low-up of 21 adult patients with acute leukemia treated with peripheral blood HPCT following RIC after being deemed inadequate for fully myeloablative conditioning. A matched comparison arm of 42 consecutive patients transplanted after full myeloablative (FMA) conditioning is also reported. **Methods:** Between 1999–2004, 21 patients were treated with Fludarabine-based conditioning prior to infusion of hematopoietic stem cells. Patients were followed for toxicities and responses. IRB approval was obtained prior to data collection. Kaplan-Meier estimates of survival were performed. Patient disease characteristics were analyzed using T-tests to determine any factors that may be associated with outcome and relapse. Significant factors on univariate analysis were placed into a Cox regression model for multivariable analysis. **Results:** Patient characteristics: 76% of patients had AML and 24% had ALL. 14% of patients had relapsed or refractory disease at transplant. A greater proportion of patients undergoing RIC, had poor-risk cytogenetics. Median age was 56 yrs. 81% of patients experienced clinically significant acute or chronic GVHD. Overall post-transplant survival was 29%. Twenty-nine percent (6/21) patients suffered relapse at a median of 4 months post-transplant. Cox proportional hazards models for overall post-transplant survival and logistic regression models for relapse and overall survival (N = 63), showed that the only significant predictor for OS and relapse was cytogenetic risk group ($P = .000$). Of note, none of the 5 ALL patients transplanted after RIC have relapsed with a median follow-up of 2.2 years (0.1–3.9 years); among 16 AML pts., 5 have relapsed with median follow-up of 0.4 years (.1–2.2 years). These results compare favorably to our cohort of pts. transplanted after full myeloablative conditioning. **Conclusion:** The role of RIC transplants for the treatment of high risk acute leukemia in older adults remains a promising therapy and warrants further study (Table).

Patient Characteristics

Characteristics	RIC pts. N = 21 (%)	Comparison arm (FMA) N = 42 (%)
ALL	5 (24%)	10 (24%)
AML	16 (76%)	32 (76%)
Median age	56 (45–68)	52 (41–62)
Male	14 (67%)	20 (48%)
Disease status at transplant:		
CR1	13 (62%)	30 (71%)
CR2–3	4 (19%)	7 (17%)
Relapsed-Refractory	3 (14%)	5 (12%)
Donor: Related	12 (57%)	34 (81%)
Unrelated	9 (43%)	8 (19%)
Cytogenetic risk:		*
Favorable	1 (5%)	4 (10%)
Intermediate	6 (28%)	19 (45%)
Poor	14 (76%)	18 (43%)

*1 Pt, cytogenetics were unknown

199

INVESTIGATING THE ROLE OF DENDRITIC CELLS IN EXTRACORPOREAL PHOTOPHERESIS USING AN IN VITRO MODEL

Holtick, U.[1], Marshall, S.R.[1], Wang, X.N.[1], Hilkens, C.M.U.[2], Dickinson, A.M.[1] 1. Haematological Sciences; 2. Rheumatology, The Medical School, University of Newcastle upon Tyne, United Kingdom.

Graft-versus-host disease (GvHD) is the major complication after allogeneic transplantation and contributes significantly to transplant related mortality and morbidity. Especially steroid refractory or steroid depending GvHD is linked to poor survival or life quality. Conventional immunosuppression has very limited success in these conditions and increases susceptibility for infection and relapse. Extracorporeal Photopheresis (ECP) is a promising therapy for acute and chronic GvHD not responding to conventional immunosuppressive therapy. ECP treatment seems not to result in a pan-immunosuppression but has quite selective effects on the pathogenic process in GvHD. The mechanisms of action of ECP in GvHD known so far include lymphocyte senescence or apoptosis and cytokine modulation. Some groups report that antigen presenting cells like dendritic cells (Dc) might be important for ECP mechanisms. We have developed an in vitro model of ECP (in vitro PUVA) to investigate ECP effects on dendritic cells. Initial experiments have shown the maturation of monocyte derived Dcs treated with in vitro PUVA (upregulation of CD83, CD86, HLA-DR as well as reduced endocytosis capacity), but also the induction of apoptosis. The stimulatory capacity of in vitro PUVA treated Dcs was strongly inhibited in autologous and allogeneic MLR. However, treatment of antigen-primed Dcs resulted in less inhibition, suggesting factors that might preserve Dc stimulatory capacities. Immature Dcs, retrieved after coculture with in vitro PUVA treated lymphocytes, show inhibited stimulatory capacity on autologous and allogeneic T cells. Currently, we are investigating the changes in phenotype and cytokine pattern which could transfer anergy or promote tolerance induction. In parallel, we are analyzing effects on monocyte-derived dendritic cells from patients undergoing ECP treatment for chronic GvHD. Dcs rendered tolerogenic could play a major role in ECP mechanisms.

200

HUMAN T LYMPHOCYTE ACTIVATION KINETICS FOR IDENTIFYING AND TARGETING ALLOREACTIVE T CELLS

Bajwa, R.P.S., McCarthy, P.L., Wallace, P.K., Wallace, S., Wu, Y., Battiwalla, M. Roswell Park Cancer Institute, Buffalo, NY.

Selective depletion of alloreactive T cells from a stem cell graft has the potential of reducing graft-versus-host disease (GvHD) while preserving graft-versus-leukemia (GvL) and third party responses. For this purpose several techniques generate and deplete alloreactive cells, which are donor-derived T cells activated by recipient tissue. The kinetics of T cell activation in donor-recipient co-culture systems is critical in optimizing the timing of depletion of alloreactive T cells. We present the T cell activation kinetics in our preclinical system. Peripheral blood mononuclear cells (PBMCs) were derived form several pairs of unrelated healthy human volunteers. 2500 cGy irradiated cells (stimulators) were co-cultured with PBMCs (responders) in a 1:1 ratio and a concentration of 5×10^6/ml in serum free medium. Stimulator cells were labeled with PKH67 and the co-cultures were, analyzed for CD3, CD4, and CD25, expression by flow cytometry on days 0 through 7, using Topro-3 to exclude dead cells. Our results show that CD3+, CD4−, CD25+ cells (alloreactive CD8 cells) increased from ≤1 % on day 0, to 6.5 percent by day 5; the addition of IL2 amplified the increment to nearly 10% by day 5. CD3+, CD4+, CD25+$^{\text{dim or total}}$ cells (CD4+ activated T cells) did not appreciably increase over time and ranged between 2 to 5%; the addition of IL2 did not have any effect. The CD3+, CD4+, CD25+$^{\text{bright}}$ cells (T regulatory cells) increased from ≤1% at baseline to 5% by day 5 and these were unaffected by the addition of IL2. The proportion of non-activated T lymphocytes, decreased with time of co-culture progression. Our results show that T lymphocyte activation, defined by CD25 expression, progressively increases through the first week of co-culture. This important observation will help in establishing the timing of allograft manipulation, for the selective depletion of alloreactive T cells in clinical hematopoietic stem cell transplantation.

201

SIROLIMUS/MYCOPHENOLATE MOFETIL (MMF) AS TREATMENT FOR GRAFT-VERSUS-HOST-DISEASE IN TWO CHILDREN WITH SEVERE RENAL AND CALCINEURIN-INHIBITOR-ASSOCIATED CENTRAL NERVOUS SYSTEM (CNS) TOXICITY

Ehlert, K., Groll, A.H., Vormoor, J. University Hospital, Department of Pediatric Hematology and Oncology, Muenster, Germany.