

Abstract# 4490

INDUCTION OF G₀/G₁ TRANSITION IN LEUKEMIC CELLS BY INTERFERON THERAPY INCREASES SENSITIVITY TO DAUNORUBICIN, BUT NOT TO CYTOSINE ARABINOSIDE (Ara-C). I. Jedema*,¹ R.M.Y. Barge,¹ R. Willemze,¹ J.H.F. Falkenburg,¹ ¹Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands.

Chemotherapy-resistance of leukemic cells may in part be due to the unresponsiveness of non-cycling cells to cell cycle dependent cytotoxic drugs. In this study we have explored the effect of manipulation of the cell cycle status of leukemic cells on the sensitivity to the anticancer drug daunorubicin. As a model we used the GM-CSF dependent human myeloid leukemic cell line AML-193. Ara-C was used as a control for S-phase specific cytotoxicity. As target cells were: a) non-proliferating, resting control AML-193 cells, cultured in the absence of cytokines (83% G₀, 4% G₁, 7% S and 6% G₂/M), b) IFN-γ or IFN-α activated AML-193 cells (55% G₀, 23% G₁, 11% S and 11% G₂/M), and c) proliferating GM-CSF treated AML-193 cells. No changes in expression of Fas, TNFR-1, Bcl-2, Bax, p53, caspases, adhesion molecules or costimulatory molecules were found after GM-CSF or IFN treatment. The leukemic cells were tested for their sensitivity to 10⁻⁶ M Ara-C or 10⁻⁶ M daunorubicin-induced apoptosis. Cell death was determined by ⁵¹Cr release assay and confirmed by Annexin-V staining and flowcytometric analysis. Results are shown as percentage specific lysis after 24 hrs in table 1 (* = p<0.01, relative to control). Treatment of AML-193 cells with IFN-γ or IFN-α was sufficient to increase the sensitivity to daunorubicin, whereas sensitivity to Ara-C was not changed by IFN treatment. GM-CSF treatment increased the sensitivity to both Ara-C and daunorubicin-induced apoptosis. In addition, we determined the absolute numbers of cells in each cell cycle fraction that had undergone apoptosis in response to daunorubicin. A fixed number of IFN treated AML cells was mixed with a fixed number of Flow-Count Fluorospheres and incubated in medium with 10⁻⁶ M daunorubicin. After 48 hours we calculated the percentages of total cells that had undergone apoptosis in each cell cycle fraction. After daunorubicin treatment, 70% of the G₀ cells were killed, compared to only 35% of G₀ cells. In conclusion, induction of G₀/G₁ transition by IFN-γ or IFN-α treatment without induction of proliferation is sufficient to increase the sensitivity to daunorubicin-induced apoptosis. A high percentage resting G₀ cells in the leukemic (precursor) cell population might be responsible for chemotherapy-resistance of leukemia. Combining interferon with chemotherapy may improve the efficacy of the treatment.

| | Control | IFN-gamma | IFN-alpha | GM-CSF |
|--------------|------------|-------------|-------------|-------------|
| Daunorubicin | 23% (n=40) | 50%* (n=20) | 36%* (n=15) | 45%* (n=40) |
| Ara-C | 8% (n=40) | 13% (n=20) | 9% (n=15) | 24%* (n=40) |

Abstract# 4491

GLUCOCORTICOID-INDUCED CELL DEATH AND PROLIFERATION IN VITRO IN CHILDHOOD ACUTE MYELOID LEUKEMIA. Gertjan J.L. Kaspers*,¹ Eric G. Haarman*,¹ Christiaan M. Zwaan,¹ Rob Pieters*,² Elisabeth R. Van Wering*,³ Anna Van Der Does-Van Den Berg,³ Ursula Creutzig,⁴ Anjo J.P. Veerman,¹ ¹Pediatric Hematology/Oncology, University Hospital Vrije Universiteit, Amsterdam; ²AZ/R Sophia Children Hospital, Rotterdam; ³Dutch Childhood Leukemia Study Group, The Hague, The Netherlands; ⁴BFM-AML Study Group, Munster, Germany.

Glucocorticoids (GC) have significant antileukemic activity in ALL, but their role in AML is less certain. We previously reported that the majority of AML samples was resistant to GC in vitro, and that in 10 out of 28 AML samples prednisolone (and dexamethasone if tested) even induced proliferation in vitro (Kaspers et al., Leukemia 1994). We have extended the number of observations and now studied the clinical relevance of resistance to prednisolone (PRD) and of PRD-induced proliferation in pediatric AML samples taken at initial diagnosis. Using the colorimetric 4 days MTT assay, 126 AML samples were tested successfully. Drug resistance was expressed in LC50 values, the PRD concentration needed to kill 50% of cells. Sensitivity to PRD was arbitrarily defined as LC50 values < 150 µg/ml, the remainder of the samples as resistant (Kaspers et al., Blood 1998). PRD-induced proliferation was defined as a cell survival greater than that in control wells not containing drug after the 4 days of culture. All patients were treated with PRD during the so-called consolidation course. Results: 15% of the AML samples were relatively sensitive to PRD (although LC50 values were still high compared to most ALL samples, data not shown). PRD sensitivity was more often seen in samples with FAB type M1 (6/14) than in non-M1 samples (13/110, p=0.002; 2 cases not evaluable). PRD-induced AML cell proliferation was seen in 32/126 samples, more often in FAB M5 (13/24) than in non-M5 samples (19/102, p<0.001). We reported previously that within AML, FAB M5 samples are relatively in vitro sensitive to most drugs, except PRD (Zwaan et al., Blood 2000). PRD sensitivity or resistance did not significantly correlate with the probability of DFS, although it seemed that in vitro sensitive patients relapsed later. Patients with PRD-induced AML cell proliferation had a lower EFS (20±10% vs 50±7%, p=0.002) and tended to have a lower DFS (30±15% vs 55±8%, p=0.08). In conclusion, the far majority of newly diagnosed pediatric AML samples is in vitro resistant to GC-induced cell death. GC-induced cell proliferation in vitro occurs in about 25% of AML samples (even in about 50% of FAB M5 samples), which phenomenon is associated with a worse clinical outcome.

Abstract# 4492

SINGLE NUCLEOTIDE POLYMORPHISM IN MDR1 GENE AMONG PATIENTS WITH ACUTE LEUKEMIA: CORRELATION OF DRUG INDUCED APOPTOSIS TO MDR1 GENOTYPE. Samir V. Kubba,¹ Mark Koury,¹ Vladimir Kravtsov,¹ John Greer,¹ Richard Kim*,² ¹Department of Hematology and Oncology, Vanderbilt-Ingram cancer center, Nashville, TN, USA; ²Department of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA.

The Multi Drug Resistance gene MDR1 product P-glycoprotein, has been shown to be expressed in leukemic cells. It has been shown to account for some of the variability in response to certain chemotherapeutic agents. Here we analyzed DNA samples derived from thirty patients diagnosed with acute leukemia, for single nucleotide polymorphisms SNPs, in the human MDR1 gene. Two exonic regions notable for common polymorphisms were detected: a single base pair substitution at G2677T which is Ala893Ser in exon 21 and a conservative substitution in exon 26 which is C3435T, Ile1145Ile. To determine a functional role of such SNPs to the effects of chemotherapeutic agents, a comparison of genotype vs. rate of drug-induced apoptosis was carried out. Genotyping was determined using a sensitive non-isotopic single strand conformation polymorphism SSCP method. Rate of apoptosis was determined using the Microculture Kinetic Assay MiCK. The MiCK assay is a validated method of determining the rate of apoptosis among patient derived leukemic cells, upon exposure to a given chemotherapeutic agent in vitro. Given that the exon 26 SNP did not encode a change in amino acid the genotype phenotype analysis was limited to G2677T SNP, encoding an alanine to serine substitution. Twelve subjects 40% were found to be homozygous for the wildtype allele GG, while thirteen 43.3% patients were found to be heterozygous GT and five 16.6% had the homozygous TT change. Genotype related differences in the extent of drug induced apoptosis were revealed by the MiCK assay. Etoposide induced apoptosis in cells with GG genotype was 12.9%, GT 13.4% and TT 15.2%. For daunomycin no differences in apoptosis levels were seen between GG and GT genotypes which was 17.4%. Cells with TT genotype had 18.2% apoptosis. While these differences failed to reach statistical significance p less than 0.05 due to small sample size, the trend towards higher levels of apoptosis in the presence of the T2677 Ser893 in MDR1 to the effects of drug substrates of P-gp such as etoposide and daunomycin suggest this SNP may be of functional relevance to leukemia therapy. It is also possible that the SNP results in variable drug resistance pattern depending on the chemotherapeutic agent as this SNP may alter substrate specificity of the transporter. Additional studies are warranted with more patients, additional chemotherapeutic agents, and comparison with clinical outcomes to fully determine the in vivo relevance of this SNP.

Abstract# 4493

BIOCHEMICAL EVALUATION OF THE RELATIONSHIP BETWEEN CONJUGATION RATIO AND APPARENT AFFINITY OF ZEVALIN™ (IBRITUMOMAB TIUXETAN) FOR THE CD20 ANTIGEN. M.J. LaBarre*, K. Hathaway*, J. Hernandez*, K. Lowery*, L.R. Salmeron*, R. Morena*, P. Chinn*, J.E. Leonard.

Zevalin™ (ibritumomab tiuxetan) is a monoclonal antibody conjugate being investigated for the treatment, following labeling with yttrium-90, of relapsed or refractory, low-grade, follicular or CD20⁺ transformed B-cell non-Hodgkin's lymphoma. The murine anti-CD20 ibritumomab is coupled to the amino-directed chelator MX-DTPA (tiuxetan) through a stable thiourea linkage to form the conjugate ibritumomab tiuxetan. Studies using fluorescein isothiocyanate and ibritumomab demonstrated that amino groups modified during conjugation were randomly distributed among accessible amino groups in the molecule. The conjugation reaction was reproducible and resulted in a heterogeneous mixture of conjugates, with resulting chelates-to-antibody ratio mixtures being generally described by a Poisson distribution function. Based on this distribution, most of the conjugate molecules should have chelate:antibody mol ratios of between 0:1 and 2:1. An ion-exchange high performance liquid chromatographic method was developed to separate the conjugate populations with 0, 1, 2, and 3 chelates per mol of antibody. The average conjugation ratio for five lots conjugate prepared during a BLA-enabling campaign ranged between 1.5 and 1.7 moles of chelate per mol of antibody. A direct relationship existed between the average conjugation ratio and the binding activity of unlabeled conjugate. The apparent Kd for binding to CD20⁺ WIL2S cells was approximately 10, 20, and 40 nM for ibritumomab tiuxetan with average chelate:antibody ratios of 1:1, 2:1, and 3:1, respectively. The apparent affinity of unmodified ibritumomab for the CD20 antigen was approximately 5 nM. The relative competitive binding activity for five lots of conjugate ranged between 91% and 123%, and the percent radioincorporation values ranged between 98% and 99%. After 8 hours at 2-8°C immunoreactivity decreased by <4% with <2% loss of radiometal; after 96 hours in human serum at 37°C immunoreactivity decreased by <10% with <3% loss of radiometal. These results demonstrated that the average conjugation reaction resulted in a 3-4 fold decrease in the apparent affinity of the conjugate for the antigen, and that radiolabeling procedures used to prepare labeled Zevalin resulted in a radioimmunoconjugate with reproducibly high binding activity, high radioincorporation and good stability.

Abstract# 4494

ANTIPROLIFERATIVE ACTIVITY OF INOSITOL HEXAPHOSPHATE (IP₆) ON NORMAL AND LEUKEMIC HEMATOPOIETIC STEM CELLS. Giorgio Lambertenghi Delilieri,¹ Federica Servida*,² Chiara Borsotti*,² Davide Soligo.¹ ¹Department of Hematology, Ospedale Maggiore, IRCCS, and University of Milan, Milan, Italy; ²Matarelli Foundation, Milan, Italy.

Inositol hexaphosphate (IP₆) is a naturally occurring polyphosphorylated carbohydrate present in plants (particularly cereals and legumes) that has significant "in vivo" and "in

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