

in blast cells. (7) Pts with classic MDR1 function more often have blasts that are T-lineage or B-lineage with co-expression of myeloid antigens than MDR1-negative pts. Because the S9400 study is ongoing, more time will be needed to determine the prognostic significance of these findings.

**Abstract# 2789**

**FLUDARABINE INHIBITS STAT1 SIGNALING IN NORMAL T CELLS AND CLL CELLS.** D.A. Frank, S. Mahajan\*, J. Ritz. Dana-Farber Cancer Institute, Boston, MA, USA.

Fludarabine, an important drug in the treatment of patients with CLL, is unique in that it is effective in cells with a low growth fraction suggesting that it must act independent of incorporation into DNA. Fludarabine also induces a prolonged and profound immunosuppression, similar to that seen in animals in which STAT1 is lacking due to gene targeting. To test the hypothesis that fludarabine acts by inhibiting signaling through STAT1, normal peripheral blood lymphocytes were incubated in fludarabine for 24 hours, then stimulated with IFN- $\alpha$ , a cytokine known to induce activation of STAT1. Fludarabine, but not the immunosuppressant cyclosporine A, caused a pronounced inhibition of IFN- $\alpha$ -induced STAT1 DNA binding. Furthermore, STAT1 tyrosine phosphorylation and translocation to the nucleus were markedly inhibited in the fludarabine treated cells. Fludarabine had no effect on the activation of Jak1 and Tyk2, the Jak family kinases activated by IFN- $\alpha$ . However, STAT1 protein levels were found to be significantly decreased in fludarabine-treated cells. By contrast, levels of other STAT proteins, Jak family kinases, and MAP kinase were unaffected by fludarabine treatment. The decreased levels of STAT1 were reflected in a dramatic decrease in STAT1 mRNA, suggesting that fludarabine inhibits production of STAT1. When normal PBLs were treated transiently with fludarabine, the loss of STAT1 persisted for a prolonged period. Furthermore, such transient exposure of PBLs to fludarabine caused a lasting inhibition in proliferation and cytokine production in response to mitogen, mirroring the prolonged immune suppression induced by fludarabine in vivo. To determine whether the loss of STAT1 seen in lymphocytes treated with fludarabine in vitro is also seen in vivo, PBLs were harvested from a patient with CLL 24 hours following administration of fludarabine. As with in vitro exposure, fludarabine treatment in patients also led to the loss of STAT1. Fludarabine-induced STAT1 loss occurred in primary CLL lymphocytes treated in vitro, and in a number of lymphoid cell lines. However, Jurkat cells, whose growth is not inhibited by fludarabine, did not display a loss of STAT1 following fludarabine treatment. These findings suggest that STAT1 may be an important target for fludarabine, and that inhibitors of STAT1 may possess anti-cancer and immunomodulatory activity.

## ADULT ACUTE LEUKEMIA — NOVEL INSIGHTS

**Abstract# 2790**

**PREDICTION OF SURVIVAL AND RESPONSES TO CHEMOTHERAPY IN ACUTE MYELOGENOUS LEUKEMIA (AML) BY THE MICRO-CULTURE KINETIC (MiCK) ASSAY OF APOPTOSIS.** V.D. Kravtsov, J.P. Greer, Y. Shyr, D.J. Haselton, J.A. Whitlock, S. Goodman, R.S. Stein, M.J. Koury. Vanderbilt University Medical Center, Nashville, TN, USA.

Chemotherapy induces apoptosis in tumor cells of patients (pts) with AML. *In vitro* measurement of drug induced apoptosis by the MiCK assay may provide a means to predict drug responses for individual pts (*Blood* 1998; 92:968). The MiCK assay was applied to study responses of tumor cells isolated from 74 AML pts to cytarabine (C), idarubicin (I), mitoxantrone (M), etoposide (E) and daunorubicin (D). Concentrations of tested chemotherapeutic agents ranged from 0.01 to 160  $\mu$ M. Apoptotic responses were monitored for 24 h and measured in kinetic units (KU) which are directly related to the percentages of apoptotic cells in the cultures. The distribution of the peak amount of apoptosis was analyzed for each drug and these peak values were separated into two groups of responsive or nonresponsive for the induction of apoptosis. Pts' median age was 43 yrs (range, 3-77); male:female ratio was 30:44. Clinical features [cytogenetics (cyto), preceding myelodysplasia (MDS), *de novo* vs relapse, and prior chemotherapy] as well as the MiCK assay results were correlated with survival and clinical response. The 1 yr survival was 35%  $\pm$  7% with a median of 257 days. The MiCK assay based on sensitivity to I, C, and M but not to D or E was statistically more significant in predicting survival than any of the clinical features.

Feature	N	1 Yr (%)	p	Drug	MiCK (KU)	N	1 Yr (%)	p
Cyto-Good	6	44		C	<2	60	31	.04
Intermediate	27	43	.42		$\geq 2$	14	52	
Poor	24	18		I	<3	41	22	.002
Prior MDS	22	18	.69		$\geq 3$	33	42	
No MDS	52	34		M	<2	32	24	.05
Relapses	21	14			$\geq 2$	42	40	
<i>De Novo</i>	53	38	.07	E	<3	64	32	.13
Prior Chemo 1*	10	22	.09*		$\geq 3$	10	64	
$\geq 2^*$	11	14		D	<3	50	36	.63
					$\geq 3$	24	29	

\* Courses of prior chemo compared with *de novo* (no chemo)

There were 29 CR, 8 PR, and 12 no responses (NR) in 49 pts evaluable for clinical response; 25 pts were not evaluable due to either bone marrow transplant (n = 17) or early death/no therapy (n = 8). The 1 yr survival for CR was 58% vs 10% for PR + NR (p = .0001). There was a significant difference in predicting response (CR vs PR + NR) for I (p < .001), C (p = .046), and M (p = .034); neither E (p = .111) nor D (p = .136) predicted response. Preliminary data indicates the

MiCK assay is a better predictor of response and survival in AML than clinical features.

**Abstract# 2791**

**A CULTURE SYSTEM BASED ON THE CELL LINE AFT024 THAT RELIABLY SUPPORTS LONG-TERM IN VITRO EXPANSION OF Ph+ ALL BLASTS.** C.M. Verfaillie, J. Barker, K. Van Overbeke\*, E. Fan\*. Division of Hematology, University of Minnesota, Minneapolis, MN, USA.

To support *ex vivo* growth of B-ALL blasts, contact with normal human bone marrow (BM) stromal feeders is imperative, although some ALL-blast populations still apoptose. *Ex vivo* culture systems are, however, required to test anti-leukemic therapies. We have shown that the murine fetal liver cell line, AFT024, supports differentiation of BM CD34<sup>+</sup>CD38<sup>-</sup> cells to pre-B cells and differentiation of BM CD34<sup>+</sup>CD10<sup>+</sup> pro-B cells to mature B cells. We therefore hypothesized that survival and proliferation of B-ALL blasts *ex vivo* may be reproducibly accomplished by cultivation on AFT024. Pre-B-ALL blasts from 5 Ph<sup>+</sup> ALL patients with overt leukemia were cultured in contact with AFT024 for 2 months. To prevent NK and T cell overgrowth, CD3<sup>+</sup> and CD56<sup>+</sup> cells were removed by FACS. Medium consisted of X-VIVO + 5% FCS, with or without Flt-3L, SCF, IL7, IL2 or IGF, alone or in combination. 89  $\pm$  2% of day 0 cells were CD34<sup>+</sup>, 91  $\pm$  3% CD10<sup>+</sup>, 65  $\pm$  8% CD19<sup>+</sup>, <2% CD7<sup>+</sup>, CD2<sup>+</sup> or CD14<sup>+</sup>. By day 14, 6-11 fold more cells were present in cultures without cytokines or with IL7 or Flt-3L alone, and the number remained stable till day 56. The viable cell number in cultures with SCF, IL2 or IGF alone, or any cytokine combination, declined after day 14 and most cells had died by day 28. Cells collected between day 3-56 from cytokine free cultures had the same phenotype as day 0 cells: >90% CD34<sup>+</sup>/CD10<sup>+</sup> and >50% CD19<sup>+</sup> whereas 10-30% of cells cultured with IL7 or Flt-3L for 2-4 weeks became CD34<sup>-</sup> but were still CD10<sup>+</sup>. All cultured cells were BCR/ABL positive. We performed limiting dilution assays to assess the absolute number of cells capable of initiating B-leukemic cultures. B-leukemic culture initiating cell frequency (wells with CD10<sup>+</sup> cells) was similar in the 3 culture conditions but decreased by 50% between week 4 and 8 (1:120-150 to 1:250-300 cells). To determine when cell proliferation commenced, cells were labeled with PKH26 and PKH staining intensity measured on day 3, 5, 8 and 14: proliferation started between day 5 and 8 and all cells had proliferated by day 14. In conclusion, we developed an *in vitro* culture system that reliably supports expansion of Ph<sup>+</sup> pre-B-ALL blasts for a minimum of 8 weeks. This should prove invaluable for studies evaluating novel therapies aimed at eliminating Ph<sup>+</sup> leukemic blasts from autografts.

**Abstract# 2792**

**CHARACTERISATION OF ACUTE PROMYELOCYTIC LEUKAEMIA (APL) CASES LACKING THE CLASSICAL t(15;17): RESULTS OF THE EUROPEAN WORKING PARTY.** D. Grimwade\*, A. Biondi, M.-J. Mozziconacci\*, A. Hagemeijer, R. Berger\*, M. Neat\*, K. Howe\*, J. Jansen\*, I. Radford-Weiss\*, D. Head, V. Liso, D. Sainy\*, G. Flandrin, F. Birg\*, M. Lafage-Pochitaloff\*. On behalf of Groupe Français de Cytogénétique Hématologique, Groupe Français d'Hématologie Cellulaire, UKCCG and BIOMED 1 European Community-Concerted Action "Molecular Cytogenetic Diagnosis in Haematological Malignancies", UK.

APL is typified by the t(15;17); this rearrangement leads to the formation of the *PML-RAR $\alpha$*  fusion gene predicting a beneficial response to retinoids and a favourable prognosis. However, it is now clear that a sizeable minority of cases with suspected APL lack the classical t(15;17); this prompted the establishment of a European working party to further characterise this group. Epidemiologic data from participating centres considering 622 cases of APL, demonstrated that the t(15;17) is not identified in 9% patients successfully karyotyped. Such cases (n = 88) were referred to a workshop held in Monza, Italy in June 1997 and subjected to central morphologic, cytogenetic and molecular review; this process excluded 22 cases, considered 7 cases with *ider* (17q) as an additional abnormality and yielded 59 evaluable cases of morphologically confirmed APL lacking the t(15;17). In the majority (42/59), molecular analyses revealed underlying *PML/RAR $\alpha$*  rearrangements and morphologic features of this group did not differ from 20 control APL cases with documented t(15;17). In 13 patients *PML/RAR $\alpha$*  rearrangements resulted from complex or 3-way translocations involving visible abnormalities of chromosomes 15 and/or 17, whilst in the remaining 29 patients molecular and cytogenetic analyses were consistent with insertion events. Metaphase FISH performed in 21 patients demonstrated that insertions most commonly lead to formation of the *PML-RAR $\alpha$*  fusion gene on 15q (16/21); in 4/21 cases *PML-RAR $\alpha$*  fusion occurred on 17q, whilst in the remaining case *RAR $\alpha$ -PML* was the sole fusion gene formed and was localised to 17q. In 9/59 workshop cases, RT-PCR analysis confirmed that APL was associated with a *PLZF/RAR $\alpha$*  rearrangement; in 8 cases the t(11;17)(q23;q21) was identified cytogenetically, whilst in the remaining case cytogenetic analysis revealed del(11)(q23) with no visible abnormality of chromosome 17. In both cases of t(11;17) associated APL in which PML immunofluorescence was performed, a wild type nuclear localisation pattern was observed distinct from the microparticulate pattern seen in cases with formation of the *PML/RAR $\alpha$*  fusion, thereby confirming that disruption of PML nuclear body structures is not a prerequisite for the pathogenesis of APL. Amongst the remaining 8 cases of morphologically confirmed APL, 2 were found to have rearrangements of *RAR $\alpha$*  and the respective fusion partners are presently under characterisation, whilst in 6 cases disruption of *RAR $\alpha$*  was excluded, indicating that alternative molecular pathways could mediate the differentiation block that typifies this disease. In conclusion, this study highlights the importance of combining morphologic, cytogenetic, immunofluorescence and molecular analyses for optimal management of APL patients and better understanding of APL pathogenesis.