# XI Congresso Nazionale A.I.F.E.G.
# Pavia-Collegio Ghislieri; 24-25 Ottobre 2013
# Abstracts

### Characterization of alternative transcripts in *MUTYH*-associated-polyposis patients carrying the p.Gly396Asp mutation

Bartolo Bono[1], Antonella Balsamo[1], Elisa Giacomin[2], Alessandra Viel[2] and Tiziana Venesio[1]
*[1]Molecular Pathology Laboratory, Institute for Cancer Research and Treatment, Strada Provinciale 142, 10060 Candiolo-Torino, Italy [2]Experimental Oncology, Centro di Riferimento Oncologico, IRCCS, Aviano (PN)*

*MUTYH* gene, coding for a glycosylase of the BER complex, is composed of 16 exons and gives rise to 15 possible different transcripts. The presence of a high number of transcripts has been recently correlated with the deletion of some exons in *MUTYH*-associated-polyposis (MAP) patients (*Plotz et al., 2012*).The aim of this study was to understand if the common c.1187G>A (p.G396D) germline mutation, localized on the 3' splice site between intron 12 and exon 13, is able to lead to alternative transcripts formation.*MUTYH* transcript was analyzed in peripheral blood RNA from health individuals and 14 MAP patients carrying c.1187G>A mutation as homozygotes (3) or heterozygotes (11). RNA of these cases was transcribed into cDNA, amplified on exons 10⎪11-16 by RT-PCR, subjected to specific "nested PCR" and characterized by sequencing, Bioinformatic methods were used to predict the splicing in this region with c.1187G>A variant.The skipping of exon 12 was found in all homozygous and one heterozygous patients. In addition, the retention of intron 12 was detected in one of the homozygous patients displaying the skipping and in 3 heterozygous patients. The analysis by ESEfinder and SpliceAid software suggested that c.1187G>A variant can alter the sequence leading to the dislocation of SR factors involved in splicing regulation.These data indicate that c.1187G>A variant exerts its pathogenetic effect not only as a missense mutation but also as a modulator of the transcriptional expression of *MUTYH*.

### TWIST1 positive cells in the stroma of colorectal cancer: tumor cells in disguise

Giuseppe Celesti[1], Giuseppe Di Caro[1], Paolo Bianchi[1], Fabio Grizzi[1], Gianluca Basso[1], Andrea. Doni[2] , Federica Marchesi[2], Giancarlo Marra[3], Massimo Roncalli[4, 6], Alberto Mantovani[2, 6], Alberto Malesci[5, 6], Luigi Laghi[1, 5]
*[1]Molecular Gastroenterology and [2]Immunology Lab, [4]Pathology and [5]Gastroenterology Dept., Humanitas Clinical and Research Center, Rozzano, Milano, Italy. [3]. Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland. [6]. Translational Medicine, University of Milan, Milan, Italy*

**Background** Cancer cells undergo an epithelial-to-mesenchymal transition (EMT) to become invasive, allowing tumors to progress. However, there is no direct evidence that human cancer cells undergo an EMT. **Aim** To demonstrate the existence of EMT-neoplastic cells in mesenchymal disguise in the stroma of human colorectal cancer (CRC). **Methods** We measured the expression of TWIST1 in human colorectal cancer (CRC) cell lines and examined the effects of overexpression or knockdown in vitro and in mice. We used immunohistochemistry to measure levels of TWIST1 in 201 colorectal tumor samples. In 20 samples, immunostaining was combined with fluorescence in situ hybridization analyses. Levels of TWIST1 messenger RNA (mRNA) were measured in blood samples from 15 patients. **Results** We documented EMT in a human cancer cell-line, CoLo741, with mesenchymal phenotype. TWIST1 is crucial in maintaining EMT and in increasing invasiveness of CRC cells. In 201 CRC specimens, TWIST1 expression in the stromal compartment was associated with advanced stage, and worse survival. Out of 20 CRC, 17 showed chromosome 7 and 20 trisomic epithelial neoplastic cells, and in all these 17 cases, we traced the same trisomies only in TWIST1+ cells in the stroma. Blood levels of *TWIST1* mRNA were significantly higher in CRC patients than in controls. **Conclusion** The stroma of human colorectal tumors contains TWIST1-positive cancer cells with mesenchymal phenotypes. Patients with CRC have higher levels of TWIST1 mRNA than healthy individuals.

### Coexisting constitutional promoter methylation and large genomic deletion of the *MLH1* gene in a patient with Lynch Syndrome

[1]Giulia Cini, [1]Elisa Giacomini, [1]Michele Quaia, [2]Maria Grazia Tibiletti, [2]Annamaria Chiaravalli,
[2] Ileana Carnevali [1]Alessandra Viel,
*[1]Experimental Oncology 1, Centro di Riferimento Oncologico, IRCCS, Aviano (PN); [2]Unit of Pathology, Ospedale di Circolo-Università dell'Insubria Varese.*

The majority of Lynch Syndrome patients have point mutations inactivating one of the four *MMR* genes. Improved analysis technologies have also led to the identification of several large genomic rearrangements, primarily deletions, in a variable proportion of cases, and rare germline epimutations consisting of constitutional promoter methylation of the *MLH1* and *MSH2* genes. While methylation of the *MSH*2 gene has been clearly linked to coexisting *in cis* genomic deletions of the neighboring *EPCAM* gene, methylation of the *MLH1* gene is believed to be primarily due to *in trans* acting elements.We report here a case with a heritable large genomic deletion of *MLH1*, which was associated with constitutional partial promoter methylation of the same gene. The large deletion, involving only exon 1 of the *MLH*1 gene, was found by MLPA analysis in a patient with colorectal and endometrial cancers (30 and 40 years) displaying MSI-H and loss of the MLH1 protein. She had a strong family history with several early onset intestinal cancers, throughout 3 generations. However, blood of this carrier also revealed an *MLH1* promoter hypermethylation, detected by 3 out of 5 *MLH1* probes of the MS-MLPA kit. The two non-methylated peaks corresponded to sequences which were included in the exon 1-deletion. Both deletion and methylation have been confirmed also in tumor DNAs of the proband and his affected brother. We hypothesize that hypermethylation is not an independent event, but it is induced by a so far unknown mechanism related to the presence of the concurrent deletion.

### *APC* and *MUTYH* Allele-Specific Expression in mutation-negative patients with multiple colorectal adenomas (MCRAS) and CRC

[1]Curia Maria Cristina, [2]De Iure Sabrina, [2]Fantini Fabiana, [3]Di Filippo Pamela, [2]Battista Pasquale, [1]Palka Giandomenico, [4]Valanzano Rosa, [4]Tricarico Rossella, [5]Martayan Aline, [5]Stigliano Vittoria, [6]Di Gregorio Patrizia, [3]Cama Alessandro, [2]Aceto Gitana.
*Departments of [1]Medical, Oral and Biotechnological Sciences; [2]Clinical and Experimental Sciences; [3]Drug Sciences, "G. d'Annunzio" University, Chieti-Pescara; [4]Clinical Pathophysiology, Medical Genetics Unit, University of Florence; [5]Clinical Pathology Unit, Regina Elena National Cancer Institute-IRCCS, Rome; [6]Division of Immunotransfusion, "SS. Annunziata" Hospital, Chieti.*

Altered germline allele-specific expression (ASE) may be a useful marker of genetic predisposition to cancer or other diseases. Germline APC mutations and ASE are associated with familial adenomatous polyposis (FAP). Also MUTYH mutations are associated to polyposis (MAP) and carriers of MUTYH bi- and monoallelic mutations have an increased risk of developing gastrointestinal tumours. In this study we analyzed whether germline APC and MUTYH ASE variations may be associated with low penetrant familial cancers and whether pathogenic APC and MUTYH mutations had impact on ASE. In peripheral blood lymphocytes (PBLs) of 53 APC-negative CRC and/or MCRA patients, we identified 14 samples heterozygous for the frequent APC coding SNP rs2229992 (c.1478C>T). Moreover, to assess the effect of APC mutations on ASE we analyzed 17 carriers of pathogenic variants. Six of these individuals were heterozygous for the rs2229992 marker and 1 of them, bearing the pathogenic APC p.Ser895X, showed allelic loss by ASE analysis. Moreover, we analyzed ASE of MUTYH among APC-negative patients. Twenty-one of these patients were heterozygous for the frequent MUTYH coding SNP rs3219489 (c.1014G>C). Three patients, APC- and MUTYH-negative, showed ASE values deviating more than 1SD from the mean ASE values measured so far in 55 individuals (21 cases and 34 out of 160 healthy donors). Our study underlines the importance of RNA-level studies in the molecular diagnosis of patients with MCRAs and/or CRC without detectable mutations in APC and/or MUTYH.

**Endometrial cancer and Lynch Syndrome: S.I.O.G. and AIFEG**

Antonino Ditto[1,2], Lucio Bertario[1], Adriano Decarli[3]
*[1]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; [2]Società Italiana Ginecologia Oncologica (SIOG); [3]Università degli Studi di Milano, Milan, Italy.*

OBJECTIVE: It is known that 2.5% of all endometrial cancer (EMCA) patients have Lynch syndrome (LS). This syndrome, formerly known as hereditary nonpolyposis colon cancer (HNPCC), is caused by a mutation in Mismatch Repair (MMR) Genes and is associated with multiple malignancies. The main cancer risks for women with LS include a 40–80% risk of colon cancer, 40–60% risk of endometrial cancer, 10–12% risk of ovarian cancer, as well as a smaller risk of urinary tract, small bowel, stomach, biliary tract and brain tumors. A questionnaire was developed to investigate whether gynecologist takes in consideration familiar and personal genetic risk and advice genetic counseling in and out-patients. METHODS: An ad-hoc synthetic questionnaire was developed and disseminated electronically to members of the SIOG to be completed online. Special queries were addressed to assess how frequently LS were suspected and diagnosed in patients with EMCA, according to the different institutions and how the approach and management was standardize, particularly considering the practice of the genetic tests. RESULTS: Five hundred members viewed the questionnaire; however, only 1/5 members fully completed it. CONCLUSIONS: The physician awareness of LS is good. The gynecologists generally investigate the familiar and personal genetic risk. There are wide variations in the use of genetic counseling. This survey could create the basis for future collaboration in research protocols.

**Genetic analysis in siblings showing the same *MUTYH* germline genotype but different morphological and clinical phenotypes**

Edoardo Errichiello[1], Antonella Balsamo[1], Paolo Fortina[2], Guglielmina N. Ranzani[3], Mauro Risio[1] and Tiziana Venesio[1]
*[1]Molecular Pathology Laboratory, Institute for Cancer Research and Treatment, Strada Provinciale 142, 10060 Candiolo-Torino, Italy; [2] Kimmel Cancer Center, 233 S. Tenth Street, Suite 1009, Philadelphia, PA 19107, USA;[3]Dept.of Biology and Biotechnology, University of Pavia, Pavia, Italy.*

*MUTYH* is a DNA glycosylase involved in the preservation of DNA from oxidative stress. Mutations of this gene are tipically linked with a specific hereditary colorectal cancer syndrome termed *MUTYH*-associated polyposis (MAP) and have been recently associated with other "oxidative diseases", in both oncological (follicular thyroid carcinoma) and neurodegenerative (age-related macular degeneration) settings. MAP carcinogenesis still remains partially unknown even if an accelerated tumour progression can be a feature of this syndrome. Our project aims to better characterize the MAP carcinogenetic pathway and to identify new predisposing/protective genetic variants that may confer a higher/lower risk of malignant transformation. We analysed two couples of sisters in two independent pedigrees, each carrying the same *MUTYH* germline mutations (p.G396D/c.1229insGG and p.Y179C/p.Y179C, respectively) and comparable individual characteristics (such as gender and age) but unexpectedly showing very different clinical, morphological and molecular (*KRAS* p.G12C and *BRAF* p.V600E mutations) features in their neoplastic lesions. Our preliminary findings suggest that other factors may be involved in MAP tumorigenesis and *MUTYH* biallelic mutations are probably not the only genetic alteration affecting the MAPK/ERK signalling cascade, whose oncogenic disruption definitely drives the malignant transformation together with the imbalance of mitochondrial oxidative phosphorylation.

**Nutritional and physical activity intervention in a family cancer clinic: a factibility study**

Giabardo C[1]., Forni C. [1], Pegoraro MC. [1], G. Schiavo[2], Padovani M. [1], Binato S. [1], Magazù M. [1], Oliani C. [1], Barana D. [1].
*[1]U.O.C Oncologia, ULSS5 Ovest Vicentino (VI); [2]Servizio di Dietetica e Nutrizione Clinica, ULSS5 Ovest Vicentino (VI)*

Overweight and obesity seems to be associated with a higher risk of developing colorectal cancer and body mass index is possibly correlated to clinical outcome in early stage breast and colorectal cancer pts. Furthermore recent studies have shown that excess body weight increases the risk of incident colorectal adenomas in people with Lynch Syndrome. Since March 2012 a Dietetics and Physical Activity Program is run at the Oncology Unit. The attendance to this project is also offered to obese /overweight patients (BMI > 25 Kg /mq) BRCA and MMR gene carriers followed in our Family Cancer Clinic. The first nutritional evaluation comprises all the clinical anamnestic data, the medical examination, the body composition measurement through bioelectric impedance analysis, the measure of resting energy expenditure using indirect calorimetry , the measure of energy expenditure through arm-band_and biochemistry tests. The SF36 quality of life questionnaire and psychological interview are also carried out. A personalized nutritional intervention and regular leisure-time exercises are therefore established. Patients are followed monthly by a dietitian and the psychologist in order to verify compliance and monitor the weight trend. After six months of treatment all the parameters and measurements at the start of treatment are reassessed in order to evaluate the efficacy of the intervention. Up to now, 7 out of 38 Lynch gene carriers fulfilled the project criteria. Three out of 7 have accepted to partecipate and have concluded the dietary program with a median 6 kg weight loss.

**Isolated loss of *MSH6* associated to a germline mutation in *MSH2*: report of the first case**

Daria Loconte[1]*, Margherita Patruno[1]*, Patrizia Lastella[2]*, Carmela Di Gregorio[3], Valentina Grossi[4], Giovanna Forte[4], Dora Varvara[1], Rosanna Bagnulo[1], Cristiano Simone [1,5], Nicoletta Resta[1], Alessandro Stella[1]
*[1] Division of Medical Genetics, Department of Biomedical Sciences and Human Oncology, Università di Bari "Aldo Moro", Bari, Italy; [2]Geriatric Unit and Rare Disease Center, University Hospital of Bari, Bari, Italy; [3] Department of Laboratory, Pathology, and Legal Medicine, University Hospital Polyclinic of Modena, Modena, Italy; [4] Cancer Genetics Laboratory, IRCCS 'De Bellis', Castellana Grotte (BA),Italy; [5] Laboratory of Signal-dependent Transcription, Consorzio Mario Negri Sud; Santa Maria Imbaro (Chieti), Italy*

Since the original discovery that Lynch syndrome (LS) is caused by germline mutations in one of the four (hMLH1, hMSH2, hMSH6 and hPMS2) DNA mismatch repair (MMR) genes more than 20 years ago, molecular testing of these four genes has been widely used to diagnose the syndrome in patients. The positive testing of suspected cases is important for patient's management and preventing strategies in order to minimize cancer risk in the proband and its relatives. However, nearly one third of the reported mutations does not lead to obvious non-functional consequences on the encoded protein. Thus, a relevant fraction of these changes has been termed variants of uncertain significance (VUS). The ability to proof their causative nature is fundamental to adopt the most appropriate surveillance strategies for the patients where they are identified. Here, we describe an LS family harboring a previously unreported germline mutation of MSH2. To our knowledge, this is the first description of an in-frame three nucleotide insertion in MSH2 (c.1249-1251 dupGTT, p.417V-418I dupV). The mutation is unique since the two available tumors from affected family members both show lack of MSH6 protein despite a wild type MSH6 gene sequence. The thorough molecular and biochemical analysis of the mutation demonstrated that it represents the first case of a causative mutation in MSH2 leading to isolated loss of protein expression in MSH6. Considering the traditional MSI-IHC-gene sequencing approach routinely used in diagnostic laboratories involved in LS patients testing, the reported case may represent a novel mechanism responsible, at least in part, for those MSI-H cases with no apparent MMR defect.

Finally, as a further unique feature of the reported family, in one tumor sample the loss of MSH6 expression was concurrent to two different "second hit" one in the MSH2 gene and a second in MSH6.

**Inherited predisposition to colorectal adenomas and carcinomas: screening for mutations in the proofreading domain of POLε and POLδ polymerases**

Monica Marabelli[1], Valeria Molinaro[1], Antonella Balsamo[2], Tiziana Venesio[2] and Guglielmina Nadia Ranzani[1].
*[1]Department of Biology and Biotechnology, University of Pavia; [2]Unit of Pathology, Institute for Cancer Research and Treatment, Candiolo, Torino.*

Patients with multiple colorectal adenomas and carcinomas are usually screened for germline mutations in *APC* and *MUTYH* genes, which underlie familial adenomatous polyposis (FAP) and MUTYH-associated polyposis (MAP), respectively. However, the known susceptibility genes do not account for all familial cases, making it challenging to provide genetic counseling. Recently, it has been shown that germline mutations in *POLE* and *POLD1* genes can predispose to multiple colorectal adenomas and/or CRC. These genes encode for the major components of the DNA polymerases ε and δ; the identified mutations map in the proofreading domain of these proteins, affecting replication fidelity and, consequently, genomic stability.
In light of this finding, from a large series of cases we selected 30 patients proved to be mutation-negative for both *APC* (by PTT, sequencing and MLPA methods) and *MUTYH* (by sequencing) genes. We are currently searching for mutations in POLε and POLδ proofreading domains by sequencing the corresponding coding exons in *POLE* and *POLD1* genes. No constitutive mutations have been identified up to now, suggesting that germline *POLE* and *POLD1* variants are rarely associated with intestinal adenomas and CRC.

**Heterogeneous molecular mechanisms underlie Hereditary Diffuse Gastric Cancer syndrome**

Valeria Molinaro[1], Valeria Pensotti[2], Monica Marabelli[1], Irene Feroce[3], Monica Barile[3], Simonetta Pozzi[3], Lucia Fini[4], Luigi Laghi[4], Davide Serrano[3], Loris Bernard[2], Bernardo Bonanni[3] and Guglielmina Nadia Ranzani[1].
*[1]Department of Biology and Biotechnology, University of Pavia; [2]Cogentech, IFOM-IEO Campus, Milan; [3]European Institute of Oncology (IEO), Milan; [4]Humanitas Clinical Institute (ICH), Rozzano – Milan.*

The inactivation of *CDH1* gene (E-cadherin) is a well-established defect in gastric cancers (GC) of both diffuse and intestinal histotypes. Germline defects of *CDH1* have been associated with Hereditary Diffuse Gastric Cancer (HDGC), an autosomal dominant syndrome (1-3% of GC cases) highly predisposing to diffuse-type GC and to lobular breast cancer.We searched for *CDH1* germline lesions in 32 HDGC probands selected according to international consensus criteria. We performed a series of complementary approaches on both DNA and RNA including: DNA sequencing, in silico analysis, MLPA, SNuPE, RT-PCR, Realtime RT-PCR and bisulfite-sequencing. All these techniques allowed us to identify different types of *CDH1* molecular defects in 19% of probands. Loss/aberrant expression of E-cadherin has recently been associated with alterations in miR-200 family members targeting the 3'-UTR of *ZEB1/2* and *SUZ12*, and in miR-101 targeting the 3'-UTR of *EZH2* and *PTGS2* genes. We searched for mutations of these miRNAs and corresponding targets sequencing the DNA of the probands without *CDH1* disease-causing defects. No germline mutations were identified, suggesting that other mechanisms may be involved in E-cadherin loss/downregulation. To find new genes possibly associated with HDGC we are currently applying next generation sequencing. Results are expected to increase mutation detection rate, improving genetic counseling and management of the families at risk.

**An unusual case of polyposis with very early symptom occurrence**

M.Ponz de Leon*, A.Bianchini°, L.Reggiani Bonetti*, M.Pedroni*, C.Di Gregorio*, A.Merighi [Δ], A.Cacciari°.
** Dipartimento di Medicina Diagnostica, Clinica e Sanità Pubblica, Università di Modena e Reggio Emilia; ° Struttura Complessa di Chirurgia Pediatrica, Policlinico, Modena; [Δ] Struttura Complessa di Gastroenterologia, Policlinico, Modena*

We report the clinical case of a young patient who manifested an "accelerated" form of Familial Adenomatous Polyposis (FAP), with development of major lesions within the first decade of life. The patient is a member of the last generation of a FAP family already described in various reports, and featured by profuse polyposis around the age of 20 years, desmoid tumors in the majority of affected individuals of both sexes, and an uncommon APC gene mutation (4394ins15del310). The proband showed features of multiple desmoids as early as age 2-3 years. In that period numerous polyps developed in the large bowel and, in addition, ultrasound and liver biopsy led to the diagnoses of hepatoblastoma. Partial liver resection was carried out with full recovery of the patient. However, the proband underwent several operations for removal of desmoid tumors from the age of 3 years. The tumors were located in subcutaneous tissues of the abdomen and of the back, as well as in the surgical scars of the previous liver resection. These removed fibrous masses were between 3 and 10 cm of major diameter. Owing to the increasing number of polyps in the large bowel, at age 9 the proband was operated of total colectomy with endorectal pull-through of the ileum. At the last control (2013, age 12) physical and mental development were normal; new desmoid, however, developed contiguous to the surgical scar and chemotherapy (Vinorelbin, Metotrexate) was initiated. No other member of the family had symptom appearance as early as the proband, and no other relative showed features of hepatoblastoma. Among the possible interpretations of this anomalous and accelerated clinical course we propose at least 3 possibilities:
1.Presence of unknown environmental factors (alimentation? Unusual leisure habits?) which might interfere with the lack of function of APC; 2.Presence and influence of a second mutation – or a polymorphism – in the APC gene; 3.Concomitant mutation in a second gene, especially among those controlling cell survival or genome instability.

**Mutazione del gene *CDH1*: due casi clinici**

Simonetta Pozzi[1], Bernardo Bonanni[2], Monica Barile[2], Davide Serrano[2], Irene Feroce[2], Loris Bernard[3], Guglielmina Nadia Ranzani[4], Valeria Molinaro[4], Monica Marabelli[4], Valeria Pensotti[3], Angelica Sonzogni[5], Bruno Andreoni[6] e Roberto Biffi[1].
*[1]Istituto Europeo di oncologia divisione chirurgia addomino pelvica; [2]Istituto Europeo di oncologia divisione di prevenzione e genetica oncologica; [3]IFOM-IEO Campus; [4]Dipartimento di Biologia e Biotecnologie,Università di Pavia; [5]Istituto Europeo di oncologia divisione di anatomia patologica; [6]Istituto Europeo di oncologia divisione di chirurgia generale*.

La mutazione germinale del gene CDH1 è responsabile per la sindrome del carcinoma gastrico diffuso ereditario, il rischio di sviluppare un tumore gastrico (TG) nei carriers è del 80% e, nelle donne, si associa il 20-40% di rischio per il tumore lobulare della mammella (TLM). Famiglia S: paziente con diagnosi di TLM a 36 anni e familiarità positiva per TLM e TG. Si esegue consulenza e test genetico: in prima istanza per BRCA 1-2 (risultato WT) poi per CDH1 (riscontro di mutazione). Alla luce del risultato si discute l'indicazione alla gastrectomia profilattica. La paziente decide per la gastrectomia profilattica, nonostante EGDS con biopsie negative, che rivela un TG pT1a pN0, G3. L'accertamento nei consanguinei ha confermato la stessa mutazione nel fratello e nello zio. L'EGDS del fratello conferma la presenza di un TG. Famiglia B: paziente affetta da linfoma di Hodgkin trattato con CT e RT all'età di 22 anni; a 32 anni gastrectomia per carcinoma indifferenziato diffuso pT3 pN1, G4. La familiarità è negativa per tumori. Per la giovane età si esegue il test per CDH1 che risulta mutato. Viene proposto l'accertamento ai genitori e alla sorella. Il padre e la sorella risultano mutati, la successiva EGDS è negativa per il padre e positiva per la sorella che effettua gastrectomia per un TG pT1a pN0, G3. La consulenza genetica è raccomandabile in soggetti con TG giovanili per un programma personalizzato di sorveglianza, ed eventuale chirurgia profilattica nei soggetti mutati.

**Methylation profiles of Lynch colorectal cancers: a multicentric AIFEG study**

Nora Sahnane[1], Francesca Magnoli[1], Ileana Carnevali[1], Monica Pedroni[2], Giulia Magnani[2], Carmela Di Gregorio[2], Maurizio Ponz de Leon[2], Luca Reggiani Bonetti[2], Lucio Bertario[3], Paola Sala[3], Stefano Signoroni[3], Anna Maria Chiaravalli[1], Maria Grazia Tibiletti[1], Fausto Sessa[1], Daniela Furlan[1]
*[1]Department of Surgical and Morphological Sciences, University of Insubria and Ospedale di Circolo, Varese, Italy. [2]Department of Diagnostic and Clinical Medicine and Public Health, University of Modena and Reggio Emilia, Modena, Italy. [3]Unit of Hereditary Digestive Tract Tumours Fondazione IRCCS – Instituto Nazionale dei Tumori, Milan, Italy*

Gene hypermethylation and LINE-1 hypomethylation are common features of sporadic colorectal cancer (s-CRC). By contrast, information about the incidence and the role of aberrant methylation in Lynch Syndrome CRC (LS-CRC) is very limited. The aim of this project was to portrait the DNA methylation pattern of LS-CRC with known pathogenetic mutations and to compare it to non-Lynch CRC. In detail we analyzed the methylation status of 38 genes by MS-MLPA and of LINE1 elements by pyrosequencing in 58 LS-CRC, 120 s-CRC (22 MSI and 98 MSS) and 23 Early-Onset MSS CRC (EO-CRC: patients ≤40ys) enrolled by three Italian centers. Samples of normal mucosa (NM) and of tumor-adjacent normal mucosa (t-NM) were used as controls. Hypermethylation levels in LS-CRC were similar to those of MSS s-CRC and EO-CRCs, while a significantly higher number of methylated genes was observed in MSI s-CRC ($p<10^{-3}$). *GATA5* (20q13) and *WT1* (11p13) genes, both mapping in known imprinted regions, displayed aberrant methylation in ≥60% of samples, without differences among the four groups. Interestingly, these genes were methylated in t-NM but not in NM control samples. LINE1 hypomethylation was observed in all groups with significantly higher levels in MSS s-CRC ($p<0.01$). Our data suggest that LS-CRC, despite their different initial genetic defect, share analogue somatic epigenetic patterns with s-CRC. Aberrant methylation of GATA5 and WT1 is an early and cancer-specific event in all the four groups of CRCs included in this study.

**FAP in paediatric age as a model to evaluate the management of the disease since its onset**

Paola Sala[1], Stefano Signoroni[1], Elena Sommariva[1], Marco Vitellaro[2], Giovanni Ballardini[3], Paolo Radice[4] and Lucio Bertario[1].
*[1]Hereditary Digestive Tract Tumor Unit, Predictive-Preventive Medicine; [2]Colorectal Cancer Surgery Unit; [3]Diagnostic Endoscopy and Endoscopic Surgery Unit; [4]Unit of Molecular bases of genetic risk and genetic testing, Predictive-Preventive Medicine; Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy.*

Purpose: To evaluate the management of Familial Adenomatous Polyposis (FAP) diagnosed in paediatric age, the impact of Guidelines, their significance and the Register role. Methodology: FAP pts (<18 years old) enrolled by the Register of Hereditary Colorectal Tumors at Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy. Clinical data and records, genetic tests results, surveillance, treatment procedures and modalities were analyzed. Results: 280 FAP patients (pts) (14%) from 2005 had diagnosis in paediatric age (< 18 yrs old), mean age was 14 yrs: no FH in 14% and M/F ratio 1.3. The genetic test was done in the 92% and APC mutation carriers were 88%. The diagnosis was done for Family History (FH) in 54%, for the genetic test in 24% and for symptoms in 22%. In 14 pts (5%) the first colonoscopy was negative. Surgery was done in190 (68%) and in 83 (44%) in the same year of the diagnosis: total colectomy (TC) with Ileo-Rectum Anastomosis (IRA) in 72%. Colorectal Cancer (CRC) was diagnosed in 4 patients and in 7 patients when surgery was postponed (>1 year). Extracolonic manifestations were found in 11% of pts and desmoids in 12%. During the follow-up, mean 12 years (1-51), 15 (7.8%) patients had rectal cancer and the risk was estimated as 25% after 25 years. 9 patients (3.2%) died for CRC, 3 (1%) for desmoids, 2 (0,7%) for other causes or malignancies. Conclusion: About 1/10 FAP pts had diagnosis in paediatric age and CRC was found in 4 of them (1.4%). The TC was done concomitant with FAP diagnosis only in 30% of pts and the decision to postpone the surgery raised CRC risk (6%). TC-IRA was the preferred surgical procedure and CRC in the rectal stump was a long-term relevant risk.

**Autophagy in DNA microsatellite stable and unstable colorectal carcinoma**

Paola Sena[1], Francesco Mariani[2], Monica Pedroni[2], Giulia Magnani[2], Carmela Di Gregorio[2], Carla Palumbo[1], Maurizio Ponz de Leon[2], Luca Roncucci[2]
*[1]Dipartimento di Scienze Biomediche Metaboliche e Neuroscienze, Sezione di Morfologia Umana; [2]Dipartimento di Medicina Diagnostica, Clinica e di Sanità Pubblica, Università di Modena e Reggio Emilia*

Recent reports indicate that autophagy plays a crucial role in many physiological and pathological conditions. During these processes, autophagy is induced to recycle cellular constituents and clear damaged organelles to help them adapting to their surrounding microenvironment. The role of autophagy in tumors remains controversial, especially the issue of its action as a tumor brake or promoter. In particular the relationship of autophagy with apoptosis in malignant colorectal tissue is still a matter of debate. This study investigated autophagy and apoptosis by assaying the expression of factors that characterize these processes, in normal colorectal mucosa and along colorectal carcinogenesis. Immunofluorescence techniques coupled with confocal microscopy and immunoblot experiments were performed on 15 samples of normal mucosa (NM), 15 microadenomas (MA) and 15 carcinomas (C) obtained from patients who underwent colonoscopy or surgical resection for colorectal cancer. Moreover, 23 carcinomas classified as DNA microsatellite stable (MSS), and 26 microsatellite unstable (MSI) were analyzed by immunofluorescence experiments. The results clearly showed a significant increasing expression of autophagic factor in microadenomas and carcinomas with respect to normal mucosa. Furthermore, in MSS carcinomas the level of autophagic factor expression was higher than in MSI carcinomas. These data support the hypothesis that autophagy represents an advantage for cancer cell survival.

**Ovarian Cancer and Lynch Syndrome. The European Institute of Oncology experience**

Davide Serrano, Irene Feroce, Monica Barile, Aliana Guerrieri Gonzaga, Antonella Puccio and Bernardo Bonanni
*Division of Cancer Prevention and Genetics; European Institute of Oncology, Milan Italy*

Approximately 10% of ovarian cancer (OC) is associated to hereditary germ-line mutations. BRCA 1-2 mutations account for 85–90% of these tumors. An exact estimate has not been established for Lynch syndrome (LS), but the lifetime risk of OC in females carrying a MMR (mismatch repair genes) mutations could be up to 12% or higher considering only MLH1 and MSH2 genes.In our High Risk Clinic we have collected 167 ovarian cancer probands. Among these women we have 69 BRCA1 mutations, 28 BRCA2 mutations, two MLH1 mutations, three MSH2 mutations and one MSH6 mutation. Four OC patients were already tested for MMR genes and resulted WT. Retrospectively we are evaluating if, among the remaining 55 WT tested for BRCA genes, there are any probands with a pedigree suggestive for LS. There are 20 probands that may deserve to be tested also for MMR. Two of them had no suggestive family history but early onset of OC, eight subjects had at least one family member with another LS related cancer and ten subjects had early onset of the OC and at least one LS related cancer within the family (in this one there is a proband with a synchronous ovarian and endometrial cancer diagnosis). The MMR test for this last one is ongoing.Our experience supports the importance to screen for LS in OC cases, and detailed pedigree allows to choose the proper molecular tests. Thus we may offer the best surveillance and prevention strategies for each probands and their families.

**_BRAF_ mutation analysis is a valid tool to implement Lynch Syndrome diagnosis in patients classified according to Bethesda Guidelines**

Stefano Signoroni[1], Paola Sala[1], Francesca Molinari[2], Andrea Lampis[3], Claudia Bertan[3], Federica Perrone[3], Paolo Radice[4], Stefano Crippa[2], Milo Frattini[2] and Lucio Bertario[1].

[1]*Unit of Hereditary Digestive Tract Tumours, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy;* [2]*Institute of Pathology, Locarno, Switzerland;* [3]*Department of Diagnostic Molecular Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy;* [4]*Unit of Molecular bases of genetic risk and genetic testing, Predictive-Preventive Medicine; Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy.*

**AIMS AND BACKGROUND:** Lynch Syndrome (LS) is caused by Mismatch Repair (MMR) genes germline mutations. Gene testing was essentially done according to clinical criteria such as Amsterdam Criteria (AC) and Bethesda guidelines (BG).

BRAF mutations are absent in patients with a MMR genes germline mutation and present in sporadic colorectal cancer (sCRC) with microsatellite instability (MSI) due to promoter hypermethylation of MLH1 gene. Our aim was to verify whether BRAF mutations may help to select patients to address to germline MMR mutations assessment. **MATERIAL AND METHODS:** We analyzed 303 formalin-fixed paraffin embedded CRC samples including 174 sCRC, 28 patients fulfilling AC, and 101 suspected-Lynch patients fulfilling BG. We analyzed MSI and BRAF mutations in all CRC samples. MLH1, MSH2 and MSH6 germline mutations were investigated in MSI patients fulfilling AC or BG. **RESULTS:** sCRC showed MSI in 20/174=11% cases. BRAF mutations were detected in 10/174=6% sCRC cases and segregated with MSI patients ($p = 0.002$). MSI was observed in 24/28=86% AC cases which were BRAF wild-type. A MMR gene mutation was detected in 22/26=85% AC cases, all showing MSI. Suspected-Lynch cases carried MSI in 41/101=40% and BRAF mutations in 7/101=7% cases. A MMR gene mutation was detected in 13/28=46% evaluable MSI patients and only in wild-type BRAF cases. **CONCLUSIONS:** The prevalence of BRAF mutations in CRC patients is low but correlated with MSI instability and BG, whereas they are absent in LS patients. BRAF mutation analysis in suspected-Lynch patients would exclude about 7% of patients from unnecessary molecular analyses.

**Cost-Effectiveness of inherited CRC identification in Varese Province**

M.G.Tibiletti[1], L.Gambino[2], S.Pisani[2], I.Carnevali[1], M.Bonfanti[3], D.Croce[3], F.Sessa[1], G.Pinotti[4]

[1]*UO Anatomia Patologica,Ospedale di Circolo-Università dell'Insubria, Varese;* [2]*Osservatorio Epidemiologico ASL Varese;* [3]*CREMS (Centro di Ricerche di Economia e Management in Sanità e nel Sociale), Università Carlo Cattaneo -LIUC Castellanza;* [4]*Divisione di Oncologia, DPT Oncologico provincia di Varese Ospedale di Circolo Varese*

Identification of hereditary colorectal cancers (HCRC) led to more effective surveillance and can prevent premature death of patients affected by the most common hereditary form of CRC.

Even if the identification of HCRC based on family history is an inexpensive method, it is limited in sensitivity and only a proportion of the expected number of HCRC patients in a CRC population is identified by family history.In order to investigate the cost-effectiveness of genetic identification of HCRC patients we evaluated the incidence of gene carrier for cancer syndrome in Varese Province.In detail, based on CRC incidence and on data from literature, 21 male and 14 female affected by HCRC were expected for year. Considering that each family have almost 4 relatives, 70 subjects gene carriers for CRC for year are expected in Varese province. The volumes of care related to treatment and follow up of HCRC were based upon the analysis of record obtained from Varese Hospital, while the cost of current care was evaluated using ROL guidelines, tariffs and DRG reimbursement provided by the Regional Healthcare Service of Lombardy Region. In this project we correlate the cost of intensive care with and without genetic testing and also current care versus intensive care in subjects affected by HCRC. In addition we also evaluated the cost of tickets that gene carrier subjects must pay for own intensive care. The genetic identification and the intensive care of subjects affected by cancer syndrome is cost effective respect to intensive care only. The benefit of intensive care including genetic testing during a long time seems result in a reduction of cancer care costs.

**Risk of cancer in a consecutive series of patients with multiple colorectal adenomas (MCRAs)**

Emanuele DL Urso [1]; Salvatore Pucciarelli [1], Marco Agostini [1], Isabella Mammi [2]; Maribel Sullivan [1], Michela Campi [1], Paola Del Bianco [3], Donato Nitti [1].

[1]*Azienda Ospedaliera-Università di Padova, Dipartimento di Scienze Chirurgiche, Oncologiche e Gastroenterologiche, Clinica Chirurgica 1^, Padova, Italy;* [2] *Istituto Oncologico Veneto; Unità per i Tumori Ereditari, Padova, Italy;* [3] *Istituto Oncologico Veneto: Servizio Sperimentazioni Cliniche e Biostatistica, Padova, Italy.*

**Background**. Few data are available on risk of colonic and extracolonic cancer in patients with multiple, non diminutive, polyps of the large bowel. Aim o the study is to investigate clinical and molecular factors related to risk of cancer in these patients (pts). **Methods**. Pts with multiple colorectal adenomas (MCRAs) were defined if >10<100 colonic polyps were found Pts were enrolled from January 2003 to August 2013. APC and MutYH sequencing was performed. Clinical, molecular data and patients family histories were collected. A logistic regression analysis of factors related to presence of colonic and extracolonic tumours was performed. **Results.** 73 pts (44 male), median age: 54 years (range: 19-73 years). Median (mean) number of polyps: 30 (44) (range 10-100). Synchronous colorectal carcinoma (CRC): 38 cases (52%); extracolonic tumor: 28 pts (38%), being the most frequent breast, prostate cancer and lymphoma: 6, 4 and 4 cases respectively. Pathogenetic mutations: biallelic MutYH (n = 14, 19%) and APC (n = 3, 4 %). The median (mean) follow-up was 41 months (median 46 months, range: 2-336 months). The following variables were investigated in relation to incidence extracolonic and breast tumours: age, gender, polyps number, mutation found, CRC, family history of CRC, family history of extracolonic cancer. Only age significantly correlates with incidence of breast tumours and family history of non colonic cancer with presence of extracolonic malignancies. **Conclusions.** High risk of malignancies in pts with MCRAs, but no clinical or molecular risk factors for tumours can be identified within this group. By this point of view the finding of a pathogenetic mutation does not help in the treatment decisions nor in the follow up planning.

**Laparoscopic Surgery in Familial Adenomatous Polyposis. 10 years' experience**

Marco Vitellaro, M.D.* Stefano Signoroni, PhD**, Paola Sala, PhD**, Lucio Bertario, M.D.**

**Colorectal Cancer Surgery Unit; **Hereditary Digestive Tract Tumor Unit, Predictive-Preventive Medicine. Fondazione IRCCS "Istituto Nazionale dei Tumori" Milano, Italy*

**Introduction: A**im of this study was to evaluate the feasibility, short and long-term outcomes of laparoscopic (Lap) prophylactic surgery in FAP patients (pts). **Methods:** Pts who underwent Lap Total Colectomy/ileal-rectal anastomosis (TC/IRA) or Proctocolectomy/ileal pouch-anal anastomosis (PC/IPAA) from 2003 to 2011were identified from the Hereditary Colorectal Tumor Registry database. Main outcomes were: major complication within 30 days; long-term malignant recurrence and intrabdominal/abdominal wall desmoids. **Results:** 74 pts, 42 male, age median (range) 27 years (9-68), BMI 23 (16-35). 60 pts were classic FAP and 14 AFAP. 58 pts had TC/IRA and 16 had PC/IPAA. 1 pt was converted to open surgery due to a desmoid. Median follow-up time was 64 (18-111) months. Morbidity occurred in 8 (10.8%) pts: anastomotic leak in 5 (6.7%) pts; pelvic bleeding in 1 pt; anastomotic bleeding in 1 pt; and a pancreatitis in 1 pt with a combined duodenal polyp removal. Median postsurgical length of stay was 6 (4–24) days. Pathology revealed severe dysplasia in 20 pts and adenocarcinoma in 10 (13.5%). There was no local malignant recurrence, and 1 mortality due to liver metastases at 24 months in a pT3N2 pt. Desmoids occurred in 3 (4.0%) pts. Small-bowel obstruction occurred in 3 (4.0%) pts. **Conclusion:** Laparoscopic approach for FAP pts appears to be safe, feasible, oncologically sound, and is a reliable alternative to open surgery. The trend of lower desmoids risk is an important related result.