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Challenges of drug development in pediatric oncology. A perspective from the pharmaceutical industry

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In the past, the pediatric evaluation of new drugs has been unsatisfactory. In pediatric oncology, an important challenge is the contrast between a very high unmet medical need concentrated in a limited number of patients, and a rapidly growing drug development pipeline. By the introduction of relevant business incentives coupled with the definition of specific regulatory obligations, FDA regulations have triggered an important increase in the number of new drugs tested clinically in children. A similar regulation is being implemented in the EU. We were interested to get a better understanding of the current practice of pediatric oncology drug trials.
Methods: Public clinical trials databases (ClinTrials.gov, NCI-PDQ) were searched on 28 June 2006 for phase 1–2 trials evaluating new anticancer drugs (terms "leukemia", "cancer", and "child"). When relevant, data were cross-checked with data from cooperative groups and drug companies websites.
Results: 71 trials were identified (45 phase 1, 26 phase 2). Sponsorship was delegated to government administrations or cooperative groups (n = 56, 79%), with 45 trials sponsored by the US-NCI (63%). The involvement of a drug company was clearly mentioned for 15 trials (21%). Trials involved the evaluation of 50 drugs (20 FDA-approved for adults, 30 not yet approved): tyrosine kinase inhibitors (32%), monoclonal antibodies or fusion proteins (20%) and cytotoxics (18%). 36 trials (51%) were with drugs not yet approved for adult use. 89% of trials were conducted in the US and 11% in EU. To estimate when pediatric trials are initiated, the time between the start of pediatric trials and the first FDA approval was analyzed. This interval could be calculated for 8 drugs and varied from 15 mo. before approval (imatinib) to 79 mo. after approval (thalidomide), with a trend for an earlier start for drugs approved after 2000.
Discussion: With the possibility of under-reporting in registries, the data indicate that a large number of new drugs are being tested in pediatric trials. Pediatric trials are started earlier with recent drugs, usually when safety and efficacy data are available from prior adult trials, and after adult phase II–III trials have been initiated. The sponsorship is commonly delegated by drug companies to cooperative groups or NCI.
Conclusions: In the context of current legislations, pediatric development needs to be systematically considered whenever appropriate. Criteria to better rationalize the decision to conduct pediatric trials (and when to start them) need to be developed, taking into account the degree of unmet medical need, the potential for development beyond phase 1 studies, and the need for relevant prior preclinical, safety and efficacy data.

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Strategies to transfer success in the development of molecularly targeted therapy to the challenge of paediatric cancers

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We have been successful in drugging the cancer genome as shown by trastuzumab, imatinib and others (Workman P. Drugging the cancer kinome: progress and challenges in developing personalized molecular cancer therapeutics. Cold Spring Harb Symp Quant Biol. 2005 70 499). The objective here is to review strategies and technologies that led to success with targeted molecular therapeutics against adult cancers and to discuss how these can be transferred to paediatric cancer. Many powerful technologies accelerated our ability to discover new targeted agents and develop them effectively. Building on decades of basic cancer research, advances in high-throughput (HT) genome sequencing and gene expression profiling have enhanced our the identification of new targets involved in malignant progression as well as diagnostic, prognostic, predictive and pharmacodynamic biomarkers which need to used to improve the decision-making throughout preclinical and clinical development. Development of minimally invasive methods based on PET and MRS/MRI is particularly important. Various HT screening methods provide leads for drug discovery. Optimization of these leads using structure-based design has been important. Optimizing pharmacokinetic properties alongside potency and selectivity is critical, aided by use of HT methods including cassette dosing. Two major strategies are recommended: (1) Identify druggable paediatric targets for development of specific agents using the above approaches; (2) Find paediatric cancers in which molecular therapeutics developed for adult cancers should have mechanism-based activity. We are taking both approaches, although the latter is restricted by the paucity of druggable targets and the latter by the lack of detailed knowledge of the precise signalling networks in paediatric cancers. The PI3 kinaseAKTPTEN-mTOR pathway is frequently deregulated in adult cancers and seems likely to be so in childhood cancers, for example paediatric glioma. PI3 kinase inhibitors such as PI103 show promising activity in adult glioma models (Workman P et al. Drugging the PI3 kinome. Nature Biotechnol 2006 24 794). We are evaluating these in the paedatric cancer models. HSP90 molecular inhibitors cause combinatorial depletion of multiple oncogenic client proteins (Sharp S and Workman P. Inhibitors of the HSP90 molecular chaperone: current status. Adv Cancer Res. 2006 95 323) and these are also under evaluation in paediatric cancers in the lab and in the clinic.

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Preclinical strategy for selecting novel compounds for pediatric malignancies: innovative therapies for children with cancer

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Introduction and Methods: Innovative Therapies for Children with Cancer (ITCC) is a European consortium of $\pm$30 pediatric oncology centres (ITCC Clinical) in 5 countries for early clinical trials and 9 labs (ITCC Biology) for the preclinical evaluation of novel (targeted) compounds for their possible use in 6 high risk pediatric tumors: neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, ALL, medulloblastoma, osteosarcoma. Expertise in gene (Affymetrix microarrays and RQ-PCR) and protein expression and in vitro/vivo models are present for all tumors using cell lines (CL) and tumor samples (TS).
The preclinical evaluation strategy consists of: 1. Target presence screening (mRNA profiles and tissue micro arrays of $\pm$ 100 TS per tumor type; mRNA profiles of CL) 2. In vitro drug efficacy screening on CL (MTT) 3. In vivo drug efficacy screening using mouse xenografts 4. Target validation by siRNA
By using Affymetrix microarrays and RQ-PCR we screened 370 TS and 82 CL for mRNA expression of the following targets: EGFR, Erb-B2, c-Kit, PDGFRα, PDGFRβ, VEGFR1, VEGFR2, IGF1R, FLT-3, Cyclin D, RAF kinase, PI3 kinase.
Results: EGFR expression was moderate to high in the majority of solid tumors, and negative in ALL. PDGFR-β has a moderate to high expression in neuroblastoma, whereas c-Kit is especially expressed in Ewing sarcoma and to a lesser extent in osteosarcoma. IGF1R expression had the lowest expression in Ewing sarcoma, and highest expression in neuroblastoma. mTOR was expressed in all tumor types. Flt-3 expression was restricted to ALL. An overview of other target expression will be presented, as well as in vitro efficacy data of several compounds.
Conclusion: The ITCC preclinical strategy for testing novel compounds is a stepwise approach of target analysis and validation, using well characterized vitro/vivo models of 6 pediatric tumor types. The Affymetrix profiles of the first 370 TS and 82 CL give a first indication of target presence, which is relevant for the selection of novel compounds for further preclinical testing. ITCC has a substantial capacity and expertise for evaluating new drugs in the various pediatric tumor models and has started collaborations with several pharmaceutical companies.

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Testing program approaches to evaluating new agents – The Pediatric Preclinical Testing Program (PPTP)

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The NCI-supported Pediatric Preclinical Testing Program (PPTP) is a comprehensive program for systematically evaluating new agents against childhood solid tumor and leukemia models. The primary goal of the PPTP is to identify new agents that have the potential for significant activity when clinically evaluated against selected childhood cancers.
The PPTP is supported through an NCI research contract to St. Jude Children's Research Hospital (SJCRH) with Dr. Peter Houghton as the Principal Investigator. The PPTP has established panels of childhood cancer xenografts and cell lines to use for in vivo and in vitro testing. These include panels for Wilms tumor, sarcomas (rhabdomyosarcoma, Ewing sarcoma, and osteosarcoma), neuroblastoma, brain tumors (glioblastoma, ependymoma, and medulloblastoma), rhabdoid tumors (CNS and renal), and acute lymphoblastic leukemia (ALL). The in vivo panels include 51 solid tumor models and 10 ALL models that suitably replicate the gene expression profiles of their respective clinical cancers.
The PPTP systematically tests 10–12 agents or combinations of agents annually against its in vitro and in vivo preclinical models. Pharmacokinetic studies are performed as necessary to determine the systemic drug exposures associated with antitumor activity, which allows comparison between the drug exposures required for activity in the childhood cancer preclinical models and those achievable in humans. When appropriate for molecularly targeted agents, the degree of target modulation associated with antitumor activity is evaluated.
Agents tested by the PPTP to date have included standard agents, as well as molecularly targeted agents such as bortezomib (proteasome inhibitor),

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.

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Cell cycle targeting in neuroblastoma

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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.

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Potential targets in Ewing tumours

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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.

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Novel targets and targeted compounds in paediatric CNS tumours

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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.

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Rational development of combination therapies for paediatric malignancies

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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.