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EDITORIAL: JUNE 2007

In this issue, we are pleased to announce the winner of the 2007 Pezcoller Foundation / AACR International Award for Cancer Research, Mina J. Bissell, Ph.D. - Distinguished Scientist in the Life Sciences Division at Lawrence Berkeley National Laboratory – California, USA.

The Selection Committee met in Boston in December 2006 and was made up of the following: Anton J.M.Berns, as Chairperson, Netherlands Cancer Institute Amsterdam, Stephen J.Elledge, Harvard Medical School Boston, Elaine V.Fuchs, Howard Hughes Medical Institute Rockefeller University, Joe W.Gray, Lawrence Berkeley National Laboratory University of California, Nancy E.Hynes, Friederich Miescher Institute Basel, V.Craig Jordan, Fox Chase Cancer Center Philadelphia, Richard M.Marais, Instuitute of Cancer Research London, Pier Giorgio Natali,Regina Elena Cancer Institute Rome.

2007 Pezcoller Foundation - AACR International Award for Cancer Research
Dr. Gios Bernardi and Dr. Mina J. Bissell

The motivation of the Award: Dr. Mina Bissell was selected for her pioneering work on the relationship between cancer genetics and the three-dimensional structure of cells and tissues. She is a recognized leader in the study of the extracellular matrix – the complex physical and biochemical environment that surrounds living tissues – and how it regulates genes in both normal organs and malignant tumors.

Dr. Bissell is an extraordinary cell biologist whose discoveries have had an enormous impact on our understanding of the mechanisms by which living cells proliferate, differentiate, became cancerous, or

die. Her studies have revolutionized our fundamental understanding of cancer biology.
Dr. Bissell gave the Pezcoller Lecture "Phenotype Overrides Genotype in Normal Mammary Gland and Breast Cancer" in Los Angeles at the AACR Meeting on April 15th 2007. She gave also a lecture in Rome on May 7th at the Regina Elena Cancer Institute. Before reaching Trento for the official Award Ceremony (Buonconsiglio Castle May 11th) she gave the Korsmeyer Lecture at the VIMM (Venetian Institute of Molecular Medicine) in Padua on May 9th to honour the memory of the late Stanley Korsmeyer who received our Award three years ago.

This year we are pleased to celebrate the tenth anniversary of the Pezcoller Foundation-AACR International Award.
In this issue we are also presenting the speakers abstracts of the 19th Pezcoller Symposium on "Hypothesis driven clinical investigation in cancer". A meeting focused on clinical investigations aimed at validating targets identified in basic preclinical studies and exploiting them through the development of rationally designed and hypothesis driven clinical trials.

On Sept.7th in Rovereto the Pezcoller Foundation will recognize Prof. Luigi Chieco Bianchi as winner of the 2007 Pezcoller Foundation–FECS Recognition for Contribution to Oncology. The winner will give the Pezcoller lecture at the ECCO 14 Congress in Barcelona on Sept. 24th 2007.

At the back we have inserted the call for the 2008 Pezcoller Foundation / AACR International Award for Cancer Research.

Gios Bernardi MD
The Pezcoller Foundation President
and Editor of the Journal

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19th Pezcoller Symposium

HYPOTHESIS DRIVEN CLINICAL INVESTIGATION IN CANCER

June 14-16, 2007 - Trento, Italy

ABSTRACTS OF ORAL PRESENTATIONS

MOLECULAR CANCER THERAPIES: INSIGHTS FROM KINASE INHIBITORS

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A central theme that has emerged from the clinical development of kinase inhibitors is the notion of "kinase-dependent" cancer, i.e. those cancers whose growth is driven by a specific kinase or set of kinases. By definition, such cancers should respond (shrink) when exposed to an inhibitor that effectively blocks the enzymatic activity of the responsible kinase. Based on current example, clinical sensitivity to a kinase inhibitor is highly likely if the tumor has a "driver" mutation in the gene encoding the target kinase that alters its biological potency. Discovery of such mutant kinases is a primary rationale for cancer genome resequencing efforts.

A growing issue complicating the success of kinase inhibitor therapy of cancer is acquired resistance, defined as disease relapse on continuous therapy after an initial response. First recognized as a significant problem initially in advanced stage CML patients, acquired resistance also occurs in chronic phase CML, GIST (gastrointestinal stromal tumors), HES (hypereosinophilia syndrome) and lung cancer. 85 percent of relapsed CML patients have mutations in the ABL kinase domain that alter drug sensitivity. 38 different ABL mutations have been reported to date. Curiously, only a small number of the mutations occur at contact residues. Rather, the majority occur at residues that appear, based on structural modeling studies, to alter the conformational flexibility of ABL such that it can no longer achieve the closed, inactive conformation required for optimal imatinib binding. These structural insights suggested that second generation ABL kinase inhibitors should bind in a less conformation-dependent fashion to retain activity against most imatinib-resistant mutants. This is the case with the dual SRC/ABL kinase inhibitor dasatinib (BMS-354825), which binds ABL in the active or inactive conformation and was recently approved for treatment of imatinib-resistant CML patients. We have also examined mechanisms of resistance to dasatinib in vitro and in patients treated on these trials. Unlike imatinib, resistance occurs almost exclusively through mutations at drug contact residues, presumably due to less conformation-stringent binding requirements. Some mutations confer resistance to dasatinib but not to imatinib; however, sequential treatment with ABL kinase inhibitors selected for compound mutants (two or more mutations in the same BCR-ABL mRNA) in

many patients. Compound mutants limited the ability to respond to re-treatment with imatinib and, in some cases, enhanced the oncogenic fitness of BCR-ABL. These data provide evidence in favor of combination therapy with these two compounds for CML and have implications for other cancers such as GIST, HES and lung cancer where analogous kinase inhibitor resistance mechanisms have been described.

We are also learning that response to kinase inhibitors can be affected by factors in addition to mutations in the target kinase, such as mutations in other "modifier" genes that reduce kinase dependence. One example is the failure of glioblastoma patients with mutant EGFR (viii EGFR) to response to EGFR inhibitors if the tumor also contains a mutation in the PTEN tumor suppressor gene. Another variable is the blockade of negative feedback loops that can paradoxically lead to hyperactivation of a signaling pathway. This concept is best illustrated by treatment with mTOR inhibitors, which can cause increased activity of Akt in some tumors, and therefore may counteract the intended therapeutic effect. Similar to the problem of acquired resistance, these scenarios may also be solved through appropriately designed combination therapy. A broader implication is that patient-tailored kinase inhibitor therapy is likely to require evaluation of a suite of molecular variables for optimal predictive power.

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SELECTIVE AND MULTI-TARGETED KINASE INHIBITORS IN GIST: LESSONS FOR RATIONAL DRUG DESIGN AND PATHWAY-DRIVEN CLINICAL DEVELOPMENT

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Mutations which induce constituently activated intracellular signaling pathways represent some of the most promising mechanisms by which certain cancers can be understood, diagnosed, and managed with pathway-directed therapeutic interventions. Our team has chosen to study in detail a reasonably simple and genomically stable form of human sarcoma known as gastrointestinal stromal tumor (GIST), with the aim of understanding this system well and using this knowledge to develop novel diagnostic and therapeutic strategies. Before 1999, the basic biology and pathology of GIST were poorly understood. The observation by Hirota, Kitamura, and

colleagues in 1998 of activating mutations in the KIT proto-oncogene served to identify a key pathway which has been a tractable target of therapy. Remarkable progress has now been made in treating GIST, starting with the development of imatinib, a selective tyrosine kinase inhibitor. The subsequent identification of mutations in the PDGFRA proto-oncogene of a subset of KIT-negative GIST further justified the development of new agents to target these mutationally-activated signaling molecules. In GIST, imatinib selectively inhibits the abnormal signaling activity from most mutated variants of KIT and a small subset of the mutated PDGFRA kinases. Although imatinib represents an effective therapy for many GIST patients, resistance to this single agent develops over time, with the evolution of secondary resistance mutations in the tumor genome. Alternative therapeutic options are required for patients with GIST following failure of imatinib due to primary resistance, secondary resistance, or for the rare subset who experience unacceptably severe toxicities with imatinib. Sunitinib (previously known as SU11248) is a multi-targeted kinase inhibitor that was approved globally in 2006 for the treatment of GIST following failure of imatinib. In a placebo-controlled, phase III trial, sunitinib significantly improved disease control and other measures of tumor response in patients with imatinib-resistant GIST or imatinib intolerance. Several other multi-kinase inhibitors are now being studied in clinical trials, and combinations of agents are being tested to inhibit other elements of signaling cascades downstream of the receptor tyrosine kinases, including PI-3-kinase and mTOR. This field has also spurred the development of novel imaging techniques and biomarkers of response, including molecular analyses of the tumor lesions to explain resistance mechanistically. Current goals of our work includes structural analyses of the mutant kinases to understand how resistance to small molecule kinase inhibitors can be explained at the molecular level. We are also exploring the inhibition of chaperone function by inhibiting the Heat Shock Protein-90 (Hsp90) system as a way to selectively destroy numerous different structural mutants of KIT or PDGFRA. A phase I trial of the water-soluble Hsp90 inhibitor known as IPI-504 is in progress with early data supporting the hypothesis that destruction of KIT is possible by this molecular mechanism. GIST is a paradigm of rational investigation and molecular pathway analysis being applied to a human solid tumor with great impact on human health and measurable impact on clinical outcomes. By understanding the lessons gleaned from GIST, further translational and clinical research efforts can be designed and implemented to control other common cancers with more complex genetic and signaling mechanisms.

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DEFINING CLINICALLY RELEVANT MOLECULAR SUBSETS OF LUNG CANCER

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The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), gefitinib (Iressa) and erlotinib (Tarceva), induce dramatic responses in certain patients with non-small cell lung cancer (NSCLC). As such, the drugs provide an unexpected tool to dissect clinically relevant molecular subsets of NSCLC. For example, using mutational profiling of tumor DNA from patients with known clinical outcomes to these drugs, we have demonstrated that somatic mutations in the tyrosine kinase domain of EGFR are associated with sensitivity to gefitinib and erlotinib (1), while mutations in KRAS, which encodes a GTPase downstream of EGFR, are associated with primary resistance (2). EGFR mutations are more commonly found in tumors from patients who never smoked cigarettes (1), while KRAS mutations are more frequent in those with significant tobacco exposure (2). Moreover, in our analysis of 300 patients with tumors resected at MSKCC and never treated with kinase inhibitors, EGFR and KRAS mutations have prognostic value, as patients whose tumors with EGFR mutations have a longer overall survival than those whose tumors have KRAS mutations. Thus, these two mutations define distinct populations of NSCLC patients with different natural histories and responses to targeted therapy.

We have also demonstrated that second site mutations in the EGFR kinase domain are found in about half of patients with acquired resistance to these drugs (3, 4). The most common (>90%) second-site mutation involves a CàT change at nucleotide 2369 in exon 20, which results in substitution of methionine for threonine at position 790 (T790M). This mutation can rarely be found before treatment and is linked to genetic susceptibility to lung cancer. We continue to re-biopsy patients who develop acquired resistance. In addition, to understand the role of the T790M mutant in lung tumorigenesis, we developed mice with inducible expression in type II pneumocytes of EGFRT790M alone or together with a drug-sensitive L858R mutation. Both transgenic lines develop lung adenocarcinomas that require mutant EGFR for tumor maintenance but are resistant to an EGFR kinase inhibitor. EGFRL858R+T790M-driven tumors are transiently targeted by hsp90 inhibition. Notably, EGFRT790M-expressing animals develop tumors with longer latency than EGFRL858R+T790M-bearing mice but in the absence of additional kinase domain mutations. Thus, in addition to conferring resistance to kinase inhibitors, the T790M mutation has gain-of-function activity in vivo. These mouse models should be useful for developing improved therapies for patients with lung cancers harboring the EGFRT790M mutation.

We are now using a variety of molecular and biological approaches to define further mechanisms of sensitivity/resistance to gefitinib and erlotinib and to identify additional molecular subsets of lung cancer that have relevance in the clinic (5).

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THE VHL TUMOR SUPPRESSOR PROTEIN: GUIDE TO NEW TARGETED AGENTS FOR KIDNEY CANCER

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Heterozygous germline inactivation of the VHL tumor suppressor gene causes VHL disease, which is characterized by an increased risk of several different tumor types including clear cell renal carcinoma. Tumor development in this setting is linked to loss of the remaining wild-type VHL allele in a susceptible cell. Approximately 75% of sporadic kidney cancers exhibit clear cell histology and ~50% of these are effectively VHL-/- due to somatic VHL mutations or hypermethylation. Restoration of VHL function is sufficient to suppress tumor formation by VHL-/- renal carcinoma cells in animal models. Collectively, these observations suggest that VHL is a 'gatekeeper' tumor suppressor gene for clear cell renal carcinoma. The VHL gene product, pVHL, is the substrate recognition module for a ubiquitin ligase complex that targets the alpha subunits of the heterodimeric transcription factor HIF (hypoxia-inducible factor) for polyubiquitylation. The interaction of pVHL and HIFα requires that HIFα be hydroxylated on one (or both) of two prolyl residues by members of the EglN family, which are oxygen-dependent enzymes. When pVHL is defective, or oxygen levels are low, HIF accumulates and transcriptionally activates 100-200 genes involved in acute or chronic adaptation to hypoxia. Including amongst these are genes such as VEGF, PDGF B, and TGFα. We and others have shown that downregulation of HIF is both necessary and sufficient for pVHL to suppress VHL-/- renal carcinoma growth in vivo. Moreover, we showed that conditional expression of a non-hydroxylatable version of HIFα phenocopies the pathological changes that occur after conditional inactivation of VHL mice. In addition, the risk of renal carcinoma linked to different VHL alleles in man correlates with the degree to which they deregulate HIF. Collectively, these observations have helped to validate HIF, and HIF-responsive gene products such as VEGF, as therapeutic targets in kidney cancer. Notably, a number of drugs that inhibit VEGF, or its receptor KDR, have now demonstrated significant activity in the treatment of renal carcinoma. HIF itself is not thought to be 'druggable' but a number of drugs have been identified that, at least indirectly, downregulate HIF including mTOR inhibitors, HSP90 inhibitors, and HDAC inhibitors. An mTOR inhibitor was recently reported to delay kidney cancer time to progression in a randomized Phase III trial. Current clinical trials are testing combinations of agents that inhibit different HIF-responsive gene products or that indirectly target HIF.

Our laboratory is focused on identifying new pVHL functions relevant to tumorigenesis as well as identifying genes that become essential for survival specifically in VHL-/- cells. In addition, we are trying to identify genetic alterations that cooperate with VHL loss to promote renal tumorigenesis. This information will hopefully inform future kidney cancer clinical trials.

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RATIONAL SELECTION OF LUNG CANCER THERAPY

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Unlike some tumor types, the majority of the common solid tumors appear not to be driven by single dominant targetable pathways. Instead, diseases such as lung cancer are likely to be much more complex and heterogeneous, with many distinct and overlapping subsets of tumors within the class, each of which will demand an in depth analysis to define the optimal therapeutic approach. These groups are starting to be defined by multiple technologies, and the simplest example is the small subset of patients with tumors expressing mutant EGFR, who achieve substantial clinical benefit from minimally toxic targeted therapy. Even for this small subset of patients with mutant epidermal growth factor receptors (EGFR), multiple resistance mechanisms have emerged requiring different salvage strategies. There also appears to be a group of patients without EGFR mutations who experience clinically significant survival benefit, and identification of this subset would also have significant clinical benefit. Toward this end, we have used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI MS) as a rapid, affordable and simple strategy that can be applied to the analysis of complex biological samples such as serum, urine and tissue. Peaks in the mass spectrum correspond to ions formed from relatively abundant species in the sample, predominantly peptides and proteins. We have used MALDI MS to study unfractionated, pretreatment sera to identify NSCLC patients with improved survival after treatment with the EGFR TKIs gefitinib and erlotinib(1). Mass spectra, independently acquired at two institutions, gave highly concordant results, and were used to generate an algorithm predictive of time to progression and survival. This prediction algorithm was then validated in a blinded manner in two independent cohorts of NSCLC patients treated with EGFR TKIs. This classification algorithm did not predict outcome in three independent cohorts of patients who did not receive treatment with EGFR TKIs. Thus, if upheld in prospective clinical trials, this simple, rapid, and inexpensive analysis of pre-treatment peripheral blood might be useful in selecting therapy for advanced non-small cell lung cancer patients.
Like the EGFR tyrosine kinase inhibitors, the COX pathway inhibitors have hints of activity in subsets of patients but overall disappointing benefit in unselected patients. We have developed assays for the non-invasive assessment of the eicosanoid expression status of individual lung cancer patients, and this appears to identify those who will benefit from COX inhibition(2). However, this pathway is complex in lung cancer, and there are known to be some COX-produced eicosanoids that have anti-tumor activities, unselective blockade of the pathway may have mixed effects. We are therefore developing selective PGE_2 receptor inhibitors

for targeting of these specific pathways with adverse effects(3). We have also defined a novel receptor target, Notch3, that is overexpressed in a large fraction of NSCLC, and are developing targeted inhibitors for this pathway(4, 5). For treatments without defined targets, candidate expression array and proteomic signatures have been developed to assist in the prediction of benefit.

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THE IMPORTANCE OF SELECTING THE RIGHT PATIENTS FOR THE RIGHT DRUGS.

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Over the past 30 years, the number of new drugs approved each year has steadily declined while the cost of developing each new drug has grown almost exponentially. It currently costs approximately US $900 million to bring a drug to market, mainly because the industry has such a high failure rate and because this failure occurs so late in development. A survey of the 10 largest pharmaceutical companies conducted between 1991 and 2000, showed that the overall failure rate for oncology drugs was 40% during phase I, 70% during phase II, 60% during Phase III and of the few showing success in phase III, 30% were not approved (Kola & Landis 2004). This means that a new oncology drug entering clinical trials has a one in fourteen chance of being used in clinical practice. The pharmaceutical and biotechnology industry cannot be sustainable with the high attrition rates currently operable in drug discovery and development.

To reduce this high attrition rate, and the associated costs, pharmaceutical companies need to be able to make confident decisions regarding efficacy and toxicity as early as possible during the drug development process and to ensure that those patients most likely to benefit from the drug are enrolled into the pivotal phase III trials. Unfortunately, there are few tools available that enable "quick to kill" and "quick to proof of concept" decisions to be made during the early phases of drug development. Most oncology trials rely on CT scan measurement of tumor size or serum tumor markers to assess early clinical response, however, tumor shrinkage or falling serum tumor marker levels are often poor predictors of survival benefit and may not reveal the clinical benefit of cytostatic drugs. Randomized phase II trials are frequently used to seek early signals of efficacy, however these are accompanied by high false positive and false negative rates, conferring considerable risk when making the critical decision to proceed, or not to proceed, to a pivotal phase III trial.

Underlying many of these issues, is the reality that many drugs only benefit a subset of patients. This results in the need to treat many patients in order to identify the few that derive clinical benefit. This traditional "trial and error" practice of drug development is being progressively replaced by targeted therapies that are coupled to biomarkers which help identify patients more likely to show a treatment benefit or experience adverse events. This approach was pioneered by Genentech in the late 1990s with the co-development and co-approval of trastuzumab (Herceptin®)

and immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) tests devised to detect amplification and over-expression of its target, HER2.

Several pharmaceutical companies and academic centers are now employing biomarkers to enrich clinical trails for patients with a higher likelihood of showing a therapeutic benefit, thereby allowing trials to be conducted with fewer subjects reducing both the time and cost of drug development. A logical extension of this concept has been captured by the term "personalized medicine" which envisages a state when therapeutic decisions will be tailored to individuals based on their unique biomarker profile. The lure of personalized medicine has spurred the application of powerful new technologies such as microarray expression profiling, serum and tumor proteome profiling and genome-wide genetic association studies, to identify biomarkers or biomarker signatures with the expectation that they will have greater predictive power than conventional single-analyte pathology tests such as IHC and FISH. Although there have been a few encouraging examples, the successful broad implementation of this approach, however, is proving to be highly challenging.

In this lecture, I shall explore some of these challenges. While archival tumor tissue samples, taken at the time of the original diagnosis, are readily available on most subjects many biomarker techniques do not work on paraffin embedded formalin fixed tissues, limiting the types of exploratory analyses that can be performed. Furthermore, the primary tumor may no longer be representative of the metastatic tumor being treated in patients who have failed multiple therapeutic regimens. The need to obtain fresh primary and / or metastatic tumor biopsy tissue for exploratory biomarker studies, which are unlikely benefit the particular patient, is often clinically and ethically unjustifiable and often becomes a barrier to patient enrollment. Similarly, surrogate biomarker measurements in more accessible blood samples may not be representative of the tumor (eg serum proteomics) or may only be informative in a small subset of patients (eg circulating tumor cells or DNA). Accordingly, few surrogate biomarkers have been adequately validated to enable decision making in clinical trials. Furthermore, studies designed to ascertain whether a drug is safe and effective may not be properly designed or adequately powered to test biomarker hypotheses or validate diagnostic tests.

This new therapeutic and diagnostic paradigm holds great promise but also creates new challenges. Using recent examples, I shall explore several issues that arose during the development of targeted therapies which highlight the need for closer collaboration between academia and companies developing targeted therapies and companion diagnostic tests and a greater awareness among patients, patient advocates, institutional review boards, clinicians, and pathologists of the critical need to obtain tumor samples in order to identify which patients are most likely to benefit.

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DOES ONCOGENES ADDICTION PROVIDE SUFFICIENT THERAPEUTIC INDEX FOR TARGETING

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The phosphatidylinositol-3-kinase (PI3K) pathway plays a crucial role in cell growth and survival and is activated in cancer. Multiple components of the pathway are frequently targeted by amplification, mutation and translocation in cancer patients. Indeed, the breadth and frequency of genomic aberrations in the PI3K pathway in cancer patients indicates a critical role in tumor initiation and progression and further validate the pathway for targeted therapeutics. The frequency of aberrations in the PI3K pathway exceed that of any other pathway with the possible exception of the p53 pathway. However, crosstalk with the p53 and retinoblastoma pathways comprises a signalling network that promotes tumour initiation and progression. Further as the PI3K pathway is activated in tumors and consists of multiple kinases, it is a target rich environment. Despite major interest in this pathway for drug discovery efforts against cancer, no drugs have yet been approved that act specifically against PI3K or the downstream regulator, Akt. However, several drugs that were developed for other purposes either directly or indirectly target PI3K signaling, such as the rapamycin analogs, ether lipids such as perifosine and miltefosine, and inhibitors of the epidermal growth factor receptor (EGFR), HER2, c-kit, platelet-derived growth factor receptor and bcr–abl. Because of the crucial role of the PI3K pathway in normal cell growth and in response to stress, the main challenge to developing PI3K drugs is to identify inhibitors with a usable therapeutic index. Tumors with aberrations in the PI3K pathway may undergo "oncogene addiction" rendering them sensitive to inhibition of the PI3K pathway providing a potential therapeutic index. It is likely that PI3K inhibitors will need to be used in combination with other drugs that cause cell stress, such as other signaling inhibitors, radio- and chemotherapy. Points at which therapeutic intervention might be appropriate in the PI3K pathway include targeting PI3K itself, the downstream regulator Akt, although inhibiting this crucial signaling node might result in toxicity, and other downstream components such as mTOR, integrin-linked kinase (ILK), phosphoinositide-dependent kinase-1 (PDK-1), p70S6 kinase and Forkhead/FOXO1. As with other molecularly targeted agents including imatinib mesylate (Gleevec) and trastuzumab (Herceptin), the success of PI3K inhibitor drugs will likely depend on the selection of cancer patients likely to be responders and non-responders based on genomic aberrations. The co-development of molecular markers determine early responders allowing triage to effective will increase utility of the targeted agents.

PHARMODYNAMIC AND RESPONSE BIOMARKERS

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We are entering the era of molecular medicine, in which a growing understanding of the biology of cancer causation and persistence is leading to the development of better and better molecular targeted therapies that can be effective in the control of cancers in individual patients. In the future, these individualized treatments

are likely to be guided by molecular imaging through the use of radiolabeled biomarkers, which will enable better patient selection, pharmacodynamics for response, and also prediction of patient outcome.

Molecular imaging in oncology means imaging the molecules and molecular based events which are responsible for producing and maintaining the malignant state. Molecular imaging can assess a number of the altered properties of tumors in vivo, and positron emission tomography(PET) is particularly well-suited for this purpose. With the widespread availability of pet CT imaging, diagnostic imaging applications to many common human tumors are facilitated. In recent years, the potential value of molecular imaging for monitoring treatment response has been recognized.

Tumor response has been studied with biomarkers such as [^{18}F] 2-Fluoro-2-deoxy-D-glucose (FDG), a tracer which measures glycolysis(1-9). Because tumors have fundamentally deranged control of growth, markers of proliferation such as [^{18}F] Fluorothymidine (FLT), are particularly useful to monitor treatment response and induce a change in growth state. The selective effect of anticancer drugs on key molecules, may also be imaged with [^{18}F] Fluordihydrotestosterone (FDHT), an androgen receptor binding agent useful in prostate cancer(10); and [^{68}Ga] Gallium-Fab'2 Herceptin (HER scan)(11), a radiotracer which binds to the human tyrosine kinase linked to growth factor receptor Her 2. Finally the pharmacology and pharmacodynamics of drugs such as tyrosine kinase inhibitors(12, 13), tumoricidal antibodies(14, 15), and adoptive immunotherapy(16) is also being studied with pet imaging.

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FUNCTIONAL IMAGING OF THE IN VIVO RESPONSE OF TUMORS TO TARGETED INHIBITORS

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The efficient clinical development of therapies aimed at inhibiting or regulating the activity of specific proteins requires a knowledge of the magnitude and kinetics of target inhibition as a function of the dose and schedule of the agents employed. Up to now, this has been difficult if not impossible with currently available methodology. Immunohistochemical assays have not been made quantitative, biopsies of solid tumors are difficult to obtain, and it is effectively impossible to obtain multiples biopsies over short time periods to ascertain kinetics of inhibition. Furthermore, tumor responses are often heterogeneous and this may be due decreased drug accumulation or target inhibition in metastatic sites. Currently, target inhibition is determined in surrogate normal tissue, which probably does not accurately reflect the effects of the drug in the tumor and, occasionally in biopsies of the tumor obtained before and after drug administration. The latter has provided significant advances in understanding, but biopsies are difficult to obtain and multiple post-treatment biopsies are not feasible. Thus, kinetics of target inhibition cannot be evaluated. This is important because of the key role of adaptation of the signaling network in response to target inhibition and for the rational design of mechanism based combination therapy.

These problems could be overcome with the development of efficient means for the isolation and analysis of circulating tumor cells, the development of serum markers that accurately and rapidly reflect changes in the tumor, or the development of methodologies for functional imaging. We have chosen to concentrate on the last of these. Two examples will be discussed, which involve imaging of the pharmacodynamics of Hsp90 inhibition and the biologic effects of inhibition of the MEK/MAPK signaling pathway.

Hsp90 is a protein chaperone that is required for the stability and conformational maturation of a subset of key signaling proteins and mutated oncoproteins. Hsp90 inhibitors cause the ubiquitination and proteasomal degradation of these client proteins, including HER2, which is the most sensitive Hsp90 client so far identified. One such inhibitor, 17-AAG, has been shown to degrade HER2 and other clients in vivo and to inhibit the growth of both xenograft and transgenic tumor models. 17-AAG is now being extensively studied in clinical trials and has been shown recently to have significant antitumor activity in patients with advanced breast cancers with HER2 overexpression. These studies have been hampered by the inability to determine the degree of target

inhibition obtained with the drug. We have used the biologic effects of Hsp90 inhibition to develop a method for assessing the pharmacodynamics of 17-AAG with PET imaging. An F-ab fragment of Herceptin with a short (2 hour) half-life in vivo was generated and chelated to a variety of positron emitting isotopes, including 68Ga. We showed that the surface expression of HER2 on tumor xenografts could be quantitatively imaged with this reagent. Furthermore, the reagent could be used to image the effects of 17-AAG on HER2 expression in tumors in vivo as a function of dose and time after administration of the drug. This technique may therefore allow the imaging of the pharmacodynamic effects of 17-AAG in patients. We have now begun using this technique in conjunction with the Phase 2 clinical trial of 17-AAG and Herceptin in patients with HER2-breast cancer whose tumors have progressed on Herceptin in combination with chemotherapy. This methodology can be utilized as a platform for imaging the effects of other targeted compounds, if a protein the surface expression of which is rapidly lost in response to the inhibition of the target is identified.

Many inhibitors of signal transduction cause cessation of tumor proliferation without inducing apoptosis or tumor regression. Such responses can be as clinically significant as regression and suggest effective target inhibition, but they are difficult to distinguish from indolent disease that has not significantly responded to treatment. We have shown that tumors with mutant BRAF are quite sensitive to pathway inhibition with MEK inhibitors. In xenograft models, inhibition is usually associated with tumor stasis, rather than regression. This is born out in early clinical trials, in which the treatment of patients with melanoma with MEK inhibitors has resulted in some objective tumor responses and many instances of stabilization of disease.

In BRAF tumors, MEK inhibition is associated with loss of D-cyclin expression, induction of p27 expression and rapid loss of RB-phosphorylation and concomitant induction of profound G1 arrest. We reasoned that, in these tumors, G1 progression was completely dependent on MAPK activity. 18-FLT is transported into tumor cells in an S-phase dependent manner. We reasoned therefore that the 18F-FLT PET would be extremely sensitive to MEK inhibition in these tumors and could constitute a marker of early response to the drug. This turned out to be the case. The FLT PET image rapidly declined after dosing with MEK inhibitors and remained low throughout the course of treatment. After discontinuation of the drug, the image did not immediately increase but stayed low, sometimes for weeks, until the tumor began to increase in size. In contrast, although the increase in the FDG PET image in growing tumors was prevented by the MEK inhibitor, no decline in signal was noted. The results suggest the FLT PET may be useful for detection of early response of mutant BRAF tumors to MEK inhibition and may allow discrimination between slowly growing tumors and tumors that have stabilized in response to pathway inhibition. This could be of great utility for assessing the clinical impact of agents that cause stable disease. This imaging technique will also be useful in assaying the heterogeneous responses of tumor metastases that occur in response to therapy. We plan to initiate FLT PET imaging as an adjunct to future phase 2 trials of inhibitors of RAF/MEK/MAPK signaling to test these possibilities in patients.

SIGNATURES FOR CANCER DISCOVERY

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The application of genomic approaches to the study of cancer holds tremendous promise for improved diagnostic and prognostic tests, and for the elucidation of new therapeutic targets by building a molecular taxonomy of the disease. Such molecular taxonomy might be built at the level of DNA, RNA, protein, or in principle, other biomolecules (e.g. metabolites). Our laboratory has focused on the use of DNA microar-

ray-based RNA profiling, defining gene expression 'signatures' of cancer.

Our initial work in this area established the proof of concept that gene expression profiling could be used to classify disease – both to discover previously unknown subclasses of cancer (class discovery) and the assignment of patient samples to previously established classes (class prediction). This initial study (1) demonstrated the feasibility of classifying acute leukemias, and formed a foundation for further classification studies involving childhood brain tumors (2), prostate cancer (3), lymphoma (4) and lung cancer (5). In each of these cases, intriguing gene expression signatures were identified with potential for future clinical utility. However, clinical implementation of such signatures will await much larger, validation studies that have yet to be conducted.

More recently, we have addressed the challenge of using gene expression data in the drug discovery setting. Traditionally, gene expression profiling is performed, and the resulting signatures studied intensively (using conventional cell biological methods) to determine which components of the signature (i.e. which proteins) represent potential therapeutic targets that might be worthy of conventional biochemically-based high throughput small-molecule screens. Unfortunately, in many cases it has proven extremely difficult to determine which of the many signature genes represent an attractive target, and in some cases there may be no single gene/protein, but rather the collaboration of a group of signature genes that is required to explain the phenotype under study. In other cases, the critical therapeutic target is identified, but is found to be of a class of 'undruggable' targets, such as transcription factors.

We have therefore asked whether gene expression signatures might be used in a different way in the context of drug discovery. That is, having defined a gene expression signature of a biological state of interest (e.g. tumor subtype or state of pathway activation), could a small molecule library be screened to identify compounds capable of modulating the signature of interest – and by inference, modulate the biological state under study. We piloted this idea, termed Gene Expresion-based High Throughput Screening (GE-HTS), and applied it to the discovery of compounds capable of inducing the myeloid differentiation of acute myeloid leukemia cells (6). Importantly, the discovery of these compounds did not require a specialized phenotypic assay, nor did it require prior knowledge of the mechanism by which differentiation occurs. We have subsequently applied this GE-HTS concept to the discovery of compounds that inhibit the activity of the Ewing Sarcoma oncogene EWS/FLI (7) and that abrogate androgen receptor signaling in prostate cancer (8). These experiments establish the feasibility of using a gene expression signature as the read-out of a primary small-molecule screen.

Extending on this concept of signature-based chemical screening, we have recently established the feasibility of using a database of gene expression profiles to systematically connect signatures of diseases to signatures of gene product function or signatures of drug action. We refer to this project as the Connectivity Map project. By querying a centrally generated database of gene expression profiles, users can find 'connectivity' between a query signature of interest and one or more treatments (perturbagens) in the database. The data and tools are available at www.broad.harvard.edu/cmap, and we have used the method to discover relevant connections in dexamethasone-resistant childhood leukemia (9), androgen response in prostate cancer (8), and connections to HDAC inhibition in various cell types (10). These experiments demonstrate the feasibility of the Connectivity Map approach, and suggest the value of creating a larger, more extensive, publicly accessible Connectivity Map database.

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THE USE OF GENETICALLY ENGINEERED MICE TO INVESTIGATE TREATMENT RESPONSE AND RESISTANCE

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The 21 kD proteins encoded by the KRAS, NRAS, and HRAS proto-oncogenes encode signal switch molecules that regulate cell fates by cycling between an active guanosine triphosphate (GTP)-bound state (Ras•GTP) and an inactive guanosine diphosphate (GDP)-bound state (Ras•GDP). Ras•GTP activates multiple downstream effector pathways including the Raf/MEK/ERK, phosphoinositol 3'-kinase (PI3K)/Akt, and Ral•GDS cascades. Signaling terminates when Ras•GTP is hydrolyzed to Ras•GDP. GTPase activating proteins (GAPs) are negative regulators of Ras output that bind to Ras•GTP and stabilize a transition state between Ras•GTP and Ras•GDP, which accelerates the t1/2 of the intrinsic Ras GTPase activity from minutes to seconds. Mammalian cells express two major GAPs – p120 GAP and neurofibromin. The latter is encoded by the NF1 tumor suppressor gene, which is mutated in persons with neurofibromatosis type 1 (NF1). NF1 is a common multi-system disease that is associated with a predisposition to specific cancers including juvenile myelomonocytic leukemia (JMML). Many tumors from affected individuals show loss of the normal NF1 allele, which is consistent with its biochemical function as a negative regulator of Ras signaling.

Point mutations of RAS genes at codons 12, 13, and 61 are detected in many in human cancers, particularly carcinomas of the lung, pancreas, and colon cars and myeloid leukemia. Each of these residues participates in GTP binding and amino acid substitutions constitutively activate the Ras pathway by increasing intracellular levels of Ras•GTP. Biochemical studies have shown that oncogenic Ras proteins demonstrate defective intrinsic GTP hydrolysis and are resistant to GAPs. In addition to activation by point mutations in RAS genes, other cancer-associated mutations such as FLT3 internal tandem duplications, mutations in the kinase domain of the EGFR receptor, BRAF and PTPN11 point mutations, NF1 inactivation, and the BCR-ABL fusion protein deregulate Ras signaling. Together, the prevalence of oncogenic RAS mutations and the existence of alternative mechanisms that result in hyperactive Ras in cancers without RAS mutations establish hyperactive Ras as a major therapeutic target. However, as Ras signaling plays a central role in normal cell growth and differentiation, developing agents with a beneficial therapeutic index is challenging. Furthermore, because Ras is a "nodal" protein that regulates multiple downstream effectors, the critical target(s) of hyperactive Ras that are required for tumor initiation and maintenance in different cell contexts are largely unknown. Importantly, the most obvious therapeutic strategy for cancers with RAS mutations – developing specific inhibitors of the mutant protein – is extremely difficult due to structural considerations and because oncogenic Ras does not have an over-active enzymatic activity. Instead, substitutions at codons 12, 13, and 61 impair intrinsic and GAP-mediated GTPase activity. An effective small molecule therapeutic would thus have to effectively restore normal biochemical activity (i.e. repair a broken enzyme).

Strains of mice carrying conditional mutant alleles of Nf1 or oncogenic Kras in the endogenous loci are novel reagents for understanding how cells remodel signaling networks in response to hyperactive Ras and for performing preclinical studies of promising agents. Use of the Mx1-Cre transgene to ablate Nf1 or activate KrasG12D expression in hematopoietic cells results in a fatal myeloproliferative disorder (MPD) that is more aggressive in Kras mutant mice (1-3). These murine leukemias are reminiscent of JMML and chronic myelomonocytic leukemia (CMML). We have recently utilized flow cytometric methodologies to interrogate signaling networks in primary hematopoietic stem/progenitor cells, and have shown that oncogenic K-RasG12D expression induces characteristic changes in downstream effectors (4).

Interestingly, downstream effector pathways that are profoundly deregulated in cell lines transduced with oncogenic RAS surprisingly showed low levels of activation in primary tumor cells from mice expressing mutant K-Ras.

We are using retroviral insertional mutagenesis (RIM) to identify mutations that cooperate with hyperactive Ras to induce progression from MPD to acute myeloid leukemia (AML), and are testing targeted agents in these strains. Many inhibitors that target effectors of mutant Ras such as Raf, MEK, and Akt are under development or in early phase clinical trials. In recent studies, we found that CI-1040, a potent inhibitor of MEK, had no beneficial effects in Nf1 mice with MPD at the maximally tolerated dose (MTD). By contrast, MEK inhibition induced objective regression of Nf1-deficient AMLs. These AMLs uniformly developed resistance to CI-1040 in vivo, despite equivalent biochemical inhibition of the target in paired sensitive and resistant clones. The pattern of retroviral insertions found in resistant AMLs is consistent with outgrowth of a pre-existing clone during CI-1040 administration, and we have isolated candidate resistance genes. In other studies, we are exposing MPDs and AMLs with RAS mutations to MEK inhibitors to determine pattern of response and resistance. These studies emphasize the importance of cell context in the response to targeted agents, and establish a tractable in vivo system for identifying genes that modulate therapeutic efficacy and for probing mechanisms of acquired resistance.

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USING CANCER GENOMICS TO INFORM TARGETED DRUG DEVELOPMENT

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Scientific advances in understanding the genetic basis of cancer, the availability of the complete human genome sequence and the development of genomic technology associated with the Human Genome Project have fueled a dramatic paradigm shift in cancer drug development. Initial successes with therapies targeted at specific molecular defects in specific cancer subtypes, such as imitanib for CML and GIST, and trastuzamab for ERBB2 positive breast cancer, changed the direction of academic medical research as well as the pharmaceutical industry. As these targeted therapies became not just scientific and clinical successes, but commercial successes as well, companies previously only interested in broad-based cytotoxic agents that might be applicable in many cancer types became willing to invest in targeted agents for specific cancer genotypes. In most major pharmaceutical companies, this transformation is now complete, with oncology drug development pipelines made up of almost entirely of molecularly-targeted small molecules. These changes also provided the basis for preclinical studies to predict which patients are most likely to respond to each drug and in turn inform clinical trials designs where targeted agents can be tested only the patients mostly likely to respond.

The central tenet of our preclinical strategy to identify response prediction biomarkers is that cancer is a disease of the genome, and all underlying abnormalities in cancer are fixed in the genome either by genetic or epigenetic alterations. These changes include mutations, amplifications and deletions, translocations, complex rearrangements and epigenetic alterations. Structural genetic changes also may be reflected in the transcriptome and proteome. It is these somatic genetic alterations in the tumor that are the targets and primary determinants of response to the current generation of targeted cancer therapeutics. Thus we have constructed a panel of 300 human cancer cell lines that represent the broad spectrum of disease found in adult cancer patients, and determined the IC50 for every compound in development in every cell line. In parallel, we have profiled all cell lines for DNA copy number at high resolution using Affymetrix 500K SNP arrays, for RNA transcript profile with Affymetrix U133 arrays and with targeted genomic sequencing to identify somatic cancer-associated intragenic mutations We also have developed a human tumor bank to determine the incidence and prevalence of predictors of cell line sensitivity in primary human tumors. Data from these analyses, which also incorporate relevant data in the scientific literature, then inform the design of clinical studies.

Like standard cytotoxic chemotherapy, it is expected that targeted agents also will be more effective in combination that as monotherapy. Thus in addition to defining the primary determinants of response to single agent therapy, we are utilizing shRNA technology to identify synthetic lethal phenotypes that predict both effective targeted combinations and new targets. Based on recent successes with combining targeted agents and standard cytotoxics, we are also evaluating those combinations. The overall effect of this new approach to cancer drug development benefits everyone, with more effective, less toxic drugs for patients and shorter development programs with lower cost and less risk of failure for the pharmaceutical industry.

KINASE SHRNA SCREENING FOR POTENTIAL DRUG TARGETS IN OVARIAN CANCER CELLS

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Ovarian cancer is a particularly difficult disease with which to grapple therapeutically. This is because the majority of patients present to their physician too late in the disease course to be candidates for curative surgery. Moreover, while platinum and taxane derivatives are effective first line chemotherapy treatment for advanced disease, they are very rarely curative. Worse still, there are few, known druggable proteins that have been established as validated targets in this disease. Therefore, we elected to initiate shRNA library screening for proteins that, when sufficiently depleted from selected human ovarian cancer cell lines, lead to significant defects in cell proliferation and, ideally, viability. Conceivably, one or more of these proteins can serve as credible ovarian cancer drug targets. With the development of a hairpin library encompassing all 92 human tyrosine kinases, we have screened three, different ov ca cell lines and detected erbB3 as a protein that, when depleted in all cases, was associated with a major proliferative defect. The erbB3 protein in these and several other such lines (but not in all that were tested) was found to be activated through Y1289 phosphorylation, and its co-immunoprecipitated with the p85 subunit of PI-3 kinase. This means that erbB3 has engaged the PI3K pathway in these cells. Analogous results were obtained with fresh tumor cells derived from paracentesis samples of a significant fraction of late stage ov ca patients. The biological significance and the implications for therapeutic strategy making of these and related results will be discussed.

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PANCREATIC CANCER MODELS AND MEDICINE

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Pancreatic ductal adenocarcinoma (PDA) is the fifth-leading cause of death due to cancer in the western world. This has been attributed to a lack of effective therapies and the inability to detect PDA in its earliest stages. Pursuits into the etiology of PDA have demonstrated that oncogenic KRAS mutations occur early in the genesis of PDA, whereas tumor suppressor gene mutations that include Ink4a, p53 and DPC4/SMAD4 occur later. We have previously generated mutant mice that develop preinvasive pancreatic cancer, termed pancreatic intraepithelial neoplasia (PanIN), by conditionally expressing an endogenous KrasG12D allele in pancreatic progenitor cells(1). Mice harboring PanINs slowly progress to invasive and metastatic PDA, and have a median survival of 16 months. Pathologically and biologically, the murine PanIN and PDA lesions closely resemble their human counterparts, with cachexia and metastasis to the same sites commonly noted in patients (liver, lung, mesentery, celiac plexus) commonly observed. Biochemical pathway analysis has revealed activation/expression of the Notch, Cox-2, erbB1/B2, and hedgehog pathways, which are potential therapeutic targets in PanIN/PDA. Furthermore, serum proteomic analysis revealed a predictive diagnostic pattern for PanIN, suggesting that such strategies may be applicable to the identification of patients with PanIN. By incorporating additional conditional mutations in tumor suppressor genes including P53(2), Ink4a/ARF(3), and DPC4(4), we have also generated models of advanced and metastatic PDA (for P53 and Ink4a/ARF) and Mucinous Cystic Neoplasia (for DPC4). The pancreatic-specific concomitant expression of an endogenous KrasG12D allele and an endogenous Trp53R172H allele resulted in a model of metastatic PDA, and cells derived from tumors demonstrate widespread numerical and structural chromosomal instability (CIN), a common observation in human PDA. Since the Kras/p53 murine model of PDA mimics the pathophysiological, genetic and genomic features of human PDA, we sought to determine whether this model would be more predictive than the current standard of tumor xenografts in the evaluation of therapeutics. We find that mice with PDA are usually refractory to Gemcitabine treatment, whereas mice harboring subcutaneous PDA tumors of human or murine origin are sensitive to this chemotherapeutic. Pharmacodynamic analyses revealed a large inhibitory effect of gemcitabine on cell proliferation in ectopic tumors, and only a minimal effect in autochthonous PDA. A potential explanation for this discrepancy between ectopic tumors and our autochthonous model is the striking difference in gemcitabine triphosphate levels in tumor samples. As gemcitabine has limited activity in pancreatic cancer patients, and this is reflected in our mouse model, we suggest that the exploration of additional therapeutics in such models is warranted (5).

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RECURRENT GENE FUSIONS IN PROSTATE CANCER

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To date, the great majority of disease-specific, recurrent chromosomal rearrangements have been characterized in hematological malignancies and mesenchymal

tumors and not in common epithelial tumors such as breast, lung, colon, or prostate cancer. Here, we employed a bioinformatics approach on a compendium of cancer gene expression data to discover candidate oncogenic chromosomal aberrations based on outlier gene expression. In addition to identifying many gene partners of characteristic rearrangements in human malignancies, this approach identified two members of the ETS family of transcription factors, ERG and ETV1, as outliers in prostate cancer. Either ERG or ETV1 was over-expressed in the majority of prostate cancers (50-70%) and were mutually exclusive across several independent gene expression datasets, suggesting that they may be functionally redundant in prostate cancer development.

By RNA ligase-mediated rapid amplification of cDNA ends (RACE), we identified a recurring gene fusion of the 5' untranslated region of a prostate-specific, androgen-regulated gene TMPRSS2 to ERG or ETV1 in prostate cancer cases which over-expressed the respective ETS family member. These gene fusions were confirmed using quantitative PCR (QPCR) and sequencing of reverse transcription PCR products. In addition, using fluorescence in situ hybridization (FISH), we demonstrated that 23 of 29 (79%) prostate cancer samples harbor rearrangements in ERG or ETV1. Furthermore, in vitro cell line studies suggest that the androgen-responsive promoter elements of TMPRSS2 mediate the aberrant over-expression of ETS family members in prostate cancer. Subsequently, we interrogated the expression of all ETS family members in prostate cancer profiling studies and identified outlier expression of ETV4 in two of 98 cases. In one such case, we confirmed the over-expression of ETV4, and by RACE, QPCR and FISH, we identified fusion of the TMPRSS2 and ETV4 loci.

Together, these results suggest a pathogenetically important role for recurrent chromosomal rearrangements in common epithelial tumors and have implications in the molecular diagnosis and treatment of prostate cancer. Importantly, these results identify three molecular subtypes of prostate cancer, TMPRSS2:ERG, TMPRSS2:ETV1 and TMPRSS2:ETV4, and suggest that dysregulation of ETS family member expression through gene fusions with TMPRSS2 may be a generalized mechanism for prostate cancer development.

In our most recent work, we explored the mechanism of ETS family over-expression in prostate tumors. Remarkably, we identified novel 5' fusion partners in prostate tumors with outlier expression of ETS family members, including untranslated regions from a prostate-specific androgen-induced gene and endogenous retroviral element, a prostate-specific androgen-repressed gene, and a strongly expressed housekeeping gene. As the commonality of these rearrangements is the aberrant over-expression of ETS genes, we recapitulated this event in vitro. We demonstrate that ETS over-expression in multiple benign prostate cells induces a marked increase in invasion, confirming the role of ETS gene rearrangements in prostate cancer development. Identification of distinct classes of ETS gene rearrangements demonstrates that dormant oncogenes can be activated in prostate cancer by juxtaposition to tissue-specific or ubiquitously active genomic loci. Subversion of active genomic regulatory elements may serve as a more generalized mechanism for carcinoma development. Furthermore, the identification of androgen-repressed and insensitive 5' fusion partners has important implications for the anti-androgen treatment of advanced prostate cancer.

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THE ENDOTHELIN AXIS IN CANCER: THE PROMISE AND THE CHALLENGES OF MOLECULARLY TARGETED THERAPY

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Improvement of current cancer therapeutic strategies is clearly relaying on the identification of suitable target molecules, which are specifically and abundantly expressed by the tumor and are accessible to bioactive agents. The endothelin (ET) axis, which includes ET-1, and the ETA and ETB receptor, may represent a novel target in tumor treatment (1). Experimental data are now available demonstrating that the ET-1 axis may directly contribute to tumor growth and indirectly modulate tumor-host interactions in various tumors such as prostatic, ovarian, renal, pulmonary, colorectal, cervical, and breast carcinoma, Kaposi's sarcoma, brain tumors, and melanoma (2). In this context during the past years we have analyzed in depth the ET-1 axis in two malignancies namely ovarian carcinoma and cutaneous melanoma., for both of which there is currently no treatment options for advanced disease. We have provided evidence that in ovarian cancer ligand-mediated ETAR engagement activates different signal transduction pathways including protein kinase C, phosphatidylinositol 3-kinase, mitogen-activated protein kinase and transactivate epidermal growth factor receptor. As a result, the ETAR mediates multiple tumor-promoting activities, including enhanced cell proliferation, escape from apoptosis, angiogenesis, epithelial-mesenchymal transition, motility and invasiveness. These findings together with recent gene expression profiles of ovarian cancer, identifying ETAR as metastasis-associated genes which correlates with resistance to chemotherapy, strongly indicate ETAR as a relevant target for effective mechanism-based therapies (3,4). The recent production of highly-selective small molecules that inhibit ETAR offers the possibility of testing this therapeutic approach in a clinical setting. Among the ETAR antagonists, the orally active ZD4054, which is in early development for cancer treatment, is likely to be effective in ovarian cancer (5). Preclinical and clinical data of ETAR antagonists in patients with ovarian and prostate cancer are encouraging and provide a rationale for the use of these molecules alone and in combination with cytotoxic drugs or molecular inhibitors. This latter strategy is likely to be more successful since cooperation between redundant biochemical pathways appears to be the main reasons for the failure of a therapy aimed at interfering with a single specific molecular target. The reported lack of clinical response to EGFR inhibition may therefore suggest that the tumor

can maintain redundant downstream signalling through alternative receptors, such as ETAR, that may override the effects of EGFR inhibition and generate resistance to anti-EGFR therapy. The evidence of cross-talk between the EGFR and ETAR pathways, along with the improved therapeutic efficacy of combined targeting of ETAR, employing ZD4054, and of EGFR, by the selective inhibitor gefitinib (IRESSA) observed in ovarian cancer preclinical models (6), provide a rationale for a clinical evaluation of this combination.

Differently from ovarian cancers, phenotypic and genotypic analyses of cutaneous melanoma have identified the ETBR as tumor progression marker, thus representing a potential therapeutic target. ET-1 and ET-3 through ETBR activation trigger signalling pathways involved in events associated with disruption of normal host-tumor interactions and with tumor progression. Pharmacological interruption of ETBR signalling by specific ETBR antagonist is effective in melanoma xenografts indicating a novel therapeutic approach in the treatment of this malignancy (7,8). In view that recent data of phase II trials demonstrates that bosentan, a dual endothelin receptor antagonist, was well tolerated and produced disease stabilization in certain patients with metastatic melanoma (9), the potential role of endothelin receptor antagonists in the treatment of melanoma should be further assessed in randomised controlled studies in combination with other anticancer drugs.

Collectively this improved knowledge of the interconnected molecular mechanism promoted by ET-1 axis underlying tumor progression will certainly fuel the interest of basic and translational scientists to critically evaluate these exciting possibilities.

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TARGETING THE KINOME USING ATP AND NON-ATP COMPETITIVE INHIBITORS

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The vast majority of kinase inhibitors developed to date target the ATP binding site of the kinase in its "active" conformation where the activation loop is phosphorylated (Type I). Recently, crystal structures of inhibitors such as imatinib (STI571), BIRB796 and sorafenib (BAY43-9006) have revealed a new binding mode that

exploits an additional binding site immediately adjacent to a region occupied by ATP (Type II). This pocket is made accessible by an activation loop rearrangement that is characteristic of kinases that are in an "inactive" conformation (Type II). Here, we present a structural analysis of binding modes of known Type II inhibitors and demonstrate that they conform to a pharmacophore model that is currently being used to design a new generation of kinase inhibitors. We also report the discovery of a new class of cellular Bcr-abl inhibitors (exemplified by GNF-2) that target the myristate-binding site and allosterically inhibit kinase activity. GNF-2 exhibits exclusive antiproliferative activity towards Bcr-abl transformed cells with an IC50 = 150 nM. GNF-2 acts synergistically with ATP-competitive inhibitors in cell culture and in a murine Chronic Myelogenous Leukemia (CML) model. We discuss the binding mode for GNF-2 to Abl and discuss the mechanism by which mutations in Bcr-abl can induce resistance to GNF-2.

MOLECULAR TARGETING OF THE RET KINASE IN THYROID CANCER

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Papillary thyroid carcinoma (PTC) is the most prevalent thyroid cancer subtype. PTC is treated by surgical resection, adjuvant radioiodine treatment and thyroid hormone replacement to suppress thyroid-stimulating hormone. However, some PTC patients may have persistent or recurrent disease with loss of responsiveness to ^{131}I therapy. Medullary thyroid carcinoma (MTC) accounts for 5%-10% of all thyroid cancers. MTC is sporadic in about 75% of cases, and in the others it is a component of the Multiple endocrine neoplasia type 2 (MEN 2) autosomal dominant cancer syndrome. MEN 2-associated hereditary MTC is bilateral and multicentric and it is usually preceded by multifocal C-cell hyperplasia. Early surgery in carriers of RET gene mutations has significantly improved the prognosis of familial MTC. However, MTC patients, particularly those with sporadic tumors, are often incurable because the cancer has already metastasized before being diagnosed.
The RET receptor tyrosine kinase could be prime target for the molecular therapy of thyroid cancer. The RET receptor is rearranged (about 30% of the cases) in PTC, generating the chimeric RET/PTC oncogenes. Moreover, germline point mutations in RET cause MEN 2 syndromes and somatic mutations in RET, mainly targeting V804, M918 and E768, are also found in sporadic MTC. RET/PTC and RET/MEN 2 are constitutively active tyrosine kinases able to induce the formation of transformed foci, anchorage independent growth and tumorigenicity in nude mice when introduced in NIH 3T3 cells. Moreover, they initiate thyroid carcinogenesis when introduced in transgenic mice.
We have searched for RET inhibitors by analysing by an in vitro RET kinase assay several known ATP-competitive tyrosine kinase inhibitors (pyrazolo-pyrimidines, cinnamomalonitrile derivaties, quinoxalines, quinazolines and anilino-quinazolines). Some pyrazolo-pyrimidines scored able to inhibit RET/PTC and RET/MEN 2 autophosphorylation in a dose dependent manner. ZD6474 (Zactima, vandetanib), an anilino-quinazoline, and BAY439006 (Sorafenib), an aryl-urea, also inhibited RET with an IC50 of roughly 100 nM. ZD6474 is a KDR and EGFR inhibitor that is already in advanced stage of clinical study for its antiangiogenic activity. BAY439006 (Sorafenib) is a multi-target kinase inhibitor approved for renal carcinoma able to affect besides RET also VEGFR, PDGFR and KIT kinases. ZD6474 had cytostatic effects in human thyroid carcinoma cells spontaneously harbouring oncogenic RET alleles and had significant activity in xenografted RET-mutation positive medullary thyroid carcinoma TT cells. X-ray

structure analysis confirmed that ZD6474 binds the ATP-pocket of the RET kinase.

One problem that could emerge with the use of these inhibitors is molecular resistance formation secondary to primary or secondary mutations in the RET kinase domain. Indeed, RET mutants in valine 804, corresponding to threonine 315 in Abl (the "gate-keeper": a residue important for resistance formation against imatinib), exert significant resistance against ZD6474.

Both ZD6474 and BAY439006 are currently undergoing phase II testing in patients with thyroid cancer. Clinical evaluation coupled with measurement of RET phosphorylation levels or RET-pathway activity under inhibitor treatment will be crucial to assess the capability of the compounds to hit the target in human patients and to establish the clinical value of RET inhibition for MTC and PTC treatment. In this context, the development of surrogate markers of RET inhibition will be of great help to assess the efficacy of the compounds.

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ABSTRACTS OF POSTERS

THE ECTOENZYME α-ENOLASE, A PANCREATIC ADENOCARCINOMA (PDAC)-ASSOCIATED ANTIGEN, ELICITS BOTH IN VITRO AND IN VIVO T CELL RESPONSES.

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To search for pancreatic adenocarcinoma (PDAC)-associated antigens recognisable by either CD4 and CD8 T cells, 2D electrophoretic protein maps derived from human PDAC cell lines were used as a bait and we screened the immunoreactivity of IgG contained in sera of 50 PDAC patients. We found that 63% of PDA patient sera contain IgG to α-enolase, an enzyme of the glycolitic cascade that also acts as plasminogen receptor. Conversely, 14% of healthy donor and 6% of non PDAC patient sera contained IgG that only weakly reacted to some α-enolase isoforms. Immunoprecipitation, confocal microscopy and immunohistochemisty experiments showed that α-enolase is expressed on the surface of PDAC cell lines and that its expression is markedly enhanced in PDAC biopsies. α-enolase-pulsed dendritic cells induced the proliferation, IFN-α secretion and lysis by autologous CD4 and CD8 T cells. Adoptively transferred human α-enolase specific T cells in nu/nu mice injected with PDAC cells, inhibited tumor cell growth. An anti-α-enolase memory T cell response had been detected in PDAC patients. Taken as a whole these data indicate that α-enolase induces an in vivo humoral and in vitro cellular response by both healthy and PDAC patients and elicits, if properly displayed, a specific T cell reactivity in vivo. These properties define α-enolase as PDAC-associated antigen exploiting in novel anti-tumor immunotherapy protocols.

DIFFERENT FUNCTIONAL ROLE OF BRAFV600E MUTATION IN NEVO-MELANOCYTIC LESIONS

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The purpose of this study was to investigate the presence of a gene expression signature in BRAFV600E nevo-melanocytic lesions compared to wild type ones, all derived from sun exposed sites.
Materials and methods: microdissected tissues from excisional biopsies of 14 acquired nevi and 15 cutaneous melanomas were analyzed to detect the presence of BRAF and NRAS mutations and to profile whole genome expression by means of oligonucleotide microarrays. Class comparison methods were used to select differentially expressed genes between wild type and mutated lesions. Real Time RT-PCR and immunohistochemistry were applied to validate differences at the mRNA and protein levels.
Results: BRAF mutations were evidenced in 64% of nevi and in 80% of melanomas. All of them consisted of the oncogenic change V600E and the mutation event was independent of Clark's level. No lesion was mutated on exons 1 and 2 of the NRAS gene.
Comparison of the expression profiles revealed no significant difference between BRAFV600E and wild type lesions when nevi and melanomas were considered together. On the other side, some differentially expressed genes were found as a function of BRAF mutation if melanomas or nevi were taken alone.
In mutated nevi there was a modulation of genes involved in cell cycle arrest, DNA repair and detoxification, in line with the idea of oncogene-driven senescence and that BRAFV600E mutation is an early hallmark of photoexposition-induced damaged DNA.
In mutated melanomas, transcription and the MAPK pathway were more activated and a number of genes involved in migration-invasion were more expressed than in wild type lesions, whereas DNA repair genes were downregulated. As far as genes associated to therapy sensitivity, BRAF wild type melanomas had a stronger expression of genes related to the immuno and inflammatory response and this suggests that immunotherapy, whose success strongly depends on the host environment, could be less effective in BRAF mutated lesions. On the other side, in such lesions a gene involved in melanoma drug resistance was strongly downregulated and this indicates that the presence of BRAFV600E substitution may be associated to a lower resistance to chemotherapy.
Conclusions: all the molecular functions affected except for the immune response, that is higher in all BRAF wild type samples, were found oppositely modulated in mutated nevi and melanomas.
Such results suggest a different functional role for BRAFV600E activating mutation in cutaneous melanomas and acquired nevi. This mutation seems to promote tumor progression at the melanoma stage, whereas, at the nevocytic level, it causes minor changes and represents a limiting factor to the nevo-melanoma transition.

ANALYSIS OF GENOMIC ALTERATIONS IN NON-SMALL-CELL-LUNG-CANCER SAMPLES BY THE MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) TECHNIQUE

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Non-small cell lung cancer (NSCLC) is a common malignancy and the major cause of cancer mortality in the world. Multiple chromosomal aberrations have been reported in NSCLC by standard cytogenetic or comparative genomic hybridization analysis. One of emerging methodologies to characterize the genomic changes in tumors is Multiplex Ligation-dependent Probe Amplifi-

cation (MLPA), a technique that allows rapid and precise quantification of multiple probes within a nucleic acid sample. In this study, we used MLPA to investigate the genomic changes occurring in a group of 40 NSCLC samples generally obtained at the time of surgery and before chemotherapy. Preliminary results indicate that tumors could be classified into 3 arbitrary groups, based on this technique: NSCLC samples with low (< 5 alterations on a total of 120 probes analyzed), intermediate (6-15 alterations), or high (> 15 alterations) genomic instability. In each case, patterns of amplification from tumor DNA were compared to those obtained from analysis of DNA derived from blood of the same patients or normal lung tissue; tumor cells were >80% in all samples analyzed, as evaluated by standard histologic analysis prior to DNA extraction. Moreover, we have also compared MLPA patterns from the primary tumor and metastatic lymph nodes in 2 samples. The genomic changes measured were highly heterogeneous, although amplification of 11q13 region - which has previously been reported in NSCLC - was found in 3 out of 8 samples analyzed. The MLPA analysis highlighted amplification of certain genes that could be involved in tumor progression, including AKT1, TERT, GNAS and FLJ20517. In the ongoing experiments we are performing MLPA analysis to the remaining samples and will investigate the impact of the degree of genomic instability detected at diagnosis by MLPA analysis on the clinical course of the disease.

β-ARRESTIN-1 AS MESSENGER OF ENDOTHELIN A RECEPTOR-DRIVEN β-CATENIN SIGNALING PATHWAY: IMPLICATION FOR AN EFFECTIVE TARGETED-THERAPY IN OVARIAN CANCER

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The endothelin A receptor (ET_AR)/endothelin-1 (ET-1) axis has a key role in ovarian carcinoma growth and progression. Among the different intracellular pathways activated by ET-1, the epidermal growth factor receptor (EGFR) transactivation represents a downstream signaling in driving ovarian cancer progression. Recently, it has become evident that β-arrestin promotes signal transduction by G-protein-coupled receptors acting as multifunctional adaptor protein. In this study, we examined the functional role of β-arrestin-1 in the cross-communication between ET_AR and EGFR to regulate cell-cell adhesion complex, β-catenin signaling and epithelial to mesenchymal transition (EMT) in ovarian cancer cells. We reported that, in HEY and OVCA 433 cells, ET-1 induced the translocation of β-arrestin 1 from the cytosol to the membrane fraction and the dephosphorylation on serine-412, leading to the formation of a membrane-associated ET_AR/β-arrestin-1/Src signaling complex ("signalplex"). By transfection with FLAG-tagged WT- or mutant S412D-β-arrestin 1, we demonstrated that this signalplex was crucial for the transactivation of EGFR and downstream signaling, such as β-catenin tyrosine phosphorylation, associated with a loss of β-catenin/E-cadherin interactions. As shown by cell fractionation technique and gene reporter assays, ET-1 induced signalplex-mediated β-catenin nuclear translocation and its transactivating ability for the TCF/LEF transcription factor. The functional consequence of these events is enhanced cell invasion, indicating that the ET_AR/β-arrestin-1/Src complex and subsequent EGFR transactivation may also account for the aggressive behavior of ovarian cancer cells. These effects were prevented by both ET_AR antagonists and ET_AR siRNA, thus validating ET_AR as the receptor involved. In human ovarian carcinoma xenografts, ET_AR

blockade by selective ET_AR antagonist, ZD4054, significantly inhibited tumor growth, peritoneal dissemination and expression of EMT effectors. The in vitro results provided the basis to test combined targeting of ET_AR by ZD4054, and of EGFR, by the EGFR inhibitor gefitinib (IRESSA). The coadministration of ZD4054 enhanced the efficacy of gefitinib leading to partial (82%) or complete tumor regression on HEY ovarian carcinoma xenografts, indicating new effective therapeutic opportunities for ovarian cancer patients. Supported by AIRC, Italian Ministry of Health, AstraZeneca.

ENDOTHELIN B RECEPTOR BLOCKADE IMPAIRS THE INTERPLAY BETWEEN ENDOTHELIN AXIS AND HYPOXIA IN MELANOMA CELLS

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Phenotypic and genotypic analyses of cutaneous melanoma have identified the endothelin B receptor (ETBR) as tumor progression marker, thus representing a potential therapeutic target. Hypoxia-inducible factor (HIF)-1α is the transcriptional factor that conveys signalings elicited by hypoxia and growth factors. We previously demonstrated that upon activation by endothelin (ET)-1, ETBR promotes melanoma progression through a mechanism that involves induction of HIF-1α under normoxic condition and to a greater extent under hypoxia. Here we demonstrated that melanoma cells expressed besides ETBR, also ET-1 and ET-3 that increased in response to hypoxic stimulus indicating, for the first time, the presence of an autocrine loop that could be amplified by the interplay between hypoxia and ET axis. Analysis of the mechanisms by which ETs induced HIF-1α activity showed that ET-1/-3 regulated HIF-1α accumulation and activity by increasing HIF-1α protein stability. In particular, ET-1/-3 decreased both mRNA and protein levels of a HIF-1α-associated prolyn hydroxylase 2 (PHD2), the critical oxygen sensor controlling the low steady-state levels of HIF-1α in normoxia. Concomitantly, ET-1 impaired HIF-1α degradation, as determined by the use of reporter protein containing the HIF-1α oxygen-dependent degradation domain encompassing the PHD-targeted prolines. These effects were blocked by the selective ETBR antagonist, BQ788, as well as by ETBR siRNA. These results demonstrate that in human melanoma cells, ET-1 and ET-3 act in an autocrine fashion through ETBR to control HIF-1α stability and activity and that the increased levels of HIF-1α, in turn, may sustain ET-1 and ET-3 expression. Moreover, in melanoma xenografts, ETBR antagonist suppressed tumor growth, neovascularization and invasion-related effectors, indicating that targeting ETBR related signaling cascade may represent a novel treatment of melanoma by impairing the positive feedback loop between ET axis and hypoxic melanoma microenvironment. Supported by AIRC, Italian Ministry of Health.

THE EFFECTS OF CHRONIC ADMINISTRATION OF ATORVASTATIN AGAINST AUTOCHTHONOUS MAMMARY CARCINOGENESIS.

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Statins are small-molecule inhibitors of 3-hydroxy-3-

methylglutaryl coenzyme (HMG-CoA) reductase (also known as HMGCR), which sits at the apex of a molecular pathway called the mevalonate cascade. As well as reducing cholesterol levels, statins can inhibit other products and reactions in the mevalonate pathway, including the generation of mevalonate and downstream isoprenoids. Several works in preclinical models of colorectal and breast cancer, and melanoma, indicates that statins have anti-cancer property. The anti-cancer property seems to be associated to statins with lipophylic structure such as atorvastatin.

Our previous data showed that electroporation of a plasmid coding for the extracellular (EC) and transmembrane (TM) domain of the rat HER-2 is able to trigger an immune response that protects BALB/c mice transgenic for the rHER-2 (BALB-neuT) from the progression of autochthonous mammary carcinomas. The protection rests on IFN-g producing CD4+ T cells and antibodies reacting to rHer-2. The induction of this protective response is remarkable as BALB-neuT mice display central and peripheral tolerance to rHer-2 as this transgene is expressed in the thymus and over-expressed it in the mammary gland starting from the 3rd week of age.

In this work we attended to study the effect of high doses of atorvastatin chronic administration alone and in combination with DNA vaccination in BALB-neuT mice. Atorvastatin alone, administered starting at 10 week of age, when in the mammary gland are present in situ carcinomas, is able to maintain tumor free all BALB-neuT mice until week 26, when all control mice already have palpable carcinomas. Surprisingly, atorvastatin combined with EC-TM vaccine reduced the capability of the DNA vaccine to confer protection in BALB-neuT mice. Indeed, in these mice antibodies induction is turned down by statin. These preliminary results indicate that atorvastatin could be used as a drug against small breast cancer lesions but it could have immunosuppressive effects.

MOTHER ANTIBODIES TO ERBB2 HAMPER GENETIC PREDESTINATION TO MAMMARY CARCINOGENESIS

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In BALB/c virgin females carrying in heterozygosis the activated rat ErbB2664V-E transgene (neu) under the mouse mammary tumor virus promoter (BALB-neuT mice), overexpression of the transgene and the onset of neu+ mammary carcinomas are already evident at 4 weeks of age. By week 33, an invasive carcinoma is palpable in all mammary glands and all mice die eventually because of tumor outgrowth. We have shown that high titers of anti-neu IgG2 antibodies elicited by intramuscular injection of DNA plasmids coding for the extracellular and transmembrane domains of rat neu protein (EC-TM plasmid) impair the progression of these autochthonous carcinomas. The efficacy of this vaccination increases in function of the earliness of its administration. Its repetition at 10-week intervals keeps these mice tumor-free for long periods, often close to their natural lifespan. To evaluate whether this devastating cancer progression is hampered in mice born from immunized mothers, wild-type BALB/c females were vaccinated twice with EC-TM plasmid and mated with BALB-neuT males. Cancer progression was significantly delayed in the neu+ offspring. However, this protection disappeared when they were lactated by untreated foster mothers, while lactation by an immunized mother was not enough to confer significant protection on neu+ offspring born from untreated mothers. The protective effect passed on by immunized mothers ceased when the mothers were KO for the µIg chain and unable to produce antibodies, or when the newborn mice were KO

for FcγRI/III. These data suggest that anti-neu antibodies delivered transplacentaly and by lactation elicit antibody-mediated-cytotoxicity by FcγRI/III+ effector cells in the newborn. The possibility to vaccinate a woman, without affect her fertility and pregnancy, and to protect her child genetically predisposed to develop mammary carcinomas should be a new way to delay tumor oncet.

THE EPIDERMAL GROWTH FACTOR RECEPTOR IN NON-SMALL-CELL LUNG CANCER XENOGRAFTS

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The epidermal growth factor receptor (EGFR) plays an important role in cell proliferation and differentiation. It is expressed in allmost all tissues and is overexpressed in many cancer types. In lung cancer the EGFR is overexpressed in 50-80% of the patients with squamous or adenocarcinomas. With the newly developed tyrosine kinase inhibitors (TKI) Gefitinib and Erlotinib as well as the monoclonal antibody Cetuximab new drugs are available for the treatment of patients with lung cancer. The evaluation of clinical trials using Erlotinib and Gefitinib revealed no correlation between the EGFR expression and the response to therapy. It was demonstrated that only a small group (women, never-smokers and people with asian origin) did benefit from the treatment with TKIs. In addition, patients with mutations in the exon 18- 21 of the EGFR gene showed a better response to a therapy with TKIs.
We have developed new lung cancer xenograft models. Fresh tumor material of patients with non small cell lung cancer (NSCLC) was subcutaneously transplanted in immunodeficient mice shortly after removal. Up to now 101 tumors had been transplanted from which 21 passagable models could be generated. It could be demonstrated that the murine passages coincide with the original tumor regarding histology, the expression of the surface proteins E-Cadherin, EpCAM, the cell proliferation marker Ki-67 and in gene profiling. The analysis of the EGFR gene revealed no mutations relating to a better response to TKIs. With the exception of two models all express a wild type EGFR. Two K-ras mutations were found in the xenografts and seven different mutations could be located in the p53 gene. Furthermore, the sensitivity of the xenografts was tested against five clinically used cytotoxic agents (Etoposid, Carboplatin, Gemcitabine, Taxol and Navelbine) and two EGFR inhibitors (Erlotinib and Cetuximab). It could be shown that there exist strong differences in responses among the xenografts.
In future the EGFR-Inhibitors will be tested in single or combination therapy in these models. The patient tumor will be analyzed by gene arrays and protein analysis in order to find genes or proteins correlating with the response of therapy in lung cancer. And the responses to therapy will be related to EGFR pathway associated molecules.

The Pezcoller Foundation
Journal
news from the Pezcoller Foundation world

2008
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 and the Pezcoller Foundation President. 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. Previously the Pezcoller Foundation, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration with the ESO-European School of Oncology.

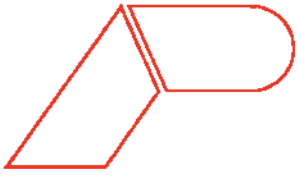
The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 25,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 60 other countries around the world.

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 12-16, 2008 in San Diego), and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy, after the AACR annual meeting (May 9, 2008). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: Friday, September 14, 2007

Questions about the nomination process – www.aacr.org - can be directed to awards@aacr.org



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