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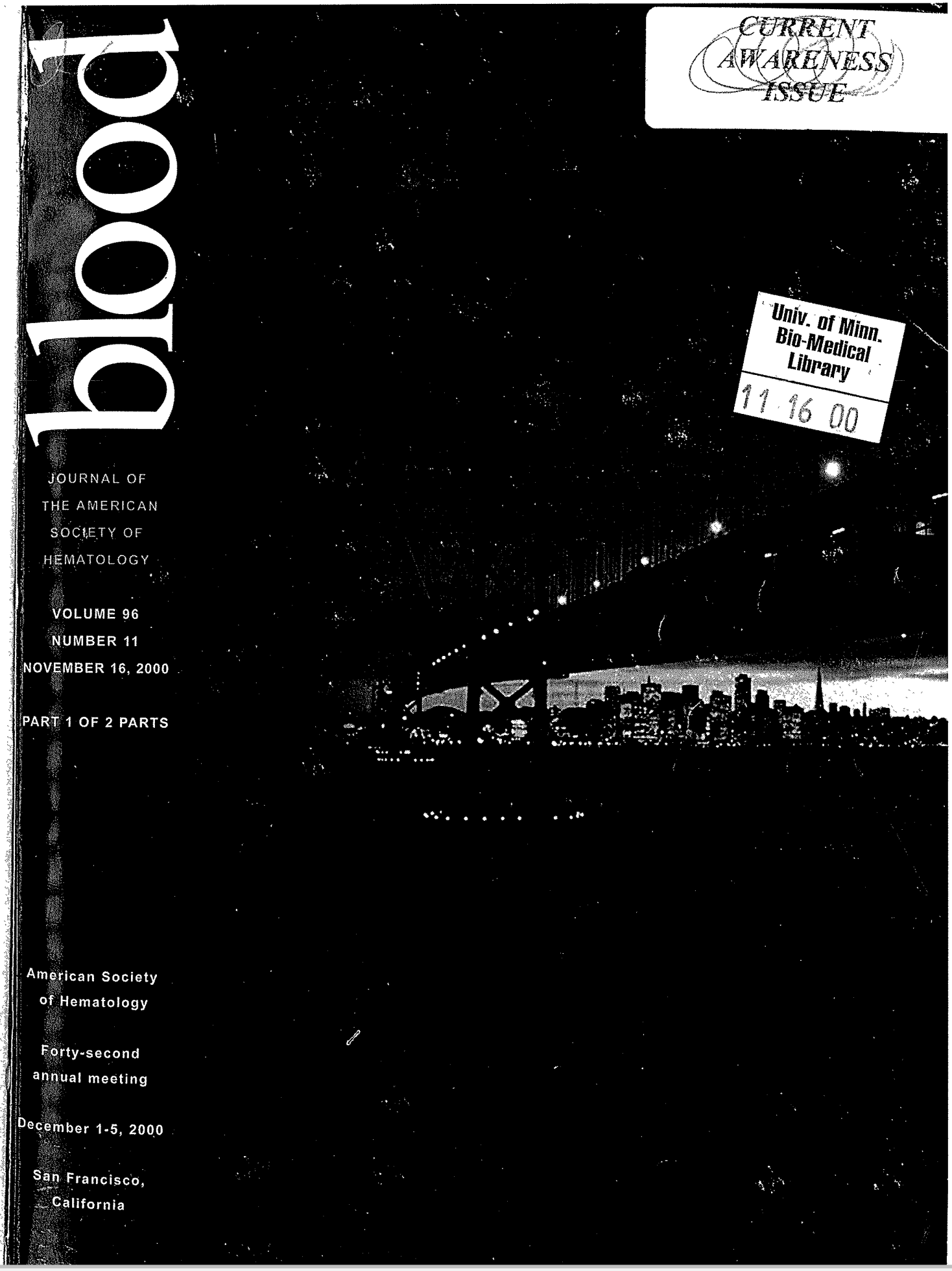

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Abstract# 1310 **Poster Board #-Session: 360-II**

DEREGULATION OF C-MYC GENE EXPRESSION BY THE HUMAN IgH 3' ENHANCER IN BURKITT'S LYMPHOMA. Lu Zhang*, Lina Somsouk*, Linda M. Boxer. *Division of Hematology, Stanford University, Stanford, CA, USA.*

The expression of the c-myc gene is deregulated by the immunoglobulin heavy chain (IgH) 3' enhancers in Burkitt's lymphoma cells with the t(8; 14) translocation. Several studies have examined the activity of the murine IgH enhancers with the c-myc promoter. To study the mechanism of activation of c-myc by the human IgH enhancers, we cloned the human B-cell specific hypersensitive sites (HHS12, HHS3 and HHS4) from the IgH enhancer Cα1 region. Using a reporter system, we tested the activation of the c-myc promoter by these HHS regions. The most 3' enhancer HHS4 showed a B-cell stage specific pattern. It increased c-myc expression 7.8-fold in both orientations in Raji Burkitt's lymphoma cells but less than 1.7-fold in DHL-9 cells. An NF-kB site was responsible for most of the activity of HHS4. Human HS3 in both orientations increased c-myc expression slightly (2.4-fold) in Raji and 1.4-fold in DHL-9 cells. Deletion analysis identified two regions that were active. Several different HHS12 forms have been cloned that differ by the number of copies of a 53-bp motif which contained a putative NF-kB binding site. However, constructs that contained different copy numbers of this repeat (1, 2 and 3 copies) showed no significant difference in activity in both Raji and DHL-9 cells. To further define the regulatory elements within the HHS12 enhancer region, deletion analysis was performed. A region 3' of the 53bp repeat showed a positive effect on transcription of the c-myc promoter. Deletion of this 30bp region decreased the activity to the basal level. UV cross-linking studies showed that two proteins (40 kDa and 80 kDa) bound to the region. EMSA competition studies showed that STAT4 is a potential candidate for binding to this site. 3' deletions also identified two other active regions in HHS12. Strong synergistic interactions were observed among the enhancers HS1,2,3, and 4 with the c-myc promoter. The HHS124 combination yielded a 26-fold, HHS34 combination a 13-fold and HHS123 combination a 7-fold induction in c-myc expression in Raji cells. The combination of all four enhancers showed the highest activity with a 42-fold induction of c-myc promoter activity in Raji cells.

Abstract# 1311 **Poster Board #-Session: 361-II**

DEREGULATION OF NOTCH2 SIGNALLING IN B-CLL. Rainer Hubmann*,[2] Josef Schwarzmeier*,[1,2] Medhat Shehata*,[1,2] Martin Hilgarth*,[1] Rudolf Berger*,[2] Klaus Lechner.[1] *[1]Internal Medicine I, University of Vienna, Vienna, Austria; [2]L. Boltzmann Inst. for Cytokine Res., University of Vienna, Vienna, Austria.*

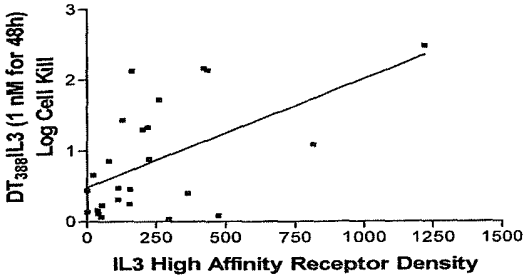
Members of the Notch gene family encode transmembrane receptors that control differentiation and apoptotic programs of many types of progenitor cells including hematopoetic precursors. Activation of Notch by its ligand causes cleavage and translocation of the intracellular domain (NotchIC) to the nucleus, where it activates the transcription factor CBF1. The aim of this study was to elucidate the mechanisms leading to the overexpression of the CD23a isoform in peripheral blood B-cell chronic lymphocytic leukemia (B-CLL) cells which is supposed to be related to B-CLL cell viability. By electrophoretic mobility shift assays (EMSA), we identified a transcription factor complex (C1) which binds to one known and four newly identified putative CBF1 sites in the CD23a proximal promoter. The significance of this complex was highlighted by the fact, that in B-cell samples the intensity of C1 correlated with their respective levels of CD23a expression. Furthermore, using Epstein Barr virus (EBV) infected BL41 cells as a model for CBF1 mediated CD23 expression, C1 was found to be EBV inducible. Since CBF1 is the nuclear target of Notch signaling, we investigated the expression pattern of different members of the Notch gene family by RT-PCR and found that Notch2IC is overexpressed in B-CLL cells. Constitutively active Notch is known to lock cells into an immature state and appears to inhibit apoptosis in certain leukemic cell lines. Therefore, deregulation of Notch2 signaling would be consistant with the nature of B-CLL cells indicating that Notch2 might play a pivotal role in the pathogenesis of this disease.

MOLECULAR PHARMACOLOGY & DRUG RESISTANCE II

Abstract# 1312 **Poster Board #-Session: 362-II**

HIGH AFFINITY INTERLEUKIN-3 RECEPTOR EXPRESSION ON BLASTS FROM PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA CORRELATES WITH CYTOTOXICITY OF A DIPHTHERIA TOXIN/IL-3 FUSION PROTEIN. Richard L. Alexander*, Jason Ramage*, Barbara Klein*, Gregory L. Kucera*, Micheal A. Caligiuri, Clara D. Bloomfield, Arthur E. Frankel. *Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; Pharmacia, St. Louis, MO, USA; Comprehensive Cancer Center, Ohio State University, Columbus, OH, USA.*

We have constructed a fusion protein ($DT_{388}IL3$) in which we fused interleukin-3 (IL3) to the catalytic and translocation domains of diphtheria toxin (DT) (Leukemia 14:576-585, 2000 and Protein Engineering 13:575-581,2000). Previously, we demonstrated that $DT_{388}IL3$ was toxic to a fraction of myeloid leukemia cell lines (Bioconjugate Chemistry 11.564-568, 2000). Next, we wanted to determine the sensitivity of patient leukemic blasts to $DT_{388}IL3$ and factors that may predict cytotoxicity. To this end, IL3 receptor density and $DT_{388}IL3$ sensitivity of acute myeloid leukemia (AML)-colony forming cells (CFC) were measured in leukemic blasts from twenty-five AML patients. The blasts had high affinity (Kd = 16 ± 15 pM n = 25) IL3 receptor densities that ranged from 0 to 1220 receptors per cell and low affinity (Kd = 7060 ± 6938 pM n = 25) receptor densities that ranged from 263 to 18250 receptors per cell. We observed greater than one log cell kill, as measured by percent colony inhibition, in nine of twenty-five AML patient samples. There was a strong correlation between $DT_{388}IL3$ log cell kill and blast high affinity IL3 receptor density ($p = 0.0044$) and no correlation between low affinity receptor density and log cell kill. These results suggest that at least one factor important in the sensitivity of patients' leukemic blasts to $DT_{388}IL3$ is its ability to bind to the cell surface. $DT_{388}IL3$ shows toxicity to patient leukemic blasts as well as cell lines and suggests that the drug merits further preclinical development. In addition, the presence of high affinity IL3 receptors on patients' blasts may predict clinical responsiveness to $DT_{388}IL3$ in clinical trials.



Abstract# 1313 **Poster Board #-Session: 363-II**

THALIDOMIDE (THAL) AND ITS ANALOGS OVERCOME DRUG RESISTANCE OF HUMAN MULTIPLE MYELOMA (MM) CELLS TO CONVENTIONAL THERAPY. Teru Hideshima*,[1] Dharminder Chauhan*,[1] Yoshihito Shima*,[1] Raje Noopur*,[1] Faith E. Davies*,[1] Yu-Tzu Tai*,[1] Steven P. Treon,[1] Boris K. Lin*,[1] Robert L. Schlossman,[1] Paul G. Richardson,[1] Deepak Gupta*,[1] George W. Muller*,[2] David I. Stirling*,[2] Kenneth C. Anderson.[1] *[1]Adult Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; [2]Celgene Corporation, Warren, NJ.*

Although Thal was initially used to treat MM due to its known anti-angiogenic effects, the mechanisms of its anti-MM activity are undefined. In this study, we assessed the activity of Thal, as well as its analogs the Selected Cytokine Inhibitory Drugs (SelCIDs) and Immunomodulatory Drugs (IMiDs), against human MM cells. Like Thal, both SelCIDs and IMiDs inhibit TNFα; SelCIDs are phosphodiesterase 4 inhibitors (PDE4I) which do not enhance T cell activation, whereas IMiDs are not PDE4I and stimulate T cell proliferation. Thal (100 μM) and IMiDs (0.1-1.0 μM), but not SelCIDs, directly induce apoptosis or G1 growth arrest in MM cell lines and patient MM cells which are resistant to melphalan, doxorubicin, and dexamethasone (Dex). Thal and the IMiDs also enhance the anti-MM activity of Dex and are partially inhibited by interleukin-6. IMiDs induced apoptosis associated with decreased p21 expression in MM.1S cells (wild type p53) versus G1 growth arrest associated with increased p21 in Hs-Sultan and patient MM cells (mutant p53). As for Dex and in contrast to gamma irradiation and Fas induced cell death, apoptotic signaling triggered by Thal and the IMiDs in MM cells is associated with activation of related adhesion focal tyrosine kinase (RAFTK). These studies therefore demonstrate direct activity of Thal and IMiDs on MM cells resistant to conventional therapy. Thal and the IMiDs therefore represent a new treatment paradigm targeting both the tumor cell and the microenvironment to overcome classical drug resistance and achieve improved outcome in MM.

Abstract# 1314 **Poster Board #-Session: 364-II**

GROWTH OF ACUTE LYMPHOBLASTIC LEUKEMIA CELLS IS SUPPRESSED BY β-1-ADRENERGIC AGONISTS AND THE NOVEL WATER-SOLUBLE FORSKOLIN NKH477. Ryosuke Ogawa,[2] Artem Bugayenko*,[1] Gregory J. Kato.[1] *[1]Division of Pediatric Hematology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2]First Department of Internal Medicine, University of Occupational and Environmental Health School of Medicine, Kitakyushu, Japan.*

Substantial evidence indicates that elevation of intracellular cyclic AMP suppresses growth and induces apoptosis in thymocytes. Data from our lab and others indicate that cultured T-cell and B precursor acute lymphoblastic leukemia cells exhibit similar sensitivity to elevation of cAMP by phosphodiesterase inhibitors. We have undertaken a survey of adenylyl cyclase activators that might provide together with phosphodiesterase inhibitors synergistic elevation of cAMP. We have previously reported such a synergy of the PDE4 inhibitor rolipram with the model adenylyl cyclase activator forskolin. A congener of forskolin, the water-soluble compound NKH477, is utilized in Japan as an inotropic agent for refractory heart failure. Using a modified MTT assay on the CEM-CCRF model of T-cell ALL, we find that this drug suppresses cell growth with 50% inhibition (IC50) provided by 40 micromolar NKH477. The addition of the PDE4 inhibitor rolipram to NKH477 provides synergistic suppression of cell growth. 20 micromolar NKH477 reverses glucocorticoid resistance in these cells, with a two to three log reduction in the dexamethasone IC50. We also investigated the growth suppressive potential of β-adrenergic agonists, which also can induce activation of adenylyl cyclase. We find that the β-adrenergic agonist isoproterenol and β-1-adrenergic agonist dobutamine potently elicit growth suppression,