received chemotherapy as primary treatment to postpone irradiation and to minimize its suspected deleterious effects on the growing CNS. Children 5 years and older received EFRT. Upon progression the corresponding treatment modality was applied in a crossover design. Brachytherapy (BT) was used in selected cases regardless of age. The Kaplan–Meier method was used to estimate overall survival (OS) and progression-free survival (PFS). PFS estimates were compared by means of the log-rank test.
Results: During a median follow-up period of 4.3 years (range 0−9.7 years), 59 patients (31%) experienced progression or relapse and 12 patients (6%) died. The 5-years PFS/OS was 60%/92% after EFRT and 69%/94% after BT.
Children with pilocytic astrocytoma achieved a PFS of 69% at 5 years. In contrast, the PFS rate of children with non-pilocytic histology was only 43% ($P < 0.05$).
Neither of the potential risk factors, such as tumour location, prior chemotherapy and age, nor the administered irradiation technique (BT versus EFRT) had a significant impact on PFS.
Escalation of the total dose of EFRT above 45 or 50.4 Gy did not result in an improved PFS.
Conclusions: EFRT plays an important role in the treatment of childhood LGG. In selected cases BT is comparably effective.
Currently the recommended dose prescriptions for EFRT range between 45 and 54 Gy. According to our data a reduction of the total dose below 50.4 or even 45 Gy seems to be feasible without compromising progression-free survival. However the optimal total dose of EFRT still needs to be defined in a prospective trial.
Non-pilocytic histology seems to worsen prognosis.

4101 ORAL

Multicentre Prospective Classification of Childhood Brain Tumours Using Magnetic Resonance Spectroscopy

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Introduction: Magnetic Resonance Spectroscopy (MRS) provides non-invasive metabolite profiles which can be used to aid diagnosis and provide prognostic markers. Previous studies of MRS for classifying childhood brain tumours have been limited by small numbers of cases and retrospective, single-centre design. The aim of this study was perform a large prospective multicentre evaluation of MRS as a tool for grading childhood brain tumours.
Method: A tool for classifying tumours into low grade vs high grade was built using single-voxel MRS (PRESS, TE/TR 30/1500 ms) acquired using two1.5 T scanners in a single centre (Centre 1) over a 5 year period up to May 2008. A total of 123 cases were accrued with grading confirmed by histopathology (N = 97) or by radiological review with no biopsy (N = 26). Of these, 81 were diagnosed as low grade (LG; WHO grade I or II) and 42 as high grade (HG; WHO grade III or IV). The MRS grading tool was constructed by processing MRS data using TARQUIN to determine metabolite concentrations, this method can account for differences in MRS data acquisition protocols that are difficult to avoid in multicentre studies; then classifier training was performed using principal components analysis followed by linear discriminant analysis. The MRS grading tool was then tested in a prospective manner on data acquired on 6 different scanners in 4 centres. The test dataset consisted of 55 cases from Centre 1 acquired between June 2008 and September 2010, and 55 cases from Centres 2−4, of which 10 were acquired on a 3 T scanner.
Results: The prospective testing gave an overall accuracy of 86%. The classification accuracy of cases from centres 2−4 was lower (80%) than that of cases from centre 1 (92%). Some cases which had an MRS classification of high grade which were low grade on histopathology behaved in an aggressive manner and responded poorly to treatment.
Conclusions: High classification accuracy for tumour grade has been shown in a prospective multi-centre evaluation of a childhood brain tumour classifier based on multivariate analysis of metabolite profiles derived from MRS. Where there is a disagreement between grade given by MRS and histopathology, MRS may aid tumour classification.

4102 ORAL

A Chemical Genetics Screen Identifies a Novel Drug That Targets Steroid Biogenesis and Receptor Signaling Leading to Growth Inhibition of Pediatric Malignant Astrocytoma Cell Lines

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Background: Brain tumours are among the leading cause of cancer-related deaths in children, with least 60% manifested as astrocytomas. Malignant astrocytomas represent 8−12% of all pediatric supratentorial brain tumours, with an overall median survival of 11−14 months. While those that arise in the brainstem represent an additional 10−20%, with a 10-year overall median survival of <10%. Despite current therapies, challenges still exist in the treatment of pediatric malignant astrocytomas, leading to the need to explore new therapies. Since a wide range of genes involved in steroid biogenesis and signaling are expressed in pediatric malignant astrocytomas, our objective was to investigate whether novel classes of drugs that target these gene products can be effective in inhibiting growth.
Methods and Results: We screened using a candidate chemical structure approach, a library of 400 drugs which can potentially inhibit steroid biogenesis and cell signaling. By using a panel of human pediatric malignant glioma cell lines established from surgical specimens, we discovered a potent drug that inhibits androsterone (male sex pheromone) biogenesis and with the ability to significantly reduce the viability of pediatric malignant astrocytomas in a dose dependent manner. Cells treated with this drug responded by undergoing apoptosis, cell cycle regulatory, and invasive changes. Furthermore, significant inhibition of transformation was noted. Cells also become increasingly radiosensitive upon drug treatment. Most remarkable, the toxicity on human astrocytes (control) was minimal.
Conclusion: We have discovered a novel drug from a chemical genetic screen which can significantly inhibit the growth of pediatric malignant astrocytomas, with minimal toxicity on non-transformed human astrocytes.

4103 ORAL

Doxorubicin Can Be Safely Omitted From the Treatment of Stage II/III, Intermediate Risk Histology Wilms Tumour − Results of the SIOP WT 2001 Randomised Trial, on Behalf of the SIOP Renal Tumours Study Group

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Aims: The SIOP WT 2001 trial aimed to test whether doxorubicin (D) can be safely omitted from chemotherapy for stage II/III, intermediate risk histology Wilms tumour (WT), in the setting of exclusion of a newly defined high risk subgroup (blastemal-type) from the randomisation.
Methods: International multicentre trial (28 countries, 261 centres) registering all children diagnosed with a primary renal tumour. Those aged 6m-18 yrs with localized tumours were treated with 4 weeks pre-operative chemotherapy with vincristine (V) and actinomycin D (A). Tumour stage and histological risk group were assigned after delayed nephrectomy. StageII/III intermediate risk WTs were randomized between 26 weeks AV or AVD (total Doxorubicin 250 mg/m^2). Stage III tumours received 14.4 Gy flank irradiation.
Statistics: A non-inferiority limit of up to 10% decrease in 2 yr EFS was considered acceptable. Probability of wrongly accepting non-equivalence was set at alpha 0.025, power 0.90 with recruitment target 550 randomised patients. Randomisation was stratified by participating group and tumour stage.
Results: 583 patients were randomized between 11/2001−12/2009, with 341 stage II and 242 stage III. Median follow up was 39 months. 94% (512/543) were confirmed as eligible by central pathology review. In intention to treat analysis, there were 22 events (20 relapses)/9 deaths among 291 randomised to AVD and 34 events (27 relapses)/7 deaths

among 292 randomised to AV, with 2 yr EFS of 92% (95% CIs:89−96%) and 89% (95% CIs:85−93%) (logrank p = 0.06) and 5 yr overall survival of 96% (95% CIs:94−99) and 96% (95% CIs:93−99) (logrank p = 0.61), respectively. The Hazard ratio for any event by 5 yrs in the experimental AV arm compared to standard AVD chemotherapy was 1.67 (95% CIs:0.98–2.85, stratified logrank p = 0.058). Analysis confined to eligible patients or by treatment received did not materially affect the results.
Conclusions: By using stage and histology after pre-op chemotherapy for risk stratification, doxorubicin can be omitted from treatment of stage II/III intermediate risk WTs.

4104 ORAL

Development of a Molecular Classification of Retinoblastoma

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Background: Although survival for retinoblastoma (RB) patients is excellent (>90%), invasion into the optic nerve or choroid is relatively frequent, increasing the potential for extra-ocular metastasis. Little is known about the molecular events which influence tumour behaviour and there are currently no molecular markers which could be used to predict prognosis. The purpose of our research is to develop a clinically relevant molecular classification of retinoblastoma and to translate this into 1H-MRS (1H-magnetic resonance spectroscopy) detectable markers which could be used for the non-invasive diagnostic assessment of retinoblastoma tumours.
Methods: Gene expression profiling (Affymetrix HuGene 1.0ST arrays) was carried out on 21 RBs. Principal component analysis (PCA) of the expression data was used for unbiased classification of molecular subgroups. Differentially expressed genes in each subgroup were identified using SAM (significance analysis of microarrays). Histopathology data from the same tumours was used to assess the clinical relevance of the molecular classfication. *In vitro* proton magnetic resonance spectroscopy (1H-MRS) was carried out on a subset of RBs to identify metabolite spectra specific for molecular subgroups.
Results and Conclusions: PCA showed a clear separation of RBs into 3 distinct subgroups. Genes contributing to this classification included many associated with retinal, and particularly photoreceptor (rod/cone) development and function. Group 1 RBs (N = 12) showed down-regulation of photoreceptor gene expression. In contrast group 2 RBs (N = 7) were characterized by elevated expression of genes associated with cone differentiation and group 3 RBs (N = 2) expressed markers of rod, cone and Müller glial cells (all of which are derived from a common retinal progenitor cell). Significantly, 67% of group 1 RBs showed extensive optic nerve and/or choroid invasion, compared with only 22% of group 2 and 3 RBs, suggesting that loss of photoreceptor differentiation may be associated with more aggressive tumour behaviour. Interestingly, recurrent chromosomal alterations characteristic of RB (1q gain, 6p gain, 16q loss) were almost entirely restricted to group 1 RBs, indicating that genes on these chromosomes may function in differentiation-related pathways and/or in the regulation of cell cycle exit. Preliminary 1H-MRS results identified several metabolites (e.g. glutamate/glutamine, taurine) which may have clinical potential as markers of RB subgroups.

4105 ORAL

Isolation of Neuroblastoma Cells as a Substrate for Pharmacodynamic Biomarker Assays to Accompany Early Clinical Trials of Neuroblastoma

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Background: Pharmacodynamic (PD) biomarkers provide proof-of-principle of target modulation and evaluate downstream biological effects of novel targeted therapeutics. Repeat tumour biopsies in children are problematic, complicating the implementation of these assays into paediatric trials. Neuroblastoma (NB) is a high-risk childhood cancer in which bone marrow (BM) metastases are frequent. Our aim is to obviate the need for biopsies by developing a methodology to obtain pure or highly enriched bone marrow-derived tumour cells as a substrate for assays.
Material and Methods: Peripheral blood (PB) and BM samples were spiked with cells from the NB Kelly cell line. MACS MicroBeads Technology was used for the cell separation: Purity and recovery of positive selection for GD2+ neuroblastoma antigen and negative selection of CD45+ cells were compared using flow cytometry.
To determine the suitability of the samples (1) total protein concentration (bicinchoninic acid assay), and (2) changes in total and phosphoprotein signals of the PI3K pathway (MesoScale Discovery) before and after the separation were compared.
Results: CD45 negative selection achieved a median 3.6-fold (range 2.0−6.3), 2.5-fold (2.0–11.0) and 6.1-fold (2.8−9.3) enrichment of NB cells in spiked PB, spiked BM and clinically involved BM samples respectively. Cell recovery with CD45 negative selection was superior to GD2 positive selection (73%±25 vs. 21%±20 cells recovered, p < 0.001).
Cellular losses were manageable permitting the realisation of protein-based assays. Each sample was lysed to a final volume of 100 μL. In these lysates, total protein concentration was 10.4 mg/mL±3.0 for samples pre- vs. 5.7±3.2 post-immunomagnetic separation (p = 0.10). Median sample volume required for our PI3K protein analyses was 13.9 μL (range 8.6–50.2).
PI3K assay ranges were tested in spiked cells. There was a moderate decrease in the total protein signals (-0.24 log difference, p = 0.35) and increase in the phospho protein signals (+0.14 log difference, p = 0.51) after the separation.
Conclusions: Immunomagnetic separation was able to obtain samples with high purity in neuroblastoma cells in spiked and clinical samples. The number of cells recovered was sufficient for protein analyses. The procedure had a moderate impact in the total and phospho-protein signals for the PI3K pathway but signals were detectable and consistent. Neuroblastoma cells isolated from BM could be a source of tissue for PD assays in future clinical trials.

4106 ORAL

Polymorphisms in Methotrexate Transport Pathway − a New Tool for Toxicity Prevention in Pediatric Acute Lymphoblastic Leukemia

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, accounting for 30% of all pediatric malignancies. Remarkable progress has been made in the treatment of acute lymphoblastic leukemia (ALL): four decades ago, the cure rate was less than 10%, today it is nearly 80%. An important component of ALL therapy is methotrexate (MTX). Treatment with MTX often causes toxicity, dose reduction or cessation of treatment being necessary. Interindividual differences in adverse reactions can be due to different factors, including polymorphisms in key genes. In the last years, several studies have investigated the relationship between genetic variation and MTX-related toxicity.
Recently, in our group, considering MTX clearance as an objectively quantifiable toxicity criterion, we have confirmed the association between SLCO1B1 rs11045879 polymorphism and toxicity, previously proposed by Treviño and collaborators. As SLCO1B1 is a hepatic transporter involved in MTX elimination, polymorphisms in transporter genes from the same pathway could also have a role in MTX toxicity.
As a result, in the present study, we have evaluated polymorphisms in 12 genes of MTX transport as toxicity predictors in pediatric B-ALL, all of them homogeneously treated according to the standardized LAL/SHOP protocol.
Material and Methods: DNA was extracted from blood samples of 150 paediatric ALL patients treated with the LAL/SHOP protocol by standard phenol-chloroform method. We genotyped 384 SNPs in 12 transporter genes (SLCO1B1, SLCO1B3, SLCO1A2, ABCB1, ABCG2, ABCC1, ABCC2, ABCC3, ABCC4, SLC19A1, SLC22A6 and SLC22A8) with Illumina Golden Gate platform and we analyzed their correlation with MTX toxicity.
Results: We confirmed that polymorphisms in transporter genes are associated with MTX clearance.
Conclusions: Our results suggest that polymorphisms in genes involved in MTX transport could be new toxicity markers in pediatric ALL.
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