EXPERIMENTAL ONCOLOGY AND MOLECULAR MEDICINE DEPARTMENT

DIRECTOR OF DEPARTMENT: Maria Grazia Daidone
+39 02 2390 2238
mariagrazia.daidone@istitutotumori.mi.it

This Department includes 10 Research Units and one AIRC-awarded start-up Unit dedicated to preclinical investigations. Its primary goal is to serve as an important conduit through which new discoveries are applied to cancer diagnosis, prognosis, and treatment. This is fostered by a collaborative environment and strong interaction among physicians and basic scientists working in different disciplines, as well as by collaborations with research groups in Italy and abroad.

The activity of the Research Units is addressed:
• to identify and validate biomolecular features associated with tumor development and progression as diagnostic, prognostic, and treatment response/resistance markers, and as molecular targets to develop new treatment approaches;
• to investigate the tumor microenvironment and extracellular matrix at a molecular and functional level;
• to elucidate the interactions between tumor cells and the immune system; with the final aims of:
  • developing highly sensitive tests (which utilize a panel of novel biomolecular markers) for a possible clinical application;
  • preclinical testing of novel drug combinations, and to develop novel therapeutic agents;
  • identifying novel therapeutic strategies based on immunomodulation, and to develop vaccination strategies, also taking advantage of the acquired competence in developing new generation recombinant antibodies.

Such studies involve multidisciplinary approaches, statistical and bioinformatic methodologies, and integration among the different high-throughput and high-resolution methodologies and functional tests. Investigations are carried out using different preclinical experimental models and validated on large series of human biospecimens.
The Department supports investigators with state-of-the-art core facilities, with shared instrumentation and trained specialists. The Department is organized in the following Units:

- **Molecular Mechanisms of Cell Cycle Control.** Research of the Unit is oriented towards: i) analysis of the ATM-dependent pathway in the cellular DNA damage response to double strand breaks and alterations of this response in tumor cells and in cancer-predisposing neurodegenerative syndrome; ii) development of pro-apoptotic SMAC-mimetic compounds with anticancer activity targeting the BIR3 domain of the inhibitor of apoptosis XIAP, frequently upregulated in tumors.

- **Molecular Mechanisms.** The Unit is involved in studies of the molecular mechanisms of thyroid carcinogenesis. The final goal of ongoing studies is the identification of markers useful for early detection, prognosis, and follow-up, as well as novel therapeutic targets through: i) the generation of in vitro models of thyroid carcinogenesis; ii) analysis of the role of selected pathways and molecules; iii) mRNA and microRNA expression analysis; iv) characterization of a thyroid tumor case collection, used both for discovery and validation studies.

- **Tumor Genomics.** The research activity covers all aspects of lung cancer with the final aim of making an impact on a disease that is a major health-care burden in terms of morbidity and mortality. The Unit uses an integrated approach that combines cellular and molecular biology, biochemistry, and pharmacology to gain new insights in the pathogenesis of lung cancer and to find novel ways to provide early diagnosis and new treatment options. The goal of translational studies is the implementation of highly sensitive molecular tests that can be included in screening programs to improve both detection and clinical management of lung cancers.

- **Immunobiology of Human Tumors.** The research activity focuses mainly on cutaneous melanoma. The main goals are: i) to understand how the adaptive immune response developed by cancer patients or promoted by immunotherapy can contribute to control tumor growth; ii) to identify new molecular targets to overcome melanoma resistance to target-specific therapy; iii) to understand the mechanisms of interaction of the tumor with its microenvironment; iv) to develop a functional classification of melanoma based on RTK expression and intracellular signaling pathway activation.

- **Molecular Immunology.** This Unit investigates the complex interplay between cells of the immune system, the extracellular matrix, and transforming tissues, following the hypothesis that the evolving microenvironment is crucial for the fate of incipient tumors and might therefore offer new therapeutic targets within stroma components. Taking advantage of knock out and transgenic mouse models, the role of immune cells (such as mastocytes, T regulatory cells, neutrophils and myeloid derived suppressor cells) and their cross-talk in the context of the tumor stroma embedded in the ECM are elucidated to understand tumor and metastasis development in different neoplasms (prostate, breast, colitis-associated cancer, osteosarcoma, lymphoma and myeloid malignancies).

- **Immunotherapy of Human Tumors.** The major goal is to investigate the cross-talk between tumor cells and the host immune systems in cancer patients, to understand their impact on disease course and response to treatments, and to identify novel therapeutic strategies based on immune manipulation. Towards this aim, the Unit includes both clinical and experimental expertise, to focus on: i) clinical and immunological effects of immune-based cancer therapies; ii) cancer-related immune regulatory pathways; iii) molecular studies on melanoma progression markers.

- **Biomarkers.** Research in this Unit is aimed at identifying and validating cancer-related and actionable biomarkers relevant for cancer progression, using molecular and cell biology, high-throughput techniques, and bioinformatic tools. Studies are mainly focused on breast cancer to investigate: i) gene expression profiles on “critical” samples (formalin-fixed paraffin-embedded material and/or limited amount of cells, such as circulating tumor cells and tumor-initiating cells); ii) microRNA as blood-derived biomarkers that are potentially
useful for early detection and risk assessment through non-invasive approaches. In order to develop sensitive and specific tests for clinical application, particular efforts have been spent in the last years to understand pre-analytical and analytical confounders for circulating markers.

• **Molecular Targeting Unit.** This Unit is currently focused on identifying new molecular targets against which to design drugs against molecular pathways sustaining breast cancer progression without disrupting normal cell functions. Research activities are directed to investigate: i) the patho-biological role of extracellular matrix components in disease progression and response to therapy; ii) therapeutic targets in triple-negative breast tumors, and mechanisms involved in the early metastatic capability; iii) activity of resistance mechanisms to HER2-targeted therapies.

• **Molecular Therapies.** Through a multidisciplinary approach and the integration of functional and analytical methodologies, the Unit aims: i) to gain insight into the molecular events involved in tumor progression; ii) identify and validate new potential targets and prognostic/predictive markers; iii) develop and validate new diagnostic/therapeutic strategies, mainly based on new generation recombinant antibodies, to target ovarian and prostate cancers.

• **Molecular Pharmacology.** The research activity is focused on: i) identification and validation of novel therapeutic targets, (i.e. G-quadruplex structures and heparanase); ii) dissection of drug resistance mechanisms in tumor cells; iii) rational design of novel anticancer drug combinations; iv) preclinical development of novel therapeutic agents, based on the availability of a large number of preclinical models of human tumors of different histologic type; v) identification and functional validation of microRNAs as novel therapeutic targets/tools.

• **AIRC Start Up Unit.** The goal is the identification and study of microRNAs involved in the most important pathways activated in human breast cancer through investigations on: i) microRNAs involved in molecular pathways associated with the different molecular subtypes; ii) biological effects of the microRNAs of interest identified in the previous task and validation of putative targets; iii) prognostic/predictive significance of selected microRNAs on series of primary breast carcinomas; iv) identification of the mechanisms regulating the expression of candidate microRNAs.

**HIGHLIGHTS**

In sentinel nodes of melanoma patients with progressing disease, CD30/TNFRSF8 is upregulated and a higher number of CD30(+) lymphocytes can be detected in nodes and peripheral blood lymphocytes from these patients. These findings reinforce the concept that sentinel nodes act as pivot sites for determining progression patterns, revealing that the presence of CD30(+) lymphocytes at those sites correlates with melanoma progression.

Unsupervised clustering analyses in independent datasets of invasive breast tumors profiled by using different platforms segregated ECM3 tumors as an independent subset in all datasets. ECM3 showed a homogeneous gene pattern, consisting of 58 genes encoding 43 structural ECM proteins. Both stromal and breast carcinoma cells can coordinately express ECM3 genes, contributing to an extracellular matrix gene expression profile defining a molecular subtype that is likely to progress.

The diagnostic performance of a noninvasive plasma microRNA signature classifier (MSC) was retrospectively evaluated in samples prospectively collected from smokers within the randomized Multicenter Italian Lung Detection (MILD) trial. The diagnostic performance of MSC for lung cancer detection was 87% for sensitivity and 81% for specificity across both arms, and 88% and 80%, respectively, in the LDCT arm. This large validation study indicates that MSC has predictive, diagnostic, and prognostic value and could reduce the false-positive rate of LDCT.

miR-205 showed to be the most down-modulated miRNA in prostate cancer (PCa) cells upon CAF stimulation, due to transcriptional repression by HIF-1, a redox-sensitive transcription factor. miR-215 replacement in PCa cells is able not only to prevent but also to revert the oxidative/pro-inflammatory axis leading to EMT induced by CAFs. Such finding sets the rationale for developing miRNA-based approaches to prevent and treat metastatic disease. Moreover, miR-205 proved to impair the autophagic flux and to enhance cisplatin cytotoxicity in castration-resistant prostate cancer cells.

Acidification proved to induce reversible anergy in both human and mouse CD8+ T lymphocytes in vivo and in the tumor microenvironment, and the administration of proton pump inhibitors, which buffer tumor acidity, can revert T-cell anergy and increase the efficacy of immunotherapy. Such findings show that acidification of the tumor microenvironment may represent a novel mechanism of immune escape that can be overcome by drugs targeting pH-regulatory pathways.
The anti-tumor activity of CpG oligodeoxynucleotides (CpG-ODN) aerosol was demonstrated in mouse lung metastases. In particular, aerosolized CpG-ODN activated a local immune response, while tumor immunogenicity and tumor-induced immunosuppressive environment represent critical factors for the success of CpG therapy in the lung. Combination of CpG-ODN, cetuximab, and cisplatin displayed high efficacy for advanced ovarian xenograft tumors. CpG-ODN combination therapies that enhance the immune response in the tumor microenvironment and concomitantly target tumor cells are highly active even in experimental advanced malignancies. Using mouse tumor and cellular models, trabectedin, a recently approved chemotherapeutic agent, proved to induce rapid apoptosis exclusively in mononuclear phagocytes.

SM83, a newly synthesized dimeric SMAC mimetic, modulates in vivo the immune system within the tumor microenvironment and, through its pro-inflammatory action, induces cancer cells to die by necrosis. Our work provides evidence that SMAC mimetics could be more therapeutically active than expected by stimulating the immune system.

The heparanase inhibitor SST0001, alone and in combination with antiangiogenic agents, showed antitumor efficacy in human pediatric sarcoma models.

Cisplatin or oxaliplatin, in combination with the MEK1/2 inhibitor CI-1040, resulted in a synergistic effect associated with enhanced apoptotic response in platinum-sensitive cells exhibiting increased phospho-ERK1/2, down-regulation of apoptosis-related factors (BAX, PUMA, FOXO1) and of phosphatases inhibiting ERK1/2 (DUSP5, DUSP6).

**KEYWORDS**

antibody-based therapy, apoptosis, blood-derived biomarkers, cancer vaccines, circulating tumor cells, drug resistance, exosomes, extracellular matrix, microRNA, preclinical drug development, regulatory T cells, solid tumors, target-specific therapy, translational medicine, tumor genetics, tumor microenvironment.
MOLECULAR TARGETING UNIT
Head
Elda Tagliafu, Biol Sci D
Research Staff
Rosaria Orlandi, Biol Sci D; Serenella Pupa, Biol Sci D
Postdoctoral Fellow
Manuela Campiglio, Biol Sci D
PhD Students
Gaia C. Ghedini, Biotech Sci D; Marta Giussani, Biotech Sci D; Alessandra Meini, Biol Sci D; Marianna Sasso, Biol Sci D; Tiziana Triulzi, Biotech Sci D
Fellows
Lorenzo Castagnoli, Biotech Sci D; Valentina Ciravolo, Biotech Sci D; Viola Regondi, Biotech Sci D; Anna Rossini, Biol Sci D; Federica Turdo, Biol Sci D
Consultants
Sylvie Ménard, Biol Sci D; Marco Sandri, Stat Sci D
Technicians
Pierangelo Aiello, Patrizia Casalini, Cristina Ghirelli

MOLECULAR THERAPIES
Head
Silvana Canevari, Biol Sci D
Research Staff
Mariangela Figini, Biol Sci D; Delia Mezzanzanica, Biol Sci D; Antonella Tomassetti, Pharmacol Sci D
Postdoctoral Fellows
Fabio Benigni, Biol Sci D; Marina Bagnoli, Biol Sci D
Fellows
Technicians
Paola Alberti, Francesco Caroli, Anna Maria Invernizzi, Elena Luison, Cristina Luna

MOLECULAR PHARMACOLOGY
Head
Nadia Zaffaroni, Biol Sci D, PhD
Research Staff
Marco Folini, Biol Sci D, PhD; Cinzia Lanzi, Biol Sci D; Paola Perego, Biol Sci D, PhD
Postdoctoral Fellows
Giovanni I. Beretta, Biol Sci D, PhD; Joanna Bidzinska, Biotech Sci D, PhD; Giuliana Cassinelli, Pharm D, PhD; Grazia Comino Reale, Biol Sci D, PhD; Michelandrea De Cesare, Vet D; Paolo Gandellini, Biotech Sci D, PhD; Laura Gatti, Biol Sci D, PhD; Alessia Lopergolo, Biotech Sci D, PhD; Marzia Pennati, Biol Sci D, PhD; Valentina Zuco, Biol Sci D
PhD Student
Valentina Profumo, Biol Sci D
Fellows
Denis Cominetti, Biol Sci D; Nicola Fenderico, Biotech Sci D; Francesca Santambrogio, Biol Sci D; Stefania Sbarra, Biol Sci D
The following core facilities are available.

**Immunohistochemistry** (Technical Specialists: Lorena Ventura and Lucia Gioiosa): histological and cytological processing, including tissue microarrays, a wide range of histological techniques, immunohistochemistry, in situ hybridization, and autoradiography.

**Cell imaging facility** (Technical Specialist: Patrizia Casalini, Biol Sci D): provides access to 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 3 channel bright field image collection, and live cell imaging.

**Flow cytometry and cell sorting** (Technical Specialist: Gabriella Abolafio; Research Fellow: Andrea Vecchi, Biol Sci D): state-of-the-art flow cytometric and cell sorting instrumentations, and software analysis.

**Microbiology** (Technical Specialist: Maria Teresa Radice): core services include media preparation; bacterial transformation; purification of plasmid DNA, BAC, YAC; freezing and storage of recombinant plasmids and bacterial strains.

**Cytogenetics and molecular cytogenetics** (Specialist: Patrizia Gasparini, Biol Sci D): 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 (in particular, lung, colon and breast cancers, and soft tissue sarcomas).


**Proteomics/mass spectrometry laboratory** (Dario Caccia, Biotech Med D, PhD; Ruben Magni, Biotech Med D, PhD and Technical Specialist: Maida De Bortoli): see Shared Research Resources, page 63.


**Laboratory animal facility**

**Administrative Personnel:** Claudia Miranda Biol Sci D, and Grazia Convertino, Simona Galluzzi, Ester Grande, Silvia Grassi, Laura Mameli, Silvia Portincasa, Luisa Rivetta, Daniela Silva, Laura Zanesi, Cristina Zanini. This team facilitates the activity of the Department by providing administrative support to research unit leaders and core facilities, coordinating the activities of graduate students and fellows, handling purchasing requests for laboratory consumables, and finance administration.

**Laboratory Management Team:** Enrico Ronchi, Domenico Di Fazio, Angelo Labori, Salvatore Venturino. This team plays an essential role in supporting the research units in the Department for maintenance of instrumentation, and management and supervision of areas for cryopreservation of stored tissues/cells/cell extracts and reagents. In addition, the team – in association with the administrative team – also oversees a cost-effective and efficient centralized system of ordering and stock control for the most widely used items.

**Supporting Personnel:** Antonietta Calcagnò, Linda Cimaglia, Antonio Illuminato, Giuseppina Liguori, Agata Mancuso, Luisa Mona, David Penni, Gisella Rivadossi, Pasquale Russo, Claudio Santagostini.

---

**AIRC START UP UNIT**

**Head**

Marilena V. Iorio, Biotech Sci D

**Postdoctoral Fellows**

Ilaria Plantamura, Biol Sci D, PhD; Claudia Piovan, Pharm Biotech D, PhD

**Phd Student**

Elvira D’Ippolito, Biotech Sci D