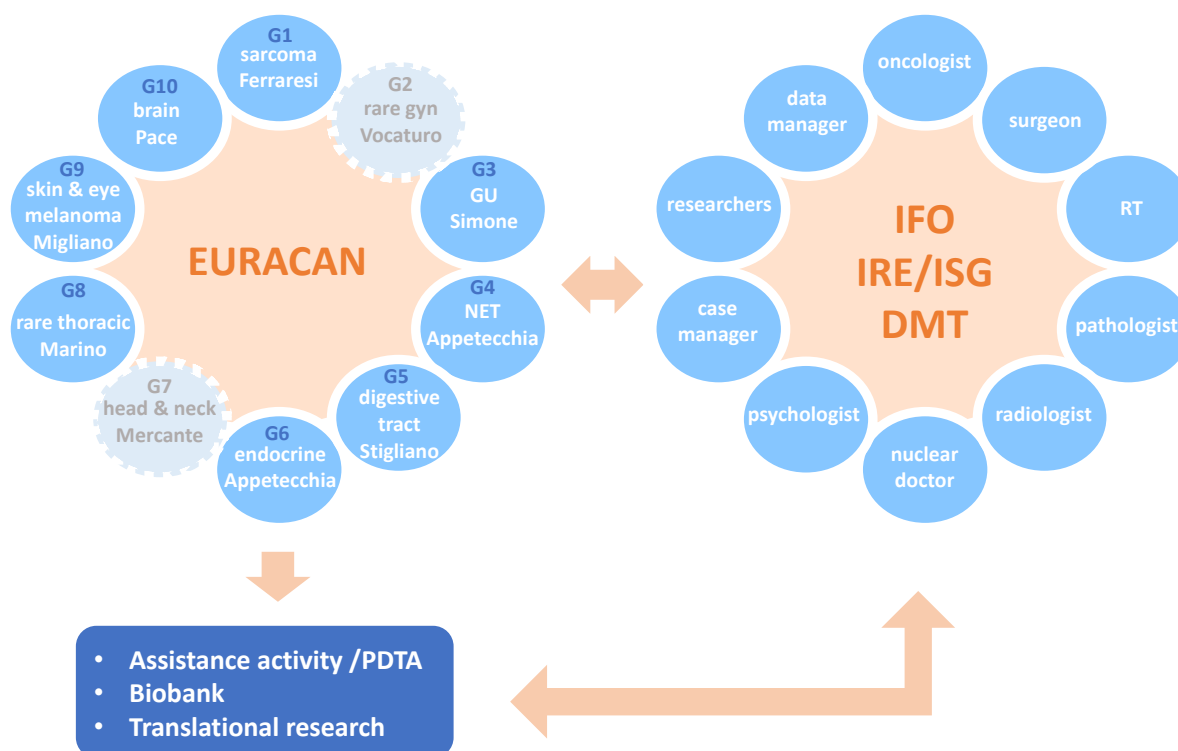


Figure 13

In 2018, the Scientific Direction finally established a set of Translational Groups on various RTs that meet about every two months to discuss current projects involving both clinicians and dedicated researchers. In the last 3 years, IFO has participated in 23 clinical trials regarding RTs and in 2018, the study groups (clinicians and researchers) on TRs produced 28 papers published in national and international journals.

Plans:

1) The Organization: a daily challenge

Considering the high annual number of patients with RT under care at IFO, the presence of team of specialists dedicated to the various RT classes making up 10 multidisciplinary groups (DMT) representing all 10 classes of Rare Solid Tumors in Adults, the presence of all the Medical and Surgical disciplines appropriate for the treatment of these neoplasia and the simultaneous broad-spectrum research activities dedicated to the RTs and coordinated by the RT Translational Group, the aims for development can be a little ambitious over time.

For 2019, the objective is to become a Regional HUB (Provider center) of the National Rare Tumor Network. From this perspective various implementation projects are in drawing to a close:

- 1) Define a PDTA Institute dedicated to RTs
- 2) Define and start-up project introducing RTs through creating a "IFO –RT Desk" with dedicated Medical, Nursing and Administrative Staff
- 3) Fully implementing a "Rare Tumor Clinical Registry - IFO" (ReCTR)
- 4) Implementing Biobanking by obtaining biological samples from all new cases under care at IFO
- 5) Broadening the "Narrative Medicine" project - IFO to Patients with RT
- 6) Organising internal and external training events (in association with the OdM of Rome and the MMG Associations) for Health care personnel as well as events offering information to Citizens.

In the diagram below (Fig. 14) you can see how the organization is planned for the end of 2019:

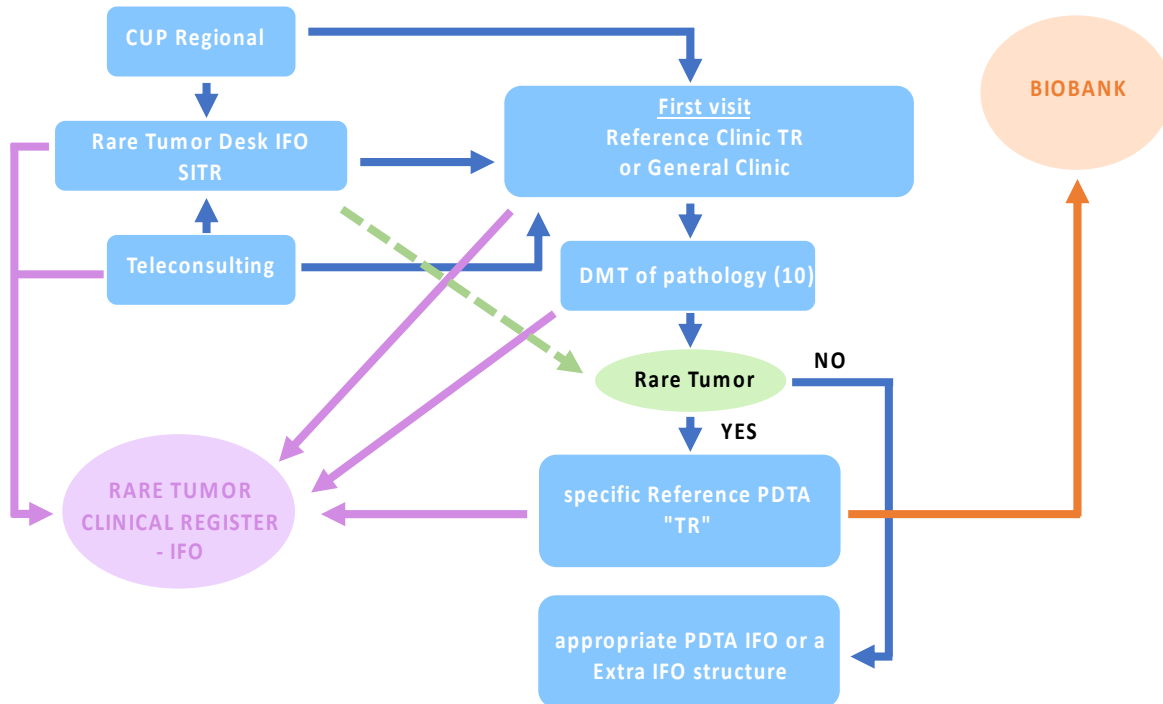


Figure 14

This plan has been developed and consolidated within the internal organization of IFO, where for years Clinicians and Researchers of the Institute have been participating in national and international research activities, with a spontaneous tendency towards a qualitative and quantitative improvement, it is therefore natural that Institute in the next 3 years will become an international Clinical and Research and Training HUB.

Obviously, given the geographical position of Rome, the scenario can only be the Mediterranean basin and the Near East.

It is in this context that the following improvement actions to be promoted are:

- 1) The creation of a Multicultural Office (obviously initially with contract staff), where future users, collaborators and international partners can establish contact with the Institute for both Assistance and for all other needs;
- 2) The creation of an IFO facility that can represent and unify the Research and Training activities in collaboration with all the Health care staff and non-Institute staff (IFO Oncology Training and Research School);
- 3) The Multicultural Office according to instructions from the Managing Direction Offices (General Direction, Medical Chief Office, Scientific Direction, ...) will promote all the possible steps to follow at the Embassies located in Italy, Foreign Health Institutions, Foreign Ministries, ... to activate cultural collaborations (School of Oncology - Research and Training) and to control / facilitate patient flows from abroad according to the financial coverage made available.

Obviously, this particularly ambitious project finds an ideal scenario of development and initial experimentation in the field of RTs. It is precisely for "orphan diseases" that support from all sectors is particularly needed and deemed important.

2) *Research: Rare Tumors, between hope and difficulty*

As it has already been pointed out, RTs share poor molecular knowledge that make innovative treatments (immunotherapy and target therapies) much less available than the more common type cancers, and moreover due to the lack of interest from companies they are less inclined to invest in "niche" type diseases.

Given the high level of expertise provided by IFO in both clinical and basic research, it will be able to invest in the study and treatment of RTs through planning translational patients care thanks to NGS genomic characterization (with high complexity gene panels or even by exome sequencing) on biopsy, re-biopsy and liquid biopsy of one or more rare tumors both for diagnostic purposes, in identifying known and unknown gene fusion products, and for therapeutic purposes. In particular, patients with Rare Tumors whose available medical treatment options are often limited and where a significant annual case history available at IFO (e.g. bone and soft tissue sarcomas, cholangiocarcinoma, etc.) may be evaluated in order to identify the presence of mutations in order to possibly discuss these patients during the Molecular Tumor Board sessions and to identify new biomarkers based, for example, on non-coding RNAs (e.g. mRNAs) as prognostic and predictive indicators of disease. The aim is to contribute to a more complete molecular classification of even the rarest tumors and to "enrich" existing treatment guidelines with possibly new therapeutic targets thanks to the contribution of advanced molecular diagnostics. IFO has also joined European projects such as the SPECTA project (Screening Cancer Patients for Efficient Clinical Trial Access) (EORTC protocol 1553) whose main objective is to create a platform for collecting biological and clinical data and performing molecular screening in neoplastic patients including those with RT.

1 Year Objectives

Clinical objectives

- PDTA
- Desk for Rare Cancers/Rare Diseases
- Study entitled: "Register rAre adult solid canceRs In iTaLY (RARITY)" (ACC)

Translational Research Objectives

- CAR-T project (part of translational research in collaboration with OBG) on bone and soft tissue sarcoma

3 Year Objectives

Clinical objectives

- Become a HUB center in the new National Network on Rare Cancers in all of Lazio
- Increase the number of patients with TR from 15-20% to about 40% regarding regional cases

Translational Research Objectives

- Phase 1-2 clinical translational studies with CAR-T in collaboration with OBG on rare neoplasms (sarcomas, brain tumors, blood and lymphoproliferative diseases)

FINANCIAL RESOURCES

Implementation of the strategic plan requires significant investment of resources both in terms of personnel dedicated to research, in infrastructures and financial support for ambitious research projects.

With respect to personnel between the end of 2019 and April 2020 about 100 units of personnel dedicated to research (either research laboratory workers and administrative support to research) previously under short term contracts renewed every year have been hired with 5 years term renewable contracts (Ministerial Decree 20.11.2019). This has represented an historical moment for the institute, by providing a greater level of stability to the research work force. This type of contract will be applied to additional resources to be hired in the next years once the appropriate Ministerial Decree will be issued.

Here below is a schematic overview of the specific research budget of Regina Elena Institute (Fig. 15). This does not include the cost of about 30 units of personnel under permanent contract whose financial support is being provide by institutional funds and whose costs account for approximately 3.5 M€

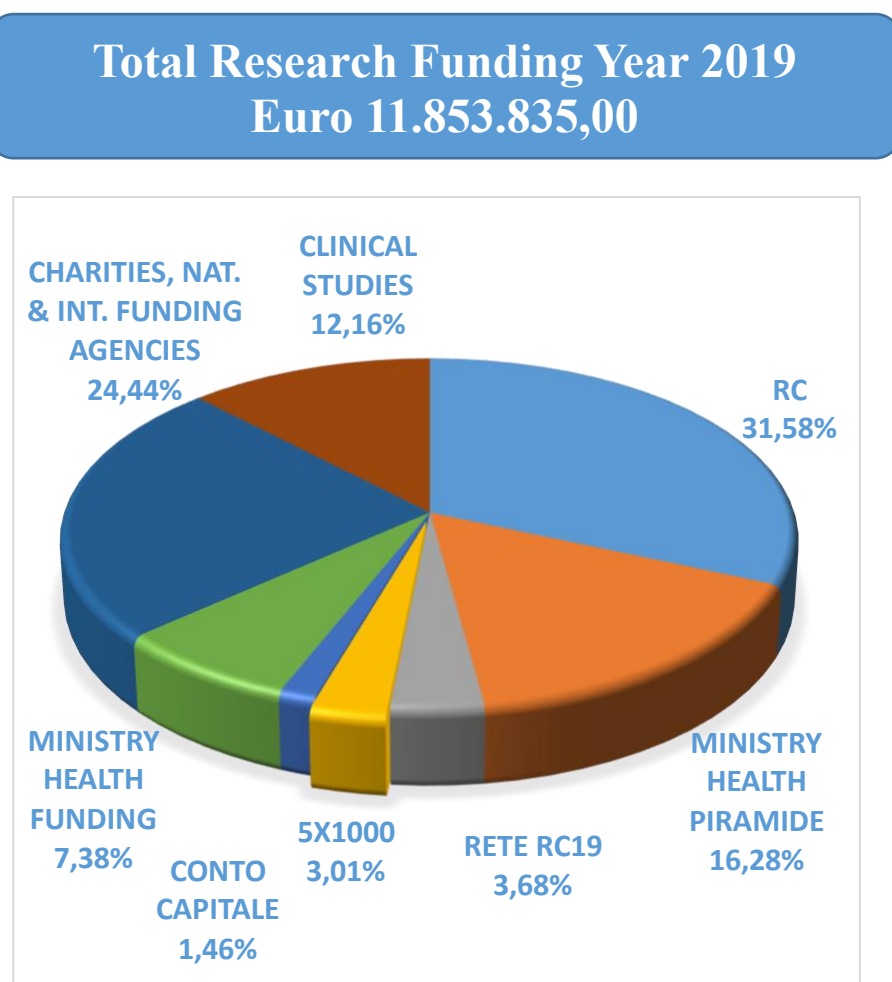


Figure 15

CONCLUSIONS

The PRESTO strategy is an integrated, multi-year strategy composed of 10 structural elements pertaining to three macro-areas which aims to transfer innovation and scientific knowledge quickly into clinical practice in order to help improve patient care responses in the field of oncology. It is a very ambitious strategy whose implementation demands high levels of coordination and internal collaboration between the institute's researchers and clinicians as well as adequate financial support but above all ongoing support from the other IFO Departments. The implementation of the PRESTO strategy can only be possible through the endorsing institutional players such as the Ministry of Health, the Regione Lazio, AIFA, collaboration with other local research bodies, constructive dialogue with pharmaceutical companies and the close ties made with cancer patient associations.