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PHARMACOKINETICS OF ORAL TACROLIMUS IN RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Tacrolimus is an immunosuppressive agent used for the prevention and treatment of graft-versus-host-disease (GVHD) in allogeneic hematopoietic stem cell transplantation (HSCT). In contract to its intravenous formulation, pharmacokinetics of oral administered tacrolimus was yet to be fully examined. **Patients /Methods:** Recipients of allogeneic HSCT with GVHD prophylaxis using tacrolimus were eligible. Tacrolimus were initiated by continuous intravenous infusion from day −1, and continued until patients became capable of taking tacrolimus orally. On the second day of oral tacrolimus administration, whole blood concentration of tacrolimus was measured by MEIA before, 30min, 1, 2, 3, 4, 6, and 12 hours after administration. **Results:** Thirteen patients were evaluable. Mean blood level before administration (C_0) was 9.85 ±1.85 ng/mL. Mean maximum concentration (C_{max}) was 19.8 ±12.3 ng/mL, and median Tmax was 3 hours (range; 1–6 hours). Two subgroups were identified in regard to the difference between C_0 and C_{max}; $C_{max} - C_0 \geq 10$ ng/mL (Group A: n = 4) and $C_{max} - C_0 < 10$ ng/mL (Group B: n = 9). Although there was no significant difference in C_0 and C_{12} between the two groups, mean area under the concentration curve for 12 hours (AUC_{0-12}) in group A was significantly greater than that in group B (204 ± 51.6 vs 136 ± 22.9 nghr/mL; $P = .045$). Mean half-life of tacrolimus in group A was significantly shorter than that in group B (16.2 ± 8.28 hr vs 42.8 ± 15.5 hour; $P = .041$). **Discussion:** Pharmacokinetics of orally administered tacrolimus did not necessarily show a uniform pattern; and a prominent feature was an unexpectedly high C_{max} and AUC observed in some patients. However, this phenomenon could not be recognized only by measuring its trough level (C_0) because of alteration in its half-life. We conclude that pharmacokinetic analysis should be performed in every patient to decide optimal dose of tacrolimus to prevent overdosing.

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DIFFERENTIAL EFFECTS OF IMMUNOSUPPRESSANTS ON ADOPTIVELY TRANSFERRED $CD4^+CD25^{high}$ REGULATORY T CELLS IN PREVENTION OF EXPERIMENTAL ACUTE GRAFT-VERSUS-HOST DISEASE

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The observation that $CD4^+CD25^+$regulatory T cells (Treg) are capable of suppressing experimental aGVHD while preserving the beneficial graft-versus-tumor effect (GVT), fueled interest in exploring cell-based immunotherapy in clinical trials. However, in contrast to experimental models of aGVHD, most clinical transplantation protocols include combinations of immunosuppressive drugs and currently there is no information on the impact of these drugs on Treg function in vivo. Therefore, we evaluated the impact of Cyclosporine A (CSA), Mycophenolate mofetile (MMF), and Rapamycin (RAPA) on Treg function both in vitro and in vivo in a murine acute GVHD model. Treg reisolated from mixed leucocyte reactions (MLR) containing CSA but not RAPA showed significantly reduced ability to suppress $CD4^+CD25^-$ T cell proliferation in secondary MLRs ($P = .025$). The CSA effect could be reversed to 85 ± 3.4% by the addition of IL-2 (50 IU/ml) to the primary culture. In vivo bioluminescence imaging (BLI) after major mismatch BMT demonstrated reduced early proliferation of donor derived luciferase-labeled conventional T-cells (Tc^{luc+}) in animals treated with Treg. The addition of RAPA and to a lesser extent MMF did not interfere with Treg function. Conversely, combining Treg with CSA led to a significantly increased Tc^{luc+} proliferation ($P = .002$) indicating loss of Treg function. Treatment with Treg/CSA was associated with increased GVHD risk and poorest survival of the Treg/drug combinations. The Treg/RAPA combination did not abrogate GVT effector function of donor derived conventional T-cells against $A20^{luc+}$ leukemia cells as determined by BLI and histology. Our data indicate that CSA, but not RAPA or MMF reduces Treg function possibly through an IL-2 dependent mechanism. In vivo, the combination of Treg with RAPA or MMF allowed for adequate GVHD suppression with retention of GVT activity which may provide guidance for clinical trials.

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PHARMACOKINETICS OF ANTI TUMOR NECROSIS FACTOR ANTIBODY (INFLIXIMAB) IN CHILDREN WITH ACUTE GVHD INVOLVING THE GASTROINTESTINAL TRACT

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Infliximab (REMICADE®, Centocor), a chimeric anti-TNFα monoclonal antibody, has been used in the management of patients with steroid-refractory acute GVHD. Though preliminary results using infliximab have been encouraging, the pharmacokinetics (Pk) of infliximab in this clinical setting have yet to be defined. A prospective trial examining the Pk of infliximab in pediatric patients with gastrointestinal (GI) GVHD was undertaken. Five subjects (median 11 yrs, range 9 mo–17 yrs) with GI GVHD were enrolled. All subjects had histologic confirmation of GVHD and had failed > 48 hours of corticosteroids prior to study entry. Subjects received a single 5 mg/kg dose of infliximab. PK and pharmacodynamic samples were drawn at 16 time points (hours 0, 1, 2, 6, 24, 48, 96, weekly days 7–42, days 56, 70, 84). Based upon existing Pk data in patients with Crohn's disease, the time required to reach a trough concentration ≤2 mcg/ml was the defined primary study endpoint. **Results:** Dosing was well tolerated in all five subjects. Infliximab concentrations are as follows: Peak concentrations (Cmax) ranged from 64-160 mcg/ml and were achieved by hours 2–6. Drug concentrations > 20 mcg/ml were maintained for > 7 days in all subjects. The primary endpoint (trough concentration □ 2 mcg/ml) was reached on days 28, 35, 21, 35, and 7(asterisk) (median 28 days, mean 25 days), respectively. Infectious complications with a possible attribution to infliximab included endocarditis on day 21 in 1 subject and CMV reactivation 1 month post infliximab in a second subject. No invasive fungal infections were seen. Durable complete responses of GVHD were seen in 2 subjects by days 7 and 28, respectively. Two subjects are alive (6 months and 16 months). **Conclusion:** In summary, pharmacokinetics of infliximab indicate that concentrations > the targeted threshold level can be maintained for several weeks following a single 5 mg/kg dose in patients with GI GVHD. A phase II clinical trial, with dosing intervals based upon these Pk parameters, will be required. This investigator-initiated study was conducted through the Pediatric Blood and Marrow Transplant Consortium. Infliximab and partial financial support were provided by Centocor, Inc.

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REDUCED-INTENSITY CONDITIONING PERMITS A SIGNIFICANT GRAFT VS LEUKEMIA (GvL) EFFECT FOR ACUTE LEUKEMIA

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Delivering effective treatment for acute leukemia to older adults remains a significant challenge. Bone marrow/hematopoietic progenitor cell transplant (HPCT) remains the only curative option for relapsed/refractory or unfavorable risk disease. Myeloablative conditioning regimens are associated with significant morbidity and mortality. The use of reduced intensity conditioning (RIC) may offer a chance for cure, but whether RIC allows for a significant GvL effect is unclear. We report a single institution fol-

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

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

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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.

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

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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.

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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

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