RESEARCH ACTIVITY

Prostate cancer program

Breast cancer: outline of clinical and preclinical research

Lung cancer program

Melanoma program

Personalized treatment of sarcomas

Novel approaches to determine prognosis and response to treatment in mature B-cell malignancies

Development of radiopharmaceuticals for tumor characterization, molecular imaging, and therapy

Pediatric brain tumors
The Prostate Cancer (PC) program is a translational multidisciplinary program with expertise in epidemiology, experimental oncology, pathology, imaging, urologic surgery, radiation oncology, medical oncology, palliative care, and psychology. Since 2005, the clinical team has been offering patients with PC a multidisciplinary approach in all stages of disease (in 2011, about 980 multidisciplinary visits were carried out as first examinations, second opinions, follow-up of patients on active surveillance or watchful waiting). Clinical cases are discussed weekly to share decisions, individualize therapeutic and observational strategies, enroll patients in trials, and verify adherence to institutional guidelines and quality assurance. Considerable attention is paid to quality of life and psychological issues. Patients and their families can rely on dedicated psychologists in the decision-making phase and throughout the course of the disease. Some of the ongoing research is summarized below.
Development of novel approaches for the inhibition of cell survival factors in preclinical models of androgen-independent PC. Based on the observation that G quadruplexes (G4) exist not only in telomeres, but also in the promoter of several oncogenes, we focused our attention on the effects exerted by a new class of naphthalene diimides, which can reversibly bind to G4 structures, on the expression of the c-myc and hTERT genes in PC cell lines. Quantitative RT-PCR data showed down-regulation of c-myc, which was paralleled by a significant reduction of the expression levels of hTERT. Inhibition of the two oncogenes seemed to represent the primary cause of drug-induced impairment of telomerase catalytic activity in PC cell lines.

miRNAs in PC: expression profiling and functional analysis. We have previously shown that miR-205 is down-regulated in PC compared to adjacent non-neoplastic tissue and acts as a tumor-suppressor in human prostate, as its reintroduction in PC cells reverts the epithelial-to-mesenchymal transition. To gain insight into this early loss of miR-205 and into the mechanisms of PC development, we investigated the physiological role of miR-205 in normal prostate. We found that miR-205 participates in a network involving Np63, an alternatively spliced isoform of p63, which is essential for maintenance of the basement membrane in prostate epithelium. At the molecular level, Np63 is able to enhance miR-205 transcription by binding to its promoter, whereas miRNA can post-translationally limits the amount of Np63 protein, mostly by affecting proteasomal degradation of Np63 rather than through a canonical miRNA/target interaction. Functionally, miR-205 is able to control the deposition of laminin-332 and its receptor integrin-4. Hence, pathological loss of miR-205, as widely observed in prostate cancer, may favor tumorigenesis by creating discontinuities in the basement membrane. We also demonstrated that therapeutic replacement of miR-205 in prostate cancer cells can restore basement membrane deposition and 3D organization into normal-like acinar structures, thus hampering cancer progression.

Cells and matricellular proteins as accomplices in PC. Mast cells (MC) are c-Kit-expressing cells, best known for their primary involvement in allergic reactions, but recently reappraised as important players in cancer promotion and inhibition. In particular, we focused on the role of MCs in PC development. In PC from both tumor-prone transgenic adenocarcinoma of the mouse prostate (TRAMP) mice and human patients, MCs are specifically enriched and degranulated in areas of well-differentiated (WD) adenocarcinoma, but not around poorly differentiated (PD) foci that coexist in the same tumors. We derived novel TRAMP tumor cell lines, representative of WD and PD variants, and through pharmacologic stabilization or genetic ablation of MCs in recipient mice, we showed that MCs promote WD adenocarcinoma growth but are dispensable for PD tumors. WD tumors rely on MCs for matrix metalloprotease 9 (MMP-9) provision, as reconstitution of MC-deficient mice with wild-type but not MMP-9(-/-) MCs was sufficient to promote their growth. In contrast, PD tumors are MMP-9 self-competent, consistent with an epithelial-to-mesenchymal transition. Such a dual source of MMP-9 was confirmed in human tumors, suggesting that MCs may be a good target for early-stage prostate cancer. Interestingly, in testing whether MC targeting could block or delay tumorigenesis in tumor-prone TRAMP mice, we observed a high incidence of early and aggressive tumors, characterized by a neuroendocrine (NE) signature and c-Kit expression. Taken together, these data underscore the contribution of MCs in tumor progression and uncover a new, opposite role of MCs in protecting against the occurrence of aggressive NE variants in prostate cancer.
Active surveillance evaluated as an alternative to radical therapies in low risk PC. Selected patients are followed-up clinically and diagnostically (PSA and biopsy), and offered curative treatment if the PC appears to progress, thus limiting overtreatment. Two protocols are open to enrollment: the PRIAS international prospective study, which, from September 2007 to December 2011, included 209 patients; the SA INT protocol, from March 2005 to December 2011, included 145 patients. The hypothesis to be confirmed is that <5% patients will develop clinical progression by a positive bone scan during their lifetime. Patients on active surveillance are proposed side protocols aimed at evaluating new biomarkers and non-invasive diagnostic tests in blood and urine samples. As of December 2011, more than 90 patients accepted to participate in the collection.

Open label Phase II study of vaccination with survivin peptides in PC patients in biochemical failure (PROVAX study). This trial, started in late 2010, is evaluating a novel vaccine combination (survivin peptides and IMP321) in 20 hormone-naïve or refractory patients with biochemical recurrence. Vaccine peptides, restricted for different HLA-I alleles, are emulsified in Montanide® ISA 51 VG and administered weekly for 8 weeks and then every 4 weeks thereafter: IMP321 is given prior to every second vaccination at the same site. In patients experiencing clinical benefit at the end of vaccination, the entire vaccine cycle will be repeated. A “safety run-in phase” was included to assess the toxicity of vaccine combination; the first three subjects were treated and observed for 4 weeks before starting enrolment. No adverse event was experienced during the first vaccinations and enrollment started in early 2011. The study is still open. In October 2011, the protocol was amended to schedule a new induction phase for patients with increasing levels of PSA (≥2 ng/ml and <5 ng/ml in two consecutive blood samples, compared to baseline values) during the maintenance phase.

Predictive models of late rectal toxicity after high-dose PC radiotherapy (RT). New RT techniques to treat PC with high doses and a high level of precision are being studied. However, a significant proportion of patients still experience acute and late toxicity, since organs at risk are unavoidably included within the high dose region. Although predicting RT morbidity can prevent further deterioration of quality of life and help introduce treatment corrections to personalize therapy, little attention and inadequate efforts have been devoted to the development of easy-to-use tools that use individual dosimetric, clinical, and genetic risk factors, and which are able to predict the side-effects of RT. The group involved in the project also participated in two studies (AIROPROS 0101 and AIROPROS 0102) that assessed predictors of rectal toxicity. The results gave important indications about the modeling of rectal bleeding in addition to other acute and late rectal complications. The data from AIROPROS 0102 were also used to develop nomograms and artificial neural network models to predict acute and late rectal toxicity after RT of PC. These are the first user-friendly tools reported in the literature for evaluating the probability of individual radio-induced rectal toxicity. A pilot study was also designed to identify genetic markers predicting late rectal bleeding. Patients were selected within the AIROPROS 0101 trial, and this pilot study showed that individual dose-volume information coupled to the patient’s genetic profile might help to explain, on the basis of dose-volume histograms, the quality and unexpected rectal bleedsers, as well as the unpredicted absence of late toxicity in some individuals.

The group is currently involved in a prospective multicenter study (DUE 01, promoted by San Raffaele Scientific Institute) focused on assessing predictors of genitourinary (GU) toxicity and erectile dysfunction (ED) after PC high dose RT. The final aim of the study is the development of predictive models for GU toxicity and ED with inclusion of dosimetric, clinical, and genetic risk factors.
In the domain of prediction of rectal toxicity, a new pilot study is recruiting patients with the aim of evaluating the correlation between toxicity insurgence and plasma levels of a panel of inflammatory markers. This study includes patients undergoing radical prostatectomy, radical RT, and adjuvant RT. Both absolute levels of inflammatory markers and their kinetics as a function of radiation dose and follow-up time are being evaluated. Results from this pilot study could be used prospectively to identify radiosensitive patients and to optimize their RT protocol.

**Keywords:** translational research, multidisciplinary approach, experimental therapeutics

**PUBLICATIONS**


Valdagni R. Prostate cancer units: has the time come to discuss this thorny issue and promote their establishment in Europe? Eur Urol. 2011; 60(6): 1193-6.
Major unresolved scientific problems surrounding breast cancer (BC) are related to: increasing incidence, prevention, early diagnosis, disease progression, treatment, and resistance to clinical treatments and their toxicity. The heterogeneity of human BC, in terms of genetic susceptibility, clinical behavior, molecular profiles, and even histomorphologic features, represents a major obstacle to the solution of more effective therapies. Investigations at the genetic and transcriptional levels have shown that such heterogeneity may be explained by: a) varying susceptibility to malignant transformation of different mammary cells; b) progression of breast carcinogenesis that is not necessarily stepwise or linear, from well-differentiated to poorly differentiated tumors, which is also complicated by the finding that; c) no single dominant pathway or histologic presentation has emerged in BC, whereas mutation within a single pathway has a dominant role during progression in virtually all tumor types. High throughput technical approaches for molecular analyses have prompted a new classification of human BC and provided a new paradigm for reducing disease complexity, unraveling biological heterogeneity. This will help to better identify those destined to develop BC among at-risk women, and, among patients, those who will develop new disease manifestations for more rational planning of therapeutic strategies.
INT has traditionally provided the highest quality and level of innovation in designing and developing new approaches to the multidisciplinary treatment of women with BC. This tradition has led to landmark accomplishments, such as the introduction of new standards of therapy that are now common practice in the field of oncology. Investigations designed and conducted at INT have demonstrated the possibility of limiting the extent of surgical removal of BC, thus avoiding mastectomy in many women with relatively small tumors without compromising the chance of successful eradication of disease. These achievements originate in an approach that merges different disciplines into a common effort for the ultimate benefit of patients. There are several key elements of success, but the most relevant are the creation of a dedicated team of clinical investigators supported by a centralized and unique team for data management and analysis, the establishment of expertise in the development of new drugs against metastatic BC, to rapidly implement new discoveries in the treatment of cases with early BC, and constant exchange among different laboratories of the Department of Experimental Oncology and Molecular Medicine and the Units of the Department of Preventive and Predictive Medicine. In all these respects, the approach to BC at INT has always been translational and multidisciplinary, and this tradition is maintained in the current organization of clinical and experimental services devoted to the treatment and study of BC, also including partnerships with pharmaceutical industry.

This project represents one of the first efforts to outline the biology underlying the distinct risk situations for BC by applying novel approaches for molecular analysis and target validation to case series recruited at INT in the last decades in the context of epidemiologic or chemoprevention studies and adjuvant/neoadjuvant treatments. Investigations are focused on: a) effects of different metabolic/nutritional factors on relevant biomolecular features; b) effects of lifestyle changes on biomarkers and molecular signatures of proven prognostic relevance; c) interaction between host (including ECM features) and tumor factors; d) new genetic risk factors, including variants of uncertain significance in BRCA genes; e) gene expression fingerprints associated to with distinct new disease manifestations and response to different treatment protocols; f) functional analyses of genes whose expression affects tumor progression and treatment resistance; g) at a preclinical level, effect of novel chemopreventive and/or antitumor therapies. To improve our understanding of BC and to develop clinically-useful strategies, we need better understanding of host factors (including age, diet, lifestyle, metabolic syndrome, environmental factors, polymorphisms, and mutations in susceptibility genes), tumor microenvironment (growth factors, infiltrating cells, and cytokines), and genomic changes occurring in cancer cells. At INT, we have a unique opportunity to investigate these aspects taking advantage of: 1) dedicated infrastructure; 2) well-annotated biological samples; 3) a well-established philosophy for cancer and normal tissue acquisition, storage, and distribution to research Units; 4) updated follow-up information and validated dietary questionnaires; 5) improvements to overcome intrinsic limitations for genomic studies due to technical artifacts and/or to limited sample size; 6) well-trained teams with specific skills in different disciplines.

Specifically, during 2011 we focused on the following:

a) Expression of androgen receptors (AR) was evaluated on more than 500 primary BC. Co-expression of AR and estrogen receptors (ER) was observed in 65% of cases, and ER negative tumors were shown to be AR positive in 45% of cases. Such findings, together with the evidence of an association between AR expression and favorable clinicopathologic features, provide support to the hypothesis that AR positive tumors are well differentiated and largely hormone responsive.
b) In this study, which is the first that is prospectively assessing the relationship between the presence of metabolic syndrome and BC risk, we observed an association in postmenopausal women. When considering different BC subtypes defined by receptor status, metabolic syndrome was associated with a significant increase of ER-positive, PgR-positive, HER2-negative tumors, but not ER- and PgR-negative, HER2-positive tumors. Hyperglycemia, defined by high serum glucose, and insulin resistance, defined by high HOMA-IR, were important risk factors for BC both in pre- and postmenopausal women, and also in those who were diagnosed after 55 years of age. For this latter subset of women, a decreased risk for elevated SHBG concentrations has also been found. The implication is that altered glucose metabolism is a risk factor for BC and should be considered in prevention initiatives.

c) Wild-type FOXP3 induction in BC cell lines through an inducible Tet-off system showed higher in vitro migration ability and increased invasion capacity of WT-FOXP3-induced compared with non-induced cells. In addition, GeneSet Enrichment analysis of differentially expressed genes between FOXP3-induced and non-induced cells indicated FOXP3-induced expression of several genes implicated in migration and metastasis, with an enrichment in molecules involved in pathways of TGF-beta signaling and the epithelial-to-mesenchymal transition, as well as in focal adhesion, strongly suggesting an involvement of FOXP3 in tumor cell dissemination.

d) In the framework of collaborative studies within international consortia, three allelic variants have been identified on chromosomes 12p, 12q, and 21q which are associated with an increased risk of BC in the general population, as well as three allelic variants, two of which are close to ESR1, associated with an increased risk of developing BC in women carrying a mutation in the BRCA1 and BRCA2 genes.

e) The investigation on differentially expressed microRNAs in different BC molecular subtypes (basal, HER2+, and luminal, as defined by the expression of genes ESR1 and ErbB2) showed downregulation of miR-190b, miR-375, and miR-342-5p in tumors belonging to the basal subtype. Using a Gene Ontology approach, these microRNA were shown to be associated with terms like mitochondrial matrix, carbohydrate catabolic process and mitochondrial respiratory chain, thus suggesting a possible alteration of carbohydrate metabolism in basal tumors. Experiments are currently ongoing to verify this possibility.

f) The effect of the combination of 4-oxo-4-HPR with paclitaxel (PTX) in BC cells showed that the retinoid is able to strongly inhibit the growth of both ER+ and triple negative cells, and to synergistically improve the cytotoxic activity of PTX. These results suggest that the retinoid could be a suitable substitute for microtubule poisons. In collaboration with the University of Milan (Prof Della Valle), chemical modification of the structure of 4-oxo-4-HPR is currently under investigation in order to increase its solubility and, consequently, bioavailability.

g) A database collecting information related to clinicopathologic features, metabolic syndrome, patient follow-up, and availability of biological specimens (tumor material, blood, serum and plasma, collected with informed consent) has been established and will represent an added value of this project for future studies.

This project enables a better working relationship among specialists in cellular and molecular biology, pathology, medical oncology, and epidemiology. Therefore, even though within an ambitious perspective, this project represents a starting point for a broader plan.

Keywords: metabolic syndrome, genetic risk, gene expression profile, microRNA profile, microenvironment
PUBLICATIONS


LUNG CANCER PROGRAM

The incidence and mortality of lung cancer have constantly declined during the last three decades in male populations of Europe and the US, mainly as a consequence of effective smoking control policies. This reduction is by far the most important determinant of the reduction in total cancer mortality observed for all sites. In the same period, however, the cure rates for lung cancer have not significantly improved, and the 5-year survival rate of all detected lung cancers remains below 15%.

Beyond the specific question of mortality reduction, a decade of clinical research on low-dose CT (LDCT) screening has markedly changed our knowledge on the natural history and biology of lung cancer. In fact, collateral studies of the Multicentric Italian Lung Detections (MILD) project have provided new insight into the genetic determinants of tobacco addiction, chronic obstructive pulmonary disease, and coronary calcification as independent risk factors for lung cancer; frequency of interstitial lung disease and bronchial diverticula, along with the value of tissue and blood biomarkers. After 15 years of extensive research on circulating DNA, we have demonstrated that miRNA signatures in plasma can not only detect lung cancer 2 years earlier than LDCT, but can also predict the aggressiveness of disease and distinguish indolent from lethal cancers. This discovery will help clarify why the most virulent forms of lung cancer elude LDCT screening and will open new perspectives in the early detection and management of lung cancer.

SCREENING AND RANDOMIZED TRIALS

Our screening program started with the pilot study on 1035 volunteers in Milan in 2000 and was followed up in 2005 by a randomized trial comparing annual or biennial LDCT with observation, namely MILD. This included 4099 participants, 1723 randomized to the control group, 1186 to biennial LDCT screening, and 1190 to annual LDCT screening. The MILD trial has now entered its 7th year. In the follow-up stopped in November 2011, we reported 9901 person-years for the pilot study and 17,621 person-years for the MILD trial. Forty-nine lung cancers were detected by LDCT (20 in biennial and 29 in the annual arm), of which 17 were identified at baseline examination; 63% were of stage I
and 84% were surgically resectable. Stage distribution and resection rates were similar in the two LDCT arms. The cumulative 5-year lung cancer incidence rate was 311 per 100,000 in the control group, 457 in the biennial, and 620 in the annual LDCT group ($P = 0.036$); lung cancer mortality rates were 109, 109, and 216/100,000 ($P = 0.21$), and total mortality rates were 310, 363, and 558/100,000, respectively ($P = 0.13$). Total mortality in the pilot study was similar to that observed in the annual LDCT arm at 5 years. There was no evidence of a protective effect of annual or biennial LDCT screening. Furthermore, a meta-analysis of the four published randomized trials showed similar overall mortality in the LDCT arms compared with the control arm.

**BIOMARKERS**

miRNAs represent a class of molecules that have the capacity for simultaneous regulation of hundreds of genes and entire networks of biological processes. We previously identified diagnostic and prognostic miRNA signatures in tissue and plasma samples of lung cancer patients detected by spiral-CT screening. In plasma samples, we also looked at signatures able to predict lung cancer in samples collected up to 2 years before lung cancer CT detection. We identified 4 signatures: i) risk to develop lung cancer (RD), ii) risk to develop the aggressive form of lung cancer (RAD), iii) presence of disease (PD), and iv) presence of aggressive disease (PAD).

One of main criticisms of our previous work concerned the use of pools of 5-6 plasma samples of disease free subjects, instead of individual samples. During the last year, we were able to enlarge the validation set and analyze the robustness of our signatures on individual samples collected from 53 CT-detected lung cancer patients and 100 disease free individuals. Our results from studying plasma and normal tissue of lung cancer patients lead us to consider the influence of the tissue microenvironment on cancer development.

Epithelial cancers are now considered not to be simply the result of abnormal growth of cancer cells, but rather the result of complex interactions of cancer cells with stromal components and cells of the immune system. In particular, it has been demonstrated that stromal "activated fibroblasts" are able to promote the development of several tumors by inducing angiogenesis, remodeling the extracellular matrix, and inducing epithelial-mesenchymal transition through the activation of several pathways such as TGF-$\beta$ or Wnt. The refined signatures, composed of a total of 24 miRNAs, were validated on samples collected from the 53 patients and 65 controls. The previous results were confirmed or ameliorated: for the two signatures of risk and diagnosis, we observed a specificity and sensitivity higher than 80% and for the two signatures of aggressiveness a very high specificity (>96%) and ~88% sensitivity. Moreover, two of the 53 patients within the validation set developed neoplasms (one bronchial alveolar carcinoma [BAC] and one benign tumor). The plasma samples collected before CT-detection were both positive for the RD signature but negative for the RAD one; the sample collected at the moment of the CT-detection in the BAC patient was also positive for the PD signature, but not for the PAD one.

We are studying the role of activated fibroblasts on tumor development. We were able to establish several fibroblast cultures from surgical specimens of lung cancer patients and to identify those with high levels of alpha-smooth muscle actin ($\alpha$-SMA), a marker of fibroblast activation. In particular, a major question concerned the plasma miRNAs that were able to predict the aggressive form of lung cancer (RAD signature), and whether they are a signal of some event in the normal lung. This hypothesis could potentially
explain why we can observe their deregulation as early as two years prior to lung cancer development. Expression of the six most deregulated miRNAs of the RAD signature was analyzed in fibroblast cell lines with high and low α-SMA levels. The three miRNAs that were most up-modulated in the RAD signature (mir-197, mir-28-3p, and mir-17) were all significantly over-expressed in activated fibroblasts. Of the three down-modulated miRNAs, two (mir-101 and mir-21) were generally under-expressed in activated fibroblasts and one (mir-451) was not detectable by qRT-PCR.

**STEM CELLS**

The identification of lung tumor cancer stem cells (CSC) and associated markers may be useful for optimization of therapeutic approaches and to provide predictive and prognostic information in lung cancer. We reported the presence of highly tumorigenic CD133+ subpopulation of cells displaying stem-like features and chemoresistance to conventional drugs in primary non-small cell lung tumors (NSCLC). We demonstrated that in vivo cisplatin treatment of lung tumor graft models, which closely resemble the features of parental primary tumors, resulted in enrichment of the chemoresistant CD133+ fraction. In particular, an increased of CD133+CXCR4+ subset was observed which we speculated is involved in metastatic process.

Furthermore, new combination strategy to overcome CSC-induced resistance to conventional cytotoxic compounds are evaluated, and the in vivo efficacy of a differentiating agent (i.e. ATRA) in depleting the tumor stem-like pool was assessed. ATRA treatment was able to partially deplete the component of CD133+/CXCR4+ cells as shown by fluorescence-activated cell sorting (FACS) analysis, an effect that may account for the increased latency in tumor re-growth after combination therapy compared to cisplatin alone.

Similar results were also obtained using an innovative in vitro model that allows the recovery of highly purified and viable tumor cells from tumor grafts that retain the features of the parental tumors which grow as spheroids in serum-free medium (cancer tissue originated spheroids, CTOS). Exploiting CTOS culture from several tumor graft models, we tested the efficacy of ATRA treatment partially depleting the CSC chemoresistant fraction.

We also investigate the role of CSC in the metastatic process, demonstrating that the induction of EMT in a primary cell line generated a subset of CD133+cells negative for the epithelial antigen EpCAM that strongly express CXCR4, a chemokine receptor shown to be involved in metastatic progression in different tumor types. The CD133+/CXCR4+/EpCAM- phenotype may identify a particular subset of lung cancer stem cells endowed with the ability to disseminate and to initiate secondary tumors. To support this theory, we used FACS to examine the presence of CD133+/ESA-/CXCR4High subpopulation in primary tumors and corresponding lymph node metastases, and we found that a fraction of CD133+/ESA- cells, strongly expressing CXCR4, were detectable in metastasis but not in primary tumors. Moreover, the phenotypic study of spontaneous lung metastases derived from subcutaneous injection of the H460 lung cancer cell line revealed enrichment for the CD133/CXCR4 double positive population in metastases compared to parental tumors. Thus, a distinct subset of CD133+ migrating CSCs, possibly generated through the EMT process, could be responsible for formation of metastases.

**INFLAMMATION AND CANCER**

It has been shown in a large study cohort of MILD participants that simple CT might provide complementary information for lung cancer risk stratification. Specifically, airflow obstruction is a strong independent predictor of lung cancer and suggests that increased mean lung density may also have an important value in determining prognosis. Conversely, no association between the features of emphysema as assessed by
automated CT analysis and lung cancer was observed. All of these findings are important to stratify the risk of lung cancer for future prospective evaluations.

Other smoking-related inflammatory abnormalities have been extensively evaluated in MILD. Although these have not been demonstrated to be linked to lung cancer, their assessment should not be avoided in the lung cancer screening setting. It has been shown that asymptomatic screening participants may develop relevant interstitial lung diseases (ILDs). Some of these ILDs should be not overlooked as they carry a prognosis even worse than many lung cancer subtypes. Although atherosclerotic vascular disease accounts for more deaths and disability than all types of cancer, the importance of detecting subclinical atherosclerosis and targeting prevention of future cardiovascular events is only now starting to be highlighted in the lung cancer screening setting. In the MILD trial, it has been shown that that routine evaluation of coronary artery calcium score is feasible and provides relevant prognostic information.

**LUNG FIBROBLASTS**

In co-injection experiments with lung cancer cells, we observed that lung fibroblasts isolated from different sources have pro-tumorgenic potential and influence composition of the extracellular matrix (ECM). Compared to tumors generated by injection of A549 cells alone, heterotypic tumors displayed strongly increased levels of COL6A3 and MMP2, slightly increased levels of SPARC and reduced CTSL and CTSC, indicating the influence of fibroblasts on ECM composition. Tumors generated by co-injections were also more likely to metastasize to the lung. Moreover, culturing of primary lung cancer cells with CAF conditioned medium (CM) also resulted in similar transcriptional regulations of ECM-related genes, and increased levels of MMP2 were detected in tumors derived from injection of CM treated cells.

Signals from the microenvironment have also been shown to modulate different aspects of carcinogenesis and, in particular, contribute to tumor heterogeneity through induction of epithelial-mesenchymal transition (EMT). This process could also result in modulation of the subpopulation of cancer cells endowed with higher tumor forming potential, operationally defined as CSC. In preliminary experiments, exogenous stimuli can regulate the CD133+ phenotype of lung cancer cells. TGF-beta treatment resulted in an increase in CD133+ cells both in A549 cells (5-fold increase) and in a primary lung adenocarcinoma cell line established in our laboratory (LT73; 10-fold increase), also confirming a link between EMT induction and acquisition of the stemness phenotype by cancer cells in lung cancer. Furthermore, stimuli from CAFs can de novo generate CD133+ cells. LT73 cells contain a small subpopulation of CD133+ cells (0.1%) which remains stable during culturing. Through FACS sorting, a line devoid of CD133 expressing cells was generated (LT73 CD133neg) and cultured in the presence of medium conditioned by CAFs (CM) or directly co-cultured with CAFs. After both treatments, appearance of CD133 positive cells could be demonstrated, while cells with this phenotype were undetectable after culturing in normal conditions. Accordingly, expression of stemness related genes (OCT4, NANOJ, alpha6ITG) was increased in LT73 CD133neg after treatment with CM from different fibroblast cultures demonstrating a direct influence of stromal components on the modulation of the CSC pool. Taken together, these data demonstrate that cross-talk between fibroblasts and cancer cells can dictate ECM composition and regulate CSC content and dissemination of lung cancer suggesting that identification of factors responsible for this cross-talk could be instrumental in devising novel therapeutic strategies.

**GENOME-WIDE ANALYSES**

Associations between clinical outcome of cancer patients and the gene expression signature in primary tumors at the time of diagnosis have been reported. We tested whether
gene expression patterns in non-involved lung tissue might correlate with clinical stage in
lung adenocarcinoma (ADCA) patients, comparing the transcriptome of non-involved
lung samples from 60 ADCA smoker patients of clinical stage I versus 60 patients with
stage >I. Five candidate genes were down-regulated in stage >I and in lung ADCA tissue
compared to non-involved tissue. Studies in vitro indicated that four of the genes
(SLC14A1, SMAD6, TEMEM100, and TXNIP) inhibited colony formation of lung cancer cell
lines transfected to overexpress these genes, suggesting their potential tumor-suppressor
activity. Individual variations in the transcriptional profile of non-involved lung tissue may
reflect individual predisposition to tumor aggressiveness in patients with lung ADCA.

Non-involved lung tissue from cancer patients surgically resected for a lung ADCA
(n=40) or for a lung metastasis from a non-lung cancer (n=40) was also analyzed by
genome-wide transcriptional analysis to identify a transcriptional signature associated
with risk of these pathological conditions. Comparison of the gene expression pattern in
the two groups pointed to a transcriptional signature enriched for genes involved in the
extracellular matrix (ECM)-receptor interaction pathway. Seven of 11 genes identified in
this pathway encode collagen chains, suggesting that individual variations in transcript lev-
els of these genes in non-involved lung tissue may modulate the risk of metastatic spread
to the lung. By qPCR, results for collagen genes COL3A1, COL4A1, and COL4A2 were
confirmed in the discovery series and validated in an independent series of 36 lung
metastases and 47 lung ADCA patients. Overall, analysis by qPCR of the two series com-
bined indicated statistically significant 1.2-fold up-regulation of COL4A1 and COL4A2
transcript levels in patients with lung metastasis of a non-lung cancer. Transcriptional up-
regulation of collagen genes in normal lung tissue may be associated with individual risk
of lung metastasis.

We have also started a study on the transcriptional profile of non-involved lung tissue
from 300 lung ADCA patients in association with clinical parameters related to chronic
obstructive pulmonary disease (COPD), and in particular with forced expiratory volume
in 1 second (FEV1), forced vital capacity (FVC), and the FEV1/FVC ratio. We are using the
Illumina microarray platform which allows detection of expression levels of about 48,000
transcripts.

CARCINOGENESIS

We carried out an extensive FCM analysis of the immunological profile of lung tissues
isolated from 52 patients. For each, the frequency (determined as % of all CD45+ leuko-
cytes) of 11 different cellular subsets was characterized in three different tissue samples
(based on an operational classification of the surgical samples as “normal/far”, “adjacent”
and “tumor” tissue). The investigated subsets included: CD3+CD4+ and CD3+CD8+ T
lymphocytes, CD19+ B cells, CD14+ monocytes, and CD4+ FOXP3+ regulatory T cells.
In addition, we looked at activated T cells expressing either HLA-DR or CD69. All tissue
samples, including those defined as “normal” and “adjacent” contained a sizeable fraction
of recently activated (HLA-DR+, or CD69+) T cells. Furthermore, the comparison of frequency of each of the subsets indicated, in several instances, a signifi-
cant skewing in the distribution in tumor tissue compared to normal and adjacent tissues.
In particular, no difference was found for any subset between normal and adjacent
tissues, while an increased frequency, in tumor tissue, of CD3+ T cell and CD19+ B cells,
activated HLA-DR+ T cells and CD69+ CD8+ T cells was found. In addition, conventional
CD4+ FOXP3+ regulatory T cells were at higher frequency in the neoplastic tissue com-
pared to normal/adjacent tissues. In contrast, significant reduction in monocytes was
observed in tumor tissue compared to normal and adjacent tissues. Interestingly, we also
found the presence of CD8+ T cells expressing FOXP3 in tumor tissue. This subset was
recently described by us in melanoma samples as an “early effector” subset. Taken
together, these results suggest that: a) both normal/adjacent and neoplastic lung tissues
are actively involved in ongoing immune responses as documented by presence of recently activated (HLA-DR+/CD69+) T cells; b) conventional regulatory T cells accumulate selectively in neoplastic tissue, but are also found in surrounding normal tissues; c) the presence of CD8+ FOXP3+ T cells is consistent with the early phases of generation of anti-tumor immunity in neoplastic tissue; d) neoplastic tissue has a specific immunological profile compared to the normal/adjacent tissues.

EXPERIMENTAL MODELS

We used p53+/- mice and replaced their hematopoietic cells with those from p53 wild type Thy 1.1 congenic mice in order to avoid the prominent lymphoid transformation. The initial idea of promoting lung cancer in such chimeric mice by lung delivery of inflammatory agents as they develop osteosarcomas at a high rate. Lung inflammation was then studied in the context of bleomycin-induced pulmonary fibrosis. Fibrosis results from inflammatory tissue damage and impaired regeneration. In the context of bleomycin-induced pulmonary fibrosis, we demonstrated that the matricellular protein SPARC distinctly regulates inflammation and collagen deposition depending on its cellular origin. Reciprocal Sparc-/- and WT bone marrow chimeras revealed that SPARC expression in host fibroblasts is required and sufficient to induce collagen fibrosis in a proper inflammatory environment. Accordingly, Sparc-/- > WT chimeras showed exacerbated inflammation and fibrosis due to the inability of Sparc-/- macrophages to down-regulate TNF production because of an impaired response to TGF-b1. Hence, the use of bone marrow cells expressing a dominant negative form of TGF-bRII under the monocyte-specific CD68 promoter, as a decoy, phenocopied Sparc-/- donor chimeras. Our results point to an unexpected dual role of SPARC in oppositely influencing the outcome of fibrosis. The pro-inflammatory condition of SPARC-KO>WT chimeras is now exploited to test whether total body radiation and bleomycin-induced lung injury render mice prone to lung cancer.

Keywords: lung cancer, early diagnosis, biomarkers, inflammation

PUBLICATIONS


Malignant melanoma continues to be a considerable medical issue because of the unsatisfactory efficacy and significant toxicities of currently-available drugs, and the rising incidence of the disease worldwide, which has doubled in the last 10 years with over 160,000 cases. Currently, over 90% of patients present with only primary melanoma at the time of first diagnosis. Although resection of the primary tumor is curative in many cases, some 20-30% of patients will eventually die of their cancer. Patients with distant metastases and/or stage IV disease have a very poor prognosis with a median survival of 8 months and a 2-year survival rate of 11%. Established in 2000, the Melanoma Program is a multidisciplinary approach involving surgeons, dermatologists, oncologists, pathologists, and experimental scientists, all involved in patient care and research. This integrated network offers patients the highest standards of care and improves issues related to melanoma diagnosis and therapy by increasing current knowledge about biology, genetics, and interaction with the host environment.

The projects of this multidisciplinary program:

• Offer melanoma patients the most promising therapeutic strategies, based on a careful selection at the individual level of tumor features and disease stage

• Perform extensive and coordinated preclinical and clinical studies aimed at understanding the mechanisms of action of novel anti-melanoma therapies and pathways of resistance, with particular attention to immune responses and immunotherapy

• Understand the strategies utilized by melanoma cells to restrain tumor immunity from the very early phase of disease and promote disease progression, and to identify pharmacological tools for the recovery of effective immune responses.
Several integrated research projects are ongoing, fostering further understanding of: a) genetic alterations that promote tumor transformation and progression; b) the role of altered intracellular signaling pathways in promoting melanoma cell survival, resistance to programmed cell death and escape from immune control; c) the role of pro-inflammatory crosstalk between tumor and stroma in promoting tumor growth and progression; d) identification of molecular markers of progression; e) identification and pharmacological targeting of cancer stem/initiating cells within the tumor population.

From a clinical point of view, crucial questions are approached with the goal of developing novel and more effective therapeutic strategies, by combining emerging treatments and drugs (including vaccines) with more conventional therapies, both in experimental models and clinical trials.

**Novel therapeutic approaches.** Phase I-II clinical trials testing the most promising therapeutic strategies have been activated within the multidisciplinary clinical program, and patient recruitment is in progress. These include vaccination strategies with tumor antigens as recombinant proteins (MAGE3, PRAME and NY-Eso1, ASCI strategy), immunomodulation of anti-CTLA4 antibodies in combination with chemotherapy (NIBIT trial), and novel BRAF and MEK inhibitors. Studies focused on evaluating the immunological effects occurring in treated patients are performed through dedicated monitoring of tumor-T cell immunity, frequency and function of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) together with cytokine/chemokine serum profiling.

Recently, based on consistent preclinical and initial clinical data, we have designed a randomized clinical protocol testing whether immunomodulating agents can synergize with standard therapies in improving specific tumor immunity to increase clinical benefits. Specifically, in patients with intradermal/subcutaneous melanoma lesions, we will test electrochemotherapy (ECT) either alone or in combination with proton pump inhibitors or intratumor injection of low dose IL-2. The study will be aimed at testing whether the combinatorial approach can induce positively modulate tumor immunity (in terms of increase in tumor-specific CD8+ and/or CD4+ T cells and decrease of immunoregulatory cells such as Tregs and immunosuppressive myeloid components of different origin) either at the systemic or local level. Clinical benefit, as improved TTP or effects on distant untreated tumor lesions, will be also determined by clinical and pathological assessment.

**Study of immunoregulatory pathways.** The evaluation of the phenotypic and functional features of immunosuppressive cells (such as Tregs and MDSCs), the molecular pathways leading to their accumulation and the impact that these cells may have on the clinical course of melanoma patients has allowed us to gain information about: i) the expression of LAG-3 as marker of activated Treg present at the tumor site, displaying stronger immunosuppressive activity; ii) the role of tumor exosomes in converting myeloid cells into MDSC, and the immunosuppressive/pro-tumorigenic properties of these cells generated either in vitro or in vivo in melanoma patients; iii) immunomodulation by drugs, such as cyclophosphamide or proton pump inhibitors (esomeprazole), on Treg and MDSC in vitro or in vivo, of treated patients. Specific studies have been conducted to study the metabolic impact, and particularly the impact of glycolysis, hypoxia, and associated pH dysregulation in the tumor microenvironment, on different arms of the immune responses. On the basis of the data produced in vitro, ex-vivo, and in different melanoma murine models, we discovered that pH regulation can profoundly influence tumor-specific immunity and that drugs interfering with such a pathway (e.g. proton pump inhibitors or the omeprazole family) may represent a valid therapeutic
strategy to recover immune-mediated tumor control. A patent about the immunomodulating role of proton pump inhibitors in cancer has been proposed and is presently under evaluation.

**Molecular studies on markers for melanoma progression.** We have analyzed the results of gene expression and microRNA profiling studies in archival sentinel lymph node biopsy (SNB) samples to evaluate transcriptional profiles for the identification of markers to be exploited for the stratification of stage III patients according to prognosis. We have evidence that tumor-positive SNB from patients with node involvement at regional complete lymph node dissection and poor prognosis at 5 years follow-up possess distinct transcriptional patterns, which are mostly due to immune response regulated genes in the sentinel lymph node. Analysis of the miRNA profiles and preliminary assessment of the miRNA-mRNA target pairs identified a set of miRNA and immune response regulated genes, thus supporting the hypothesis that miRNA can influence melanoma progression by regulating immune response processes ongoing in the sentinel lymph node. In order to evaluate mutated BRAF as a circulating molecular marker for disease progression, we have analyzed mutated BRAF in metastatic melanoma lesions and in plasma of melanoma patients. About 50% of melanoma tissues are positive for the T1799A variant (V600E) by sequence analysis, while the mutation is detected in 60% of cases by allele-specific real-time PCR-based detection with improved sensitivity. Circulating BRAFV600E is detected in 70% of patients, further indicating intratumor heterogeneity for the mutation.

**mRNA and miRNA profiling of MDSC induced in vitro by melanoma exosomes.** Preliminary results pointed out a role for mRNA and miRNA carried by melanoma exosomes in modulating molecules involved in regulation of immune processes in monocytes. Monocytes treated with melanoma exosomes show a transcriptional pattern involving IL-6 and HIF1α. Melanoma exosomes carried miRNA targeting genes involved in the MDSC response and mRNA regulating TGF-β signaling.

**Mechanism of action of ipilimumab in metastatic lesions.** The availability of the anti-CTLA-4 mAb ipilimumab, for therapy of advanced melanoma, has led to the first randomized phase III study showing improved survival in metastatic disease. The use of ipilimumab is based on the hypothesis that CTLA-4 blockade may suppress a negative pathway of immunity, which is thought to be dominant in neoplastic tissues of advanced cancer. Therefore, blockade of CTLA-4 by ipilimumab is expected to activate T-cell–mediated responses at the tumor site. However, immunologic monitoring of neoplastic tissues from patients treated with ipilimumab has not been previously performed. To address this issue we carried out a detailed immunohistochemical analysis of metastatic lesions removed before and after ipilimumab therapy from a melanoma patient. We found that objective tumor response, seen in one of two post-therapy lesions, was associated with a specific immunological profile, which was not seen in either the pre-therapy or post-therapy non-responding lesion. The neoplastic tissue of the regressing lesion was characterized by increased infiltration by activated (HLA DR+) CD3+ T cells that expressed cytolytic effector markers, by the presence of CD1a+ dendritic cells, and by a lower frequency of FOXP3+ lymphocytes. These results suggest that ipilimumab promotes infiltration of neoplastic tissues by dendritic cells and T cells and promotes functional differentiation of T cells to cytolytic anti-tumor effectors.

A subset of melanoma characterized by selective expression of the AXL receptor tyrosine kinase (RTK). The identification of new subsets of melanomas based on analysis for RTK expression may lead to the development of new and targeted therapies based on the usage of specific RTK inhibitors. In this study, we found that a subset of melanomas (38% of 58 cell lines investigated) express RTK AXL, a member of the TAM (Tyr3, AXL, and MER) family of receptors. AXL was specifically expressed by a subset of MITF-negative melanomas lacking expression of melanocyte differentiation antigens.
AXL was functional in these tumors as shown by analysis of downstream signaling pathway activation following stimulation with the ligand (GAS6). Inhibition of AXL expression by siRNA, or treatment of melanoma cells with a selective inhibitor (R428), suppressed motility, invasiveness, and ability of neoplastic cells to heal a wound and migrate across endothelial cell layers. These results suggest that AXL is a potential therapeutic target in a subset of MITF-negative melanomas.

Keywords: tumor vaccines, immune escape, BRAF/MEK inhibitors, melanoma stem cells

PUBLICATIONS


PERSONALIZED TREATMENT OF SARCOMAS

OVERVIEW

Soft tissue and bone sarcomas, including GISTs, are rare and highly heterogeneous tumors. Heterogeneity is firstly related to their ubiquitous anatomical origin. In this regard, applying good surgical principles to all sarcoma primary sites is not feasible. In particular, retroperitoneal and thoracic sarcomas have a poorer prognosis, primarily for surgical reasons. It is realized that new therapeutic avenues should be explored for these tumors, exploiting all disciplines, from surgery to radiation and medical therapy.

Heterogeneity is also related to the pathology of these tumors, as there are dozens of histological subtypes. It is well-known that the natural history of sarcomas differs substantially depending on the histological subtype. In the last few years, it has also become clear that histologic subtypes possess distinct cytogenetic and molecular profiles that affect sensitivity to medical therapies, including cytotoxic and targeted agents. These latter have mechanisms of action that are more specifically dependent on the molecular profile of the tumor. This poses new challenges to medical therapy of sarcomas, reinforcing the need to personalize medical treatment, mainly exploiting knowledge of deregulated molecular profiles. In this regard, GISTs constitute a tumor model that the sarcoma medical community has had the privilege to explore, and the model can now be tested in other types of sarcomas. In particular, it is clear that the current approach to sarcomas should be highly personalized, on a highly multidisciplinary basis. GISTs constitute an advanced platform, but other sarcomas are catching up rapidly, as new targeted agents are becoming available at a steady pace.
The aim of this group is to strengthen the institutional research platform, aimed at gaining new insights on the antitumor activity of cytotoxics and molecular targeted agents in selected types of sarcoma, including the rarest ones. New insights will also be gained on how to incorporate these medical therapies into multidisciplinary treatment strategies, with insights even in the most challenging clinical presentations of sarcoma. New avenues in the methodology of clinical research will be attempted, so that an additional value of the project will be the assessment of new strategies for clinical research, potentially serving as a model for other rare tumors. The patient populations include all patients affected by sarcoma, which for practical reasons are mainly divided into adult soft tissue sarcoma, GIST, small blue round cell sarcoma, osteosarcoma, chordoma and chondrosarcoma, and rare histological types.

The group has the goals of:

- Strengthening the institutional infrastructure for clinical studies on new agents in sarcoma. The aim of the infrastructure is to place the INT in the best position to launch and/or to join phase II and phase III clinical studies on new agents in sarcoma.
- Supporting the institutional infrastructure for translational research related to the targeted use of new molecular agents in sarcoma. The infrastructure will aim to place the INT in the best position to perform translational research on the use of new agents in sarcoma in tailored manner.
- Supporting the sarcoma networking coordinated by the INT to allow prospective and retrospective studies even on the rarest sarcoma subtypes and presentations. The INT coordinates the Italian Network on Rare Tumors, which is a powerful means to perform prospective and retrospective studies on rare presentations, with regards to their natural history and sensitivity to treatments.
- Studying the clinical utility of new targeted agents for treatment of sarcoma. Targeted agents imply considerable rethinking of clinical methodology (e.g., with regard to tumor response, assessment etc.). However, only centers with a large number of patients can embark on such a project, through systematic clinical observations and studies focusing on treatment strategies.
- Developing and testing new methodologies for clinical research in rare tumors. An effort will be made to test new options firsthand at least on the rarest presentations.

**RELEVANT OUTPUT**

During the third year of the project, global international collaboration among sarcoma centers was strengthened. The ESMO International Conference on Sarcoma & GIST was organized by INT and planned for March 2012; beyond the educational intent, the event is a crucial moment for the sarcoma community, aimed to share new investigational studies and methodologies in sarcomas.

Several clinical trials on rare sarcoma subtypes are ongoing or just completed:

1. Phase II trial on nilotinib in pigmented villonodular tenosynovitis. This is a rare condition in which tumor growth is sustained by CSF1R/CSF1 through an autocrine/paracrine loop that is potentially inhibited by nilotinib. Enrollment has just been com-
pleted and data analysis is ongoing. Results on the activity of imatinib were published in collaboration with other European centers.

2. Phase II trial on the activity of NGR-TNF, a fusion protein derived from NGR and TNF. We postulated that vascular sarcoma histotypes could show a particular sensitivity to this compound.

3. Two phase II trials in imatinib-pre-treated chordoma: a) lapatinib, an EGFR inhibitor; the final results will be presented at ASCO 2012 b) everolimus + imatinib, currently enrolling.

4. Four phase II trials on GIST: a) everolimus + imatinib in imatinib-naïve patients with a PDGFR A D842V mutation; b) regorafenib, a BRAF inhibitor, as third line therapy; the results of this completed international trial will be presented at ASCO 2012; c) BKM 120, a PI3K-inhibitor, as third line therapy; d) third generation TKI-inhibitor (Dovitinib) in high dose imatinib pretreated patients.

5. Phase III trial in advanced pretreated liposarcoma/leiomyosarcoma: eribulin versus dacarbazine.

6. Phase II trial on sirolimus + sorafenib in advanced pretreated osteosarcoma: it is an Italian Sarcoma Group trial, planned on the published results on the activity of sorafenib alone in this setting of patients.

7. Phase II multicentric international trial on denosumab in bone giant cell tumor: interim analysis of this enrolling trial was presented both at CTOS 2011 and ASCO 2011.

8. Phase II randomized vs placebo double-blinded trial con IPI926 in advanced chondrosarcoma.

9. Activity of sunitinib in advanced sarcoma patients treated at our institution was updated in a presentation at CTOS 2011. The animal model is under study in order to identify the mechanism of action of the drug.

We first published our data on sunitinib in a lveolar soft part sarcoma, confirming its antitumor activity, based on biological rationale. We reported that sunitinib is active both by a direct antitumor effect and an antiangiogenic mechanism.

We established the activity of imatinib in chordoma, with a publication on the final analysis of the phase II study.

We reported on the activity of dacarbazine +/- doxorubicin in clear cell sarcoma in advanced setting in a pooled series treated within the project of the National Rare Cancer Network.

Regarding myxoid liposarcoma, the results of the Italian Sarcoma Group phase II study on neoadjuvant trabectedin was accepted for publication in 2012. A pooled retrospective analysis (INT, Royal Masden London) of the activity of trabectedin in advanced uterine leiomyosarcoma was published, and the activity of ifosfamide in well differentiated/dedifferentiated liposarcoma was confirmed and presented at ASCO 2011.

A position paper on the surgical approach of retroperitoneal sarcomas was published, due to the complexity of surgery in this disease and the lack of a standardized approach. Based on the consensus statements from European and North American expert sarcoma surgeons, the paper was aimed to describe a reproducible and standardized approach to these tumors. A detailed description of the different procedures according to the variety of different presentations was made.
Finally, the results of the phase III Italian/Spanish Sarcoma Groups were accepted for publication in 2012: in high risk localized sarcoma patient population, randomized to receive three or five cycles of chemotherapy in addition to local treatment, with no difference was detected in overall survival.

**Keywords:** sarcomas, rare sarcoma subtypes, genetic alterations

**PUBLICATIONS**


NOVEL APPROACHES TO DETERMINE PROGNOSIS AND RESPONSE TO TREATMENT IN MATURE B-CELL MALIGNANCIES

OVERVIEW

Multiple myeloma (MM) and chronic lymphocytic leukemia (CLL) are mature B-cell cancers with an incidence that increases with age. Despite novel drugs, MM remains incurable for the vast majority of patients, with a highly variable outcome in different prognostic subgroups. The same applies to CLL: the disease is incurable and prognosis is extremely variable depending upon several clinicopathological factors. The selection of the most appropriate treatment (chemo-immunotherapy or stem cell transplantation [SCT]) must include knowledge of the clinical and genetic prognostic factors of the patient. For example, the limited duration of response after autologous SCT for MM patients with t(4;14)(p16;q32), t(14;16)(q32;q23) and 17p13 deletions highlights the need to develop a risk-adapted treatment strategy. The same is true for CLL patients carrying the 17p13 deletion. From an idealistic point of view, treatment strategies should be tailored based on clinical risk determination, adverse genetic prognostic factors, and host features. Although there is some data from gene expression profiling studies, there are no clear and firm conclusions on prognostic relevance and applicability in a clinical setting. While flow cytometry, cytogenetics, and molecular analyses may provide important prognostic information for both MM and CLL, they are not routinely used, and not frequently used together in a prospective fashion. Modern treatment protocols lead to complete remission (CR) in a considerable proportion of patients with MM and CLL. However, many patients will ultimately relapse, implying that the achievement of a clinical CR is compatible with a significant amount of residual malignant cells. For these reasons, a comprehensive approach prospectively integrating clinical and laboratory data is required for better stratification of patients, which will in turn translate into better allocation of healthcare resources.
Our major objective is to analyze the pre-treatment clinical and biological features of patients to determine their value in predicting disease response and survival. In particular, focus is placed on four main research areas: i) the genetic features of tumors (cytogenetics and FISH studies of MM cells, detection of MM stem cells, validation of the 17-gene expression-based risk-stratification model, identification of circulating MM specific miRNAs, use of a proteomic strategy on both leukemic cells and plasma specimens to identify novel proteins as biologic indicators of prognosis and response to treatment for CLL); ii) the immunological evaluation of the host and tumor interaction (evaluation of the T cell, natural killer cell and myeloid derived suppressor cell compartment); iii) monitoring of minimal residual disease (multiparameter flow cytometry and molecular methods); iv) assessment of bone lesions using a novel imaging method [whole body magnetic resonance imaging (DW-MRI)]. A fundamental goal of this project is to find a panel of biomarkers that can be easily and routinely used in a clinical setting.

**RELEVANT OUTPUT**

Promising results have been obtained during the last year of activity. In particular, we have demonstrated that:

- The number of lesions revealed by diffusion weighted MRI (DW-MRI) is significantly higher than that revealed using standard radiological examinations. There was a significant difference in the number of lesions detected in patients with symptomatic MM compared to patients in follow-up. Complex image analysis defined a diffusion coefficient (ADC, apparent diffusion coefficient) that is inversely correlated with tumor cellularity. In particular, most patients with MM had lesions characterized by a median ADC of 0.7, with a narrow standard deviation. The ADC value increases with response to treatment. Monitoring of the ADC value allows functional and morphological evaluation of bone lesions, indicating the enormous potential of applying DW-MRI for evaluation of MM.

- Circulating miRNAs can be detected and analyzed by quantitative RT-PCR in peripheral blood plasma samples of MM patients. We have characterized a specific circulating miRNA signature that differentiates MM patients from healthy subjects. It was possible to identify a correlation between the levels of specific circulating miRNAs and prognostic factors defined by ISS and cytogenetics. Further studies are aimed at identifying the role of differentially-expressed miRNAs in the plasma of myeloma patients. Preliminary results indicate that miRNAs present in the peripheral blood could be used for disease monitoring in MM patients. The same methodology is being used to study smoldering MM.

- Elevated levels of serum amyloid A (SAA) protein in plasma of CLL patients were identified using a proteomic strategy. To detect new plasma biomarkers in CLL patients, surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) mass spectrometry was applied. The analysis revealed statistically significant differences in SAA expression between the plasma of LLC patients and healthy subjects (P=0.002). In addition, SAA levels are elevated in the plasma of patients with an unfavorable cytogenetic profile (t(12;17) del(17) del(11)) compared to patients with normal or other
cytogenetic status (P=0.02). In CLL patients, SAA plasma levels also positively correlated with a peripheral lymphocyte doubling times of less than one year (P=0.009). Although preliminary, these data suggest that elevated SAA levels are associated with unfavorable prognostic markers and support the view that inflammation is implicated in development of CLL.

- Clonogenic MM cells can be stained using the Hoechst side population and Aldefluor assays. These methods are used to quantify MM stem cells and assess their role as a surrogate marker for clinical response during therapy.

We have started a collaboration with the experimental department of our Institution with the aim of applying array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays to study new emerging molecular subgroups with prognostic significance for MM patients enrolled in clinical trials.

A new phase III trial aimed at comparing bortezomib, cyclophosphamide and dexamethasone versus lenalidomide cyclophosphamide and dexamethasone in patients with multiple myeloma at first relapse or primary refractory has initiated. With this project, we plan to integrate clinical and biological data from patients enrolled with the ultimate goal of finding a panel of biomarkers that can be easily and routinely used in a clinical setting. Samples are being collected by each REL (Rete Ematologica Lombarda, Lombardy Hematology Network) center participating in the project and sent to our laboratory for biological studies. Since the beginning of the trial (April 2011), 45 patients have been enrolled. Peripheral blood and BM samples are being collected at study entry, after the first three cycles during the induction phase, at the end of the induction phase, at the end of the consolidation phase, and thereafter every 4 months for at least one year. We have therefore collected 75 vials of positively selected CD138+ plasma cells. Fifteen patients have completed the first 3 cycles and four patients the 6 cycles, and all biological samples have been collected. The plasma samples from the PB of all patients have been stored for miRNA analysis. Using multiparametric flow cytometry, we are studying fresh blood samples to assess the presence of MM progenitor cells that may be used as a surrogate marker for clinical response to a given drug combination. We are also assessing the correlations between the presence of specific T-cell subsets, natural killer (NK) cells, myeloid derived suppressor cells (MDSC), and the response to proposed therapies to provide data regarding a potential tumor-promoting (or tumor-inhibiting) role for each subset of MM patients.

**Keywords:** multiple myeloma; response to therapy; biomarkers; chronic lymphocytic leukemia
PUBLICATIONS


DEVELOPMENT OF RADIOPHARMACEUTICALS FOR TUMOR CHARACTERIZATION, MOLECULAR IMAGING, AND THERAPY

All the radiopharmaceuticals used in Nuclear Medicine for tumor imaging provide not only morphological data, but also important information on the biology of cancer tissue such as aggressiveness, proliferative activity, hypoxia, and amino acid uptake. All these parameters can be considered as prognostic factors and may be useful at tumor presentation for selection of the most effective treatment. Metabolic imaging in monitoring treatment response can overcome the intrinsic limitations of morphologic assessment of tumor response. In recent years, many new radiopharmaceuticals were developed and investigated based on specific recognition and binding to tumor targets. The same radiopharmaceuticals proposed for tumor imaging, when labeled with radioisotopes with high activity, may be able to deposit a sufficient amount of radiation energy to kill cancer cells. The radioisotope therapy includes different steps: choice of a specific target on cancer cells; selection of a metabolic 'bullet' that can bind the target with high affinity; radiolabeling of the probe to localize disease; and finally, identification of the radionuclide with the best physicochemical characteristics (positron, beta or gamma emission). In the case of thyroid cancer, which in advanced stages is currently treated with radioiodine, the development of an individual dosimetry and the better knowledge of its biology could greatly improve treatment strategies. This project is focused on the development of: a) \(^{3'}\)-deoxy-\(^{3'}\)-\(^{18}\)F-fluorothymidine (\(^{18}\)F-FLT) as a radiopharmaceutical for clinical PET; b) radiolabeled somatostatin analogues for radioreceptor therapy of neuroendocrine tumors (NET); c) a radiolabeled monoclonal antibody fragment directed to prostatic specific membrane antigen (PSMA) both as diagnostic tracer and therapeutic agent for prostate cancer; d) novel techniques for radiopharmaceutical and nuclear medicine, and to provide biological insights to optimize therapy of thyroid cancer.
Development of 18F-FLT as a radiopharmaceutical for clinical PET. The major objective of this study is to differentiate malignant from normal tissues and measure cancer cell proliferation. The tracer under investigation is 18F-fluorothymidine (18F-FLT), which can be used for diagnosis and monitoring the effectiveness of anticancer treatments. In particular, this tracer will be assessed in both pre-clinical and clinical studies in patients with locally advanced breast cancer undergoing neoadjuvant chemotherapy.

New radioreceptor therapy for NETs. The radiolabelling of somatostatin analogues with lanthanides or lanthanide-like radiometals has already been optimized using beta emitters such as 90Y and 177Lu, and with 68Ga for PET. Here, the objective is to evaluate whether the combined treatment with 177Lu-DOTA-TATE and 90Y-DOTA-TATE can exploit the different emission energy of the two radionuclides to improve the killing effects on both micro- and macro-metastases.

Development of immunological probes against PSMA. PSMA is a transmembrane protein that is overexpressed in prostatic cancer. The anti-PSMA single chain Fv, after radiolabeling, can detect prostate cancer cells better than traditional anti-PSA antibodies due to its binding to the cell surface. Both positron and electron emitting radioisotopes are considered for radiolabeling since the reliable tracers used for diagnostic imaging can potentially be utilized for therapy when conjugated with 131I, 90Y, or 177Lu.

Novel approaches for treatment of thyroid cancer. Here, the first step will be to investigate the expression of IGFBP7 and TIMP3, rearrangement of the oncogenes RET/PTC, and the mechanism of tumor response to combined treatment with gimatecam and HSP90 or the MEK inhibitor CI-1040. When radioisotope therapy with 131I is used, pre-treatment dosimetry is essential to establish the optimal activity and to maximize the dose of irradiation.

Original method for 18F-FLT synthesis. An easy production of 18F-FDG has been patented. The radiopharmaceutical fulfills the requirements of the Pharmacopea (sterility, pyrogenicity, pH, chemical and radiochemical purity, analysis of residual reagents). Animal models bearing breast cancer tumors have been studied to compare the uptake of 18F-FLT with 18F-FDG and to determine 18F-FLT uptake and behavior, and in particular its relationship with the biology of cancer tissue (cell proliferation, necrosis, prognostic markers). Studies using micro-PET have confirmed the feasibility of an in vivo approach. A clinical protocol has been approved by an Independent Ethics Committee, which entails evaluation of early response to primary chemotherapy (AT+C MF) in patients with locally advanced breast cancer; stage T2-4,N0-3,M0. A PET evaluation with 18F-FLT is planned at the beginning of treatment, after the first cycle, and at the end of the treatment prior to surgery. 18F-FLT uptake will be compared with pathological response, prognostic parameters, proliferative indices, and other modalities of diagnostic imaging. This ongoing study aims to validate the use of 18F-FLT PET as an early predictor of response to chemotherapy. At present, 11 patients have entered the clinical study; our preliminary observations show that tumor lesions (both primary cancer and lymph node metastases) take up 18F-FLT with different intensity, and the tracer was also useful to localize distant metastases.

Radioreceptor therapy of NETs. These investigations have continued with tyr(3)-octreotate (TATE) bound to DOTA with 90Y or 177Lu radioisotopes (90Y-DOTA-TATE and 177Lu-DOTA-TATE). Having noted the individual effect of therapies with 90Y and 177Lu labeled somatostatin analogues, and taking into consideration the different properties
Multidisciplinary Programs

of both radionuclides, combination treatments with $^{90}$Y and $^{177}$Lu peptides is being evaluated, especially in tumors with heterogeneous properties. The limiting factor in this case is the combined toxicity caused by the radiolabelled peptides. Data from animal studies have shown that the association of different radioisotopes was more effective in the overall survival of mice. The combination of $^{90}$Y-DOTA-TATE and $^{177}$Lu-DOTA-TATE determined a 62% survival rate at 150 days after therapy compared to the same rate of survival at 88 days after $^{90}$Y-DOTA-TATE alone and 96 days after $^{177}$Lu-DOTA-TATE alone. Preliminary results in our group on patients treated with four therapeutic cycles alternating 5.55 GBq $^{177}$Lu-DOTA-TATE and 2.6 GBq $^{90}$Y-DOTA-TATE suggested that the treatment is well tolerated with only rare cases of transient and mild hematological toxicities. The protocol adopted (repeated cycles with 5.5 GBq of $^{177}$Lu-DOTA-TATE and 2.6 GBq of $^{90}$Y-DOTA-TATE) resulted in an objective response in 21 of 26 cases (80.1%), and included 8 partial responses (30.1%), one complete response (3.8%), and 12 stable disease (46.1%). Side effects were limited to transient leucopenia in a few cases; no renal toxicity was described. On the basis of these results, it is planned to increase the activity of the radiopharmaceuticals injected (7.4 GBq for $^{177}$Lu and 3.7 GBq for $^{90}$Y) to further improve clinical response in patients with advanced disease that is refractory to traditional approaches.

Radiolabeled monoclonal antibody fragment against PSMA. The murine monoclonal anti-PSMA engineered fragment (D2B) has been radiolabeled with different radionuclides that are suitable for diagnostic imaging ($^{111}$In) and therapy ($^{131}$I and $^{90}$Y). The conditions for radiolabeling have been studied along with the stability of the fragment and its affinity, all with satisfactory results. Animal models based on cell lines having different expression of the antigen of interest (PC3-PSMA, cells transfected with PSMA, and PC3-WT, the same line not expressing the antigen) have been prepared. Initial data have shown that the biokinetics of the D2B fragment is better than the intact antibody, and tumor visualization is very clear within 6 hours after injection, and the tumor/background ratio is more favorable. In addition, the specificity of D2B uptake results very high with the fragment, which detects only lesions expressing PSMA. Pre-clinical studies in animal bearing tumor expressing (LNCaP/PC3-PSMA) or not expressing (wtPC3) the antigen have been performed. Preliminary experiments of tumor localization have been performed with the anti-PSMA scFv fragment (D2B) fluorescently labeled with cys5.5 dye. Tumor visualization was evident within 3 hours after injection and tumor visualization is very clear within 6 hours after injection with reduced background compared to localization with the entire antibody.

The same antibody fragment has been radiolabeled with different radionuclides suitable for diagnostic imaging ($^{111}$In and $^{131}$I) and therapy ($^{131}$I). Radiolabeling conditions are now well established and we demonstrated stability and maintained reactivity of the fragment after labeling. In vivo studies with the above murine model using a $^{131}$I radiolabeled scFv fragment have been performed indicating that the optimal tumor/background ratio is obtained at 24 hours. Ongoing further experiments with $^{131}$I and $^{111}$In radioisotopes will hopefully enable to optimize these reagents for diagnostic imaging of prostate cancer.

Biological characterization of thyroid cancer and new therapeutic approaches. In particular, the genetic mechanisms in thyroid cancer are being studied (BRAF mutations, RET/PTC and TRK oncogenes in papillary cancer, and PAX8/PPARgamma rearrangements and RAS mutations in follicular cancer). The development of new anti-cancer drugs is ongoing by identifying the role of the RET/PC (TPC-1) translocation, the BRAF V600E (BCPAP and NIM1) mutation, and the double lesions BRAF V600E/PI3K E54K (K1). The antiproliferative activity of gimatecan, a topoisomerase inhibitor, and several agents that affect the transduction pathway such as the HSP90 inhibitor 17-AAG and the MEK 1/2 inhibitor CI-1040 are under investigation. Considering the radiometabolic treatment of differentiated thyroid cancer, individualized dosimetry has been developed to demonstrate the potential clinical advantages in
maximizing the therapeutic activity of $^{131}$I according to the pre-treatment bone marrow dosimetry (Benua Leeper method). A multicenter Italian study was also carried out to calculate the absorbed dose both in bone marrow and in lesions in patients treated with fixed activity ranging from 3.7 to 12 GBq. The median [range] of the absorbed dose to bone marrow was: 0.50 [0.20–1.50] Gy. This implies that the fixed activity currently used in these types of treatment could be increased for most patients. Lesion evaluation in the multicenter study gave an indication of a strong reduction of lesion uptake in two subsequent cycles. If this is confirmed with further observations, a convenient treatment strategy of metastatic patients should be based on unequivocal staging of metastatic patients before the first treatment ($^{124}$I-PET), followed by maximized administrations.

**Keywords:** molecular imaging, radiopharmaceutical development, radiometabolic therapy

**PUBLICATIONS**


Pediatric Oncology manages and studies pediatric age cancers with a theoretic age range of 0-15 years (21 years in Northern Europe and USA). In common practice, the 15-21 year limit is for those otherwise typically pediatric cancers also presenting in adults (neuroblastoma, nephroblastoma, medulloblastoma) that are best studied and treated in a pediatric oncology setting.

The multidisciplinary goals are:

- Improving treatment strategies and number of cured children
- Reducing the risk of relapse
- Improving salvage treatment
- Understanding clinical and biological prognostic factors
- Providing adequate and continuous follow-up
- Organizing tailored rehabilitation
- Preventing, recognizing, and treating late-effects
- Providing, whenever needed, genetic counseling

The size of the problem is that 1-2 children under 15 years/10,000 each year suffers from cancer. In Italy, there has been a definite increase in the incidence of 2% per year in the last 20 years, and the expected new cases/year are now around 1800. The increase is especially related to more leukemias, brain tumors, and neuroblastoma. Childhood cancer remains, however, a rare disease representing 1-2% of all cancers.

Dying for cancer is the first cause of death for disease in childhood, but survival has increased from 65% in 1983-85 to 75% in 1992-1994 and over. Childhood cancer is not a disease but rather a “world of disease”, different for histology, site of origin, race, sex and age, among others. A large interest is presently concentrated on reducing the costs in late-effects for cure.
**PEDIATRIC BRAIN TUMORS AS A FIRST MODEL OF MULTIDISCIPLINARY CARE**

Brain tumors are the most common solid tumor in childhood and are the second most frequent childhood malignancy after leukemia. They account for 15% to 20% of all primary brain tumors. These tumors are now leading cause of death from childhood cancer. Around 350-400 new cases are diagnosed yearly in Italy. The INT, and in particular the Pediatric and Radiotherapy Units, have a long-standing experience in the management of pediatric brain tumors. Every year, about 60-70 new patients are submitted to adjuvant treatment after surgery consisting of radiotherapy and chemotherapy depending on histological diagnoses, extent of disease, and age of patients. Around 400 patients cured for brain tumors are in active follow-up consisting of thorough clinical examination, MRI, neurological, endocrinological, psychiatric, and psychologic neuro-cognitive counseling when requested. Patient accrual during the last three years (2009-2011) is shown in the figure.

![Patient accrual graph](image)

**MAIN TRIALS**

**Medulloblastoma.** Among pediatric brain tumors, medulloblastoma (MB) represents the most frequent malignant entity. Our efforts have been concentrated in the previous 10 years on the creation, application, and evaluation of an innovative trial for metastatic tumors combining, in the adjuvant setting, intensive chemotherapy, tailored craniospinal irradiation delivered according to a non-conventional technique called HART (hyperfractionated accelerated radiotherapy), and post-radiation chemotherapy whose intensity has been determined by pre-radiation response to drugs. This trial, which has become the most used treatment in Europe for this patient sub-setting, allows an overall survival at 10-years of 70% versus compared to 40% for historical data. The actions in the multidisciplinary setting have allowed to determine: chemo-responsiveness during the early phase of treatment whose results have driven total craniospinal HART doses and post-HART treatment, thus sparing those children with a more curable tumor from higher radiation doses and more intensive chemotherapy, including, for those who needed, two myeloablative thiotepa courses. Questions about different prognoses in metastatic tumors are also being explored from pathological/biological points of view with front-line central review differentiating MB subtypes and looking at markers in both immunohistochemistry and FISH that can further stratify tumor features and out-
come, such as p53, MYCN and MYC, nuclear beta-catenin, survivin, GAB, YAP, and FILA.

- The multidisciplinary team including Pediatrics, Pediatric Radiotherapy and Radiodiagnostic MRI will lead in Italy, together with other extra-institutional partners for neurosurgery and neuro-pathology, the upcoming standard risk MB protocols. Moreover, in the upcoming European protocol for standard risk MB, which will further stratify patients according to biologically-different prognostic subgroups, we are preparing to centrally check the quality of radiotherapeutic plans using a software system called VODCA that will be disseminated to two other complementary pediatric radiotherapy centers in Italy, in order to assure the best available treatment in all the participating centers.

**Ependymoma.** It is the second most common malignant tumor in children. In the last 18 years, our Unit has coordinated clinical national trials, pathological and biological studies, and neurocognitive outcome evaluation and has provided much guidance for the forthcoming SIOP (International Society of Pediatric Oncology) study. This study is a comprehensive program to improve the accuracy of diagnosis and explore different therapeutic strategies in children, adolescents, and young adults with a primary diagnosis of intracranial ependymoma. It will include a centralized review of pre and postoperative imaging to confirm that removal of the tumor is complete and to get advice from a panel of key opinion leaders for second look surgery if needed. It will also include a central review of pathology to confirm histological diagnosis and to prospectively identify disease subgroups and/or correlate patient response to treatment. After surgery and central review of imaging and pathology, patients will be enrolled in one of three clinical studies according to the outcome of the initial surgical resection (residual disease vs no residual disease), according to age or eligibility/suitability to receive radiotherapy.

- Also in this setting, apart from front-line central pathology review, use of VEC (vincristine, etoposide, cyclophosphamide) chemotherapy as a standard, solicitation of second-look surgery, which were all derived from Italian experience, a special role will be given to hypofractionated radiotherapy boost for postoperative residual disease. This adjuvant treatment was originally conceived in our Institute and the first results will be presented in June at the ISPNO (International Symposium of Pediatric Neuro-oncology). Here again, in the context of the multidisciplinary efforts, the indications for radiotherapy need to consider a better local control by applying non-conventional fractionation on a smaller volume and using all the technological developments required by any single patient with any individual disease presentation. The possibility to give a conformational treatment with an accurate neoplastic target that limits therapeutic dose only to tumoral tissue arises from the availability of high-quality imaging, reproducible immobilization systems, computerized system for virtual simulation, imaging fusion, three dimension treatment plans, and technology miniaturizing. As for the MB protocol, the quality of radiotherapy will be nationally checked by us.

### BIOMARKERS

**Cerebrospinal fluid proteome from Central Nervous System (CNS) pediatric tumors: patient related pattern.** Cerebrospinal fluid (CSF) cytology is the gold standard for diagnosing leptomeningeal dissemination, which provides crucial information for a correct therapeutic approach in brain tumors. Sensitivity of combined CSF cytology and neuroimaging studies, however, remain relatively low. Changes in CSF protein composition have been shown to reflect pathological processes in the CNS, such as tumor growth.

- We seek to characterize the CSF proteome of pediatric primary CNS tumors to identify CSF biomarkers predictive of tumor proclivity to leptomeningeal spread or related to patient clinical outcome. We have preliminarily piloted a label-free shotgun approach based on a high sensitive nano-LC-MS/MS linear ion trap-FT mass spectrometry to define the CSF protein pattern from four children with MB. A total of 257 proteins and 147 non-redundant proteins were detected with extremely high
stringency (confidence 99-100%). Protein functional classification based on Gene Ontology indicated that many of these proteins are involved in CNS disorders. These preliminary results indicate that the annotation capabilities presented provide specific information about CSF proteins and are a convenient method to design iterative and targeted follow-up experiments. We will apply the above-described proteomic strategy on a initial series of 30 children with primary CNS tumors, whose CSF has been already prospectively banked at diagnosis and at different timing of their disease course, upon institutional review board and ethical committee approval. A comparison of CSF from patients and controls will be done using highly sensitive proteomic approach for protein mapping, classification and identification in minimal amounts of CSF. Correlation with patient clinical and histological variables will also be explored. One of the points of major clinical relevance will be elucidating possible correlation between putative tumor-specific CSF proteins and presence/development of leptomeningeal dissemination. A larger cohort of patients, prospectively recruited from the host institution and other Italian and European collaborating institutions, will be characterized for CSF proteome once the first phase of the proposed project has identified reliable CSF candidate markers of disease progression/recurrence. It is believed that markers predictive of early tumor progression or leptomeningeal metastasis can allow for timely and appropriate treatment of high-risk children with primary CNS tumors, or even more importantly, for sparing unnecessary therapies in low-risk patients. Standardization and validation of putative biomarkers are needed to give widespread acceptance.

**Identification of microRNA biomarkers from cerebrospinal fluid.** These will be investigated in a number of brain tumors using CSF from pediatric lymphoma patients as controls. We will compare the expression pattern of the miRNAs analyzed in relation to cancer type, pathologic and molecular subtype, relevant clinical and pathologic features (gender; age, tumor size, location, WHO grade) as well as outcome parameters (surgical management, response to therapy, survival). Validation of the obtained results will be performed in an independent cohort of body fluid samples from pCNS patients subsequently collected throughout the course of the project.

**Pediatric malignant glioma.** Progress starting from the worst case scenario of diffuse intrinsic pontine glioma. Pediatric gliomas are the third most common malignant tumors in children and comprise a heterogeneous collection of CNS neoplasms distinct from adult gliomas based on histopathological, clinical, and molecular characteristics. Although the distinctive molecular features of pediatric gliomas are rapidly emerging, this information has not yet resulted in improved patient benefits. The present proposal aims at retrospectively and prospectively evaluating relevant prognostic and predictive biomarkers that may impact stratification criteria of patients with glioma. Both tumor-derived and circulating markers will be considered, with particular emphasis on diffuse intrinsic pontine gliomas (DIPG), a dreadful glioma subgroup showing less than one year of median survival, with less than 10% of patients surviving for two years or longer. We established a novel, promising treatment protocol of DIPG including ERBB1-targeted monoclonal antibody administration that will be launched as a phase II trial with the aim of ameliorating patient outcome and correlating biomarkers of treatment response. Finally, a novel pharmacologic approach to systemic treatment of CNS tumors will be assessed in pre-clinical models in order to evaluate whether temporary and reversible opening of the blood-brain barrier may improve antitumor drugs uptake in these chemoresistant tumors. The present project will thus explore novel treatment approaches to pediatric gliomas and provide evidence for the use of molecular biomarkers for prognostic and therapeutic stratification purposes. To achieve these goals, we will:

- Evaluate the prognostic and predictive potential of known and novel tumor-derived molecular markers frequently involved in pediatric glioma
- Evaluate the novel treatment strategy of DIPG children with radiotherapy, nimotuzumab and vinorelbine
• Assess circulating molecules (micro-RNAs and serum-soluble proteins) as prognostic/predictive biomarkers in DIPG patients
• Assess a pre-clinical model of pharmacological strategies to overcome the blood-brain barrier and enhance CNS tumor sensitivity to cytotoxic drugs.

NEW DRUGS

A phase 1 study of LDE225 in pediatric patients with refractory or recurrent medulloblastoma or other tumors potentially dependent on the Hedgehog signaling pathway. Our Unit has been selected as the only Italian center for this first-in-children trial. Aberrant activation of the hedgehog (Hh) signaling pathway is linked to the pathogenesis of several types of cancer, such as MB, basal cell carcinoma (BCC), and rhabdomyosarcoma. Hh signaling has also been described to play a role in neuroblastoma, hepatoblastoma, high-grade glioma, and osteosarcoma. Aberrant Hh signaling is involved in tumorigenesis through dysregulation of the cell-cycle, protection against apoptosis, and modulation of angiogenesis. Smoothened (Smo) is a positive regulator of Hh signaling and therefore may be an important therapeutic target. Approximately 25% of sporadic MBs are reported to have mutations that activate the Hh pathway through loss of function mutations in protein patched homolog (also known as patched [PTCH] or suppressor of fused [SuFu]); or gain of function mutations in Smo.

In addition, patients with Gorlin Syndrome, a condition characterized by a PTCH mutation, have an increased tendency to develop BCC, MB, rhabdomyosarcoma, and ovarian carcinoma. LDE225 is a potent, selective, and orally bioavailable Smo antagonist. Suppression of Gli1 mRNA expression in skin, a marker of Smo inhibition, has been observed in a dose/exposure-dependent manner. The primary purpose of this study is to evaluate the safety of LDE225 in a pediatric patient population and to define the maximum tolerated dose (MTD) of LDE225 in children with advanced solid tumors that are potentially dependent on the Hh signaling pathway (recurrent or refractory MB, rhabdomyosarcoma, neuroblastoma, hepatoblastoma, high-grade glioma, or osteosarcoma) and have progressed despite standard therapies or for which no standard treatment options exist. Pediatric malignancies driven by Hh aberrations represent an area of high unmet medical need, and LDE225, a specific and potent Smo antagonist, may be an effective treatment for these diseases. A total of 6 patients have been treated so far reaching the forth stratum.

OUTCOME

Diffusion Tensor Imaging to study radiation-induced damage and its correlation with neuro-cognitive outcome. This study is being conducted on a selected population of cured children from at least 3 years and submitted, among other treatments, to focal radiotherapy (RT). The intrinsic toxic effect of tumor, surgery, and adjuvant therapies, especially radiotherapy, on brain tumors is well known and appears at a temporal distance with characteristic cognitive deficits (IQ, attention, memory, etc). White matter (WM) can be better characterized by a particular application of MRI called DTI (diffusion tensor imaging). By the study of the direction in which water molecules diffuse in brain parenchyma, DTI is able to give information about the ultrastructural integrity of the WM (myelinization, fiber diameter and consistence, inter cellular spaces, cytoskeleton). Literature data has shown that, after RT, DTI parameters and median diffusivity are abnormal even after ending treatment.
• The aim of this research is evaluation of the association between neuro-cognitive/psychological deficits after treatment, and in particular to focal and WM damage quantified by DTI. Studying patients treated with focal RT would allow correlation of RT volume and doses with MRI alterations and intellectual damages to identify possible critical areas in the genesis of cognitive deficits. The identification of more damaged structures in patients treated with RT could theoretically contribute to better RT planning aimed at saving more fragile areas and reducing the incidence of cognitive and learning problems, and thus the need for rehabilitation and school assistance, facilitating social reentry. Statistically significant association of such parameters could bring new knowledge about the pathogenesis of WM damage.

PSYCHOLOGICAL SUPPORT

Internalizing problems, anxiety, depression, withdrawal, and consequent social problems are frequently observed in children with brain tumors. Effective (complete, truthful, consistent, comprehensible, gradual and continuous, and tailored) communication to the child about his/her condition is associated with better psychological outcome. To fulfill this goal, support for parent-child communication was created, which consists in a booklet able to explain the normal functioning of the brain and the possibility of its damage in tumors and treatments. This tool has been distributed to a consistent number of patients and the effects will be assessed with tailored instruments (Child Behavior Checklist [CBCL]) and a semi-structured interview to understand the difference in the quantity and quality of internalizing problems, comparing a new patient group with a previously reported group of 64 children that were not given this special support.

AUXOLOGIC SUPPORT

The Nuclear Medicine Unit has a long-standing experience in diagnosis and treatment of endocrine complications of oncological treatments. More than 300 pediatric patients treated for brain tumors or other solid tumors with an endocrine morbidity are now in active follow-up as outpatients for endocrine monitoring. Among these, over 100 patients are undergoing growth hormone replacement therapy. In addition to routine activities, several research projects studying modifications of endocrine profiles in patients with neoplasms are ongoing.

Keywords: brain tumors, tailored treatment, functional outcome, prognosis

PUBLICATIONS