Studies of childhood tumors aimed at improving prognosis and reducing adverse treatment effects; studies focused on the prevention, early diagnosis and management of long-term cancer- and treatment-induced effects; when cure is no longer possible, focus on support for patients and family to ensure they are not abandoned but supported (through control of physical and psychological symptoms) and accompanied along the terminal phase of the disease.

**AIMS:** Integrating longer survival and improved quality of life. For the most important childhood tumors (neuroblastoma, Wilms’ tumor, Ewing’s sarcoma), studies will seek to identify new therapeutic targets and thereby new approaches to biological drugs, as well as assessing iatrogenic sequelae with respect to thyroid, cardiac, pulmonary and gonadal function in long-term cancer survivors.

**PROJECTS**

- Tumors of the central nervous system (*Maura Massimino*)
- Adolescents with cancer in Italy: from local projects to a National coordinated program (*Andrea Ferrari*)
- Genetic and biomolecular characterization of Wilms’ tumor and detection of predictive markers of poor prognosis (*Daniela Perotti*)
- Introduction of new drugs in the treatment of pediatric cancer (*Michela Casanova*)
TUMORS OF THE CENTRAL NERVOUS SYSTEM

OVERVIEW AND SCIENTIFIC GOALS

CNS neoplasms are the leading cause of cancer death in children. The incidence is 2.4 cases per 100,000 per year, with approximately 450 new cases in Italy. While high-grade malignant astrocytomas (WHO grade III and IV, WHO) are prevalent in adult histologies, in pediatric patients low-grade gliomas and embryonic tumors (PNET and medulloblastoma) outweigh other histotypes, which represent, respectively, 50% and 20% of brain tumors under the age of 15 years. The signs and symptoms by which a tumor of the CNS occurs should not be underestimated either by the physician, parents or teachers in order to allow a prompt diagnosis and suitable therapy. In fact, at present, more than half of children who are diagnosed with a brain tumor have a chance for cure and become an adult, but the price of healing is often high in terms of sequelae in terms of neuro-cognitive deficits and endocrine, metabolic and somatic growth. Aspects of prevention, rehabilitation and correction of these deficits are thus an integral part of the treatment plan for these children.

For some histologies or presentations of disease (i.e. in the first case: the atypical rhabdoid tumor and, in the second case, the intrinsic neoplasms of pons-DIPG) or relapse of the disease, however, the chances of recovery are still an open challenge and treatment with new drugs or combinations of drugs with biological and molecular represent the focus of future therapies. The aims of our activity include to launch new therapeutic clinical protocols for diseases where a treatment standard already exists (medulloblastoma and ependymoma in the European context), experimental strategies in disease with poor prognosis (DIPG, glioblastoma), evaluation of surrogate biochemical markers to understand disease nature and history in those tumors where biopsy is not common or probably not definitely descriptive of tumor heterogeneity as in DIPG, and evaluation of long-term effects in cured patients through novels bio-engineering tools.

PROGRAM HIGHLIGHTS

Medulloblastoma (MBL) with no residual post-surgery, without metastasis and without biological risk factors (anaplastic / large cell, amplification of myc). The academic protocol for standard risk MBL medulloblastoma for which our center is the national coordinator within the SIOP (International Society of Pediatric Oncology) has already passed the stage of approval AIFA and the Ethics Committee of the INT. Once awarded the contract by the University of Hamburg, we will provide activation in other centers.

MBL at relapse. The phase 3 randomized protocol with the inhibitor of the pathway sonic hedgehog - LDE225 was opened. The results of steps 1 and 2 have already been presented in international conference venues.

Metastatic MBL. Ongoing debate at the European level in relation to the establishment of the protocol. The basis on which this Protocol is built, however, are the results with the strategy published by us in the Journal of Clinical Oncology in 2009.

Ependymoma. Similar stage as for the protocol of medulloblastoma. It obtained the approval of the AIFA and procedures should start at the ethics committees. Our center is also the national coordinator for this protocol.

Malignant gliomas. Our center, coordinator for Italy of the Protocol, which includes the randomization of temozolomide and radiotherapy versus radiotherapy, temozolomide + bevacizumab. Eight patients have been enrolled so far and ours is the center with the largest recruitment compared with 81 activated centers around the world including Canada and Australia. A futility report should be made available shortly.)
PROGRAM MEMBERSHIP

Veronica Biassoni, Clinical researcher, responsible for Italy of the DIPG network
Elisabetta Schiavello, Clinical researcher
Lorenza Gandola, Senior pediatric radiotherapist
Emilia Pecori, Junior pediatric radiotherapist
Emanuele Pignoli, Senior physicist
Fulvia Gariboldi, Rehabilitation physician
Alfonso Marchiano, Senior radiologist and coordinator of pediatric diagnostics

SELECTED RECENT PUBLICATIONS


ASSOCIATED CLINICAL TRIALS

Randomized trial for pediatric malignant glioma (Herby)
Ependymoma 2nd AIEOP trial
Nimotuzumab, vinorelbine and radiotherapy for DIPG, Tensor imaging evaluation of focially irradiated children

SELECTED RECENT MAJOR GRANTS

AIRC grant 2012-2014
Lombardy Region Grant 2011-2013 for Diffuse Tensor imaging evaluation (co-responsible, PI Geraldina Poggi in IRCCS Eugenio Medea, Bosisio Parini)
Grants by patient charities: Associazione Bianca Garavaglia, Fondo di Gio

KEYWORDS

Childhood brain tumors, prognosis improvement, marker surrogate, late-effects decrease
ADOLESCENTS WITH CANCER IN ITALY: FROM LOCAL PROJECTS TO A NATIONAL COORDINATED PROGRAM

OVERVIEW AND SCIENTIFIC GOALS

Adolescents with cancer are a unique group, with special characteristics. Patients in this age group seem to inhabit a “no man’s land”, belonging neither to the pediatric nor to the adult worlds of oncology. Their optimal management (e.g. coping with their complex psychological and social needs, providing age-appropriate facilities, and their inclusion in clinical trials) remains a challenge that requires broad-based processes. In particular, a lack of improvement in survival rates compared to other age groups has been reported, and survival rates of adolescents are poorer than those of children with the same disease, partially due to differences in biology but also to treatment delivered (limited access to optimal cancer services and low accrual to clinical trials).

The Youth Project of the Pediatric Oncology Unit at the INT in Milan was launched in 2011, as a dedicated program within the pediatric oncology unit (with no upper age limit for admitting patients with pediatric cancers to the pediatric unit) focusing on clinical aspects (e.g. inclusion in clinical trials, psycho-social support, fertility preserving facilities), but also with the view of creating dedicated multifunctional spaces and special events.

Thereafter, the Youth Project group leaded the Committee on Adolescents of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP), which successively evolved from a pediatric oncology-based Committee to a comprehensive national broad task force dedicated to adolescents and also to young adults, involving various stakeholders and in particular the adult oncology scientific societies: a new society has been created, called SIAMO (Società Italiana Adolescenti con Malattie Onco-ematologiche - Italian Society for Adolescents with Onco-hematological Diseases), which should be the official structure to achieve the support from the National Health Service and other organizations.
PROGRAM HIGHLIGHTS

Youth Project of the Pediatric Oncology Unit at the INT in Milan (www.ilprogettogiovani.it)
- A new model of specific culture, with the challenge to deal not only with disease, but with the life of patients, and their normality, creativity, and strength
- Developed within the pediatric oncology unit, as an offshoot of our existing activities, without requiring major changes to organization or new professional staff
- Double objective: 1) to improve and standardize clinical aspects (e.g. inclusion in clinical trials, psycho-social support, school and job support, fertility preserving facilities, access to care after cancer therapy), 2) to make the hospital a special place for our teenagers, by creating dedicated multifunctional spaces (including a 30 m² gym) and special activities, events, and courses (e.g. arts, photography, music, new technologies), involving various professionals working with the patients: in particular, B.LIVE, the stylist collection and the fashion parade (2012 project), the song Clouds of Oxygen (2013 project), sport (hospital & outdoor activities, e.g. sailing).
- A project on public information using new technologies easily accessible for teenagers (e.g. YouTube) has been developed with the aim to reduce the delay in diagnosis often observed in adolescents and young adults (www.infoadolescentietumori.it)

SIAMO - Società Italiana Adolescenti con Malattie Onco-ematologiche (www.progettosiamo.it)
- A comprehensive national program dedicated to adolescents (and young adults) with cancer. For the first time, the pediatric cooperative group Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) and the Federation of Parent Associations for pediatric onco-hematology (Federazione Italiana Associazioni Genitori Oncoematologia Pediatrica - FIAGOP) joins the adult cooperative groups Associazione Italiana di Oncologia Medica (AIOM) and Società Italiana di Ematologia (SIE)
- SIAMO moves from a pediatric oncology-based committee on adolescents to a forward-thinking national broad-based task-force dedicated to adolescents and young adults, and wants to represent the official structure to achieve the support from national health service organizations and governments.
- SIAMO involves not only physicians (from both the pediatric and the adult medical oncology world), but also various stakeholders such as nurse groups, psychologists, social workers, advocacy organizations, and survivor groups.
- SIAMO wants to definitely face up to the strong necessity to bridge the gap in the quality of professional care for adolescents with cancer. This is the major challenge, and requires broad-based schemes able to involve the public and its awareness, healthcare providers, cooperative groups running clinical trials, university, and also the national government.
- SIAMO wants to cooperate with the other international specific groups, starting from the European Network for Teenagers and Young Adults with Cancer (ENTYAC).

PROGRAM MEMBERSHIP

Andrea Ferrari, Responsible of the Youth Project and coordinator of Italian society SIAMO
Maura Massimino, Director of the Pediatric Oncology Unit
Carlo Alfredo Clerici, Psychologist
Laura Veneroni, Psychologist dedicated to adolescents
Monica Terenziani, Responsible for the Fertility Program
Filippo Spreafico, Responsible for the Sport Project

SELECTED RECENT PUBLICATIONS

Ferrari A.: The challenge of access to care for adolescents with cancer in Italy: national and local pediatric oncology programs. International Perspectives on AYAQ, Part 2, Journal of Adolescent and Young Adult Oncology 2013; 2(3): 112-117

SELECTED RECENT MAJOR GRANTS

Financial support is provided by the Associazione Bianca Garavaglia and the Near/Magica Cleme Foundation.

KEYWORDS

Adolescents, young adults, access to care, national program.
GENETIC AND BIOMOLECULAR CHARACTERIZATION OF WILMS TUMOR AND DETECTION OF PREDICTIVE MARKERS OF POOR PROGNOSIS

OVERVIEW AND SCIENTIFIC GOALS

Wilms tumor (WT) is a pediatric renal malignancy characterized by a high degree of histological and genetic heterogeneity. While most WTs are sporadic, familial cases have also been described. In addition, approximately 5% of WTs are associated with syndromic conditions.

At present, the genetic factors responsible for WT predisposition and development have been elucidated only in part. One of the difficulties in the study of WT is represented by the substantial lack of reliable models of the genesis of this disease. However, a strategy allowing the propagation of primary WTs in vivo has recently been developed (Shakked et al. EMBO Mol Med 2012, 4:1-20) that warrants more comprehensive investigations on WT-derived cells.

Overall, WTs display good prognosis and, thanks to a multimodal clinical approach, the 5-year overall survival rate approaches 90%. However, a survival rate <50% is expected in patients who relapse. In addition, a significant proportion of long-term survivors suffer from severe late therapy-related complications. At present, validated risk factors in use for therapeutic stratification are tumor stage and diffuse anaplasia. Studies aimed at identifying genetic factors predictive of adverse prognosis have revealed that loss of heterozygosity (LOH) at chromosomes 1p and 16q occur more frequent in WT s of relapsing cases, although the relative low incidence of 1p/16q losses renders their predictive value relatively low. Gain of chromosome 1q has also been reported as an adverse prognostic factor by several groups, but this finding needs prospective validation. Global gene expression analyses of WTs reported to date have failed to identify a molecular signature related to prognosis.

Specific aims of this research program are:

1. Identify genes involved in hereditary susceptibility to WT by massive parallel DNA sequencing.
2. Identify and validate genetic signatures predictive of poor prognosis by genome wide approaches, including single nucleotide polymorphisms (SNPs), gene expression, and miRNA profiling.
3. Verify the accuracy of selected genetic markers in predicting the clinical outcome of WT patients.
4. Obtain WT-derived cell cultures as models recapitulating the disease. Prospective studies and investigations addressing the molecular pathways leading to relapsing disease will be performed by engrafting fresh minced WTs into mice and, following serial passages, by recovering single cell suspensions maintaining both in vivo tumor expansion and in vitro differentiating capabilities.
5. To study functional aspects, relevant to WT, of genes and non-coding RNA misexpressed in tumor specimens. Moreover, as WTs are of embryonic origin, cancer and kidney-derived cell lines will be exploited as read-out models for the study of genes/miRNAs depletion or induction to assess their role on cell morphology and transcriptional regulation of early developmental genes.
PROGRAM HIGHLIGHTS

1. Genome-wide gene expression analysis comparing favorable histology WTs from relapsing and non-relapsing patients has been performed in collaboration with the Functional Genomics service. Approximately 700 differentially expressed genes and 20 miRNAs were identified as significantly associated with relapse. Strikingly, mRNA levels of genes expressed in the early stages of kidney development and in the blastemal component of WT tissues were found to be reduced in specimens from relapsing compared to non-relapsing patients. Moreover, the embryonic stem cell (ESC)-like signature, characterizing cancer-initiating cells, appeared to be frequently lost in relapsing WTs. Conversely, in these tumors an increase in the level of transcripts of genes associated with more differentiating steps of kidney organogenesis was observed. The set of genes whose expression was found to be predictive of poor prognosis included SPP1, MAOA, MUC1, CLDN1, and MYC. Further investigations on additional WT samples are necessary to verify the prognostic significance of markers emerging from these studies, and to help stratifying WT patients for risk of relapse thus allowing tailored therapeutic regimens.

2. A total of 125 unilateral favorable histology WTs registered between 2003 and 2008 in the Italian cooperative clinical protocol on WT were examined at microsatellite markers mapping to chromosomes 1p, 7p, 11q, 16q, and 22q. In line with previous findings, loss of heterozygosity (LOH) at 1p was significantly associated with a worse disease-free survival (probability 0.67 for patients with 0.92 for those without 1p LOH, $p = 0.0009$), as confirmed by multivariate analysis adjusting for tumor stage and patient age at diagnosis. There was no difference in disease-free survival probability among children with LOH in the other chromosomal regions tested. The worse outlook for children older than 2 years at diagnosis did not seem to be influenced by the LOH patterns considered (Spreafico et al., J Urol. 2013;189:260-266).

3. A family with two cases of WT, the mother and her son, was investigated molecularly. A previously unreported frameshift mutation of the WT1 gene, c.983delC (p.P328QfsX53) causing the loss of the C-terminal of the protein, was detected in both affected family members. This WT pedigree adds to the few already reported in which a role of WT1 mutations has been established (Melchionda et al., Pediatr Blood Cancer. 2013;60:1388-9).

4. A pediatric renal tumor tissue bank has been established with approximately 500 cases from multiple centers throughout Italy to date, with matched clinical and histological data, to support biological studies.

PROGRAM MEMBERSHIP

Daniela Perotti
Research staff scientist - 'Molecular bases of genetic risk and genetic testing’ Research Unit, Department of Preventive and Predictive Medicine. Molecular biologist, involved in the molecular characterization of WT, co-responsible for biological studies of the National Clinical Protocol on WT.

Filippo Spreafico
Staff clinician - Pediatric Oncology Unit, Department of Hematology and Pediatric Onco-Hematology. Chair of the National Clinical Protocol on pediatric renal tumors.

Antonio Fiorino
Associated researcher - ‘Molecular bases of genetic risk and genetic testing’ Research Unit, Department of Preventive and Predictive Medicine. Cellular and molecular biologist, involved in functional studies on WT.

Paolo Collini
Staff clinician - Anatomic Pathology Unit, Department of Pathology and Laboratory Medicine. Pathologist reviewing histological diagnoses within the National Clinical Protocol on WT.

Paolo Radice
Head of ‘Molecular bases of genetic risk and genetic testing’ Research Unit, Department of Preventive and Predictive Medicine. Expert in cancer genetics.

SELECTED RECENT PUBLICATIONS


ASSOCIATED CLINICAL TRIALS


SELECTED RECENT MAJOR GRANTS

Associazione Bianca Garavaglia
Project title: Bio-molecular characterization of Wilms tumor
Principal investigators: Filippo Spreafico, Paolo Radice
Duration: Jan 2012 – Dec 2014

KEYWORDS

Wilms tumor, genetic markers, prognostic factors, functional studies
Despite major progress in the past 40 years, 20% of children with cancer die from their disease, and 40% of survivors have late adverse effects. Cancer remains the most common fatal disease of childhood. The improvements in cure rates seem to have slowed down during the past decade, probably because we have reached the level where it is difficult to further improve outcomes with currently available treatment. Innovative, safe, and effective medicines are urgently needed. More than 100 drugs are approved for the treatment of malignancies, and among these about 30 are currently used in pediatric oncology and only 15 have been labeled for use in children. Possible reasons for the disparity between adult and pediatric drug approvals include the relative rarity of childhood malignancies, the histopathologic and biologic differences between many adult and pediatric tumors, and the limited number of pediatric patients eligible for pharmaceutical trials. Although regulatory initiatives in the past 15 years in the USA and Europe have been introduced, new drug development for children with cancer remains insufficient. Many pharmaceutical companies consider the adult population as their key market, and the development of a drug for pediatric use only or primarily is done to comply with regulatory obligations. Most children with cancer are still largely denied access to innovative drugs in Europe. In 2003, a European academic Network (42 academic institutions in 9 European countries) was created to properly address pediatric drug development; ITCC (Innovative Therapies for Children with Cancer) consortium was established through institutional resources, developing partnerships, collaboration, and clinical and biological research projects. This shows the clear commitment of all partners to work together, to combine expertise and strength, and to create a critical mass that is well integrated in the European pediatric oncology research area. Within this European Network (ITCC Consortium), our Institution is the most active center in Italy and recognized internationally. The main objective of the project is to increase preclinical and early clinical evaluation of new drugs in children and adolescents with cancer, with the final aim to increase the number of patients being cured and improve the quality of cure. Pediatric-specific needs include:

- Increased knowledge on the pharmacology in pediatric patients (especially in the very young) in order to improve dosing, tolerance and efficacy
- Age-appropriate formulations
- Evaluation of long-term sequelae in survivors following the use of new drugs.
Thanks to the collaboration with ITCC, the number of clinical trials with innovative drugs was significantly implemented and several phase I – first in children – studies have been opened recently.

In most of the ongoing studies, our Institution plays a pivotal role; it is the only Italian center selected for participation, and in others it acts as coordinating center.

As an example, in 2011 we started the phase I–first in children – of LDE225, an oral, potent and selective inhibitor of Smo, a key positive regulator of Hedgehog signaling; it was a dose-escalation study to characterize the safety, tolerability, pharmacokinetics, and pharmacodynamics of the drug in children with recurrent or refractory medulloblastoma, or other tumors potentially dependent on Hedgehog (Hh) signaling pathway. At that time, our institution was the only Italian center open for accrual. The drug went subsequently entered in phase 2 at is now in phase 3 in patients with recurrent/ refractory medulloblastoma. In the phase 3 study, only patients with activated Hh-pathway are included, who are identified on the basis of a 5-gene signature defined during the phase 1/2 study. Our institution is the coordinating center for Italy, which is currently the country with the highest enrollment. The results of the phase 1/2 studies, already presented in several meetings, will be published soon.

Also in the ongoing phase I – first in children – trial on LDK378, a selective inhibitor of ALK, our Institution is the only open center in Italy. Thanks to the positive interaction with the manufacturer, we also received the compound for preclinical evaluation. In vitro and in vivo experiments have been performed and are ongoing.

**SELECTED RECENT PUBLICATIONS**


**ASSOCIATED CLINICAL TRIALS**

Phase II open-label, randomized, multi-center comparative study of bevacizumab-based therapy in pediatric patients with newly diagnosed supratentorial high-grade glioma (Herby)

Open-label, multi-center, randomized phase II study evaluating the addition of bevacizumab to chemotherapy for childhood and adolescent patients presenting with metastatic rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma (the Bernie study)
SELECTED RECENT MAJOR GRANTS
Grant from the ROL (Rete Oncologica Lombarda) for the clinical management of the academic trial - International randomized phase II trial of the combination of vincristine and irinotecan with or without temozolomide (VI or VIT) in children and adults with refractory or relapsed rhabdomyosarcoma – as national sponsor and coordinating the activities of Italian centers
Grants by patient Charities (Associazione Bianca Garavaglia)

KEYWORDS
New drugs, Phase I studies, Early phase II studies