

**48th National Meeting of the Italian Cancer Society – Bari, October, 1-4 2006:
Meeting Report**

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After some decades, the National Meeting of the Italian Cancer Society (SIC) has been held in Puglia, where several Institutions and laboratories involved in cancer research field are present. About 250 people took part in the event composed for 65% of young researchers. In fact, the Scientific Board of the SIC decided to focus main attention to the involvement of new researchers in the society life paying great attention to their needs and problems. The meeting was held in a pleasant area of the city and focused on several sessions for most part looking at translational application of cancer research. Another important aspects has been the presence of sessions in collaboration with other scientific societies, such as the Italian Human Genetic Society (SIGU), the Italian Society of Anatomopathology and Cytology (SIAPEC), the Metastasis Research Society (MRS), the Italian Society of Medical Oncology (AIOM). In this meeting, for the first time, a pipeline where several pharmaceutical companies presented new drugs and new technologies has also held with lunch and dinner meetings dedicated to specific topics. Thirteen speakers from Europe and USA held lectures keeping the great attention of the whole floor.

The first session dealt with **Genetic and epigenetic changes** of tumors and has been chaired by **A. Fusco** (University “Federico II” Naples, Italy) and **S. Tommasi** (Istituto Tumori “Giovanni Paolo II” Bari, Italy). **M. Rocchi** (University of Bari, Italy) started describing Genome plasticity and its influence on cancer. Since the discovery of the 9/22 translocation in the chronic myeloid leukemia, several additional human neoplasias have been shown to be generated by genomic rearrangements.

Following the completion of the human genome sequence, many studies have focused on peculiar features of human genome architecture, segmental duplications in particular, that are directly or indirectly involved in triggering genomic disorders. In addition, recent studies have disclosed an unpredicted high level of copy number variation, involving also functional sequences, in the human population. This plasticity played an important role in the evolution. In this context, Rocchi

focused on the consequences of this plasticity on tumors and underlined that such biological phenomena can be fully understood only if studied in an evolutionary frame.

R. Sgarra (University of Trieste, Italy) dealt with HMGA proteins which constitute a group of architectural nuclear factors involved in chromatin dynamics. In particular, they participate in specific protein-DNA and protein-protein interactions that induce both structural changes in chromatin and formation of stereospecific complexes called “enhanceosomes” on the promoter/enhancer region of several genes. Several experimental data have demonstrated an involvement of these factors in the process of neoplastic transformation mostly due to their post-translational modifications. Sgarra showed, by an LC/MS-based screening, that methylation and phosphorylation of HMGA are differentially modulated in the various cell lines analysed. Furthermore, HMGA proteins not only have a different expression patterns, but are also differentially modified, suggesting different functions in the transformation process.

V. Corbo (University of Verona, Italy) reported his data which aimed to clarify the role of *MEN1* gene in sporadic Pancreatic endocrine tumors (PETs). Mutations they found in this gene results in a functional loss of menin and suggest the involvement of *MEN1* gene in the pathogenesis of about one-third of sporadic NF-PETs. As we observed a different incidence of mutations in clinically different tumors, the existence of different molecular events underlying the pathogenesis of these tumors subtypes is suggested.

On the other hand, **M. Widschwendter** (Breast and Gynaecological Cancer Institute for Women’s Health University College London, UK) described the importance of methylation in the inactivation of oncogenes and tumour suppressor genes. In particular, he showed the role of methylation in most of the pathways involved in breast cancer. A strict interaction has been demonstrated between DNA methylation changes in breast cancer and HR status or response to hormonal therapy that was not previously appreciated. These results suggest exciting opportunities for the development of robust assays for clinical diagnosis and for predicting response to antiestrogen therapy in the adjuvant setting. Furthermore, he underlined also the importance of

DNA methylation changes both in cancer cells and tumor stroma with a higher prevalence in HER-2/neu positive breast cancers with respect to the HER-2/neu-negative ones. These alterations could help to explain the higher aggressiveness and resistance to antihormonal therapies of HER-2/neu-positive cancers. Indeed, epigenetic markers can be useful for diagnosis, prognosis and prediction of breast cancer when studied in serum, nipple fluid aspirate or tumor, but they can also predict breast cancer risk when studied in WBC and NFA of healthy people. In this context, he hypothesized to use methylation signature of stem cells as target of sporadic breast cancer predisposition.

P. Parrella (Istituto di Ricovero e Cura a Carattere Scientifico “Casa Sollievo della Sofferenza,” San Giovanni Rotondo, FG, Italy), showed data of her group on gene methylation in preneoplastic and neoplastic lesions of breast cancer. She demonstrated that APC seems to be methylated at the early stages of progression, whereas CDH1 and CTNNB1 methylation occurs later. Thus, these genes are more informative than other methylation markers for early breast cancer detection. Indeed, her results indicate a direct involvement of methylation of the APC, CDH1, and CTNNB1 CpG island promoter in the early stages of breast cancer progression, and suggest that these molecular alterations might be involved in the transition to an invasive phenotype.

The joint session with the Italian Society of Human Genetics, **Joint Meeting SIC-SIGU** focused on “**Familiarity and prevention**” chaired by **R. Dolcetti** (Centro di Riferimento Oncologico, Aviano-PN, Italy) and **L. Larizza** (University of Milano, Italy) **A. Ashworth** (Institute of cancer Research, London, UK) reported that individuals harboring germ-line mutations in the *BRCA1* or *BRCA2* genes are at highly elevated risk of a variety of cancers. Ten years of research has revealed roles for BRCA1 and BRCA2 in a wide variety of cellular processes. However, data have been accumulated indicating that both BRCA1 and BRCA2 proteins are critically important in the repair of double-strand DNA breaks by homologous recombination. Despite this increasing knowledge of the defects present in BRCA-deficient cells, *BRCA* mutation carriers developing

cancer are still treated similarly to sporadic cases. Prof. Ashworth proposed that the functional role played by BRCA proteins in DNA repair could be exploited in the treatment of BRCA-deficient cancers by targeting the tumors with drugs that create DNA damage highly reliant on BRCA1 or BRCA2 for repair. Poly(ADP-ribose) polymerase 1 (PARP1) is one possible therapeutic target, being an enzyme critical to the base excision repair pathway. In fact, PARP1 inhibition leads to severe, highly selective toxicity in BRCA1- and BRCA2-defective cells, with selectivity being several-fold higher than for conventional chemotherapy drugs. Chemical inhibitors of PARP1 are in the early stages of clinical trials and it will be relevant to assess their potential efficacy as treatment for BRCA-deficient tumors. Prof. Ashworth also discussed how therapies developed to treat *BRCA* mutant tumors might be applied to some sporadic cancers sharing similar specific defects in DNA repair.

L. Varesco (Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy) addressed the topic of the prevention of colorectal cancer (CRC), which is usually based on the recognition and removal of (detectable) polyps ('secondary prevention'): the goal is to detect and remove lesions at high risk of cancer progression. Secondary prevention is directed to healthy people and it involves a series of choices that have to be made in order to establish which prevention program is more suitable for that individual. A lot of scientific information is needed to answer each of the related questions and, more, a correct interpretation of existing knowledge has to be made. The goal is to be able to balance benefits and harms of (different) prevention programs for each individual. A key factor in this process is to estimate the "a priori risk" of disease for that specific person making possible to use the most appropriate 'package' of information. The main risk factor for CRC is age. After 50 yrs CRC becomes a 'common' disease. The second recognized risk factor is 'family history' (FH), including hereditary predisposition. Several epidemiological studies indicate that FH confers a higher risk of developing CRC. Different risks are given here for different grade of relationship, age and number of affected relatives. The term 'familial cancer' (FC) is often used. It can be defined as 'the presence of more than 1 case of the same type of cancer within a nuclear family'. Many genetic

models are proposed to explain familial recurrence but we don't yet know 'FC genes'. Hereditary cancer can be defined as a cancer due to (conditional to) the presence of a hereditary mutation in a single gene. Many features help us to recognize it but none is pathognomonic. Several Hereditary-CRC (HCRC) genes have been identified in the last 10-15 years giving us the possibility to make a molecular diagnosis of HCRC, irrespective of clinical history. Considering numbers, it is important to remember that HCRC are rare, representing a small proportion of CRC and even a limited proportion of FH-CRC. However, their identification is important because cancer risk for individuals from HCRC families is high and not limited to the colon-rectum. Based on this cancer risks estimates, options for preventions are all 'major' decision to take for at risk (young) individual, ranging from strict surveillance, prophylactic surgery to preimplantation diagnosis. Therefore, to provide the 'best' answer to the questions of families with suspected HCRC, the health care system needs to create a real network, or better a 'network system' of local, regional and national nodes in which each node has a defined function (triage, genetic counselling and molecular diagnosis, clinical surveillance) and also connects with proper nodes according to family/individual needs.

S. Tommasi (Istituto Tumori "Giovanni Paolo II", Bari, Italy) showed a new bioinformatic approach to analyze aCGH data. She analyzed 124 breast cancer patients tumor tissue utilizing a high-resolution array Comparative Genomic Hybridization based on 2464 bacterial artificial chromosome clones. Then, statistical and neural approaches have been used to extrapolate profiles for the familial and sporadic groups which were investigated by the Matchminer database and Gene Ontology cluster analysis to evidence gained and lost genes and to group them on the basis of specific pathways. The peculiarity of the neural network used permitted to evidence the functional relationship among genes in specific pathway active in familial but not in sporadic breast cancer.

MC. Scaini (University of Padova, Italy) reported on the functional characterization of the CDKN2A Gly23Asp mutation, detected in a melanoma-prone family with 3 melanoma cases. Consistently with published findings, also in this family the Gly23Asp mutation did not co-

segregate with the disease. The analysis showed that, compared with wt protein, the p16 protein carrying the Gly23Asp mutation was less efficient in inducing G₁ cell cycle arrest, less effective in its ability to inhibit cell proliferation, suppress colony formation, and bind to CDK4. On these grounds, the Gly23Asp mutation could be considered a pathogenic mutation. The final contribution was from **M.Pensabene** (University “Federico II”, Napoli, Italy), who presented the preliminary results of an extensive analysis carried out in families with inherited or familial breast and/or ovarian cancer aimed at determining the related cancer spectrum. On the basis of family history, pedigrees were classified in three distinct subgroups: hereditary with aggregation, hereditary without aggregation, and familial. The frequency of distinct tumor types in families with BRCA1/2 mutations has been also recorded. Completion of the analysis (228 pedigrees) will allow the identification of significant associations correlations.

As regards the **JOINT MEETING SIC – SIAPEC on Molecular taxonomy of cancer**, chaired by **G. Bevilacqua** (University of Pisa, Italy) and **A. Scarpa** (University of Verona, Italy) two lectures and three selected oral presentations have been presented. In the first lecture, entitled “Prediction of response to chemotherapy: a new challenge in tumor pathology”, **H. Hoefler** (Technical University of Munich, Munchen, Germany) reported his studies aimed to the identification of genetic markers, on the genomic, mRNA, or protein level that could predict response of upper gastrointestinal carcinomas to neoadjuvant chemotherapy. In fact, only 30-40% of the patients with locally advanced adenocarcinomas of the upper gastrointestinal tract respond to the multimodal neoadjuvant treatment protocols including 5-FU and cisplatin. He reported the examples of putative markers identified in esophageal and gastric cancer. In esophageal carcinomas, higher levels of MTHFR gene expression, as well as caldesmon and of the two drug carrier proteins MRP1 and MDR1 were associated with response to therapy. In gastric carcinomas, mutations of the p53 gene revealed no association with response or survival, but tumors with a high rate of loss of heterozygosity (LOH), determined by microsatellite analysis, showed a better response to a

cisplatin-based chemotherapy. Analysis of expression of several 5-FU- and cisplatin-related genes, demonstrated an association of DPD expression with response to therapy and longer survival. In particular, the combination of TP and GADD45 gene expression data showed the most relevant association with response to therapy in this tumor type. Prof. Hoefler underlined that the many studies that are being performed in this field will furnish a database of potential novel markers that will need a validation in multicenter prospective studies. The real challenge of potential clinical application will be the capacity of efficiently organize these trials.

In his lecture on the role of “Surgical pathology in the XXIst century”, **A. Cavazzana** (Azienda Ospedaliera Universitaria Pisana, Pisa, Italy) pointed out that surgical pathologists are nowadays facing novel challenges in their routine practice, which must take into account the results of the recent studies dealing with the discovery of novel markers as those reported by Hoefler. In fact, although the morphologic diagnosis still represents the most rapid and cost-effective way to diagnose a cancer, it only allows to group diseases and does not provide information regarding the response to therapy of the single patient or disease. Whenever feasible the final diagnosis should contain all those information that may anticipate the behavior of a given tumor in a specific patient as well as the response to therapy. In this effort to improve the overall diagnostic efficacy, the routine application of new as well as relatively old techniques, such as immunohistochemistry, in situ hybridization, molecular analysis on microdissected cells may provide new solutions to old problems. Using several examples, he showed how a “morpho-molecular approach” to the diagnosis is to be expected to furnish the technical and cultural solution to this new challenges.

In the first oral presentation entitled "Early detection of bladder cancer by urine telomerase activity", **Sanchini. MA** (Morgagni-Pierantoni Hospital, Forli', Italy) et co-workers investigated in a prospective case-control study, the diagnostic relevance of a novel test they developed for the early detection of bladder cancer. They reported that their simple and relatively inexpensive test allowed to measure the telomerase activity in urine with a 90% sensitivity and 88% specificity. They further showed that the specificity of the test increased to 94% for individuals less than 75 years of

age and, more important, that the same predictive accuracy of telomerase activity levels was observed for patients with low-grade tumors or negative cytology. These results seem to indicate urine telomerase activity as an important non invasive diagnostic innovation that could be used for bladder cancer detection in high-risk groups such as symptomatic individuals or workers exposed to carcinogens.

Vincenzi. B (University Campus Bio-Medico, Rome, Italy) and co-workers, in the presentation “Angiogenesis modifications are predictive of response and outcome advanced colorectal cancer patients during therapy with cetuximab plus irinotecan”, reported the results of a trial conducted on 45 pretreated metastatic colorectal cancer patients treated with a weekly combination of cetuximab plus irinotecan, designed to investigate if circulating levels of VEGF could be related with clinical response and outcome. They reported that patients with at least 50% reduction of VEGF levels had a response rate significantly higher than that of patients unreduced VEGF levels. The median time to progression and overall survival were also longer in patients who showed VEGF reduction.

In the third oral presentation entitled “AFP together with squamous cellular carcinoma antigen (SCCA) as serological markers for HCC diagnosis. study of 961 patients”, **Fransvea. E** (University of Bari Medical School, Bari, Italy) and co-workers reported the results of their study aimed to discover new noninvasive biomarkers helpful to HCC diagnosis. The study was performed measuring the levels of several antigens, including AFP, squamous cellular carcinoma antigen (SCCA), SCCA immuno-complex (IC) and AFPIC in the serum of 421 HCC and 368 liver cirrhosis (LC) patients. The combined use of AFP and SCCAIC allowed to correctly diagnose HCC in 72% of patients, with 26% false positives. The combined use of AFPIC, SCCA and SCCAIC identified 79% of HCC patients classified as LC according to AFP alone, and supported the diagnosis of HCC in 82% of the patients with moderately increased AFP. These results point that the combination of all these markers may be used as a surveillance strategy significantly increasing the diagnostic accuracy of HCC.

Eliminato:

For the first time in the history of the SIC congress, Bari hosted an oral poster session entirely dedicated to presentations made by young investigators in cancer research: the **Under35 investigator's meeting**, chaired by **L. Manenti** (MolMed, Biotech Farmaceutica, Milan, Italy) and **G. La Rocca** (University of Palermo, Italy). The breadth of the session was extremely wide, with presentations centred on clinical and preclinical studies as well as advanced basic research. With the higher number of presenters (sixteen researchers from different national institutes), the session was a successful significant starting point for future initiatives involving the active participation of younger scientists to the Society projects, which is one of the main commitments of SIC.

A. Rizzo (Regina Elena Cancer Institute, Rome, Italy) presented the results of a study on telomere-directed inhibition of tumour growth. The investigators used a G-quadruplex ligand, which resulted in the inhibition of growth in five different types of tumour xenografts. The inhibitory effect was due to the induction of short-term apoptosis mediated by telomere disruption.

P. Gandellini (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy) showed a targeting of human telomerase reverse transcriptase (hTERT), through the use of a panel of small interfering RNAs (siRNAs), with a degree of telomerase activity inhibition in PC-3 and DU145. The data presented suggest siRNA-mediated hTERT down-regulation as an efficient strategy to impair prostate cancer cell growth.

L. Ciuffreda (Istituto Regina Elena per lo Studio e la Cura dei Tumori, Rome, Italy) showed the promising results of PD0325901, a MEK-1/2 inhibitor, against human melanoma. The authors demonstrated an inhibition of cell growth (due to cell accumulation in G₀/G₁ phase). More interestingly, the molecule administration resulted in inhibition of VEGF secretion by tumour cells, showing a promising additional anti-angiogenic effect for this compound.

T. Gelardi (University Federico II, Naples, Italy) presented the PKC β 2-selective inhibitor, Enzastaurin in several human cancer cell lines. In all cancer cell lines tested Enzastaurin induced

apoptosis (not affect cell cycle distribution), suppressed GSK3 β phosphorylation and reduced VEGF expression and secretion.

F. Pastorino (G.Gaslini Children's Hospital, Genoa, Italy) presented the data of a multi-centred study on liposomal chemotherapy of Neuroblastoma. In particular, the addition of NGR peptides (which target CD13 isoforms expressed by angiogenic endothelial cells) to the surface of doxorubicin-loaded liposomes resulted in tumor regression and vascular destruction. Moreover, combination therapy with GD2 targeted liposomes resulted in long-term survivors.

L. Porcelli (National Cancer Institute, Bari, Italy) provided evidences on the possibility to utilise clofibric acid derivatives with PPAR α/γ agonist activity in several cancer cell lines. All agents showed an IC50 ranged between 20 μ M and 100 μ M; among them, the main active ones were LT127 and LT160 (two enantiomers). After 2 days drugs exposure, PTEN was strongly inhibited with a consequent increase of p-Akt. Preliminary results, suggest an inhibition of cell proliferation through PPAR γ dependent, and independent signals.

G. Buda (University of Pisa, Italy) presented a study on the association between *SLC19A1* and *TYMS* polymorphisms with multiple myeloma outcome in 120 patients. The *TYMS A-227A* genotype correlated with poor response, while *SLC19A1 T-233T* was associated to a longer overall survival, therefore suggesting that pretherapeutic determination of such polymorphisms should help in the choice of the therapeutic protocol.

S. Cattaruzza (University of Parma, Italy) presented RNAi knockdown of NG2 in sarcoma cells specifically impairs their vessel invading, motile and invasive capabilities in response to collagen type VI (Col VI) matrices. Host NG2 contributes to tumour propagation. Specific antagonists of the cell signalling pathways engaged by NG2 are currently exploited.

R. Sasanelli (University of Bari, Italy) showed the results of *in vitro* experiments on the inhibitory effects of novel platinum complexes with biphosphonate analogues on different matrix metalloproteinases. The results were extremely interesting, since the novel compounds inhibited the activity of MMP-9, MMP-3 and MMP-12, while SMP, cisplatin or carboplatin failed in doing so.

A.M.L. Coluccia (University of Bari Medical School, Bari, Italy) correlated high levels of Bcr-Abl in CML-BC with beta-catenin nuclear accumulation and its transcriptional activation in fresh CML-BC patient samples. Imatinib impaired the beta-catenin related transcription allowing a glycogen synthase kinase3 (GSK3)-mediated S/T-phosphorylation of beta-catenin. Moreover, the authors established a functional link between Bcr-Abl kinase activity and beta-catenin nuclear signaling. The ability of Bcr-Abl to recruit and phosphorylate beta-catenin could contribute to extend the lifespan of leukemic cells.

S. Caporali (Istituto Dermopatico dell'Immacolata-IRCCS, Rome, Italy) presented the results of in vitro experiments on activation of AKT in response to temozolomide in the lymphoblastoid cell line TK6. The results were confirmed on colon carcinoma cell lines with the use of siRNAs. Therefore, the authors demonstrated a novel role of ATR as upstream activator of AKT

L. Carrassa (Mario Negri Institute, Milan, Italy) down-regulated by siRNA the expression of Chk1 and/or Chk2 in the HCT-116 colon carcinoma cell line. The inhibition of Chk1 but not Chk2 caused a greater abrogation of the G2 block and a greater sensitization to different DNA damaging agents in the cell lines with a defective G1 checkpoint.

F. Ratti (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy) showed the results of a study on the anti-apoptotic effects of Seladin-1 expression in metastatic melanoma. Seladin-1 was expressed at higher levels in metastatic cells compared to primary tumours, and showed a protective role against H₂O₂-induced apoptosis. Moreover, Seladin-1 expression was associated to higher intracellular levels of ROS.

M.S. Pino (Regina Elena Cancer Institute, Rome, Italy) characterized nine wild-type EGFR human PDAC cell lines for the expression of EMT markers and hMena. Cell lines which express the epithelial protein E-cadherin showed greater sensitivity to erlotinib. In contrast lines having undergone EMT, expressing N-cadherin and/or vimentin were relatively resistant. These findings may have important implications for the development of novel, molecularly-targeted approaches for the treatment of PDAC.

M. Donadelli (University of Verona, Italy) showed the results of a study on the inhibitory effects of PDTC on growth of pancreatic adenocarcinoma cells. Growth inhibition of p53-negative cells was caused by cell-cycle arrest in S-phase, mainly due to p21WAF1/CIP1 induction, as confirmed by silencing experiments. The hypothesis was further confirmed by *in vivo* experiments on xenografted mice.

I. Cantiani (Istituti Ortopedici Rizzoli, Bologna, Italy) showed a significant inhibition of migration and angiogenesis after treatment of TC-71 cells with NVP-AEW541. A decrease in animal weight, urea and glucose blood serum was recorded. After initial loss of weight all treated animals started to grow again. Combination of the NVP-AEW541 and vincristine did not change the toxicity pattern.

Such a number of high interest reports showed the high potential of young cancer researchers in Italy. We hope that the Society will continue in pursuing such initiatives which should deserve a higher attention.

The session on **Tumors from bone and to bone**, chaired by **M.P. Colombo** (Fondazione IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy) and **F. Silvestris** (University of Bari, Bari, Italy) provided an overview on genes and related pathways involved in bone-related cancers with prognostic or therapeutic relevance. The bone provides a favourable microenvironment for the proliferation and survival of tumor cells because of its abundance of growth factors. In her lecture **Teresa A. Guise**, (University of Virginia, Charlottesville, VA) illustrated the molecular mechanisms responsible for bone metastasis. Tumor factors disturbing the interplay between osteoclasts and the osteoblasts contribute to generate a bone-forming (osteoblastic) or bone-lysing (osteolytic) phenotype.

Bone metastases are common in lung, kidney, breast and prostate cancers. However, while breast cancers usually give osteolytic lesions, prostate cancer is unique in that bone is the only clinical detectable site of metastases, and the resulting tumors tend to be osteoblastic rather than osteolytic.

Prostate cancer cells through production of specific growth factors can induce osteoblasts that, on turn, may promote metastases, driving a vicious cycle that fuel tumor growth. **T. Guise** overviewed these mechanisms and focused mainly in the action of the vasoactive peptide, endothelin-1 (ET-1), which is produced by prostate cancer and stimulated the new bone formation associated with osteoblastic metastases via its effects on the endothelin A receptor in mice and humans. ET-1 is a mitogenic factor for osteoblasts and also stimulates osteoblast production of factors such as CCN proteins and IL-6 that might further enhance tumor growth in bone. Atrasentan, an ET-1 receptor antagonist, was developed based on these findings. Guise showed that Atrasentan prevents osteoblastic bone metastases in a mouse model and reduces skeletal morbidity in men with advanced prostate cancer, giving therefore another piece of evidence that inhibition of osteoblast function may be part of the therapy against prostate cancer in bone.

The session continued with **K. Scotlandi** (Istituti Ortopedici Rizzoli, Bologna, Italy) who focused on osteosarcoma, a mesenchymal tumor thought to derive from osteoblastic precursors. This tumor retains the expression of early markers of osteoblastic differentiation and the ability to produce an aberrant matrix, which is instrumental for the correct diagnosis of osteosarcoma. By contrast, late markers of osteoblastic differentiation, such as osteocalcin, are poorly or not expressed in the great majority of osteosarcomas. Because osteoblast differentiation is quite well known, osteosarcoma represents an excellent model for testing the relationships between differentiation and transformation. Scotlandi presented data on the expression of CD99, a cell adhesion molecule that is highly expressed in bone-lining and mature osteoblasts, but nearly absent in osteosarcoma, where it acts as oncosuppressor. Molecular findings point to overexpression of caveolin-1 and down-modulation of c-src kinase activity as possible mechanisms inducing reversion from malignancy by CD99 replacement via gene transfer. Studies on c-src specific inhibitors will say whether they can be used to treat osteosarcomas.

Other studies on sarcomas were presented by the group of **VM Fazio** (IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy) and of **L Pazzaglia**, Istituti Ortopedici

Rizzoli, Bologna, Italy. A mutational analysis of EXT1 and EXT2 genes in a large cohort of Italian multiple osteochondromatosis families revealed up to 27 new mutations. This makes possible to support the genetic origin of the disease, which represent the largest group of benign tumors of bone, thus reducing the group of unclassifiable cases.

The group of **F. Dammacco** (University of Bari Medical School, Bari, Italy) provided data supporting the use of antiangiogenic drugs, particularly VEGF and bFGF inhibitors, in the therapeutic management of multiple myeloma, the most common bone-affecting cancer.

The session ended with studies related on the role of NG2 proteoglycan in sarcomas. NG2, serving as a primary mediator of the tumour cell-host interaction within the microenvironment, is widely expressed in a variety of tumors. Recent evidences suggest that NG2 is a marker associated to primary malignancy and metastasis in melanoma other than sarcomas. It promotes tumour dissemination and interactions with collagen type VI. Its targeting might have promising therapeutic prospective.

The **Joint meeting SIC-MRS on Microenvironment and metastasis** has been chaired by **A. Albini** (Istituto Nazionale Ricerca sul Cancro, Genova, Italy) and **A. Vacca** (University of Bari, Italy). The symposium came from the concept that it is necessary to consider the microenvironment of a cancer as a functional whole with the neoplastic cells. In addition to existing efforts directed at dysfunctional transformed cells, it is essential to develop strategies to target to the tumor microenvironment, in order to control the process of carcinogenesis, and prevent cancer spread and metastasis. The idea that the microenvironment is a crucial regulator of metastasis was originally proposed by Paget in his “seed and soil” hypothesis. Carcinogenesis and tumor metastasis result not only from the interaction of cancer cells with endothelial cells but for the fact that stromal and inflammatory cells and tissue play a critical role in directing the formation of metastasis. In the presentation entitled “Metastasis suppressors alter cellular responses to microenvironmental signals”, **D.R. Welch** (Birmingham, Alabama) has reported novel data on two major genes,

BRMS1 and Kiss1 that are involved in the suppression of metastasis. Both of them induce alterations of the interaction of metastasis to the host.

The product of the KISS1 gene suppresses metastasis of several tumor models without blocking tumor growth. The KISS1 protein is secreted and processed to a fragment called kisspeptins, which binds to a G protein-coupled receptor. KISS1 secretion is required for metastasis suppression via GPR54 receptor and for maintenance of disseminated cells in a dormant state. BRMS1 is a member of HDAC transcription co-repressor complex and suppress breast cancer and melanoma cells metastasis in animal models. BRMS1 regulates osteopontin (OPN) expression. OPN is a tumor-metastasis activator, and now demonstrated to be a crucial downstream target of BRMS1. Suppression of OPN could be one of the mechanisms of BRMS1-dependent suppression of tumor metastasis.

Another crucial gene for metastasis is c-met, the receptor of the growth factor HGF, This molecule is expressed in tumor and in endothelial cells and promotes scatter, motility and angiogenesis. **S. Giordano** (IRCCC Candiolo Torino, Italy) has reviewed novel data on the role of met and the signaling pathways. Metastasis follows the re-activation of the program termed invasive growth, which is physiological during embryonic development. The MET proto-oncogene, which is expressed in stem and cancer cells, is a regulator of invasive growth. The MET tyrosine-kinase receptor is phosphorylated upon binding to the ligand and is involved in cell invasion and metastasis through transcriptional activation of various genes. The deregulated activation of Met is crucial for the acquisition of tumorigenic and invasive phenotype. The involvement of MET in human tumors has been definitively properties and can be achieved through various mechanisms, including MET interaction with other membrane receptors. Co-players of met are integrins, plexins, other RTKs, CD44, FAS. Interfering with Met activation is a new approach to prevent tumorigenesis and metastasis.

Novel therapeutic approaches based on the use of silencing RNA towards the met oncogene have been advanced in the presentation.

Colon cancer is one of the most challenging neoplasms, among the 4 big killers; in colon cancer the microenvironment is particularly crucial and it has been shown that inflammation and infections can dramatically promote carcinogenesis. **Marchiò S** (University of Torino) have presented in a selected contributions data on an extracellular signature of metastatic colon cancer: molecular and pharmacological approaches.

Besides the importance of genomics and the prediction derived from the signatures in gene and protein profiles, the immunological approach is one of the most crucial to cancer metastasis.

In recent years several groups in Italy and abroad have devoted attention to chemokines and their receptors in cancer. Chemokines and their binding proteins belong to three major classes. The receptor for Fractalkine is important in pancreatic cancer, one of the most life-threatening pathologies. This phenomenon has been reported by **Marchesi F** (Dept. Immunology Istituto Clinico Humanitas, Rozzano, Italy) in their presentation entitled “Expression and function of CX3CR1 receptor for CX3CL1/FRACTALKINE on pancreatic tumour cells and its involvement in pancreatic cancer neural tropism”.

We should not forget in the complex picture of metastasis and its relation with the microenvironment the importance of apoptosis and the signaling associated with death pathways.

Giorgini S (Regina Elena Cancer Institute, Rome, Italy) in the talk “Upregulation of BCL-XL in tumor cells increases CXCL-8 expression and enhances angiogenesis” have correlated the overexpression of an apoptosis related gene, BCL-XL, to the expression of an angiogenic chemokine, CXCL-8, and the consequence of an increased angiogenic phenotype. The group also in the past has investigated how apoptosis and angiogenesis are somehow interrelated phenomena, linking again the cancer to its microenvironment.

The 2006 Annual meeting of SIC has hosted for the first time a session entirely dedicated to the pipeline of new drugs under development in the major pharmaceutical companies: **Drug company pipelines** chaired by **G. Tortora** (University Federico II, Naples, Italy). In this first meeting, the

guest speakers were representative of GlaxoSmithKline, AstraZeneca, Nerviano Medical Sciences and Roche.

B. Costa (GlaxoSmithKline Medical Leader Oncology, Verona, Italy) from the Medical direction of GSK, illustrated the agents under development for cancer treatment, focusing on two anticancer agents, lapatinib and pazopanib, and two drugs for the support and preventive therapy, relacatib and eltrombopag. Lapatinib is a small molecule, quinazoline derivative, reversible inhibitor of both EGFR and HER2 that is orally available. Lapatinib is active against several types of cancer, particularly breast cancer, and has rapidly progressed through the clinical evaluation phases. Lapatinib in addition to capecitabine confers an advantage in the survival of trastuzumab-, anthracycline- and taxane-refractory breast cancer patients overexpressing HER2, as compared to capecitabine alone, and seems also active in preventing brain metastases. These results favoured the recent approval of lapatinib for the treatment of this setting of patients. PTEN deficiency is a major cause of resistance to EGFR/HER2 inhibition and confers resistance to trastuzumab; interestingly, lapatinib has shown activity in inflammatory breast cancer, a particularly severe disease, irrespective of PTEN status. Several clinical trials are now ongoing to evaluate lapatinib activity in breast cancer either in combination with or in comparison to trastuzumab. Other studies are ongoing or planned in other diseases or in combination with chemotherapeutic or targeted drugs. Pazopanib is a novel orally available, multitargeted antiangiogenic drug that inhibits all three VEGFRs, PDGFR and c-kit. Pazopanib is active against several tumor models in preclinical studies and blocks VEGF-mediated endothelial cell proliferation. Evidence of activity, particularly in renal cancer, emerged from early clinical studies. Studies combining pazopanib and lapatinib have provided preliminary evidences of cooperativity and are in the clinical plan of development. Relacatib is a novel inhibitor of cathepsin K, an enzyme that digests bone proteins in the osteoclasts-tumor cells microenvironment of bone metastasis. Preclinical studies and early clinical studies demonstrated that relacatib antagonizes the induction of osteolytic lesions by tumor cells. Eltrombopag is an orally available small molecule which induces megakaryocyte proliferation and

differentiation. Short term treatment of healthy volunteers and thrombocytopenic patients has shown an increase in the platelet count.

E. Mari, from the Medical Direction of AstraZeneca, presented several anticancer agents under development. Vandetanib (ZD6474) is a multitarget small molecule inhibiting VEGFR2 (KDR), EGFR and RET oncogene. On this basis vandetanib has shown activity against a wide range of tumor types, including those resistant to common EGFR inhibitors. Vandetanib has proven effective in non small cell lung cancer (NSCLC) patients progressing after first-line chemotherapy failure and phase III trials are ongoing in this disease. Other studies are ongoing in colon cancer patients and in medullary thyroid cancer, based on the selective inhibition of RET, a pathogenetic lesion in this neoplasia. AZD2171 is a small molecule antiangiogenic agent targeting all VEGFRs and c-Kit. It has shown activity against several tumor models and has completed phase I studies to evaluate the toxicity. ZD4054 is a small molecule specific Endothelin-A receptor (E_A-R) antagonist. E_A-R is a recently discovered receptor playing a critical role in the proliferation of different types of cancer, particularly of prostate and ovary. ZD4054 is active in mice studies against different types of cancer in addition to prostate and ovarian. Moreover, it reduces angiogenesis, osteoblast proliferation in prostate cancer and pain sensation. Is currently under development in clinical studies conducted in prostate cancer. AZD6244 is a potent inhibitor of MEK active at nanomolar concentration in different tumor types. Early clinical studies have been conducted and are ongoing especially in melanoma. AZD0530 is a specific Src/abl kinase inhibitor. It has shown the interesting ability to inhibit pFAK, invasion and metastasis formation in vivo in tumor models including orthotopic pancreatic cancer, while apparently it has modest antiproliferative activity. It also prevents src-dependent bone resorption. Phase II studies are undergoing evaluating src kinase as pharmacodynamic marker. AZD1152 is a specific inhibitor of aurora kinases, an attractive target for the treatment of cancer since inhibition of Aurora kinase B leads to a distinct phenotype (failure of cell division) that is unique and different from that seen with classic 'anti-mitotic' agents. In rodent

models AZD1152 produces profound and durable anti-tumour effects. AZD1152 has the potential for activity in multiple tumour types and is in Phase I clinical trials.

F. Colotta (Director of Research and Development at Nerviano Medical Science center, Nerviano, Italy) illustrated the structure, the human resources and the development plan of the largest Italian industrial center of oncology. Dr. Colotta highlighted several agents that are currently evaluated in Phase I-II clinical trials. Nemorubicin, a topoisomerase I inhibitor, is active on a broad spectrum of tumour models, including those resistant to other agents and is now evaluated in several phase I-II studies. Brostallicin is a synthetic second generation agent that binds DNA minor groove. Brostacillin has a potent antitumor activity and a proapoptotic effect. It is noteworthy that it is active also in chemo-resistant tumor models. Brostacillin is currently evaluated in clinical studies. An array of agents that interfere with Aurora kinases function on mitotic spindle is under development. The Aurora inhibitors are currently in different phases of development. Finally, NMS has a portfolio of different inhibitors of Cyclin-dependent kinases (Cdks). They have shown antiproliferative activity in tumor models and are currently undergoing clinical development.

G. Ross (Clinical Science Leader of Oncology-Roche in Welwyn, UK) described the main results obtained and the plans for the development of different anticancer agents, focusing mainly on bevacizumab and partly on erlotinib. Bevacizumab, an antibody targeting all VEGF-A isoforms, is the first targeted agent approved for clinical use in first line colon cancer patients and one of the most successful targeted agents developed so far. Bevacizumab improves the effect of chemotherapy by pruning the vasculature and by increasing the drug delivery in the tumor. Bevacizumab in combination with different schedules of chemotherapy including fluoropyrimidines, irinotecan and oxaliplatin increases the survival of patients affected by colon cancer. It has also shown improvement of survival in first line NSCLC in combination with chemotherapy, in patients with anthracycline refractory breast cancer in combination with paclitaxel and in renal, pancreatic and ovarian cancer patients. Many studies are ongoing to evaluate its activity in combination with other targeted agents, including cetuximab, trastuzumab and erlotinib. An important effort is made

to evaluate the timing and length of administration and possible pharmacodynamic markers able to monitor its activity and select candidate patients. Erlotinib is a prototype small molecule EGFR inhibitor approved in NSCLC patients refractory to chemotherapy. The double blockade of EGFR and VEGF is an attractive strategy to enhance tumor targeting and to overcome resistance to anti-EGFR drugs. A clinical proof has been provided combining bevacizumab and erlotinib in the second line of NSCLC.

As regards the **Homeostasis and tumor progression** session, chaired by **F. Bussolino** (University of Torino, Italy) and **S.J. Reshkin** (University of Bari, Italy), the elegant model proposed by Folkman, in which tumor cells release molecules able to recruit capillaries from surrounding tissues, has been enriched by new evidences and hypotheses. For instance brain tumors develop around pre-existing capillaries mainly recruited by cancer stem cells and then, when central necrosis occurs, sprouting angiogenesis take places (cooption model). The contribution of bone-marrow precursors (vasculogenic model) or of cancer cells (vasculogenic mimicry) has been described in several situations. It is emerging in tumor progression the role of lymphangiogenesis that partially shares some regulatory cues with angiogenesis. Finally new sub-sets of myelomonocytic cells have been identified in tumors allowing a more accurate definition of their role in the different steps of cancer progression including capillary formation and the tumor intravasation. Finally, an even more puzzling observation is that the cancer malignancy does not always parallel the vascularization degree. Therefore the scenario of the angiogenic switch caused by an increasing number of genetic lesions and an unbalance between angiogenic and anti-angiogenic factors is changed being more complicated.

The therapeutic rationale derived from Folkman's model, "starve the tumor to death", is somewhat overwhelmed. The previous observation that anti-angiogenic therapy is not interested by resistance has not been widely confirmed. Tumors that lose p53 expression become less responsive to anti-angiogenic compounds, most probably for their acquired ability to survive in hypoxic conditions.

Preclinical evidences for antiangiogenic drug evasion by alternate pathways of angiogenesis in tumour cells has been described in pre-clinical models when tumors are treated with highly specific targeted antiangiogenic drugs. Angiogenic inducers produced in response to hypoxia may act to shield tumour EC. The recent observations that hypoxia stimulates metastasis spreading may implicate that antiangiogenic drugs may promote metastasis and invasion. Therefore the therapeutic window of an anti-angiogenic therapy has to be carefully checked. It also becomes apparent the fact that the efficacy of angiogenesis inhibitors depends on tumor stages. Normalization of tumor vasculature is an emerging evidence that certain antiangiogenic molecules can also transiently normalize the architecture of tumor capillaries before to induce their regression. This observation has two relevant consequences: i) it reduces hypoxia and favors conventional therapies, ii) cell motility and vascular remodeling seem to be the final targets of this therapeutic effect.

Three very interesting contributions presented in the section homeostasis and tumor progression tackle this modified vision of the impact of angiogenesis on tumor progression. Albini's group ([Istituto Nazionale Ricerca sul Cancro, Genova, Italy](#)) presented data showing that the chemopreventive retinoid N-(4-hydroxyphenyl)retinamide (4HRP) exerts its anti-tumor effect by stabilizing the transcription factor hypoxia-inducible factor -1 α (HIF-1 α). HIF-1 α is a key player in tumor angiogenesis induced by hypoxia and indeed this paradoxically effect could be explained, as suggested by the authors, by a modification of cell redox state that favors mitochondrial-dependent apoptotic pathway. However, the HIF-1 α stabilization by 4HRP should be utilized according to the normalization concept of tumor vasculature proposed by Rakesh Jain.

Another point of complexity in the anti-angiogenesis therapy concerns the differences in phenotype of vascular cells from different anatomical sites, including cancer tissues, that reflect a differentiated gene expression programs. By a differential gene expression analysis in endothelial cells (EC) isolated from tumor and normal tissues, the group of Raffaella Giavazzi (Mario Negri Institute, Bergamo, Italy) identified four new putative markers of tumoral EC. The impact of these

data is extremely wide and tools exploited from these genes may be used in pre-clinical models of target therapy and in ameliorating diagnostic capacity.

Finally the group of **G. Persico** (Institute of Genetics and Biophysics “Adriano Buzzati-Traverso” CNR, Napoli, Italy) presented a new variant of Placental growth factor (PlGF) that loosed the property to activate VEGFR-1, but retained the property to form heterodimers with VEGF-A. This allows depleting VEGF-A homodimers. Because VEGF-A-PlGF variant heterodimer does not activate VEGF receptors, this mutant is a useful compound to inhibit VEGF-A - dependent angiogenesis. By using tumor xenograft models the authors support and confirm their hypothesis. PlGF has been often considered an irrelevant molecule in the context of tumor angiogenesis. This report together emerging data from literature highlights a new relevance for this growth factor in tumor angiogenesis.

During the **Vaccination and tumor therapy session**, chaired by **A. Amadori** (University of Padova, Italy) and **M. Guida** (Istituto Tumori “Giovanni Paolo II”, Bari, Italy), centered on the issues raised by the poor outcome of the current vaccination approaches in cancer patients, **S. Ferrone** (Roswell Park Cancer Institute, New York, USA) and **G. Parmiani** (Fondazione IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy) presented results of clinical trials with melanoma antigens in patients. In these lectures, a special focus was placed on the numerous mechanisms which may interfere with effective immune responses to tumor antigens. These include poor immunogenicity of tumor-specific antigens, immunosuppression by bone marrow-derived cells, both myeloid and lymphoid, and emergence of "stealthy" tumor variants. According to both speakers, a major hurdle is represented by the down-regulation of HLA antigen expression on tumor cells, which compromises their recognition and eventually precludes effective neoplastic cell killing by tumor antigen-specific CD8⁺ T cells. As pointed out during the meeting, IFN- γ improves MHC expression in a fraction of melanoma cell lines, and future clinical trials are going to address the efficacy of vaccination approaches involving combinations of appropriate peptides and the IFN- γ

gene. In this setting, it is possible that IFN- γ as well may act as an efficient adjuvant, in view of its ability to enhance HLA class I expression; in addition, IFN- γ may also exert different activities, among which the anti-angiogenic potential has not to be overlooked. The selected presentations highlighted different aspects of vaccination and tumor therapy. Valzasina focused on the tumor-driven expansion of regulatory T cells from CD4⁺CD25⁻ peripheral T cells and suggested that triggering of OX40 on these cells may specifically block their inhibitory activity, thus favoring tumor rejection. Cappello and coworkers showed new data indicating that the ectoenzyme α -enolase is a pancreatic adenocarcinoma-associated antigen, capable of eliciting T cell responses in both mice models and patients. Finally, Banzato et al. expanded their previous work with hyaluronic acid conjugates and showed evidence of therapeutic efficacy of a novel radiotherapy approach based on ¹⁸⁸RE-hyaluronic acid for the treatment of primary liver tumors and liver metastases.

In conclusion, a great scientific relevance from this meeting and a great hope for the future. In fact, the young investigators which met in Bari already organized a forum discussion on the treated issues which will have a development during this year and will be realized in the next SIC meeting in Aviano in November 26-29 2007.