

Abstract# 3128

Poster Board #-Session: 592-III

DENDRITIC CELLS FUSED WITH ACUTE MYELOGENOUS LEUKEMIA (AML) BLASTS INDUCE ACTIVATION OF ANTITUMOR CYTOTOXIC LYMPHOCYTES. G.-Andre Banat*,¹ Nurguel Usluoglu*,¹ Oliver Christ*,¹ Hans Pralle.¹ ¹Hematology & Oncology, University, Giessen, Germany.

Several observations indicate that T-lymphocytes can recognize AML blasts and thereby mediate antileukemic reactivity. For example, it is documented that T cells are involved in the pathogenesis of antileukemic graft versus host disease. Moreover, AML blasts often have chromosomal abnormalities which could encode for leukemia specific antigenic peptide sequences probably presented in the context of self MHC molecules. In the most patients AML blasts express both MHC class I and II molecules for antigenic presentation. The expression of costimulatory molecules on AML blasts is very heterogeneous and could be downregulated during one cell cycle, suggesting the ability to initiate an antileukemic T cell response which differs not only between individual patients, but may also result in T cell anergy. Dendritic cells (DC) are potent antigen-presenting cells. In this study we fused AML blasts with autologous DC by polyethylene glycol. The fusion cells were positive for MHC class I and II, B7-1, B7-2 and adhesion molecules. Most of the fused cells exhibited typical DC morphology with veiled processes and dendrites. In a mixed lymphocyte hybrid reaction we could demonstrate that the fusion cells retained the functional potency of DCs and were able to induce proliferation of autologous T cells compared to the most naive AML blasts. Moreover, the autologous T lymphocytes could be primed by the fusion cells to induce cytotoxicity against autologous AML blasts up to 50% in a effector/target ratio of 10:1. Antibody blocking assays could show that the lysis is mediated by CD8 positive T lymphocytes in a MHC class I dependent reaction.

Abstract# 3129

Poster Board #-Session: 593-III

CHEMOTHERAPY OF MYELOID LEUKEMIA DIRECTED BY A MICROCULTURE KINETIC (MiCK) ASSAY FOR APOPTOSIS. Vladimir D. Kravtsov,¹ Victor Priego*,² Garret J. Reilly,² Harold Sethi*,² James Cooke*,² William Smith*,² Mark J. Koury.¹ ¹Pathology and Medicine, Vanderbilt University, Nashville, TN; ²Suburban Hospital, Bethesda, MD.

Our recent studies on 74 AML patients showed that the MiCK assay of apoptosis can predict chemotherapy outcome in acute myeloid leukemia (*Blood*, 92:677a, 1998). In our ongoing project, the MiCK assay is used to individualize treatment protocols for AML patients. Here we report five cases in which treatment of leukemia patients was based on MiCK assay results. Blast cells were purified from bone marrow or peripheral blood of four AML patients and one CML patient in blastic crisis (bc). Drug responses to 7 doses (ranging from 0.01 to 160µM) of etoposide (E), cytarabine (C), daunorubicin (D), mitoxantrone (M), and idarubicin (I) were tested using the MiCK assay as described previously (*Blood*, 92:968, 1998). For each agent, peak amount of apoptosis was detected and expressed in Kinetic Units (KU). As we reported previously, drug responses of greater than 3 KU for I and D or greater than 2KU for E, M and C correlate well with complete remission. MiCK assay for Pt 1 and Pt 2 was done after they failed to respond to induction therapy with C+D (7&3). For these Pts, M was indicated by the MiCK assay as the only effective apoptosis-inducing agent. Based on MiCK assay results, M was selected for the re-induction therapy of Pt 1 and Pt 2 with outcomes of CR. MiCK assay for Pt 3 and Pt 4 was done at the time of diagnosis. Extent of the response to M of leukemic cells of Pt 3 was almost two-fold over the cut-off level of this agent. Based on this prominent *in vitro* response, Pt 3 received a single agent induction therapy with M which resulted in CR. Leukemic cells of Pt 4 were most sensitive to I, although the extent of the response to I was in a "gray" zone (slightly less than cut-off level of 3KU). This Pt received C (100mg/m²) and I (12mg/m²) with an outcome of CR. For Pt 5, M was the best apoptosis inducer, although *in vitro* responses to all tested agents were less than cut-off levels. Pt 5 received C (100mg/m²) and M (12mg/m²) and had only partial response. These results indicate that the MiCK assay may find clinical use in customizing chemotherapy for both *de novo* and relapsed acute myeloid leukemia patients.

Pt No	Diagnosis	Age/ Sex	Best Drug Responses (KU)	Treatment protocol	Outcome
1	CML, bc	32/M	M(2.5), D(0.6), C(0.6)	M(20mg/m ² x 3d)	CR
2	AML, M4	85/F	M(2.5), I(1.5), D(0.9)	M(12mg/m ² x 3d)	CR
3	AML, M1	64/M	M(3.9), I(3.2), D(1.3)	M(20mg/m ² x 3d)	CR
4	AML, M5	69/M	I(2.7), M(1.4), C(0.5)	C+I (7&3)	CR
5	AML, M4	70/F	I(1.6), M(1.2), C(1.2)	C+M (7&2)	PR

Abstract# 3130

Poster Board #-Session: 594-III

INHIBITION OF THE TRANSFORMING ACTIVITY OF FLT3 INTERNAL TANDEM DUPLICATIONS DERIVED FROM AML PATIENTS BY A TYROSINE KINASE INHIBITOR OF THE TYRPHOSTIN CLASS. Kam-Fai Tse*,¹ Jeffrey Allebach*,¹ Frank D. Bohmer*,² Donald Small.¹ ¹Pediatric Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; ²Max Planck Society, Friedrich Schiller University, Jena, Germany.

FLT3 is a receptor tyrosine kinase which may play a role in a fraction of leukemias. In addition to being aberrantly expressed in many leukemias, internal tandem duplications (ITDs) of the FLT3 gene has been detected in 20% of patients with AML. AML patients with FLT3 ITDs have a poor prognosis. The FLT3 ITD and downstream signaling proteins, including ERK and STAT 5, are constitutively activated in the absence of FLT3 ligand (FL) stimulation. As a result, the development of novel chemotherapeutic interventions specifically targeted to FLT3 activity is desirable. AG1296 is a tyrosine kinase inhibitor of the tyrphostin class which shows inhibitory activity for FLT3, in addition to the PDGF and c-KIT receptors.

We examined the inhibitory effects of AG1296 on two FLT3 ITDs isolated from AML patients in the IL-3 dependent cell line, Ba/F3. Immunoprecipitation and immunoblotting demonstrated that both FLT3 ITDs were constitutively phosphorylated in the absence of FL in Ba/F3 cells. The activated FLT3 ITDs were blocked by AG1296 with an IC₅₀ of approximately 2 µM. Both FLT3 ITDs induced the constitutive phosphorylation of STAT 3, STAT 5A, STAT 5B, CBL, SHC and SHP-2 in Ba/F3 cells. The phosphorylation of these downstream signaling molecules was potently suppressed in the presence of AG1296. Submicromolar concentrations of AG1296 inhibited the IL-3 independent growth of Ba/F3 cells expressing the FLT3 ITDs. Thus, AG1296 may represent a novel therapeutic approach, alone or in synergy with other chemotherapeutic compounds, to treat AML patients presenting with the poorly prognostic FLT3 ITDs.

Abstract# 3131

Poster Board #-Session: 595-III

PHASE I STUDY OF TEZACITABINE, AN ANTIMETABOLITE DEOXYCYTIDINE ANALOG, IN PATIENTS WITH RELAPSED AND REFRACTORY HEMATOLOGIC MALIGNANCIES. S. Faderl, Deborah A. Thomas, Jorge Cortes, Francis Giles, Guillermo Garcia-Manero, Susan O'Brien, Charles Koller, Michael J. Keating, Fernando Cabanillas, Andreas Sarris, Vickie Grigsby*, William Plunkett, E. M. Kantarjian. *The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA.*

Tezacitabine [(E)-2'-deoxy-2'-(fluoromethylene)cytidine; FmC] is a nucleoside analog with potent antitumor efficacy *in vitro* and in animal tumor models *in vivo*. Tezacitabine is phosphorylated intracellularly and is relatively resistant to metabolic deactivation by cytosolic deaminases. It is an irreversible ribonucleotide reductase inhibitor and DNA chain terminator. Antiangiogenesis and induction of apoptosis are other mechanisms of action. In phase I studies in solid tumors, dose levels were 16-630mg/m² q3wk, 32-270mg/m² q2wk, and 112mg/m² qwk or 2x/wk, with myelosuppression as the DLT. We performed a phase I study in patients (pts) with relapsed/refractory hematologic malignancies using a 3+1 design. We used short i.v. infusions on a once daily x 5d q3-4wks schedule. Pts with leukemias (AL) were entered and evaluated separately from pts with lymphoproliferative disorders. In pts with AL, myelosuppression was not considered as DLT unless it persisted for ≥42 days with ≤5% BM blasts. So far, 20 pts have been entered at dose levels of 16 and 6mg/m². 13 pts had AML (1 CML-MBC, 2 biphenotypic, 1 RAEB), 4 pts had ALL, 2 pts had NHL, 2 pts received 3 courses (1 AML, 1 NHL), and 3 pts received 2 courses of AML, 2 NHL. The median age for pts with ALL was 52 yrs (range 18-77) and for AML 66 yrs (65-78). Pts with AL had a median of 3 previous regimens (range 1-4). Pts with ALL received a median of 9 prior regimens (range 9-11). All 3 NHL pts developed myelosuppression with neutropenia being the most frequent (grade 3/4 in 3), followed by anemia (2). 1 pt developed a drug-related rash. In AL pts, the most common tezacitabine related adverse effects (AE) were drug fever (57%, grade 3/4 in 2 pts), GI effects (nausea, vomiting, mucositis) (31%), and skin rash (19%). 1 pt experienced a seizure-like event (m²). 4 AL pts died on study (1 AML with AMI [1mg/m²], 3 AML with PD). One pt achieved a partial response and 2 had stable disease. Among pts with AL, 14 pts had SD prior to progression, and 1 achieved a complete remission at 6mg/m². Response could not be assessed in 2 pts. 2 pts with ALL had a significant reduction of blasts in the marrow and BM. We conclude that tezacitabine is a novel nucleoside analog with activity against hematologic malignancies.

HODGKIN'S LYMPHOMA

Abstract# 3132

Poster Board #-Session: 596-III

PROGNOSTIC SIGNIFICANCE OF BCL-2 PROTEIN EXPRESSION IN ADVANCED HODGKIN'S DISEASE. M. Diviné*, J. Briere, Meignin*, Y. Kerneis*, O. Reman*, J. Troncy*, D. Assouline*, Kohser*, F. Berger*, P. Brice, E. Lepage*, C. Fermé, For the GHM. *Hôpital Henri Mondor, Créteil, France.*

Intracytoplasmic bcl-2 protein expression is detected in Reed-Sternberg cells (RS) in 30 to 65% of Hodgkin's disease (HD) according to the series published. Some of us pointed out that bcl-2 protein expression might be predictive of a worse prognosis. We prompted us to analyze the distribution of the bcl-2 protein in tissue sections of 266 HD (pts) with clinical stage IIIB/IV HD. These pts were uniformly treated with MOPP hybrid chemotherapy in the H89 protocol (Fermé et al. *Blood* 2000;95:2246). Among pts with histologically reviewed HD, material of 169 pts was available. The main characteristics of this group were similar to those of the remaining group of 97 pts who were involved in the H89 protocol. Staining for bcl-2 was performed on deparaffinized sections using an indirect immunoperoxidase method and a specific monoclonal antibody (Ab) 124, Dako SA, Glostrup, Denmark). Optimum labeling was obtained by microwave pretreatment. Scoring for bcl-2 was independently performed by two of us without knowledge of the clinical outcome. Tumors with any detectable bcl-2 in RS cells were called positive and those without any detectable bcl-2 in RS cells except in some infiltrating small lymphocytes (internal control) were called negative. 69% of the pts were males. Median age was 33 years, with 24% of the pts being older than 45 year. Ann Arbor stage was I in 45%, IVA in 9%, and IVB in 46%. Histology was nodular sclerosis (NS) in 69%, mixed cellularity (MC) in 28%, and unclassified in 3%. Anemia was seen in 35%, low hemoglobin in 29%, lymphopenia in 15%, and white blood cell ≥ 15 G/l in 23%. bcl-2 was detectable in RS cells of 57 pts or 34% of all pts. Expression of bcl-2 was 40% in NS, 23% in MC (P=0.03). With a median follow-up of 46 months, the 5-year estimates of overall free survival, event-free survival and overall survival were 80%, 74% and 79%, respectively.

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Abstract# 3133
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Abstract# 3134

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