

## Research Facilities at Fondazione IRCCS Istituto Nazionale dei Tumori

The Fondazione IRCCS "Istituto Nazionale dei Tumori" of Milan (INT) is a comprehensive cancer center whose activities range from epidemiology (both descriptive and molecular) to rehabilitation and palliative care, through innovative prevention, diagnosis and treatment. INT is provided with 482 beds; in 2018, INT hospitalized a total of 18.049 inpatients (of which 4250 in day hospital) and carried out a total of 1.238.267 consultations.

As an Institute for scientific research, treatment and care (Istituto di Ricovero e Cura a Carattere Scientifico, IRCCS), in accordance with its institutional mission, INT is in a privileged position to conduct translational research. To this end, multidisciplinary groups, including both basic scientists and clinicians, and also bioinformaticians and statisticians, work together to plan and perform major research projects concerning all type of cancer but adult Central Nervous System.

Since 2014 INT has been designated as "Comprehensive Cancer Centre" by the Organization of European Cancer Institutes (OECI), a qualification that INT shares with world-renowned European Institutions, and in 2016 joined Cancer Core Europe (CCE), the network of excellence among 7 European Cancer Institutes that brings together the expertise and critical mass required to effectively move translational research into the clinic, bringing breakthrough discoveries to benefit patients at an appropriate cost.

Educational activities are available for clinical and experimental researchers.

### Research facilities

The following resources are available at the Departments of Experimental Oncology of the Scientific Directorate.

• **Platform of Integrated Biology (PIB).** PIB is a multi-disciplinary group that includes biologists, bioinformaticians and laboratory technicians which supports researchers through all the experimental and analytical steps of genomics, transcriptomics, epigenomics and metagenomics studies, including study design, sample processing, quality controls, data generation, analysis and integration.

Specifically, the PBI performs:

- ✓ DNA and RNA extraction from FFPE/frozen tissues, cell lines, liquid biopsies (e.g. blood, plasma, urine), saliva and stools specimens;
- ✓ quantification and quality assessment of nucleic acids;
- ✓ labeling and hybridization methodologies required for microarray analyses;
- ✓ library preparation, quantification and sequencing for NGS approaches;
- ✓ Quality control, processing and bioinformatic analysis of data.

PIB activities are conducted using the following state-of-the-art equipment:

- ✓ QIAcube and QIASymphonySP (Qiagen) for nucleic acid extraction and purification;
- ✓ 4200 TapeStation (Agilent), Nanodrop and Qubit (Thermo Fisher) for quantity and quality control of nucleic acids;
- ✓ M220 Focused-ultrasonicator (Covaris) for DNA shearing;
- ✓ JANUS (Perkin Elmer) and STARlet (Hamilton) automated workstations for liquid handling;
- ✓ iScan (Illumina), SureScan Microarray Scanner (Agilent) and Gene Chip System 3000 (Affymetrix, Thermo Fisher) platforms for microarray analysis including mRNA, miRNA and lncRNA expression, DNA methylation, CGH and CNV, SNP genotyping;
- ✓ QuantStudio 12K Flex Real-Time PCR System with OpenArray, Accufil and Twister Automation Robot for quantitative real-time PCR (Thermo Fisher);
- ✓ Ion Chef Instrument, Ion PGM and Ion S5XL (Thermo Fisher) and NextSeq 500 (Illumina) for NGS applications;
- ✓ 10X chromium single cell sequencing.

All samples manipulated by PIB are tracked using dedicated software provided by TwinHelix.

The bioinformatics team of PIB provides accurate and exhaustive analysis of microarray and sequencing data using cutting-edge methods, combining open-source and third party software (Ingenuity Pathway Analysis, QIAGEN). PIB set-up bioinformatic pipelines for standard analyses such as read alignment, data pre-processing, variant calling, differential expression, gene set enrichment and pathway analysis. In addition, advanced and custom analysis workflows can be designed to integrate multi-omics data and/or to tackle the needs of specific projects. PIB maintains a computational infrastructure for bioinformatics data analysis that includes:

- ✓ Two Ion Reporter local servers for analysis of Ion Torrent NGS data
- ✓ One BaseSpace local server for analysis of Illumina NGS data
- ✓ One high-performance computing cluster with a total of 150 core and 350 GB RAM

PIB is certified according to the standard UNI EN ISO 9001:2015.

- **Immunohistochemistry:** performs histological and cytological processing by a wide range of histological techniques, immunohistochemistry, in situ hybridization, and autoradiography.
- **Cell imaging facility:** provides access to the BioRad Radiance 2000 and Leica SP8 AFC AOBs WLL HyD laser confocal microscopes allowing for a wide range of fluorescent dye use, sequential and simultaneous up to 8 channel bright field image collection, and live cell imaging. A MALDI imaging is also available.
- **Flow cytometry and cell sorting:** using state-of-the-art flow cytometric instrumentation, and software analysis.
- **Microbiology service** for plasmid DNA purification; mutagenesis; bacterial strains transformation and storage.
- **Laboratory animal facility.** Our Animal facility is equipped for housing transgenic and immunodeficient mice under specific pathogen-free conditions (SPF) and it is composed by 9 independent rooms that host the mice (360 Individually Ventilated Cages (IVC) per room) and 4 laboratories equipped with laminar flow hoods, centrifuges, refrigerators, scales and stereo microscopes.

Our goal is to guarantee animal welfare reducing any source of stress and distress. For this purpose animals are provided with various environmental enrichment materials including nesting material, paper tubes, running wheel and acrylic mouse houses.

Animal health care is provided by the Animal Welfare Manager and by a Veterinarian specialized in laboratory animals. We monitor our animal to identify potential health threats within our facility according to FELASA recommendations. Researchers and technicians working with animals must attend appropriate education and training according to specific topics required by the EU Directive 2010/63/EU "on the protection of animals used for scientific purposes".

**In Vivo Imaging:** In vivo imaging techniques allow us to observe the processes of our interest in live animals in a non-invasive way without having to sacrifice them. Imaging helps us to reduce the number of animals used to achieve our purpose as we can get more information from each animal. Our facility is equipped with IVIS Spectrum (Bioluminescence, Fluorescence, 3D images) and micro-ultrasound (Vevo™, Visualsonic).

**Biological X-ray irradiator:** Understanding the interactions of ionizing radiation with tissues and cells is crucial for translational research. Our facility is equipped with: a) RS 2000 Small Animal and Cell Irradiator; b) X-RAD SmART Small Animal Image Guided Irradiator

**Micro-PET:** In the Nuclear Medicine Department of our Institute a dedicated micro-PET scanner (GE Healthcare eXplore VISTA) has been installed. The technical characteristics of this scanner are specific to obtain metabolic imaging of small animals.

- **Tissue and cell repository:** Departments of Pathology and Experimental Oncology have implemented and maintain a large bank of frozen and FFPE normal, tumor tissues and blood/plasma/serum samples, collected and stored within a short time from removal following SOPs. Thousands of well-annotated clinical specimens of different tumor histotypes, linked to dedicated databases of patho-biological and clinical information, are currently available. Patients sign an informed consent which allows INT investigators to use the leftover material of biological samples collected during standard surgical and medical procedures for research purposes. Aliquots are attributed to individual studies after approval of Institutional Review

Board and specific requests to the Ethical Committee. All leftover material is stored in the Institutional BioBank for at least 20 years from the collection, including residual material of specific project studies.

- **DEPArray platform** combines imaging technologies with the ability to manipulate and recover individual, viable or fixed rare cells from a heterogeneous sample for subsequent culture or molecular analyses. DEPArrayNext, currently available, allows a significant increase in the number of recoveries of selected cell compared to the previous platform (from 32 to 96 recoveries for each sample), also offers one additional fluorescence channel for a better cell selection thus representing a valuable resource for circulating tumor cells (CTCs) and for all rare-cell studies at single-cell level.

The INT single-cell workflow platform includes Parsortix, a flexible micro-fluidic technology employing a disposable cassette for marker-independent capture and harvesting of CTCs from whole blood based on their less deformable nature and larger size as compared to other blood components. Parsortix also allows isolation of CTC clusters for studies on the interaction between CTCs and the microenvironment. Besides being instrumental for liquid biopsy studies at single cell level, the DEPArray also offers applications at tissue level allowing digital sorting of pure cells derived from FFPE sections that can be used for high resolution molecular analyses.

- **Cytogenetics and molecular cytogenetics** with state-of-the-art instruments, approaches of classic and molecular cytogenetics (fluorescent in situ hybridization and karyotype analysis using spectral karyotyping) and dedicated software allows identification of specific chromosomal alterations that are potentially useful for cancer diagnosis and as targets for novel treatments and/or associated with drug resistance in several solid tumor types.

- **Proteomics/Metabolomics facilities.** A fully equipped laboratory is dedicated to process biological material and perform analyses in clinical biochemistry, high resolution mass spectrometry for lipidomics, HPLC/mass spectrometry for hormonal and pharmacological analyses, Gas chromatography/mass spectrometry for metabolomics analyses, LC Orbitrap, ICP mass spectrometry for elemental analyses. The availability of the dedicated software LipidSearch, together with the skills of laboratory staff, allow for lipidomics data processing to help identify etiological, early detection or drug response biomarkers.

**Medical Statistics, Biometry and Bioinformatics.** The Units provide high quality statistics and quantitative support to clinical and translational research and also conducts independent methodological research and consulting, offering significant input into design, data collection and analysis in the areas of biostatistics, clinical epidemiology, basic, oriented and translational research.

**Epidemiological research platforms** including 1) large prospective studies on the association between environmental, metabolic and genetic factors and cancers (e.g. European Prospective Investigation into Cancer and Nutrition <https://epic.iarc.fr/>), 2) European population-based database on cancer incidence and survival for all cancers including the rare ones (i.e. surveillance of cancer patients in Europe <http://www.eurocare.it/> and <http://www.hrstudies.eu>; information network on rare cancers <http://www.rarecarenet.eu/>) and 3) birth defects registry (i.e. Lombardy registry) on the relationships between adverse birth outcomes and cancer. These platforms have been and are still instrumental to generate and testing hypotheses about risk factors associated to cancer onset as well as to study cancer prognosis, prognostic factors and to explain survival differences across countries. INT has a long lasting experience in analyzing large population-based database as well as in undertaking methodological research and interventional, descriptive and analytical studies based on population based/real world data.

**Breast cancer clinical registry.** The breast cancer clinical registry at INT weekly collects data on cancer patients receiving breast surgery at INT since 1<sup>st</sup> October 2011. Presently in the registry the pathological and clinical data from more than 8800 patients are available and are connectable with INT repositories (blood biobank). 73% of them had a diagnosis of primary malignant breast cancer. A cancer registry in a comprehensive cancer center can be used for research purposes, eg estimate of the number of cases needed for clinical studies, assessment of biobank specimens with specific characteristics, evaluation of clinical practice and adhesion to clinical guidelines, comparative studies between clinical and population sets of patients, studies on cancer outcomes and prognosis, and studies on cancer survivorship.

**Data warehouse.** At INT, the implementation of a clinical data warehouse (DWH) is ongoing. DWH is centralizing all the patient-oriented clinical and anagraphical data collected routinely during the daily activities for clinical, organizational and management aims in the ICT (Information & Communication Technology Unit) applications. Every night data are stored from the applications of the following systems: Central anagraphic, Imaging, Surgery, Discharge, Laboratory, and Pathology. In 2020, also data from Electronic clinical record, Chemotherapy and Radiotherapy will be included. The DWH can be used for example for prospective and retrospective studies. Patient enrolment performed by a specific study (for example using the Electronic Clinical Record) can be flagged in DWH patients with the code/name of the project so that data collected during daily clinical practice useful for the study can be downloaded from the DWH.

Other extensive and diverse facilities are available for diagnostic and research purposes in the Units of Pathology, Radiology and Nuclear Medicine, Hemathology and Clinical Laboratory.

The Institute is equipped with cutting-edge equipment such as:

- 2 dual-head gamma-cameras (GE Infinia and Millennium MG) installed at the Nuclear Medicine Department;
- 1 6-slice SPECT-CT (Single Photon Emission Computed Tomography combined with Computed tomography) scan (SIEMENS Symbia Intevo 6) installed at the Nuclear Medicine Department;
- The PET unit is also equipped with various automated modules for synthesis of radiopharmaceuticals, 1 micro-PET for preclinical imaging and visualization of living animals for research purposes (GE Explorevista) and 2 PET-CT (Positron emission tomography combined with Computed tomography) whole body scanners (PHILIPS Gemini TF 64 slices and GE Discovery 710);
- 3 Computed Tomography (CT) scanners (PHILIPS 6-slice Brilliance installed at the radiotherapy department and PHILIPS 64-slice Brilliance and SIEMENS 128-slice Somatom Definition, both of them installed at Radiology Department);
- 3 1.5 Tesla Magnetic Resonance Imaging (MRI) scanners (SIEMENS Magnetom Avanto, PHILIPS Achieva and Ingenia, all of them installed at Radiology Department);
- The Radiotherapy Department is also equipped with 6 linear accelerators to deliver a wide range of cancer treatments (VARIAN DHX, Unique, DBX, Truebeam and Trilogy, ELEKTA Preciseplus);
- 2 automated robotic systems designed for the preparation of drugs (Health Robotics iv Station) installed at the Pharmacy Department.
- Personal Genome Machine and Ion S5XL (Life Technologies) are available for NGS at the Pathology Department.
- Ventana Benchmak Hybridization for automated in situ Hybridization
- Leica Laser Capture Microdissector, enabling users to isolate specific single cells or entire areas of tissue
- State-of-the-art flow cytometric instrumentation
- Luminex, a bead-based multiplexed immunoassay system in a microplate format.

**Clinical Trials Center.** The Clinical Trials Center supports Clinical Researchers in many aspects of investigational clinical studies, such as study design, statistical analysis/validation, data management, submission to Ethics Committees/regulatory authorities, budget and contract related issues, pharmacovigilance by dedicated personnel including data managers, statisticians, research nurses and administrative personnel. At present the CTC oversees and manages around 667 clinical studies, 432 of which are experimental studies that included more than 182.180 patients.

**Grant Office.** The INT Grant Office provides timely advice and information to researchers on funding opportunities; coordinates the participation of the research projects to funding programs; provides information on the internal procedures for submissions of project proposals; supports researchers to the submission and the final financial report and audit processes.

**Biomedical Library.** The INT Library is affiliated to the European Association for Health Information and Libraries. It offers a large collection of basic science journals and reference books, and electronic access to the full text of scientific and clinical journals, databases and books.

**Technology Transfer Office.** The INT Technology Transfer Office (TTO) was created in 2009 to address two requirements: improve research results in a scientific and economic key and optimize processes in technology transfer and intellectual property management. The TTO offers support services for patent activities (from the beginning of a new invention to the filing and maintenance of the correspondent patent), spin off evaluation and dissemination of IP culture within researchers.