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IRB Protocol Write-up

Relationship Between Whole Blood Tacrolimus Levels and the Incidence of Acute Graft-Versus-Host Disease (aGVHD) in Pediatric Patients After Allogeneic Hematopoietic Stem Cell Transplant (AlloHCT)

A. Study Purpose and Rationale:

The success of allogeneic hematopoietic stem cell transplantation (AlloHCT) continues to be limited by the incidence of acute graft-versus- host disease (aGVHD), a serious and sometimes fatal complication. aGVHD occurs in the first 100 days post-transplant, and results from effector –T cells in the donor tissue targeting antigens in the immunosuppressed host, which then causes cellular destruction in the skin, GI tract, or liver (Welniak, Blazar et al. 2007). The incidence of aGVHD grades II-IV in the pediatric population ranges from 19-85% (Jonsohn, 2008). Prevention of aGVHD after transplant and optimization of prophylactic regimens is therefore a major concern.

The mainstay of prophylaxis against GVHD is a calcineurin inhibitor (CNI) such as cyclosporine or tacrolimus, in combination with either methotrexate or mycophenylate mofeteil. In many centers, tacrolimus has replaced cyclosporine as the CNI of choice, likely in response to two multicenter phase III trials demonstrating superior prevention of aGVHD and improved side effect profile when using tacrolimus and methotrexate as compared to cyclosporine and methotrexate (Ratanatharathorn, Nash et al. 1998, Nash, Antin et al. 2000).

Tacrolimus is isolated from the growth medium of *Streptomyces tsukubensis* and prevents GVHD by inhibiting T-cell activation through down-regulation of IL- 2. Major toxicities of tacrolimus include hypomagnesemia, nephrotoxicity, hypertension, hyperglycemia and tremor (Yanik, Levine et al. 2000), which necessitates close monitoring of drug levels throughout administration. In order to minimize these toxicities, a suggested target range for whole blood tacrolimus levels post-AlloHCT is 10-20 ng/mL(Przepiorka, Devine et al. 1999) .

Few studies have investigated the optimal whole blood concentration of tacrolimus in preventing aGVHD after AlloHCT. In a large retrospective study in adult patients, Ram and colleagues demonstrated that tacrolimus levels following myleoablative conditioning were not correlated with incidence or severity of GVHD, but that higher tacrolimus levels following non-myeloablative conditioning were associated with a decreased incidence GVHD grades III-IV (Ram, Storer et al. 2012). A later study by Mori et al. reported a significant association between aGVHD incidence and mean tacrolimus concentrations < 15ng/mL (Mori, Kato et al. 2012).

Even fewer published reports have addressed target levels of tacrolimus in the pediatric population after AlloHCT. Many of these studies have been in adult patient populations

and applying their results to the pediatric population is not ideal, as children have demonstrated increased metabolic clearance of tacrolimus when compared to adults (Mehta, Beltz et al. 1999, Przepiorka, Blamble et al. 2000). In patients < 18 years of age, a single study has shown a significant correlation between whole blood tacrolimus levels and incidence of aGVHD (Watanabe, Matsumoto et al. 2009).

The proposed study would be the largest retrospective analysis of the association between whole blood tacrolimus concentrations and incidence of aGVHD in pediatric patients to date. We will also evaluate the time required for these patients to reach a therapeutic steady state level of tacrolimus and perform a risk factor analysis for those patients who were diagnosed with aGVHD.

Hypothesis:

We postulate that lower whole blood tacrolimus levels during week 2 after transplant can predict incidence of aGVHD in our population of pediatric patients treated with AlloHCT. We also postulate that patients who were diagnosed with aGVHD, took more days on average to achieve a steady state tacrolimus level in the therapeutic range (defined in this study as >11 ng/mL).

B. Study Design and Statistical Analysis:

Conceptual and Operational Definitions:
Predictor variables are median whole blood tacrolimus levels for days 1-7, 8-14, 15-21, 22-28 and 1-28, and number of days to reach steady state therapeutic level (>2 days with level >11 ng/mL). The primary outcome variables are diagnosis and grade of aGVHD as defined by the currently accepted consensus criteria. Confounding variables include patient age, donor age, donor sex, HLA disparity, preconditioning regimen, and malignant vs. non-malignant underlying disease.

Study Design:
This study is a retrospective chart review of 150 patients treated with AlloHCT from related or unrelated donors, for malignant and non-malignant disorders at Columbia University Medical Center from 2005 to 2012.

Statistical Analysis:
Patients will be divided into 2 groups depending on whether they had myeloablative or non-myeloablative conditioning. Within these groups, to compare aGVHD and no aGVHD groups, we will use an unpaired two-tailed T-test for means of median tacrolimus levels during week 2 after transplant.

We will also use ROC to determine level at which there is the greatest sensitivity and specificity in predicting incidence of aGVHD. We can then use this level (~11) to compare mean lengths of time for patients with and without aGVHD to be above that level for >2 days. (unpaired T test)

Cox regression analysis will be utilized for our entire sample population to estimate the effects on incidence of aGVHD by other covariates including patient age, donor age, donor sex, HLA disparity, preconditioning regimen, and malignant vs. non-malignant underlying disease.

Sample Size:
Myeloablative conditioning regimen:
At $\alpha = 0.05$ and Power = 0.8, we could see an effect size of 1.5, with 30 patients in the GVHD group and 30 patients in the no GVHD group assuming a standard deviation of 2.0.

Non-myeloablative conditioning regimen:
At $\alpha = 0.05$ and Power = 0.8, we could see an effect size of 1.3, with 30 patients in the GVHD group and 60 patients in the no GVHD group assuming a standard deviation of 2.0.

C. Study Procedure:

For patients in this study, tacrolimus was started between day -9 and -1 prior to AlloHSCT and initiated via an intravenous infusion at a rate of 0.03 mg/kg/day. The dose was adjusted in accordance to the institutional protocol with the goal of maintaining whole blood tacrolimus levels between 10 and 20 ng/mL. Once patients were able to tolerate oral medications, they were transitioned to oral tacrolimus. Whole blood samples were collected with each patient's regularly scheduled morning labs while they were on a continuous intravenous drip and prior to the patient's morning dose once they were transitioned to oral tacrolimus. In the first month after transplant, most patients had levels measured on a daily or near daily basis. Once aGVHD was diagnosed, patients were either started on topical or systemic glucocorticoids, which were tapered after completion of induction therapy and improvement in GVHD symptomatology.

Data collection will be performed by manual chart review. All tacrolimus levels obtained through day +28 will be collected, as well as data regarding underlying diagnosis, GVHD diagnosis and grade, day that GVHD was diagnosed, day converted to PO, preconditioning regimen, age, sex, HLA disparity, donor source, and donor gender. We will also collect data regarding use of any drugs that may affect tacrolimus levels such as phenytoin, azole medications, flagyl and caspofungin.

D. Study Drugs:
None

E. Medical Device:
None

F. Study Questionnaires:
None

G. Study Subjects:
Inclusion criteria include: (1) T-cell depleted bone marrow transplantation from HLA-A, B and DR matched donors, (2) use of tacrolimus and mycophenolate mofetil ± methotrexate for GVHD prophylaxis, (3) administration of tacrolimus for at least 4 weeks after transplantation, (4) age 21 years or younger, and (5) measurement of blood tacrolimus levels at least four times per week. Exclusion criteria include: (1) engraftment syndrome, (2) discontinuation of tacrolimus prior to the end of week 2 post-transplant, (3) acute GVHD diagnosed prior end of week 2 post-transplant, and (4) use of drugs known to interact with tacrolimus metabolism.

H. Recruitment of Subjects:
The medical records of patients who have been treated within the pediatric bone marrow transplant department at New York Presbyterian Columbia will be utilized. There will be no recruitment of medical records from outside facilities unless those records had been previously transferred to New York Presbyterian Columbia with patient care.

I. Confidentiality of Study Data:
Waiver of informed consent is requested due to the fact that this is a retrospective chart review which does not require subject contact and therefore involves no more than minimal risk to the patients involved. Waiver of consent will not adversely affect the rights or welfare of the subjects involved because no personal patient identifiers (i.e. initials, individual case studies) will be used. Data will be stored in a secure location, accessible only to investigators.

J. Potential Conflict of Interest:
None

K. Location of the Study:
Pediatric bone marrow transplant division at New York Presbyterian Columbia University Medical Center

L. Potential Risks:
The only risk to subjects is disclosure of identifying medical information to personnel outside of the research team. If medical information is disclosed to personnel outside the medical teams, it will be immediately reported to the IRB with a corrective action plan as a protocol deviation for further review. Ensuring all computers with patient information are password protected will ensure this risk remains low.

M. Potential Benefits
The results of this trial could lead to identifications of the factors in tacrolimus dosing and administration that are associated with aGVHD. Once we identify the risk factors and high risk patients, we may be able to create a set of recommendations that will help clinicians to use a standardized tacrolimus dose and administration plan. Information

obtained can also lead to future prospective trials studying tacrolimus dosing and administration. Information from this study leading to the development of future protocols may also lead to practice changes.

N. Alternative Therapies:
None

O. Compensation of Subjects:
None

P. Costs to Subjects:
The subjects will not incur any additional costs as a result of participation in this study.

Q. Minors as Research Subjects:
The study population does include minors, however, their active participation is not required as this is a retrospective chart review.

R. Radiation or Radioactive Substances: None
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