Conclusions: Our results indicate that BAFF-induced inflammation-related signal transduction can lead to BCL10 nuclear translocation and NF-κB activation. The autocrine BAFF signal transduction pathway may contribute to the HP-independence of gastric MALT lymphoma without t(11;18)(q21;q21).

9233 POSTER

Pharmacokinetic Intra-individual Variability of Imatinib − Consequences for Therapeutic Drug Monitoring in Chronic Myeloid Leukaemia

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Background: Imatinib is a competitive inhibitor of protein tyrosine kinase Bcr-Abl and is currently used for the treatment of chronic myeloid leukaemia (CML) and other digestive malignant pathologies. Trough imatinib plasma levels are associated with major molecular response in CML. Due to important interpatient variability, monitoring of imatinib plasma levels can be very useful especially in the case of treatment failure. The aim of this work was to determine intra-individual variability in imatinib plasma concentrations to a better knowledge of therapeutic drug monitoring (TDM) of imatinib in the real life.
Methods: Imatinib plasma concentrations were determined by high-performance liquid chromatography with UV detection at 262nm after liquid-solid extraction. Limit of quantification was set at 200 ng/mL. Blood samples were collected at steady-state (trough values before drug administration). Samples were collected and analysed over a period of 3 years with an interval of at least 3 months between each sample. Dosage regimens ranged from 200 mg/day to 600 mg/day. Results were expressed as mean ± standard deviation and variability in plasma concentrations were presented as a CV expressed in percentage. A minimum of three measurements was needed to calculate CV%.
Results: 21 patients were evaluated with a mean number of 5 measurements per patients (3−10) and a total of 108 samples. Sex-ratio (M/F) was 0.48, mean age and weight were respectively 55±16 years and 76±19 kg. Concerning the 400 mg/day group of patients (61 samples), mean imatinib plasma concentration was 1130±640 ng/mL, ranging from 250 to 2800 ng/mL(total CV of 57%) with 18 subtherapeutic concentrations (a plasma threshold of 1000 ng/mL is associated with major molecular response). For all dosage regimens (21 patients), mean intra-individual variability of imatinib was 31%, ranging from 9.7% to 70%. Possible causes for pharmacokinetic variability are multiple: drug/drug interactions (due to metabolism of imatinib with CYP3A4 and transport by Pgp), poor compliance, genetic polymorphisms. A value of 31% for intra-individual variability can be considered low.
Conclusion: The high interindividual and relatively low values of intrapatient variability in plasma support therapeutic drug monitoring. Nevertheless mean plasma concentrations were often closed to therapeutic threshold and our data suggest the need of regular TDM measurement.

9234 POSTER

Cytogenetic Abnormalities in the Spleen Detected by FISH in Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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Background: In chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), the prognostic importance of cytogenetic abnormalities in blood and bone marrow is well established. However, only limited data has been published regarding frequency and prognostic impact of aberrations in other tissues.
Materials and Methods: We identified 62 patients with CLL (n = 57) and SLL (n = 5) who underwent splenectomy between 1989–2010. Fluorescence in situ hybridization (FISH) could be successfully performed on spleen tissue in 60 cases for detection of the cytogenetic abnormalities 11q-, 13q-, 17p- and trisomy 12. The results were compared with available FISH analyses on blood and/or bone marrow. To avoid false-positive results due to incomplete nuclei present in the tissue sections, the cut-off rate for the deletions was set to 40%. For trisomy 12 the cut-off was set to 5%.
Results: Cytogenetic aberrations were detected in 75% of the patients; in 31 cases a single abnormality and in 16 multiple aberrations. The most common aberration was 13q-, detected in 69% of cases. There was a significant correlation between the frequency of 13q and 11q deletions in spleen and blood/ bone marrow.
In 4 of 27 cases, new abnormalities were detected in blood/bone marrow samples after splenectomy. Two cases with a heterozygous 13q deletion in the spleen developed homozygosity for 13q- in blood and a new 11q- clone was found in two patients. One patient had both homo- and heterozygous 13q- clones in the blood before splenectomy as well as in the spleen, but in repeated blood samples taken nine to ten years later only a heterozygous clone remained.
After a median follow-up of 43 months after splenectomy, 23 of 62 patients are alive. Median time to next therapy was 9 (range 0–255+) months. Cytogenetic aberrations had a significant impact on overall survival dividing patients into three categories, (1) 13q- as a sole abnormality, (2) normal karyotype or trisomy 12 and (3) abnormalities involving 11q- or 17p- ($p < 0.05$). Patients with 11q- and/or 17p- did also have shorter therapy-free survival ($p < 0.01$).
Conclusions: In our study on splenectomized patients, cytogenetic aberrations could be reliably detected by FISH on paraffin-embedded sections and did influence overall and therapy-free survival. Clonal evolution could be found in a few patients.

9235 POSTER

BCL2 Expression Correlates With Surface Immunoglobulin Levels and Prognosis in Follicular Lymphoma Patients

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Background: Almost 90% of Follicular Lymphoma (FL) carry a t(14;18)(q32;q21), which juxtaposes the immunoglobulin heavy chain (IgH) enhancers to the Bcl2 gene and results in an overexpression of the BCL2 protein. Most cases of FL show heterogeneous intensity of IgH or BCL2 protein. The aim of this study was to correlate IgH and BCL2 expression and to ask whether different levels of BCL2 were associated to resistance to therapy and prognosis in FL patients.
Matherial and Methods: We analyzed 103 freshly isolated FL cases by flow cytometry and immunohistochemistry to asses BCL2 levels, and correlate BCL2 expression to survival. Isolated cells from FL patients and cell lines were studied for BCL2 regulation and resistance to apoptosis.
Results: We found a strong positive correlation between IgH and BCL2 expression in FL cases ($P < 0.0001$) as well as in subpopulations of FL cells within individual patients. A concordant regulation of both IgH and BCL2 in lymphoma cells carrying a t(14;18) translocation was found. Remarkably, primary FL cells expressing high BCL2 were more resistant to Rituximab or doxorubicin than FL cells with low BCL2 isolated from the same patient. Finally, patients with low BCL2 levels had significantly higher probability of survival as compared to patients expressing high levels of BCL2 ($P < 0.05$).
Conclusion: We show a strong correlation between IgH and BCL2 expression in FL cells, suggesting mechanisms that concordantly regulate both IgH and BCL2 transcription in translocated cases. Heterogeneous expression of BCL2 affects sensitivity to therapy and overall survival.

9236 POSTER

Phosphatidylinositol-3 Kinase I Inhibitor BKM120 Induces Cell Death in B-chronic Lymphocytic Leukemia Cells In-vitro

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Background: B-chronic lymphocytic leukemia (CLL) is characterized by the accumulation of CD5+ B-lymphocytes that are long-lived *in-vivo* but die quickly by apoptosis when cultured *in-vitro*. The phosphatidylinositol-3 kinase (PI3K) cascade is a critical component of survival signalling including PI3K-activated Akt (phosphorylated Akt) which inhibits cell death pathways by inactivating pro-apoptotic proteins. However,PI3K has increased activity in CLL lymphocytes as compared to normal B lymphocytes. There are three classes of PI3Ks of which class I is the most clearly implicated in human cancer. The PI3K-δ inhibitor, CAL-101, promotes caspase-dependent apoptosis and abrogated protection from spontaneous apoptosis induced by CD40 in primary CLL lymphocytes *in-vitro*. Thus PI3K pathway appears to play a critical role in B-CLL cell survival. BKM120 is a pan class I PI3K inhibitor developed by Novartis. Phase I trials demonstrated that plasma concentrations of 3−5 μM can be obtained. In view of the critical role of PI3K in CLL homeostasis, the activity of BKM120 was examined in CLL lymphocytes.
Material and Methods: BKM120 cytotoxicity was assessed by the MTT assay in primary B-CLL lymphocytes and the MEC-2 B-CLL cell line.

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.