(cGVHD) was 54% and 34%, respectively. Probability of OS was 62% (CI_{95}: 48.3–78.4). There was >10-fold interpatient variability in AUC_{0-6} and MPA trough (C_0). Mean PK are shown in Table 1.

Table 1. Mean pK Parameters

	IV MMF administration			MMF PO 1st sample	MMF PO 2nd sample
Mean pK values	Day +1 (SD) [N=27]	Day +7 (SD) [N=31]	Day +14 (SD) [N=32]	Day +45-100 (SD) [N=15]	Day +45-100 (SD) [N=9]
C_{max} (mg/L)	15.4 (19.71)	12.31 (7.99)$^{\Psi}$	16.54 (13.24)$^{\Psi}$	13.15 (12.04)	13.20 (8.86)
T_{max} (h)	1.87 (0.47)	1.92 (0.55)	1.75 (0.44)	1.7 (0.98)	1.33 (0.83)
C_0 (mg/L)	0.33 (0.29)*	0.68 (0.56)*	0.72 (0.62)	1.45 (1.46)	1.43 (1.37)
C_{ss} (mg/L)	5.45 (6.34)	4.73 (2.22)	6.46 (4.07)	5.38 (3.55)	6.54 (3.55)
AUC_{0-6} (mg•h/L)	32.06 (38.09)	26.82 (12.35)$^{\Psi}$	33.71 (16.86)$^{\Psi}$	26.50 (19.03)	26.70 (15.81)
CL_{ss} (L/h•kg)	1.46 (1.04)	1.40 (0.63) *	1.17 (0.63) *	2.21 (1.43)	1.46 (1.01)
V_{ss} (L/kg)	3.04 (1.91)	3.35 (1.58)$^{\lambda}$	3.00 (2.05)$^{\lambda}$	9.77 (8.93)	18.33 (35.74)
$T_{1/2}$ (h)	1.02 (0.69)	1.35 (1.01)	2.49 (6.77)	3.17 (2.63)	10.19 (18.06)

*P=0.002.
$^{\Psi}$P=0.0003.
$^{\lambda}$P=0.001

MPA steady state clearance (CL_{ss}) (cohort 1 vs 2 vs 3 = 1.65 vs 1.45 vs 0.88 L/h·kg, P = 0.0039) and volume of distribution (V_{ss}) (cohort 1 vs 2 vs 3 = 3.87 vs 3.22 vs 2.44 L/kg, P = 0.052) were higher in pts <6 yrs. There was also a trend toward higher CL_{ss} in pts receiving MA vs NMA (1.55 vs 1.15 L/h·kg, P = 0.054). There was a significant difference in most PK values on Day +7 vs Day +14: higher AUC_{0-6} & C_{max} and decreased CL_{ss} & V_{ss} on Day +14 (Table 1). These differences suggest improved mucosal healing and/or increased enterohepatic recirculation on Day +14 vs +7. Mean MPA C_{ss} in this study was significantly higher (>5 mg/L) than previously reported in other adult and pediatric AlloSCT trials (1.6–4.8 mg/L) (Nash et al, 2005; Jacobson et al 2008). This can be explained by >2-fold higher dose of MMF (~30 mg/kg q6h) compared to others (15 mg/kg q6-8h). The incidence of aGVHD and cGVHD were comparable to previous reports. These results suggest a need for more frequent (q6h) MMF dosing in pediatric AlloSCT recipients, especially those <6 years. However, the optimal MMF dose (mg/kg) and relationship between MPA exposure and risk of acute GVHD remain to be elucidated.

371

HIGH BUDESONIDE BIOAVAILABILITY IN PATIENTS WITH GASTRO-INTESTINAL (GI) GRAFT VERSUS HOST DISEASE (GVHD) AND/OR *CLOSTRIDIUM DIFFICILE* INFECTION

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Background: Therapy of acute intestinal GVHD is routinely treated with immunosuppressive agents, including systemic corticosteroids. Several reports have shown responses of using "topical" therapy with budesonide for GI GVHD. Budesonide is a potent steroid with low oral bioavailability due to high first pass metabolism by CYP 3A4. In patients with Crohn's disease, bioavailability is approximately 10–20%. No data are available in hematopoeitic stem cell transplant recipients. Several reports using budesonide for the treatment of GI GVHD have noted responses in other GVHD sites, thus questioning if active absorption was occurring. As a quality measure at our center, several patients who had GI GVHD and/or *C. Difficile* were evaluated for systemic availability of budesonide.

Methods: Random plasma budesonide levels were analyzed by HPLC/Mass Spectrometry in 8 patients with a history of GI GVHD receiving Enterocort brand of budesonide.

Results: A total of 11 budesonide plasma levels were measured in 8 patients. Seven patients had active GVHD, 5 patients had active GVHD and concurrent C. Difficile infection, and 2 patients had C. Difficile without active GVHD at the time of plasma measurements. Four of the 8 levels were measured on a budesonide dose of 9 mg TID, two on 9 mg once daily, three on 6 mg TID, and two on 3 mg TID. The mean budesonide plasma level was 0.86 mcg/dl (0.08–1.5), 0.60 mcg/dl (0.09–1.1), 0.97 mcg/dl (0.2 – 1.1) and 0.65 mcg/dl (0.2–1.1) respectively. Mean budesonide levels were higher if patients were on voriconazole (1.05 mcg/dl) than on micafungin (0.47 mcg/dl). The mean budesonide bioavailability was 37% in all patients and 50% if on Voriconazole. Thus, budesonide doses ranging from 3 mg TID to 9 mg TID would represent a potency equivalent to 40–162 mg methylprednisolone/day.

Conclusion: Systemic budesonide levels were significantly elevated in patients with acute GI GVHD. Patients on voriconazole had higher plasma levels of budesonide, possibly attributable to CYP450 inhibition. The use of oral budesonide in patients with gastro-intestinal GVHD may lead to systemic absorption and subsequent additive systemic corticosteroid effects.

IMMUNE RECONSTITUTION

372

THYMIC EPITHELIAL CELLS AND DENDRITIC CELLS MEDIATE THYMIC RENEWAL THROUGH CCL25 PRODUCTION

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Impaired thymopoiesis contributes to immune deficiency following hematopoeitic stem cell transplantation (HSCT). Clinical data suggest that thymic epithelial cell (TEC) damage from the preparative regimen may compromise thymic recovery. We have previously shown that TEC are critical for thymic renewal in the setting of androgen withdrawal. These UEA+ medullary TEC provide a niche for early thymic progenitor (ETP) entry and thymocyte development by increasing the production of CCL25. Following androgen withdrawal, CCL25 accelerates thymocyte development and enhances ETP entry. Blockade of this ligand completely abrogates these affects, preventing thymic renewal. We now demonstrate that cytotoxic preparative regimens may impair thymic reconstitution by damaging stroma, decreasing CCL25 availability. In this study, we examined the effects of cyclophosphamide (CY) (240 mg/kg/mouse) or total body irradiation in female mice on TEC and medullary dendritic cell (mDC) populations and CCL25 production. At ½ myeloablative dose of 750 cGy, using flow cytometry of TEC, we show that both CY and radiation significantly deplete UEA+ medullary TEC while sparing Ly51+ CD45- cells. However, the effects of these two agents differed with regards to the proportions of TEC within the Ly51+ fraction. Radiation led to an increase in fibroblasts (CD45- Ly51+ MHCII-) with a marked reduction of cortical TEC (CD45- Ly51+ MHCII+), while this TEC subset was spared following CY administration. Furthermore, mDC were decreased following radiation while unaffected by CY. After irradiation, these alterations significantly impair CCL25 production as shown by decreased mRNA production of TEC enriched preparations and thymocyte fractions (in which only mDC produce CCL25). In contrast, CCL25 production was unaffected following CY administration, consistent with the hypothesis that the spared TEC populations preserve CCL25 production. These data suggest that cytotoxic preparative regimens may impair thymic renewal by destroying TEC and mDC populations thereby reducing available CCL25, a ligand necessary and essential for thymic recovery. Furthermore, these data suggest that different preparative regimens may impair thymic recovery to varying degrees due to discrimination of injured TEC and mDC populations, and the preservation of thymic CCL25 production. Investigation of alternative preparative regimen agents may present an opportunity to improve thymic renewal following HSCT.

373

HUMAN COLON CARCINOMA CELLS EXPRESSING CMVPP65 ANTIGEN: AN IN-VIVO MODEL FOR ADOPTIVE IMMUNOTHERAPY OF CMV DISEASE

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Adoptive transfer of large doses of CMV-specific T cells (CMV-CTL) can effectively prevent CMV disease in HSCT recipients. However, data regarding the relative potency of T cells (TC) specific

for different epitopes and the doses required to eradicate disease are lacking. We have developed an *in-vivo* model to assess specificity and efficacy of CMV-CTL and establish TC doses required for treatment using colon carcinoma cells transduced with CMVpp65 as a surrogate system. HLA A0201$^+$ human colon carcinoma cells transduced with CMVpp65 and GFP-firefly luciferase (CoCa-pp65) were injected subcutaneously (s.c.) into NOD/Scid-IL2Rgc-KO/J mice (NSG) at doses ranging from 10^4–10^6 (4 mice /group) and tumor growth followed for 4 weeks using bio-luminescent imaging and tumor measurements. Consistent engraftment was observed with 3×10^5 cells. Groups of NSG mice were then injected s.c with CoCa-pp65 (3×10^5) mixed with HLA A0201-restricted CMV-CTL (A2-CMV-CTLs) at E:T ratios of 50:1, 10:1, 2:1 and 0.4:1. To ascertain antigen specificity of A2-CMV-CTLs, groups of 4 NSG mice were subsequently co-injected s.c. with 3 tumor-TC combinations (E:T = 1:1) as follows: (i) A2-CMV- CTLs + 3×10^5 CoCa-pp65 (ii) A2-CMV- CTLs + A2 tumor without CMVpp65 (iii) A2-Flu-CTLs + 3×10^5 CoCa-pp65. Control animals were injected with the 2 tumors without TC. IL-2 (2000 U i.p) was given 2 × a week, growth followed by imaging x 4 weeks. No growth of CoCa-pp65 was detected in any animal co-injected with A2-CMV-CTL at any E:T ratio, with consistent tumor growth over 4 weeks in controls. Current experiments are assessing E:T ratios lower than 0.4:1. Tumor growth was observed in control groups (ii) and (iii) with no detectable growth in (i) by imaging or examination of autopsied tissues. These studies demonstrate that CMV-CTL can eradicate clonogenic tumor cells expressing an immunogenic viral antigen *in-vivo* at E:T ratios that are significantly lower than those required *in-vitro*. This model also allows for comparison of cytotoxic activity of TC directed against different immunogenic epitopes and permits comparisons of virus-specific and tumor specific TC for their immunotherapeutic potential.

374

OVERCOMING ANTIGENIC COMPETITION TO PRODUCE MULTISPECIFIC CYTOTOXIC T LYMPHOCYTE LINES FOR ADOPTIVE TRANSFER

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HSCT recipients are susceptible to lethal opportunistic infections, which are often not amenable to conventional therapies. We have shown that adoptive transfer of just 2×10^5/kg in vitro expanded multivirus-specific cytotoxic T lymphocyte lines (CTLs) targeting EBV, CMV, and Adv can protect against these infections. Within each CTL line, however, Adv-specific T cells were a minority of the total. Furthermore, epitope analysis showed a small number of Adv epitopes recognized by the trivirus lines, potentially limiting their antiviral potency. This low frequency and restricted epitope recognition is likely due to antigen/epitope competition and we have now devised a means to overcome the problem so that we can extend the approach to produce CTLs targeting additional viral pathogens. We proposed that antigenic competition could be overcome by promoting the survival of T cells with subdominant specificities using IL-4 and IL-7. Co-culture in the presence of these cytokines individually or in combination increased total T cell expansion by 2–6 fold. Further, within 9 days of culture, cytokine-supplemented CTLs had enhanced reactivity (measured by IFNg ELIspot) against multiple immunodominant and subdominant viral epitopes from EBV, CMV, and Adv. For example, Adv specificity in the trivirus CTL detected responses against 5.25 hexon-peptide pools (range 4–12) vs 1.5 pools (range 0–4) in "standard" CTL from the same donors (n = 6). Reactivity to CMV-pp65 was also broader (10 pools (range 8–15) vs 4 (range 4–5). Anti-viral specificities were sustained over multiple rounds of in vitro stimulation, and the CTLs were cytolytic in chromium release and co-culture assays. In contrast, standard CTLs were only cytolytic against immunodominant antigens. To date, addition of these cytokines has allowed us to produce single cell lines containing effector cells specific for multiple epitopes in EBV, CMV, Adv, and BK antigens. This technology will allow us to produce a single T cell product which can safely protect against a wide range of pathogens simultaneously.

375

EXPANSION OF REGULATORY T CELLS WITH ULTRA LOW-DOSE IL-2 AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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CD4$^+$ CD25$^+$ FoxP3$^+$ regulatory T cells (Tregs) are of increasing interest in hematopoietic stem cell transplantation (HSCT) since they may help prevent graft-versus-host disease (GvHD). Murine models demonstrate that infusion of donor-derived Tregs to HSCT recipient mice can prevent GvHD by suppressing alloreactive T cell responses, while human recipients of allografts with low Treg numbers have increased rates of GvHD. We and others have previously given low-dose IL-2 to HSCT recipients in an attempt to enhance antitumor immunity. Although no definitive antitumor effects were observed, the patients were noted to have low rates of GvHD. Retrospectively, it has been demonstrated that patients who received IL-2 therapy had higher levels of FoxP3$^+$ T cells (i.e., Tregs) than controls, suggesting that IL-2 preferentially expands Tregs *in vivo*, thereby preventing GvHD. We have therefore initiated a phase I/II clinical trial to evaluate the efficacy and toxicity of low-dose IL-2 injections following allogeneic HSCT. The intent is to promote Treg expansion *in vivo* and prevent acute GvHD. Thus far, 5 patients who received a HSCT for hematologic malignancies have been treated with ultra low-dose IL-2 (100,000 to 200,000 units/m^2 subcutaneously three times weekly) beginning day +7 to day +28 after HSCT, for 6 to 12 weeks. Median age at time of transplant was 21 years (range, 9 to 56 years). Two patients received matched sibling donor transplants and three received alternative donor transplants with Campath 1-H for *in vivo* T-cell depletion. Flow cytometric analysis of blood from all patients demonstrated a rise in the percentage of CD4$^+$ CD25$^+$ FoxP3$^+$ Tregs by 6 weeks following initiation of IL-2 therapy with mean of 4.7% (range, 0 to 9.8%) pre IL-2 to a mean of 17.7% (range, 7.8 to 31.1%) after IL-2 treatment. Functional analyses of these CD4$^+$ CD25^{bright} cells showed suppression of thymidine uptake in mixed lymphocyte cultures. No grade 3 or 4 toxicities occurred while on IL-2. No patient developed > grade I acute GvHD. One patient who did not complete the full 12 weeks of IL-2 therapy developed extensive, chronic GvHD two months after stopping IL-2. In conclusion, low-dose IL-2 is relatively well tolerated and may expand a CD4$^+$ CD25$^+$ FoxP3$^+$ Treg population *in vivo*. Their effects on GvHD and relapse remain to be determined.

376

RECOVERY OF IMMUNE CELL SUBSETS POSTTRANSPLANT IS ASSOCIATED WITH INFECTION RISK BUT NOT SURVIVAL, RELAPSE OR NON-RELAPSE MORTALITY

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Background: Late recovery of total lymphocyte, CD4 T cell, CD8 T cell, B cell or monocyte counts post transplant has been associated with poor transplant outcomes, including infections, in some but not all studies involving relatively small numbers of patients. We set out to determine whether transplant outcomes could be predicted from immune cell subset counts in a relatively large group of patients (n = 98) with long follow up (4 ½ years).

Methods: In recipients of matched sibling myeloablative transplant performed in 1996–2000 we prospectively measured the following immune subsets at day 30, 80, 180 and 365: total B cells, IgD+ (naive) B cells, IgD- (memory) B cells, CD4+ T cells, CD8+ T cells, CD4+CD8+ cells, CD4-CD8- T cells, CD28+ (costimulation-competent) or CD28- CD4 or CD8 T cells, naïve (CD45RAhigh) or memory (CD45RAlow/neg) CD4 T cells, naïve (CD11alow) or memory (CD11ahigh) CD8 T cells, NK cells and monocytes. Outcomes determined from a retrospective chart review included total, definite (microbiologically documented), bacterial, fungal, viral, HSV, VZV, and CMV infections in the intervals of days 30–80, 81–180, 181–365, and >365 post transplant, as well as overall survival, relapse, non-relapse mortality and fatal infection.