MEC-2 cells carried a p53 deletion which corresponds to CLL patients with the worst prognostics. Inhibition of PI3K was assessed by detection of phosphorylated Akt (S473) by FACS analysis. Induction of apoptosis was determined by AnnexinV/propidium iodine staining and detection of caspase-3 cleavage.
Results: The IC_{50}s (concentration of BKM120 resulting in 50% cell death) are below the obtainable plasma concentration in 60% of the samples and slightly greater than 5μM in 16% of the samples. BKM120 IC_{50}s negatively correlate with somatic mutations in the immunoglobulin variable region genes (IgVH) in our set of patients. Moreover, treatment with BKM120 results in decrease phosphorylated Akt (Ser473) and increase apoptosis in the B-CLL samples tested.
Conclusions: Despite the development of new treatments, CLL remains an incurable disease encouraging development of new strategies targeting signal transduction pathways essential to CLL lymphocytes survival such as the PI3K/Akt pathway. In view of the critical role of PI3K in CLL homeostasis, the activity of BKM120 suggests that this drug will have activity by itself in this disease.

9237 POSTER

Analysis of the Glucocorticoid Resistance Mechanism in Children With Acute Lymphoblastic Leukemia Using DNA Microarrays

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Background: The main aim of this study was to analyse glucocorticoid receptor polymorphism and evaluation of the prognostic and predictive significance of this polymorphism in patients with childhood acute lymphoblastic leukemia (ALL). DNA microarray technology is able to determine the gene expression profile of a whole genome which is currently approximately 23 thousand genes. These methods also can determine expressed gene variation, gene polymorphism and chromosome changes.
Material and Methods: Gene polymorphism was analysed in 6 pediatric patients with ALL (3 good prednisolone responders, 3 bad prednisolone responders). The RNA and DNA were isolated using the phenol-chlorophorm method from bone marrow samples before treatment (day 0) and after the prednisolone monotherapy (day 8). The in vitro chemoresistance test (MTT) using prednisolone (PRED), dexamethasone (DEX) and combination of PRED/DEX were done for each patient. The DNA microarray analysis was performed using the GeneChip Human Gene 1.0 ST Array and Cytogenetics 2.7M Array (Affymetrix). The statistics were analysed using the R and Bioconductor pack.
Results: The pilot data of the project will be presented. The DNA microarray analysis was performed on 24 samples from the 6 patients with ALL. In total, 137 genes were differentially expressed ($p < 0.001$ or $\log FC < -2$ or $\log FC > 2$) in good and poor prednisolone responders. We anticipate benefits and provide a perspective on DNA microarray methods and their impact on individualized therapy in children with ALL.
Conclusion: DNA microarray methods prove able to identify gene expression profiles which relate to patient chemosensitivity/chemoresistance. Owing to correlations with the MTT glucocorticoid test we can eliminate interindividual variability in metabolism, pharmacokinetics, pharmacodynamics and genetics. Glucocorticoid chemoresistance prediction can individualize corticoid therapy in children with ALL, improve therapeutic protocols and reduce treatment side effects.
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9238 POSTER

Allogeneic Stem Cell Transplantation Induces Autoantibodies Against Cancer Testis Antigens in Multiple Myeloma Patients

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Background: Allogeneic stem cell transplantation (alloSCT) is thought to induce immunological graft-versus-myeloma (GvM) effects in multiple myeloma (MM), however, the specific tumour targets recognized by the anti-myeloma immune responses are unknown. Cancer-testis antigens (CTA) are characterized by their tumour-specific expression and high immunogenicity and are very frequently found in MM. Unfortunately, little is known about immune responses against CTA such as NY-ESO-1 and SSX-2 in MM and it is unclear how such immune responses behave over time.
Methods: We performed the first comparative, longitudinal and functional study of spontaneous NY-ESO-1- and SSX-2-specific antibody responses analyzing 1094 peripheral blood and 200 bone marrow (BM) plasma samples from 194 MM patients.
Results: Of all MM patients, 2.6% and 3.1% evidenced antibody responses against NY-ESO-1 and SSX-2, respectively. Importantly, equally strong CTA-specific antibodies were detectable in the BM of the seropositive patients indicating the presence of humoral immunity in the immediate tumour environment. We found the NY-ESO-1 specific antibodies to target a number of different epitopes while all SSX-2-specific antibody responses were restricted to a single epitope covering amino acids 81−90 of the whole protein sequence. NY-ESO-1-specific, but not anti-SSX-2 antibodies, underwent affinity maturation over the course of the patients' disease. NY-ESO-1- and SSX-2-specific antibodies were of the IgG1/IgG3 and IgG3 subtypes, respectively, and were both capable of activating complement. Correlating humoral immune responses with clinical events we observed that the development and/or maintenance of humoral responses against NY-ESO-1 was related to progression of myeloma while anti-SSX-2 antibodies were induced shortly after alloSCT and were preferentially present in phases of clinical remission.
Conclusions: Our results suggest that CTA represent targets for spontaneous humoral responses in MM and that they might also be of relevance for the immune control of MM as part of an alloSCT-induced GvM effect.

9239 POSTER

Rapid Method to Measure Thioguanine Incorporation Into DNA

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Background: The thiopurine drugs, 6-mercaptopurine, azathioprine, and thioguanine, are used in the treatment of acute lymphoblastic leukaemia (ALL). During treatment the thioguanine nucleotides formed are incorporated into the DNA, causing apoptosis due to the cells inability to repair the resulting damage. This mechanism is believed to be important for the effects of thiopurine drugs. We have developed a novel method for the determination of thioguanine incorporation into DNA which is both faster and cheaper than earlier methods.
Monitoring the effects of thiopurine treatment by measuring thiopurine metabolites in erythrocytes has proven to be elusive due to the lack of good correlation between measured concentrations and thiopurine effects. If the incorporation is the main mechanism of thiopurine action, a reliable method capable of measuring the incorporation in an ordinary blood sample, such as the method we have developed, should provide a significantly better correlation with treatment effect.
Material and Methods: Briefly, DNA extracted from buffy coat is degraded using nuclease P1 and alkaline phosphatase to produce free nucleosides which are purified by filtration. Thioguanosine and thymidine are separated and detected using an LC-MS/MS system and the ratio between the bases provides a measurement of the extent of thioguanine incorporation in DNA. The method has been successfully applied to cell culture samples as well as samples from patients treated orally with thiopurines.
Results: In 8 inflammatory bowel disease patients treated with azathioprine the measured incorporation ranged from 2.2 to 8.4 thioguanine bases for every 10 000 thymidine bases (median 5.2). This is in agreement with earlier reports on incorporation in childhood leukemia patients.
Conclusions: With the presented method it is possible to determine the incorporation of thioguanine into DNA during thiopurine treatment in a cost effective manner, but further research is needed to determine if there is a place for this type of methods in the monitoring of thiopurine treatment.
An ongoing study aims to compare the incorporation to treatment effects as well as conventional measurements of erythrocyte metabolite levels. By this study we hope to determine if incorporation is a more reliable measurement to predict treatment effect and if the erythrocyte metabolite levels correlate with the incorporation.