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14. ABSTRACT

1. Contingency Preparedness: Collect information from transplant centers, build awareness of the Transplant Center Contingency Planning Committee and educate the transplant community about the critical importance of establishing a nationwide contingency response plan.

2. Rapid Identification of Matched Donors: Increase operational efficiencies that accelerate the search process and increase patient access are key to preparedness in a contingency event.

3. Immunogenic Studies: Increase understanding of the immunologic factors important in HSC transplantation.

4. Clinical Research in Transplantation**:** Create a platform that facilitates multicenter collaboration and data management.

15. SUBJECT TERMS
Research in HLA Typing, Hematopoietic Stem Cell Transplantation and Clinical Studies to Improve Outcomes

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DEVELOPMENT OF MEDICAL TECHNOLOGY

FOR CONTINGENCY RESPONSE TO MARROW TOXIC AGENTS

QUARTERLY RESEARCH PERFORMANCE REPORT

SUBMITTED October 13, 2022

Office of Naval Research

And

The National Marrow Donor Program®

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Minneapolis, MN 55401

I. Heading

PI: Jeffrey Auletta, M.D.

National Marrow Donor Program

N00014-20-1-2832

Development of Medical Technology for Contingency Response to Marrow Toxic Agents

II. Scientific and Technical Objectives

The main goal of all activities funded through this grant is to develop, test and mature the ability of the NMDP Coordinating Center and NMDP contracted network sites network sites to address contingency events wherein civilian or military personnel are exposed to marrow toxic agents, primarily ionizing radiation or chemical weapons containing nitrogen mustard. As a result of prior efforts in this regard a solid foundation has been established. The proposed new activities will continue to enhance and expand our capabilities in each of the four focus areas. Contingency preparedness activities will continue to integrate NMDP's role with federal, state and local agencies.

An accident, a military incident, or a terrorist act in which a number of individuals are exposed to marrow toxic agents will result in injuries from mild to lethal. But the extent of individual injuries and the likelihood of recovery in many cases will not be apparent until days or weeks after the event. Casualties will be triaged by first responders, and those with major marrow injuries who will need aggressive medical support and may be ultimately candidates for hematopoietic cell transplantation (HCT) will need to be identified. While these patients are being supported, HCT donor identification activities will be initiated because it will not be initially clear which ones may ultimately require HCT. NMDP-approved transplant centers will provide a uniform and consistent clinical foundation for receiving, evaluating and caring for casualties. NMDP Coordinating Center will orchestrate the selection and testing necessary to rapidly identify the best available donor or cord blood unit for each patient utilizing its state-of-the-art communication infrastructure, sample repository, laboratory network, and human leukocyte antigen (HLA) expertise. NMDP's on-going immunobiologic, bioinformatics and clinical research activities promote studies to advance the science and technology of HCT transplantation to improve outcome and quality of life for the patients.

Importantly, most individuals with near-lethal marrow toxic injuries will recover their own marrow function provided they receive intensive supportive care from the medical professionals that are part of the contingency response community.[1] These professionals can save the lives of persons with severe marrow suppression using the knowledge and skills practiced every day to treat patients undergoing HCT coordinated through the NMDP.

III. Approach

A. Contingency Preparedness

HCT teams are uniquely positioned to care for the casualties of marrow toxic injuries. The NMDP manages a network of centers that work in concert to facilitate unrelated HCT. The Radiation Injury Treatment Network (RITN), comprised of a subset of NMDP's network centers, is dedicated to radiological disaster preparedness activities and develops procedures for response to marrow toxic mass casualty incidents.

B. Development of Science and Technology for Rapid Identification of Matched Donors
Disease stage at the time of transplantation is a significant predictor of survival, decreasing the time to identify the best matched donor is critical. Methods are under development to rapidly provide the best matched donor for HCT.

C. Immunogenetic Studies in Transplantation
Improving strategies to avoid and manage complications due to graft alloreactivity is essential to improve the outcomes of HCT. Research efforts are focused on strategies to maximize disease control while minimizing the toxicity related to alloreactivity in HCT.

D. Clinical Research in Transplantation
Clinical research creates a platform that facilitates multi-center collaboration and data management to address issues important for managing radiation exposure casualties. Advancing the already robust research capabilities of the NMDP network will facilitate a coordinated and effective contingency response.

IV. Updates

A. Contingency Preparedness

Maintain the Radiation Injury Treatment Network (RITN) to prepare for the care of patients resulting from a hematopoietic toxic event.

RITN and the Radiation Emergency Assistance Center/Training Site (REAC/TS) developed a new 2.5-day in-person training course which targets a new audience (healthcare coalition members). The course includes lecture, hands on demonstrations, skill practice, and an exercise. The pilot courses are tentatively scheduled for September in the cities of Philadelphia and San Francisco and attendance will be offered to local hospital coalition members. Due to increased COVID travel and in-person meeting restrictions in San Francisco, CA and Philadelphia, PA, these courses were postponed. The University of San Francisco and Stanford University successfully hosted the rescheduled course June 28-30, 2022. The Children's Hospital of Philadelphia, Thomas Jefferson University, and the University of Pennsylvania are also successfully hosted their rescheduled course July 12-14, 2022.

B. Development of Science and Technology for Rapid Identification of Matched Donors

Expand the genetic diversity of the registry through continued addition of adult donors and cord blood units, utilizing high volume HLA typing methodologies.

A total of 115,619 newly registered volunteer donors were HLA typed and added to the Be The Match Registry during the performance period.

Modeling and analysis of registry coverage for the Warfighter

Activity under this grant is complete and will continue under a subsequent award.

Development of science and technology for rapid communication of HLA data

Activity under this grant is complete and will continue under a subsequent award.

Use of population genetics and machine learning to automate the donor selection process

Activity under this grant is complete and will continue under a subsequent award.

C. Immunogenetic Studies in Transplantation

Evaluate HLA disparity and impact on HCT by adding selected pairs to the Donor/Recipient Pair project utilizing sample selection criteria that optimize the new data generated by the typing project.

Activity under this grant is complete and will continue under a subsequent award.

Develop and mature typing characterization of immunogenetic regions from underserved populations to improve matching and transplant outcomes for more diverse patients

Activity under this grant is complete and will continue under a subsequent award.

Determine the frequency and risks associated with donor clonal hematopoiesis of indeterminate potential in HCT.

Evaluating the impact of donor clonal hematopoiesis of indeterminate potential (CHIP) on HCT outcomes

Completed the analysis for the study entitled "GV19-01: Exploring the link between donor engrafted clonal hematopoiesis and adverse outcomes in allogeneic HCT: Pilot study. The study found no associations between donor CHIP and any outcomes. An abstract describing the results of the pilot study was presented as a poster at the 2022 BMT Tandem Meetings. A manuscript describing the results of the pilot study is under development. The lack of an association between CHIP and outcomes resulted in a reevaluation of this line of inquiry under this grant. Funds allocated for this effort have been reassigned to address the more pressing topics noted below.

Evaluation of Unrelated Donor Peripheral Blood Stem Cell (PBSC) Graft Composition and Impact on Allogeneic HCT Outcomes

While allogeneic HCT offers potentially curative therapy to patients with a variety of benign and malignant diseases, both acute and chronic GVHD continue to plague the field and often limit the longevity and quality of life for patients. The composition of PBSC grafts has been evaluated in multiple studies to attempt to discern associations between various cellular subsets and outcomes. The BMT CTN 0201 randomized trial of bone marrow versus PBSC found that PBSC grafts were associated with a higher risk of cGVHD and worse quality of life following unrelated donor HCT compared to BM. A correlative study of graft immunophenotype failed to identify any associations between PBSC graft composition and outcomes. However, the PBSC cohort included only 147 evaluable products limiting the power to evaluate various cellular subsets. The association between PBSC graft immunophenotype and outcomes remains unclear.

The primary aim of this study is to evaluate PBSC graft stem cell and associated immune cell composition and to determine at 12-months of follow-up how either the comprehensive graft cellular composition profile or specific graft composition elements influences the primary outcomes of time to neutrophil engraftment and overall survival. Secondary outcomes of interest include, but not limited to, incidence of acute and chronic GVHD, primary disease relapse, TRM, and DFS. The study will evaluate approximately 2,100 PBSC products over a 3-year accrual period with 1,100 collected in the U.S. through the NMDP and an additional 1,000 collected in Germany for U.S. based patients through the DKMS. The U.S. testing will be supported through this grant and DKMS will support testing of German collected products. Data will be merged with CIBMTR collected clinical outcomes for analysis and correlation with clinical outcomes.

Analyses include:

- Stem cell subset composition (not just number) influences time to engraftment and immune reconstitution.
- Both conventional and novel unconventional T cell subsets within the graft influence GVHD, relapse, infection and immune reconstitution after transplant.
- Natural killer cells have a role in transplant biology and number and phenotype in the donor graft influence GVHD, relapse, infection and immune reconstitution after transplant.

- The myeloid/antigen presenting cell compartment of the graft influences infection risk and immune reconstitution, thus play a role in long term patient outcome.

The secondary aims of this study are:

- Explore potential associations of favorable PBSC graft composition features that may be predicted by analysis of peripheral blood samples at time of unrelated donor work-up such that these biomarkers could be incorporated into donor selection algorithms.
- Evaluate graft composition association with >12-month outcomes for overall survival, primary disease relapse, DFS and the incidence of late transplant effects including, but not limited to, chronic GVHD, diseases of the cardiovascular, pulmonary, and endocrine systems, dysfunction of the thyroid gland, bone diseases and the development of secondary primary malignancies.
- Establish a cohort of pre-transplant recipient and pre-donation adult unrelated donor biologic samples (whole blood, plasma, viable PBMC and viable donor PBSC graft mononuclear cells) collected prospectively from donors and patients enrolled on this study. This important biospecimen resource will be critical for the support of additional protocol team defined allogeneic HCT related correlative studies that will extend the knowledge gained from the primary study.

During the past quarter accrual continued for U.S. based donors. A total of 212 product samples were received and tested through September 30, 2022 with 67 tested in the last quarter. Testing costs are covered under this grant while staff support is funded under a subsequent grant. The DKMS laboratory continued efforts to establish the standardized immunophenotyping panel for testing of Germany based donors. The study team met several times to finalize plans for concordance testing between the U.S. and DKMS laboratories. Concordance testing using known controls supplied by the NMDP testing laboratory will be performed in the next quarter. Accrual of German donors has begun on a limited basis and will be expanded following successful completion of the concordance evaluation to ensure that data is being consistency captured in both the U.S. and German laboratories. Testing of German donors will be fully funded by DKMS.

A national framework for introducing measurable residual disease testing into the clinical care of AML patients undergoing allogeneic transplantation

While allogeneic HCT is a curative therapy for many patients with acute myeloid leukemia (AML), the risk of relapse even after achieving a cytomorphological complete remission (CR) is the most common form of treatment failure and death. Transplant-related morbidity and mortality is a major obstacle for the effective use of alloHCT, resulting in the potential under- or over-utilization of conditioning regimen intensity to prevent AML relapse. The presence of residual leukemic burden, known as measurable residual disease (MRD), prior to transplant is associated with worse outcomes after transplantation. AML MRD testing is not standardized, and no clear path to translate findings from research laboratories to clinical transplant settings currently exists.

Planning continued on a project designed to address this issue by developing a coordinated national framework to 1) allow collection of leftover initial AML diagnosis material from patients who have received alloHCT in US centers, 2) prospectively collect samples from AML patients after unrelated donor alloHCT to determine optimal timing and method for post-alloHCT MRD monitoring and 3) implement findings from phases 1 and 2, together with a central reference laboratory, to allow local centers to perform standardized MRD testing pre or post alloHCT. This would allow both selection of conditioning intensity, but also inform post-transplant maintenance and allow patient selection for novel clinical trials.

During the past quarter the protocol team continued to meet regularly to finalize plans to launch the IRB approved and ClinicalTrials.gov registered study protocol entitled, "MEASURE: **M**olecular **E**valuation of **A**ML patients after **S**tem cell transplant to **U**nderstand **R**elapse **E**vents". To date, 15 centers have committed

to participate in the study and combined plan to enroll >250 patients per year. Eight of 15 sites have received local IRB approval for the protocol. All sites have initiated submission of regulatory documents required for participation. Site initiation visits were completed for 3 sites with 2 more scheduled for early next quarter. One site has fully opened the study and has enrolled 3 patients. The study will continue under a subsequent grant.

Even when patient and donor are HLA matched, post-transplant complications occur, therefore, other loci may play a role

During the past quarter a KIR genomic typing project completed its 4th phase with 200 samples using a KIR capture and long-range sequencing protocol[6] developed under this grant. This phase, combined with the previous phases of 8, 12, and 48 samples brings the total to 268 individuals and demonstrates the ability to scale this assay to clinical cohort levels.

The KIR genes encode proteins containing two or three extracellular immunoglobulin-like domains that recognize human leukocyte antigen (HLA)/peptide complexes and other ligands. This recognition helps initiate inhibitory or activating cytotoxic signaling in natural killer (NK) cells. NKs and their KIR receptors are essential to human health and their genes impact infections (including HIV), pregnancy, autoimmune diseases, transplantation, and immunotherapy.

KIR genes have expanded via tandem duplication and evolved in primates over the last 30–40 million years. They are 10–15 000 bp long and separated by ~1000 bp. Homology is a characteristic of the region, and a pair of alleles of any two genes are ~85–98% identical. The recombination rate is high in this system, and dozens of gene-content haplotypes are seen in Europeans. It is a transposon-rich region, which provides the primary mechanism for recurrent meiotic recombination events. These characteristics have made the region intractable and essentially invisible to whole genome sequencing based on short read-length methods (e.g., shotgun sequencing). Targeted typing methods using NGS generate results that are highly ambiguous and incomplete and require assumptions about the underlying genomics to interpret.

Many studies have published associations with KIR and human health. They are not necessarily consistent, but they all are based on low-resolution factors such as presence/absence of gene region or short motifs in certain genes. Higher-resolution genomic analysis has not been possible due to lack of sequencing capability until now. The new "clonal" genomic sequencing method developed under this grant provides the ability to annotate and associate at any resolution or feature. The relevance of establishing this at scale is that it means for the first time the clinical relevance of the entire KIR system can be studied instead of small fractions. Analysis of this data is still underway, but it has already contributed 27 new haplotypes to the human genome reference which now contains more alternate references for the KIR region than any full chromosome, 47% of which are from African Americans.

D. Clinical Research in Transplantation

Conduct clinical outcomes research using the CIBMTR research database and repository.

Observational Research

- Published 156 manuscripts in peer-reviewed journals during the grant period.
- Additional research activity will be reported under a subsequent grant.

Support for the Clinical Transplant-Related Long-term Outcomes of Alternative Donor Allogeneic Transplantation (CTRL-ALT-D) trial

BMT CTN 1702: Clinical Transplant-Related Long-term Outcomes of Alternative Donor Allogeneic Transplantation (CTRL-ALT-D) trial has accrued 1758 subjects through September 2022. A total of 2 patients were accrued in the past quarter and closed to accrual on August 10, 2022. Study follow-up will continue under a subsequent grant.

Publications

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* The American Society of Blood and Marrow Transplant was renamed as The American Society of Transplant and Cellular Therapy in 2020. The change led to an update to the name of the society journal from Biology of Blood and Marrow Transplant (Impact Factor: 3.9) to the Journal of Transplant and Cellular Therapy resulting in a reset of the impact factor.