Acute Myeloid Leukemia (AML) not in remission at time of HSCT carries a very poor prognosis. We previously reported a single institution experience indicating significant activity for myeloablative clofarabine/busulfan conditioning (CloBu4) in refractory hematologic malignancies, particularly in AML (Blood. 2011 Oct 13; 118(15)). In an effort to better establish efficacy of HSCT with CloBu4 for AML not in remission we initiated a multi-center phase II study with a target accrual of 75 pts. Only pts with active AML defined as >5% myeloblasts on bone marrow examination within 14 days of registration were eligible. AML was required to be refractory to two lines of intensive induction, one line of induction following relapse (CR > 6 months), or untreated relapse (CR < 6 months). Pts receiving previous HSCT were excluded. Busulfan was administered as a single daily dose of 3.2 mg/kg IV x 4d (days -5 to -2) targeting a plasma AUC of 4500-5500 μM.min/day and clofarabine as a single daily dose of 40 mg/m2 IV x 5d (days -6 to -2). An 8/8 HLA matched related or unrelated donor was required. GVHD prophylaxis was per institutional preference, but included calcineurin inhibitor together with either mycophenolate mofetil or methotrexate. Twelve centers across US and Canada are participating. At time of interim analysis there were 60 pts with 56 evaluable for toxicity and response with additional pt accrual expected at time of abstract submission. One pt enrolled but withdrew before conditioning. Pts had median age of 56 yrs (range: 3-65 yrs), received a median of 2 inductions and had a median of 26% bone marrow blasts (range: 5-94%) within 14 days of enrollment. Matched unrelated donors were utilized in 46% of pts. Pts engrafted at a median of 13 days (range: 9-25) for neutrophils and 16 days (range: 8-72) for platelets. The most frequent grade 3-4 non-hematologic toxicities reported by day + 100 as related to conditioning were infections (N=6; 11%) and aGVHD (N=4; 7%). Grade 5 non-hematologic toxicities possibly related to conditioning include neutropenic enterocolitis (N=1), aGVHD (N=1), pneumonia (N=1), alveolar hemorrhage (N=1), ascites/veno-occlusive disease (N=1), and metabolic acidosis / sepsis (N=1). Morphologic remission was observed 92% of pts with response assessment at day + 30 (N=53). The cumulative incidence of aGVHD grades II-IV by day + 100, accounting for relapse and death as competing risks, was 37% [95% CI: 24, 50%]. Non-relapse mortality and relapse at one year was 25% [95% CI: 11, 38%] and 32% [95% CI: 27, 58%], respectively. The estimated one year OS and DFS are 39% [95% CI: 24, 64%] and 32% [95% CI: 20, 52%], respectively. In summary, preliminary analysis of a multi-center study of CloBu4 conditioning in AML not in remission exhibits impressive early responses with NRM and relapse incidence similar to previous trials utilizing this regimen.

221 Induction of a CD8+ T Cell Response to Tumor Antigens Is Associated with Improved Survival in Patients Transplanted for Acute Myeloid Leukemia
Sanjeev Nagra1, Oliver Charles Goodyear2, Josephine Khan3, Nadira Yasmin Jilani4, Paul Ferguson4, Nigel Russell5, Mike Dennis6, Paresh Vyas7, Shamala Siddique8, Charles Croadock2,4, Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, United Kingdom; 2Department of Immunology, University of Birmingham, Birmingham, United Kingdom; 3Cancer Research UK Clinical Trials Unit, University of Birmingham, Birmingham, United Kingdom; 4School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom; 5Nottingham City Hospital, Nottingham, United Kingdom; 6Department of Haematology, The Christie Hospital, Manchester, United Kingdom; 7MRC Molecular Haematology Unit and Department of Haematology, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

Disease relapse and graft-versus-host disease (GVHD) are the major causes of treatment failure after allogeneic stem cell transplantation (SCT). The DNA methyltransferase inhibitor azacitidine (AZA) possesses significant anti-tumor activity in myelodysplasia and acute myeloid leukemia (AML). Recently its administration post-transplant has been shown to both induce a CD8+ T cell response to a broad range of tumor antigens and augment reconstitution of T regulatory cells. We therefore prospectively studied the impact of post-transplant AZA on the outcome of patients allografted for AML. 37 patients were transplanted using an alemtuzumab based fludarabine/melphalan conditioning regimen. 36 mg/m2 of AZA was administered for five consecutive days at four weekly intervals until 12 months post-transplant. AZA was commenced at a median of 54 days (range 40-194) post-transplant and was well tolerated. 31 patients completed three or more cycles. 16 patients relapsed at a median time of 8 months post-transplant. No patient developed chronic extensive GVHD. AZA induced a CD8+ T cell response to tumor-specific peptides in 16 of the 31 patients who received more than three treatment cycles. No such response was observed in matched controls who received no post-transplant AZA. Induction of a CD8+ T cell response was associated with an increased one year overall survival of 81% versus 41% in patients with no CD8+ T cell response (p= 0.02). An increased number of T regulatory cells was detected in 16 patients compared with matched controls. In conclusion administration of AZA post-transplant is associated with a low incidence of GVHD and induces a CD8+ T cell response to tumor antigens which correlates with a reduced risk of relapse. These data demonstrate the ability of AZA to improve the outcome of allogeneic SCT, potentially through epigenetic manipulation of the alloreactive response, and require validation in a randomised clinical trial.

222 Allogeneic (Allo) Stem Cell Transplantation (SCT) for Acute Myeloid Leukemia (AML) in First Complete Remission (CR1) with a Reduced Intensity Conditioning (RIC) of Fludarabine/Flu (Bu2) and Horse ATG (hATG): The UMass Experience
Rajneesh Nath1, Jan Cerny2, Mythologu Ramanathan3, Glen Raffel4, Mridula George5, Zeina Al-Mansour4, Laura Petrello-Deluca6, 7Tzafra Martin7, Zankar Desai8, Jayde Bednarik9, Aimee Kroll-Desrosiers10, Alan Rosmarin11, 1Hematology/Oncology, Section BMT, UMass Memorial Medical Center, Worcester, MA; 2Division of Hematology/Oncology, University of Massachusetts, Worcester, MA; 3Hematology/Oncology Section BMT, UMass Memorial University Campus, Worcester, MA; 4Hematology/Oncology Section BMT, UMass Medical Center, Worcester, MA; 5Hematology/Oncology, University of Massachusetts Medical Center, Worcester, MA; 6Hematology/Oncology, UMass Memorial Medical Center, Worcester, MA; 7Hematology/Oncology Section BMT, UMass Memorial Medical Center, Worcester, MA; 8Stem Cell Laboratory, University of Massachusetts Medical Center, Worcester, MA; 9Pharmacy, UMass Memorial Medical Center, Worcester, MA; 10University of Massachusetts, Worcester, MA; 11Hematology/Oncology, UMass Memorial, Worcester, MA

Background: AML patients are frequently consolidated with Allo-SCT in CR1. There is no consensus about the regimen intensity (RIC versus ablative) in this setting.
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.

224

Extended Dose-Total Body Irradiation (186Gy) Followed By an Allogeneic Cell Transplantation for the Treatment of Refractory Acute Myeloid Leukemia: Early Results

Mitchell Sabloff1,2,3, Sultan Alturki1,3, Harold Atkins1,2,3, David S. Allan1,2,3, Jason Tuy1,2, Mai Le1,2, Linda Hamelin1,2, Rajiv Samant1,2, Dawn Sheppard1,2,3, Lothar H. Huebsch1,2,3, Tim Ramsay1,2, Christopher N. Bredeson1,2,3, 1 The Ottawa Hospital Research Institute, Ottawa, ON, Canada; 2 The University of Ottawa, Ottawa, ON, Canada; 3 Clinical Epidemiology, Ottawa Hospital Research Institute, Ottawa, ON, Canada.

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

223

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

Pritesh Patel, Dolores Mahmud, Annie Oh, Damiano Rondelli. Department of Medicine, Section of Hematology-Oncology, University of Illinois Hospital & Health Sciences System, Chicago, IL.

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)/ Anthraccline (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 morbidity 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 10^6/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%. 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).

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.

Kaplan-Meier Plots of Survival Time by Type

Survival Probability

Time (Years)

Type: OS PFS