(n=3). Adult donors were HLA-matched related (n=9), HLA-matched unrelated (n=7), HLA-mismatched unrelated (n=4). Conditioning was myeloablative in 22 patients and reduced intensity conditioning in 6.

Results: All patients except one engrafted. The rate of grade II-IV acute graft-versus-host disease (GVHD) at 100 days was 26% (95%CI 11-44%). The rate of chronic GVHD at 2 years was 8% (95%CI 1-24%). The cumulative incidence of relapse/progression and non-relapse mortality at 2 years was 8% (95%CI 1-22%) and 12% (95%CI 3-29%), respectively. As of July 2013, with a median follow-up among survivors of 26 months (range 2-134 months), 22 of 27 HCT recipients were alive; 2 died of relapse, 1 of GVHD and 2 of multiorgan failure. At 2 years, OS for patients receiving a HCT was 80% (95% CI 58-91%) and PFS was 80% (95%CI 58-91%) (Figure 1).

Conclusions: Our data indicate that HCT represents an important salvage therapy associated with extremely favorable outcomes for patients with relapsed CBF AML and for those with high risk features at presentation. Additional studies in larger patient cohorts are needed to determine the optimal transplant strategy for this group of patients.

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Relapse after Allogeneic Hematopoietic Stem Cell Transplantation (HCT) for Acute Myeloid Leukemia (AML)/Myelodysplastic Syndrome (MDS) Following Intravenous Busulfan Plus Fludarabine Based Conditioning: Outcomes and Monocyte Chemo-Attractant Protein -1

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Introduction: Reduced and full intensity conditioning (RIC, FIC) regimens employing intravenous (i.v.) busulfan plus fludarabine (Bu-Flu) have resulted in improved treatment related mortality and comparable overall survival in patients undergoing allogeneic HCT for AML/MDS who are not candidates for more intense regimens. However, relapse after HCT remains a leading cause of treatment failure after such conditioning regimens.

Methods: In Order to assess relapse following allogeneic HCT for AML/MDS , a retrospective analysis was performed to evaluate the outcomes of 55 consecutive patients with AML/MDS (49/6) who received i.v. Bu-Flu based conditioning. Blood samples were collected post HCT in a subset of those patients (30 patients). Serum values of 42 biological markers were measured at day 30 post HCT (2/30 patients were day 60 samples) using multiplex Luminex assay. Patients characteristic are shown in Table-1. Patients received single daily dose of iv Bu 3.2 mg/kg for 2 days (RIC, Bu2-Flu) or 4 days (FIC, Bu4-Flu) based on age, older or younger than 65 respectively. Fludarabine was given as a single daily dose of 40 mg/Kg for 4 days. Graft versus host disease prophylaxis was Tacrolimus/Methotrexate in FIC recipients and Tacrolimus/Mycophenolate in RIC recipients. Low dose thymoglobulin of 4.5 mg/kg was used in unrelated donor HCT recipients.

Results: With a median follow up of 18 month, the overall survival (OS) at 1 & 2 years was 73±6% and 67 ± 7%, respectively, (Fig 1). Similarly, disease free survival at 1 and 2 years was 64 ± 7%. As expected, there was low cumulative incidence of treatment related mortality of 8 ± 3% at 1 and 2 years while the cumulative incidence of relapse was 28.0 ± 3% and 31± 2% at 1 and 2 years respectively, (Fig 2). Cumulative incidence of grade II-IV acute GVHD was 54% with grade III-IV of 25% at day 100. Cumulative incidence of chronic GVHD was 49, 54% at 1 and 2 years respectively. In a subset of patients where chemokine analysis was performed (30 patients), only MCP-1 levels at day 30 post HCT were predictive of relapse out of the 42 biological markers tested. The 7 out of 30 patients who relapsed in this subset (23%) had higher mean level of MCP-1 at day 30 of 537, SD ±213 versus 324, SD ± 160, *P=0.007*, (Fig 3). MCP-1 was predictive of leukemic relapse 82 days in advance on average prior to overt hematological relapse. Full chimerism (>95%) was detected at Day 30 in 5/7 patients who relapsed in the biological marker group.

Conclusion: Bu-Flu based conditioning regimens result in improved OS in patients with AML/MDS but do not impact relapse rate after allogeneic HCT. Serum MCP-1 levels in the early post-transplant period were predictive of relapse in subset of patients where post HCT biomarkers were available. Future larger studies may find potential role of MCP-1 in

Table 1

Patient Characteristics	n, (%)
Age, median (range), years	58 (26-73)
Patient 55 years of older	30 Patients (54.5%)
Gender	27 Female, 28 Male
Donor type	
Unrelated donor (MUD)	39 (71%)
Related donor (RD)	16 (29%)
Conditioning Regimen	
Bu4Flu (FIC)	36 (65%)
Bu2Flu (RIC)	19 (35%)
Status at transplant	
CR1	36 (65%)
CR2	5 (9%)
PIF	14 (35%)
CIBMTR risk	
Low	39 (71%)
Intermediate	4 (7%)
PIF	14 (22%)
Cytogenetic risk	
Low	33 (60%)
High	22 (40%)

CR=complete remission, PIF=primary refractory

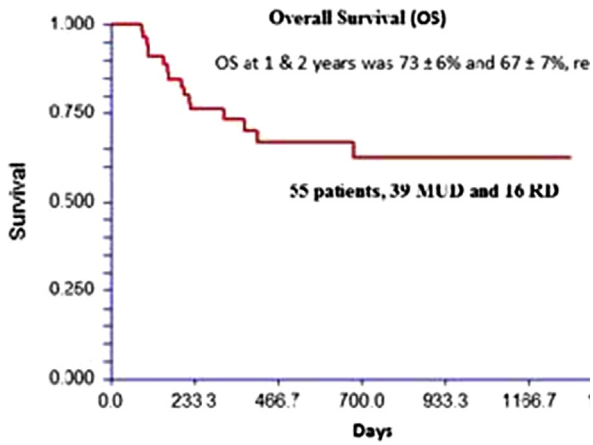

Fig 1.

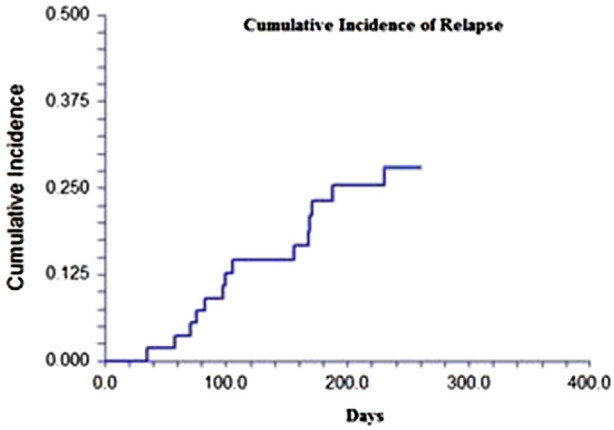

Fig 2.

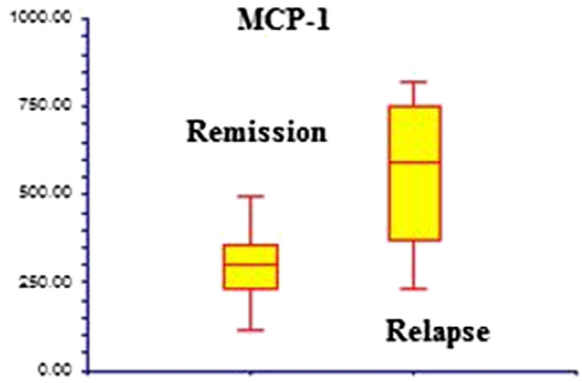

Fig 3.

predicting relapse in patients at risk after HCT with Bu-Flu for AML/MDS.

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A New Class of Antigen T-Cells That Redirect Bystander T-Cells to CD19 Positive Malignancies

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Background: Immunotherapy with T cells expressing chimeric antigen receptors (CARs) has shown promise for the immunotherapy of CD19+ malignancies in early clinical studies. However, clinical efficacy depends on significant *in vivo* expansion of adoptively transferred T cells, which can be difficult to achieve. Genetically modifying T cells with bispecific T-cell engagers, which are able to recruit other T cells locally, amplifying antitumor effects, could potentially overcome this problem. Consistent and prolonged synthesis of engagers by T cells should also be superior to the intermittent direct infusion of the protein, both because these molecules have short half-lives and do not accumulate at tumor sites. The goal of this project was to generate T cells secreting CD19-specific T-cell engagers (CD19-ENG T cells) and to evaluate their effector function in vitro and in vivo.

Methods: A CD19-specific T-cell engager gene, consisting of two single chain variable fragments specific for CD3 and CD19, was synthesized and subcloned into a SFG retroviral vector in front of an IRES and mOrange. CD19-ENG T cells were generated by retroviral transduction and we determined their effector function in coculture and cytotoxicity assays, and in the Ph+ ALL BV173/xenograft model.

Results: Post transduction 50-60% of T cells were positive for transgene expression. In coculture assay CD19-ENG T cells recognized CD19+ lymphoma (Daudi, Raji) and acute leukemia (BV173) cells as judged by IFN-γ and IL-2 secretion in contrast to CD19- K562 cells. None of the targets were recognized by non-transduced (NT) T cells or T cells secreting engagers specific for an irrelevant antigen (EphA2-ENG T cells). Antigen-dependent recognition was confirmed in standard cytotoxicity assays. In transwell assays containing inserts that do not allow T-cell migration, only CD19-ENG T cells redirected NT T cells in the bottom well to CD19-positive tumor cells, demonstrating the ability of a diffusible product from CD19-ENG T cells to redirect NT T cells to CD19-positive tumor cells. To assess the anti-tumor activity of CD19-ENG T cells in vivo we used BV173 cells that were genetically modified with fire fly luciferase (ffLuc; ffLuc-BV173) to allow for serial bioluminescence imaging. NSG mice were injected iv with ffLuc-BV173 cells, and received an iv dose of CD19-ENG or EphA2-ENG T cells and an ip dose of IL2 on days 7, 14, and 21 post leukemia cell injection. Untreated mice served as controls. CD19-ENG T cells had potent anti-leukemia activity in contrast to EphA2-ENG T cells resulting in a significant survival advantage of treated animals.

Conclusions: We have generated CD19-ENG T cells with the unique ability to direct bystander T cells to CD19+ malignancies. CD19-ENG T cells had potent anti-leukemia activity, and may present a promising alternative to current CD19-targeted immunotherapy approaches.