p = 0.27). Cause of death was relapse 23%, aGVHD 2%, cGVHD 1%, infection 2%, MSOF/ ARDS 3% and other 2%. In conclusion: the use of t-Bu/Flu in AML patients, with a daily AUC targeted at 5300 ±10% is a tolerable regimen with low NRM. It is also well tolerated regardless of age. Patients in a first CR, even those with URC, have very good outcomes. Relapse is still an issue in these AML patients and post transplant therapy should be considered, particularly for patients not in remission at the time of transplant. Currently we are assessing the maximum tolerated AUC of Bu in an ongoing trial.

168

SEQUENTIAL THERAPY WITH NONABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION, POST TRANSPLANT IMATINIB AND DONOR LYMPHOCYTE INFUSION FOR CHRONIC MYELOID LEUKEMIA

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Allogeneic stem cell transplantation (ASCT) is a potentially curative therapy for patients(pts) with CML. Its use has declined because of the favorable results of tyrosine kinase inhibitors (TKIs), and concerns regarding toxicity and GVHD. The efficacy of ASCT is largely due to the immune graft-vs.-leukemia effect. Nonmyeloablative conditioning has reduced toxicity, and post transplant treatment with imatinib and donor lymphocyte infusions can produce durable molecular complete remissions in pts with residual disease. We prospectively studied use of a reduced intensity preparative regimen involving fludarabine, busulfan and Thymoglobulin followed by ASCT from an HLA identical or one antigen mismatched related or unrelated donor. Pts who do not achieve molecular CR (mCR) after 3 mo received TKI treatment. Those without mCR after an additional 3 mo received escalating doses of donor lymphocyte infusion (DLI). 42 pts, median age 42 yrs (range 14–69) were entered. All were previously treated with imatinib, but with detectable disease. 20 had early disease (chronic phase or isolated clonal evolution) and 22 had advanced CML (prior accelerated or blast phase). 4 early and 9 advanced pts had a cytogenetic CR (CyCR) at the time of transplant. The regimen was well tolerated without life-threatening toxicity except for one pt with reversible transplant related microangioapathy. Two pts had graft failure, one required a second transplant and the other had autologous recovery with Ph- hematopoiesis. Only one pt died within 100 days post transplant. At 3 months, all of the chronic phase pts and 21 of the 18 advanced pts achieved CyCR and 7 early and 5 advanced pts achieved a mCR. 22 pts received treatment with TKIs post transplant; mCR was achieved in 7 of the 9 early pts and 2 of 13 advanced pts. 16 pts subsequently received DLI; 2 of 2 pts with early disease achieved mCR with one other pt too early to evaluate; 2 of 13 advanced pts have a mCR. 31 pts are alive. This includes 18 of 20 (90%) transplanted with early disease, (all in cCyR 13 in cMR) and 13 of the 22 (59%) with advanced disease (7 in cCyR and 9 in cMR). This sequential strategy of nonmyeloablative ASCT, post transplant TKIs, and DLI is associated with a low treatment related mortality and deserves further study to produce durable mCR in patients with a suboptimal response to imatinib. Most favorable results were achieved in pts without overt transformation.

169

REDUCED INTENSITY CONDITIONING (RIC) REGIMEN FOLLOWED BY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN ADULT PATIENTS (PTS) WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: RIC regimens are less myelosuppressive, but still allow for successful engraftment with acceptable treatment-related mortality (TRM) in frail pts who otherwise would not be suitable candidates for HSCT. This is particularly relevant in ALL, since pts often sustain toxicity from dose-intense regimens, or may be diagnosed in advanced age. The antitumor effect of this approach is not well-established in ALL.

Methods: We evaluated 30 ALL pts (19 M/11 F) treated from 1996 to 2008 with fludarabine 120 mg/m^2, melphalan 140 mg/m^2 and unmanipulated stem cells. GVHD prophylaxis consisted of tacrolimus and mini-methotrexate. Anti-thymocyte-globulin was added to matched unrelated pts.

Results: Median age was 44 years (range 23–64). ECOG status at time of HSCT was 0 (n = 16), 1 (n = 10) or 2 (n = 4) with median co-morbidity score of 3 (range 0–7) by Charlson Comorbidity Index. 24 pts had B-lineage and 6 had T-lineage disease. Cytogenetic data were available for 26 pts; 19 had high-risk cytogenetics, including 9 with Ph+ disease. Disease stage at time of study entry was CR1 (n = 5), ≥CR1 (n = 12), or primary or refractory relapse (n = 13), with median 2 prior chemotherapy regimens (range 1–4); five pts had a prior allogeneic HSCT. Donor type was matched related (n = 13) or matched unrelated (n = 17) and stem cell source was bone marrow (n = 14) or peripheral blood (n = 16). The median total nucleated cell dose and CD34+ cell dose were 3.80×10^8 cells (range 0.68 – 17.16) and 4.15×10^6 cells (range 1.78 – 12.03), respectively. Median time to ANC 0.5×10^9/L was 13 days (range 10–24). Median time to platelet count 20×10^9/L was 18 days (range 10–57). Eight pts were alive at a median follow up of 12 months from HSCT (range 3–59). OS and DFS were 32% and 29%, respectively, at 1 year. Of note, only 1 among 5 pts in CR1 had disease progression, compared to 8 among 13 with refractory disease at time of HSCT. The cumulative incidence of acute GVHD, grades II-IV and III-IV were 40% and 13%, respectively, and chronic GVHD was 22% (7% for extensive). The cumulative incidence of TRM at 100 days and 1 year were 17% and 33%, respectively. There were 22 deaths: recurrence 14, infection 4, GVHD 4.

Conclusion: RIC HSCT can provide disease control in patients with ALL, and merits further evaluation. Alternative treatment strategies need to be explored in pts with advanced disease. The observed TRM rate is comparable to previously reported rates in in heavily pre-treated leukemia patients.

170

EPIGENETIC REGULATIONS INFLUENCE CML CELL SENSITIVITY TO BUSULFAN: RELEVANCE TO PRETRANSPLANT CONDITIONING THERAPY

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Busulfan (Bu) is a DNA-alkylating drug which is commonly used in myeloablative pretransplant conditioning therapy in patients with CML. A major obstacle to successful treatment is inherent or acquired cellular Bu-resistance. Knowing that DNA hypermethylation may contribute to drug resistance, we hypothesized that cellular Bu-resistance can be reversed by altered epigenetic regulation. We established a Bu-resistant CML cell line B5/Bu250^6 which is 4-fold more resistant to Bu than the parental B5 cells but 4-fold collaterally sensitive to 5-aza-2'-deoxycytidine (DAC). Real-time PCR analysis of the expressions of known DNA methyltransferases showed upregulation of *DNMT3B* in B5/Bu250^6 cells relative to the parental B5 cells without significant changes in *DNMT1*, *DNMT2*, and *DNMT3A*. This result suggests that B5/Bu250^6 cells might be addicted to DNMT3B to epigenetically regulate expression of genes involved in Bu resistance; exposure to DAC might inhibit DNMT3B enzyme and sensitize cells to Bu. Indeed, exposure to DAC synergistically increased Bu-mediated cytotoxicity in B5/Bu250^6 cells as evaluated by the MTT assay and median-effect analysis (combination index < 0.5). The DAC-induced sensitivity to Bu of B5/Bu250^6 cells was associated with PARP1 cleavage, phosphorylation of histone 2A and activation of caspases 3, 8 and 9 suggesting induction of apoptotic response. Real-time PCR and immunostaining analyses of the expressions of various viability-related genes, which are known to be epigenetically regulated, showed significant increase in the expression of *CDKN2A* (*p16^{INK4A}*) and *XAF1* (X-linked inhibitor of apoptosis protein (XIAP)-associated factor 1) genes in B5/Bu250^6 cells exposed to DAC. Analyses of their promoter regions by methylation-specific PCR showed a significant decrease in their methylation status which correlates with the alteration in their gene expression in the presence of DAC. The