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

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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