17-DMAG (Hsp90 inhibitor), AZD2171 (VEGFR2 inhibitor), dasatinib (Src inhibitor), and ispinesib (kinesin spindle protein inhibitor).
To facilitate interactions between pharmaceutical sponsors and the PPTP, the PPTP utilizes a model material transfer agreement (MTAs) developed in collaboration with pharmaceutical sponsors and academic research centers. The model MTA has been accepted by all of the PPTP sites, which markedly expedites testing of agents through the PPTP. Additional information about the PPTP is available at http://ctep.cancer.gov/resources/child.html.
By facilitating development of a more reliable pediatric new agent prioritization process, the PPTP contributes to the goal of identifying more effective treatments for children with cancer.

240 INVITED

Cell cycle targeting in neuroblastoma

H.N. Caron, J. Molenaar, M. Ebus, R. Versteeg. *Academic Medical Center, Paediatric Oncology, Amsterdam, The Netherlands*

CCND1 (Cyclin D1) regulates G1 cell cycle progression by activating CDK4/6 kinase activity and thus controlling the phosphorylation of the retinoblastoma protein (pRb). Microarray analysis of 131 neuroblastoma tumors and cell lines revealed a high expression of CCND1 in the majority of tumors. We identified a low frequency of genetic abberations of the CCND1 gene in neuroblastoma. Immunohistochemical staining of tissue arrays with 183 neuroblastomas confirmed high expression of CCND1 in 75% of the tumors on a protein level.
We subsequently analyzed the aberrantly expressed CCND1-pRb pathway for possible therapeutic targets. We therefore inhibited several cell cycle genes using transient siRNA. Inhibition of CCND1 and CDK4 in neuroblastoma cell lines caused a G1 arrest followed by neuronal differentiation. Inhibition of CDK2 caused a G1 arrest followed by massive apoptosis. In addition, we transfected neuroblastoma cell lines with inducible siRNA constructs against CCND1, CDK4 and CDK2. siRNA-induction time courses of these cell lines were analyzed using MLPA and Affymetrix microarrays. We thereby identified the apoptotic pathway components activated by CDK2 silencing. Finally we evaluated the pro apototic effect of CDK2 silencing in neuroblastoma cell lines using five different small molecule CDK inhibitors. For several CDK2 specific inhibitors we could show a G1 arrest followed by the induction of apoptosis. Evaluation of CDK2 inhibiting small molecules in combination with established cytostatics and new drugs is now in progress in vitro and in vivo.

241 INVITED

Potential targets in Ewing tumours

O. Delattre. *Institut Curie, Pathologie Moleculaire des Cancers, Paris, France*

Ewing tumor is the second most frequent bone tumour in children and young adults. It is characterized by the fusion between EWS on chromosome 22 and one of five ETS members being FLI-1 most frequently or ERG, ETV1, E1AF and FEV. The role of EWS-ETS fusion in tumor development has been clearly documented through a variety of cell models. Indeed, ectopic expression of EWS-ETS can transform NIH3T3 cells or mesenchymal stem sells. The resulting tumors closely resemble Ewing tumors. Reciprocally, the silencing of EWS-ETS expression by RNA interference in Ewing cells leads to a cell cycle arrest and to apoptosis. This indicates that EWS-ETS is necessary for Ewing cell growth and that it may constitute by itself a valuable target for new therapeutic approaches. However, targeting the EWS-ETS fusion in vivo raises the yet unsolved concern of the delivery of specific siRNA to the tumor cells. Another approach may rely on the development of small molecules inhibitors of the EWS-ETS protein. Unfortunately, EWS-ETS is a transcription factor, a class of peptide that, at the difference of proteins with catalytic domains, cannot be easily inhibited by pharmacological agents. It has also to be kept in mind that to be efficient and to induce cell death, the inhibition of EWS-ETS has to be complete since a minimal activity of EWS-ETS, although insufficient to promote cell proliferation can prevent apoptosis. Other innovative therapeutic approaches may arise from the understanding of EWS-ETS function and particularly knowledge of the downstream pathways altered by EWS-ETS. This may lead the identification of critical proteins absolutely required for EWS-FLI to exert its oncogenic action and for which specific drugs may already exist or may be developed in the short term. Among others, one such pathway is IGF1 that is strongly activated in Ewing cells, in particular as a result of EWS-ETS action.

242 INVITED

Novel targets and targeted compounds in paediatric CNS tumours

C. Lau, X. Li, J. Su, Q. Shu, N. Ahmed, S. Gottschalk. *Texas Children's Hospital, Hematology – Oncology, Houston, TX, USA*

Despite advances in multi-modality therapy, the prognosis for malignant brain tumors in children remains poor. Even among the survivors, there are long-term neuro-cognitive sequelae of the disease compounded by the adverse effects of therapies on the developing brain and other organ systems. Thus there is an urgent need to develop more specific therapies that can optimize survival while minimizing the toxic effects on the normal brain tissue. In the last few years, molecular genetic studies and genomic screening have provided a better understanding of the biology of medulloblastoma and helped identify novel therapeutic targets. These include the recognition of the involvement of the sonic hedgehog (SHH) pathway in the pathogenesis and the association of the over-expression of ERRB2 with poorer prognosis in medulloblastoma. Pre-clinical studies using inhibitors of SHH pathway (e.g. cyclopamine and HhAntag691) and ERRB2 tyrosine kinase inhibitors (e.g. OSI-774) are now underway. More recently the role of the Notch signaling pathway in medulloblastoma was also described and interference of this pathway with gamma secretase inhibitors leads to depletion of cancer stem cells in medulloblastoma. In addition, induced differentiation is also a very attractive therapeutic strategy since normal brain tissue will theoretically be spared the deleterious effects of cytotoxic agents. Some of the differentiation inducers under investigation include retinoids, phenyl butyrate, and other histone deacetylase inhibitors such as valproic acid. Furthermore, recent evidence suggest that telomerase plays a significant role in the pathogenesis of medulloblastoma and primitive neuroectodermal tumor and that inhibition of telomerase function represents a novel experimental therapeutic strategy. Similarly, high-grade gliomas in children were found to have increased activation of various signal transduction pathways involving the receptors of epidermal growth factor (EGFR) and platelet-derived growth factor (PDFGR) as well as the RAS pathway. Inhibitors of these pathways, e.g. ZD1839 (Iressa) for EGFR, Gleevec for PDGFR and farnesyl-transferase inhibitors, are now under intense investigation. Finally, novel methods used to exploit these potential targets such as the development of cancer vaccine based on chimeric T-cell receptors against ERRB2 will also be discussed.

243 INVITED

Rational development of combination therapies for paediatric malignancies

P. Kearns. *Depts. of Clinical Sciences and Cellular and Molecular Medicine, University of Bristol, Bristol, UK*

Innovative Therapies for Children with Cancer (ITCC) is a European consortium comprising a network of 9 laboratories undertaking pre-clinical evaluation of targeted anti-cancer compounds in 6 high-risk paediatric tumour types and a network of over 30 European clinical centres, across 5 countries, collaborating in undertaking the early phase clinical trials.
Until recently, drug development has focussed primarily on adult cancers and the potential efficacy of novel agents in childhood malignancies was not considered. The rapid progress in understanding of the molecular basis of carcinogenesis and the cellular processes which maintain the malignant phenotype have led to the development of more exquisitely targeted therapies. The molecular targets identified may not always have relevance in paediatric malignancies. Conversely, many molecular targets of specific relevance to paediatric malignancies are lost in the translation to therapeutically useful targets. However, a considerable overlap exists between adult and paediatric oncology in target overexpression and activated signal transduction pathways. The principal aim of the ITCC Consortium is the development of novel therapies for paediatric malignancies through pre-clinical evaluation in models of specific relevance to childhood malignancies and well executed paediatric Phase I/II studies.
The primary focus of early clinical evaluation of novel compounds is as single agents, investigating their individual PK and PD parameters, alongside efficacy and toxicity profiles. However, the successful advancement of new compounds into clinical practice will be as combination therapies. Pre-clinical and clinical activities have been launched towards the evaluation of combinations of novel compounds.
An overview will be presented of the potential combinations within the field of paediatric oncology:
– Combination of conventional cytotoxics and "new" cytotoxics;
– Combination of conventional cytotoxics and targeted compounds;
– Combination of targeted compounds (or multi-targeted compounds).
The rationale of combining novel therapies will be discussed taking into account the current data of single drug activity of these compounds in paediatric tumours, the toxicity and the limitations of PK/PD analysis in young children.