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

I. Stratford, K. Williams. *University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Ground Floor – Stopford Building, Manchester, United Kingdom*

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

types. Pharmacokinetic studies of these drugs will finally prepare them for evaluation in future clinical studies in childhood cancer patients.

9 INVITED

Targeted therapies for paediatric brain tumours

B. Geoerger. *Institut Gustave Roussy, Department of Pediatrics and "Pharmacology and new treatments in cancer", Villejuif, France*

Tumors of the central nervous system (CNS) as an entity represent the most common solid tumors in childhood. Despite an intensive multi-disciplinary treatment approach combining surgery, radiation therapy and chemotherapy (including high-dose regimens), 45% of children with CNS tumors still die of their disease. Moreover, current treatment protocols are at their limit of cumulative toxicities and induce significant sequelae. The recent development of targeting agents, particularly tyrosine kinase inhibitors and monoclonal antibodies, opens a new horizon for the treatment of children. Cell survival pathways through epithelial growth factor receptor (EGFR), platelet-derived growth factor (PDGFR) and insulin-like growth factor receptor 1 (IGF1-R) and downstream mediators are activated in childhood CNS tumors, although the molecular mechanism may be distinct to those found in adults. EGFR inhibition, by the small molecule tyrosine kinase inhibitors, gefitinib and erlotinib, as well as the anti-EGFR antibody nimotuzumab are currently being evaluated in children with malignant brain tumors, particularly malignant gliomas, alone or in combination with radiation therapy. Furthermore, the dual inhibition of EGFR and ErbB2 may be of advantage to reduce cell activation through their hetero-dimerization and a clinical phase II study of lapatinib is ongoing in children with relapsing brain tumors. The role of PDGFR tyrosine kinase inhibitors, such as imatinib mesylate, is not yet determined although it may be useful in PDGFR-driven tumors such as medulloblastoma and oligodendroglioma. Combination studies with irradiation or other chemotherapeutic agents will be more favored than single agent treatment. Pediatric malignancies are excellent targets for anti-angiogenic treatment and preliminary results of the first approved monoclonal anti-VEGF antibody bevacizumab and irinotecan in recurrent adult GBM are promising. Other targeted agents such as mTOR, hsp90 and PI3 kinase inhibitors as well as DNA repair modulators e.g. Poly(ADP-Ribose) polymerase (PARP)-1 inhibitors are currently under evaluation in preclinical or adult trials and may be interesting for combination treatments in children. Whether the inhibition of the Notch or SonicHedgehog signaling pathways through gamma-secretase and smoothened inhibitors, respectively, may lead to enhanced response and survival in children with primitive neuroectodermal tumors/medulloblastoma needs further evaluation, as the inhibition of cyclin D1 or multiple growth factor receptors present in the hSNF5/INI-1 deficient atypical teratoid/rhabdoid tumors, and therapies to disrupt CNS cancer stem cells. Advances in genetics and molecular biology led to the development of these targeted agents and will determine future new targets with a potential to change significantly the treatment of cancer. With a close dialog between basic researchers and clinicians, these new therapies promise the ability to improve treatment and survival in children with malignant brain tumors.

10 INVITED

Targeted therapies for acute leukaemias in children

P. Kearns. *The institute of Child Health, Birmingham, United Kingdom*

Stepwise optimisation of treatment protocols over the last 2 decades has led to major improvements in the survival in children with acute leukaemias, however, high risk sub-groups remain refractory to currently available therapies. Future progress may be achieved by focussing more targeted therapies at refractory high risk sub-groups and reducing the burden of therapy for those children cured with current intensive multi-agent treatment protocols. Recent developments in the understanding of the molecular basis of leukaemogenesis and the cellular processes maintaining the malignant phenotype has increased interest in the possibility of exquisitely targeted therapies. Several genetic aberrations have been identified which define poor risk sub-groups of ALL and AML which may be exploited as therapeutic targets, the most advanced examples being the BCR-ABL fusion protein and activating mutations of FLT3. Nevertheless, defining potential targets for therapeutic exploitation remains a considerable challenge with the additional complexity in leukaemia of the inherent heterogeneity of the disease. Both AML and ALL comprise a wide range of phenotypic and genotypic sub-types. Moreover, there is increasing evidence of sub-populations of leukaemic cells with properties of self-renewal, multi-potentiality and proliferative capacity, thought to constitute 'leukaemia stem cells'. To date, this has been best characterised for AML and chronic myeloid leukaemia. Unique molecular features are being defined in putative leukaemia stem cells, which may provide important novel approaches to treatment in the future. New targeted agents are being considered for potential application in the paediatric setting including several tyrosine kinase inhibitors; for example dasatinib and nilotinib in philadephia positive disease, CEP 701 and PKC 412 in FLT3 mutated disease and the antigen-directed immuno-conjugate gentuzumab ozogamicin in AML. In addition, an exciting range of new cellular targets is emerging, including within aberrantly activated signal transduction pathways implicated in the pathogenesis of acute leukaemias, for example the RAS mediated and PI3K-Akt / mTOR pathways. The successful translation of targets within these pathways into effective new treatment modalities is awaited. An overview of the potential application of novel therapeutic approaches in the management of paediatric acute leukaemias will be presented.

11 INVITED

Challenges in recruiting patients for early clinical trials

B. Morland. *Birmingham Children's Hospital, Department of oncology, Birmingham, United Kingdom*

There are many challenges facing clinicians recruiting patients into clinical trials. These challenges are compounded further when the patients being recruited are children. There are a number of separate issues to be considered. Firstly the regulatory environment in which patients are being treated. Many regulatory authorities permit the undertaking of Phase I clinical trials in children, but not all. European advice, until relatively recently did not specifically identify the importance of early clinical trlals in children for example. In many situations clinicians need to be able to demonstrate clear "benefit" to the patient being treated. With early clinical trials however this is a real challenge. The endpoint of a Phase I study, by definition is normally to reach an dose determined by toxicity, not efficacy. Indeed the concept of effectiveness as measured by conventional oncology endpoints, response etc are normally not seen in such studies.
The ethical question of conduct of Phase I trials in children often blurs into the regulatory requirements. The ethical challenges are in some senses common to all trials involving minors. The term "informed consent" is used by most clinicians involved in the process, but how many of us have stepped back from the process and asked how vaild the consent porcess is. Things are compounded further when parents act as a proxy for their child's consent, particulary in the challenging area of multiply relapsed patients where every "last ditch" effort needs to be explored by the parent. But is this in the best interest of the child? Here the clinician may have a personal dilemma acting as the advocate for the child, whilst wishing to increase recrutment into specific trials.
One area which is perhaps less understood is the whole area of cultural differences in acceptance of experimental therapies in childen. We are working in an increasingly international environment and it is invitable that some cultural differences in general approach to clinical trials are allowed for and accepted as part of the study design. Within the European consortium running early clinical trials, Innovative Therapies for Children with Cancer (ITCC) we are successfully conducting Phase I and early Phase II studies across 5 EU Member States. In so doing we have to deal with a variety of regulatory, ethical and cultural differences. We believe that well established networks such as ITCC can assist individual families and clinicians in participation in these challenging studies. In adition we believe we can be a source of shared experience and good practice for regulatory and ehtical committees.

Special session (Mon, 24 Sep, 13:30−14:30)
Increasing sensitivity of whole body imaging in oncology – a blessing or a curse?

12 INVITED

Increasing sensitivity of body imaging in oncology – a blessing or a curse? Expectations of the oncologist

I. Judson. *The Institute of Cancer Research, CR UK Centre for Cancer Therapeutics, Sutton, United Kingdom*

The so-called "Will Rogers phenomenon" is based on his quote "When the Okies left Oklahoma and moved to California, they raised the average intelligence level in both states". Stage migration occurs when more sensitive imaging techniques identify hitherto unsuspected disease, placing better prognosis patients in a worse stage category and improving the outcome in both stage groups without any change in treatment. We have to ask what such imaging improvements actually achieve in clinical management.

1. Is "stage" a useful concept outside clinical trials?
2. Is it valuable to identify more advanced stage disease in order to treat it more intensively?