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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