for the first 5 years following surgery ... but lose their prognostic power beyond year 5.
This observation calls for the need to reinforce the study of circulating tumor cells and disseminated tumors cells, which constitute a window into the metastatic process ... and a potential way of grasping the biology of putative breast cancer stem cells.
If the cancer stem-cell theory is confirmed, it will be important to identify, among CTC and DTC, which cells are capable of generating metastases. Genotyping and phenotyping of these cells should provide insight into the metastatic process and should lead to the discovery of new therapeutic targets.
The need for a revolution in the design and conduct of cancer clinical trials: As science is now catching up with clinical needs, a profound revolution needs to take place in the way clinical trials are being designed, conducted and financially supported. The trials should no longer be designed for the whole BC population, but indeed, should be tailored at relevant molecular subtypes. A much more intense cross-talk with basic scientists needs to occur – early on – with molecular hypothesis (for example of reduced or enhanced treatment benefit) being incorporated upfront and served by adequate statistical power.
Every possible effort at gathering patient and tumor material has to be implemented, given the parallel development of a variety of high-throughput genomic and proteomic platforms that should allow for a much more comprehensive picture of the biology of the tumor as well as the particularities of the host.
The ensuing costs of these "clinical-omic" trials will be substantial but this is the price today for moving from increasingly expensive empirical oncology treatments to tailored therapies that might be cost-saving in many instances.
The financial burden of these clinical-omic trials of the 21st century should be shared by governments, health insurance companies and pharmaceutical industry.

Monday, 24 September 2007

Symposium (Mon, 24 Sep, 10:45−12:45)
Angiogenesis and vascular targeting

3 INVITED

Signalling pathways as targets for therapy in angiogenesis and metastasis

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Cell motility, proteolysis and interactions with extracellular matrix (ECM) underpin angiogenesis and invasion, key determinants of tumour progression. These processes provide a rich source of molecular targets for cancer therapy as inhibitors may restrain both angiogenesis and metastasis with activities complementary to cytotoxic therapies. Oncogenic receptor tyrosine kinases (RTK) and angiogenic RTK such as VEGFR-2 on endothelial cells (EC) and VEGFR-3 on lymphatic endothelial cells (LEC) activate signaling cascades including phosphatidylinositide 3-kinase (PI3K), phospholipase C (PLC)g and mitogen activated protein kinases (MAPK). We are exploring the therapeutic potential of inhibitors of these pathways in in vitro functional assays and human tumour xenograft models. PI3K antagonists inhibited chemomigration and haptotaxis of a wide variety of tumour cells in vitro and downregulated specific matrix metalloproteinases and angiogenic cytokines. Novel inhibitors also showed activity in human tumour xenografts (including orthotopic, metastatic models) with clear downregulation of biomarkers of response. Tumours with activated PI3K pathways due to PTEN loss, upregulated RTK or P110a mutations were equally sensitive. The compounds also inhibited EC proliferation, migration, tubular differentiation in vitro and tumour angiogenesis in vivo indicating, as predicted, additional indirect therapeutic effects. Secondly, we showed that PLCg1 plays a major role in tumour cell and EC motogenic responses to both activated RTK and ß1 integrins. Validation of PLCg1 as a therapeutic target was obtained using stable and inducible RNAi vectors in vitro and in vivo in an orthotopic, metastatic prostate carcinoma xenograft model. We are now developing inhibitors of this potential new therapeutic target, and will aim to disable both PLCg and PI3k pathways since there is evidence of compensatory activation. Heat shock protein 90 (HSP90) chaperones key oncogenic proteins, and inhibitors can thus effectively and simultaneously disrupt several parallel signalling pathways. 17AAG downregulated client proteins in human tumour cells and EC and inhibited haptotaxis, chemomigration, invasion and uPA production. In vivo, EC client proteins (including all three VEGF receptors) were downregulated by 17AAG and inhibition of growth and metastasis of human tumour xenografts was associated with reduced microvessel density. Future work will identify optimal combinations of novel inhibitors for the prevention and treatment of disseminated disease.

4 INVITED

Clinical anti-angiogenesis

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Angiogenesis, the formation of new blood vessels, has been validated as a target in several phase III clinical trials in which conventional chemotherapy with or without inhibitors of VEGF has been compared. Studies in colorectal cancer, renal, breast and non-small cell lung cancer have demonstrated a survival advantage in favour of combination therapy. While, most of these results have been associated with the anti-VEGF antibody, bevacizumab, recent data in hepatocellular and renal carcinoma have demonstrated a survival advantage with oral VEGF receptor tyrosine kinase inhibitors, highlighting the potential of this class of molecule.
It is clear that VEGF is a valid target in oncology and that VEGF inhibitors have a vascular mode of action. However, this is a complex issue as it appears that anti-angiogenic drugs might have a direct effect on blood vessels as well as on circulating endothelial cells and their precursors. There is an additional confound in that VEGF inhibitors might also have an anti-tumour effect.
There remain significant questions about the optimum use of VEGF inhibitors. For instance: VEGF inhibitors are postulated to cause reductions in vascular permeability, normalization of the vasculature and reductions in interstitial pressure. These parameters are potentially important in terms of scheduling of combination therapy. On the other hand in the single agent, maintenance therapy of cancer it is not clear how long to continue therapy and in particular whether we should continue treatment beyond progressive disease. Indeed the mechanisms of escape from VEGF inhibitors are being defined now and this will be an important area for future research.
Emerging data have shown that combinations of VEGF and EGF inhibitors can induce significant anti-tumour response rates in heavily pre-treated patients, prompting the question of how active these non-cytoxic regimens will be in the first line setting. One problem with this approach is the cost of combination therapy and it will be critical to establish biomarkers that predict benefit or progression so that these drugs can be used optimally. Finally, as new classes of anti-angiogenic agents emerge we will need to focus on their mechanisms of action of the compounds to ensure that particular pathways are optimally inhibited.

5 INVITED

Role of haematopoietic cells in tumour angiogenesis: from discovery to targeted cancer gene therapy

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We recently described a population of monocytic cells characterized by the expression of the Tie2 receptor (Tie2-Expressing Monocytes, TEMs). These TEMs specifically home to tumours and promote tumour angiogenesis and growth. Remarkably, the selective elimination of TEMs by a Tie2-driven suicide gene completely prevented human glioma neovascularization in the mouse brain and induced substantial tumour regression (De Palma et al., Cancer Cell 2005). In this model, TEM elimination did not affect myelopoiesis, nor it prevented recruitment of other haematopoietic populations to the tumours, suggesting that TEMs represent a distinct lineage of proangiogenic monocytes. To substantiate this concept, we used cell sorting and real time PCR-based low-density arrays to compare the gene expression profile of TEMs with that of other tumour-infiltrating and tissue-derived myeloid cells. We found that although TEMs have typical features of tumour monocytes/macrophages, a significant fraction of the interrogated genes were differentially expressed in TEMs vs. tumour macrophages. Some of these genes have critical roles in angiogenesis, tissue remodelling and immunity, which suggests that TEMs may also create an immune-privileged environment that promotes tumour growth. Remarkably, we identified Tie2-expressing monocytes also in human peripheral blood and cancer, suggesting that these cells may have a role in human pathology, possibly representing a novel pharmacodynamic marker to monitor angiogenesis or new targets of anti-cancer therapies.
Given their marked tumour-specificity, TEMs might be used as selective gene delivery vehicles for the transport of gene therapy to tumours.

In fact, because Tie2 is only expressed by TEMs among the progeny of hematopoietic stem cells (HSCs), transplanting HSCs transduced by a lentiviral vector (LV) containing the Tie2 promoter would provide for selective transgene expression at the tumour site. We hypothesised that TEM-mediated delivery of IFN-α might achieve locally effective concentrations while minimising its toxic effects.
We transduced HSCs with LVs expressing the potent anti-angiogenic factor alpha-interferon (IFN) or the GFP gene under the control of either the Tie2 or the ubiquitously active PGK promoter, and transplanted the transduced cells into nude mice. All PGK-IFN mice died of graft failure, indicating that ubiquitous expression of IFN in the HSC progeny was severely myelotoxic. In contrast, Tie2-GFP and Tie2-IFN mice were reconstituted by the transduced HSCs and remained healthy until the end of the studies. In order to compare TEM-mediated delivery with systemic expression of IFN, a group of Tie2-GFP nude mice also received an intravascular injection of PGK-IFN LV, which led to sustained IFN expression in the plasma.
Six-eight weeks post-transplant, we either injected human glioma cells intracranially or mammary carcinoma cells subcutaneously (s.c.) in the transplanted mice and monitored tumour growth for 3−12 weeks. We observed significant inhibition of tumour growth in all tumour models tested. In nude mice challenged with intracranial human gliomas, the majority of Tie2-IFN mice were either tumour-free or had tumours barely detectable by magnetic resonance imaging or at necropsy. Tumours detected in Tie2-IFN mice had lower cell proliferation rate, increased apoptosis and greatly reduced vascular area as compared to those grown in GFP mice. In this xenograft model, we observed that the interferon response was specifically targeted to the tumour stroma. Surprisingly, sustained plasma levels of IFN not only failed to inhibit glioma growth, but also induced body wasting and severe myelotoxicity. In Tie2-IFN FVB (immunocompetent) mice challenged with syngeneic s.c. mammary tumours, we observed tumour rejection at 2−3 weeks post-injection. Interestingly, these tumours were extensively necrotic and massively infiltrated by T cells, which, together with a transcriptional profile of tumour-infiltrating hematopoietic cells, suggested an immune cell-mediated antitumour response.
In conclusion, targeted delivery of IFN by TEMs achieved substantial antitumour activity in the absence of systemic toxicity, while ubiquitous expression in BM-derived cells or sustained expression in the plasma were not efficacious and were highly toxic. Taken together, these results provide proof of principle of a new gene therapy paradigm in which ex vivo transduction of HSCs can be used to safely deliver potent anti-cancer molecules in a tumour-targeted fashion.

6 INVITED

Vascular targets – from concept to development

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Many strategies currently exist to target angiogenesis and/or vascular function in tumours. However, in order to use these new approaches optimally there is a need to understand how they will interact with conventional therapy. In this presentation we will show the importance of drug scheduling when combined with radiotherapy. The examples we will use are the PARP inhibitor TPI14361 and the VEGF receptor antagonists ZD6474 and ZD2171
AG361 is a potent inhibitor of the DNA repair enzyme PARP; however, this nicotinamide analogue can also alter endothelial cell function such that, in solid tumours, perfusion is improved and tumour oxygenation increased. This reflects itself in the tumours being substantially more responsive to treatment with radiation.
ZD6474 and ZD2171 inhibit VEGF receptor II. Following treatment with these agents tumour growth is slowed and this is accompanied by a decrease in vessel area/number in the tumours. This can result in a change in the level of tumour oxygenation which will reflect itself in a change in tumour radioresponsiveness. Hence, when combining these drugs with radiotherapy, how the two modalities are schedules could profoundly alter outcome of therapy.
The final part of the presentation ill focus on a novel antiangiogneic agent, opticin. This protein is a Class III member of the SLRP family of proteoglycans. It exists in the (avascular) vitreous tumour and has been shown to have marked inhibitory effects on endothelial cell proliferation, migration and tube/sprout formation when stimulated with a range of different pro-angiogenic growth factors. Further, opticin inhibits tumour cell proliferation in vitro and has a marked effect on the growth of experimental tumours in vivo.

Symposium (Mon, 24 Sep, 10:45−12:50)
Will the new European paediatric medicine regulation improve access to new and well-evaluated drugs for children with cancer?

7 INVITED

Angiogenesis as a target for paediatric malignancies

J. Rössler. *University of Freiburg, Division of Pediatric Hematology and Oncology, Freiburg, Germany*

Angiogenesis is a crucial process in tumor progression and metastization. The origin of neo-vessels within the expanding tumor tissue is considered to be the result of sprouting and co-option of neighbouring pre-existing vessels. More recently, it has been shown that mobilization and functional incorporation of other cells, including circulating endothelial cells and progenitor endothelial cells are also involved.
In pediatric oncology, accumulating data points towards the important role and impact of tumor vessels on the aggressive phenotype and on the mechanisms of proliferation as well as the pattern of metastization of solid tumors. Tumor endothelial cells and expression of angiogenic factors have been identified in several embryonic tumors. Therefore, the angiogenic growth factor VEGF and subsequent VEGF receptors represent interesting targets for therapy directed against the tumor vasculature.
After more than 30 years of pre-clinical research on tumor angiogenesis, the first anti-angiogenic drug - the anti-VEGF antibody bevacizumab – was approved by the FDA in 2003 and has demonstrated since preliminary benefits for adult cancer patients. Until today, however, clinical use of anti-angiogenic agents in children with cancer has been very limited. Initial data on phase I trials are available for bevacizumab, VEGFR tyrosine kinase inhibitors and metronomic, low dose combination chemotherapy. More importantly, differences in toxicity profiles in children compared to adults with special regard to the cardiovascular system and the developing organism must be worked out.
In accordance with the successful use of anti-angiogenic agents in combination with chemotherapy in adult patients, phase II and III studies in pediatric oncology are urgently wanted.

8 INVITED

KidsCancerKinome; Looking for new targets in paediatric cancers

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In this lecture I will present an update on the activities of the European KCK (KidsCancerKinome) consortium. Nine European research centers devoted to molecular-biologic, pharmacologic and clinical studies of childhood cancers and two SMEs are engaged in the KidsCancerKinome consortium. The research centers already have an established collaboration for pre-clinical evaluation of anti-cancer compounds in the European 'Innovative Therapies for Children with Cancer' consortium (ITCC).
The KidsCancerKinome consortium will make a comprehensive analysis of the human protein kinase family in childhood tumors, as protein kinases are excellent targets for small inhibitory molecules designed for adult tumors, and many more of such drugs are currently in development. Six aggressive childhood tumors, killing ~2000 children in Europe annually, will be addressed, i.e. medulloblastoma, osteosarcoma, Ewing sarcoma, neuroblastoma, rhabdomyosarcoma and ALL.
The KCK consortium has gene expression profiles (Affy U133plus2 arrays) of >500 tumor samples form those six tumortypes. We have performed extensive analyses of mRNa expression of human kinases. Preliminary data on expression patterns of the human kinome will be presented. Detailed analyses for the first 5 kinases for which targetted drugs are available, i.e. PI3K, IGF1R, mTOR, CDK2 and EGFR, will be presented.
Lentiviral shRNA mediated inactivation of these kinases in cell lines will be used to validate suitable kinases as drug targets. The first round of lentiviral RNAi knockdown is currently ongoing for the CDK2 gene.
Many novel kinase inhibitors are under development for adult oncology and KCK will test their in vitro activity against the tumor-driving kinases identified in this program. For those kinases that have no small molecule inhibitors, a novel generation of siRNA based nucleic acid drugs (LNAs), produced by the Santaris company, will be applied and tested in vitro.
Successful small molecule inhibitors and LNAs will be taken further to in vivo validation in established xenograft models of the six childhood tumor