Wednesday 8 November 10:15–12:00

WORKSHOP 4

New targets in angiogenesis

14 INVITED

Vascular targeting

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One avenue towards the development of more selective anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of binding molecules (e.g., human antibodies) specific for tumor-associated markers.
The targeted delivery of therapeutic agents to newly-formed blood vessels ("vascular targeting") opens a broad palette of biomedical opportunities. Angiogenesis, i.e., the proliferation of new blood vessels from pre-existing ones, is an important process not only in cancer, but also in relevant diseases such as certain blinding ocular disorders and rheumatoid arthritis. The ability to selectively target and occlude neovasculature promises to be useful for the diagnosis and treatment of angiogenesis-related diseases.
In collaboration with Philogen SpA and with Luciano Zardi (Genova), my laboratory has developed human monoclonal antibodies, capable of selective targeting of neo-vascular structures in solid tumors and in a number of angiogenesis-related diseases. Three derivatives of these antibodies (i.e., two immunocytokines and a radiolabeled antibody) are currently being investigated in clinical trials.
The identification of novel vascular markers of pathology and the development of novel methodologies for the isolation of high-affinity small organic binding molecules are possibly the most pressing technological challenges for future developments in the field of vascular targeting. In the first research area, we have developed a novel chemical proteomic methodology based on the in vivo perfusion of tumor-bearing animals with reactive ester derivatives of biotin, followed by purification of the biotinylated vascular proteins in normal organs and tumors and by a comparative mass spectrometric analysis, for the identification of novel accessible markers of pathology. In the second research area, my laboratory has developed novel DNA-encoded chemical library technologies (e.g., encoded self-assembling chemical libraries), which allow the construction and screening of chemical libraries of unprecendented size.

References

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15 INVITED

New hypoxia targets

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Hypoxia is common in human cancer and via hypoxia inducible factor 1 induces many proangiogenic cytokines, including VEGF, PDGF, TGFB3, adrenomedullin, leptin, endothelin 1, tie2. Receptors on endothelial cells are upregulated such as transferrin receptor, VEGF receptor 1, VEGF receptor 2, CXCR4 and Hepatocyte growth factor receptor. These already provide many targets for therapy. We discovered a novel endothelial pathway regulated by hypoxia, the expression of Delta4 and endothelial specific notch ligand, in human tumour vasculature and shown it is upregulated in bladder, breast and renal cancer endothelium. In vitro work indicates the important role of Delta4 in maintaining many endothelial functions necessary for angiogenesis and we are now investigating approaches to inhibit notch signalling in vivo.
Copper has been known for many years to be essential for angiogenesis although the mechanism has been unclear. Copper uptake is stimulated by hypoxia. We have carried out gene array analysis of endothelial cells and found that copper chelation induces free radical stress and inhibits Superoxide Dismutase 1 function. RNAi for Superoxide Dismutase inhibition also had similar effects, indicating this may be a key pathway controlling angiogenesis. Lysyl oxidase is another copper dependent hypoxia inducible enzyme critical for angiogenesis. We have therfore carried out a phase I trial of ATN224, an orally available copper chelator and shown it has effects on circulating endothelial cells and produced stable disease for a number of patients, at the highest dose in a phase I trial. Phase II trials are planned. Although VEGF inhibition has now shown marked effects in at least four major tumour types improving survival, clearly there are other pathways that interact or may synergise and amongst these are the notch pathway above and copper chelation. Combined approaches will be investigated in preclinical models initially.

16 INVITED

New drugs in early clinical development

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Tumours must develop and sustain a vascular network to grow beyond a few millimetres. Vascular endothelial growth factor (VEGF) is recognised as a pivotal stimulus of tumour angiogenesis and vascular permeability, and inhibition of VEGF/VEGF receptor signalling is considered to be an attractive therapeutic strategy. Indeed, recent clinical studies using the anti-VEGF-A monoclonal antibody bevacizumab in combination with certain chemotherapies have demonstrated the clinical value of targeting the VEGF signalling axis. An alternative anti-angiogenic approach is represented by the development of small-molecule inhibitors of VEGF receptor tyrosine kinase activity. Examples of this novel class of agent include AZD2171 and vandetanib (ZACTIMA™; ZD6474). AZD2171 is a highly potent inhibitor of VEGF signalling and angiogenesis. Early clinical data show that AZD2171 has encouraging antitumour activity across a broad range of tumours with a side effect profile that appears to be predictable and manageable. Vandetanib has potential for potent and broad antitumour activity by selectively targeting VEGFR and EGFR signalling, two clinically validated pathways in non-small-cell lung cancer. Vandetanib has additional activity against RET kinase, which is important for the growth and survival of certain types of thyroid cancer. Both agents have pharmacokinetic properties that support once-daily oral dosing. Data from the ongoing clinical development programmes for each agent will be presented at the meeting.
ZACTIMA is a trademark of the AstraZeneca group of companies.

17 INVITED

Metronomic antiangiogenic chemotherapy: recent preclinical and clinical advances

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"Metronomic" (antiangiogenic) chemotherapy refers to the close regular administration of low (non-toxic) conventional chemotherapy drugs, in the absence of any prolonged drug-free break periods, over long periods of time, even several years [1]. Unlike "dose-dense" and intensive chemotherapy it is minimally toxic and thus does not usually require supportive care drugs [1]. The preclinical anti-tumor effects of certain metronomic chemotherapy regimens can be surprisingly effective, especially when used in combination with concurrent administration with a targeted biologic antiangiogenic agent. It is thought that the anti-tumor basis of metronomic chemotherapy is mainly via antiangiogenic mechanisms as a result of the local targeting of dividing endothelial cells in the growing tumor neovasculature, and also the systemic targeting of bone marrow derived circulating endothelial progenitor cells (CEPs) [1]. Maximum tolerated dose (MTD) chemotherapy may, in some circumstances, also target CEPs but a hemopoiesis-like pro-angiogenic acute CEP 'rebound' can occur immediately afterwards which nullifies this potential antiangiogenic effect. Shortening or eliminating the drug-free break periods compromises this robust repair process. This CEP rebound phenomenon may also help explain the ability of certain antiangiogenic drugs such as bevacizumab (Avastin®) to enhance the efficacy of some conventional chemotherapy regimens, i.e., by preventing the systemic CEP rebound [2].
Several phase II metronomic chemotherapy clinical trials, some randomized, have been completed, most using daily low dose (e.g. 50 mg) oral cyclophosphamide, in conjunction with a targeted biologic agent such as bevacizumab or letrozole [3] for treatment of either advanced or early stage breast cancer, or celecoxib for advanced non Hodgkins lymphoma, with encouraging results despite the obvious drawback of the empiricism associated with metronomic dosing. However, advances, both preclinically and clinically are being made in the discovery of surrogate markers to monitor biologic activity of metronomic chemotherapy and help determine the optimal biologic dose. These markers include circulating apoptotic endothelial cells [1] and CEPs [4].

References

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WORKSHOP 5

Chemoprevention and biomarkers

18 INVITED

Angiogenesis as a target for chemoprevention

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Angiogenesis is necessary for solid tumor growth and dissemination, a promising target not only in cancer therapy but also in prevention. We have shown that various molecules, such as flavonoids, antioxidants and retinoids, act in the tumor micro-environment inhibiting the recruitment and/or activation of endothelial cells and phagocytes of innate immunity. N-acetyl-cysteine, the green tea flavonoid epigallocatechin-3-gallate (EGCG), and Alpha lipoic acid (ALA) all prevent angiogenesis in the Matrigel sponge angiogenic assay in vivo and inhibit the growth of the highly angiogenic Kaposi's sarcoma tumor cells (KS-Imm) in nude mice. The synthetic retinoid 4-hydroxyfenretinide (4HPR) also showed anti-angiogenic effects. Taken together, these data indicate that angiogenesis is a common and key target of most chemopreventive molecules, where they most likely suppress the angiogenic switch in pre-malignant tumors, a concept we termed "Angioprevention". Functional genomics analyses of gene expression regulation by anti-angiogenic chemoprevention compounds in primary human umbilical endothelial cells (HUVEC) in culture through Affymetrix GeneChip arrays identified overlapping sets of genes regulated by the anti-oxidants. In contrast, the ROS-producing 4HPR induced members of the TGFβ-ligand superfamily, which, at least in part, explains its anti-angiogenic activity. NAC and the flavonoids all suppressed the IkB/NF-kB signalling pathway even in the presence of NF-kB stimulation by TNFα, and showed reduced expression of many NF-kB target genes. A selective apoptotic effect on transformed cells, but not on endothelial cells, of the anti-oxidants may be related to the reduced expression of the NF-kB dependent survival factors Bcl2 and Birc5/survivin, that are selectively over-expressed in transformed cells, by these factors. Inflammation is increasingly recognized as an angiogenic staimuilus in cancer, the repression of the NF-kB pathway suggests anti-inflammatory effects for the anti-oxidant compounds that may also have an indirect role in angiogenesis inhibition. For example, the green tea flavonoid EGCG inhibits inflammation-associated angiogenesis by targeting inflammatory cells, in particular neutrophils.

19 INVITED

"Integrative epidemiology" – from risk assessment to outcome prediction

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We propose an integrative epidemiologic approach to studying the entire cancer spectrum from exposure, to predisposition, early diagnosis and ending with cancer outcome, by applying the principles and perspective of epidemiology to the notable advances in molecular biology. This approach begins with epidemiologic case-control studies in which biologically intensive studies can be carried out usually on surrogate tissues (lymphocytes, plasma or serum). Common genetic variants that modulate behavior (eg CYP2A6) may also modify predisposition through altered metabolism of carcinogenic exposures. Tumor DNA can be isolated from serum or plasma, as a useful source for screening specific transcripts or mutations in mitochondrial or nuclear-DNA sequences. Identification of protein patterns in serum using high throughput proteomics linked to novel bioinformatics approaches can be useful for early detection. As we move along the continuum from surrogate to intermediate and target tissues, the procedures become more invasive, the size of the study more restricted, and we apply the case series approach. Pharmacogenetic profiles can be used to individualize therapy and to understand the functional consequences of chemoprevention, chemotherapy, or radiotherapy response. Again, common genetic variants may affect both risk and outcome, eg, matrix metalloproteases. At this level we can also correlate the genetic and epigenetic spectrum of changes in tumor tissue with epidemiologic data, and with surrogate tissue phenotype and genotype data. The converse direction also applies in that genes demonstrated to contribute to tumorigenesis provide a rich source of candidates for analysis related to exposure, to predisposition, early diagnosis. For example, polymorphisms in the cyclin D1 protooncogene, which is frequently elevated in tumors, are predictors of risk of development of lung cancer. This allows leveraging of the power of approaches arising from the completion of the human genome project that facilitate analysis of genetic changes in tumors on a global basis. This is a powerful new approach that cannot be successfully accomplished by any discipline independently. Integrating these new approaches requires a combination of the rigor of data and sample collection and validation inherent in epidemiologic research with the ability to perform global, unbiased analysis of genetic aberrations in tumors.

20 INVITED

The cancer preventive agent identification and early development program at the NCI

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Increasing knowledge of the molecular biology of cancer and improving methods of screening and early detection provide opportunities to prevent cancer initiation and to reverse or delay premalignant progression. To this end the Chemopreventive Agent Development Program of the Division of Cancer Prevention, U.S. National Cancer Institute has established a systematic research and development program to identify, develop, and qualify potential cancer preventive agents for clinical trials. The preclinical stages of the program encompass processes to identify potential agents and molecular targets, in vitro mechanistic and in vivo efficacy screening assays, in vivo intermediate endpoint and cancer incidence/multiplicity testing, and pharmacology and toxicology assessments. With chemical synthesis, manufacturing, and formulation data, also generated by the program, the preclinical in vitro and in vivo results are assembled into Investigational New Drug applications to the U.S. Food and Drug Administration for clinical studies. Clinical studies are initiated as single and repeat-dose phase 1 safety and pharmacokinetic studies and move forward to phase 2 biomarker modulation and efficacy studies. In this presentation, the processes of agent identification and development will be reviewed, particularly emphasizing newer computational approaches to agent identification using QSAR tools to mine chemical libraries for potential leads, toxicities, and molecular targets and to pharmacological evaluations using systems biology to study combinations of agents. Examples of agents currently in development will be presented to illustrate the challenges presented by complex botanical mixtures, biological peptide vaccines, single chemical entities, and combinations of agents. Experimental data illustrating the established methodologies for efficacy and toxicity assessment will be cited. Whether implemented as medical interventions in high risk subjects, dietary and supplement recommendations to population subsets, or changes in screening algorithms, the continued governmental commitment to cancer prevention promises to reduce significantly the economic and medical burden of cancer.

21 INVITED

Development of cancer chemopreventive agents post-coxibs

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The realisation that the long term use of the selective COX-2 inhibitors such as celecoxib can be associated with cardiac complications has led to the re-consideration of the risk-benefit assessment associated with their potential use in cancer chemoprevention. The coxib experience has engendered a subtle re-orientation of cancer chemoprevention drug development activities. There are indications according to which the adverse effects of coxibs are dose-dependent, so pre-clinical drug discovery and development efforts have been increasingly directed at the exploration of combinations of low dose coxibs with other drugs e.g. atorvastatin. An alternative development strategy is based on the expectation that diet-derived agents and age-old herbal remedies may have *a priori* a favorable safety record. Thus the characterisation of mechanisms of action and efficacy of naturally occurring agents with potential cancer chemopreventive properties has gained considerable momentum. Our group explores the chemopreventive efficacy, mechanisms, pharmacokinetics and pharmacodynamics of naturally occurring phytochemicals exemplified by silibinin from milk thistle, anthocyanins contained in fruits and berries and tricin from rice bran in the Apc^{Min+} mouse model of gastrointestinal carcinogenesis. All three agents reduced Apc^{Min+} mouse adenoma multiplicity. Measurement in clinical pilot studies of agent levels and biochemical changes germane to anticarcinogenesis in blood and tissues of humans who ingest these agents helps to ascertain whether the levels achieved in the preclinical