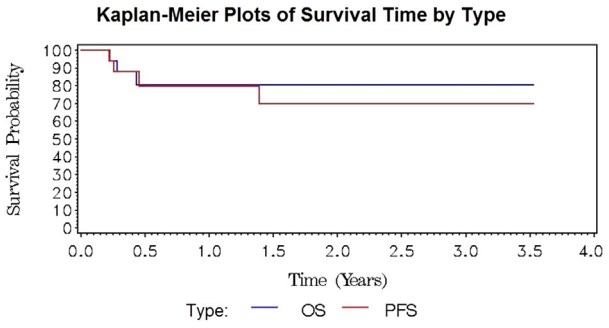

Methods: We retrospectively analyzed the outcomes of all AML patients in CR1 who underwent Allo-SCT with RIC regimen (Flu-Bu2-hATG) at UMass since 2010.

Results: 18 patients (12 males; 6 females) with a median age of 67.5 (range 24-83) years were identified. Twelve (66%) had prior MDS or poor prognostic cytogenetics. Induction chemotherapy consisted of High dose Ara-C (HIDAC)/ Anthracycline (n=17) and Decitabine (n=1). Five patients required reinduction to achieve a CR. Fourteen (78%) received post CR therapy: HIDAC (n=5), HIDAC/Hypomethylating agent (n=3) and Hypomethylating agent alone (n=6). Seven (39%) patients had inadequate recovery of counts (CRi). Median time from diagnosis to SCT was 130.5 (range 33 -384) days. Median Hematopoietic (H) SCT comorbidity index (CI) was 2.5 range (1- 9). SCT donors were sibling (n=2) and unrelated (n=16). Seventeen (94%) patients were 8/8 HLA match and one (6%) was 7/8 HLA match. Stem cell source was peripheral blood (n=17) and G-CSF primed marrow (n=1). RIC dosing was Bu (3.2 mg/kg day x 2), Flu (30mg/m2 x 6) and hATG (20mg/kg x 3). Graft versus Host Disease (GvHD) prophylaxis was calcineurin inhibitor/ mycophenolate-mofetil (MMF) (n=16) and Sirolimus/MMF (n=2).

Median CD34 cells infused were 5 x 10e6/kg range (2.5-6). All patients engrafted with a median time to neutrophil engraftment of 15 days (range 10-22) and a median time to platelet engraftment of 15 days (range 0-21). Non relapse mortality at 100 days was 5.9%. Cumulative incidence of grade 2-4 acute GvHD was 39%. For patients alive beyond 6 months the cumulative incidence of chronic gvhd was 64%. Kaplan Meier estimate of 2 year overall (OS) and progression free survival (PFS) was 80.5 % (95% CI 50.6-93.3%) and 69.9 % (95% CI 36.7-88.0%) The median follow-up for the survivors was 509 (59-1288) days. No patient with HSC-CI < 5 died while all 3 patients with HSCT-CI > 6 expired ($p < .001$). Presence of chronic gvhd was associated with a better PFS (100% versus 32.1%) ($p< .02$) and a tendency for better OS (100% versus 57.1%) ($p= .06$) indicating a preservation of graft versus leukemia effect with this regimen.

Conclusion: Flu-Bu2-hATG RIC regimen has low early mortality and improved PFS and OS for AML patients undergoing Allo-SCT in CR1. The regimen should be evaluated in a prospective clinical trial.

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Synergistic Cytotoxic Effect of the PARP Inhibitor ABT888 and Busulfan in Acute Leukemia Cell Lines

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The alkylator Busulfan (Bu) is used in conditioning regimens for acute leukemia prior to allogeneic stem cell transplant. Multiple DNA repair mechanisms including mismatch repair and base excision repair have been implicated in resistance to Bu. The enzyme PARP is central to base excision repair. We hypothesized that treatment of acute leukemia cell lines with both ABT888 (Veliparib), an inhibitor of PARP 1 and 2, and Bu would lead to synergistic cell kill and that this effect is maximal in mismatch repair deficient cells.

Two mismatch repair proficient cell lines (K562 and HL60) and 2 mismatch repair deficient cell lines (NB4 and REH) were treated with ABT888 alone, Bu alone or a combination of both. In single drug experiments, doses of drug treatment ranged from 0-400mcg/ml. In combination experiments a fixed dose of ABT888 of 1.25mcg/ml was utilized with Bu doses varying from 0-200mcg/ml. This dose of ABT888 was chosen as it approximated to patient blood levels in clinical trials. After 24 hours of treatment, cells were washed and resuspended in fresh medium. Proliferation of cells was measured by standard 3H-thymidine uptake assay at 48 hours. Sigmoidal dose response curves and GI50 values were then calculated. In addition, cells were tested for apoptosis by flow cytometry using activated caspase 3 and annexin/ PI staining at 24 and 48 hours after treatment.

All 4 cell lines were found to be resistant to single agent ABT888. Despite mismatch repair deficiency in REH cells, therapeutic doses of ABT888 did not cause significant decreases in proliferation. The effect of ABT888 was, as expected, much less evident in the mismatch repair proficient K562 cells. These cells were also relatively resistant to single agent Bu in vitro. The combination of Bu and ABT888 was synergistic in all cell lines with GI50 (micromoles/ml) for Bu decreasing from 67.8 to 45.7 in K562, from 23.3 to 8.0 in HL60, from 46.6 to 36.1 in NB4 and from 34.4 to 17.0 in REH cells. The Combination Index was <1 in all cell lines indicating synergy. Dose Reduction Index, indicating the factor by which the dose of Bu can be decreased to achieve the same treatment effect size, ranged from 1.45 to 3.1. The synergistic effect was greatest in the mismatch repair deficient cell line REH (Combination Index 0.53, Dose Reduction Index 3.1). As expected the synergistic effect observed did not correlate with increased apoptotic death of leukemic cells.

To our knowledge, this is the first study to show synergy of a clinically available PARP inhibitor with Bu. We believe this data warrants further study with the potential clinical application of increasing the anti-leukemic effect of stem cell transplantation conditioned with Bu containing preparative regimens.

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Extended Dose-Total Body Irradiation (18Gy) Followed By an Allogeneic Cell Transplantation for the Treatment of Refractory Acute Myeloid Leukemia: Early Results

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Introduction: Overall survival (OS) after a standard allogeneic hematopoietic cell transplantation (alloHCT) for refractory

AML (rAML) is poor (<20%). Higher TBI doses have been shown to reduce AML relapse for patients in CR1. Cyclophosphamide in the conditioning is suspected of contributing to significant toxicity. Preliminary work with high doses of TBI (16Gy), without cyclophosphamide, followed by an HLA matched related or unrelated donor (MRD or MUD)-alloHCT has resulted in long-term remissions in 2 out of 4 patients. Based on these encouraging results we initiated and report on the preliminary results of a phase 2 study of single agent extended dose TBI (ED-TBI) (18Gy).

Methods: Patients (18-60 years old) with rAML and an HLA MRD or MUD received an alloHCT after ED-TBI, 2.25Gy BID (days -4 to -1) x 8 fractions (total 18Gy). Donor cells were infused on day 0. Tacrolimus (day -3) and mycophenolate mofetil (MMF) (day 1) were used for graft vs. host disease (GVHD) prophylaxis. Toxicity was measured using the CTCAE v.4 and the LENT-SOMA scales. The primary end point was treatment related mortality (TRM). Secondary endpoints included: engraftment, morbidity/mortality at 30, 100 and >180 days, incidence of GVHD, relapse rate and one year progression-free (PFS) and OS.

Results: Patients: Five patients have been enrolled. One patient had rAML following two induction attempts. Another patient had rAML (secondary) after one induction attempt and three had relapsed (<6 months) rAML. All had persistent leukemia at the start of ED-TBI, with 4 out of 5 having > 50% blast cells in the marrow.

Toxicity: <30 days, 2 patients had grade 3 mucositis. Two patients required parenteral feeding. One patient experienced reversible veno-occlusive disease. All patients developed diarrhea. Two patients suffered from grade 3 dehydration, requiring prolonged intravenous hydration (days 37-107).

Engraftment: Neutrophil engraftment occurred between days 15-25 and the patients were discharged from the hospital between days 19-30.

GVHD: Immunosuppression was completely tapered at day 101 in 1 patient. One patient continues to taper off at day 217, due to persistent chronic GVHD (cGVHD) and 3 did not complete tapering before relapse. Acute GVHD occurred in 1 patient <day 30, treated with prednisone. cGVHD occurred in all patients >day 100, involving the GI tract (n=2) and liver (n=2). One patient has persistent cGVHD beyond 4 months involving the mouth and lungs.

Response: Of the 4 patients who had circulating blast cells, all were cleared by day 1. Two patients are in continuous CR on days 217 and 338. Three patients have relapsed at days 96, 110 and 139 and died on days 106, 145 and 257, respectively.

Conclusion: rAML is controlled by ED-TBI followed by an alloHCT. The toxicity of ED-TBI is acceptable and comparable to other alloHCT conditioning regimens. Safety and efficacy are continuing to be assessed with a goal to recruit 20 patients.

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Synergistic Cytotoxicity of the Multikinase Inhibitor Sorafenib with the DNA Alkylating Agent Busulfan, and Nucleoside Analogs in Human FLT3-ITD-Positive Acute Myeloid Leukemia Cell Lines

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The nucleoside analogs clofarabine (Clo) and fludarabine (Flu), together with the DNA alkylating agent busulfan (Bu), are commonly used as part of the pre-transplant conditioning regimen in allogeneic hematopoietic stem cell transplantation (allo-HSCT). The multikinase inhibitor sorafenib (Sor) has clinical activity in FLT3-ITD-positive acute myeloid leukemia (AML). We previously reported that a combination of [Bu+Clo+Flu] has a synergistic cytotoxicity in AML cells. We now hypothesized that Sor, if combined with [Bu+Clo+Flu], will further enhance this cytotoxicity. We exposed the FLT3-ITD-mutated AML cell line MV4-11 to low concentrations of Bu (1 μg/ml), Clo (10 nM), Flu (2.5 μM) and Sor (1 nM) alone or in various combinations. Exposure of the cells to Bu, Clo, Flu or Sor alone, or to two-drug combinations [Bu+Clo], [Bu+Flu] and [Clo+Flu], did not significantly affect cell proliferation or apoptosis relative to control cells. The combination of [Bu+Clo+Flu] resulted in 20% inhibition of cell proliferation and demonstrated 10% increase in apoptosis. Addition of Sor to the [Bu+Clo+Flu] mixture further enhanced the inhibition of proliferation by 60%, and increased apoptosis to 50%, suggesting synergistic cytotoxicity. Biochemical analyses suggest that this cytotoxicity may be attributed to (1) activated DNA-damage response (2) histone 3 modifications, (3) inhibition of different kinases, and (4) activation of the intrinsic apoptosis pathway. The phosphorylation of kinases, including FLT3, MEK and AKT, was significantly inhibited when cells were exposed to [Bu+Clo+Flu+Sor]. FLT3-ITD-activated STAT5 and its target gene *Pim 2*, a serine threonine kinase, were both down-regulated when cells were exposed to Sor alone, [Bu+Clo+Flu] and [Bu+Clo+Flu+Sor]. The level of the pro-apoptotic protein PUMA increased, while the pro-survival proteins MCL-1 and Bcl-xL were down-regulated when cells were exposed to the four-drug combination. The levels of phosphorylated and total P53 increased in the mitochondria when cells were exposed to [Bu+Clo+Flu] or [Bu+Clo+-Flu+Sor]. The changes in the levels of these proteins involved in mitochondrial control of apoptosis may consequently cause mitochondrial outer membrane permeabilization (MOMP). The mitochondrial membrane potential (MMP), a marker of MOMP, decreased by 20% and 60% when cells were exposed to [Bu+Clo+Flu] and [Bu+Clo+Flu+Sor], respectively. This drug-mediated decrease in MMP may have caused leakage of cytochrome *c*, SMAC/DIABLO and AIF from the mitochondria to the cytoplasm and/or nucleus, leading to caspase activation, nuclear fragmentation and cell death. These results provide a mechanistic basis for investigating the addition of sorafenib in future clinical trials of (double) nucleoside analog-busulfan combinations in pre-transplant conditioning therapy for patients undergoing allo-HSCT for FLT3-ITD positive AML.

LYMPHOMA/MULTIPLE MYELOMA

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High-Dose Chemotherapy and Autologous Hematopoietic Cell Transplantation Early during the Course of Disease Appears to Improve Outcomes of Patients T Cell Non-Hodgkin Lymphoma: Results of a Single-Institution Experience

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