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To date there are no published data on pharmacokinetics (PK) of MMF in children undergoing AlloSCT. The objective of this study is to evaluate effects of age on the PK of MMF in pediatric AlloSCT pts. From 1/04-9/05 we enrolled 26 pediatric AlloSCT with 23 being evaluable: mean age 7.6 yrs; wt 31.6 kg; M:F = 11:12; NBL PR (n = 3), SCD (n = 2), AML (CR1 [n = 3], CR2 [n = 1], CR3 [n = 1], relapsed/induction failure [n = 2]), SAA (n = 4), CML CP (n = 1), ALL (CR1 [n = 1], CR2 [n = 2], CR3 [n = 1]), HD CR2 (n = 1), ALCL refractory (n = 1); donor sources: MFD (6/6 PBSC [n = 6], 6/6 BM [n = 2], 5/6 PBSC [n = 2]), 6/6 related CB (n = 1), UCB (6/6 [n = 2], 5/6 [n = 2], 4/6 [n = 7]), and 8/10 MUD PBSC (n = 1). Cohort 1 (<6 yrs) (n = 8); 2 (6-12 yrs of age) (n = 8); 3 (12-16 yrs) (n = 7). GVHD prophylaxis included tacrolimus (starting on day −1 or 1st day of conditioning to maintain concentrations 5-20 ng/mL) and MMF (900 mg/m^2/dose IV Q6h starting on day +1, then converted to PO [same dose] after day +14). Serum samples for MPA assay were drawn on day +1, +7, and +14 at hour 0, 0.5, 1, 2, 3, 4, and 6 post-dose. MPA plasma concentrations were determined by reverse-phase HPLC. MMF dose was adjusted to maintain MPA trough 1-3.5 mg/L. The mean CD34$^+$ cell dose/kg = 25.8 × 10^5, TNC dose/kg = 48 × 10^7. Time to neutrophil (ANC ≥500/mm^3 × 2 d) and platelet engraftment (untransfused count ≥20K × 7 d) were 22 d and 39 d. The mean f/u was 278 d. Mean MPA PK on day +14: Cmax = 12.4 mg/L, total MPA trough = 0.9 mg/L, AUC_{0-12} = 32.7 mg · hr/L, Css = 2.7 mg/L, $T_{1/2}$ = 1.5 h , Vss = 1.8 L/kg, and CL = 1.4 L/kg/h at a mean MMF dose of 1080 mg/m^2 IV Q6h. The breakdown of age cohorts is shown in Table 1. Incidence of GI adverse events attributable to MMF was 65% (nausea/vomiting [n = 12], diarrhea [n = 7], abdominal pain [n = 3], pneumatosis intestinalis [n = 1], colitis [n = 1]). Incidence of grade II-IV aGVHD was 59% (13/22 evaluable pts) and cGVHD was 26.3% (5/19 evaluable pts). Kaplan-Meier probability of 1 year OS was 70.4% (CI: 49.7-91.1%). In comparison to MMF PK in adult AlloSCT pts receiving cyclosporine/MMF (Nash et al, *Biol Blood Marrow Transplant*, 2005), children have significantly higher MMF clearance rates (1.4 vs 0.54 L/kg/h). MMF doses >3-fold higher than those used in pediatric SOT recipients were required to achieve AUC_{0-12} = 30-60 mg · h/L. Short half-life (1.5 hrs) and rapid clearance of MMF in pediatric AlloSCT pts may be related to a lack of enterohepatic recycling and enhanced UDP-glucuronosyltransferase activity (Table 1).

Table 1. *Mean Age-Related IV MMF PK on Day +14*

Age Group	Cmax (mg/L)	Total MPA Trough (mg/L)	Free MPA Trough (ng/mL)	AUC 0–12 (mg · hr/L)	Total Css (mg/L)	T1/2 (hr)	CL (L/kg/hr)
<6 y.o. (n = 6)	12.2	1.0	18.7	34.0	2.8	1.8	1.6
6–12 y.o. (n = 5)	11.8	0.4	14.8	29.5	2.5	1.1	1.4
12–16 y.o. (n = 2)	14.4	1.4	17.9	39.9	3.3	1.5	0.6

Cmax = peak concentration; MPA = mycophenolic acid; AUC = area under the curve; Css = steady state concentration; T1/2 = half-life; CL = clearance.

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THE ROLE OF MHC CLASS II IN CD4$^+$CD25$^+$ T CELL-MEDIATED FACILITATION OF ALLOGENEIC HEMATOPOIETIC ENGRAFTMENT AND SUPPRESSION OF GVHD

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In recent years there has been significant interest in the potential of CD4$^+$CD25$^+$ regulatory T cells to suppress GVHD and promote allogeneic engraftment. In a MHC-mismatched model utilizing C57BL/6 (B6) T cell-depleted bone marrow and sublethally conditioned (7.0 Gy TBI) BALB/c recipients, we have demonstrated that co-transplanted donor CD4$^+$CD25$^+$ T cells are capable of supporting multi-potential and lineage-committed donor progenitor activity as well as long-term chimerism and tolerance. Little is currently understood regarding the antigen recognition initiating regulatory cell function during hematopoietic transplantation, and we have employed our *in vivo* model toward elucidating the antigenic requirements involved in the initial promotion of allogeneic engraftment. Transplanting BALB/c × B6 F1 CD4$^+$CD25$^+$ T cells (1 × 10^6) with B6 marrow (2 × 10^6) significantly increased B6 CFU-GM in BALB/c recipients seven days post-BMT ($P < .001$ vs. BM alone), demonstrating that donor CD4$^+$CD25$^+$ T cells did not require alloreactivity to support hematopoietic progenitors. Furthermore, B6 CD4$^+$CD25$^+$ T cells failed to augment MHC-disparate C3H/HeJ CFU-GM in BALB/c recipients ($P > .05$ vs. BM alone), suggesting that donor CD4$^+$CD25$^+$ T cells might require recognition of syngeneic MHC for progenitor support. Indeed, augmentation of donor CFU-GM was abrogated when B6 CD4$^+$CD25$^+$ T cells were co-transplanted with B6-MHC class II$^{-/-}$ marrow into BALB/c recipients ($P > .05$ vs. BM alone). CD4$^+$CD25$^+$ T cell-mediated augmentation of donor chimerism two months post-BMT was also abrogated by co-transplantation with MHC II$^{-/-}$ marrow. In order to compare the role of MHC class II in progenitor support vs. suppression of GVHD, a model of CD8-mediated GVHD was utilized: co-transplanted BALB/c CD4$^+$CD25$^+$ T cells were able to prevent the GVHD mediated by highly purified BALB/c CD8 T cells in B6-wt recipients, but failed to control the GVH reaction that occurred in B6-MHC II$^{-/-}$ recipients. Therefore, while donor CD4$^+$CD25$^+$ T cells required co-transplantation with syngeneic MHC II to support donor hematopoietic progenitors, allogeneic recipient MHC II was required for suppression of GVHD. In conclusion, donor CD4$^+$CD25$^+$ T cells capable of promoting long-term engraftment and tolerance may have distinct antigenic requirements from those responsible for GVHD suppression, and clinical protocols for allogeneic transplantation involving CD4$^+$CD25$^+$ T cells should account for their distinct antigenic requirements.

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ROLE OF IL-2, IL-7, AND IL-15 IN ALLOGENEIC GRAFT-VS-LEUKEMIA AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA IN A NOD/scid CHIMERIC MURINE MODEL

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We studied the GVL effects of human alloreactive CTL against ALL in a chimeric NOD/scid mouse model. CTL were generated from random blood donor PBMCs stimulated with the 697 human ALL cell line and supplemented with IL-2, −7, or −15. CD8 positive T cells comprised the majority of the cultures in each group: 46% for IL-2, 52% for IL-7, and 45% for IL-15 cultured CTL (n = 13). CTL grown in each cytokine resulted in similar in vitro cytotoxicity: IL-2 41.3%, IL-7 37.7%, IL-15 45.3%, n = 12-15, and had statistically similar intracellular perforin and granzyme-B expression. IL-7 and IL-15 CTL had statistically higher bcl-2 levels than IL-2 CTL suggesting better anti-apoptosis and survival potential. NOD/scid mice were injected with 697 ALL cells followed by 5 × 10^6 CTL. Mice were sacrificed seven days following CTL injection and residual leukemia was measured in the bone marrow and spleen via flow cytometry. There were two groups of experiments with different degrees of leukemia engraftment seen at day 21. In one group (low engraftment) mice not receiving CTL had a baseline leukemia burden of 2.01% and 0.15% in the bone marrow and spleen, respectively (n = 15). Mice treated with IL-15 cultured CTL had a reduction in tumor burden to 0.2% (n = 13, $P = .01$) and 0.05% (n = 13, $P = .01$) in bone marrow and spleen, respectively. Those treated with IL-2 or IL-7 cultured CTL showed no significant difference in leukemia burden