whereas no effect of dCK and CDA variants has been observed. Reconstitution times were measured after all cycles of consolidation therapy leading to similar results.
Conclusion: These findings indicate that functional genetic variants in cytarabine transport may alter the individual susceptibility for cytarabine related side effects. Ongoing work will study the role of recently reported genetic variants from genome-wide association studies on cytarabine toxicity.

9243 POSTER

Ex vivo assessment of variability in cytarabine cytotoxicity: influence of transport genetics

S. Parmar[1], A. Seeringer[1], K. Pitterle[1], D. Denich[1], F. Gärtner[1], E. Lebedeva[1], J. Kirchheiner[1]. [1]*Institute of Pharmacology of Natural Products and Clinical Pharmacology, Clinical Pharmacology, Ulm, Germany*

Background: Recent whole genome approaches on cytosine arabinoside (AraC) cytotoxicity have evaluated the role of genes within the metabolic pathway of AraC and resulted furthermore in new candidate genes found to be associated with AraC toxicity in human lymphoblastoid cell lines (LCL). Since LCLs might vary from native cells in apoptosis behavior and in gene expression profiles due to the EBV transformation, we intended to study variability of AraC toxicity in an ex vivo assay of native peripheral blood mononuclear cells from hundred healthy donors.
Methods: Cells were isolated using ficoll density gradient and incubated for 48h with 3 μM AraC or 3 μM AraC + 150 nM S-(4-Nitrobenzyl)-6-thioinosine (NBMPR). The addition of 150nM NBMPR causes specific inhibition of the human equilibrative nucleoside transporter 1 (hENT1), which mediates 80% of the AraC influx. Cells were double stained for annexin-V-FITC and propidium iodide and analyzed by flow cytometry to determine early and late apoptosis. AraC specific toxicity (AST) was calculated as: AST [%] = ((vital $cells_{control}$ [%] / vital $cells_{0h}$ [%]) – (vital $cells_{treated}$ [%]/vital $cells_{0h}$ [%])). Transporter and other candidate gene mRNA expression levels were determined by Real-time PCR using SYBR-Green chemistry.
Results: Within-subject variability in AraC specific cell toxicity was 12.4% whereas between-subject variability was 43.3%. Mean AST without transport inhibition was 14.7% (2.3−29.4%) and 3.4% (0.0−17.1%) after specific inhibition of hENT1. Higher hENT1 expression correlated with higher AraC induced apoptosis after transport inhibition ($p = 0.03$).
Conclusion: Higher expression of the hENT1 transporter correlated with less effect of transport inhibition on AraC cell toxicity. A less saturable transport capacity in subjects with genuine higher hENT1 expression levels might lead to enhanced cell toxicity of AraC. Further studies will elucidate pharmacogenetic mechanisms leading to individual differences in hENT1 expression.

9244 POSTER

Expression and immunogenicity of cancer-testis antigens in acute myeloid leukemia

T. Luetkens[1], B. Kloth[1], G. Fuchs[1], Y. Hildebrandt[2], S. Kobold[1], K. Bartels[1], S. Meyer[1], N. Kröger[2], C. Bokemeyer[2], D. Atanackovic[2]. [1]*Universitätsklinikum Hamburg-Eppendorf, Oncology/Hematology, Hamburg, Germany;* [2]*Universitätsklinikum Hamburg-Eppendorf, Stem Cell Transplantation, Hamburg, Germany*

Background: Only 30% of patients with Acute Myeloid Leukemia (AML) over 60 years receive conventional induction therapy due to therapy-related mortality in up to 50% of these patients. An evaluation of novel therapeutic approaches is required. In order to identify target structures for antigen-specific immunotherapies, we performed a comprehensive analysis of CT antigen expression, its dependence on epigenetic mechanisms, and associated humoral immune responses in AML.
Methods: 10 AML cell lines and bone marrow (BM) samples from 10 healthy donors were screened for the expression of 22 CT antigens by RT-PCR and Western blot. Cell lines were further evaluated by RT-PCR following stimulation with 5-Aza-2-Deoxycytidine and Trichostatin A. Expression of 16 selected antigens was analyzed in BM and peripheral blood (PBL) samples from 98 patients with AML and sera from 42 patients were screened for antibodies against NY-SAR-35.
Results: RT-PCR showed the expression of 9 of the 22 investigated antigens in at least one untreated cell line. Only PRAME was detected in more than two cell lines. Treatment with 5-Aza-2-Deoxycytidine led to a two-fold increase in the average number of CT antigens expressed per cell line while treatment with Trichostatin had no significant effect on cumulative antigen expression. Investigating the expression of 15 antigens in samples from patients with AML by RT-PCR, PRAME was most frequently detected (52.1%) but expression did not correlate with clinicopathological parameters or survival. Interestingly, the only other antigen we were able to detect repeatedly (17%) in patients with AML was NY-SAR-35. Two putative transmembrane domains of the theoretical NY-SAR-35 protein indicate that this antigen, which showed an extraordinary tissue restriction, might be directly accessible to immunotherapeutic approaches. Accordingly, fluorescent staining of AML cell lines revealed a homogenous presence of NY-SAR-35 on the cell surface. Despite this finding, none of the investigated AML patients showed a significant humoral immune response against this antigen.
Conclusions: Only tumor antigen PRAME is commonly expressed in AML cell lines, but patient samples further revealed a frequent expression of CT antigen NY-SAR-35. We show for the first time that expression of this antigen does not lead to antibody responses in patients with AML, but argue that targeted therapies remain an option due to its restricted expression, inducible expression changes and its ideal localization for immunotherapeutic approaches.

9245 POSTER

Notch pathway genetic signature is associated to Clofarabine resistance in pediatric T-Acute Lymphoblastic Leukemia (ALL)

F. Melchionda[1], A. Astolfi[1], S. Formica[1], M. Franzoni[1], G. Paone[1], V. Libri[1], S. Serravalle[1], A. Pession[1]. [1]*Pediatric Oncology and Hematology, Pediatrics, Bologna, Italy*

Background: Despite several progress obtained in pediatric T-ALL treatment, this subtype of leukemia is still associated with higher relapse rate and resistance, thus T-ALL constitutes a significant clinical challenge and identification of new drugs and drug combinations has become a priority in the field. The study evaluated clofarabine (CLOF) anti-tumor activity in ALL cells obtained from pediatric ALL pts and identified genes associated to sensitivity and resistance.
Material and Methods: 17 ALL samples were isolated from bone marrow at diagnosis (10 T-ALL and 7 B-ALL). We evaluated CLOF antitumor activity (EC50) by vitality test (WST-1, Roche) and correlated it to dexamethasone (DXM) activity in vitro and to clinical data, as WBC at diagnosis and response in vivo to prednisone, collected according to ALL Pediatric Prot. AIEOP-BFM ALL 2000. Gene expression profiling of sensitive (S) vs resistant (R) samples was conducted using Affymetrix microarray HGU133 plus 2.0.
Results: While B-ALL generally responded to low doses of CLOF (median EC50 = 0.04 uM) and with a low variability among samples (range=0.013−0.086uM), CLOF EC50 in T-ALL showed a large variability (range=0.009−289 uM). T-ALL samples were stratified into two groups: S (n = 4) vs R (n = 6), according to the median EC50 value and to CLOF plasmatic concentration reached upon administration of the MTD (0.01 uM). No clinical data (prednisone response, WBC at diagnosis) correlated to in vitro CLOF response and no correlation was found with DXM response in vitro ($p = 0.325$). Microarray analysis identified differential gene expression in CLOF-R vs CLOF-S patients, particularly anti-apoptotic genes and immune response genes. Pathway analysis showed that Notch signaling was the pathway more significantly correlated with CLOF resistance in T-ALL ($p < 0.005$), with the differential expression of many genes, as NOTCH2, CTBP2, NOTCH3, MAML3, HES1, DTX3. Validation of genechip analysis was done by real-time PCR of NOTCH2 and HES1 genes, confirming the increased expression of NOTCH2 ($p = 0.037$) and HES1 ($p = 0.042$) transcripts in all R vs S patients.
Conclusion: CLOF response in T-ALL appears to correlate with Notch pathway signaling. Our data explore the rationale to identify cases more likely to respond to CLOF and to design new therapeutic strategies for T-ALL

9246 POSTER

Evolution of the accelerated and blastic phases of chronic myeloid leukemia: molecular, cytogenetic, flowcytometric and electron microscopic studies

N. Kholoussi[1], A. Khorshed[2], A. Soliman[2], N. Abdel Wahab[2], S. Ibrahim[2], K. Emara[2], R. Rashed[2], T. Mansour[2]. [1]*National Research Center, Immunogenetics, Cairo, Egypt;* [2]*National Cancer Institute, Clinical Pathology, Cairo, Egypt*

Background: chronic myeloid leukemia (CML) is a clonal disease that results from an acquired genetic change in a pluripotential hemopoietic stem cell. Molecular abnormalities and mutations usually accompany the accelerated and blastic crisis phases of CML. This study was conducted to explore the possible ultrastructural, molecular cytogenetic, apoptotic and morphological abnormalities that may contribute to the progression of chronic phase to accelerated and blastic crisis phases in CML patients.
Material and Methods: The study included thirty CML patients newly diagnosed and under treatment presenting to the Medical Oncology department of the National Cancer Institute and ten age-matched subjects