not in CR at SCT. Randomized studies are needed to determine the role of RIC in MDS/AML pts in CR at SCT. The novel mod-MAC regimens are relatively well tolerated even in pts not eligible for MAC and may be more effective than standard RIC in refractory disease.

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ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (HCT) FOR PROLYMPHOCYTIC LEUKEMIA (PLL)

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Although PLL is indolent in some people, most affected persons die within 1 year. Several case reports suggest a benefit to allogeneic HCT. We sought to determine if this hypothesis is correct in the observational database of the Center for International Blood and Marrow Transplant Research. We retrospectively reviewed data from 47 subjects transplanted between1995 and 2006. Median age was 54 y (range, 30–75 y). Karnofsky performance score was >80% in 30 (75%). Immunophenotype was B-cell in 11 (23%), T-cell in 21 (45%), and unknown in 15 (32%). Six patients (13%) had a splenectomy and 16 (36%) were in complete remission (CR) at the time of transplant. Donors were HLA-matched siblings or well-matched unrelated donors in 34 (70%). Conditioning regimens included high-doses of radiation or busulfan in 19 (40%), low-doses in 14 (30%), and neither in 14 (30%) including melphalan (N = 7) and fludarabine-containing regimens (N = 6). Grafts were blood cells in 31 (66%). Grade 2–4 acute graft versus host disease (GvHD) developed in 52% (95% confidence interval (CI) 38–66%) of transplant recipients. 1 year incidence of chronic GVHD was 42% (CI 28–57%). Treatment related mortality (TRM) was 28% (CI 16–42%) and occurred within 1 yr of transplant. Common causes of non-relapse mortality were infection (N = 4; 12%), GvHD (N = 5; 15%), and pneumonitis/ARDS (n = 3;9%). PLL progression was the most common cause of death (N = 16; 49%). Median progression-free survival (PFS) and overall survival (OS) is 5 and 11 mos, respectively. Median PFS for 16 subjects transplanted in CR is 8.9 mo. Median PFS for patients with matched siblings or well-matched donors (N = 34) is 5.2 mo. With a median follow-up of survivors of 13 mos, the 1y PFS and OS is 33% (CI 20–47%) and 48% (CI 33–62%), respectively. At 2y, the PFS is also 33% (CI 20–47%) and OS is 35% (CI 21–50%). These data provide information on anticipated outcomes of allotransplants for PLL.

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PROLONGED SURVIVAL IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) FOLLOWING REDUCED INTENSITY CONDITIONING WITH CORD BLOOD OR SIBLING DONOR TRANSPLANTATION

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The optimal therapy for adult ALL patients >35 years or intolerant of myeloablative allotransplantation is uncertain. Twenty-two adult ALL patients received reduced intensity conditioning (RIC) followed by allogeneic transplantation. All patients were high risk with 64% having Ph^+ ALL and 46% in $\geq 2^{nd}$ remission, 20/22 were in cytogenetic CR. Following a uniform preparative regimen (fludarabine 40 mg/m2/day × 5, cyclophosphamide 50 mg/kg/day and 200 cGy total body irradiation) patients received either umbilical cord (UCB, n = 18) or matched related (MRD, n = 4) donor grafts. All patients reached neutrophil engraftment (median 10 days) and 100% donor chimerism (median day 23). Overall survival, treatment related mortality and relapse were 50% (95% confidence interval [CI], 27–73%), 27% (95%CI, 9–45%), 36% (95%CI, 14–58%) at 3 years, respectively. There were no relapses beyond 2 years. OS, TRM and relapse following cord blood HCT were 49% (95%CI, 23–75%), 28% (95%CI, 8–48%) and 33% (95%CI, 9–57%). The cumulative incidence of acute (grade II-IV) and chronic graft versus host disease was 55% and 45%. HCT in CR1 led to less TRM (8% vs 50%, $p<0.04$) and improved OS (81% vs 15%, $p<0.01$). For ALL adults in CR1, RIC allografting using UCB or MRD results in modest TRM, limited risk of relapse and promising leukemia-free survival.

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ADOPTIVE T CELL THERAPY USING EDUCATED T CELLS GENERATED BY THE SEQUENTIAL STIMULATION WITH DC/TUMOR FUSION CELLS AND ANTI-CD3/CD28

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Acute leukemia is susceptible to T cell mediated killing as evidenced by the graft versus disease effect following allogeneic transplantation. The development of adoptive immunotherapy to more selectively target tumor cells is an area of interest. Ligation of the CD3/CD28 complex results in expansion of T cells ex vivo with a phenotype that is dependent on the immunologic milieu during stimulation. We have developed a cancer vaccine in which autologous tumor cells are fused with dendritic cells (DCs). We postulated that stimulation with fusions followed by anti-CD3/CD28 would result in the expansion of activated T cells targeting tumor antigens. Tumor was obtained from peripheral blood or bone marrow of patients with AML. DCs were generated from adherent mononuclear cells cultured with rhIL-4, GM-CSF and TNFand fused with tumor cells by coculture in polyethylene glycol. T cells were stimulated by fusions prior to or following exposure to antiCD3/CD28 antibody coated plates. Stimulation by fusions followed by anti-CD3/CD28 resulted in the synergistic expansion of T cells, manifested by a greater stimulation index (SI) compared to stimulation by either pathway alone (SI 8.2 versus 3.3 with fusions alone). A rise in CD4+/CD25+ cells was noted following sequential stimulation with fusions and anti-CD3/CD28 (9.3% vs. 2.7% with fusions alone). An higher percentage of CD4+/CD25+ cells expressed IFNγ when exposed to fusions followed by anti-CD3/CD28 (7% compared to 2% with fusions alone). As compared to un-stimulated T cells, stimulation with fusions or anti-CD3/CD28 resulted in a 3.3 and 3.8 fold increase in CD8+ T cells expressing granzyme B, respectively. In contrast, sequential stimulation with fusions and anti-CD3/CD28 induced a 19-fold expansion of granzyme B+ cells consistent with enhanced cytolytic capacity. In spectratyping analysis, T cells undergoing sequential stimulation with fusions and anti-CD3CD28 demonstrate greater skewing of CDR3-size usage in the T cell receptor as compared to T cells stimulated by fusions or anti-CD3/CD28 alone. The pattern of gene expression in T cells stimulated sequentially by fusions and anti-CD3CD28 is being assessed. In conclusion, sequential stimulation of T cells by fusions and anti-CD3/CD28 results in the expansion of tumor reactive activated T cells. A clinical trial evaluating the use of T cells generated by sequential stimulation with DC/tumor fusions and anti-CD3CD28 for patients with acute leukemia is planned.

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IMPAIRED PRE-TRANSPLANT FEV1 IS ASSOCIATED WITH REDUCED OVERALL AND DISEASE FREE SURVIVAL FOLLOWING ALLOGENEIC TRANSPLANT FOR ACUTE MYELOGENOUS LEUKEMIA

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Introduction: Pulmonary function testing (PFT) is routinely performed prior to allogeneic hematopoetic stem cell transplant (HSCT), but its relationship to survival is poorly described. In this report, the post-transplant survival outcomes of Acute Myelogenous Leukemia (AML) patients are examined with respect to pre-transplant pulmonary function.

Methods: Two hundred fifty one patients with AML receiving HSCT between January 2001 and May 2008 at the University of Michigan were retrospectively analyzed for pre-transplant PFTs by forced expiratory volume at one second (FEV1), forced vital capacity (FVC), and diffusion capacity (DLCO). PFT function parameters FEV1 (≥80% vs. <80%), FVC (≥80% vs. <80%), and DLCO (≥65% vs. <65%) were correlated with overall (OS) and disease free survival (DFS). Using a multivariate Cox regression analysis, OS and DFS were modeled for the following risk factors: age at

transplant, disease status, donor type, absolute neutrophil count, absolute lymphocyte count (ALC), presence of circulating blasts, stem cell source, CD34+ cell dose, conditioning intensity, cytogenetic profile, antecedent myelodysplastic syndrome (MDS) or secondary AML, and development of acute graft versus host disease (GVHD). Deaths attributable to relapse versus non-relapse causes were recorded for each PFT group.

Results: Pre-transplant characteristics included a median age of 49 yrs (range 7–69 yrs), intermediate/high risk cytogenetics (95%), CR1/ CR2 (93%), circulating blasts (8%), full intensity conditioning (78%), related donor (48%), FEV1< 80% (12%), FVC < 80% (15%), and DLCO < 65% (34%). Diminished FEV1 (< 80%), disease status, regimen intensity, ALC, and circulating blasts were associated with a significantly reduced disease free survival (Table). Though FEV1 < 80% and FVC < 80% were both associated with a poor DFS (p = 0.001 and p = 0.027) and OS (p = 0.005 and p = 0.017) by univariate analysis, only FEV1 < 80% remained associated with DFS and OS in the multivariate Cox regression analysis. Pre-transplant DLCO < 65% did not reach significance for either DFS or OS by univariate or multivariate analysis. Relapse related mortality was more common in patients with a pre-transplant FEV1 ≥80% (50%) then patients with an FEV1 <80% (39%).

Conclusion: Pre-transplant FEV1 < 80% is associated with reduced DFS and OS in patients with AML undergoing allogeneic HSCT and may provide valuable prognostic information in pre-transplant evaluations.

Pre-Transplant Factors Associated with Survival

	Overall Survival		Disease Free Survival	
Risk Factor	Risk Ratio	p-value	Risk Ratio	p-value
FEV1≥ 80	0.519	0.019	0.467	0.006
Disease Status[1]	2.144	0.000	1.924	0.004
Regimen Intensity [2]	2.174	0.001	2.047	0.003
ALC ≥ 0.5	0.513	0.003	0.544	0.008
Peripheral Blast +[3]	-	-	2.220	0.018

[1]Disease >CR2 or in relapse.
[2]reduced intensity conditioning (RIC).
[3]not associated with overall survival.

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ALLOGENEIC STEM CELL TRANSPLANTION IN RELAPSED/REFRACTORY ACUTE LEUKEMIA: A LONG TERM SINGLE INSTITUTION EXPERIENCE

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Introduction: The clinical outcomes of relapsed and refractory acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are dismal in adults treated with conventional therapy. The majority of patients with relapsed ALL and AML who are subsequently cured are transplanted in second remission or, occasionally, beyond second complete remission. A minority of patients, in the range of 10 to 20%, with relapsed/refractory disease are cured by an allogeneic bone marrow transplant.

Objective: We retrospectively examined 137 patients with relapsed and/or refractory acute leukemia who were recipients of allogeneic transplants (matched related [MRD], matched unrelated [MUD] and umbilical cord blood [UCB]) at our center from August 1986 to August 2008 to determine the outcome in advanced leukemia.

Methods: One hundred thirty seven patients (AML, n = 95 and ALL, n = 42) received allogeneic transplants including: 75 MRD, 39 MUD, and 23 UCB. Conditioning regimens were mostly myeloablative using combinations of cyclophosphamide(Cy), busulfan (Bu), etoposide(VP) and total body irradiation (TBI): TBI/Cy/VP, n = 31; TBI/Cy, n = 66; other TBI based, n = 10; Bu/Cy, n = 20;.other, n = 9. GVHD prophylaxis varied over the years but included a calcineurin inhibitor with methotrexate for MUD/MRD transplants and steroids for UCB transplants.

Results: The median age at transplant was 45 years for AML (13–72) and 32 years for ALL (1–66). Results are shown in the table below and compared to the survival data (extrapolated) from the NMDP/ CIBMTR database.

Table 1 Transplant Outcomes in Relapsed and/or Refractory Acute Leukemia

	N	100 day survival	1 year survival	2 year survival	NMDP/ CIBMTR -MRD	NMDP/ CIBMTR -MUD
					1 year–2year	1 year–2year
AML	95	62%	33%	27%		
MRD	75	68%	30%	22%	40%–30%	
MUD	39	54%	30%	26%		30%–20%
UCB	23	48%	26%	16%		
Survival by Age						
<40yrs	35	74%	48%	38%		
40–55yrs	36	56%	29%	26%		
>55yrs	24	54%	12%	12%		
ALL	42	57%	21%	11%	35%–20%	30%–15%

Conclusion: Our single institutional experience in allogeneic transplantation for advanced leukemia appears comparable to the CIBMTR/NMDP database. Younger patients had the best outcomes. MUD recipients had comparable outcomes to MRD. Therefore, we recommend that younger patients with advanced leukemia be strongly considered for allogeneic transplant, regardless of stem cell source, as the only potentially curative option.

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SELECTIVE REPROGRAMMING OF CD19-SPECIFIC T CELLS WITH IL-21 AND CD28 SIGNALING FOR ADOPTIVE IMMUNOTHERAPY OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Adoptive transfer of T cells has been used to treat and prevent malignancies and opportunistic infections. To improve therapuetic efficacy, investigators initially redirected specificity through the introduction of immunoreceptors. Early-phase trials are now underway to demonstrate the safety and feasibility of CD19-specific chimeric antigen receptors (CARs) which recognize antigen independent of MHC. However, it is recognized that improving *in vivo* persistence will be needed to enhance therapeutic potential of CAR^+ T cells. To this end we developed two approaches: (i) *intrinsic:* altering the signalling pathway of T cells to improve persistence and (ii) *extrinsic:* altering the culturing milieu to numerically expand CAR^+ T cells with a proliferative advantage. Previously we reported on a 2nd generation CAR (designated CD19RCD28) which activates T cells through CD3-ζ and CD28 endodomains, to sustain proliferation of CD19-specific T cells. One extrinsic factor to be assessed is IL-21, a member of the common γ-chain receptor cytokine family that can signal $CD8^+$ T cells in conjunction with CD28 to support proliferation and acquisition of desired effector functions. We now report that the addition of exogenous IL-21 favors the microenvironment to selectively propagate CAR^+ $CD8^+$ T cells with ability to kill $CD19^+$ tumor targets. To evaluate this role for IL-21, peripheral blood-derived T cells were electroporated with CD19RCD28 CAR expressed as a *Sleeping Beauty* (SB) transposon and propagated in the presence of IL-2 and/or IL-21 on γ-irradiated $CD19^+$ artificial antigen presenting cells (aAPC). There was a selective outgrowth of CAR^+CD8^+ T cells when IL-21 was present in the culture medium, compared with predominant outgrowth of CAR^+CD4^+ T cells when IL-21 was absent (Table). The propagated CAR^+ T cells produced more IFN-γ in response to $CD19^+$ stimulator cells compared to cells cultured in parallel only on IL-2, and displayed a central memory surface phenotype while retaining an ability to exhibit CD19-dependent cytolysis.