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CLINICAL CELLULAR THERAPY

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The Clinical and Financial Cost of Preemptive Management of CMV Disease – Implications for Immunotherapy

Natasha A. Jain, Sawa Ito, Pawel Muranski, Kit Lu, Jan Haggerty, Catalina Ramos, Lisa Cook, Christopher S. Hourigan, Richard Childs, Minoo Battiwalla, A. John Barrett. Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, MD

Although CMV infection only rarely leads to direct infectious mortality, and early CMV reactivation may reduce relapse rates in patients with myeloid malignancies, the management of CMV by preemptive antivirals may itself induce toxicity and financial burden which need to be quantified to provide justification for antiviral cellular immunotherapy. In this study, we analyzed CMV reactivation in 134 (72 males, 62 females) patients undergoing allo-SCT at our institute between 2006 through 2012, comparing outcomes and cost after viral reactivation based upon the risk of CMV reactivation. 102 subjects were transplanted for malignant diseases; and 32 were transplanted for non-malignant diseases. 81 subjects received CD34+ selected myeloablative, 12 received UCBT, and 41 had T-replete non-myeloablative transplant. Median age at transplant was 40 years and the median follow-up is 4.25 years. 119 (88.8%) were at risk for CMV by virtue of either the donor or recipient being sero-positive prior to transplant. Of these, 90 (75.6%) had CMV reactivation in the blood (>250 copies/ml by PCR) including 4 (3.4%) who had CMV organ disease (involving the GI=3 , lung=2, CNS=1) and all received antivirals. Median time to first CMV reactivation was 34 days (range: 8-105 days). 11% reactivated before d14+ and 92% by d100+. For comparison, we defined two groups: "Antiviral group" consisting of the 90 subjects (reactivation and/or organ disease) and a "No therapy group" consisting of 44 subjects (29 at risk subjects who did not need antiviral treatment, and 15 who were not at risk). The Antiviral group averaged 12.6 days of IV antiviral therapy and 13.9 additional days of hospitalization (P<0.02). We calculated the additional cost for the Antiviral group to be between $55,000 to $71,000 per patient. We also evaluated the impact of CMV risk on overall survival and on NRM but found no statistically significant impact. However, in multivariable modeling of NRM, CMV reactivation >250 copies/ml (OR=3, P<0.048), total duration of inpatient IV antiviral therapy (OR = 1.04 ,P<0.001), type of transplant (T-deplete vs. T-replete) (OR=4.65 ,P<0.017), were found to be significantly associated; the model excluded age, sex, diagnosis (malignant vs. non malignant) and CMV risk status.Our findings document that although direct infectious mortality from CMV has been eliminated, there is a significant cost of therapy in terms of drug and inpatient hospitalization as well as a strong association with NRM. Implications for antiviral cellular therapy are that early administration even prior to day 14 is necessary to meaningfully impact reactivation and that cost savings exceeding $25,000 are possible with even moderate (50%) reduction in the rate of reactivation requiring antiviral therapy.

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Performance Validation of Revolution Centrifugal Force (RCF) for Plasma Depletion on Hematopoietic Progenitor Cell Apheresis Product Using Sorvall ST40R Centrifuge

Sauvai chang-Fong [1], Deborah Lamontagne [2], Kristin Rathmann [3], Anginett Batista [2], Joanne Sullivan [2,3], Melhem Solh [4]. [1] Florida Hospital Cellular Therapy Lab, Orlando, FL; [2] Florida Hospital Cancer Institute, Orlando, FL; [3] Florida Hospital Cellular Therapy Laboratory, Orlando, FL; [4] Florida Center for Cellular Therapy, University of Central Florida, Orlando, FL

Background: Original Performance Qualification on Sorvall ST40R Centrifuge for plasma depletion on Hematopoietic progenitor cell (HPC) product was set at 300g Revolution Centrifugal Force (RCF) 10 minutes at 20°C to achieve ≥ 80% Total Nucleated Cell (TNC) recovery. Cellular Therapy Lab (CTL) noticed the spun down product has unclear supernatant plasma, loosely packed cellular sedimentation with difficulty for separation. CTL has decided to re-validate the process to find an optimal RCF for a clear & defined separation between plasma and Buffy coat, maintaining ≥ 80% TNC recovery.
Study Design and Methods: Research performed on practice recommendations and reference materials lead to select three variables of RCF: 700g, 1000g, and 1300g for this study. Ten samples were collected from different sources: single donor whole blood; pooled whole blood; pre HPC,Apheresis peripheral blood; HPC product and Donor Lymphocyte product. Sample size varied from 21ml – 120ml. Each sample was divided into three equal portions centrifuged for 10 minutes at 20°C at the selected RCF. White blood cell (WBC) count was tested by the Sysmex cell counter. Viability was tested with Trypan Blue method. The following data and test results were recorded for each portion pre and post centrifugation: sample volume, WBC, packed cell volume, plasma volume, Post plasma WBC, calculated TNC, Viability%, TNC % recovery, Clarity of spun sample, and Appearance of Buffy-coat. One way analysis of Variance was run to assess the P value for statistical significant difference. Optimal RCF was selected after this study. Validation of the selected RCF was performed on 5 HPC products from prior transplant recipients.
Result: The study found no statistically significant difference among the 3 RCF variables with P=0.67. The difference between Pre & Post Viability was less than 3 % which indicates minimal cell damage during centrifugation. Observation found hazy plasma and loose Buffy Coat with 700g, clear and defined with 1000g and 1300g. CTL selected RCF 1300g for plasma depletion. Validation study on 7 processes found post TNC plasma range of 0.01-0.10% of pre TNC, difference in Pre & Post Viability is 0-1%, five patients with successful absolute NC and platelet engraftment. Results of retro study compared to 7 random processes using prior RCF 300g found P= 0.352.

RCF selection Result: TNC % Recovery	RCF 700g	RCF 100g	RCF 1300g
Range	91-106	91-100	93-100
Median	95.5	98.5	97.0
Standard Deviation	4.22	2.62	2.41

Comparison Result: TNC % Recovery	Prior RCF 300g	Implemented RCF 1300g
Range	85-93	85-98
Median	88	90
Standard Deviation	3.13	3.99

Conclusion: The study found no significant difference in TNC % recovery using 700g, 1000g and1300g. There is defined cell

pellet and clear plasma with higher RCF for ease of separation which help to harvest the final concentrated cellular product for cryo-preservation; especially using small volume bag.

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Optimal Cryopreservation Conditions to Preserve Viability, Proliferation, and Lytic Function of

Zehra E. Cobanoglu [1], Dean A. Lee [2], Vladimir Senyukov [1].
[1] Experimental Pediatrics, University of Texas MD Anderson Cancer Center, Houston, TX; [2] Pediatrics, University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Natural killer (NK) cells are a promising tool for adoptive immunotherapy in many disease settings. The availability of large quantities of functionally effective NK cells represents one of the major challenges for immunotherapeutic trials, and a major objective of our laboratory is to enhance NK cell number and function ex vivo for subsequent adoptive immunotherapy. We previously published a robust approach for ex vivo propagation of NK cells that has been validated for healthy donor (Denman, 2012) and patient peripheral blood (Liu, 2013), cord blood (ASH 2012), and embryonic/pleuripotent stem cell sources (Knorr 2013) and has the potential to generate far more NK cells than can be infused at one time. Cryopreservation of NK cells provides significant advantages over infusion of fresh products by 1) reducing timing constraints in matching GMP manufacturing to patient need, 2) enabling multiple infusions from single large-scale expansions, and 3) ensuring uniform characteristics for aliquots, all of which are essential for generating off-the-shelf products. However, NK cells are known to have poor survival and function after cryopreservation, and this was recently confirmed in clinical trials using standard clinical GMP cryopreservation media containing Plasmalyte, dimethyl sulfoxide (DMSO), and human serum albumin (HSA). In contrast, our preclinical data with expanded NK cells cryopreserved in media, fetal bovine serum (FBS) and DMSO showed excellent function and no significant difference between fresh and frozen cells (Liu, 2013). Therefore, we hypothesized that serum may provide protective factors for NK cells during cryopreservation.
Methods: NK cells from four healthy donors were expanded with weekly addition of irradiated K562 Clone9.mbIL21 feeder cells as previously described. After three weeks, cells were cryopreserved with freezing media of 40% Plasmalyte and 10% DMSO, with varying ratios of HSA and human AB serum (hAB) comprising the remainder. Cryopreserved NK cells were thawed and assessed for cell recovery, viability, proliferation, and function at 3, 24, 48, 72,120, 168 hours after thawing. Cytotoxicity was determined against 721.221 target cells.
Results: A linear relationship was observed between hAB content and recovery, viability, and cytotoxicity, plateauing at 40% hAB. Unstimulated proliferation after thawing was associated with higher hAB content, but re-stimulation with Clone9.mbIL21 was similar in all conditions.
Conclusion: In the present study, we aimed to establish the benefit of including serum in cryopreservation of expanded NK cell. We confirmed a significant effect in preserving viability, cytotoxicity, and proliferation, justifying its use in clinical trials.

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[18F]-Fhbg-PET/CT Imaging of CD34-TK75+ T Cells in Allogeneic HSCT Recipients after Donor Lymphocyte Infusion (IND#11917; Clinicaltrials.Gov Identifier: NCT00871702)

Linda G. Eissenberg [1], Michael P. Rettig [2], Julie K. Ritchey [1], Robert Fulton [3], Farrokh Dehdashti [4], David Piwnica-Worms [5], John F. DiPersio [2]. [1] Internal Medicine, Washington University School of Medicine, St. Louis, MO; [2] Bone Marrow Transplantation & Leukemia Section, Division of Oncology, Washington University School of Medicine, St. Louis, MO; [3] Genome Institute, Washington University School of Medicine, St. Louis, MO; [4] Radiology, Washington University School of Medicine, St. Louis, MO; [5] Cancer Biology, M.D. Anderson, Houston, TX

Background: Allogeneic transplantation is associated with both GvL and GvHD. Elimination of donor T cells results in less GvHD, but also less GvL and engraftment. To eradicate infused T cells if GvHD develops, our suicide gene based therapy uses a CD34-Herpes Simplex Virus-1-thymidine kinase (CD34-TK75) chimera (Rettig et al, Mol Ther 2003 and J Immunol 2006). We enrich and purify retrovirally transduced cells via cell surface expression of the extracellular domain of CD34. Modified TK75 both mediates cell suicide upon treatment with ganciclovir and enables tracking of modified cells that entrap the substrate, [^{18}CT/CT.
Methods: Following production and release testing of CD34-TK75 transduced and affinity-purified T cells (88-98.8% CD34+), we began a phase I pilot and feasibility study. Cells were infused as a DLI (0.1-1.3 x 10^6 CD34-TK75+ T cells/kg) into 8 patients (relapsed after allo-HSCT). Since no toxicities were observed during treatment of our first 2 patients, we initiated trafficking studies using [^{18}F]-FHBG-PET/CT imaging at baseline, d15, and d30 after infusion of CD34TK75+ T cells in patients 3-8.
Results: No acute toxicities were associated with administering either genetically modified T cells or [^{18}F]FHBG. Using real-time quantitative PCR, we detected the CD34-TK75 transgene in the circulating CD34-TK75+ T cells of all but one patient. GvHD developed in only one patient (grade III GI) who did not respond to a 10 day treatment with I.V. ganciclovir (10 mg/kg). CD34-TK75 transgene could not be detected in the peripheral blood of this patient at the time of GvHD. Patient samples were also tested for replication competent retrovirus and for integration sites both by Ligation Mediated-PCR and hybrid capture. Baseline levels of [^{18}F]FHBG were low, primarily characterized by renal excretion, which should permit detection of T cell expansion in the lymphoid organs or intestines. However, pharmacokinetics of the radiolabel were similar in the one patient that did and those that did not develop GvHD (Fig. 1). In parallel experiments, NOD/SCIDg mice were injected (i.o.) with 2 x 10^6 CD34-TK75+ T cells (patients 2-8; n=2 mice per donor). Control mice received conditioning only. Using micro-PET/CT

SHAPE * MERGEFORMAT

Baseline scan
14-Day scan
30-Day scan
TK04 (no GvHD)
ANT
ANT
ANT
POST
POST
POST
Baseline scan
14-Day scan
30-Day scan
TK07 (Liver GvHD)
ANT
ANT
ANT
POST
POST
POST