Annual Progress Report 2005-2006

Oncology and Genetics

Doctoral School

Molecular Biology Department

and

Human Pathology and Oncology Department
Information Engineering Department
Pediatrics, Obstetrics and Reproduction Medicine Department
Surgery Department
Surgery and Bioengineering Department
This new initiative is aimed to spread the information on the research activities of PhD students in our academic community.

The pamphlet is in English in order to promote Doctoral Schools of our University at international level, with particular attention to those foreign institutions with which we have signed international cooperation agreements. Moreover, it could also be useful to foster new agreements with foreign partners.

The Rector
Prof. Silvano Focardi
This pamphlet was created to regroup and present together the research activities of the students of the Doctoral School in Oncology and Genetics in order to exploit the work of the students and to promote the collaboration on research projects.

The first pages illustrate the activity of the “annual progress report day”. This day takes place at the end of each academic year and it is dedicated to the presentation of both the research projects proposed by the new entered students and the annual progress reports of the older students. The pamphlet continues with the presentation of the research abstracts of the 24 PhD students. Finally, the last pages are dedicated to the “thesis discussion days”. In the last session of thesis discussion, the qualification of “Doctor Europaeus” was conferred to two students.

I wish to dedicate this pamphlet to the PhD students who with their continuous daily work, their perseverance and motivation represent the “mainstay” of the Institution that we call University.

The director of the School
Prof. Alessandra Renieri

[Signature]
The Doctoral School in Oncology and Genetics is constituted of 4 sections or "education trainings":
1) Medical Genetics coordinated by Alessandra Renieri
2) Oncological Genetics coordinated by Antonio Giordano
3) Colorectal and Gastroesophageal Diseases coordinated by Gabriello Tanzini
4) Hepatobiliarypancreatic Diseases and Multitumoral Syndromes coordinated by Francesco Cetta

In addition to the four above mentioned coordinators, the Faculty Board is composed by teachers from the University of Siena: Antonio Acquaviva, Alfio Andronico, Monica Bianchini, Alessandro Cappelli, Anton Ferdinando Carli, Maddalena Cioni, Giuseppe Pasquale Cito, Serenella Civitelli, Antonio De Martino, Paolo Frezzotti, Theodora Hadjistilianou, Marco Lorenzi, Sergio Mancini, Giuseppe Marzocca, Clelia Daniela Anna Miracco, Roberto Ponchietti, Francesco Salvestrini, Francesco Tani, Walter Testi, Paolo Toti, Luigi Verre; and by teachers from other Universities: Maurizio Genuardi from the University of Florence, Pier Paolo Pandolfi from the Cornell University, New York, Hans van Bokhoven from the University of Nijmegen, The Netherlands.

On the basis of research activity the School has signed 6 International Cooperation Agreements with the following Universities:
Radboud University of Nijmegen, The Netherlands
Greenwood Genetic Center, Greenwood, South Carolina, USA
Bilkent University, Ankara, Turkey
University of Kentucky, Lexington, USA
University of Duisburg-Essen, Germany
St. Kliment Ochridski University, Sofia, Bulgaria

The Doctoral School in Oncology and Genetics at the University of Siena trains students to carry out research in Medical Genetics and in Clinical and Molecular Oncology over a four years program. The aim of this Doctoral School is to train researchers who will be able to plan and develop competitive research proposals. The School has a dedicated web site at the following address: http://www.unisi.it/ricerca/dottorazioneweb/genetica_medica/. In this site one can find general information on the School, seminar activities, research projects, and PhD students scientific "identity card".
Annual progress report day

- Ariani Francesca
  Identification of MECP2 large rearrangements in Rett syndrome by Real Time qPCR

- Artuso Rosangela
  Modifier genes and Rett syndrome

- Barellini Leonardo
  Papillary carcinoma of the thyroid and FAP

- Bernini Andrea
  Anorectal Melanoma

- Caselli Rossella
  The CDKL5 gene and the early onset seizures variant of Rett syndrome

- Causarano Vincenza
  Premature Ovarian Failure

- Chessa Antonella
  Rectal Cancer surgery

- Dhamo Armand
  Hilar versus common duct cholangiocarcinoma

- De Robertis Alessandra
  Pim plays a role in activating gene transcription in cooperation with Myc

- Grassi Irene
  The familiarity in the colorectal carcinoma

- Katzaki Eleni
  Adult-onset primary open-angle glaucoma and the WDR36 gene

- Malagnino Giuliana
  Extracolonic manifestations of Familial Adenomatous Polyposis

- Mancino Mario
  The CR1 gene and mammary carcinoma

- Mari Francesca
  Clinical genetics of Rett syndrome and mental retardation

- Mariani Federico
  Intraductal Papillary mucinous tumours of the Pancreas

- Ottimo Federica
  Identification of new genes in the Rett syndrome

- Pescucci Chiara
  The array-based Comparative Genomic Hybridization

- Roberti Annalisa
  Retinoblastoma and drug resistant osteosarcoma

- Sampieri Katia
  Mutational screening of the RB1 gene in patients with retinoblastoma

- Scala Elisa
  Array CGH deletion mapping excludes the involvement of VCX-A in mental retardation

- Speciale Caterina
  The Cohen syndrome and the COH1 gene

- Squillaro Tiziana
  Neural differentiation of mesenchimal stem cells in Rett syndrome

- Vignoli Marina
  Functional variants in genes involved in the metabolism of the 5-fluorouracil

- Vogiatzi Paraskevi
  Molecular characterization of gastric adenocarcinoma

Thesis discussion days
Annual Progress Report
Oncology and Genetics Doctoral School
October 14, 2005  room 12 Centro Didattico S. Maria alle Scotte, 13.00

13.00 Presentation of the PhD students programme of the XXI cycle (3 minutes for each one)

Artuso Rosangela (A. Renieri)
Modifier genes and Rett syndrome

Chessa Antonella (G. Tanzini)
Rectal Cancer Surgery

Grassi Irene (G. Tanzini)
The familiarity in the colorectal carcinoma

Katzaki Eleni (A. Renieri)
Adult-onset primary open-angle glaucoma and the WDR36 gene

Malagnino Giuliana (F. Cetta)
Extracolonic manifestations of Familial Adenomatous Polyposis

Mancino Mario (A. Giordano)
The CR1 gene and mammary carcinoma

Ottimo Federica (A. Renieri)
Identification of new genes in the Rett syndrome

Squillaro Tiziana (A. Renieri-U.Galderisi)
Neural differentiation of mesenchimal stem cells in Rett syndrome

Tirone Andrea (F. Cetta)
Exocrin pancreas tumours

Vignoli Marina (M. Genuardi)
Functional variants in genes involved in the metabolism of the 5-fluorouracile

14.30 Final report of the XVII cycle (15 minutes for each one)

Bon Giuseppina (F. Cetta)
Genetic alterations in the hepatoblastoma and hepatocellular carcinoma associated with familial adenomatous polyposis

Mari Francesca (A. Renieri)
Presentation of the PhD thesis: "Clinical genetics of Rett syndrome and mental retardation"

Pescucci Chiara (A. Renieri)
The array-based Comparative Genomic Hybridization

15.30 Progress report of the XVIII and XIX cycles (10 minutes for each one)

3rd year

Ariani Francesca (A. Renieri)
Identification of MECP2 large rearrangements in Rett syndrome by Real Time qPCR

Bernini Andrea (F. Cetta)
Anorectal Melanoma
De Robertis Alessandra (S. Oliviero)
Pim plays a role in activating gene transcription in cooperation with Myc

Vogiatzi Paraskevi (A. Giordano)
Molecular characterization of gastric adenocarcinoma

2nd year

Caselli Rossella (A. Renieri)
The CDKL5 gene and the early onset seizures variant of Rett syndrome

Dhamo Armand (F. Cetta)
Hilar versus common duct cholangiocarcinoma

Mariani Federico (F. Cetta-E. Pinto)
Intraductal Papillary mucinous tumours of the Pancreas

Scala Elisa (A. Renieri)
Array CGH deletion mapping excludes the involvement of VCX-A in mental retardation

17.45 Progress report of the 1st year, cycle XX (5 minutes for each one)

Angelini Elena (F. Cetta)
Familial adenomatous polyposis

Barellini Leonardo (F. Cetta)
Papillary carcinoma of the thyroid and FAP

Calamati Giulia (F. Cetta)
Hepatic metastasis

Causarno Vincenza (A.Renieri-D. Toniolo)
Premature Ovarian Failure

Roberti Annalisa (A. Giordano)
Retinoblastoma and drug resistant osteosarcoma

Sampieri Katia (A. Renieri)
Mutational screening of the RB1 gene in patients with retinoblastoma

Speciale Caterina (A. Renieri)
The Cohen syndrome and the COH1 gene

19.15 Closing session and attribution of credits by the faculty board

A copy of the minutes is available at http://www.unisi.it/ricerca/dottoratiweb/genetica_medica/accessing the "Minutes" link
Identification of MECP2 large rearrangements in Rett syndrome by Real Time qPCR

Rett Syndrome (RTT, OMIM# 312750) is a progressive neurological disorder primarily affecting females with an incidence of approximately 1:15,000 born females. Mutations in the MECP2 gene are found in 70-80% of classic RTT cases and in 20-40% of variant patients. Missed mutations may be due to the limited sensitivity of the methodology. In a double copy gene, such as MECP2 in females, traditional methodologies (DGGE, SSCP, DHPLC, direct sequencing) are prone to miss gross rearrangements. For this reason, we developed a reliable, single tube, quantitative PCR assay for rapid determination of MECP2 gene dosage (Ariani et al. Hum Mut 2004). This new method allowed us to investigate the presence of large rearrangements in a group of 95 RTT patients without any detectable MECP2 point mutation. We found large deletions in 8 out 19 classic patients (app.32%) and a duplication in 1 out 23 Preserved Speech Variant cases (app.1%). We did not find any gross deletion/duplication in the 13 patients affected by the other RTT variants. These data suggest that there is specific association between the classic form of the syndrome and MECP2 rearrangements. Future Perspectives: Recently, Van Esch et al. (Am. J. Hum. Genet. 2005) demonstrated that MECP2 duplication occurs frequently in male patients with a severe form of mental retardation (MR). Consequently, we plan to use the quantitative PCR assay to determine MECP2 gene dosage in a large group of MR males (120 patients).

![PCR amplification plot of MECP2 and RNAaseP (endogenous control) in multiplex single tube assay. On the left, one of the 8 classic RTT female with a MECP2 deletion. On the right, the PSV female with a MECP2 duplication.](image)

Part of this work was published in:
Modifier genes and Rett syndrome

Rett syndrome is an X-linked progressive neurodevelopmental disorder and one of the leading causes of mental retardation in girls. In addition to the classic form of the syndrome, several variants have been described. The MECP2 gene has been found mutated in both classic and variant cases. Recently, another gene named CDKL5, has been found mutated in a small fraction of RTT patient with the early onset seizures variant of the syndrome.

In order to investigate the cause of phenotypic variability in patients with MECP2 mutations, we decided to study the role of specific genes (CDKL5 and AF4) as phenotypic modifier.

In particular, we analyzed a group of 115 RTT patients with MECP2 mutations to establish the genotype of one specific CDKL5 polymorphism (c.2372A>C, p.Q791P). The $\chi^2$ analysis demonstrated that the polymorphism is not significantly associated with any specific RTT phenotype.

My research project is aimed to study whether two intronic polymorphisms (IVS15+8T>C and IVS15-16T>C) in the AF4 gene can modulate the RTT phenotype, analyzing these sequence variations in the same group of patients.

**IVS15+8T>C**

**IVS15-16T>C**
**Papillary carcinoma of the thyroid and FAP**

Familial adenomatous poliposis (FAP) is an autosomal dominant disease with variable expressivity. Papillary thyroid carcinomas may be part of the clinical spectrum of FAP. This study has been planned together with the Department of General Surgery of Pisa University (Chief Prof. Paolo Miccoli). The aim of the study is to identify which percentage of patients affected by papillary thyroid carcinoma are affected by FAP. Materials and methods: patients, aged below 30 years, submitted to total thyroidectomy for papillary thyroid cancer in the period 2000-2005 are enrolled for the study. All these subjects will receive, by mail, a questionnaire with the intent to find in this population patients affected by FAP. They will be submitted to an interview to obtain a pedigree. Furthermore, their specimens will be reviewed and a genetic study of the RET proto-oncogene will be accomplished. The study is currently in the sending questionnaire step. 6-12 months will be necessary to have conclusions.
Anorectal Melanoma

Anorectal melanoma is a highly aggressive malignancy and long term survival is considered exceptional - 5 years survival after abdomino-perineal resection (APR) and inguinal lymphadenectomy is accounted between 0.5 and 1% (Bullard KM, et al. J Am Coll Surg 2003 196. 206-211). Since aggressive and demolitive treatment does not lead to significant results most surgeons have started to adopt a less radical approach like trans anal excision (TAE).

However the exiguous number of cases reported in all series does not allow to assess the supremacy of any technique. According some meta-analysis, TAE seems to offer good control of the disease and much less complications than APR but long term survival remains exceptional whatever treatment is chosen. Therefore most centres progressively switched from APR to TAE in the treatment of this malignancy.

In our series we report 5 patients with survival longer than 5 years all treated with APR. We studied retrospectively 15 patients (6 men, 9 women) all treated in a single Institution (Koloproctologie, Rhur Universität). All patients were stage I-II disease (N0 M0). Patients with local or distant metastases at the moment of diagnosis were not considered. Follow-up was obtained contacting directly the patients or their relatives and when feasible patients were re-admitted in hospital and fully re-evaluated.

Symptoms of presentation of disease were: bleeding (9 pts) sensation of peri-anal lump (5 pts). Interestingly 13 patients requested medical assistance and then refereed to a surgeon for haemorrhoids. Mean duration of symptoms prior diagnosis and treatment was 4 months, average follow up was 54 months (range 36-160 months).

8 patients are still alive without disease recurrence. In this cases pathology stage was T3 in 3 pts and T4 in 5 pts. Surgical treatment was APR in 6 pts and TAE for 2 pts. 5 patients, alive disease free have a mean follow up of 105 months (range 83-123 months) and were all surgically treated with APR. No other series ever published reported such prolonged survival in a significant number of cases.

Long term survival seems to be possible. Relationship between survival and surgical treatment adopted but all long term survivors were treated with APR.
CDKL5 gene and early onset seizures variant of Rett Syndrome

Rett syndrome (RTT) is a severe neurodevelopmental disorder almost exclusively affecting females and characterized by a wide spectrum of clinical manifestations. Most patients affected by classic RTT and a smaller percentage of other variants have a mutation in the methyl CpG binding protein 2 gene (MECP2). In the variant with early onset of seizures MECP2 mutations have not been reported. Our study has identified mutations in CDKL5 gene (Scala et al, J Med Genet.2005), encoding a putative kinase, in 4 female patients with early onset seizures variant: 2 early truncating mutations interrupting the catalytic domain and 2 late truncating mutations. The clinical course of these patients is strikingly similar and they fulfill the criteria for early onset seizures variant: they all showed convulsions very early in life (in the first months) and then developed typical characteristics of RTT. Given that MECP2 and CDKL5 mutations cause similar phenotypes we investigated whether the 2 proteins belong to the same molecular pathway. In collaboration with the research group of prof. Landsberger (Insubria University) we demonstrated in vitro and in vivo a direct interaction between MECP2 and CDKL5. Functional characterization of CDKL5 showed that CDKL5 harbours an auto-phosphorylation activity, demonstrating that indeed it is a kinase. Our experience suggest that CDKL5 mutations are associated only with a particular RTT phenotype and trace out a molecular link between MeCP2 and CDKL5. Further studies are necessary to establish whether MeCP2 is the main target of CDKL5 in vivo and whether the biological significance of the interaction is limited to phosphorylation.

Part of this work was published in:
Premature Ovarian Failure

Premature Ovarian Failure (POF) is a disorder of ovulation characterized by primary or secondary amenorrhea (before 40 years of age), or early menopause (before 45 years of age) and elevated serum gonadotropins level.

Known causes of POF include environmental factors, such as radiation, chemotherapy and infections. A genetic component of the disorder is demonstrated by cases associated with X chromosome rearrangements and by a significant fraction of familial cases. POF is thought to be due to the contribution of several genes acting as risk factors.

DIAPH2 is one of the three human homologues of the Drosophila melanogaster dia gene. In the fruit fly, the gene is responsible for defects in oogenesis and spermatogenesis and causes sterility in both sexes. The DIAPH2 gene was found interrupted by the breakpoint of a balanced X;12 translocation in a POF familial case. Mutation analysis on a large cohort of Italian patients failed to demonstrate any causative variant.

A large region of DIAPH2 gene is contained in a linkage disequilibrium block where an intronic variant (L2) was associated with POF. We searched for a functional variant in this linkage disequilibrium block that may represent the risk factor for POF: we looked for this variant in 50 bp intervals upstream and downstream from splice sites, in peri-exonic and intronic conserved sequences and in promoter region.

We analyzed 91 Italian POF patients presenting the L2 variant. For SNPs having a frequency higher than 5%, we extended the analysis to consider a set of 188 control DNAs. The analysis was performed by DHPLC. We found 25 SNPs fulfilling the previous condition. Up to now, none of the variants that we found is significantly associated with POF.

The figure is a schematic representation of DIAPH2 gene.
Rectal Cancer surgery
In the last years, rectal cancer surgery has improved by the increasing contribution of multidisci- 
nary team management, ranging a better postoperative survival and quality of life. Total mesorec-
tal excision and pelvic nerve preservation have allowed to reduce recurrence rates from 30% to less 
than 10% and to maintain a better quality of life in terms of functional results especially in middle 
– low rectal cancer in the respect of oncologic radicality.
The goals of rectal cancer treatment are sphincter saving surgery with local control, long term sur-
vival, genitourinary and sexual preserved functions.
Sphincter saving surgery nowadays is thought to stay on a better quality of life than abdomino-peri-
neal amputation, but some clinical trials report that permanent stoma keepers have a similar or bet-
ter quality of life than patients treated with sphincter saving surgery. Preoperative quality of life as 
absence or presence of a concomitant pathology is a predictive factor for one year-survival. Age, 
sex, operation and surgical technique influence quality of life after rectal cancer surgery.
Has been underlined that quality of life changes in postoperative period in short and middle follow 
up and a permanent stoma is not the only determinant of health related quality of life.
The role of surgeon and counselling are the main factors influencing the quality of life of patients 
who undergo rectal cancer surgery.
We propose a perspective study involving rectal cancer surgery patients to assess oncologic results 
and quality of life. Periodic clinical and instrumental follow-up according to generally accepted pro-
tocols and with standardized questionnaires (European Organization for Research and Treatment 
of Cancer QLQ-C30, QLQ-CR38) before surgery and at 3,6,9,12 months from surgery are going to 
be administered.
The knowledge of these data can contribute to address treatment in order to improve rectal can-
cer surgery outcome.
Hilar versus common duct cholangiocarcinoma
Twenty-seven patients with cholangiocarcinoma (CC) of the main ducts (excluding gallbladder carcinomas, ampullomas and intrahepatic CC) were admitted to and treated by a single Institution between 1996 and 2001 in a Western country, such as Italy. (There were 19 M and 8 F, mean age 64, 3 years, range 31-86). 20 patients had hilar CC (5 type IIIA, 6 IIIB, 4 type IV, 2 type II, 1 type I), whereas 6 patients had CC involving the common duct (CD) and 1 had multinodular CC involving the CD (CDCC).
"Radical" surgical procedures, including major liver resection plus caudate lobe removal or DPC for distal tumors, were performed in 14 patients. Mean survival of patients with CDCC was 12.4 months, whereas it was 33.7 months in patients with upper third tumors, with 2 patients still living without recurrence more than 5 years. In particular, the former patient, presently 71 years-old, had removal of a tyroid tumor, ductal breast carcinoma, hilar cholangiocarcinoma and colon (sigmoid) carcinoma, suggesting a possible new inherited multitumoral syndrome, including cholangiocarcinoma as a part of the syndrome.
Present data show that accurate diagnosis and radical liver resection, including caudate lobe removal, give good results in terms of both total survival and disease free survival, even in patients with type IIIA or IIIB hilar cholangiocarcinomas. On the contrary, patients with non hilar CC, i.e. involving the common duct, had lower survival, despite apparent "radical" treatments, such as DCP. Whether this different survival reflects a different biological behaviour of hilar versus common duct CC, i.e. a reduced perineural and lymphatic invasion in perihilar versus CC involving the lower portion of the CD, is not yet elucidated.
Pim plays a role in activating gene transcription in cooperation with Myc
The serine/threonine Kinase Pim-1, following growth factor, translocates to the nucleus where it contributes to the activation of Myc-target genes (FOSL1, ETS2, ID2 and JUNB) by phosphorylating S10-H3 histon at its E-box.
RNAi experiments demonstrated that both Myc and Pim-1 expression is essential for S10-H3 phosphorylation.
Experiments demonstrated a synergistic effect of Pim-1 and Myc in lymphomatogenesis and we also observed that Pim-1 forms a nuclear complex with Myc able to bind to and to phosphorylate H3 in vitro on reconstitute nucleosome at the FOSL1 enhancer.
These results demonstrate a new function of Pim-1 as an epigenetic modifier of the nucleosome at Myc- target loci and suggest that nucleosome phosphorylation, at the enhancer, is required triggering to transcriptional activation.
Familiarity in colorectal carcinoma

Colo-rectal carcinoma (CRK) is the second most common cause of death for neoplasia; its incidence is constantly increasing, even though surgery and anesthesia have contributed to reduce peri-operatory mortality but they did not influence the natural history of the disease; In fact patients survival time after 5 years is below 50%. CRK onset is consequent to the interaction between environmental and genetic risk factors which act through the common mechanism of DNA damage. If environmental factors were the only causes of CRK onset we should expect an elevate incidence in cohabiting non consanguineous relatives of affected patients. On the contrary, there are familial CRK cases in which the frequency is typical of mendelian dominant conditions. An higher risk of large intestine carcinomas and adenomas is found in first degree relatives of CRK patients. It is estimated that up to 15% of CRK are hereditary. This suggest that CRK is one of the most frequent genetically determined neoplasias. Hereditary CRK is classified as Familial Adenomatous Poliposis (FAP), HNPCC and familial CRK.

FAP is due to mutations in APC gene which causes an autosomal dominant transmission with high penetrance (80-93%); a positive family history is present in 55-56% of cases. In the other cases the mutation is de novo. About 80% of carriers develop clinically visible adenomas from 5-10 years, even if symptoms appear later. The risk of CRK is almost 100% at about 40 years. The presence of a well recognizable phenotypic marker (poliposis) facilitate the clinical diagnosis. For FAP a molecular diagnosis is possible. APC gene is screened for mutations on DNA isolated from peripheral leukocytes. HNPCC is classified as Lynch syndrome type I (colon CRK only) and Lynch syndrome type II (CRK in colon and other localizations). Clinically HNPCC present the following characteristics: they are located in proximal colon (70%), they are frequently multiple they are scarcely differentiated, they have mucous appearance and compared to sporadic CRK they have a better prognosis. In some HNPCC families germ line mutations in genes involved in DNA repair have been found (hMSH2, hMLH1, hPMS1, hPMS2).

Materials and methods: In order to identify possible CRK among patients surgically treated in our Institute, we will employ the following criteria positive family history, early onset, possible previous neoplasias, and other (district, histology).

Aim: the aim of this project is to identify possible molecular alterations in patients coming in our institutes with a suspected hereditary CRK; this will allow us to better follow-up and ménage patients and their families.
Adult-onset primary open-angle glaucoma and the WDR36 gene

Glaucoma is a group of ocular disorders characterized by a specific pattern of optic nerve and visual field defects. This condition is one of the two leading causes of blindness, affecting over 67 million people worldwide. Open-angle glaucoma is usually asymptomatic until the late stages of the disease, when significant and irreversible optic nerve damage has already taken place. As the sensitivity of current diagnostic techniques is suboptimal, the diagnosis of glaucoma is usually made once an irreversible damage has already occurred. As glaucoma related visual loss is preventable in many cases, there is an urgent need to diagnose glaucoma at its early stages and to institute appropriate neuroprotective management of the ganglion cells. Mapping, cloning and identification of novel mutations involved in the etiology of glaucoma provide a significant opportunity for presymptomatic diagnosis, improved prognosis and better understanding of the etiology of this blinding condition.

During the last decade, a total of six genetic loci (GLC1A 1q23-q24, GLC1B 2q1-q13, GLC1C 3q21-q24, GLC1D 8q23, GLC1E 10p14-p15 e GLC1F 7q35-q36) and two genes have been reported for POAG: myocilin (MYOC) is primarily mutated in juvenile-onset subjects, whereas optineurin (OPTN) is mainly mutated in low pressure POAG individuals. In 2005 (S. Monemi et. al) it has been identified a new glaucoma causative gene (WDR36) located in 5q22.1.

My research project is:
- Analysis of the WDR36 gene in 53 probands with glaucoma and high ocular pressure and in 40 probands with normal ocular pressure, using Denaturing High Performance Liquid Chromatography (DHPLC). The WDR36 gene consists in 23 exons but first of all it will be analyzed exon 17 in which a recurrent mutation: c.1973A>G, p.D658G has been identified. In case of negative results a further analysis will be performed in the other exons.
Extracolonic manifestations of Familial Adenomatous Polyposis

Familial Adenomatous Polyposis (FAP) is a multitumoral syndrome due to germ-line mutation of the APC gene (mapped at 5q21). In addition to hundreds to thousands of colonic polyps, which to transformation invariably into malignant tumors before age 50, the syndrome includes: gastric and duodenal polyps, perianpillary tumors, primary liver tumors, carcinoma of thyroid, tumors of the central nervous system and desmoid tumors. In addition, benign alterations such as CHRPE (Congenital Hypertrophy of the Retinal Pigment Epithelium), osteomas and dental abnormalities have been reported. In particular the research will focus on FAP associated papillary thyroid carcinoma and liver tumors. In 22 of 24 patients with FAP associated papillary thyroid carcinoma (PTC) who had genetic analysis, germ-line mutations of the APC gene, the tumor suppressor gene responsible for FAP, clustered before codon 1220, in the same genomic area usually associated with a non malignant alteration such as congenital hypertrophy of the retinal pigment epithelium (CHRPE). Interestingly, 4 of 5 of these patients had activation of the RET gene in the thyroid tumor tissue (ret/PTC1 isoform). Research: 1) Studies of genotype-phenotype correlation, i.e. correlation between the site of APC mutation and occurrence of CHRPE and PTC in different families; 2) Search for LOH of the APC gene in the peripheral tissue of FAP patients, either malignant (thyroid tumors) or benign (CHRPE), to confirm or exclude the two-hit Knudson hypothesis in the occurrence of these manifestations; 3) Analysis of ret/PTC isoform in different families with FAP associated PTC. Patients with FAP associated Hepatoblastoma (HB) had detection of their APC germ-line mutations by PCR-SSCP and sequencing. APC mutations were at codon 1061 (n=3), codon 213, 279, 554 and 1105, respectively. Adding 9 other patients from literature, who had detection of APC germ-line mutation, all 16 patients with FAP associated HB had their mutations out of the mutation cluster region (MCR, codons 1286-1513). Five of 16 (i.e. 1/3) had APC mutations at codon 1061 (n=4) or 1105, which determine the same truncated protein. On the contrary, patients with liver tumors other than HB had mutations 3’ to codon 1444. Aims of the research: 1) Studies of genotype-phenotype correlation, i.e. correlation between the site of APC mutation and occurrence of HB in different families; 2) Search for LOH of the APC gene in the liver tumoral tissue, to confirm or exclude the two-hit Knudson hypothesis; 3) Study of the Wnt-pathway by analysis of â-catenin in the liver tissue of patients with HB.
The CR1 gene and mammary carcinoma

Human Cripto-1 (CR-1) is the founding member of the epidermal growth factor (EGF)-Cripto-1/FRL1/cryptic (EGF-CFC) family of proteins. The EGF-CFC family consists of extracellular and cell-associated proteins that have been identified in several vertebrate species. The EGF-CFC family has been implicated in embryogenesis and in carcinogenesis. Overexpression of CR-1 has been reported in several types of human carcinomas, including colon, pancreas, ovary and breast while peri-tumoral normal tissues have been shown to be negative or weakly positive for CR-1 expression. In conclusion, inappropriate expression of CR-1 in adult tissues can lead to cellular proliferation and transformation and therefore may be important in the etiology and/or progression of cancer.

My research program is:
- Identification and characterization of the promoter region of human CR-1 using a full length CR-1 luciferase promoter reporter construct and several deletion constructs of the promoter region.
- Regulation of CR-1 expression assessing the effect of different growth factors, cytokines and hormones (Activin A, Activin B, TGF β-1, Retinoic Acid, BMP-2 and BMP-4) on full length CR-1 luciferase promoter reporter construct and deletion constructs of the promoter region in human embryonal carcinoma Ntera2/D1 cells and human breast cancer cells.
Clinical genetics of Rett Syndrome and Mental Retardation

The thesis focused on my personal experience of genetic counseling in Rett syndrome (RTT) and mental retardation. I had the opportunity to personally see almost 100 RTT families. Some of them were met only once and others were followed for years in order to reach a definitive molecular diagnosis or to provide the parents with pre-conceptual counseling and eventually with prenatal diagnosis. I became familiar with the preserved speech variant of RTT (PSV) and for the first time I contributed to describe a duplication of the MECP2 gene in a PSV patient. By adopting the clinical approach many hints for research became evident. I was able to demonstrate that beside MECP2, the CDKL5 gene is involved in RTT and it would appear responsible for a specific phenotype: the early seizure variant of RTT. RTT is mostly caused by “de novo” mutations in the MECP2 gene and thus the recurrence risk for the following pregnancy of a couple with a RTT girl is considered very low. The recurrence risk is not null since very few cases of germ-line mosaicism have been described. I witnessed the first case of germ-line mosaicism in RTT identified by prenatal diagnosis. The couple with the positive prenatal test decided to interrupt the pregnancy and to devolve fetal tissues of the affected fetus for research purposes to our Institute. Thanks to the availability of these tissues we are designing a panel of experiments aimed at the evaluation of the consequences of MECP2 absence on brain development at this stage. Since the RTT phenotype is almost exclusively seen in female patients, it has been thought for a long time that MECP2 mutations could be lethal in males. In the near past I contributed to describe a pathogenic MECP2 mutation in two related male patients with severe mental retardation and progressive spasticity. This report demonstrated that, in males, MECP2, can be responsible for severe mental retardation associated with neurological disorders. In the thesis I described a family with two brothers with microcephaly and mental retardation and some female relatives with isolated microcephaly. Probands were tested for MECP2 mutations and resulted negative. A possible X-linked semi-dominant inheritance was hypothesized and a linkage study on the X chromosome was performed. During my clinical activity I had the opportunity of seeing many sporadic or familial cases with mental retardation, either syndromic or not. In the last part of this thesis I reported on a male patient with severe mental retardation and mild dysmorphic features in whom an interstitial deletion of the short arm of chromosome 3 was identified. I discussed the phenotype and I reviewed the literature of the very few cases with deletions overlapping with that of our patient. Given the enormous and precious number of patients we currently visit during genetic counselling we decided to create a bank for both RTT and XLMR patients, which I described in detail. Biological samples (lymphoblastoid cell line and DNA) of each patient are stored and are available for researchers after intent statement. The bank also contains clinical and genetic information of each patient. Selected data are also accessible to the general public without a password. RTT bank includes only patients afferent to our Institute, while XLMR bank contains patients seen in clinical centers, including ours, spread all over Italy.

Part of this work was published in:
Intraductal Papillary mucinous tumours of the Pancreas

Cystic lesions of the pancreas has been steadily increasing with use of CT and US, accounting for 5-15% of cystic pancreatic masses. The first four cases of IPMT were recognized by Ohhash et al. only in 1982 and classified in IPMA (intraductal papillary-mucinous adenomas), IPMB (intraductal papillary-mucinous borderline), IPMC (intraductal papillary-mucinous carcinoma), nIPMC (non-invasive type) and IPMCs (muc vs. Tub) in 1997 by WHO. IPMT (Intraductal papillary mucinous tumours) and MCT (Mucinous cystic tumours) share rare incidence, mucin production, cystic mass and better prognosis vs. DC (ductal carcinoma) but IPMT have unique clinicopathologic findings. Completely resected MCT without evidence of invasive carcinoma do not recur or metastatize, IPMT due to its diffuse involvement and multifocality may recur after partial pancreatic resections. Different CT/US/RNM features seem to be present in IPMT and the role of ERCP is mandatory. e-US, pancreatic-duodenoscopy and intraductal-US have been also used. The presence of the major apomucin and cell-cycle regulatory proteins, immunohistochemical mucins (MUC1, MUC2, MUC5AC), p53 and ki-67 expression seems to be related with invasivity, but at present there is not a sensitive and specific marker of invasive carcinoma. Treatment criteria is not yet well defined. Different procedures have been proposed (DCP, total pancreatectomy, pancreatic resections guided by frozen section, conservative approach, follow-up). 31 patients with pancreatic carcinoma who underwent surgical resections between January 2001 and May 2005 at Human Patology and Oncology Department, Surgical Oncology Unit, Università degli Studi di Siena were retrospectively reviewed. We performed 5 distal pancreatectomies, 1 total pancreatectomy and 25 Whipple’s procedures. We found five cases of IPMT, 4 males and 1 female, mean age 65 years (range: 42-83). A fresh specimen and blood samples were obtained from all patients with pancreatic cancer. Intraductal growth and secondary chronic pancreatitis are associated with IPMT. Larger median cyst size and dilated main pancreatic duct have been found to be predictive factors of malignancy but Ca 19.9 does not predict invasivity. Mucin expression profile is related to biological and clinical characteristics but only MUC1 seems to be related to malignancy. MUC5 seems to be related to a benign course. Mucin expression profile is related to prognosis and can guide follow-up.
Identification of new genes in the Rett syndrome

Rett syndrome (RTT, OMIM#312750) is an X-linked progressive neurodevelopmental disorder that affects almost exclusively girls and it is characterized by a wide spectrum of clinical manifestations. The phenotype consists of microcephaly, stereotypic hands movements, loss of speech, autism, seizures and somatic hypoevolutism.

There is a classic form of Rett Syndrome caused by mutation in MECP2 (90%) but in 10% of cases the cause is unknown.

There are also 5 variants:
- * Preserved speech variant
- * Forme fruste
- * Congenital variant
- * Late Regression Variant
- * Early Onset Seizures Variant

For each form, the table showed the mutation detection rate in known genes.

In order to evaluate the negative cases we analysed candidate genes involved in pathogenesis of RTT; in particular we considered AF4 and HIPK2.

AF4 is located in the chromosome 4q21; it is implicated in childhood acute lymphoblastic leukemia. Mutation forme cause neurodegeneration in mouse.

HIPK2 is situated in the chromosome 7q33-34; it is a serine-treonine kinase implicated in transcriptional repression of sensory neuron survival.

<table>
<thead>
<tr>
<th></th>
<th>MECP2</th>
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<td>10%</td>
</tr>
<tr>
<td>Preserved Speech Variant</td>
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<td>0%</td>
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</tr>
<tr>
<td>Forme Fruste</td>
<td>10%</td>
<td>0%</td>
<td>90%</td>
</tr>
<tr>
<td>Congenital variant</td>
<td>10%</td>
<td>0%</td>
<td>90%</td>
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<tr>
<td>Late regression variant</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Early Onset Variant</td>
<td>0%</td>
<td>50%</td>
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</tr>
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</table>
The array-based Comparative Genomic Hybridization

Comparative Genomic Hybridization (CGH) was developed to measure copy number variations throughout the entire genome in a single experiment (Kallioniemi et al. 1992). As originally developed, CGH employs the comparative hybridization of differentially labeled genomic DNA from two (or more) cell populations to metaphase chromosomes (Fig.1A). The DNAs are usually labeled with fluorochromes. The ratio of the hybridization intensities along the chromosomes gives a measure of the relative copy number of DNA sequences in the genomes that hybridize to each location on the chromosomes. One of these genomic DNAs is obtained from a normal genome (reference), so that ratios directly map copy number variations in the test genome relative to the cytogenetic map provided by the chromosomes (Fig.1A). Microarray formats for array CGH have been developed over the last 10 years (Salinos-Toldo et al, 1997). Array CGH can provide improved quantitative accuracy, resolution, and dynamic range compared to chromosome CGH, and the measurements can be referenced directly to positions on the genomic sequence (Fig.1B and 1C). With the introduction of microarray-based CGH (aCGH), the main limitation of conventional CGH, a low resolution, is overcome. In array CGH, the metaphase chromosomes are replaced by cloned DNA fragments (±100–200 kb) with a known chromosomal location. This allows the detection of aberrations in more detail and, moreover, makes it possible to map the changes directly onto the genomic sequence. Microarray-based CGH builds upon the conventional CGH procedure, by the use of differentially labeled test and reference DNAs. Both DNAs are hybridized to cloned fragments, most often genomic DNA or cDNA, which are spotted on a glass slide (the array). The DNA copy number aberrations are subsequently measured by detecting intensity differences in the hybridization patterns of both DNAs. In array CGH, the resolution is determined by the distance between consecutive clones and the size of the used cloned DNA fragments. Thus, theoretically, arrays can be constructed covering any region of interest with any desired resolution. Recently, it has been demonstrated that up to 5% of patients with mental retardation and dysmorphisms presents submicroscopic chromosome imbalances (Flint et al. 1995; Knight et al. 1999; Biesecker 2002; de Vries et al. 2003). These findings underscore the potential importance of submicroscopic chromosomal anomalies as a major cause of human mental retardation and malformation. Array CGH is a suitable technique to detect the presence of these changes in patients with complex phenotypes. We plan to use high resolution whole genome array-CGH to analyze 17 patients with a complex phenotype suggestive of chromosomal submicroscopic imbalances and X-chromosome specific arrays to investigate 35 cases of familial X-linked mental retardation (http://www.biobank.unisi.it).
Retinoblastoma and drug resistant osteosarcoma

Genomic methylation patterns are frequently altered in tumor cells resulting in functional inactivation of several tumor-suppressor genes that is due to aberrant methylation in their promoter regions. Unlike genetic changes, epigenetic changes are pharmaceutically reversible using DNA de-methylating agents. Candidate genes regulated by transcriptional silencing due to promoter methylation are numerous. So it is possible to think that de-methylating agents allow to reactivate the expression not only of the genes improperly methylated in cancer but also of the constitutively methylated ones. CDNA microarray analysis allows to evaluate the global effect of this treatment on the different cell pathways and consequently to establish its real efficiency. I have worked out the results achieved by cDNA microarray analysis performed on a drug resistant human osteosarcoma cell line (HosDXR150) treated with de-methylating and de-acetylating drugs in order to identify gene expression pathways influenced by these treatments. The screening of the hierarchical clustering data of HosDXR150 treated with de-methylating and de-acetylating drugs, has identified a broad activation of genes related to tumor suppression, apoptosis, cellular differentiation and inhibition of angiogenesis. The re-expression of these genes could explain the effective proliferation decrease of HosDXR150 cell line after treatment with 5-aza-dC and TSA. On the opposite, genes related to cancer development, tumor progression and angiogenesis are generally down-regulated. Because HosDXR150 cell line decreases its proliferation and reactivates apoptotic pathways, it is possible to suppose that 5-aza-dC and TSA treatments reverse the MDR phenotype. On the same experimental base I have evaluated the effect of 5-Aza-dC treatment on a retinoblastoma cell line (Wer-Rb1). I assayed the proliferation index of Wer-Rb1 cell line treated with 5-AZA-2-deoxycytididine at different times (24, 48 and 96 hours). From the proliferation assay, the treatment has triggered an inhibition on cellular proliferation. I performed a FACS analysis in order to establish if this inhibition is due to G0-arrest or to activation of apoptotic pathway. FACS analysis revealed that reduction in cell number corresponds to an increase in the amount of apoptotic cells (27.71% of treated cells vs 6.7% of control). Total RNA samples were isolated from treated and untreated cells to assess the total effect of de-methylating drugs on this cellular line, and they will be analyzed in coming experiments.
Mutational screening of the RB1 gene in patients with retinoblastoma

Retinoblastoma is the most common intraocular tumour in infancy and early childhood. Constitutive mutations in the RB1 gene predispose individuals to RB development. I performed a mutational screening of the RB1 gene in Italian patients affected by RB referred to the Medical Genetics of the University of Siena. In 35 unrelated patients, we identified germline RB1 mutations in 6 out of 9 familial cases (66%) and in 7 out of 26 with no family history of RB (27%). Using the single-strand conformational polymorphism (SSCP) technique, 11 novel mutations were detected, including 3 nonsense, 5 frameshift and 4 splice-site mutations. Only two of these mutations (1 splice site and 1 missense) were previously reported. The mutation spectrum reflects the published literature, encompassing predominantly nonsense or frameshift and splicing mutations. RB1 germline mutations were detected in 37% of our cases. Gross rearrangements outside the investigated region, altered DNA methylation, or mutations in non-coding regions, may be the cause of disease in the remainder of the patients. Some cases, e.g. a case of incomplete penetrance, or variable expressivity ranging from retinoma to multiple tumours, are discussed in detail. In addition, a case of pre-conception genetic counselling resolved by rescue of banked cordonal blood of the affected deceased child is described.

Part of this work is published in:
Array CGH deletion mapping excludes the involvement of VCX-A in mental retardation

The project focuses on the molecular analysis of mentally retarded patients with putative X-chromosome deletions. VCXA has been recently proposed as a candidate mental retardation (MR) gene. To better define its involvement in MR, we have performed array-CGH deletion mapping using a 200 kb resolution X chromosome specific BAC array. We have compared the deletion present in a familial case with X-linked ichthyosis (XLI) and MR to that present in a sporadic case with XLI and Kallmann syndrome (KAL). In the case with XLI and KAL, array-CGH analysis showed the presence of a large deletion of approximately 3 Mb. The deletion includes the STS gene, the KAL1 gene (responsible for KAL) and also VCXA, VCXB, VCXB1 and VCXC genes. In the case with XLI and MR the analysis revealed the presence of a smaller deletion, approximately 0.6 Mb, spanning from clone RP11-483M24 to clone RP11-645P06. The deletion does not include the VCXA gene, which has been previously suggested to be involved in mental retardation. The deletion involves the STS gene responsible for XLI (OMIM#308100) and only a second gene, named HDHD1A. This gene, also known as GS1 (OMIM#306480), has been reported deleted in patients with isolated XLI. The deletion map in these two cases excludes the involvement of the VCX genes in mental retardation. Additional studies are necessary to exactly define the deletion break-points and to clarify the phenotypic differences in the family. The pedigree could help us to explain this case.
The Cohen syndrome and the COH1 gene
My project concerns the clinical and molecular analysis of patients with Cohen syndrome. Particularly, I will perform the genetic counselling for patients who will be referred to our clinical ambulatory. The molecular analysis is performed by DHPLC by the Medical Genetics laboratory of Siena.

Cohen syndrome is an autosomal recessive syndrome characterised by non progressive mental retardation and peculiar facial features represented by microcephaly, high vaulted palate, short philtrum, prominent maxillary central incisors, micrognathia. Other physical characteristics are hypotonia, joint hyperlaxity, truncal obesity with slender extremities (narrow hands and feet, elongated fingers and toes), retinochorioidal dystrophy, early onset progressive miopia and, at blood level, a characteristic recurrent neutropenia.

The Cohen syndrome gene has been identified in May 2003 and it has been named COH1. It spans 62 exons and 5 isoforms have been described. It encodes for a 4022 aminoacids protein which is probably involved in vesicle-mediated sorting and intracellular protein transport.

Clinically, we can distinguish “mayor diagnostic criteria” (non progressive mental retardation, facial gestalt, retinochorioidal dystrophy and recurrent neutropenia) and “supportive criteria” (early onset progressive myopia, truncal obesity with slender extremities, microcephaly and joint hyperextensibility and/or slender fingers). We consider the diagnosis as “certain” when the patient presents 3 out of 4 mayor criteria plus 3 out of 4 supportive criteria; “probable” when the patient presents 3 out of 4 mayor criteria plus 2 out of 4 supportive criteria; “possible” when the patient presents 3 out of 4 mayor criteria plus 1 out of 4 supportive criteria. These grading allows us to classify patients on the basis of the clinical features.

At present, we have identified 17 patients; 6 have been recognized as “certain”, 6 as “probable” and 5 as “possible” Cohen patients. All patients undergo molecular analysis.

We have analysed 20 exons and we have identified 3 point mutations: p.Q721fsX744; p.R1143X; p.R2535X (recurrent in the German population). We have not found the polymorphism p.C1117fsX1124 prevalent in the Finnish population.
Neural differentiation of mesenchimal stem cells in Rett Syndrome

Rett Syndrome (RTT) is a progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females (1 in 10,000/15,000). RTT is an X-linked dominant disease with lethality in hemizygous males. This syndrome is caused by mutations in MeCP2 gene that codify a transcription regulating protein. MeCP2 protein contains a methilcytosine binding domain (MBD) that binds methylated cytosine and a transcriptional repressor domain (TRD) that interacts with the co-repressor Sin 3A, which in turn binds HDACs 1 and 2. This interaction allows MeCP2 to repress transcription by stimulating the remodelling of chromatin from an active, acetylated state to an inactive, deacetylated state.

The mutated MeCP2 protein might alter neural differentiation by de-regulating the “physiologic” gene expression at the different stages of maturation process.

The aim of my research project is to study the role of MeCP2 gene in neural differentiation of mesenchimal stem cells (MSCs) withdrawn from patients affected by Rett Syndrome. The MSCs culture offer the possibility to study in vitro the various stages of “cell commitment” and differentiation. MSCs can represent a good experimental model to analyze the alterations of the neural differentiation process in patients affected by RTT. In fact, the “relative” facility with which the MSCs can be withdrawn from the patients and their remarkable somatic plasticity allow to prepare cellular cultures that will allow to analyze in vitro the different stages of neural differentiation. In detail, the neural differentiation process in MSCs obtained by healthy relatives will be compared to RTT patients one by immunocitochemistry assayes, Western blots and semi-quantitative RT-PCR.

The expected results are:
- to identify the altered biological processes in MSCs of patients affected by RTT
- to identify “master genes” whose expression is altered as an effect of mutation in MeCP2 gene.
Functional variants in genes involved in the metabolism of the 5-fluorouracil
Fluoropyrimidine prodrugs are the drugs of choice for the treatment of colorectal, breast and head and neck cancers. The conversion of 5-Fluourouracil (FU) into fluorodeoxyuridine monophosphate (FdUMP) leads to the inhibition of thymidylate synthase, the key enzyme of de novo deoxythymidine 5'-monophosphate synthesis, and subsequent DNA synthesis arrest. Although the palliative treatment of advanced colorectal cancer prolongs the medium survival, long-term survival is rare. Very frequently, drug side effects lead to the death of the patient. Moreover there is a great inter-individual variation in drug response among patients that can be due to specific clinico-pathological features, environmental factors and in particular individual’s genetic profile. Polymorphisms in drug targets can alter the sensitivity of patients to treatment, changing the pharmacodynamics of drug response. The aim of this project is to analyse the entire coding sequence of genes involved in 5-FU metabolism in patients treated with this drug, in order to identify polymorphisms of these genes and then evaluate the relationship between genetic variants and drug response. Firstly the analysis will be focused on two genes: TYMS and OPRT. TYMS is located on chromosome 18 (18p11.32), consists of seven coding exons and codes for thymidylate synthase that is the target of 5-FU antitumor activity. OPRT is located on chromosome 3 (3q13), consists of six coding exons and codes for orotate phosphoribosyltransferase, responsible for the activation of 5-FU. The study will be conducted on DNA obtained from colonic mucosa or blood of patients treated with fluoropyrimidine-based chemotherapy. The coding sequence will be analysed with DHPLC method and further sequencing.
Molecular characterization of gastric adenocarcinoma

Gastric adenocarcinoma is the fourth most common cancer in the world, the second only to lung cancer in the number of deaths it causes. It presents peak incidence at 50-70 years and is more frequent in males. Our study in Tuscany was focused to immunohistochemical analysis of gastric adenocarcinomas, to evaluate the expression of RB2/p130, AKT-P, p27, cyclin D1, E, and A, and to correlate immunohistochemical data and diagnostic factors (i.e. anamnesis, TNM). E-cadherin (CDH1) gene was also studied by automatic sequencing, Western blot and CpG Island Methylation analysis on mutation-free samples using MSP (in progress). By these studies we intend to suggest a new surgical strategy for prevention of gastric cancer based on the presence of CDH1 mutations and CDH1-promoter methylation. In particular, Western blot analysis for E-cadherin, in 3 gastric cell lines (N-87, KATO-III, AGS), revealed that AGS cell line does not present CDH1 product. Western blot for the tumor suppressor gene RB2 has been performed for the three lines, demonstrating that N-87 cell line has higher expression levels of the hypophosphorylated (active) pRb2/p130 form. Further Western analysis will be done for CDH1 and RB2 genes after treatment of the above lines with demethylating agents. In our future research, cDNA-microarray screening of a pool of gastric cancer cell lines under treatment with DNA methyltransferase inhibitors (Azacitidine) may help in the identification of new diagnostic and prognostic markers in gastric carcinomas.
Thesis discussion
Doctorate in Medical Genetics
May 30, 2005 room 7 Centro Didattico S. Maria alle Scotte, 12.30

12.30 Entering of the PhD dissertation board composed by

- Prof. Romano Tenconi, (President)
  Professor of Medical Genetics, University of Padova, Italy
- Prof. Orsetta Zuffardi, (Member)
  Professor of Medical Genetics, University of Pavia, Italy
- Prof. Alessandra Renieri, (Secretary)
  Professor of Medical Genetics, University of Siena, Italy

12.45 Thesis discussion in English language

- "Clinical genetics of Rett syndrome and mental retardation", Francesca Mari cycle XVII

13.30 Awarding of the PhD degree in Medical Genetics

13.45 End of session

A copy of the thesis is available at http://www.unisi.it/ricerca/dottoratiweb/genetica_medica/
accessing the "PhD student" link and then "PhD student in Medical Genetics".
Starting from April 13, 2006, it is possible for a PhD student to get the additional title of “Doctor Europaeus”. This title can be conferred during the final examination by the University of Siena, which is one of the Italian pioneer Universities in this field, when the following criteria are fulfilled:

- the authorization to the final PhD dissertation is accorded in the light of the reports on the thesis compiled by at least two professors belonging to two superior education institutions of two member states of the European Community different from that in which the doctorate is held;

- at least one member of the PhD dissertation board which confers the PhD qualification belongs to a superior education institution of one member state of the European Community different from that in which the doctorate is held;

- the PhD dissertation is carried out at least partially in a language of the European Community different from the national one of the state in which the doctorate is held;

- the PhD thesis must have been prepared partially following a research stay of at least three months in one member state of the European Community different from that in which the doctorate is held.

Thus, starting from April 2006 each candidate for the PhD degree could be evaluated in relation to the above reported criteria in order to decide the bestowal of qualification of Doctor Europaeus.
Thesis discussion
Doctorate in Medical Genetics
June 27, 2006 room 7 Centro Didattico S. Maria alle Scotte, 16.30

16.30 Entering of the PhD dissertation board composed by

- Prof. Lucio Luzzatto, (President)
  Istituto Toscano Tumori Scientific Director, Florence, Italy;
  Professor of Hematology, University of Genoa, Italy
- Prof. Hans Van Bokhoven, (Member)
  Human Genetics, Radboud University, Nijmegen, The Netherland
- Prof. Mario De Marchi, (Secretary)
  Professor of Medical Genetics, University of Turin, Italy

16.45 Thesis discussion in English language

- "Molecular defect in MECP2-negative Rett patients", Francesca Ariani cycle XVIII

17.45 Thesis discussion in English language

- "Genomic deletions in patients with complex phenotype: from cytogenetics to array-CGH", Chiara Pescucci cycle XVII

18.45 Evaluation of both candidates for qualification of Doctor Europaeus

The PhD dissertation board took into account the report of the external PhD theses reviewers, who were:
- Dr. Guy Froyen, Dept. Human Genetics, Catholic University of Leuven, Belgium
- Prof. Vaidutis Kucinskas, Professor and chairman, Dept. of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
- Dr. Lina Florentin-Arar, Director, Molecular Biology and Cytogenetics Center, Athens, Greece

19.15 Awarding of the PhD degree in Medical Genetics and of the qualification of Doctor Europaeus

19.20 End of the session

A copy of the thesis is available at http://www.unisi.it/ricerca/dottorationweb/genetica_medica/ accessing the "PhD student" link and then "PhD student in Medical Genetics"
Doctor Europaeus
Awarding of qualification in Medical Genetics
June 27, 2006 room 7 Centro Didattico S. Maria alle Scotte, 16.30