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Editorial: June 2005

In this issue we are pleased to announce that Lewis Cantley Ph.D., Prof. Department of System Biology, Harvard Medical School, Boston, USA is the winner of the 2005 Pezcoller Foundation-AACR International Award of Cancer Research.

The Selection Committee met in Trento in November 2004 and was made up of the following: President: Carl-Henrik Heldin – Ludwig Institute for Cancer Research, Sweden. Members: Jos Baselga – Vall d'Hebron University Hospital, Barcellona; Brian J.Druker – Oregon Health & Science University, Portland USA; Raffaella Giavazzi – Mario Negri Institute of Pharmacological Research, Bergamo; William J. Gullick – University of Kent, Canterbury, England; Lynn M. Matrisian – Vanderbilt University School of Medicine, Nashville USA; John Mendelsohn – UT M.D. Anderson Cancer Center, Houston USA; Eric J. Stanbridge – University of California, Irvine USA

The motivation for the Award was: "The 2005 Pezcoller-AACR International Award for Cancer Research is given to Dr Lewis C. Cantley for his outstanding contributions to the field of signal transduction, including his discovery of phosphatidylinositol-3′-kinase and elucidation of its role in signal transduction, as well as for his establishment of methods for unbiased determination of protein-protein interactions and kinase specificity."

2005 Pezcoller Foundation - AACR International Award for Cancer Research
Dr. Bernardi and Dr. Cantley

Lewis Cantley held his important lecture on the opening day of the 2005 Meeting of the AACR in Anaheim CA and was given the prize during an official ceremony at the Buon Consiglio Castle in Trento on Friday 6 May. During this ceremony, last year's winner Dr. Stanley Korsmeyer was remembered by a moving minute of silence.

The following pages are holding the posters of the 17th Pezcoller Symposium on "Molecular Understanding of Solid Tumors".

Tha call fo 2006 Pezcoller Foundation – AACR International Award for Cancer Research have also been inserted towards the end of this copy.

Gios Bernardi M. D.
The Pezcoller Foundation President and Editor

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17th Pezcoller Symposium
MOLECULAR UNDERSTANDING OF SOLID TUMORS
June 16-18, 2005 - Trento, Italy

ABSTRACTS OF ORAL PRESENTATIONS

Screens of all protein kinases for somatic mutations in human cancer

Dr Stratton, *the Wellcome Trust Sanger Institute Cambridge, UK*

All cells acquire somatic mutations as their DNA is replicated. These can be point mutations, rearrangements or copy number changes. A cancer occurs when a single cell acquires the requisite number and combination of mutations in a subset of human genes known as "cancer genes" (a cancer gene is defined as a gene with mutations that are causally implicated in oncogenesis). Our ongoing Census of Human Cancer Genes indicates that approximately 350 protein coding genes are currently known to be cancer genes.

Mutations in cancer genes confer selective growth advantage upon the cell in which they occur and may be termed "driver" mutations. All cells, including cancer cells, will also carry "passenger" mutations (sometimes known as "bystander" mutations). These do not confer selective growth advantage upon the cell in which they occur. The number and pattern of passenger mutations in a cancer will depend upon the number of rounds of DNA replication in the normal and neoplastic cellular lineage from the fertilised egg to the cell that is the progenitor of the last major clonal expansion of the cancer. It will also depend upon the mutation rate at each round of replication, which in turn may depend upon past or current defects in DNA repair or mutagenic exposures.

The advent of the finished human genome sequence provides the opportunity for systematic searches for somatic mutations in cancer. In principle these searches may uncover new "driver" mutations and hence new cancer genes. These searches may also, however, provide information on the number and patterns of "passenger" mutations which will inform on the mutagenic processes operative during the history of the cancer cell. Examples of both types of information emerging from systematic screens for point mutations will be presented.

In the Cancer Gene Census the most commonly encoded protein domain is the protein kinase. Protein kinases are usually activated by mutations in cancer and function as dominant cancer genes, but a minority are inactivated and may be recessive cancer genes. A number of recent studies have demonstrated that activated protein kinases are tractable targets for the development of new anti cancer drugs. The protein kinase gene family is the second largest encoded by the human genome. We have carried out systematic mutational screens in several human cancer types for point mutations in the exons and splice junctions of the 518 genes known to encode protein kinases. In these screens, approximately 1.3Mb DNA is screened in each cancer sample, the largest amount of DNA screened until now.

The 518 protein kinases have been screened in 26 breast cancers, 33 lung tumours (of which 27 were carcinomas and 6 were carcinoids), 13 testicular cancers, 20 gastric cancers and 30 colorectal cancers. There was considerable variation in the number of somatic mutations observed between cancer types. For example, no mutations were found in the 6 lung carcinoids and only a single mutation was found among the 13 testicular cancers. By contrast, 188 somatic mutations were found among the 27 lung carcinomas. There was also marked variation within cancer types. For example, among primary breast cancers most had no mutations, a couple had one or two mutations and one had approximately 50 mutations.

The number of silent mutations observed in these screens indicates that most somatic mutations detected are "passenger" mutations. There is, therefore considerable variation in the rate of passenger mutations between individual cancers and cancer types. This may be due to variation in the number of DNA replications each cancer has been through or the mutation rate at each

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replication. The large number of mutations generally seen in lung cancer (and similarly in gastric and colorectal cancer) suggest that cancers derived from surface epithelia with high turn over that are exposed to exogenous mutagens accumulate more somatic point mutations than other tissue types.
Analysis of the patterns of somatic mutation can also be informative. For example, although most primary breast cancers had few somatic mutations, one had approximately 50. This is likely due to a new mutator phenotype. The mutations in this cancer were characterised by a high proportion of C:G>G:C changes which arose in a distinctive sequence context, specifically at TpC (GpA) nucleotides. At least one other breast cancer had a similar pattern of mutations suggesting that this phenotype may be present in a significant proportion of breast cancers. The cause of this mutator phenotype is not currently known, however, it is unlikely to be due to BRCA1 *or* BRCA2 *mutations. The mutational screens of breast cancer, lung cancer and testis cancer have not yielded a protein kinase that is frequently mutated in any of these cancer types. However, in both the breast cancer and lung cancer protein kinase screens there is evidence for an excess of non-synonymous mutations (ie mutations that change the amino acid sequence) compared to that expected by chance on the basis of the number of silent (synonymous) mutations. One interpretation of this observation is that there are several "driver" mutations which are contributing to oncogenesis, but that they are dispersed among many cancer genes, each of which is mutated infrequently. Consideration of individual mutations provides evidence in support of this notion. If correct, this would suggest that there is great diversity of routes to oncogenesis. Moreover, it would highlight problems for the development of targeted therapies, if there are a substantial number of potential targets, each of which is operative in only a small proportion of human cancers.*

SNP array based analyses of somatic genetic and the identification of *MITF* as a lineage survival oncogene amplified in malignant melanoma

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Cancer is fundamentally a disease arising through the acquisition of specific somatic genetic alterations. We have approached the systematic identification of large-scale cancer associated somatic genetic alterations using single nucleotide polymorphism (SNP) arrays and of small-scale genetic changes via exon re-sequencing of primary human tumor DNA. As part of these efforts we have interrogated the structural alterations across the NCI60 panel of cancer cell lines using high-density SNP array to develop genome-wide genetic maps. Integration of such maps with gene expression signatures led to identification of MITF *as the target of a newly-recognized melanoma amplification.* MITF *amplification was associated with metastatic disease and with decreased overall survival. In melanoma cell lines,* MITF *amplification was typically accompanied by* BRAF *mutation. Ectopic MITF expression was required in conjunction with BRAF(V600E) to transform primary human melanocytes; thus,* MITF *is a human melanoma oncogene. Thus,* MITF *represents a distinct class of 'lineage survival' or 'lineage addiction' oncogenes that are required for both tissue-specific development and tumor progression. Targeting MITF alone or in combination with BRAF may offer a therapeutic avenue into this highly chemotherapy resistant disease.*

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Addressing the Complexity of the Oncogenic Process

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Genome-scale measures of gene expression derived from DNA microarray studies has the potential for adding enormous information to the analysis of biological phenotypes. Perhaps the most successful application of this data has been in the characterization of human cancers, including the ability to predict clinical outcomes. Nevertheless, most analyses have used gene expression profiles to define broad group distinctions, similar to the use of traditional clinical risk factors, leaving considerable heterogeneity within the broadly defined groups. To fully realize the clinical potential of genome-scale information requires a paradigm shift in the way complex, large-scale data is viewed, analyzed and utilized. Individual risk factors, be they genetic, clinical genomic, or other, represent only single or low-dimensional snapshots of the disease process and state. Taken further, single gene expression profiles also exhibit limitations. An example can be seen in a recent study that re-analyzed the work of van'T Veer and colleagues that described the use of a DNA microarray-based 70-gene expression profile as a prognostic factor in breast cancer (van'T Veer et al., 2002). This new study (Ein-Dor et al., 2005) demonstrates, one key point: the original 70-gene predictor is not unique in that multiple such predictors can offer comparable predictive performance. Ein-Dor and colleagues identified at least eight separate gene sets whose aggregate expression patterns can stratify the same patient sample into comparable high risk and low risk categories, suggesting that multiple forms of information derived from this dataset can equally well stratify patients according to recurrence risk. While this may surprise some, the result is perfectly consistent with the experience of multiple studies in breast cancer and other diseases. Various previous studies that focused on predicting breast cancer outcomes have yielded largely distinct gene sets from different data sets and expression platforms, and also from within-study analyses such as the Ein-Dor et al. study. Heterogeneity in patient populations, methods of assay, varieties of array technologies and other factors certainly underlie some of the differences. The fundamental reason is, however, simply the diversity, scale and complexity of genetic and genomic factors defining this disease. Our own work has demonstrated that the most robust predictive models, consistent with the concept of a complex, high-dimensional risk spectrum, make use of multiple gene expression profiles as well as clinical data combined together in integrative "clinico-genomic" models (Pittman et al., 2004). The most relevant predictive models are generated by the combined use of these multiple informative signatures, together with the relevant clinical data. Such models, which begin to address the true complexity of the disease, point the way toward predictions for individual patients. This is certainly the ultimate goal: to define integrative models that, by building on multiple aspects of information that reflect the individual circumstances, lead to personalized predictions, rather than stratification into broader patient subgroups.

An important additional aspect of the overall goal of personalized medicine is the ability to direct the use of the most appropriate therapeutic. In the case of prognosis, the goal is to identify an individual's risk to then gear therapy accordingly. Likewise, efforts to predict response to specific chemotherapies have the goal of identifying those patients most likely to respond. In each case, it remains true that patients predicted at highest risk, or likely to fail a given therapy, require additional options that are not currently obvious. The challenge is to identify characteristics of the tumor of the resistant or high risk patient that might suggest unique opportunities for therapy. With this in mind, we have applied similar strategies of gene expression profiling to identify signatures that characterize the activity of various oncogenic signaling pathways including Ras, Myc, Src, b-catenin, and Rb-E2F pathways . These gene expression profiles have been shown to accurately predict the activity of these pathways, including in the context of mouse tumor models involving the deregulation of these pathways. We have now applied the ability to predict the deregulation of a series of pathways to the analysis of human cancers – breast, ovarian, and lung. We have identified patterns of pathway deregulation that coincide with distinct clinical outcomes suggesting a capacity of the pathway predictions to identify biologically

significant characteristics of the tumors. Moreover, these pathway predictors have also been applied to the analysis of a series of breast cancer cell lines to profile the status of each pathway. This is then matched with assays measuring sensitivity of each cell line to several targeted therapeutic agents. We find that there is a relationship between the prediction of pathway deregulation and sensitivity to a drug that targets a component of the pathway. We suggest that the ability to profile the status of various oncogenic pathways may be an approach to guiding the use of targeted therapies in those patients unlikely to benefit from standard therapeutic protocols. The combined use of risk stratification or chemotherapy response prediction, together with pathway analysis, has the potential for forming an integrated approach to more effectively treating patients with advanced stage disease.

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Modeling and Analyzing Cancer Genomes: High Resolution Genomic Profiles Of Human Lung Cancer.

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Non Small Cell Lung Carcinoma (NSCLC) is the leading cause of cancer related mortality in the United States and in the world, accounting for more than one-fourth of all cancer fatalities in 2004 in the US. NSCLC is subdivided into three major histologically subtypes: adenocarcinoma (AC), squamous cell carcinoma (SCC) and large cell carcinoma, with AC and SCC subtypes representing over 85% of NSCLC cases. While these NSCLC subtypes exhibit distinct pathological characteristics, the treatment approaches have remained generic and largely ineffective despite advances in cytotoxic drugs, radiotherapy, and clinical management. For all stages of NSCLC, the 5-year survival rate has remained fixed at 15% for the last 15 years. The recent success of molecularly targeted therapies for limited subset of cancer genotypes has solidified the view that a more detailed knowledge of the spectrum of genetic lesions in cancer will lead to therapeutic progress. Among the approaches recently developed to elucidate the multifaceted aberrations present in the cancer genome, integrated array Comparative Genomic Hybridization (aCGH) and expression profiling have emerged as an effective platform for novel cancer gene discovery, providing a high-resolution view of the regional gains and losses throughout the cancer genome and their associated copy number-driven changes in gene expression.

Identification of known and novel CNAs in the NSCLC genome

We have analyzed forty-four primary NSCLC tumors, 18 adenocarcinomas (ACs) and 26 Squamous Cell Carcinomas (SCCs), using either an oligo array platform with a median resolution of 50 Kb or a cDNA array platform with a median resolution of 72.7 Kb. In addition to the primary tumors, aCGH profiling was performed on a panel of 34 NSCLC cell lines. Expression profiling using an Affymetrix 2.0 Plus platform was performed on the majority of the samples.

These profiles revealed a NSCLC genome that is highly re-arranged, harboring large numbers of distinct copy number aberrations (CNAs = 319). Some of these CNAs were recurrent across different samples, allowing for definition of a minimal common region (MCR) of gain/amplification or loss/deletion. There were 93 MCRs – 74 amplifications and 19 deletions with a median size of 1.53 Mb. These MCRs included all known regional gains and losses, specifically the known gains at 1q31, 3q25-27, 5p13-14, and 8q23-24 as well as the known deletions at 3p, 8p22, 9p21-22, 13q22, and 17p12-13. Virtually all of the genes implicated in the pathogenesis of NSCLC were contained within the MCRs including

$p16^{INK4A}$ and RB1 tumor suppressor genes and MYC, EGFR and KRAS2 oncogenes. The most notable feature of this MCR dataset was a subset (21 out of 93) of highly focal MCRs that spanned less than 0.5 Mb and possessed a median of only 5 genes. Several of these genes have already an established role in cancer (e.g. ERBB3) or possess domains suggestive of a potential role in cancer.
Common and distinct genomic features in AC and SCC.
Next, we sought to determine whether there exist genomic events characteristic of either SCC or AC. Surprisingly, only one region of gain/amplification on the long arm of chromosome 3, from 180 Mb to 199 Mb, corresponding to 3q26 to 3q29, was significantly targeted in the SSCs. Therefore, despite strikingly distinct histological presentations, SCC and AC are remarkably similar on the genomic level and are likely driven by many of the same oncogenes and tumor suppressor gene mutations. Interestingly, among the few genes located in this region that showed significant overexpression in SCCs versus ACs, even in the absence of gene copy number gains on 3q in SCCs, there was p63, a gene that exerts a seminal role in squamous tissue development and that is specifically linked to squamous cancer subtypes.
Cross tumor type genomic comparisons.
Finally, to identify genetic lesions that could be of general significance in epithelial carcinogenesis, we compared the lesions present in the lung dataset with aCGH results from another epithelial tumor type, pancreatic adenocarcinoma (PDAC). These epithelial cancers are known to share similar clinical aggressiveness, several signature mutations and high genomic complexity. In addition to expected common genomic alterations such as KRAS, c-MYC and INK4a/ARF, there were additional 20 shared novel loci. One amplicon shared in the NSCLC and PDAC datasets mapped to 20q11.2 harboring BCL2L1 (previously BCL-xL) and 4 other genes, ID1, COX4I2, BCL2L1, TPX2 and MYLK2. Interestingly, TPX2 was the only gene showing high-level copy number-driven expression in most lung cell lines and primary tumors tested, suggesting a role for TPX2 as a potential candidate oncogenes targeted for amplification in both lung and pancreas cancers. Another shared locus was a focal amplification at 8p12 including FGFR1, a cancer-relevant gene not previously implicated in lung cancer. However, detailed mapping positioned FGFR1 outside of the telomeric boundary. Only 2 annotated genes were included in this MCR, WHSC1L1 and LETM2. SiRNA-mediated knock-down of WHSC1L1 resulted in 50% reduction in the number of soft agar colonies in a cell line harboring this amplicon, while near complete FGFR1 depletion had no impact on colony formation in soft agar. These findings argue against a role for FGFR1 in lung cancers presenting an 8p amplicon and points to WHSC1L1 as a potential target of this amplification event.
In conclusion, using gene-specific CGH platforms, custom bioinformatics tools, along with integration of expression profiles, we have identified many new recurrent amplifications and deletions in the NSCLC genome. The high degree of NSCLC genomic complexity, the recurrent nature of these lesions, and preliminary functional characterization of resident genes suggests that a large number of important oncogenes and tumor suppressor genes remain to be identified, opening attractive possibilities to model this disease, as well as potential therapeutic and diagnostic opportunities for this dismal cancer.

Acute promyelocytic leukemia, a model for oncogene-targeted combination therapy

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APL is rare, but severe condition, since standard chemotherapy rarely yield more than 30 % cure rates. In 1988, the group of Pr. Wang showed that this disease could undergo complete remissions upon treatment with retinoic acid through induction of differentiation, identifying the first example of differentiation therapy. In collaboration with L. Degos and A. Dejean, I showed in 1990 that this translocation involves a fusion of the retinoic acid receptor _ with PML gene to yield a PML/RARA fusion protein. The exquisite sensitivity of the disease to retinoic acid, whose receptor is rearranged, made APL a real paradigm for oncogene-targeted therapies. One should, however, note the highly paradoxical finding of an altered receptor in a disease sensitive to the hormone. It was demonstrated that what was believed to be sensitivity, is in fact relative resistance

to retinoic action, since PML/RARA was shown to be a super repressor of nuclear receptor target genes, due to its tighter binding of transcriptional corepressors resulting from PML-induced RARA dimerisation.

We have demonstrated that PML is bound to a specific nuclear subdomain and that PML/RARA expression delocalises the components of this domain. These structures, whose exact functions are still unclear are the focus of a great interest having been associated to senescence, transformation, apoptosis or viral infections. Interestingly, PML -/- animal are susceptible to infections, have an impaired apoptosis and are tumour susceptible. Many reports have implicated PML in apoptosis or senescence control, possibly through the control of P53 function. Yet, this is a highly disputed area and the exact mechanism through which PML could control P53 function is unclear. Yet, PML/RARa expression was shown to induce apoptosis resistance and we have identified major and consistent abnormalities of PML expression in a variety of human tumours. Hence, the molecular pathogenesis of APL could be accounted both by a repression of nuclear receptor target genes involved in myeloid differentiation, and by an altered apoptosis control associated with nuclear body disruption.

In APL cells, retinoic acid induces both the transcriptional activation of PML/RARA and the relocalisation of PML towards nuclear bodies, linking the repression and NB disruption to the transformed state. Such nuclear body relocalisation results from the degradation of PML/RARA upon RA treatment and likely results in the restoration of the pro-apoptotic functions of PML. We have analysed the degradation pathways involved in RA-induced PML/RAR__catabolism and showed that it involves both a specific cleavage by caspases and the proteasome, through an interaction between the 19S component SUG-1 and the RA-activated AF-2 transcriptional activator function. PML/RAR_ targeting by RA involves its AF2 domain, both for its transcriptional activation and ligand dependent degradation. Taken the thigh links between activation and catabolism of nuclear receptors and transcription factors at large, it is difficult to separate these two effects.Arsenic trioxide induces a significant number of complete remissions in APL. Arsenic induces both differentiation and apoptosis. The respective contribution of each process to clinical remissions is currently unknown. We have first demonstrated that arsenic, similar to RA, induces PML/RARA degradation and accordingly restores nuclear bodies. In contrast to RA, that targets the RARA moiety of the fusion, arsenic targets its PML moiety, since it also degrades PML. This catabolism is preceded by the targeting of nucleoplasmic PML onto matrix associated nuclear bodies. We have shown that arsenic can enhance the proapoptotic effects of PML, suggesting that the targeting of NB-associated protein such as Sp100, Daxx or p53 onto the nuclear matrix can greatly sensitise the cells to apoptosis. PML can be modified by a family of ubiquitin-related peptides, sumo, in an arsenic enhanced manner. It was proposed that PML sumolation is involved in its nuclear matrix targeting and the recruitment of associated proteins onto nuclear bodies. We have demonstrated that the situation is considerably more complex. Arsenic induces the matrix targeting of PML independently from sumo, sumolation occurs secondarily and is responsible for the recruitement of associated proteins onto the matrix. Among these, we have identified the 11S complex of the proteasome. This is particularly important because PML degradation occurs after sumolation and proteasome recruitment. Hence, sumolation on lysine K160 is a prerequisite for PML degradation induced by arsenic. Our work implies that nuclear bodies could be sites of catabolism, which could account for the astonishing variety of associated proteins.

In an attempt to yield an arsenic-resistant APL we have developed transgenic mice where the critical sumolation suite implicated in arsenic response, PML lsyine 160, was mutated. Strikingly, this sumolation site is absolutely required for the APL-specific differentiation block. Viral transduction of hematopoietic progenitors or expression of the fusion protein in trangenic mice fail to recapitulate APL development if this critical sumolation site in PML is mutated. Ex vivo *studies suggest that the function provided by this sumolation site is transcriptional repression. These observations suggest that PML is the recurrent fusion partner of RARA in APL because, in addition to enforced dimerisation, it fuses a repression domain to the DNA binding domain of RARA.*

RA-resistant leukemic cells are arsenic sensitive and vice versa. *Some groups had described an antagonism between the two agents, at least in cell lines. We have*

identified a major synergy between the two drugs, both in cell-lines and in an animal model of the disease. In the animal model, combining the two agents suffice to eradicate leukemia. The in vivo *synergy was later confirmed by two other groups, strongly favouring combined treatments in APL patients. In that respect, data from the Chinese group has demonstrated that blast clearance is greatly accelerated in dually treated patients and that these all reach complete remissions, stressing the relevance of our observations to the cases of APL patients. Moreover, these observations provide the first example where cancer therapy was optimised in the mice and where the optimised treatment was then successfully transferred to patients.*

More recently, we have shown that cAMP can trigger differentiation of APL cells both ex vivo *and* in vivo. *Cyclic AMP greatly enhances the differentiation mediated by RA or arsenic. In addition, cAMP can even completely reverse resistance to RA that is conferred by a point mutation in the binding pocket of the RARA moiety of PML/RARA. This likely reflects a cAMP-induced conformational change in the RARA ligand-binding domain and suggests, as demonstrated in embryonal carcinoma cells, that the cAMP and RA signalling pathways both converge onto the same transcription factor: RARA. In fact, a very strong evidence for this model was recently provided by our identification of PML/RARA-specific reporter genes. Using these we have observed that only the combination of RA and cAMP activates/derepresses PML/RARA-dependent transcription in a RA-resistant leukemia, which demonstrates a functional cooperation of these two signalling molecules directly on PML/RARA. Finally, we have shown that agents that stabilize endogenous cAMP are of clinical value in RA-resistant APL patients.*

Altogether, our work has shown that the PML/RARA oncogene can be successfully targeted by 3 different agents which directly modulate PML/RARA function and/or stability. Using transgenic mice, that express PML/RARA point mutants known to impair response to these agents, we are analysig the in vivo *cross-talks between the 3 therapies. The results of this modelling in mice have lead to an optimal treatment that induces durable complete remissions in all patients, providing a decisive model for the future of cancer therapy.*

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Mouse models for cancer

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We have made a significant investment in developing new mouse models for a variety of tumors, using Cre/Lox mediated switching of tumor suppressor genes and oncogenes.

Lung tumors*. Lung tumors can be efficiently induced by Adeno-*Cre *mediated switching of a floxed mutant* Ki-Ras *allele or by inactivation of both* Rb *and* p53 *floxed alleles. Interestingly, while the same subset of cells are infected by the Adeno-*Cre *virus, activation of* Ki-Ras *or inactivation of* Rb *and* p53 *results in very*

different tumors. Somatic activation of Ki-Ras *results in adenocarcinomas resembling non-small-cell lung cancer (NSCLC). Somatic inactivation of Rb and p53 yielded tumors with all the features of metastatic small cell lung carcinoma (SCLC). We have established a series of cell lines from these mSCLC tumors. We performed array CGH analyses using a 1 Mb BAC array. Interestingly, we observed frequent amplification of L-Myc in these tumors, in agreement with the frequently observed L-myc amplification in human SCLC. Cells with amplified L-myc showed more distinct neuro-endocrine differentiation as revealed by expression array analysis. L-myc amplification was not observed in the clones with more "stem cell like" features. This is in agreement with the earlier reported mutual exclusiveness of high Myc expression and stem cell characteristics. As a prelude for therapeutic intervention studies we have established an orthotopic grafting model with these cell lines and shown that they reproduce the in vivo phenotype seen in the spontaneous tumors.*
Pituitary tumors *are efficiently induced in* $Rb^{flox/flox}$ *mice upon pituitary-specific expression of Cre recombinase. We have tested the utility of this* in vivo *tumor model system to follow tumor growth and response to therapy* in vivo *by luciferase-based imaging and monitored response to chemotherapeutic intervention. The study shows the critical requirement for timing of administration of drug regimens and illustrates the need to test these in suitable model system.*

Analysis of cell motility and transformation by oncogenic Ras using novel functional genomics approaches

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Transformation of cells by Ras and other oncogenes leads to profound changes in their gene expression programmes. Two decades of intense study of signaling pathways has led to a good understanding of many of the early signaling events induced on stimulation of oncoproteins such as Ras. In addition, recent advances in microarray technology have provided much information about the changes in transcription that accompany transformation. However, the key medium term events required to set up the transcriptional programme underlying malignant transformation are much less well defined, but may include a number of good therapeutic targets.
We have set out to study a number of aspects of cellular transformation using large-scale RNA interference libraries, including the 8000 gene NKI library (Berns et al., 2004). This library is in a retroviral vector that can be used either in selective screens in a pooled format, or in phenotypic screens in a high throughput format. To find novel signalling proteins regulated by Ras, we have undertaken a selective RNA interference screen for inhibitors of Ras induced senescence in human ovarian surface epithelial cells using pools of vectors targeting 96 genes each. A number of known critical Ras activated kinases have been identified in this way, including PI 3-kinase p110a and p70 S6K1. In addition, proteins not previously associated with Ras signaling have also been identified. One of these is MINK, a MAP kinase kinase kinase kinase which we show to be activated by Ras through the Raf/ERK pathway. The role of MINK in Ras induced senescence appears to be through its ability to activate the p38 stress activated kinase. This leads to induction of expression of the cyclin-dependent kinase inhibitor p21, which promotes cell cycle arrest. While PI 3-kinase p110a and p70 S6K1 are required both for Ras-induced senescence and Ras-induced transformation, MINK appears to play a significant role only in Ras-induced senescence.
Another screen has been carried out in a high throughput mode (one gene at a time) to study genes involved in the enhanced motility of invasive lung cancer cells carrying an activating mutation in K-Ras. Cells were transiently transfected with the RNAi vectors and a GFP vector and then their movement tracked by time-lapse video microscopy. In this way a number of novel proteins have been identified that affect cell motility, plus many proteins already known to be involved in this process. Subsequent characterisation has been carried out on a transcription factor that unexpectedly appears to play an important role in promoting invasiveness in human breast cancer. CUTL1 is important in providing the transcriptional programme needed for cells to move rapidly in two and in three dimensions, particularly being a regulator of integrin a6 expression. Low levels of CUTL1 expression prove to be a significant beneficial prognostic factor for long-term survival in breast cancer patients.

The use of this large-scale RNA interference library that targets a quarter of the human genome thus shows considerable promise in uncovering novel targets for cancer therapy. Potential problems occurring in the use of these new functional genomics tools will also be discussed.

Cell death or survival in response to p53

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p53 is a tumor suppressor protein with potent cell cycle arrest and apoptotic functions, both of which contribute to the inhibition of malignant development. Most cancer cells show loss of p53 function, and there is evidence that reactivation of p53 is more likely to induce apoptosis in tumor cells than their normal, unstressed counterparts. This differential response underlies the hope that p53 may be an effective therapeutic target. The choice of response to p53 is determined – in part – by differential activation of gene expression by p53. We have recently identified ASPP1, a protein that acts as a cofactor to allow p53-induced expression of apoptotic target genes, as a transcriptional target of E2F1. Activation of ASPP1 may therefore contribute to the ability of deregulated E2F to favor the activation of apoptosis in response to p53.
p53 can also play a role in allowing cells to repair and recover from genotoxic insult, and it is clear that p53-inducing stress signals do not always result in cell death. Understanding what regulates the choice of response to p53 is important when considering reactivation of p53 as a tumor therapy, and how to achieve maximum differential between the death of cancer cells and survival of normal cells.
Modulation of the levels of p53 can contribute to the extent of the response, and stimulation of the activity of HDM2, an E3 ligase that regulates p53 stability, can contribute to cell survival. For example, phosphorylation of HDM2 on serine 166 by AKT (which can mediate survival signals) can lead to an enhancement of HDM2 activity, resulting in increased degradation of p53. We have found that MAPKAP kinase 2 (a downstream target of p38MAP kinase) can phosphorylate HDM2 on serine 157 and serine 166, and that modification of both of these sites can modulate the activity of HDM2 towards p53. These results indicate a role for MK2 in negatively regulating the p53 response, and we find that cells deficient for MK2 show elevated levels of p53 protein, enhanced stabilization of p53 in response to UV treatment and increased sensitivity to apoptosis. Together our results suggest that MK2 may act to dampen the extent and duration of the p53 response following exposure to p38-inducing stress signals.
Interestingly, in addition to the induction of pro-apoptotic targets, p53 can induce expression of several proteins that show anti-apoptotic activity. It is clear that p53-target genes can be differentially activated and so the balance of death and survival signals induced by p53 may be critical in determining the ultimate response to p53 activation. We have found a novel p53-target gene – JAVA *- that encodes a protein with similarity to the phosphatase domain of the bifunctional enzyme PFK-2/FBPase-2, one of the principal regulators of glycolysis. Expression of JAVA may enhance the oxidative branch of the pentose phosphate pathway, hence conferring resistance to oxidative stress by enhancing NADPH production which provides the necessary reducing equivalents to restore reduced glutathione (GSH) levels. Therefore, ectopic expression of JAVA protects cells from both ROS and p53-induced cell death. We are proposing that JAVA belongs to a group of p53-inducible genes that contribute to the survival of cells undergoing oxidative stress.*

The VHL tumor suppressor gene: Kidney cancer gatekeeper

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inactivating VHL *mutations cause von Hippel-Lindau disease, which is characterized by an increased risk of clear cell renal carcinomas (RCC), hemangioblastomas, and pheochromocytomas. Tumor development in VHL disease is linked to somatic inactivation or loss of the remaining wild-type* VHL *allele in a susceptible cell. Somatic* VHL *mutations are also common in sporadic RCC, which is the most common form of kidney cancer. The* VHL *gene product (pVHL) is part of a ubiquitin*

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ligase complex that targets the alpha subunit of the heterodimeric transcription factor HIF (hypoxia-inducible factor) for destruction in the presence of oxygen. Under low oxygen conditions, or in the absence of pVHL, HIF accumulates and HIF target genes such as VEGF, PDGF B, and TGFa are transcriptionally activated. Downregulation of HIF target genes is both necessary and sufficient for the suppression of VHL (-/-) renal carcinoma tumor growth by pVHL and drugs that inhibit VEGF are showing promise as treatments for kidney cancer in man. The interaction of pVHL with HIF is oxygen-dependent because HIF must be hydroxylated on specific proline residues by members of the EGLN prolyl hydroxylase family in order to be recognized by pVHL. The oxygen atom of the hydroxyl group is derived from molecular oxygen and EGLN activity is sensitive to changes in ambient oxygen over a physiologically relevant concentration range. We recently made a mouse that ubiquitously expresses a HIF-luciferase fusion protein. This strain has allowed us to image the activity of this pathway in vivo *and is faciliatating the development of small molecule HIF agonists for the treatment of anemia and ischemic diseases.*

Hedgehog signaling in tissue regeneration, stem cell renewal, and neoplasia

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Cellular responses elicited by the Hedgehog (Hh) family of secreted signaling proteins play an important role in the growth and patterning of multicellular embryos, from insects to mammals. Deranged activation of Hh signaling pathways in contrast is associated with neoplastic growth, as established by association of pathway-activating familial mutations in Gorlin's Syndrome with an increased incidence of basal cell carcinoma, medulloblastoma, and rhabdomysarcoma. More recent studies with specific Hh pathway antagonists such as the plant-derived teratogen, cyclopamine have revealed an ongoing requirement for pathway activity in growth of additional tumor types that include small cell lung cancer, and carcinomas of the esophagus, stomach, pancreas, biliary tract, prostate, and bladder[1-3]. In contrast to the ligand-independent pathway activation associated with tumors found in Gorlin's Syndrome, Hh pathway activity in these cancers, which together account for as many as 25% of cancer deaths, depends upon expression of Hh signaling proteins.

Recent studies point to renewal and maintenance of tissue stem cells as normal post-embryonic roles for activity of the Hh signaling pathway and its sister, the Wnt pathway. These findings are of relevance to cancer because of the possible derivation of cancer stem cells, the minority of cells within a cancer that are capable of its propagation, from adult tissue stem cells. Pathway activity and expansion of progenitor or stem cell pools also are associated with response to tissue injury. Chronic tissue injury furthermore results in increased risk for cancers of the types associated with Hh and Wnt pathway activity[4]. These observations suggest that cancer growth resembles an active state of injury repair, and that continuous Hh pathway activity in cancers may result from a failure of stem cells to return to a quiescent state, as normally occurs following regeneration[4]. This hypothesis will be discussed in the light of recent studies of Hedgehog pathway activity in response to tissue injury and in carcinogenesis.

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From cancer Genomics to cancer clinic.

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cDNA microarray technologies have enabled us to obtain comprehensive data for gene expression profiles of human cancers. To isolate novel targets for diagnosis (predictive marker for the efficacy of treatment as well as tumor marker) and for treatment of cancer (molecular-targeting drug, cancer vaccine, antibody), we have been comparing expression profiles of cancer cells originated from various organs with their corresponding non-cancerous tissues using a cDNA microarray that consists of more than 30,000 genes. These experiments disclosed a number of genes that appeared to be involved in development and/or progression of cancers in those tissues. So far, we have analyzed more than 1,000 cases of clinical cancer samples of the liver, pancreas, stomach, colon, esophagus, bile duct, uterus, lung, ovary, kidney, urinary bladder, testis, prostate, breast, and soft tissues as well as acute and chronic myeloid leukemias. We have selected hundreds of candidate genes by the criteria as follows; (1) gene expressions were transactivated in a large proportion of cancer tissues in comparison with their corresponding normal tissues and (2) expression was not observed or hardly detectable in any important vital organs. The further functional analysis identified dozens of genes that are likely to function as oncogenes in various cancers. The suppression of expression of such genes with small-interfering RNAs (siRNAs) induced cell cycle arrest, apoptosis, or suppression of anchoring-dependent cell growth.

For example, one of the good candidates that we identified as a molecular target for development of drugs was SMYD3, *a gene that is over-expressed in the majority of colorectal and hepatocellular carcinomas. Introduction of* SMYD3 *into NIH3T3 cells enhanced cell growth, whereas genetic knockdown with siRNA in cancer cells resulted in significant growth suppression. SMYD3 formed a complex with RNA polymerase II through an interaction with the RNA helicase HELZ and transactivated a set of genes that included oncogenes, homeobox genes and genes associated with cell-cycle regulation. SMYD3 bound to a motif, 5'-CCCTCC-3', present in the promoter region of downstream genes such as* Nkx2.8. *The SET domain of SMYD3 showed histone H3-lysine 4 (H3-K4)-specific methyltransferase activity, which was enhanced in the presence of the heat-shock protein HSP90A. Our findings suggest that SMYD3 has histone methyltransferase activity and plays an important role in transcriptional regulation as a member of an RNA polymerase complex.*

We also developed antibodies that showed growth-suppressive effect in vivo *and/or* in vitro. *Through the genome-wide expression profiles of renal cell carcinomas (RCCs) using a cDNA microarray, we identified that hypoxia-inducible protein-2 (*HIG2) *was expressed exclusively in RCCs and fetal kidney. Induction of* HIG2 *cDNA into mammalian cells led to secretion of the gene product into culture media and resulted in enhancement of cell growth. Small interfering RNA (siRNA) effectively inhibited expression of* HIG2 *in human RCC cells that endogenously expressed high levels of the protein, and significantly suppressed cell growth. Moreover, addition of polyclonal anti-HIG2 antibody into culture media induced apoptosis in RCC-derived cell lines. ELISA analysis of clinical samples identified secretion of HIG2 protein into the plasma of RCC patients even at an early stage of tumor development, whereas it was detected at significantly lower levels in healthy volunteers or patients with chronic glomerulonephritis. The combined evidence suggests that this molecule represents a promising candidate for development of molecular-targeting therapy and could serve as a prominent diagnostic tumor-marker for patients with renal carcinomas. These results indicated that systematic expression analysis should be a very effective approach for identification of molecules that are potential targets for development of novel therapeutic drugs and diagnostic tools.*

The Shp2/Gab2 Pathway in Human Cancer.

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The SH2 domain-containing protein-tyrosine phosphatase Shp2 is required for activation of the Ras/Erk pathway in response to most growth factors and cytokines. Shp2 also has variable, receptor- and cell context-dependent effects on other downstream signaling cascades. All of these actions require recruitment of to one or more binding proteins, including scaffolding adapters such as Gab proteins.

Consistent with its positive signaling role, germline autosomal dominant mutations of Shp2 cause the human genetic disease Noonan syndrome (NS). A subset of NS patients develop myeloproliferative disorders (MPD), particularly, Juvenile Myelomyelogenous Leukemia (JMML), but also Acute Lymphoblastic Leukemia (ALL). Somatic Shp2 mutations affecting many of the same residues have been found in 35% of sporadic JMML and at lower frequency in other MPD and lymphoid leukemias. Their location within Shp2 and the genetics of NS and JMML suggest these are gain-of-function mutants. Whether all NS phenotypes result directly from Shp2 mutation, the mechanism(s) by which NS mutations perturb development and the relationship between particular Shp2 mutations and NS phenotypes remain unclear. To address these issues, we previously characterized a large allelic series of recombinant Shp2 mutants and generated murine models for NS and JMML. Our results show that disease-associated Shp2 mutants have distinct biological properties, and indicate that leukemogenic transformation requires recruitment to Gab2 and results from hyper-activation of the Ras/Erk, PI3K/Akt/Tor, and Stat 5 pathways.
In contrast to the fairly frequent occurrence of Shp2 mutations in leukemia, such mutations are rare in solid tumors. However, in previous work, we observed that Gab2 is over-expressed in 25-30% of human breast tumors, and is located within the 11q13 region amplified in 10-15% of such tumors. We investigated the role of Gab2 over-expression ex vivo and in mouse models. Gab2 expression drives increased expression of MCF10A mammary epithelial cells in 3D culture, and can collaborate with anti-apoptotic genes to cause luminal filling reminiscent of a ductal carcinoma in situ-like phenotype. Gab2 can also collaborate with Her2/Neu to confer a loss of acinar polarity and an invasive like phenotype. Experiments with Gab2 mutants and Shp2 siRNAs indicate that Gab2 acts via Shp2 and drives hyper-activation of Erk. Treatment with Erk inhibitors can block Gab2-evoked proliferation and collaboration with HER2. Furthermore, the level of Gab2 (as set either by Gab2 transgenic or knockout mice) modulate susceptibility to Her2/Neu-evoked murine mammary tumors. Finally, FISH analysis indicates that amplification is a major cause of Gab2 over-expression in vivo. Our results suggest that assessment of Gab2 levels may important prognostic/therapeutic information in human breast cancer patients. Moreover, increased activation of the Shp2/Ras/Erk pathway, caused either by Shp2 mutation or by over-expression of Shp2 binding proteins, is a common theme in neoplastic disease.

Exploiting cell signalling for pharmacological intervention

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In the past, the development of cancer drugs has been based heavily on the cell-based screening of compounds cytotoxicity and the synthesis of analogues of cytotoxic agents (1). Now, the progressive elucidation of the molecular and genomic abnormalities that are responsible for the initiation and progression of the malignant phenotype open up new opportunities for the design of drugs that will act selectively on cancer cells. Since many of the molecular abnormalities that drive malignancy results in deregulation of signal transduction pathways that control cell cycle progression and apoptosis, targets on these pathways are particularly attractive for pharmacological intervention. The development of trastuzumab, imatinib and gefitinib (in which this author was involved) has established the validity and clinical utility of the approach. Agents are now being developed against a wide range of molecular targets and signalling pathways.

Some of the lessons learned from the development of the first generation of molecular cancer therapeutics are:

- *These agents, particularly those acting as oncogenic kinase targets, are effective but their development remains challenging: The design of agents with appropriate potency and selectivity is often readily achievable with new technologies, but the production of drug-like agents with appropriate pharmacokinetic and pharmacodynamic properties in vivo is not trivial. Prioritisation of new molecular targets is difficult: There is a need to balance the desire to move rapidly to initiate a drug discovery project against a new molecular target against the importance of ensuring*

that the correct choice of target is made, bearing in mid both the extent of scientific validation for the target and the practical nissues of drug development.

- *Selecting patients based on evidence of molecular dependence is critical: This allows those who are most likely to respond to be selected for treatment, while those unlikely to respond can be spared and offered more suitable therapy.*
- *Developing molecular biomarkers is essential: In addition to diagnostic and prognostic biomarkers of molecular dependence which are crucial, it is also necessary to develop pharmacodynamic biomarkers that can be used to demonstrate proof of concept for target evaluation as well as to select the best drug schedule and dose.*
- *The development of resistance is a continuing to a problem with the new molecular therapeutics, as it was with cytotoxic drugs: This often occurs by mutation and overexpression of the target.*
- *Combinatorial treatments may be essential for cancer driven by multiple oncogenic abnormalities and to prevent resistance arising: Judicious combinations, multitargeted kinase inhibitors or by drugs acting on single targets that modulate many oncogenic genes and proteins, eg Hsp90 molecular chaperone, proteasome and histone deacetylase inhibitors, will be necessary.*
- *Prioritising drug combinations is difficult: Combinations can involve mixtures of new targeted molecular therapeutics and the use of such agents alongside cytotoxics. Better models are required to select the best combinations and prioritisation based on extensive molecular characterisation rather than simple pragmatism should be encouraged, but the latter should not be ignored.*

Against this background, the presentation will provide our latest results, and especially learning points, from our experience of drugging the cancer kinome. Recent progress in the development of inhibitors of cyclin-dependent kinases (2-4), PI3 kinase (6-8) and the Hsp90 molecular chaperone inhibitors (9-22) will be described.

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HER2 oncogene overexpression in clinical management of breast carcinoma

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Overexpression of the human epidermal growth factor-2 (HER2) oncogene in human breast carcinomas has been associated with a more aggressive course of disease. The reason for this association, found only in node-positive patients is still unclear, although it has been suggested to rest in increased proliferation, vessel formation, and/or invasiveness. Clinical and experimental data raised the possibility that surgical removal of the primary tumor promotes the growth of metastatic lesions.

Accordingly, wound drainages and post-surgical sera from breast carcinoma patients were found to specifically stimulate the in vitro growth of HER2-overexpressing breast carcinoma cells, whereas treatment with trastuzumab, if given before the growth stimulus, abolished this proliferation.

HER2 has been also associated with sensitivity to anthracyclins and resistance to endocrine therapy, suggesting that tyrosine kinase receptor and hormone receptor pathways represent two major proliferation pathways exclusively active in breast carcinomas, one sensitive to chemotherapeutic drugs and the other to anti-estrogens. Accordingly, the level of expression of hormone receptor was found to be inversely proportional to the level of HER2, demonstrating a cross-talk between the two pathways.

HER2 currently represents one of the most appropriate target for specific therapy. Indeed, trastuzumab, a monoclonal antibody directed against the extracellular domain of HER2, is therapeutically active in HER2-positive breast carcinomas. Efficacy of the treatment with trastuzumab was found in patients with metastatic breast carcinomas. Our recent results in neo-adjuvant setting showed that trastuzumab activity in patients does not occur through downmodulation of HER2 but through Antibody-dependent cell cytotoxicity. This finding will help in optimization of the treatment by increasing ADCC capability and selecting patients with appropriate immune system.
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Breast cancer predisposing alleles in Central & Eastern Europe

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Molecular basis of inherited predisposition to cancer has been one of the most successful areas of biomedical research in the recent two decades. Over 40 cancer susceptibility genes have been identified, typically those involved in high penetrance syndromes. Inherited mutations of 'stability genes' (genes involved in the maintenance of genomic integrity and DNA repair) account for majority of hereditary breast cancers (BRCA1, BRCA2, TP53, CHEK2 and ATM). Out of these, only BRCA1, BRCA2 and TP53 can confer strong predisposition to breast cancer. Mutations in TP53 are associated with young onset breast cancer in the context of the Li-Fraumeni syndrome.
BRCA1 and BRCA2 with inherited mutations are the key players in conferring hereditary predisposition to breast cancer. Current information on BRCA1 and BRCA2 mutations is available through the Breast Cancer Information Core (BIC) on the World Wide Web: http://research.nhgri.nih.gov/bic/
Attempts to identify a third breast cancer susceptibility locus (BRCA3) have so far been unsuccessful. This is probably because no single high risk gene but several low to medium risk genes (such as CHEK2) can account for the remainder of families that show a high incidence of breast cancer that is not associated with BRCA1 or BRCA2.
Positive association of mutations in CHEK2 and increased risk of breast cancer has recently been established in several countries including Poland and Hungary (1).
In the years immediately after the identification of BRCA1 and BRCA2, several research teams undertook the categorization of mutations in different populations. In the first wave of number, our group identified mutation spectrum and recurrent mutations in breast cancer families from Hungary (2). Five mutations were seen more than once (BRCA1: 5382insC, C61G/300T>G, 185delAG and BRCA2: 9326insA, 6174delT). All these mutations but 6174delT were prevalent in the Hungarian population series of 500 women with breast cancer unselected for family history (3).
In order to determine the population genetics of inherited variants in the BRCA1 and BRCA2 genes in the region we have conducted an international collaborative study in the frame of Central- and Eastern European Cancer Genetics Network. Mutation searching was carried out using a combination of different methods that included DHPLC, PTT, SSCP, HDA for a preliminary screen. For the BRCA-negative families MLPA analysis was undertaken. Mutations were confirmed by direct DNA sequencing. In these studies, 155 deleterious BRCA mutations (127 BRCA1 and 46 BRCA2 mutations) were observed among 438 breast and/or ovarian cancer families (33%) from seven countries: Hungary, Poland, Czech Republic, Serbia-Montenegro, Latvia, Greece and Turkey. Thirty-seven distinct BRCA1 and 49 BRCA2 mutations were detected. In addition, 29 distinct BRCA1 and BRCA2 sequence variants (rare missense mutations) with unknown biological effects were identified. Eight recurrent BRCA1 and three recurrent BRCA2 mutations showed clustering and association with ethnicity in the region. The most frequent BRCA allele of the region was 5382insC. Recurrent BRCA1 mutations accounted for 59% of all BRCA1 mutations identified, and for 53% of all BRCA2 mutations. (4-7). 'Founder' mutations are prevalent in certain Central- and Eastern European populations, e.g. in Hungary and Poland. Other reports came to support this conclusion (8-9). In these countries, it might be useful to identify common mutations before undertaking an extensive (and expensive) genomic search for cancer-causing mutations.

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In the ten years since the discovery of BRCA1 and BRCA2, genetic testing for breast cancer susceptibility has become integrated into the practice of clinical oncology. Yet, much controversy surrounds the estimation of cancer risk that is associated with a particular BRCA mutation. New risk figures have derived from a meta-analysis of large population-based studies including those from Central- and Eastern-Europe (10-11). There are still intense research efforts to resolve related questions on breast cancer predisposition. These include (i) the better understanding of the function and interactions of the proteins of the susceptibility genes; (ii) the discovery of new breast cancer susceptibility genes that have a causal or modifier role in neoplasia; (iii) the elaboration of new approaches for estimation of cancer risk in carriers; (iiii) the development of new ways to exploit this knowledge for the benefit of patients.
Ongoing research (including our group) and achievements on the field will be discussed.

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Genomic Dissection of Estrogen Receptor Biology

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We have used the estrogen receptor as a model system to dissect the regulatory control of transcription by a nuclear hormone receptor. Using both ChIP-on-CHIP and ChIP-cloning approaches we have identified and validated a large number of estrogen receptor binding sites. The computational analysis between EREs that are ER binders and EREs that do not bind ER alpha revealed an extended palindrome that defines the optimal estrogen response element. Our computational algorithm allows for high specificity in de novo prediction of ER binding. We then developed a novel technology called gene identification signature (GIS) that, when coupled with chromatin

immunoprecitpation (ChIP)-cloning allows for the precise identification of binding sites of any transcription factor in a genome wide scale. With this approach, we identified over 1000 high quality binding sites and confirmed that the consensus ERE is the primary recognition site for estrogen receptor alpha. These binding sites were found up to 100 kb upstream and downstream of known genes, and away from CpG islands. There was a predominance of up regulated genes with ER binding sites in the 5'region responsive genes, but no such enrichment in downregulated genes. In ER responsive genes, multiple binding segments spanning many kilobases could be found. Wider examination of the cis-regulatory regions flanking the transcriptional start sites showed species conservation in mouse-human comparisons in only 6% of predicted EREs. Thus, only a small core set of human genes, validated across experimental systems and closely associated with ER status in breast tumors, appear to be sufficient to induce ER effects in breast cancer cells. That cis-regulatory regions of these "core" ER target genes are poorly conserved suggests that different evolutionary mechanisms are operative at transcriptional control elements than at coding regions. These results predict that certain biological effects of estrogen signaling will differ between mouse and human to a larger extent than previously thought. We extended this genome-wide analysis to ERbeta. It has been observed that ERbeta has growth inhibitory effects on cells compared to the growth supporting effects of ERalpha. We found that, in the presence of ERalpha, over-expression of ERbeta alone mimics hormone treatment in ligand-unexposed cells. However, ERbeta expression specifically down-regulated a small subset of genes involved in cell proliferation, including CDC2, CKS2, NEK2, PCNA, and CDC6. Comparative analysis of hormone response in the presence and absence of ERbeta revealed greater perturbation of ERbeta-mediated transcriptional regulation by ERbeta following estrogen treatment. Taken together, these findings indicate both agonistic and antagonistic effects of ERbeta on ERbeta functions and suggest that the alpha/beta ratio within a given target cell type may have a great impact on receptor complex functions and that these ERbeta _effects will differ in the presence or absence of hormone ligands. Our studies point to the advantages of genome-wide studies in reassessing the functions of estrogen receptor function.

Concluding remarks

Carlo Gambacorti *MD*
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I want to acknowledge the high quality of all presentations. The two days spent here in Trento have been incredibly enriching and stimulating. As Bill Sellers rightly put in the opening of the meeting, we have to decide whether to direct our efforts toward "controlling, containing" cancer versus "understanding its pathogenetic mechanisms and curing it".
I divided the presentations at the meeting into 4 categories as follows:

I *Identification of new early events:*
Michael Stratton
*Bill Sellers**
Anton Berns
Giovanni Tonon

II *Assessment of gene expression patterns in cancer*
Joseph Nevins

III *Mechanisms of action of oncogenes/TSG*
*Hugues De The**
Pier Paolo Pandolfi
*William Kaelin**
Julian Downward
*Karen Vousden**
*Philip Beachy**
Benjamin Neel

*IV** *Clinical development*
Paul Workman
Sylvie Menard
Edith Olah
Edison Liu

*The presentations marked with an * contain a component aimed at translating the new knowledge obtained into innovative treatment approaches; while this can be expected form the "clinical" presentations, the fact that 4 out of 7 presentations dealing with the mechanism of action of oncogenes/TSG involved such a component, highlights an important and positive attitude toward applying without delay the results of basic studies. However some words of caution need to be said: we listened to excellent presentations focused*

on potential therapeutic applications in very common cancers, such as the PTC pathway in GI and prostate cancer, P53 refolding, HIF inhibition in renal cancer... If only one third of these projects will be successful, we will see a major breakthrough in cancer therapy in a short time and in common cancers. As the excellent presentation of Pier Paolo Pandolfi on NPM pointed out, when a single gene/protein is analyzed at different levels (gene, protein, cells, animals), very different results can be obtained; thus prudence is mandatory.

The high throughput sequencing or different types of analyses (SNPs, CGH, insertional mutagenesis) aimed at identifying new genes structurally altered in cancers also represents an essential tool in our strive to shed light on the molecular mechanisms that generate cancer.

In this context, the type of presentations that I missed here (and which I recommend for a future edition of this wonderful meeting) have been those designed at validating structurally altered oncogenes as therapeutic target. We know now several mutated genes in different solid tumors (e.g. BRAF in Melanoma, RET in Thyroid cancer, Shp2 in Juvenile MyeloMonocytic Leukemia, beta-catenin in Colorectal carcinoma). With the exception of Imatinib and BCR/ABL, no such validation has been performed up to now. The knowledge and validation of these early events in specific cancers will render translational research more focused and promising.

In this perspective, I looked with interest at the presentations of this morning, focused on breast cancer. This disease has been studied extensively, and treatment made important progresses, as the three presenters showed. Our understanding of the role of estrogen receptors and of estrogen mediated signaling as well as of Her2/Neu as therapeutic target has greatly increased. However it has to be remembered that, in spite of these achievements, we do not know yet what is/are the pathogenetic lesion(s) that originates breast cancer, and this fact continues to haunt me.

I will close with a list of open questions to be considered as take home messages:

What is the significance of targeting early pathogenetic events versus "less early" "less pathogenetic" pathways? Curing vs. controlling cancer? Inducing apoptosis versus mere cytostasis? Is there a difference in targeting different types of early events, such as for example, kinases versus transcription factors?

We as scientists always need to be critical and to pose ourselves more questions than answers.

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ABSTRACTS OF POSTERS

Dual role of stroma-produced SPARC/ 0steonectin in neoplastic transformation and tumor progression.

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SPARC (secreted protein, acidic and rich in cysteine), also known as osteonectin or BM-40, is a Ca2+-binding matricellular glycoprotein involved in development, wound healing and neoplasia. However, the role of SPARC in tumors is ill defined mostly because it is expressed by both tumor and stroma cells, especially inflammatory cells.
SPARC-/- mice originally on a mixed 129 background and outbreed mice expressing the transgenic rat oncogene c-erB2 (HER-2/neu) driven by the mouse mammary tumor virus (MMTV) promoter, were backcrossed 12 generation to BALB/c mice. Female mice carrying the activated rat HER-2/neu oncogene present high numbers of breast tumor nodules characterized as lobular carcinomas with lobes defined by connective septa containing collagen type IV which were initially used as the source of mammary carcinomas and cell lines in the present study.
We analyzed the respective roles of host- and tumor-derived SPARC in wild type and congenic SPARC-/- mice injected with SPARC-producing mammary carcinoma cells as well as chimeric mice expressing SPARC only in donor bone marrow (BM)-derived cells. Our results revealed impairment of growth, vascularization and stroma formation in tumors implanted into SPARC-/-mice. Reduced lobular structure and intervening stroma in the absence of host-produced SPARC were associated with reduced collagen type IV, an event that might favor leukocyte infiltration of tumor parenchyma. Transplanting BM from SPARC+/+ donors reversed this phenotype. Thus, the organization of tumor stroma depends on SPARC produced by host leukocytes rather than on tumor-derived SPARC at least in the case of lobular carcinoma. Although this finding was obtained using transplanted primary tumors that are so sensitive to environmental factors of failing to grow when transplanted outside the mammary fat pad we challenged our finding by evaluating spontaneous carcinogenesis. To this end SPARC-/- and control SPARC+/+ BM were transplanted into SPARC+/+ mice carrying the MMTV-rat c-erB2 oncogene. The sole defect of SPARC production in donor leukocyte was sufficient to determine tumor outcome depending on the age of recipient mice. Female transplanted at 5 weeks show accelerated transformation while those transplanted at 15 weeks of age had reduce multiplicity of tumor nodules characterized by low growth rate. We are challenging the hypothesis that transplantation before tumor onset favors transformation by removing the anti-angiogenic, bone marrow-derived SPARC effect. Differently, transplantation at 15 weeks of age when atypical hyperplasia is already present would confirm the need of leukocyte-derived SPARC as supporting stroma for tumor progression. Together our results point to a dual role of SPARC produced by stroma cells in neoplastic transformation and tumor progression.

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DOWN REGULATION OF DNA Double stRand repair genes expression in early stage breast cancer.

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A number of intricate networks have evolved in eukaryotic cells to respond to exogenous and endogenous genotoxic stimuli. Genes involved in these pathways play crucial roles in maintaining genomic integrity, and a defect in these processes may result in hypersensitivity to DNA damaging agents, genomic instability, and may lead ultimately to tumorigenesis. Of the many types of DNA damage, the Double-strand breaks (DSBs) are the most dangerous, because of the intrinsic difficulty of their repair as compared with other types of DNA damage. In this study we have analyzed genes expression of the main genes involved in DNA Double Strands repair pathways in 20 early stage (pT1cN0M0) breast cancers. A total of 13 genes implicated in both homologous recombination (HR) and Non-homologous End Joining (NHEJ) mechanisms were analyzed on a 7700 Sequence Detection System (Applera Italia) by Quantitative Reverse Transcription Polymerase Chain Reaction, using MGB probes chemistry. For each case matched pathologically normal breast tissue and tumor were analyzed. Expression of the target gene was normalized by the expression of the housekeeping gene GAPDH and for each gene mRNA levels were determined as relative expression (RE) in the tumor as compared with matched normal tissue (RE in the normal equal to 1). Thus RE value <1 indicate reduced expression and RE >1 indicate an increased expression. A substantial reduction in mRNA relative expression was found for the majority of the genes tested. Approximately 70% of the tumors showed down regulation for MRE11, RAD52, BRCA1, G22P1, and XRCC5. ATR, NSB1, RAD50 and RAD54, and Artemis were down regulated in approximately 60% of the cases. The up-regulated genes were RAD51 (70% of the cases), BRCA2 (60%) and ATM (50%). The majority of the cases showed RE value from 0.5 to 1.5 corresponding to a 50% down or up-regulation in the tumor as compared with matched normal tissue. However, a subgroup of breast cancer (approximately 50% of the cases) showed a marked reduction of RE expression levels for ATR, NSB1, MRE11. For these genes, RE value were between 0.062 e 0,315 corresponding to reduction in mRNA levels from 94% to 70% as compared with matched normal breast tissue. Overall our data suggest a substantial down-modulation of both mechanisms involved in DNA double strands break repair in a relative early stage of breast cancer progression. Moreover our data indicates a marked down-regulation of the MRE11 complex (MRE11/NSB1/RAD50) in at least half of the tumors. This complex plays a role in each of the aspects of chromosome break metabolism acting as a break sensor, in the activation and propagation of checkpoint signalling pathways and in promoting recombination between sister chromatides.

Role of caveolin-1, follistatin and cadherin-13 in the malignancy of osteosarcoma

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Osteosarcoma, the most common bone tumor, is a very aggressive disease currently treated with neoadjuvant chemotherapy and surgery. The most recent improvements in the cure rate of these patients have been achieved by dose-intensification, therefore paying the price of severe toxicity and high rate of life-threatening late events, such as secondary malignancies. This poses serious quality of life issues due to the young age of the patients and asks for identification of molecular markers to be used for risk-based stratification of patients. Genetic profiles of a series of osteosarcoma cell lines with a lower metastatic ability have pointed out at least three molecules that appeared to be over-expressed in cells with reduced metastatic potential: caveolin-1, follistatin and cadherin-13. The clinical relevance of these markers was analyzed in a series of 84 primary osteosarcoma samples. Expression of caveolin-1 and follistatin appeared to be associated (P-value=0.02). With respect to clinico-pathological parameters, significant inverse associations were observed for caveolin-1 over-expression and tumor volume (P-value=0.04) and for follistatin expression and sex (P-value=0.014) with only 4% of females expressing the protein. A significant association with clinical outcome was observed only for caveolin-1 over-expression (P-value=0.02). Transfection of caveolin-1 in U-2 OS cells confirms that over-expression of the molecule induces reduction of migration and anchorage-independent growth, further supporting the view of caveolin-1 as a marker of prognostic value that may be exploited for risk-based treatment design.

Heparin-binding Epidermal Growth Factor (HB-EGF): a target for cancer treatment with CRM197

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HB-EGF is a glycoprotein of approximately 22 KD that is initially synthesized as a membrane bound precursor (pro-HB-EGF) .[1] A soluble form of HB-EGF (s-HB-EGF) is released from the cell membrane by ectodomain shedding of pro-HB-EGF.[2] The molecule is a heparin-binding member of the EGF family and activates two EGF receptor subtypes, HER1 and HER4. Pro-HB-EGF is a juxtacrine growth and adhesion factor, while s-HB-EGF is a potent mitogen and chemotatctic factor for several cell types. HB-EGF is involved in physiological processes such as wound healing and in pathological ones such as tumor growth. Moreover, pro-HB-EGF has the unique property of acting as the specific cell membrane receptor for diphtheria toxin (DT). Interestingly,HB-EGF is overexpressed in a variety of tumor derived cell lynes including prostate, breast, colon, melanoma, and pancreas. Moreover, many other tumor cells (for instance, ovary, lung, kidney, muscle and connective tissue-derived cells) are sensitive to DT suggesting the synthesis by these cells of pro-HB-EGF [3]

Cross-reacting material 197 (CRM197) is a mutant nontoxic DT (58,422 Mr*) that shares the ability of the native counterpart to bind to pro-HB-EGF and to be a strong immunogen .[4] The following considerations prompted us to test CRM197 as an antitumor agent in advanced cancer patients. First, the frequent overexpression of pro-HB-EGF in cancer suggests that the administration of the mutant toxin to cancer patients may result in a preferential binding of the molecule to malignant cells. Second, the high permeability of tumor vasculature [5] may allow a preferential extravasation in the tumor area of a large molecule such as CRM197. Third, the binding of CRM197 to pro-HB-EGF may be followed by an immune reaction involving the tumor. Fourth, it is known that the binding of CRM197 to pro-HB-EGF inhibits the mitogenic activity of the growth factor .[6, 7]*

We recently reported the results of two Phase I trials performed with CRM197 in advanced cancer patients. The data of these trials confirm that CRM197 is a promising candidate for the role of anticancer agent .[8, 9]

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The PRL-3 phosphatase is implicated in the growth of ovarian cancer cells.

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The phosphatase PRL-3 has been found expressed at higher levels in metastasis than in primary tumors in patients with colorectal cancer. We have studied the expression of PRL-3 in ovarian cancer patients analysing the expression in early and late tumor stages. PRL-3 phosphatase expression was evaluated in 84 ovarian tumor samples frozen immediately after surgery in liquid nitrogen. In these samples, PRL-3 expression seems to correlate with disease progression, being higher in advanced (stage III) than in early (stage I). By in situ hybridisation measurements, PRL-3 expression was found to be confined to the epithelial neoplastic cells. When the expression of PRL-3 was analysed in primary tumors (stage III) and metastasis derived from them, we did not find significant differences.
To try to understand whether the expression of PRL-3 could be relevant for the growth of ovarian cancer cells, we used ovarian cancer cell lines growing in vitro which have high levels of expression of this phosphatase. We have used specific siRNAs to knock down PRL-3 expression in these ovarian cancer cell lines and, for comparison, in a human colon carcinoma cell line, HCT116, which expresses low levels of PRL-3.
The siRNA specific for PRL-3 inhibited the expression of PRL-3 protein without affecting the expression of the closely related homologue PRL-1. This reduction was associated with a strong inhibition of cell growth in vitro. Intriguingly, the growth of human colon carcinoma cells expressing lower levels of the PRL-3 was not affected by the PRL-3 knock down.
Altogether these results show that PRL-3 expression is associated with ovarian cancer progression and point to a key role for this phosphatase in the control of ovarian cancer cells growth. This suggests that PRL-3 could be considered as a target for the design and discovery of new anticancer agents to be tested against this malignancy.

Quantitative p73 transcripts analysis in early-stage of ovarian carcinoma.

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p73 is a novel gene that shares high structural homology

with the tumor suppressor protein p53. There are many evidences supporting that p53 and p73 play different role in tumorigenesis. Many studies on human cancers reported increased expression levels of wild-type p73 and the absence of any gene mutation. Moreover, unlike p53 deficient mice, p73 knock out mice do not develop spontaneous tumors. These data can be explained by the recent discovery of N-terminal truncated isoforms of p73 (DTAp73), that are the result of both alternative splicing (DN'p73, Ex2p73 and Ex2/3p73) and the use of a second intronic promoter (DNp73). Whereas the TAp73 protein has a p53-like tumor suppression function, the DTAp73 proteins act as oncogene. DTAp73 isoforms are able to compete with both wild-type p53 and transactivation-competent TAp73 mediated transactivation of target genes involved in apoptosis and cell cycle regulation.
A recent report showed that the expression levels of p73 transcript isoforms driven from the TA promoter are significantly increased in late-stage of ovarian carcinomas compared to normal adjacent tissues.
From these results, we decided to investigate the different expression of p73 isoforms in order to elucidate whether there could be a role of this gene in earlier stages of tumor progression.
With this aim we analyzed the expression profile of p73 isoforms in 99 stage I ovarian carcinoma samples by isoform-specific reverse transcription real-time PCR.
Preliminary data seem to confirm that DTAp73 transactivation deficient isoforms are expressed also in stage I tumors. However the TAp73 isoforms are still present at highest levels than the DTAp73 isoforms. The ratio between level of DTAp73 and TAp73 isoforms could represent a relevant determinant of tumor progression and malignancy in ovarian cancer.

BN80927: A novel homocamptothecin that inhibits proliferation of human tumor cells *in vitro* and *in vivo*.

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BN80927 belongs to a novel family of camptothecin analogues, the homocamptotecins, developed on the concept of topoisomerase (Topo) I inhibition and characterized by a stable 7-membered b-hydroxylactone ring. Preclinical data show that BN80927 retains Topo I poisoning activity in cell-free assay as well as in living cells, where in vivo *complexes of topoisomerases experiments (ICT) and quantification of DNA-Protein-Complexes (DPC) stabilization, have confirmed the higher potency of BN80927 as compared to the Topo-I inhibitor SN38. In addition, BN80927 inhibits Topo II-mediated DNA relaxation* in vitro *but without cleavable-complexe stabilization, thus indicating catalytic inhibition. Moreover, a Topo I altered-cell line (KBSTP2) resistant to SN38, remains sensitive to BN80927, suggesting that part of the antiproliferative effects of BN80927 are mediated by a Topo I independent pathway. This hypothesis is also supported by* in vitro *data showing an antiproliferative activity of BN80927 on a model of resistance related to the non-cycling state of cells (G0/G1 synchronized).*
In cell growth assays BN80927 is a very potent antiproliferative agent with IC50s consistently lower than those of SN38 in sensitive as well as in their related drug resistant tumor cell lines. The 2 compounds are concentration and contact-time dependent. However 1h contact time is sufficient for BN80927, at 1μM, to kill cells; SN38 need more than 6h to reach similar activity. Furthermore BN80927 shows high efficiency in vivo *in a large panel of tumor xenograft models.*
The overall profile of BN80927 and, in particular, the mechanistic differences with respect to related molecules, lead to clinical evaluation of the compound ; phase I trials are on-going.

Molecular characterization of gastrointestinal stomal tumours (GIST): detection of novel stop codon mutation.

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GastroIntestinal Stromal Tumours (GISTs) are the most common mesenchymal tumours of the gastrointestinal tract. GISTs strongly express the receptor tyrosine kinase KIT (CD117). Mutations in c-KIT cause constitutive activation of the this kinase that lead to development and growth of the tumour. Approximately 35% of GISTs lacking c-KIT mutations have activating mutations in PDGFRA. Type of mutation appears to play an important role in response to treatment. The aim of this study was to establish the frequency and the type of mutations of c-KIT and PDGFRA genes in GISTs and to correlate immunohistochemical (IHC) with molecular analysis.
DNA was extracted from 46 paraffin-embedded GIST samples. Mutational analysis for c-KIT (exons 9, 11, 13 and 17) and PDGFRA (exons 12 and 18) was performed by direct sequencing. KIT expression was studied in tissue microarray blocks by IHC using the anti CD-117 Ab with and w/o antigen retrieval.
Forty-two of 46 (91,3%) GISTs were mutated for c-KIT or PDGFRA. c-KIT and PDGFRA were mutated in 34/46 (73.9%) and 8/46 (17.4%) cases respectively.
Mutations in c-KIT accounted for 80,9 % of all mutations (34/42) and were located in exons 11 (94.2%), 9 (2.9%) and 17 (2.9%). Deletion was the most frequent mutational event in our series accounting for 58.8% of cases (20/34). In two cases the mutation led to a stop codon. The remaining cases were missense mutation. A mutational hot spot was identified between codon 550 and 560 of exon 11.
Eight cases of 46 (17.4%) were mutated in exon 18 of PDGFRA.
Forty-four patients were submitted to IHC analysis and 42 (95,4%) were positive for KIT, including the cases showing a stop codon in exon 11; 34 of 42 (75%) KIT positive samples had c-KIT mutation and 6 of 42 (14,3%) had PDGFRA mutation. The 2 KIT negative cases showed a PDGFRA mutation.
Mutations of c-KIT or PDGFRA are very frequent in GISTs (89%) and mutually exclusive. c-KIT mutations are localized mostly in exon 11. A stop codon mutation may explain a predictable insensitivity to imatinib and only 65.2% of cases with activating c-KIT mutations are predicted to respond. CD117 positivity is very useful to confirm the diagnosis of GIST, nevertheless do not correlate with mutation pattern. The molecular characterization appears to be an essential adjunct to diagnosis in the clinical management of the patients.

Cisplatin resistance in ovarian cancer using gene expression microarrays

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***Indroduction**. Ovarian cancer represents the leading cause of death from gynecological cancer in the United States and Western Europe. Cisplatin resistance represents one of the major problems of failure in ovarian cancer treatment. Cisplatin pharmacogenomics by microarray technology gives more informations about new genes involved in cellular signalling which conduct to development of malignant phenotype.*
***Material and methods**. For this study we used ovarian human cells (sensitive and resistant to Cisplatin) and human xenografts obtained from these cells injected at nude mice. We analysed the mRNA levels of gene expression profiles (transcriptome) boths* in vitro *and*

in vivo *systems. For analyses we used an Agilent Technologies Platform for microarray, using the 44.000 K array (Whole Human Genome).*
Results *The gene expression was selected for p values <0.001 and for values of fold change > 1.5 and <-1.5. For* in vitro *model, we found 1611 genes with different expression between the both cell lines (sentitive and resistant to Cisplatin). For* in vivo *model, we found 1459 genes with different expression between the both xenografts obtained from cell lines (sensitive and resistant to Cisplatin). We found 630 identically genes for boths, cell lines and xenografts.*
Conclusions *Our results demonstrate that tumor cell's resistance of cisplatin are more complex that is known in this moment. We found many identically genes for boths* in vitro *and* in vivo *systems as well as also many different genes for in vivo systems comparative with cell lines. The genes invoved in Cisplatin resistance are found in cellular signaling: apoptosis (CLU), growth factor mecanisms (PLAB and CYR 61), cellular signal transduction (YWHAH, AHR).*
Key words: pharmacogenomics, Cisplatin, microarray

An integrated immunogenomic and bioinformatic approach to identify new tumor-associated antigens in the immunological prevention of mammary cancer

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The question whether a vaccine can inhibit carcinogenesis is rarely addressed. Progression of a preneoplastic lesion is a lengthy process that may be hampered by mechanisms that are not efficacious when confronted with the unnatural speed of transplantable tumors. Carcinogenesis is a multistep process in which distinct gene products are involved at each stage. Identification of overexpressed gene provides an unprecedented opportunity to address immunity against antigens typical of defined stages of neoplastic progression. The Her-2/neu oncogene is a prototype of deregulated oncogenic protein kinase membrane receptors. BALB/c female mice transgenic for the rat Her-2/neuT (BALB-neuT mice) display one of the most aggressive forms of Her-2/neu carcinogenesis. The protein product of the rat Her-2/neu oncogene (rp185neu) is already overexpressed by 3-4 week-old mice. At 6 weeks, rp185neu cells give rise to a widespread mammary atypical hyperplasia which progresses to form an invasive carcinoma that becomes palpable in all ten mammary glands between the 22nd and the 27th week of age. During mammary hyperplasia, Her-2/neu positive stem cells metastasize in the bone marrow. Our previous demonstration of persistent inhibition of autochthonous mammary carcinogenesis in this transgenic mouse model by a combined DNA and cell vaccine (1), and the curing of Her-2 transplantable tumors by DNA vaccination (2) indicates that efficient immunological inhibition of Her-2 carcinogenesis can be achieved. We have also shown that two transcriptional profiling studies based on different immunization protocols in BALB-neuT mice (1, 3) are integrable (4). Since the progression of Her-2 mammary carcinomas may become independent from Her-2-driven signals, definition of an additional set of tumor-associated antigens (TAA) expressed by most mammary carcinomas would increase the efficiency of a vaccine. To address this question, a meta-analysis was performed on two transcription profiling studies (1, 3) of BALB-neuT mammary carcinomas to identify a set of new TAA targets to be used instead of or in conjunction with Her-2. This study identified 12 putative TAA. Three were discarded because they were expressed in some normal human tissues and four because their expression was too low in human cancer specimens. Tes, Rcn2, Rnf4, Cradd and Galnt3 were retained for further investigations. This work was based on in silico analysis of published transcriptional profiling studies. Its results must now be referred back to the in vivo pre-clinical experimentation.

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p53 gene status in patients with advanced serous epithelial ovarian cancer in relation with response to paclitaxel- plus platinum-based chemotherapy and long-term clinical outcome

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The retrospective study assessed whether p53 gene status had any predictive and prognostic relevance in 46 patients who underwent surgery followed by paclitaxel- plus carboplatin-based chemotherapy for advanced, poorly differentiated serous epithelial ovarian cancer. Twenty-three patients (50%) showed p53 mutations at exons 5 to 9. Sixteen (34.8%) patients had a polymorphism at codon 72 in exon 4 (SNP codon 72): 10 were Pro/Pro homozygous and 6 Pro/Arg heterozygous. Four polymorphic patients had a second mutation at exons 5 to 9. An inverse correlation was evidenced between SNP codon 72 and mutations at exons 5 to 9, with the latter more frequently found in wild-type (Arg/Arg) codon 72 (19/30 versus 4/16, 63.3% versus 25.0%; P = 0.03) cases. A clear trend to a higher response rate and longer progression-free and overall survival was observed in wild-type p53 and Pro/Pro polymorphic patients as compared to patients with mutant p53. In conclusion, the addition of paclitaxel to carboplatin does not appear to overcome the negative predictive and prognostic significance of p53 gene mutations in serous ovarian cancer. Nevertheless, the comprehensive analysis of p53 genotype, also including SNP codon 72, warrants further investigation in order to envisage individual responsiveness to cancer therapy.

APC methylation in colorectal cancer

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Germ-line mutations in the tumor suppressor gene APC are associated with hereditary familial adenomatous polyposis (FAP), and somatic mutations are common in sporadic colorectal tumors. We studied 24 colorectal carcinomas which promoted from benign adenomas for the methylation in the promoter region of this gene in order to establish if it is an alternative mechanism for gene inactivation in colon and other tumors of the rectum. The APC promoter is hypermethylated in 21%

of primary sporadic colorectal carcinomas (n = 24) . Methylation affects only wild-type APC in 85% of cases and is not observed in tumors from FAP patients who have germ-line APC mutations. As with APC mutation, aberrant APC methylation occurs early in colorectal carcinogenesis. We evaluated the p53 expression of all the cases in order to establish if any correlation can be made between the two genes.It seems that methylation of the APC promoter is not restricted to the colon but is present in tumors originating elsewhere in the gastrointestinal tract but rarely in other tumors. Our data suggest that hypermethylation of APC provides an important mechanism for impairing APC function and can play an important role in APC pathway in colorectal tumorigenesis.

Interference of NAMI-A with the TGF-ß machinery: a potential explanation for its anti-invasive activity

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*TGF-§ is a multifunctional cytokine involved in several cellular processes; in particular, in late stages of tumorigenesis, it acts as a prometastatic factor inducing tumour vascularization and epithelial to mesenchymal transdifferentiation (EMT) in tumour cells that gain migratory and invasive ability. TGF-§ can therefore be a good target for the antimetastatic therapy. The peculiar antimetastatic activity of the ruthenium compound NAMI-A shows many points of contact with the TGF-§ machinery. NAMI-A, [ImH][*trans-*RuCl*$_4$*(DMSO)Im], acts via the selective disturbance of the relationship between metastatic cells and host environment. In vivo it shows a profibrotic activity and antiangiogenic effects. In vitro it reduces tumour cell invasion and migration with a F-actin dependent proadhesive effects and down-regulates tumour gelatinases. Therefore the question is whether the effects of NAMI-A on tumour cells can depend upon modulation of the complex TGF-§ machinery.MDA-MB-231 and MCF-7, respectively a highly-invasive and poorly-invasive breast cancer cell line, and HBL-100, a normal mammary epithelial cell line are used. All cell lines are positive to T§RII, the receptor that directly binds TGF-§ and starts the signalling cascade; NAMI-A or TGF-§1 treatments do not modify its expression. On tumour cells, TGF-§1 induces the formation of cell protrusions, similar to lamellipodia. NAMI-A induces a spread condition, much different from that of TGF-§1: cells are more broad and crushed to the substrate, as to whether they cannot move. Contrary to TGF-§1, NAMI-A treated cells became resistant to trypsin detachment. When TGF-§1 and NAMI-A are given simultaneously to the same cells, the effect of NAMI-A on cell morphology is predominant over that of TGF-§1. In this case, cells have the same adhesive characteristic of NAMI-A treated cells. On HBL-100, both TGF-§1 and NAMI-A induce cell protrusions and maintain cell bodies in a round shape, with NAMI-A less effective on cell detachment with trypsin.*
In line with its anti-invasive effect, NAMI-A decreases the adhesion ability of MDA-MB-231 cells and it cancels the opposite effect of TGF-§1, that plays a pro-invasive role. These effects are much smaller in the non metastatic MCF-7 cells, and in the non tumour HBL-100 cells, where also TGF-§1 exerts no significant effects. On MDA-MB-231 cells, low and high concentrations of NAMI-A respectively reduce and increase the total TGF-§1 production, whereas, on MCF-7 cells, it reduces significantly TGF-§1 production, independently on the concentration used. On the non tumour HBL-100 cells, NAMI-A is devoid of effect on TGF-§1 production. These modifications are not followed by Smad 2/3 phosphorylation, the principal pathway involved in TGF-§ signalling, but we observe modulation of p38MAPK phosphorylation, a co-accessory TGF-§ pathway responsible for the EMT process, cell adhesion, migration and invasion. These data point out the relevance of the modulation of the TGF-§1 machinery in the NAMI-A selective inhibition of pro-adhesion and

invasion of metastatic cells. This effect does not go through the principal Smad2/3 pathway, but is dependent on the modulation of co-accessory p38MAPK pathway.

A tumor protease-activated conjugate based on a sea anemone toxin: its cytotoxic activity on cancer cells

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Immunotoxins and mitotoxins are chimeric molecules in which specific ligands are coupled to toxins. If the ligand moiety is specific for a tumoral cell line, the chimeric toxin can kill those cells selectively. The toxins usually used for conjugation are derived either from bacteria or from plants. Both types of toxins kill cells by inhibiting protein synthesis. To exert their effect these complexes must enter the cell, where only a portion of the internalized immunotoxin escapes degradation and reaches the cytoplasmic target.
In some cases internalization can be a rate-limiting step, which reduces the immunotoxin efficacy. One way to overcome this problem is to use membrane-active toxins, such as hemolysins and cytolysins. These pore forming toxins attack the lipid phase of cell membranes disintegrating vital membrane functions. They could provide very powerful antitumoral drugs, when conjugated with an appropriate targeting molecule.
However, the main problem of most of the immunotoxins constructed with membrane active proteins is the poor cellular specificity associated to the capacity of the toxin to bind to almost any cell membrane.
The purpose of this study was to overcome much unspecificity. To this aim we present the construction of a modular inactive conjugate between a sea anemone cytolysin (the EqTII-I18C mutant) and avidin and its activation mediated by tumor cells proteases. To construct this tumor protease-activated pore-forming toxin we chose two classes of proteases, cathepsins and metalloproteinases (MMPs), which are known to be associated to several tumors. After having measured the enzymatic activity of fibrosarcoma and breast carcinoma cells, we analysed the cytotoxic effect of the conjugate on the same lines and on human red blood cells (HRBC) as controls.
This approach is intended as a conceptual proof to demonstrate that a relatively simple chemistry could be used to conjugate a cytolysin to a ligand moiety via a peptide containing cleavage sites of tumor-associated proteolytic enzymes. We further demonstrated that the conjugate could be specifically cleaved by these enzymes, leading to its possible application as antitumoral drug. The advantage of the activable pore-forming system, for the construction of immunotoxins, is the reduction if the unspecific activity. In this way two mechanisms of target would be present in a single hybrid molecule, the recognizing of tumor cell through the ligand moiety and the specific activation of the toxin by the tumor-associated proteases.
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Increased copy number of the epidermal growth factor receptor gene is associated with clinical response of colorectal cancer to cetuximab or panitumumab

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Background. *Most patients with metastatic colorectal adenocarcinoma (mCRC) have no response to the monoclonal antibodies cetuximab or panitumumab that target the epidermal growth factor receptor (EGFR). However, ~10% of patients have rapid and often dramatic clinical response. The biologic mechanisms underlying sensitivity to cetuximab or panitumumab are unknown.*

Methods. *Tumours from 31 patients with mCRC who had either an objective response (n=10) or stable disease or progressive disease (n=21) after treatment with cetuximab or panitumumab were screened for genetic changes in* EGFR *or its immediate intracellular effectors. Specifically, we assessed the* EGFR *copy number and the mutation profile of the* EGFR *catalytic domain and of selected exons in* KRAS, BRAF, *and* PIK3CA.

Results. *Eight of nine of patients with objective responses who were assessable by fluorescence in-situ hybridisation (FISH) had an increased* EGFR *copy number. By contrast, one of 21 non-responders assessable by FISH had an increased* EGFR *copy number ($p<0.001$ for responders* vs *non-responders, Fisher's exact test). The mutation status of the* EGFR *catalytic domain and its immediate downstream effectors* PIK3CA, KRAS, *and* BRAF *did not correlate with disease response. In colorectal-cancer cell lines, the concentration of cetuximab that completely inhibited proliferation of cells with amplified* EGFR *copy number did not affect proliferation of cells with unamplified* EGFR.

Conclusions. *In mCRC response to cetuximab or panitumumab is significantly correlated with increased copy number of* EGFR. *These results provide a first insight into molecular mechanism for anti-EGFR therapies of mCRC based on monoclonal antibodies, supporting a strategy to identify patients who are likely to benefit from cetuximab or panitumumab.*

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2006 Pezcoller Foundation-AACR International Award for Cancer Research

The Pezcoller Foundation-AACR International Award for Cancer Research is given annually to a scientist anywhere in the world who has made a major scientific discovery in the field of cancer, who continues to be active in the field, and whose ongoing work holds promise for future substantive contributions to cancer research. The Award recognizes extraordinary basic or translational cancer research. The Award will be presented to a single investigator for his or her highly original work. In extraordinary circumstances, two individuals may be selected to share the award when their investigations are clearly related and have resulted in prizeworthy work. The Awardee will be selected by an International Committee of AACR members appointed by the AACR President with the agreement of the Council of the Pezcoller Foundation. The selection will be made solely on the basis of the Awardee's scientific accomplishments without regard to race, gender, nationality, geographic location, or religious or political views.

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Over the past decade the Pezcoller Foundation, in collaboration with the ESO-European School of Oncology, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 22,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 60 other countries around the word.

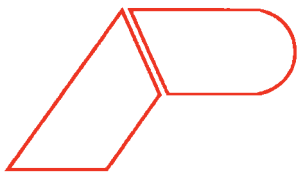
The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of this Award. This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (April 1-5, 2006 in Washingston) and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy, after the AACR annual meeting (May 5, 2006).

The award consists of a prize of ¨ 75.000 and a commemorative plaque.

Nomination Deadline: Friday, September 16, 2005

Questions about the nomination process – www.aacr.org - should be directed to the AACR Office (Philadelphia) via Fax at (215) 440 9372- Telephone (215) 440 9300 - or E-mail awards@aacr.org



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