

**NATIONAL
MARROW
DONOR
PROGRAM®**

Entrusted to operate the C.W. Bill Young Cell Transplantation Program,
including Be The Match Registry®

July 21, 2011

CDR Sheri Parker
Office of Naval Research (ONR 342)
875 N. Randolph St.
Arlington, VA 22203-1995

Subject: Final Report of the National Marrow Donor Program®

Reference: Cooperative Agreement #N00014-06-1-0859 between the Office of Naval Research
and the National Marrow Donor Program

Dear CDR. Parker:

In accordance with the requirements of the Referenced Cooperative Agreement, the enclosed subject document is provided as the Final Report for each statement of work task item of the Cooperative Agreement for the period of November 1, 2005 through October 31, 2007.

With this submittal of the Final Report, the National Marrow Donor Program has satisfied the all reporting requirements of the above referenced Cooperative Agreement.

Should you have any questions as to the scientific content of the tasks and the performance activity of this progress report, you may contact our Chief Medical Officer – Dennis Confer, MD directly at 612-362-3425.

Please direct any questions pertaining to the cooperative agreement to my attention (612-362-3403 or at cabler@nmdp.org).

Sincerely,



Carla Abler-Erickson, M.A.
Sr. Contracts Representative

Enclosure: One (1) copy of subject document

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ACRONYM LIST

AABB	American Association of Blood Banks
ABMDR	Australian Bone Marrow Donor Registry
AFA	African American or Black
AML	Acute Myelogenous Leukemia
API	Asian Pacific Islander
ARS	Acute Radiation Syndrome (also known as Acute Radiation Sickness)
ASBMT	American Society for Blood and Marrow Transplantation
ASEATTA	Australasian and South East Asian Tissue Typing Association
ASH	American Society of Hematology
ASHI	American Society for Histocompatibility and Immunogenetics
ASPR-HHS	Assistant Secretary of Preparedness and Response, Department of Health and Human Services
B-LCLs	B-Lymphoblastoid Cell Lines
BMDW	Bone Marrow Donors Worldwide
BMT CTN	Blood and Marrow Transplant - Clinical Trials Network
BRT	Basic Radiation Training
C&A	Certification and Accreditation
CAU	Caucasian
CBMTG	Canadian Blood and Marrow Transplant Group
CBB	Cord Blood Bank
CBC	Congressional Black Caucus
CBU	Cord Blood Unit
CCM	Central Contingency Management
CHTC	Certified Hematopoietic Transplant Coordinator
CIBMTR	Center for International Blood & Marrow Transplant Research
CIMS	Crisis Information Management System
CLIA	Clinical Laboratory Improvement Amendment
CME	Continuing Medical Education
CREG	Cross Reactive Groups
CSRS	Critical Staff Recovery Site
CSS	Custom Search Support
CT	Confirmatory Testing
DC	Donor Center
DIY	Do it yourself
DNA	Deoxyribonucleic Acid
DOD	Department of Defense
D/R	Donor/Recipient
EBMT	European Group for Blood and Marrow Transplantation

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ACRONYM LIST (Continued)

EFI	European Federation for Immunogenetics
EM	Expectation Maximization
EMDIS	European Marrow Donor Information System
FDA	Food and Drug Administration
FMHQ	Family Medical History Questionnaire
FOCIS	Federation of Clinical Immunology Societies
Fst	Fixation Index
FTA	Whatman FTA®
GETS	Government Emergency Telecommunications Service
GCSF	Granulocyte-Colony Stimulating Factor (also known as filgrastim)
GVHD	Graft vs Host Disease
HCT	Hematopoietic Cell Transplant
HCV	Hepatitis C virus
HHS	Health and Human Services
HIEDFS	HLA Information Exchange Data Format Standards
HIS	Hispanic
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HML	Histoimmunogenetics Mark-up Language
HPS	Health Physics Society
HR	High Resolution
HRSA	Health Resources and Services Administration
HSC	Hematopoietic Stem Cell
IBWC	Immunobiology Working Committee
ID	Identification
IDM	Infectious Disease Markers
IHIWS	International Histocompatibility and Immunogenetics Workshop
IND	Investigational New Drug
IPR	Immunobiology Project Results
IRB	Institutional Review Board
IS	Information Services
ISBT	International Society Blood Transfusion
IT	Information Technology
KIR	Killer Immunoglobulin-like Receptor
LR	Low Resolution
mHA _g	Minor Histocompatibility Antigens

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MHC	Major Histocompatibility Complex
MOU	Memorandum of Understanding
MRQ	Maternal Risk Questionnaire
MTE	Marrow Toxic Event
MUD	Matched Unrelated Donor
MULTI	Multiple Race
NAM	Native American Indian/Alaskan Native
NAIAD-DAIT	National Institute of Allergy and Infectious Diseases, Division of Allergy, Immunology, and Transplantation
NAT	Nucleic Acid Test
NCI	National Cancer Institute
NIH	National Institutes of Health
NK	Natural Killer
NLM	National Library of Medicine
NMDP	National Marrow Donor Program
OCR/ICR	Optical Character Recognition/Intelligent Character Recognition
OISR	Office of Investigator Sponsored Research
ONR	Office of Naval Research
OTH	Other (Race)
PBSC	Peripheral Blood Stem Cell
PCR	Polymerase Chain Reaction
PDF	Portable Document Format (Adobe Acrobat)
QA	Quality Assurance
QC	Quality control
RadCCORE	Radiation Countermeasures Center of Research Excellence
RCI	Resource for Clinical Investigations
REAC/TS	Radiation Emergency Assistance Center/Training Site
REMM	Radiation Event Medical Management
RFP	Request for Proposal
RFQ	Request for Quotation
RITN	Radiation Injury Treatment Network
RR	Relative Risk
SAT	Search Assistance Tool
SBT	Sequence Based Typing
SCID	Search Coordinator Maintenance Tool
SCTOD	Stem Cell Therapeutics Outcome Database
SIP	Search and Invoice Pricing
SOP	Standard Operating Procedure
SSA	Search Strategy Advice
SSOP	Sequence Specific Oligonucleotide Probes

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ACRONYM LIST (Continued)

SSP	Sequence Specific Primers
STAR®	Search, Tracking and Registry
TAT	Turn Around Time
TC	Transplant Center
TNC	Total Nucleated Cell
TOPOFF4	Top Officials 4 a Federal Exercise
VPN	Virtual Private Network
WebEOC	Web Emergency Operations Center
WHO-REMPAN	World Health Organization, Radiation Emergency Medical Preparedness and Assistance Network
WMDA	World Marrow Donor Association
WU	Work-up
XML	Extensible Markup Language

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Executive Summary

In 1986, Congress appropriated funds to begin development of the National Bone Marrow Donor Registry. Today, 22 years later, the National Marrow Donor Program (NMDP), as the contractor for the Registry, has built a racially diverse donor registry of over 7 million donors; facilitated more than 30,500 hematopoietic stem cell transplants; developed comprehensive research programs to improve post-transplant outcomes and established a network of transplant centers (TCs) capable of treating casualties resulting from military or terrorist actions, as well as patients suffering from leukemia, aplastic anemia and other life-threatening diseases.

Contingency Preparedness Planning

Navy funding has supported scientific research and technologic advancements that are key to ensuring an effective response during a marrow toxic exposure event. During this funding period, projects were completed to expand critical components of a contingency response program, including the Radiation Injury Treatment NetworkSM (RITN). Several physician training sessions and educational seminars were organized to help ensure that optimal care plans were designed and implemented. The RITN Steering Committee worked to develop treatment guidelines, standard operating procedures and education programs that are accessible on-line. The connectivity and capability of the RITN network to respond was tested during two federal exercises in June and October 2007. Other mission-critical network components including testing laboratories, donor centers (DCs) and cord blood banks (CBBs) also participated in drills.

The NMDP has developed an operational continuity plan and standard operating procedures for Coordinating Center and Network staff to use in emergencies and contingency situations. The critical functionality of the NMDP's communication infrastructure is protected through off-premises facilities that can be used as hot sites to reconstruct information systems in the event that the Coordinating Center or the entire geographic areas surrounding the Coordinating Center are affected.

To help ensure that NMDP systems would be fully capable of supporting the Network in the event of a radiation exposure, numerous computer applications required for donor identification and procurement facilitation were enhanced

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Rapid Identification of Matched Donors

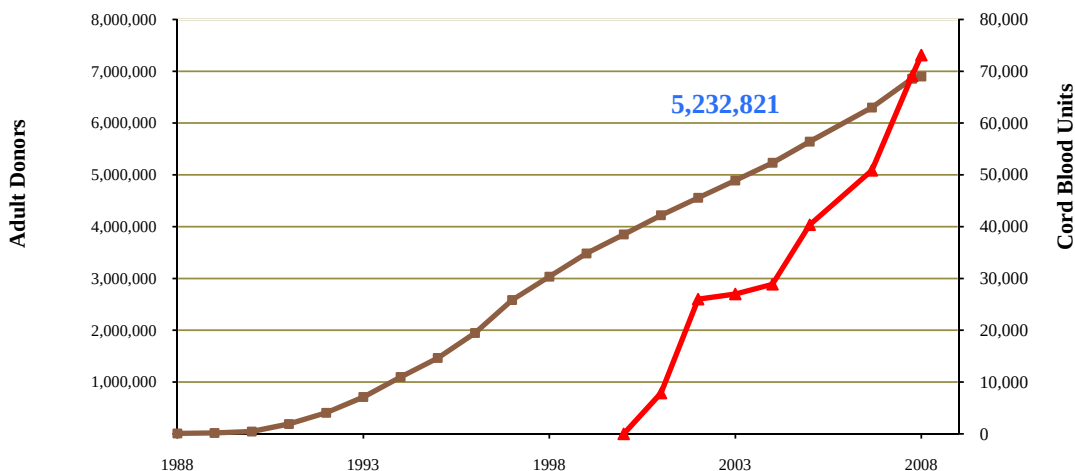
Published research data have clearly defined the relationship between human leukocyte antigen (HLA) matching and optimal patient outcomes following unrelated adult donor transplantation. In order to increase the application as a treatment option there is a significant need to expand the genetic diversity of the volunteer file - especially with individuals from U.S. minority populations. This fact, combined with the attrition rate of Registry donors, necessitates continued large-scale donor recruitment activities.

Navy funding has enabled dramatic growth of the total donor file and, importantly, the minority donors registered during the course of this grant. The transition to collection of newly recruited donor DNA by buccal swabs began during this project period. This collection method provides significant advantages over the previous methods of whole blood and filter paper spots including:

- Obviating the need for trained phlebotomists,
- Gaining better reception from new donors,
- Opening new opportunities for live donor drive venues, and
- Providing significant cost savings.

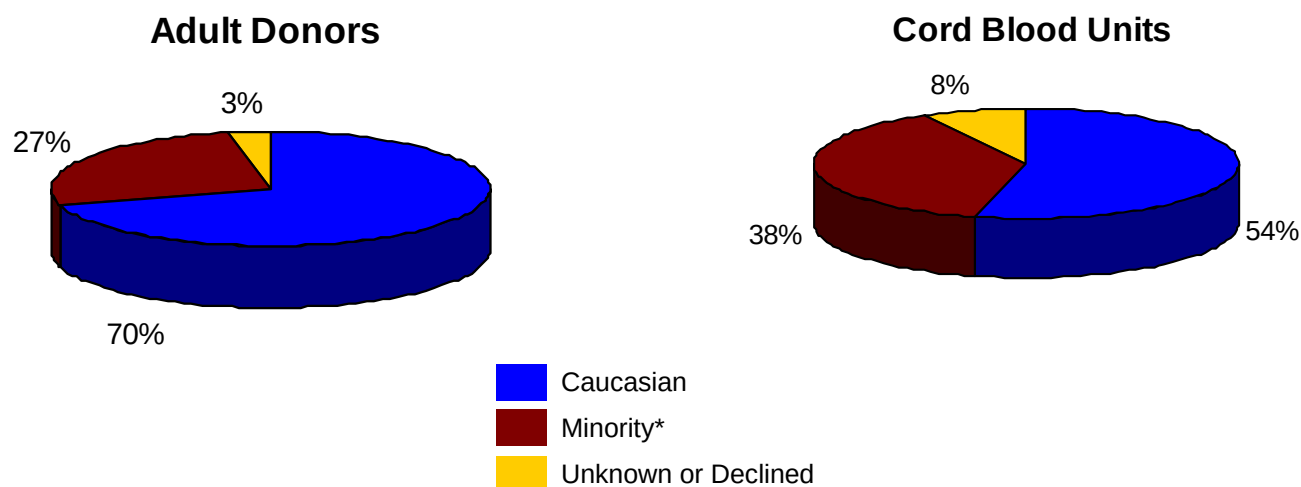
The following graph and pie chart demonstrate the increase in the size and diversity of the NMDP's donor file between 1988 and 2008:

National Marrow Donor Program Adult Donors & Cord Blood Units– Jan, 2008



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Diversity of the NMDP Registry 2007



* Minority includes donors who identified their race or ethnicity as:

- Black or African-American
- American Indian or Alaska Native
- Asian
- Native Hawaiian or Other Pacific Islander
- Hispanic or Latino

Data continue to emerge defining the use of umbilical cord blood as a source of stem cells. The reduced need for highly specific matching in order to achieve transplant success has increased the demand for this product type and allowed transplantation for patients without a well-matched adult donor. It also has the benefit of rapid availability, making it a suitable product for an emergency situation. The dependence on adequate cell dose has led to protocols for infusion of multiple units thus putting additional pressure on banks and registries world wide to expand the inventory. During this reporting period, the NMDP added a total of 30,800 new cord blood units (CBUs) and negotiated participation agreements with two new CBBs.

Research to improve the ability of HLA experts and transplant center (TC) staff to rapidly identify, select and test the optimal donor/CBU resulted in significant advances in systems, processes, and services developed by the NMDP. Most notable was the launch, in February 2006, of the matching algorithm HapLogicSM. This innovative enhancement provides allele level matching predictions at HLA-A, B, and DRB1 for both donors and CBUs most likely to match

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searching patients. A significant amount of population genetics research was completed that provided the foundational data and formats for race-specific genotype list matching. Navy-funded high resolution (HR) patient and donor HLA typings generated unbiased datasets for the creation of multi-locus haplotype frequency calculations. In addition, the final innovative approach was integration of the stored primary data that had been collected over an eight-year period from the Department of Defense and other laboratories under contract to the NMDP. These unique data, at the nucleotide level, further increased the specificity of the matching predictions. The corresponding validation by HLA Expert Advisors and the customer satisfaction survey were integral components measuring the accuracy and value of this algorithm enhancement.

Network communication systems were expanded to accommodate the enhanced matching algorithm and the additional associated modifications to improve donor and CBU selection. These changes also provided logic to support and enhance NMDP services for TCs such as expert HLA Consultation and Centralized Search Service. Most importantly, these efforts significantly increased the ability of the NMDP and the Network to respond effectively to a contingency event.

Immunogenetic Research

The HR HLA typing of paired donor and recipient samples continued to provide substantive data to increase the understanding of the impact of matching on patient outcome. The project data were also used to assess genetic diversity within the NMDP transplant population and Registry and fed into the HapLogic matching algorithm. The analytical models developed for the project data were described in several abstracts and two manuscripts. Seventy-three abstracts and forty-three manuscripts have been published using data generated from the HR Donor/Recipient Pair Project since the inception of the project.

HLA genes other than those characterized in the Donor/Recipient Pair Project and non-HLA genetic factors may all influence the suitability and success of allogeneic stem cell transplants. To further elucidate the impact on matching, another important research study funded by this grant investigated the allelic diversity of the Killer Immunoglobulin-like Receptor (KIR) ligands. During the period typing was completed on 270 Caucasian donor samples for 14 KIR genes (2DL1-5, 2DS1-5, 3DL1-3 and 3DS1). The study encountered a high degree of genetic polymorphism and allelic ambiguity in the KIR loci. Efforts are continuing to resolve all discrepancies, analyze allelic ambiguities, characterize new alleles and analyze and assign KIR haplotypes.

Funding during the project period also supported initial development of a critical resource for the evaluation and integration of non-HLA immunobiological testing results into the NMDP research program, the Immunobiology Project Results (IPR) database.

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Clinical Research in Transplantation

Improving strategies to avoid and manage graft-versus-host disease (GVHD) is an essential step in improving the outcomes of transplantation and, consequently, the ability to incorporate transplantation as an effective therapy in a variety of settings including contingency situations. The goal of the research activities funded through this grant has been to increase the understanding of the immunologic factors important in hematopoietic cell transplantation (HCT).

During this grant, the NMDP moved forward its Prospective Research program. Within the Center for International Blood & Marrow Transplant Research (CIBMTR) affiliation, the NMDP established the Resource for Clinical Investigations in Blood and Marrow Transplantation (RCI BMT). The goal of this program was an avenue for investigators to obtain statistical and data management support for prospective trials focusing on addressing various transplant issues. These were the major accomplishments:

- Assembled a Clinical Trials Advisory Committee to provide scientific review and recommendations on clinical trial proposals.
- Established a Data Safety Monitoring Board for all trials facilitated through this program.
- Received and reviewed nine proposals since the program was established.
- Initiated two trials with all appropriate approvals:
 - Unrelated Allogeneic Transplant for Renal Cell Carcinoma
 - Adult Double Cord in Patients with Hematologic Malignancies
- Approved two trials for development:
 - A study to assess safety of donation in related donors
 - Lenalidomide after allogeneic hematopoietic cell transplant for Myeloma
- Designed RCI BMT page on CIBMTR.org Web site

Additionally, the Office of Investigator Sponsored Research (OISR) was created to support donor-focused research for studies proposed by investigators outside the NMDP. During this grant, the OISR infrastructure was developed and the first study was implemented.

During the grant period, funds were used to contract with an Immunogenetic Biostatistician at the Medical College of Wisconsin to provide support to NMDP Scientific Services and the CIBMTR Immunobiology Working Committee (IBWC). The biostatistician helped to conduct and direct research to advance models for registry composition analyses, haplotype frequencies, predictive algorithms, and automated donor selection algorithms. To further stimulate

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completion of immunobiology studies within the CIBMTR, grant funds were used to provide monetary support to investigators whose studies require modest funding for completion. Three of these grants were awarded during the grant period. Grant funds also supported significant outreach efforts by the IBWC leadership to increase exposure for the IBWC to basic scientists. In addition, the IBWC continued work on the 33 active studies in the committee, accepted three new proposals for analysis and submitted five studies for publication.

END – EXECUTIVE SUMMARY

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II.A. Contingency Preparedness – Hypothesis 1:

Recovery of casualties with significant myelosuppression following radiation or chemical exposure will be optimal when care plans are designed and implemented by transplant physicians

Aim A.1.1: Secure Interest of Transplant Physicians

In working to accomplish this Aim, the NMDP focused on the education of transplant physicians and the inclusion of a subset of physicians in the development of the RITN.

In fall 2005, Dr. Confer attended the European Acute Radiation Syndrome (ARS) treatment consensus conference in Paris, France. This conference afforded the NMDP the opportunity to develop many important contacts with the international transplant community that is focusing on the response to a radiological event.

As part of development of this portion of the research, it was necessary to provide investigators information on the underlying biology and medicine of ARS. A training course, NMDP Basic Radiation Training (BRT) course, was created and distributed to physicians to provide a basic understanding of ARS. The BRT course contains four sections and a 29-question exam that is submitted via the Internet. To date, over 750 people have successfully completed the BRT.

The sections of the course are:

Section 1: Radiation basics

Section 2: Biological effects of radiation

Section 3: Exposure vs. contamination and shielding

Section 4: What does all this mean for the HCT community?

Training Objectives of the course are:

- Describe the three main types of radiation
- Understand the difference between radioactive material and radiation
- Explain the basic measurement of radiation
- Name the four primary sources of radiation
- Understand the basics of biological effects of radiation
- Be able to describe symptoms of ARS
- Explain the difference between internal and external contamination and internal and external exposure
- Explain the concept of shielding

To assist RITN centers in the dissemination of this training material, a presentation format was created in the fall of 2007 with the assistance of the TC staff at the University Medical Center in

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Tucson, Arizona. This allowed multiple centers to train a large group of staff in a short period of time.

The RITN Steering Committee consists of transplant physicians from the 13 original TCs (see Aim 2.1 for further details), experts in the field of transplantation, government and non-governmental partners. During the period of performance, five (5) meetings were held:

- September 2005
- February 2006
- September 2006
- February 2007
- September 2007

Outcomes of these meetings included:

- Definition of the RITN Purpose Statement:
The RITN provides for development of comprehensive evaluation and treatment strategies for victims of radiation exposure or other marrow toxic injuries. The RITN develops treatment guidelines, educates health care professionals, works to expand the network, and coordinates situation response. The RITN is a cooperative effort of the NMDP and The American Society for Blood and Marrow Transplantation (ASBMT).
- Creation of the RITN Acute Radiation Syndrome Treatment Guidelines:
 - These guidelines specify the monitoring of blood counts to determine if the patient was exposed to a significant dose of radiation, then utilization of granulocyte-colony stimulating factor (GCSF) as an initial step in the treatment ARS as well as the monitoring of blood counts to observe the efficacy of GCSF. NMDP evaluation of the safety of GCSF has identified severe events and adverse events associated with administration.
 - Additionally, Preparatory Regimen Guidelines were created to define best practices in preparing a victim for transplant if required.
- Discussion of non-transplant treatment guidelines as part of the adoption of the RITN ARS Treatment Guidelines.
- Development of Donor Search Criteria:
A search for a suitable, HLA-compatible related or unrelated donor or CBU should be initiated whenever there is evidence of severe hematopoietic toxicity. The criteria further specify exposure levels and blood counts that would warrant the initiation of a search. These criteria will be revised over time, as warranted, based on published clinical transplant research by NMDP, CIBMTR and others.

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- The importance of data collection during an incident was discussed and key data fields were identified and later incorporated into the NMDP standard data collection form. Response to a radiation incident will result in both lifesaving therapies and the collection of data for analysis to improve responses to future incidents.

The culminating event during this performance period was the execution of the RITN educational seminar in Bethesda, Maryland, held on September 25, 2007. This seminar, titled “Medical and Organizational Challenges Resulting from a Radiological/Nuclear Emergency” drew over 150 attendees. Attendees were solicited through the membership lists of ASBMT, American Society of Hematology (ASH), Health Physics Society (HPS), as well as physicians from the NMDP Network. The seminar provided information on potential radiation casualties in a highly organized review by experts in their fields.

The final seminar agenda was:

The Threat:

- Threat Assessment: Brooke Buddemeier, C.H.P. - Lawrence Livermore National Laboratory
- A Possible Scenario for Nuclear Casualties: Carl Curling, Sc.D. - Institute for Defense Analysis
- Lessons from the Past: Chernobyl: Allan Shapiro, M.D. - Food and Drug Administration
- Mass Casualty Event Case Studies:
 - o David Rutstein, M.D. - Health and Human Services
 - o Nelson Valverde, M.D. - State University of Rio de Janeiro

Biology:

- Introduction to Radiation Biology: Michael Robbins, Ph.D. - Wake Forest University School of Medicine
- Biodosimetry: Albert Wiley, M.D. - REAC/TS & WHO Collaborating Center at Oak Ridge

Acute Radiation Syndrome:

- ARS Hematologic Syndrome: Theodor Fliedner, M.D. - Ulm University
- ARS Skin Syndrome: Viktor Meienke, M.D. - Bundeswehr Institute of Radiobiology
- ARS Gastrointestinal Syndrome: Martin Hauer-Jensen, M.D. - University of Arkansas for Medical Sciences
- Radiation-induced Brain Injury: Michael Robbins, Ph.D. - Wake Forest University School of Medicine
- Multi-organ Failure: Marc Benderitter, M.D. - Institut De Radioprotection et de Surete Nucleaire

National Response

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- National Response Process - the HHS “Playbook”: C. Norman Coleman, M.D. - National Institute of Health
- RITN Overview: Cullen Case – NMDP
- New Approaches to Therapy Next Generation Medical Countermeasures: Nelson Chao, M.D. - Duke University

Aim A.1.2: GCSF in Radiation Exposure

This Aim focused on non-transplant treatment guidelines and patient assessment related to the use of GCSF for patient treatment as a result of a marrow toxic mass casualty event such as radiation exposure.

The RITN Executive Committee developed and the RITN Steering Committee later adopted the non-transplant treatment guidelines. These guidelines are titled RITN Acute Radiation Syndrome Treatment Guidelines. These guidelines specify the use of GCSF as an initial step in the treatment of ARS as well as the monitoring of blood counts to observe the efficacy of GCSF.

Guidelines were developed instead of protocols to facilitate the distribution and incorporation into emergency response plans. Hospitals participating in the RITN would be hard pressed to keep a treatment protocol active with no accrual of patients. This would foster an unhealthy relationship with the Institutional Review Board at the hospital. When packaged as treatment guidelines, this conflict was eliminated. Also, as a set of guidelines, locally available medications could be used for treatment (e.g. the hospital could use its preferred anti-fungal medication that staff are accustomed to using). This does not mean that recommended medications and doses are omitted from the guidelines; rather, the treating physician has the option to provide the best intensive supportive care possible within the confines of his/her clinical practice.

Aim A.1.3: Patient Assessment Guidelines

Efforts related to this Aim focused on the development of transplant treatment guidelines and the associated support systems, including the refinement of guidelines for patient assessment and the operational and educational aspects of rapid product selection and transplant as necessary in a marrow toxic mass casualty event such as radiation exposure.

The RITN worked closely with the Department of Health and Human Services - Radiation Event Medical Management (REMM) team in the development and release of Web-based transplant treatment orders. These treatment orders, as well as all information on the REMM Web site, can be downloaded and saved on a computer to facilitate access during a national disaster.

REMM Web site: www.remm.nlm.gov

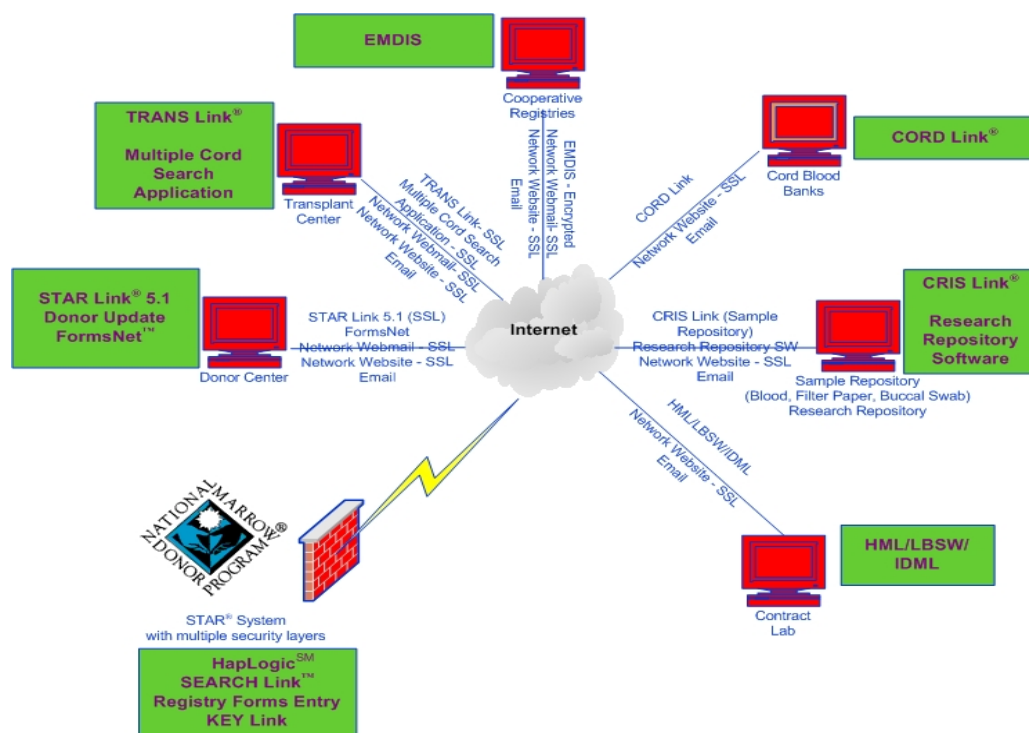
REMM Web site download link: <http://www.remm.nlm.gov/download.htm>

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The REMM Web site is not limited to hematopoietic system reconstitution after a marrow toxic incident. It is a comprehensive medical planning site with information for first responders, hospital decontamination teams, cutaneous medical specialists and many more specific areas that would come into play as a result of a mass casualty scenario. Of note is that marrow transplant specialists were the first group asked to provide input for inclusion in the site.

Diagram 1 illustrates the Network application and communication infrastructures of the STAR System and Web-based ancillary systems; STAR Link, CORD Link, TRANS Link, Multiple Cord Search Application, CRIS Link (Repository software), and FormsNet. Along with SEARCH Link and the HapLogic matching algorithm (both of which are internal to the NMDP), these systems provide rapid electronic communication and enable the NMDP to handle the high volume of transactions each day.

Diagram 1: Network Center Communication



To help ensure that the NMDP systems would be fully capable of supporting the Network in the event of a radiation exposure, the following work was performed:

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CORD Link

This application supports requested services from CBBs and was enhanced as follows during this grant period:

- Added an alert to give cord banks immediate notification of new CBU requests like patient-directed confirmatory testing (CT) and product orders through e-mail and text pages.
- Developed features to allow the cord banks to perform and track prospective CT requests.
- Modified workflow management screens to indicate incomplete cord data. This feature allows the cord bank to enter partial information as it becomes available rather than waiting until all testing information is complete, thus allowing for report card analysis earlier in the process.
- Deployed functionality to allow cord banks the ability to manage entry and error corrections on the Family Medical History Questionnaire (FMHQ), Maternal Risk Questionnaire (MRQ), and Infectious Disease Markers (IDM) forms.
- Developed code permitting cord banks the ability to view all data modification requests submitted to the NMDP. CBBs that currently take advantage of the NMDP's Optical Character Recognition (OCR) entry process gained the ability to view scanned forms in PDF format within the CORD Link application making the data available online versus on paper.
- Added features to support New Food and Drug Administration (FDA) Guidance for IDM Nucleic Acid Test (NAT) for HIV/HCV (Human immunodeficiency virus/hepatitis C virus). (On January 24, 2007, the FDA issued guidance mandating maternal IDM NAT for HIV/HCV to be performed using individual testing methods per manufacturer's requirements instead of pooled testing. This impacted CBUs collected on or after May 25, 2005. Each NMDP CBB has identified their affected inventory. On February 22, 2007, the affected inventory was labeled with a new Report Card Status: "PreOrder Condition").
- Developed features to support Local IDs and International Society Blood Transfusion (ISBT) ID fields. This allowed the CBBs to enter their entire Local CBU ID and Local Maternal ID or ISBT ID into the CORD Link application without truncation. Features were developed to permit future updating to support new Network partners.

Also, staff worked to convert data from the following banks to CORD Link :

Active Banks:

- CBB 173, M.D. Anderson CBB
- CBB 172, New Jersey CBB
- CBB 171, Cryobanks International, Inc.
- CBB 178, CORD:USE

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- CBB 158, Texas Cord Bank
- CBB 180, University of Colorado CBB
- CBB 192, Singapore CBB, Ltd.

Pending User Acceptance Testing:

- CBB 193, Sheba CBB
- Gift of Life CBB

A total of 30,800 cords were registered through CORD Link during the reporting period.

CRIS Link, the software that supports all services at the NMDP Repository, had the following modifications:

- The Research Repository component was enhanced to add the ability to select samples from the Research Repository inventory for shipment to a contract tissue culture lab for cell transformation and expansion. The feature required repurposing the shipment architecture. In addition, enhancements were made to support the consolidation of the inventory and creation and population of an off-site master repository. *[See Aim IIC.2.2 for further details]*. Changes were critical to optimize use of repository samples for search and development of accelerated matching systems for transplantation.
- Features were added to CRIS Link to support utilization of buccal swabs for new donor recruitment and for Customized HLA Typing programs. *[See Aim IIB.1.4 for further details]*. Changes support improved access to transplants for the maximum number of patients and casualties.

Aim A.1.4: National Data Collection Model

The focus of this Aim was to define and develop a national data collection and management model to collect data resulting from a mass radiological exposure event.

During this period, the RITN Steering Committee conducted the initial steps necessary to collect data connected to a mass casualty event resulting in marrow toxic injuries. The Executive Committee reviewed NMDP protocols and forms for data collection and then modified the data collection fields where necessary to ensure desirable information would be obtained. Later, the RITN Steering Committee reviewed and adopted these protocols and forms. These include:

- NMDP Protocol for a Research Database for Allogeneic Unrelated HCT and Marrow Toxic Injuries
- Necessary data collection elements from victims of marrow toxic injury have been incorporated into the routine data collection protocol for HCT recipients. This will make it easier to maintain active protocols at each center.

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- NMDP Research Database for Unrelated Donor Transplant Marrow Toxic Injury Subject Research Consent Form

These protocols and this form will be reviewed annually by participating NMDP Institutional Review Boards, and therefore, will be in place and ready for any event resulting in marrow toxicity.

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II.A. Contingency Preparedness – Hypothesis 2:

Coordination of the care of casualties who will require hematopoietic support will be essential in a contingency situation.

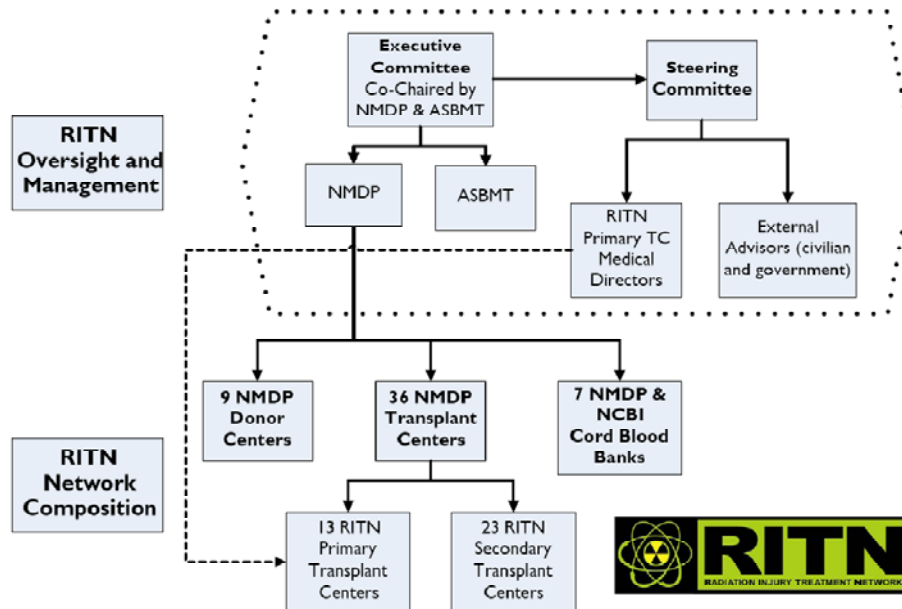
Aim A.2.1: Contingency Response Network

The RITN and its efforts were the focus of this Aim. The Radiation Injury Treatment Network was organized to develop comprehensive evaluation and treatment for victims of radiation exposure or other marrow toxic injuries. The RITN develops treatment guidelines, educates health care professionals, works to expand the network, develops coordination for situation responses and prepares table top exercises to evaluate planned responses. The RITN is a cooperative effort of the NMDP and the ASBMT. RITN development is integrated into other NMDP research programs to develop the science and technology of unrelated donor transplantation. All of these laboratory and clinical research capabilities are used in transplants performed every day and are used to demonstrate and improve radiation response contingency capabilities.

RITN centers include TCs, DCs, and CBBs. Partner organizations with clinical experts participate through the RITN Steering Committee (see figure below); this committee also includes identified primary centers (currently the original 13 TC medical directors or their delegate). Secondary RITN centers are comprised of all other participating centers. The RITN Steering Committee typically meets twice a year to discuss the further development of the RITN, its treatment procedures, training materials and other related products. An Executive Committee (comprised of NMDP and ASBMT representatives) meets monthly by conference call.

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Organization of RITN

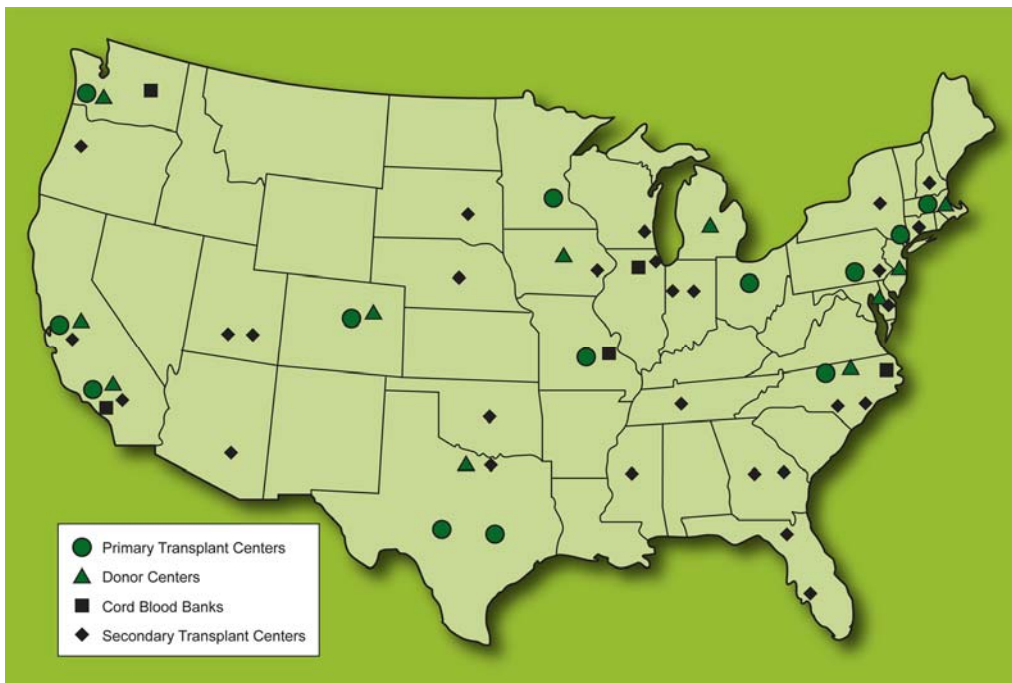


Participating centers serve as subject matter experts on the treatment of radiation victims resulting from a marrow toxic event (MTE) as well as work to establish a documented process for the care and management of mass casualty patients from an MTE. To prepare for such an event, RITN centers develop, maintain and improve standard operating procedures of how their organization would respond to such an event. Some centers are selected to work on special projects. These projects have included protocol development, education material development, presentation development, and contingency related research opportunities where applicable.

RITN centers adopt, to the extent practical, the treatment guidelines, donor selection criteria, data collection plan, and other related documents developed by the RITN Executive and Steering Committees. Where applicable, RITN centers will either obtain local Institutional Review Board (IRB) approval for these protocol(s) or participate in an IRB designated by the NMDP for these protocol(s).

In developing the RITN, the NMDP began in 2005 with 13 TCs. These centers then grew to include 53 total participating centers distributed across the United States: 36 TCs, nine (9) DCs, and seven (7) CBBs (see figure below). Each of these centers created standard operating procedures describing how their organization would respond to an MTE and participated in regular drills and an annual tabletop exercise, as well as conducted Basic Radiation Training of staff.

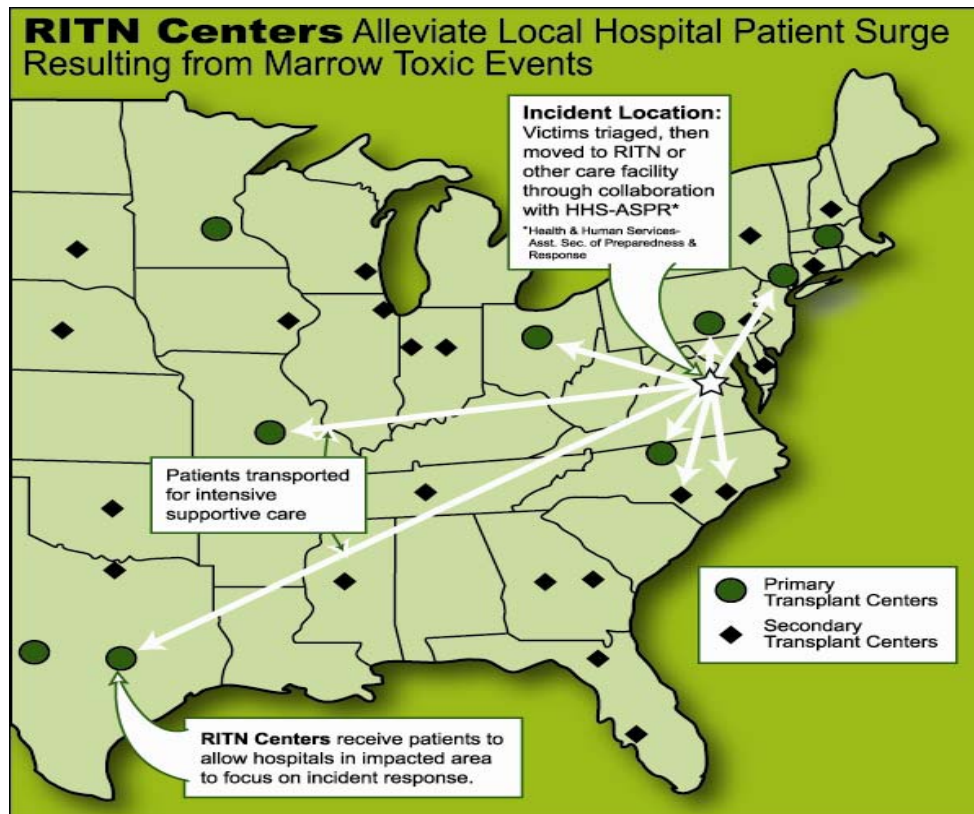
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RITN Center Distribution by Type

It is imperative to keep in mind that the RITN is not a first responder organization. All participating centers are preparing to respond to an event that occurs in another city or even coast. The NMDP anticipates that the RITN will receive patients from another part of the country to alleviate their medical load and to provide the best care possible for the victims of an MTE (see figure below).

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The RITN would not be able to succeed in response to a mass casualty event without the support of partner organizations. The NMDP has worked diligently to develop these relationships so that when an event occurs no one will need to exchange business cards, but rather will already know who to call. Two levels of partnerships have been developed, formal and informal. Formal relationships are documented through a Memorandum of Understanding (MOU). The NMDP currently has an MOU with the Office of the Assistant Secretary of Preparedness and Response, Department of Health and Human Services (ASPR-HHS). The NMDP is in the process of completing MOUs with the ASBMT and the American Association of Blood Banks (AABB). Organizations that hold informal partnerships include:

- Radiation Countermeasures Center of Research Excellence (RadCCORE) at Duke University
- National Institute of Allergy and Infectious Diseases, Division of Allergy, Immunology, and Transplantation (NAIAD-DAIT)
- National Library of Medicine, Radiation Event Medical Management (NLM-REMM)
- National Cancer Institute (NCI)
- Radiation Emergency Assistance Center/Training Site (REAC/TS)
- World Health Organization, Radiation Emergency Medical Preparedness and Assistance Network (WHO-REMPAN)

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In developing the informal relationship with the REMM, RITN physicians provided the detailed treatment guidelines required for the REMM treatment protocol, posted on www.remm.nlm.gov.

As a result of the formal relationship with ASPR-HHS, the RITN participated in two (2) federal exercises in 2007. In June 2007, the Department of Health and Human Services conducted a functional exercise (Pinnacle 07) by activating their emergency operations center. The NMDP participated in this exercise by submitting RITN capabilities information to ASPR-HHS. In October 2007, the Department of Homeland Security conducted an exercise (TOPOFF 4) involving over 15,000 federal personnel across the United States and the countries of Australia, Canada and the U.S. territory of Guam. Again, the NMDP participated in this exercise by testing its entire emergency response process, ultimately involving four (4) NMDP officers, 32 staff, and 14 RITN centers.

To facilitate distribution of RITN materials and information to participating RITN centers, a RITN Web site (www.ritn.net or www.ritn.org) was created. This resource provides many materials for RITN centers to better prepare for an emergency, including:

- RITN participating centers list
- RITN background and overview presentations
- Meeting minutes
- Copies of messages sent to RITN centers
- Guidelines and protocols
- SOP templates for TCs, DCs, and CBBs, including an example TC SOP implemented by a RITN center
- Reference materials:
 - Education and training materials
 - Example tabletop exercises
 - Copies of or links to published articles of interest
- A contact list with RITN point persons, key federal agencies and state emergency operation centers

In addition to this RITN Web-based resource, an Emergency Preparedness Section was created for the NMDP Network of centers on the Network web site. In order to provide information for NMDP staff, an Emergency Preparedness Section was created on the NMDP staff Intranet site.

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Aim A.2.2: Develop and test standard operating procedures, in conjunction with core TCs, to manage the activities required to HLA type siblings of casualties to evaluate their potential as donors for their affected family member.

The focus of this Aim was to develop and test standard operating procedures that would manage the activities required to HLA type and evaluate transplant suitability of siblings of casualties.

An NMDP multi-functional team evaluated the requirements of conducting sibling typing on a large scale for victims. Upon completion of review, it was determined that the project would require functionality not presently available. The enterprise architecture unified data model project will provide the ability to properly segregate sibling donors from volunteers as well as a means to view those relatives only on specific patient searches. As a result, this project will be completed as the data handling capabilities of the NMDP are expanded to include this requirement.

Cord Blood Contingency Preparedness Drill

A contingency drill was conducted in September 2006 to test the preparedness of the NMDP Network in responding to an urgent event. Specifically evaluated in this drill were the CBBs, the Cord Blood CT typing laboratory, the expert Search Strategy Advisors and NMDP Scientific Services staff. The 14 CBBs were notified ahead of time to anticipate a drill without knowing the exact date. They were given the expectation that for the drill, the CBU samples should be pulled and shipped within two (2) days. The CT typing laboratory was also pre-notified that a higher than normal volume of samples with urgent requests may be coming. Seven newly activated patient searches were used for the drill, taking care to select only searches from TCs not currently using a cord blood transplant protocol (to avoid potential duplication of efforts). The Search Strategy Advisors selected 27 CBUs with high Total Nucleated Cells (TNC) and greater than or equal to 4 of 6 match grade ensuring allocation of units among all participating banks. The majority of processes during the drill happened outside of the usual STAR systems, including the CBU activations from the patient searches, the sample requests to the banks, and the HLA typing requests to the lab.

Overall, the drill was extremely successful, with 14 of the 14 banks pulling samples the same day and 12 of 14 actually shipping samples in one (1) day, exceeding the minimum requirement of two (2) days. Two (2) of the banks had difficulties with shipping arrangements that were resolved on the second day. At both locations, corrective action plans have been put into place to avoid these same issues in the future. 26 of the 27 CT typing results were reported by the lab within two (2) business days, exceeding the requirement of three (3) days. Under normal circumstances, the Search Strategy Advisors' reviews are received in five (5) business days, but the consultations were completed within one (1) day during the drill.

This drill indicated that the Network is prepared to accelerate the tasks surrounding CBU selection, pulling, shipping, and HLA typing in the event of a contingency. Also demonstrated was the ability to work efficiently outside of normally utilized systems such as Search Link or Cord Link which might occur in an actual contingency event.

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II.A. Contingency Preparedness – Hypothesis 3:

The NMDP's critical information technology infrastructure must remain operational during contingency situations that directly affect the Coordinating Center.

Aim A.3.1: NMDP Continuity Planning / Disaster Recovery

NMDP Continuity Planning:

The cohesive and efficient operation of the NMDP Network is dependent upon staff and systems at the Coordinating Center. To help ensure that NMDP operations are able to continue despite interrupting events, e.g. fire or other disaster, an Operational Continuity Plan was required. The NMDP Continuity Plan protects the organization by mitigating exposure and helping to ensure that critical operations continue (or are resumed as quickly as possible) in the event of business interruptions.

In February 2006, the NMDP hired an Operational Continuity Planner to ensure the proper focus could be placed on this important area of operations recovery.

At the same time, the NMDP initiated a Critical Staff Recovery Site (CSRS) project. This facility would provide a location for staff to work from in the event that the Minneapolis Coordinating Center was rendered uninhabitable. This project was led by the Operational Continuity Planner and developed by a multi-functional team with staff from five (5) departments who determined the requirements for a CSRS and created a comprehensive Request for Quotation (RFQ) for external vendor fulfillment of this need. Members of the multi-functional team met with vendors and toured multiple locations.

Ultimately, eight (8) options were identified as possible solutions for a CSRS:

- In-sourced Site (located in WI or MN)
- In-sourced Site (located in the Minneapolis-St. Paul metro)
- In-sourced Site dual-use (located in Charlotte, NC)
- Mixed Hotel/Home Office Solution
- Home Office Only Solution
- Hotel Only Solution
- Vendor Fixed Site (located outside the Minneapolis-St. Paul metro)
- Vendor Fixed Site with Trailer Option
- Vendor Fixed Site St. Paul
- Vendor Trailer Only Option

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Upon presentation of the options and the associated costs, the NMDP Officers determined the best option based on requirements was to in-source in the Minneapolis-St. Paul metropolitan area.

To improve the ability of the NMDP to continue operations in the event of a serious operational interruption, ongoing operational continuity planning occurred. A hazard vulnerability assessment took place for both the Coordinating Center and the Repository Services facility. Several hazard mitigation recommendations were approved for the Repository Services facility, including staff fire extinguisher training and the purchase of shelter-in-place materials.

In the event of a operational interruption, staff would need to be notified of the situation quickly and concurrently. To meet this need, the NMDP renewed the contract for an Emergency Notification System service which allows pre-designated groups of staff to be contacted at the same time by a pre-recorded telephone message, fax, email, text message and pager. This system will ensure all staff receives the same message, preventing personal bias and reducing the possibility of miss-communications by allowing NMDP senior management to record a message and deliver it immediately.

Effective communications are essential when responding to any disaster, emergency or operational interruption. During this grant, the NMDP strengthened its ability to communicate during a disaster. To fortify emergency communication capabilities, the NMDP now has 127 Governmental Emergency Telecommunications Service (GETS) cards that allow users to place phone calls during times of significant telephone network congestion. These cards are issued to Coordinating Center staff, NMDP operated center staff and RITN participating centers. In addition, there are generic cards available at the Coordinating Center to be provided to any center within the NMDP Network in the event of an emergency. The NMDP has procured 30 additional satellite phones for a total of 60. Most of these portable phones were issued to RITN members, and the remaining are maintained at the Coordinating Center for use during a disaster.

To quickly consolidate Network center status reports during a disaster, the Web Emergency Operations Center (WebEOC) contract was renewed in 2007. WebEOC is a Crisis Information Management System (CIMS). This system allows all Network centers to provide detailed status information via the Internet. Once submitted, the NMDP will be able to review individual center information or a rollup of the entire Network. This capability will allow NMDP staff to ascertain information such as the number of available HCT beds, available personnel, level of supplies, and other key center operating parameters or issues. WebEOC was used in the NMDP Emergency Operations Center during the functional exercise as part of TOPOFF 4, a national readiness exercise, to send and receive capability reports from RITN participating centers.

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Disaster Recovery:

During this grant period, the NMDP maintained, further developed and tested a disaster recovery site in Kansas capable of supporting all systems. Modifications, upgrades and drills were performed to help ensure fluid system recovery as follows:

- Purchased, configured and deployed additional network bandwidth. This bandwidth is a prerequisite to remotely operate and manage the site from the NMDP's Minneapolis or New Brighton, MN, facilities. It also facilitates real time replication of critical data.
- Conducted Disaster Recovery Test #16 on April 10, 2006. This test utilized the additional bandwidth by VPN between Kansas and New Brighton. As a result, only three (3) staff members were required to fly to the Kansas data center, while all others, including application users that participated in user testing, conducted their work from New Brighton, MN.
- Increased cooling capacity of the server room to keep the servers operating efficiently.
- Purchased standby database licenses to allow real-time data replication between the recovery site and the Coordinating Center.
- Purchased remote management cards to enable effective system management from Minneapolis in a contingency situation. (The remote management capabilities keep the Kansas server room operating without being staffed by IT).
- Installed dedicated Internet virtual private network (VPN) connectivity to enable replication of data flow.
- Tested the emergency notification system. This phone-based notification system is able to communicate (by recorded voice message or email) to all NMDP staff at the same time. During this test, 92% of 556 staff members were reached and all discrepancies were resolved through a subsequent test of the system.
- Performed the 17th Disaster Recovery Test in January 2007. This utilized the servers and replicated databases that are in place at the data recovery site in Kansas. The majority of the recovery work was performed remotely from the NMDP Repository Services site in New Brighton, MN. This was the first test to take advantage of real time data replication of critical databases. As a result, the recovery efforts were completed 33% faster than the previous test.

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II.B. Rapid Identification of Matched Donors – Hypothesis 1:

Increasing the resolution and quality of the HLA testing of volunteers on the Registry will speed donor selection.

Continued advances in laboratory methods and supporting equipment have positively impacted the level of typing resolution for newly recruited volunteer donors. Beginning in 2005, one contract laboratory implemented HR, Sequence Specific Oligonucleotide Probes (SSOP)-based DRB1 testing (in addition to intermediate resolution HLA-A and B) that resulted in 90% allele level typings for their distribution of the new donor pool. In 2006, high throughput sequence based typing (SBT) was implemented by another contract laboratory that provided an average of 60-70% HR results for HLA-A, B, and DRB1. The combinations of these efforts have resulted in 45% of the new volunteer donors receiving HR DRB1 results at the time of recruitment. The per-sample cost of these typings was comparable to the intermediate resolution typing from other HLA typing methods. Over the past 10 years, the NMDP successfully reduced the cost of HLA typing by nearly 70% while simultaneously improving the precision, increasing the resolution and enhancing the quality of the typing to include the continuously increasing number of newly identified HLA alleles (see Table 1 below). The vision and efforts of the Navy to continually press the HLA community in this direction and to lead the advancement and testing of these technologies has been instrumental in achieving these accomplishments.

Table 1

Cost Decreases for ABDR		
Year	Price (Dollars)	Percent Price Decreases
1997	\$134.75	-
1998	\$73.50	45.5%
2000	\$62.20	16.4%
2002	\$56.02	10.0%
2003	\$53.80	4.0%
2004	\$53.29	0.1%
2006	\$45.78	14.1%
2007	\$45.52	0.2%
TOTAL DECREASE	\$89.23	66.2%

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If a patient does not find a matched donor and is in urgent need, patient-focused drives can be held and the donor registration process can be expedited. During this grant, 35,612 new volunteer donor samples recruited at patient-focused drives flowed through the expedited process shortening the length of time for listing from 6-8 weeks to 3 weeks. This process includes time to enter demographic data, confirm financial coverage, ship and receive the samples and complete the HLA typing. Demographic data are entered within 72 hours for expedited samples, and they are shipped the next scheduled day, Monday through Thursday. In case of a contingency event, high volumes of samples could be processed and shipped quickly using this established process. This is part of the integration of the RITN capabilities and improvements within the NMDP research and development.

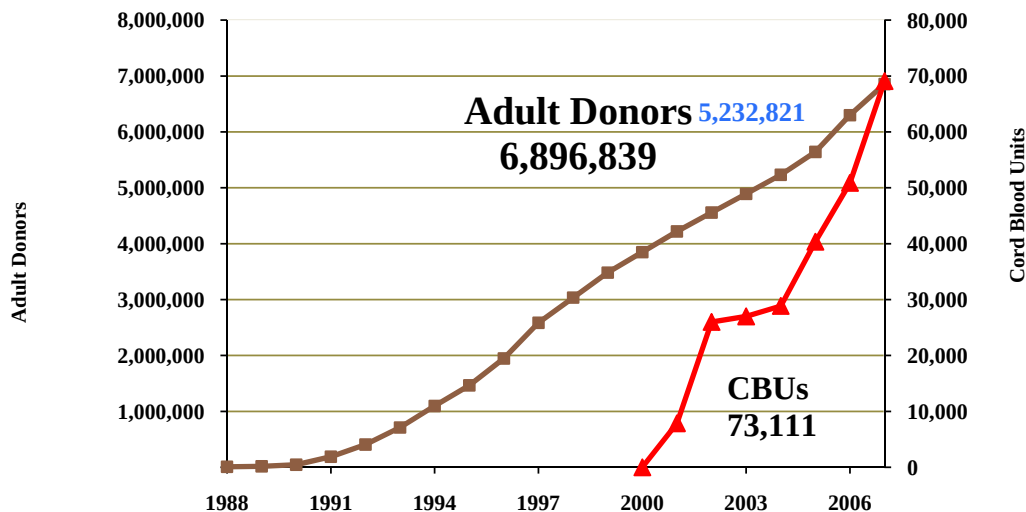
The NMDP's comprehensive quality control program has supported the successful increase in the quality of HLA typing received through the contract laboratory network. The method of inserting blind quality control samples into each laboratory's shipment of volunteer donor samples has provided more than 12 years of data, tracking the accuracy of high volume HLA typing. Errors that have been investigated and corrected due to our quality control program include clerical, false negative and positive probe scores, software, sample switches, contamination and training issues. Over this time, the accuracy rates have continued to improve, as documented by decreased monthly error rates and decreased discrepancies as the donors are selected for patients and retyped by other laboratories. The effectiveness of this program and the efforts of a uniquely qualified high-volume HLA typing laboratory network have resulted in a combined HLA class I and class II Quality Control (QC) accuracy rate during this time frame of 99.9%, a level of precision not possible prior to this research.

Aim B.1.1: Increase Registry Diversity

Continually working to increase the genetic diversity of the Registry helps to ensure that more patients will be able to locate a suitably matched stem cell product for a transplant. During this time frame, NMDP DCs (including DoD) and recruitment groups added 316,679 minority race and 352,881 Caucasian adult donor volunteers that were typed for HLA-A, B and DRB1. Navy funding supported the typing of 190,198 of these culturally diverse new donors. 30,800 additional cords were registered through CORD Link taking the total registry to >73,000.

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Registry Growth: Adult Donors and Cord Blood Units



An
RFQ

was released December 22, 2005, to the six (6) contract laboratories. The goal was to capture any cost savings associated with the pilot project which evaluated a reduction in the number of beads used in the Luminex technology (from 100 down to 20) for HLA typing of volunteer donors.

One year contracts were negotiated with five HLA laboratories and began March 26, 2006. The NMDP obtained a 14.1% reduction in the overall price per sample for HLA-A, B, DRB1 donor recruitment typing, in part due to the 20 bead pilot project and by reducing the number of contract laboratories, thus increasing the weekly volume to the remaining laboratories and taking advantage of pricing reductions for higher volume processing. One laboratory is currently performing SBT on approximately 20% of the newly recruited samples and reports an average of 60-70% HR results for class I and II. On average, 45% of new donors will have both antigens reported at allele level for class II from the three different methodologies used for this typing project: membrane bound SSOP, LABType® SSOP and SBT. A new sample collection method, buccal swabs, was implemented in April 2006 for DC/Recruitment Group sponsored drives and online Do-It-Yourself (DIY) recruitment drives. In September 2007, a buccal swab kit collection mechanism was implemented for donors who do not have a stored Repository specimen and are selected by patients for further DNA testing. *(For additional details on the implementation of buccal swabs, see Aim B.1.4.)*

To support the efficient management of the processes for new volunteer registration and tracking of tasks related to the donor CT and work-up stages, the following enhancements were made to STAR Link:

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- Completed the design and coding for DIY donor registration through www.marrow.org in August 2006. During the grant period, registered 13,659 donors through the on-line system.
- Developed automated features to support the donor network. These automated features allowed for an effective transmission of the data provided from DIY through to STAR Link and onto STAR.
- Deployed functionality to display health history information on DIY registered donors making it available to the DCs when the donors are assigned to them.
- Added functionality to support use of DIY "Promotion Codes" or "Coupons" for sponsored payment of recruitment costs (donors with a promotion code can register free).
- Added features to enhance the ability for a DC coordinator to order buccal swab kits to be sent from the Repository Services facility. These are shipped either to the donors directly or to the DC depending upon the type of request.
- Enhanced the Kit Maker application to prioritize kits for expedited patient-focused recruitment drives. This allows for guaranteed same-date processing at the Repository when the kits are returned.

This is one of the major opportunities to increase the number of donors on the NMDP file and increase the genetic diversity, making it possible for more patients or potential casualties to rapidly find closely matched donors.

Aim B.1.2: Evaluate HLA-DRB1 High Res typing

To further understand the impact of providing higher resolution typing for newly recruited donors, the NMDP developed a stratified typing program in 2005. Five HLA laboratories performed HLA-A, B and DRB1 typings at a minimum of intermediate resolution on 228,239 new volunteer donors. One of the laboratories was contracted to resolve 90% of the DRB1 typings to allele level for their randomly distributed portion of these new donors (76,935). Donors who were recruited in 2005 were analyzed in September 2007 to count the total number that had been selected on behalf of searching patients. Request types included HR typing, CT, and WU. Donors with HR DRB1 were selected 1.5, 1.5, and 2.8 times more often for HR, CT, and WU, respectively, than donors with intermediate resolution DRB1 typed during the same time frame. Pearson Chi-square analysis for each request type demonstrated a preference for donors typed at HR DRB1 with p-values <0.0001 in all cases. Although HR DRB1 typed donors represented only 34% of the newly recruited donors typed in 2005, these donors accounted for 43% of the HR activity, 44% of the CT activity, and 58% of WU activity. HR DRB1 results at recruitment had a significant impact on the likelihood of a donor being selected for further evaluation on behalf of a searching patient. However, the progression of a patient to transplant is a complex process with numerous factors being considered. And, in this study it could not be demonstrated that the positive effect of HR HLA-DRB1 typing on the donor evaluation process also has a positive impact on reducing the overall search time frame. We plan to continue to

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evaluate the benefits observed in this study, along with the significant contributions of HapLogic matching predictions to understand the impact going forward.

Patient-Directed Typing Request	IR HLA-DRB1 Cohort (N = 151304)		HR HLA-DRB1 Cohort (N = 76935)		Chi square value	p-value
	Count	%	Count	%		
HR	1227	0.81	912	1.19	76	<.0001
CT	2675	1.77	2107	2.74	224	<.0001
WU	261	0.17	364	0.47	168	<.0001

This study was presented at the 2007 annual meeting of American Society for Histocompatibility and Immunogenetics (ASHI) ¹

Aim B.1.3: Evaluate HLA-C Typing of Donors

This study was designed to assess the value of providing HLA-C typing in addition to HLA-A, B, and DRB1 at the time of recruitment. The funding supported a statistical evaluation of 20,182 Department of Defense donors registered during 2003 and evaluated through September 2006. The rates of donor selection were compared for patient-directed HR HLA typing requests, CT requests, and WU requests between unrelated volunteer donors who had HLA-C typing available and those who did not have HLA-C typing available. An exposure time analysis was used to calculate the rates of various donor selection endpoints (HR, CT, or WU) between the two otherwise equivalent groups. The two donor cohorts (no HLA-C typing vs. HLA-C typing) were then compared using the relative risk (RR), which is the ratio of the rate of the event for the HLA-C typing group divided by the rate of the event for the no HLA-C typing group.

Overall, there were *significantly fewer* HR requests among donors with HLA-C typing. In contrast, there were approximately 1.7 times more CT requests among donors with HLA-C typing. Also, among donors who had a CT request, those with HLA-C typing were twice as likely to have a subsequent WU request as those without HLA-C typing. This relationship was especially significant in the minority donor population. The results of the exposure analysis tend to support the general perception that more HLA typing information is preferred by TC staff when evaluating adult donors for their patients. However, the progression of a patient to transplant is a complex process with numerous factors being considered. Also, in this study, it could not be demonstrated that the positive effect of HLA-C typing on the donor evaluation process had a positive impact on reducing the overall search process time frame.

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Comparison of Patient-Directed Event Rates

	Group	Outcome		
		HR	CT	WU
Number of Events	No C typing	213	451	59
	C typing	60	349	55
Person-years of Exposure Time on Registry	No C typing	45359	45358	889
	C typing	19577	19577	433
Event rate per person-year (95% CI)	No C typing	0.0047 (0.0041,0.0053)	0.010 (0.009,0.011)	0.066 (0.049,0.083)
	C typing	0.0031 (0.0023,0.0038)	0.018 (0.016,0.020)	0.127 (0.093,0.161)
RR (95% CI)		0.653 (0.482,0.873)	1.793 (1.555,2.066)	1.914 (1.301,2.812)
p-value		0.003	<0.001	<0.001

This analysis was presented as a poster at the 2007 annual meeting of ASHI. ²

In a separate study, intermediate resolution HLA-A, B and C typing was prospectively performed on selected donors if the original B antigen was one which could have multiple C associations. The hypothesis was that in these cases, performing a low resolution C typing would provide information on the allele level B without actually updating the B typing. This could be a cost effective mechanism to utilize known strong B-C linkage disequilibrium to increase the “assumed” specificity of selected donors on the registry. To enhance the cost-effectiveness of the prospective typing, only donors that had recently submitted demographic updates were included in the donor pool to help ensure that updated HLA typings were applied to confirmed interested donors. Additional selection criteria were as follows:

- Typed by serology at A/B with intermediate resolution DRB1 typing or
- Typed by serology at A/B without DRB1 typing
- Never previously selected for a patient-directed typing request
- No HLA-C
- Must have a stored frozen blood sample
- Must carry one or more of the following B antigens: 7, 13, 15 (62, 63, 75, 76, 77), 18, 27, 35, 16 (38, 39), 40 (60, 61), 41, 12 (44, 45), 48, 5 (51, 52), 22 (55), 17 (57, 58), 67, 81

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A total of 38,139 donors were typed between 9/30/2004 and 7/30/2007. Eighty-four percent of the donors were Caucasian (CAU), 5% were Hispanic (HIS), 4% were Asian Pacific Islander (API), 4% were African American or Black (AFA), and 1% were Native American Indian/Alaskan Native (NAM). Donors with race codes of Multi, Other, Declined and Unknown were excluded from the analysis because the numbers were small.

Methods

Intermediate resolution HLA typings from 62,317 B-C haplotypes were evaluated to determine whether the donor's specific B allele could be predicted based solely on allele frequency data. If an intermediate resolution allele code contained two common alleles, the code was designated 'indeterminate.' For example, B*44ABWX includes B* 4402/4403/4407/4413/4426/4430/4432. Since both B*4402 and B*4403 are common alleles in all race/ethnic groups, the code was designated indeterminate. The donors with this type were subsequently evaluated to determine whether the presence of intermediate resolution C typing assisted in predicting the B allele.

Allele codes that excluded a secondary common allele were designated 'known/expected.' For example, B*44ABYR includes 4403/4407/4413/4426/4430/4432, so the expected allele is B*4403 based on allele frequency data. In these cases, the B type could be predicted without C.

The B-C and C-B haplotypes for each donor were also analyzed to determine whether the linkages were expected/unexpected using the B-C haplotype frequencies from the manuscript titled, "High-resolution HLA alleles and haplotypes in the United States population" by Maiers, et al.³ Haplotypes could not be assigned for 2% of CAU, 6% of HIS, 7% API, 6% AFA and 9% NAM. The larger percentage of failures for minorities was mainly due to smaller HR typed sample sizes available to calculate baseline haplotype frequencies and to a lesser extent higher genetic diversity within those groups relative to CAU. The B-C and C-B haplotypes were used to establish common linkages for the purposes of this analysis.

Results

The addition of prospective C typing was useful in predicting the B allele 4% of the time, primarily for B39 and B44. A total of 9% of donors had intermediate resolution B typings that contained multiple common allele possibilities (indeterminate). This occurred most frequently in the groups of B7, B35, B39 and B44. (See Figure 1, Pg 35). However, B*0702 vs. B*0705 is expected in all race groups based on allele frequency data. In addition, since multiple B35 alleles are commonly associated with Cw*0401 in the majority of race/ethnic groups, the addition of C typing is not as helpful in determining a specific B allele in this antigen group. C typing was most useful for predicting a specific B35 allele for donors with HIS ethnicity. (See Figure 2, Pg 35).

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C typing was also somewhat helpful for other B antigen groups for specific race/ethnic groups; however, the total number of donors fitting this description was negligible. For example, both B*1301 and B*1302 are common in API. B*1301 is not common in any other race/ethnic group. B*1301 is commonly associated with Cw*0304, while B*1302 is commonly associated with Cw*0602. However, only 2% of the B13 indeterminate intermediate resolution typings were from donors with a self-described API race designation. B*1302 is the expected allele in all other race/ethnic groups based on allele frequency data. (See Figure 3, Pg 36).

91% of the donors' HLA-B typings could be predicted using allele frequency data, due to the increased resolution of the typing provided by the reagents utilized by the testing laboratory. The secondary haplotype analysis established that intermediate C resolution typing could have been helpful in predicting approximately 40% of B alleles, if the typing received by the lab was low resolution (serology or XX level).

Overall, the additional C typing information was not helpful in predicting a specific B allele.

Conclusions

Based on the outcome of this analysis, modification of the selection criteria to include only donors carrying B39 or B44 antigens may be a consideration, since these are the B antigens that benefit most from the addition of C.

While this information was not necessarily helpful in predicting a donor's specific B allele, the inclusion of intermediate resolution C typing at recruitment was very helpful from a search strategy perspective, since multiple C alleles are commonly associated with one B allele for the following B antigen groups: B62, B18, B27, B44, and B51. If funding was available, a strategy to continue typing donors carrying B39 and B44 may prove beneficial for rapid identification of donors most likely to match a searching patient.

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Figure 1: Haplotype Distribution

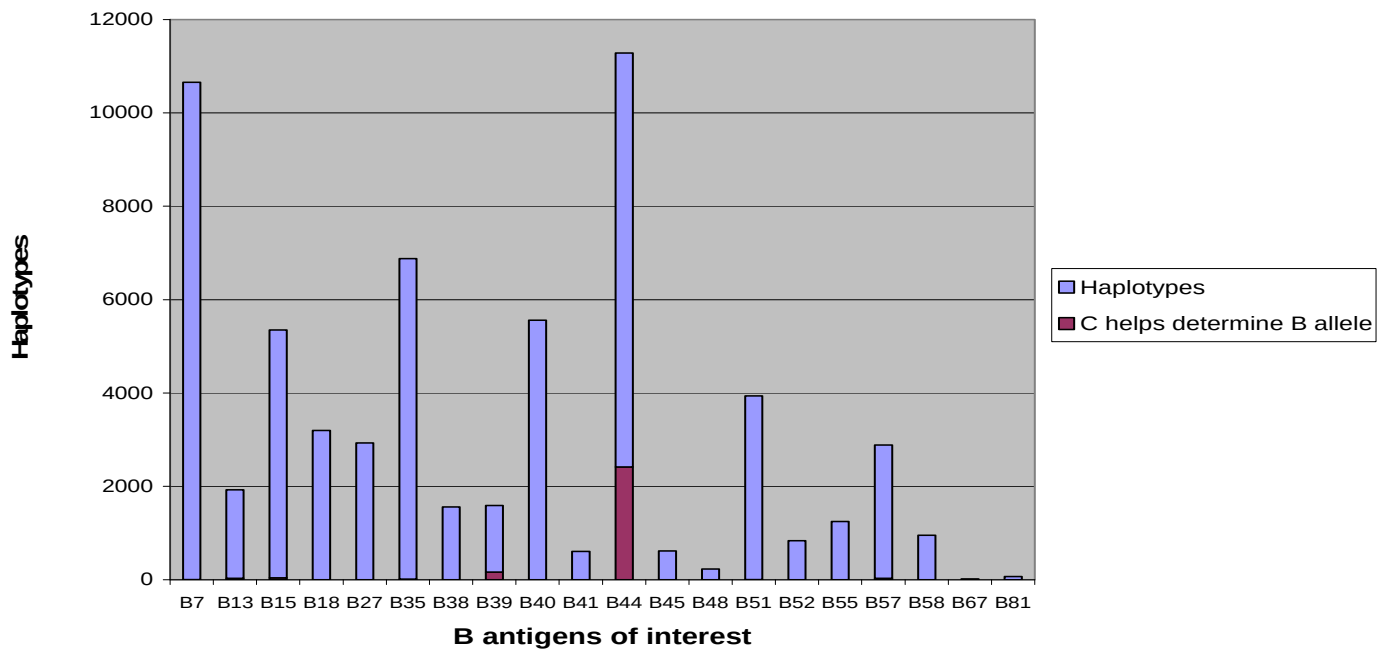
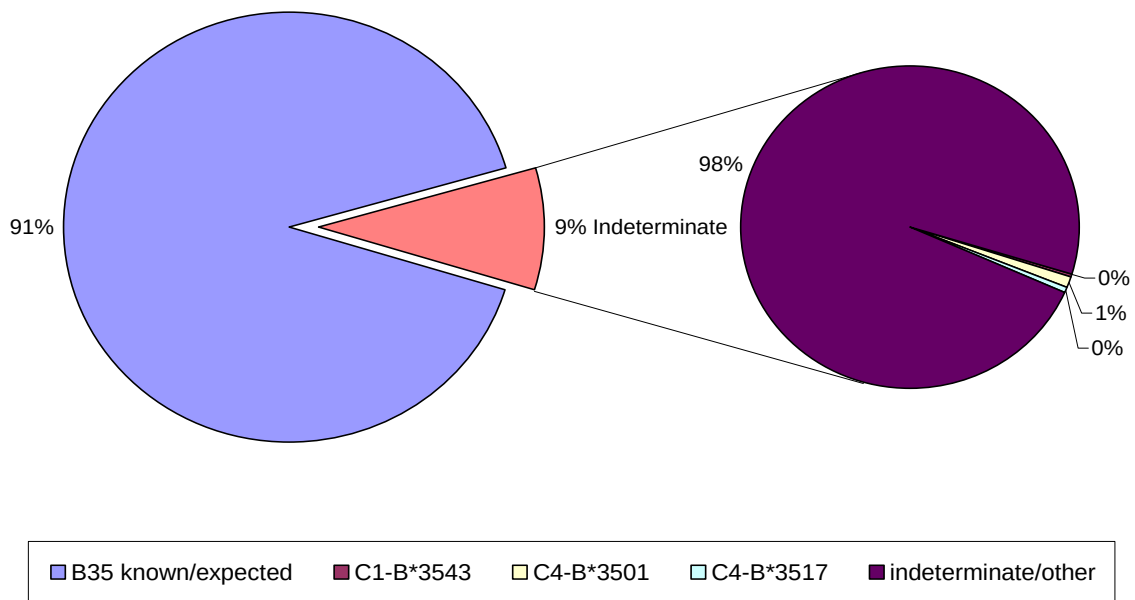
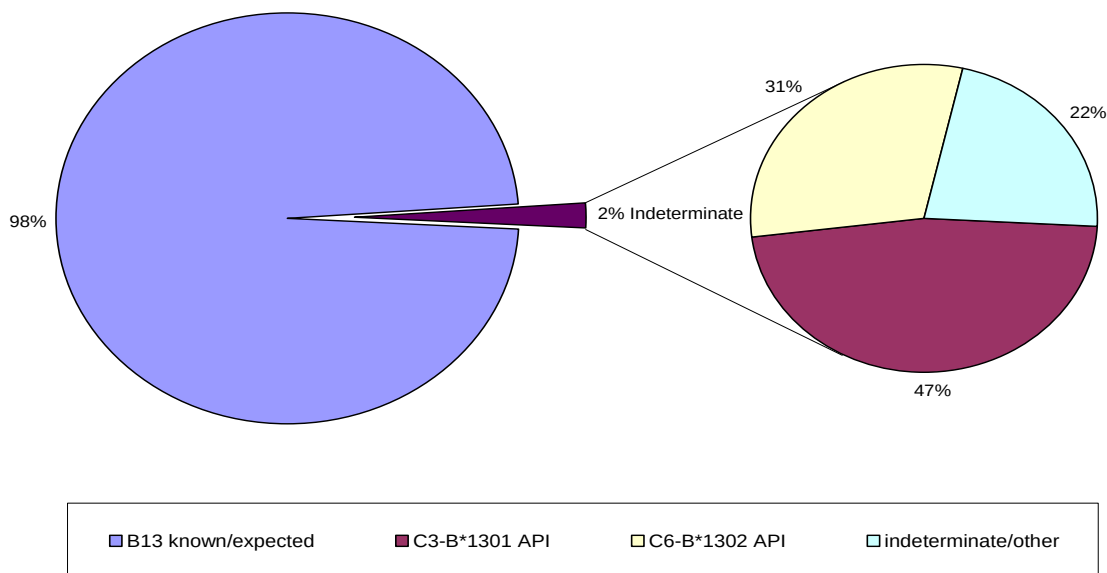


Figure 2: B35



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Figure 3: B13



Aim B.1.4: Evaluate Buccal Swabs

A pilot project was initiated in 2006 to test the ability to collect sufficient cells and perform HR, multi-locus DNA typing using buccal swabs. Three different collection methods were evaluated for complete and accurate HLA results:

- Foam-tipped swabs transferred to an Whatman FTA® Indicating Micro Card
- Foam-tipped swabs transferred to an Schleicher & Schuell 903® filter paper card
- Cotton swabs, air dried and placed into an envelope

Twenty-five samples of each type were sent to two separate laboratories and tested for HLA-A, B, C, DRB1 by two different methodologies. The results are below:

		Buccal cells on swabs, transferred to FTA 25 samples	Buccal cells on swabs, transferred to 903 25 samples	Buccal cells on swabs, no transfer 50 samples
Failure of DNA to amplify	Lab 1 SSOP	6%	6%	0%
	Lab 2 Luminex	9%	0%	0%
Percentage of repeats	Lab 1	14%	2%	0%
	Lab 2	17%	15%	3%

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100% of HLA typing results from all three methods of buccal cell collection were accurate, but the FTA paper had the highest failure rate and number of repeated tests. The 903 cards were intermediate in their amplification failure and repeat rates and the plain cotton swabs performed the best. To evaluate the feasibility, process flow, and donor perceptions of the buccal swab collection method, two large patient-focused donor drives (150 donors each) using cotton swabs were conducted. Collection of 4 swabs took from 2-4 minutes, 95% of the donors preferred collecting buccal swabs over a blood sample collection, and the HLA results were consistent and accurate. A cost benefit analysis showed a cost savings per donor of \$7.30 as compared to blood spotted onto filter paper. The NMDP transitioned to the collection of DNA from newly recruited adult volunteers using buccal swab kits in April 2006. That same month, the NMDP presented the results of these studies at the World Marrow Donor Association meeting in Cape Town, South Africa. DNA on buccal swabs from over 390,000 donors was successfully HLA typed through October 31, 2007.

The initial design of the buccal swab barcode label proved to be difficult to attach consistently at the donor drives and to process at both the Repository and HLA contract laboratories. A team worked to redesign the labels to improve functionality. To support the NMDP Blind QC program, swabs containing well characterized DNA needed to be created. Purified genomic DNA is a common substrate for DNA testing and is easily extracted from the cotton swab. From the existing Master QC inventory, over 150 different donor blood samples were available. Initially, the quality control buccal swab samples were dipped into DNA extract solutions prepared from frozen whole blood QC samples. Testing was performed by two laboratories utilizing filter capture technology for DNA extraction with complete and accurate results. Subsequently, contract laboratories using magnetic bead DNA extraction procedures reported that they were unable to capture enough purified genomic DNA from the extraction solution for accurate HLA testing. Four new QC buccal swab sample types were then evaluated: 1) purified DNA with supplemental protein, 2) B-Lymphoblastoid cell lines (B-LCL) provided by the NMDP Research Repository, 3) peripheral blood mononuclear cells, and 4) buccal cells from cheek swabs performed by QC volunteers. Parameters evaluated included availability and ease of obtaining sample type, ability to obtain accurate and complete HLA results, diversity of genotypes and cost. B-LCL cell lines were selected as the optimal QC sample type and 26 cell lines were expanded to provide cells for cotton swabs. Thousands of cell lines with complete HLA characterization are available for expansion. These cell lines can provide a diverse panel of QC samples to support all HLA typing projects.

A Sample Storage Research Study was developed and presented to the NMDP Histocompatibility Committee for their approval. The objective is to evaluate the impact of long-term humidity-controlled storage on the DNA from three (3) types of samples. All appropriate approvals were received prior to initiation of the study. In September 2007, blood and buccal swab samples were collected from 30 current HLA typed volunteer QC donors. A sufficient quantity of samples for the five (5) year study was generated and stored at the NMDP Repository. Fresh blood, blood spotted onto Whatman® (formerly Schleicher & Schuell) 903

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filter paper, and buccal swabs for each donor were sent to two laboratories for the Time = 0 initiation of the study. One laboratory was contracted to perform HR typing for HLA-A, B, C, DRB1, and DQB1 loci. The second laboratory was contracted to perform intermediate resolution typing for HLA-A, B, C, and DRB1 loci. The second laboratory was also contracted to evaluate the quantity and quality of DNA within each sample type. The first set of results was received from each of the two laboratories. All typing results were 100% accurate, and the evaluation of the DNA was complete and thorough. These results from the initial time point will be the basis for determining the stability and usefulness of the DNA stored for each sample type over the next 5 years.

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II.B. Rapid Identification of Matched Donors – Hypothesis 2:

Primary DNA typing data can be used within the registry to improve the quality and resolution of volunteer donor HLA assignments.

Aim B.2.1: Collection of Primary Data

The NMDP primary data management system was developed originally to represent SSOP typing reagents. Typing kits are stored as a combination of amplification primers and a set of reagents. Software has been developed to generate reactivity (probe hit) tables for verification and processing of the primary data. This system was enhanced during the grant period to represent Sequence Specific Primers (SSP) and SBT typing kits. Fifty-five SBT kits (primer sets) and 19 SSP kits have been registered in this system.

The Extensible Markup Language (XML) messaging format Histoimmunogenetics Mark-up Language 0.3, or “HML 0.3” was released on the NMDP bioinformatics Web site (bioinformatics.nmdp.org) for public comment. Specifications for enhancing the NMDP message processing system to accept HML 0.3 messages were defined and programming was completed to process primary data from SSP and SBT methods.

A presentation was given at the KIR Polymorphism Workshop in September 2005 to describe this messaging format and demonstrate its use with KIR typing results. An abstract describing HML 0.3 was accepted and a poster describing the system was presented in October 2005 at the ASHI annual meeting.⁴ And finally, a manuscript describing the HML 0.3 format for representing primary SBT typing results was published in the final report of the 14th International Histocompatibility and Immunogenetics Workshop (IHIWS).⁵

Since the implementation of this system, several labs have adopted the standard and have sent SSP data through the system. However, the goal of having comprehensive retrospective and prospective collection of these data has not been met (see Aim 2.2 for further details). Furthermore, although the system is capable of processing SBT results and has been used to store primary data from this typing method, the local interpretation and validation was not implemented during the period of this grant.

Aim B.2.2: Validation of Logic of Primary Data

The NMDP has performed a complete re-analysis of the HLA-A and B primary data collected over the course of its history. This validation project provided a categorization of re-interpretation results in terms of comparison with the laboratory typing and an investigation of disparities between the NMDP-interpreted result and the laboratory’s reported type. In particular, a comprehensive analysis of primary data was performed by evaluating results submitted by contract laboratories on all of the blind QC samples (with known HR HLA typing). This analysis confirmed that the system was operating correctly and was able to detect a small

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number of instances where the laboratory result (2-digit resolution) passed the standard QC check but the allele-level genotypes that resulted from re-interpreting the primary data had discrepancies at the 4-digit level of resolution. *[For a complete report, see Aim IIB. 3.1]*

The NMDP database was enhanced substantially during the period of this grant to improve the utilization of the primary data. One component was the development of a flagging mechanism to track primary data that have gone through complete validation. The resulting genotype list representation can be rapidly retrieved as the typing of record for the matching algorithm for both CBUs and adult donors. As of January 1, 2006, there were 1.7M HLA-A and B results and 2.6M HLA-DRB1 results for a total of 6M sets of primary data stored as genotype lists. A number of tools have been developed to make this genotype list data available to applications such as Web tools, validation scripts and the HapLogic matching algorithm.

The NMDP did not meet the goal of completion of partial primary data records by working with the laboratories to define supplemental reagents that were utilized at the original time of the typing. The system for processing these data has been implemented (Aim IIB.2.1), but the work of actually collecting these data from the laboratories was confounded by the lack of an efficient system for merging results from two different systems. For example, many labs use a commercial SSOP system for their primary typing and then apply results of an SSP system to resolve remaining ambiguities. Due to a lack of standards and IT integration in the laboratory, the laboratories were not able to develop a system to bring together the data from these two sources and report them to the NMDP as one message.

Recognizing this challenge as a community-wide barrier to automation and comprehensive data report, the NMDP created the HLA Information Exchange Data Format Standards (HIEDFS) Working Group. This is a consortium of commercial and government entities collaborating to develop interoperability standards for HLA bioinformatics. This includes not only the reporting of typing results and primary data in standardized formats but open, public standards for representing typing kits (SSOP/SSP/ST), reactivity tables, WHO alleles and NMDP multiple allele codes.

A manuscript describing the system and its application for Class I HLA genes is in development.

Aim B.2.3: Reinterpretation of Primary Data

Reinterpretation of primary data to improve the level of resolution of previously reported donor typings.

This purpose of this Aim was to upgrade previously reported XX-level class I HLA typings on the Registry with multiple allele codes for all cases where primary data had been collected. Contract laboratory reporting software defaulted to the lower resolution “XX” results even though the reagents produced a higher level of specificity. Due to early success in implementing matching directly based on genotype lists (Aim IIB2.4) the work under this Aim was not

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completed. An analysis of the impact of generating these new allele codes was performed and it revealed that over 200,000 new allele codes would need to be created. Given that many laboratories still work with this list manually (from a printout) it was decided not to move forward with this approach.

Aim B.2.4: Genotype Lists & Matching Algorithm

The theory underlying this Aim was that the interpretation of the primary data into genotype lists and the utilization of these instead of search determinants could provide a more rapid and more specific matching logic. The genotype list database developed as a result of this Aim has been used as the foundation for a new matching algorithm HapLogic that directly applies DNA typings of any complexity into the up-front search. The matching algorithm was modified to convert all donor and cord blood typing data (alleles, allele codes, XX-codes, serology, genotype lists) into a universal format. This allowed development of a much simpler matching algorithm with many new features. The new algorithm produces two match grades per locus instead of one and aligns the match grades to the patient's HLA types so that search tools like TRANS Link can easily use custom criteria filters to find mismatches at a particular type. The new algorithm replaces much of the early XX-codes submitted at recruitment by NMDP contract labs with genotype lists interpreted to a recently published allele list. This allows the algorithm to 1) incorporate any potential new alleles in the donor or cord type, 2) identify phase mismatches where both of the patient's alleles are possible in the donor but not on the same genotype, and 3) compute logical solutions for cross-group HLA types in the match algorithm.

Many HLA typing reagents produce results that have ambiguity at the first two digits. Laboratories attempt to resolve these by the addition of group specific reagents. As stored HLA types are reinterpreted over time, based on larger allele lists, many more types have this kind of ambiguity. The designer search determinants system was a short-term solution to the problem because it allowed multiple specificities to be assigned to a donor typing. With HML 0.3, the new genotype list database and the HapLogic matching algorithm, there is a complete solution for reporting, storing and matching without regard to the two digit groups. This development provides the foundation for future design of typing kit scoring systems that are based on allele frequencies of searching patients. This has the potential to guide the development of typing reagents that focus on resolution of high frequency alleles and exclude rare ambiguities that cross two-digit groups.

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II.B. Rapid Identification of Matched Donors – Hypothesis 3:

Registry data on HLA allele and haplotype frequencies and on the nuances of HLA typing can be used to design computer algorithms to predict the best matched donor.

Aim B.3.1: Phase I of Expectation Maximization (EM) Haplotype Logic

During the grant period, we completed validation of the final datasets which were used in the HapLogic algorithm for allele match prediction. HR allele and haplotype frequencies have been incorporated to provide probabilities of allele match on a patient/donor pair level. This first phase uses haplotype data across the A-B-DRB1 loci as well as DRB1-DRB3 and DRB1-DRB5 haplotypes in the five main race groups (CAU, AFA, API, HIS, NAM). This also includes providing DRB1 allele match probabilities for donors with only HLA-A and B typing.

The prediction system was tested on a cohort of 9,600 CT typings performed in 2003 to compare the predictions of the algorithm with the high-resolution typing performed by the TC. The results of this validation were the following statistics that quantify the accuracy:

Sensitivity	Specificity	Accuracy	Positive Predicted Value	Negative Predicted Value
0.7427	0.9238	0.8236	0.9236	0.7433

The accuracy was higher in Caucasians versus minorities and in general would be improved by the inclusion of more HR typing data into the Registry. The predictions were found to be so useful in prioritizing donors for further typing that these data were used in the Phase I release of allele-match prediction. This work lays a foundation for a Phase II allele-match prediction that extends to HLA-C and DQB1.

An abstract of HLA analysis of the high-resolution typed donor cohort used for HapLogic was accepted for a talk at the 2006 ASHI meeting⁶ and a manuscript for publication was completed.³

HapLogic Algorithm

The introduction of HapLogic algorithm was an innovative enhancement that provided valuable allele level matching information on donors and CBUs most likely to match searching patients. This enhancement included the addition of the following information:

- Prediction calculations for individual loci that indicate the potential to match the both alleles at HLA-A, B and DRB1

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- Composite predictions for the potential to match the patient's six (6) (HLA-A, B, DRB1) alleles based on A/B/DRB1 haplotype frequencies and the race/ethnicity of the donor or CBU:
 - Pr(6) – potential to match all six (6) of the patient alleles
 - Pr(5) – potential to match five (5) of the patient alleles
 - Pr(4) – potential to match four (4) of the patient alleles (cord blood only)
- Interpretation logic that uses primary data received from the laboratories to refine the genotype lists used in HLA matching. It provides indicator symbols (+, -, #) when primary data are being used:
 - + potential to match the patient allele
 - – does not have potential to match the patient allele
 - # potential to match one patient allele at that loci, but not both alleles
- Two match grades at each locus (P = Potential, A = Allele match, M = antigen mismatch, L = allele mismatch)

HapLogic Validation Results

Prior to the implementation of HapLogic, extensive validation was performed against the data and algorithm to help ensure the accuracy of the patient searches. NMDP Scientific Services, working closely with IT Quality Assurance and Bioinformatics performed a number of validation steps as follows:

Interpretation of Primary Data

- Verified that the interpretation logic correctly reads and produces HLA results from the binary primary data. 99.6% of class I and 99.3% of class II primary data strings had been correctly submitted and re-interpreted by the NMDP logic
- Validated a statistically significant subset of 29,000 class II QC typings. For each of these cases, the lab reported primary data plus an HLA result. The analysis consisted of two steps:
 - The NMDP's re-interpretation of the primary data and generation of an HLA result, which was compared to the lab reported HLA result, and
 - The NMDP interpreted HLA result was compared to the known HR HLA type of the QC sample:
 - All cases where the NMDP-interpreted HLA result was discrepant with the lab's HLA result were investigated and it was found that the discrepancy could not be attributed to the NMDP logic.
 - All cases where the NMDP-generated HLA result was discrepant with the known QC type were investigated and it was found that the discrepancy could not be attributed to the NMDP logic (i.e., lab errors).

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- Validated a statistically significant subset of 40,000 class I QC typings. For each of these cases, the lab reported primary data plus HLA-A and B results. The analysis consisted of two steps:
 - o The NMDP's re-interpretation of the primary data and generation of HLA-A and B results which were compared to the lab reported HLA results, and
 - o The NMDP interpreted HLA results were compared to the known HR HLA types of the QC sample.
 - All cases where the NMDP-interpreted HLA result was discrepant with the lab HLA result were investigated and it was found that the discrepancy could not be attributed to the NMDP logic.
 - All cases where the NMDP-generated HLA result was discrepant with the known QC type were investigated and it was found that the discrepancy could not be attributed to the NMDP logic (i.e., lab errors).

Individual Locus HLA-A, B, DRB1 and Composite Predictions

- Validated a statistically significant randomly selected subset of 9156 haplotype calculations for HLA-A, B, DRB1 individual locus scores and composite Pr(6), Pr(5) scores. This dataset was composed of approximately 70% Caucasian donors and hereafter is referred to as "random subset". A specifically selected subset of non-Caucasian donors was also validated and is hereafter referred to as "non-CAU"
 - o Defined Data Sets:
 - 224 = HLA-A and B at antigen level; HLA-DRB1 at HR
 - 444 = HLA-A, B, and DRB1 all at HR
 - 444x = HLA-A, B, DRB1 plus DRB3, DRB5 associations
 - 444xmin = HLA-A, B, DRB1/3/5 calculated with minority haplotypes
 - o Individual locus (A, B, DRB1) results:
 - A and B calculations for the 444 vs. 444x data sets:
 - over 99% passed validation. The remaining 1% is pending further investigation
 - DRB1 calculations for the 224 vs. 444 vs. 444x vs. 444x min:
 - ♦ 224 vs. 444: 70% of the calculations were not significantly changed by the higher level of specificity utilized. However 30% of the 444 results had increased accuracy (18% in the random subset and 44% in non-CAU subset). The benefit was most distinct in the non-CAU donors.
 - ♦ 444 vs. 444x: A total of 40% of the 444x showed improved calculations (22% in the random subset and 74% in non-CAU subset). The benefit was most distinct in the non-CAU donors.
 - ♦ 444x vs. 444x min: A total of 11% of the 444x showed improved calculations (0% in the random subset and 22% in non-CAU subset). The benefit was seen only in the non-CAU donors.

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- ◆ Pr(6) composite scores: Reviewed and verified calculations based on 444 and 444x haplotypes
- ◆ 444 and 444x haplotypes: 96% good. The remaining cases are pending review by Bioinformatics staff.
- Composite Scores:
 - Pr(5) composite scores: Reviewed calculations based on 444 and 444x haplotypes
 - ◆ 444 and 444x haplotypes: 97% passed validation. The remaining cases are pending review by Bioinformatics staff
 - A, B only typed donors Pr(6) and Pr(5) composite scores-two sets were evaluated: 1) random, and 2) serologic/low resolution (LR) typing :
 - ◆ Pr(6): 92% passed (97% for random, 87% for LR). The remaining cases are pending review by Bioinformatics staff.
 - ◆ Pr(5): 75% passed (62% for random, 97% for LR). The remaining cases are pending review by Bioinformatics staff.
- Completed a comparison of predicted vs. actual HLA-A, B and DRB1 typings on 2500 minority donor samples with the following composition – 31% AFA, 26% API, 39% HIS and 4% NAM.
- For a large fraction of individuals in high HLA diversity minority groups, the 4-digit DRB1 composition could be accurately estimated from lower level HLA information.

Training

The NMDP performed HapLogic training at the NMDP Annual Council Meeting in November 2005. TCs attended three (3) seminars and an interactive training session which described the scientific basis for the changes to the search algorithm, application of the enhanced algorithm for patient searches, and software enhancements related to HapLogic. After the Council Meeting, NMDP staff began developing continuing education materials, such as Web seminars and educational tools that will be used in concert with the launch of the new software.

Customer Satisfaction

In September 2006, the NMDP initiated a survey to measure TC satisfaction with HapLogic. The survey was designed to measure four key areas:

- Improvements to the search process
- Value of data elements provided on the search report
- Comparison of the revised search reports to other search reports
- Effectiveness of education programs to prepare for use of HapLogic

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The survey was implemented using Zoomerang™ Web-based survey services. TC coordinators, medical directors, and physicians were invited to participate in the survey. The survey took approximately 5-8 minutes to complete during pre-testing.

As of September 28, 2006, there were 81 completed surveys. When asked to rate their overall satisfaction with HapLogic where 1 is very dissatisfied and 5 is very satisfied, the preliminary results show an overall satisfaction rating of 4.26 with “Very Satisfied” (5) and “Satisfied” (4) each selected by 46% and 37% of respondents respectively (total of 83%). These preliminary results were presented at the NMDP Council Meeting in November 2006. The full results were analyzed and reported to the Navy in the first quarter of the 2007 fiscal year. Information from the survey was used to identify additional education needs, to design new changes to the search reports, and to guide future enhancements to the algorithm.

Aim B.3.2: Enhancement of EM Algorithm

Three sets of haplotype frequency datasets were produced during the grant period for use within the HapLogic algorithm (A-B-DRB1, DRB1-DRB3, DRB1-DRB5). These datasets were based on high-resolution typed donors from the Registry with a number of measures taken to control for bias. These included 1) considering alleles unique to the antigen binding domain (exons 2 & 3 for class I and exon2 for class II), 2) considering alleles above a frequency threshold based on the NMDP Rare Allele Tally, 3) including only one donor per patient for donors who have high-resolution based on patient directed typing, and 4) including prospective high-resolution typings such as the 2,500 EM validation dataset performed in Spring 2005. Despite these attempts to minimize the bias, this was still a biased dataset because it over-represented frequent types, but these data far exceed any others available at this time.

The results of this work were widely disseminated. A talk was given at the 14th IHIWS meeting in Melbourne, Australia, describing the methods used to develop these haplotype frequencies and in particular the modifications made to the EM algorithm. A manuscript describing the version of the EM algorithm used is in preparation with Dr. William Klitz. Another manuscript specifically addressing the high-resolution haplotype frequencies described here has been completed and includes the publishing of these frequencies on the NMDP Bioinformatics Web site.³ And finally, an abstract was submitted and accepted for an oral presentation at the ASHI Annual meeting on HLA analysis of a cohort of multiethnic donors.⁷

More work was done in preparation for completing manuscripts on the EM algorithm and its application in analyzing several populations as follows:

- Nei's genetic distance measure was implemented for comparison of populations
- Automatic phylogram creation scripts were made for all genetic distance measurements (G, Fst, Nei, Wn) for each locus, 2-locus haplotype, and full haplotype
- 2-locus haplotype Hardy-Weinberg calculations were added to the EM

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- The W statistic was implemented for showing deviation from expected Hardy-Weinberg proportions
- Total observed and expected homozygosity and heterozygosity were added to the EM output spreadsheet
- Detailed tables for systematic analysis of homozygote phenotypes were created to identify locations of deviation from expected Hardy-Weinberg proportions

C-B and DRB1-DRB3/4/5-DQB1 haplotype frequency tables were produced for future extensions to the HapLogic algorithm.

Two typing programs were initiated to generate additional haplotype frequency data for the enhanced HapLogic algorithm. The programs utilized either randomly selected minority adult donor samples or unbiased incoming recruitment stage CBU samples. Two laboratories were selected to provide the HLA typing of these samples at HR for HLA-A, B, C, DRB1/3/4/5 and DQB1.

Approximately 2200 stored minority donor samples were shipped to the laboratories during August and September of 2007. The target enrollment for newly recruited CBUs was 825, which shipped between July and October. HLA results for both programs were completed in October. The resulting haplotypes will be calculated and the data validated in the next project grant. Automated quarterly updates to all HapLogic haplotype frequency tables are in development to provide a mechanism for uploading these data to increase the accuracy of the probability predictions on search reports.

A second objective of CBU typing was to evaluate the utilization of the HR HLA-A, B, C, DRB1/3/4/5 and DQB1 typed CBUs versus the standard low resolution HLA-A, B and HR DRB1 recruitment typed CBUs. Patient-directed activity will be measured at six month intervals to determine whether the HR typing at recruitment impacted CBU selection practice at the TCs and time to transplant.

Aim B.3.3: Optimal Registry Size Analysis

During the grant year, work was performed to generate a new registry Benchmark Analysis using 8/8 allele matching. The software simulation tools developed for previous registry size analyses were extended to produce 8/8 allele match predictions by extending the 3-loci framework to 4-loci and also from antigen level types to allele level types. This work required a complete re-write of the simulation program (called “project”) from Fortran to Perl.

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The executive summary of that report is provided here:

Project I: New Registry Benchmarks

This work extended the existing 6 of 6 (A/B/DRB1) antigen level patient match rates to 8 of 8 allele level (A/B/C/DRB1) match rates. For each patient broad race category, a projected match rate was provided based on the registry size and composition as of February 2006. Models were also developed that factored in donor availability rates into these estimates to provide the likelihood that each patient would have an available 8 of 8 allele-matched donor. Projections to larger registry sizes are included as a tool to evaluate the impact of simulated recruitment scenarios on the rates.

Results:

Potential patient 8 of 8 match rates calculated with current adult donor availability data for the five (5) broad race groups of AFA, API, CAU, HIS and NAM were 36.1%, 52.4%, 80.3%, 46.4% and 61.6% respectively. As expected, in all racial/ethnic groups these rates were significantly lower than the previously calculated rates for 6 of 6 antigen level matching.

It is currently thought that this 8 of 8 allele-match simulation over-estimates the match rate primarily because there are too few HR typed donor phenotypes available. Next steps are to find a way to correct for the lack of high-resolution data or to try a different approach, such as a benchmark where "patients" are randomly selected, run through the search algorithm, and all allele-level potentially matched donors are typed to see how many real HR matches there are at four (4) loci.

An abstract on 8 of 8 allele match rate prediction was submitted and accepted for oral presentation at the 2006 ASHI Annual meeting.⁸ 7 of 8 and 4 of 6 match rate predictions were added to the registry size program. This work was also used to determine match rates for patients searching the cord registry using 6 of 6, 5 of 6, 4 of 6 match criteria who don't find matches in the adult donor registry using 8 of 8, 7 of 8 allele match criteria.

A manuscript for publication of a Greek Registry Size study with Dimitri Monos, M.D. is in progress. An abstract was submitted and accepted for a poster at the 2007 ASHI conference on the same topic.⁹

Aim B.3.4: Target Under-represented Phenotypes

The objective of this project was to link donor zip code information with HLA variables including haplotype assignments and integrate them into standard Geographic Information Systems software for visualization. A meeting was held at the NMDP Coordinating Center in March 2006 involving a panel of experts in geographical analysis of HLA. This meeting was a kickoff of the NMDP GeoCoding project to perform geographical HLA analysis of NMDP donors.

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A Bayesian classifier was developed to assign race/ethnic categories to individuals using haplotype frequencies from donor populations and the priors of the relative size of the donor populations. This algorithm gives the probability for each race/ethnicity overall and for each haplotype, allowing for multiple-race individuals. An abstract was submitted for a talk at the 2006 ASHI conference on this topic.¹⁰

The executive summary is provided here for three projects involving geographical analysis and recruitment:

Project I: Patient Profiling

This project consisted of two (2) separate analyses performed on the searching patient pool. The first analysis looked at non-HLA variables to explore correlations with success in identifying matched donors. These variables included race/ethnicity, age, sex, disease category, date of diagnoses, and geography (TC, zip code, geographical region). The second analysis was an in-depth study of the HLA types of searching patients with a focus on patients who had high haplotype frequencies but few or zero matched donors.

Results:

Of the demographic variables analyzed, only TC, race/ethnic category, registration date and birth year were found to be statistically significant when correlated with patient match rates. The geographical analysis identified state of residence to be significantly correlated with match rates for most race groups and match levels. A regional analysis of 880 zip code regions identified areas with searches that performed substantially above and below expectations base on national averages. The HLA analysis involved the development of computational methods to cluster or classify patients simply on the basis of HLA. The results from this Bayesian classifier were used to further evaluate the HLA of patients finding few or zero matches. Those with haplotypes from more than one ancestral population found fewer matched donors than those with haplotypes from single racial/ethnic groups.

Project II: Donor Genetic Clustering and Geographical Coding

Two separate studies focused on donor demographic and HLA factors. The first analysis applied the Bayesian classifier to a large donor cohort. The second analysis was an in-depth study of the geography of HLA types in the NMDP U.S. donor pool. The approach to this analysis was to look at donor allele, haplotype and phenotype frequencies to provide a comprehensive dataset that could be utilized to develop recruitment strategies.

Results:

A method for assigning haplotypes based on donor phenotypes that used information from reference populations was demonstrated. The geographical analysis of the current composition of the Registry as compared to the U.S. census provided information on areas where over and under representation of population proportions existed. Allele,

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haplotype and phenotype frequencies were analyzed geographically and demonstrated several instances where HLA varied considerably by region.

Project III: Recruitment Analysis

Using haplotype and phenotype frequency data, a diversity scoring mechanism was developed to evaluate five (5) different recruitment drive types. Donors from each of the drives types were scored according to their genetic utility for the searching patient pool. This analysis provided a way to evaluate overall recruitment according to genetic factors rather than by numbers of new donors added.

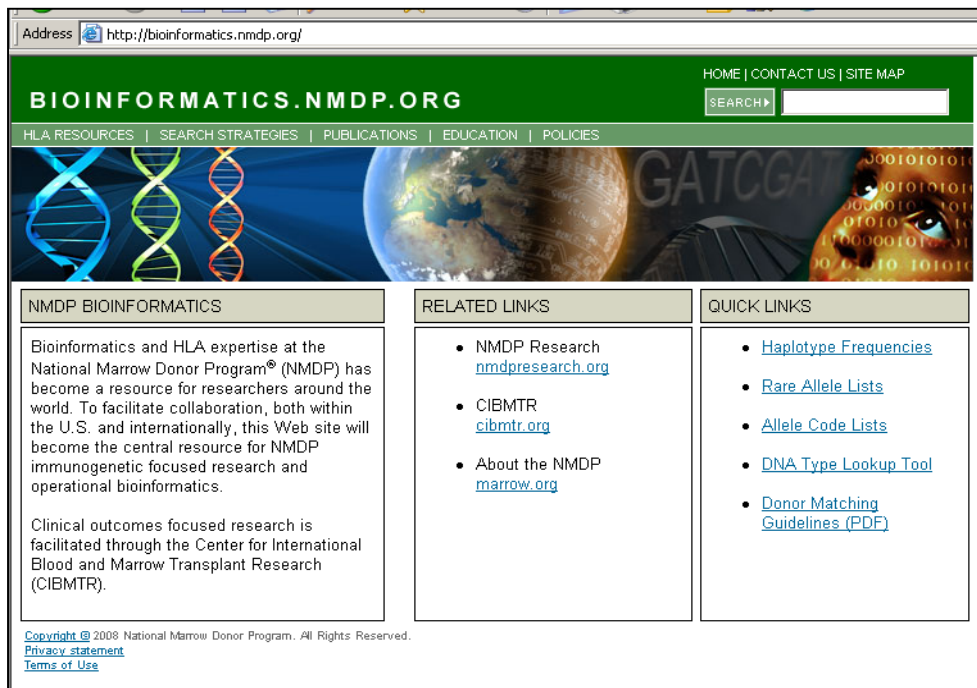
Results

Statistically significant variation of donor diversity was identified in drives from all five (5) categories (minority or patient focus, setting, type, event). This variation was not always observed when stratifying these by race. There was statistically significant variation by DC, likely influenced by geographic location. Most importantly, the effect of race was found to be more substantial than any of the other drive characteristics.

Aim B.3.5: Bioinformatics Web Site

The bioinformatics Web site (bioinformatics.nmdp.org) was developed under the period of this grant with a public release on January 31, 2006. The goals were to provide operational bioinformatics resources for researchers around the world and to serve as a location for public dissemination of NMDP generated research data. The Web site (see image next page) was developed and approved by the working group which included staff from the IT Web team, Scientific Services and Bioinformatics.

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The data from the rare allele tally were updated and added to the new Bioinformatics Web site. These data accompany the manuscript on rare alleles and the haplotypes where they are found.¹¹

Aim B.3.6: Maximize Software using Consultant Data

The expert HLA advisors were an integral part of the design and validation of HapLogic and served as beta testers for the precursory applications. During the grant period the first external version of the Search Assistance Tool (SAT) was released [v1.0]. This was a Web application that allowed viewing of a prototype of the new match algorithm (HapLogic) incorporating likelihood of DRB1 allele match into the donor listing. It also included a number of standard allele and haplotype frequency tables and displayed the information based on the patient's HLA type.

A second version of this application [v2.0] was developed for internal validation of the full HLA-A, B and DRB1 results of the new matching algorithm. This program made haplotype frequency data available on a per-patient basis and also provided a preliminary view of the new HapLogic algorithm for use by the internal HLA experts. It was later made available to external HLA consultants and continues to be enhanced as the platform for the next-generation matching algorithm. Many of the allele/haplotype frequency statistics tools are being made available on the new bioinformatics Web site as a public resource.[See Aim B.3.5]

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MultiCord Application

This SAT application was also the platform for the “MultiCord” application which was beta tested by TCs who were doing cord blood transplantation. This application identifies the degree of match between the patient and multiple CBUs where the level of resolution can be customized (allele or antigen match) and individual CBU HLA information from any worldwide registry can be added to the list. There is an option to easily include BMDW cords in the application. Any of these cords can be selected and the match between the patient and each cord, and the match between each cord will be calculated. The TNC/kg is calculated for any individual cord or any cord combination selected. Several different combinations can be created, saved and printed during any single login period to help the TC determine the best overall dose and match blend.

Search Strategy Advice (SSA) Program

The HLA consultation service was re-named as ‘Search Strategy Advice’ to more accurately reflect the information provided to the TCs. The external HLA experts and the internal donor selection team were re-named Search Strategy Advisors. The SSA program had a high level of activity and to meet the needs of the program the internal advisors became resources for the SSA service. These internal advisors had developed HLA expertise by providing support for previous search strategy programs (Ultra Urgent, Tiered Search, Preliminary Search Reviews and Formal Search Review programs) and all sat for and passed the Certified Hematopoietic Transplant Coordinator (CHTC) exam. An aggressive QC program was implemented during this period that included a well characterized process for evaluating the quality of the internal and external SSA reviews. This QC program is managed by an independent HLA expert team. The quality control results are reported quarterly to the advisors.

During this time there were 1465 SSA reviews completed which included 897 (61%) performed by external advisors and 568 (39%) by internal advisors. The required turnaround time was five (5) business days for the program, the actual was at 3.7 days (internal advisors achieved a TAT of 2.6 days).

SSA Principle Investigator Meeting

A principle investigators’ meeting was held in which all external advisors and internal advisors participated. Best practices were discussed along with future changes including: HapLogic II, revised serologic C matching, Antigen Recognition Site matching, proposed changes to Confirmatory Typing requirements, current cord blood research protocols, newly published haplotype/allele frequency data and new NMDP software (HaploStats and MatchView).

HapLogic vs. Search Strategy Advisors

A study was initiated to evaluate the quality of the predictions made by Haplogic, data will be analyzed to determine how often the selections made by a SSA (gold standard) agree with the best-matched donor identified by the algorithm. During the grant period, the SSA recommendations, Haplogic sort order and final donor selection from 118 searches were archived for future comparison. This analysis will serve as an important baseline for comparing future HapLogic enhancements and assessing improvements to the sort order. The results of this analysis will be reported during the next grant period.

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II.B. Rapid Identification of Matched Donors – Hypothesis 4:

Reducing the time and effort required to identify closely matched donors for patients in urgent need of HCT will improve access to transplantation and patient survival in the context of a contingency response and routine patient care.

Aim B.4.1: Expand Network Communications

During the grant period, the Information Technology department focused on support of the processes and tools utilized by the Search and Transplant department at the NMDP. This was accomplished by developing efficient modifications to systems, processes, and reporting capabilities, as well as ongoing enhancements of the applications. These applications and enhancements allow for increased speed and accuracy in the exchange of data transactions in the processes of identifying the best potentially matched donor. In addition, the collection of research data are essential for research studies to improve the clinical outcome for the patients.

FormsNet

Most work under this Aim focused largely on the construction of a module to support the receipt of donor IDM results from testing labs. In addition, the FormsNet 2.0 project was initiated to combine the existing legacy Registry data entry system into the Web-based FormsNet data entry application. In the area of forms harmonization, the following results were achieved:

- Completed the harmonization of the Recipient Baseline and Transplant Data Collection form.
- Continued effort to harmonize the 100-day, six-month, and annual Recipient Follow-up forms.
- Reviewed the post-transplant disease inserts. No harmonization effort was necessary, as a decision was made to use the existing CIBMTR forms.

These efforts improved efficiency by eliminating duplicate applications and QA processes, allowing for the sharing of code and resources within the applications.

EMDIS

As of October 31, 2007, 44,970 DR/HR/CT request transactions were sent and received between the NMDP EMDIS system and participating cooperative registries. The NMDP EMDIS system has been fully compliant with EMDIS Implementation Package 4 (IP4) since June 26, 2007. At the EMDIS technical committee meeting held during the WMDA conference in Bern, Switzerland, April 15-17, 2008, final plans for implementation of Implementation Package 5 (IP5) were discussed for a May 2008 deployment. Full EMDIS communication with ABMDR (Australia) has successfully commenced during this period.

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SEARCH Link and TRANS Link

The SEARCH Link V2.5.0 and TRANS Link V4.5.0 applications were upgraded on April 30, 2006. The main focus of this upgrade was to implement changes for the Cord Blood Confirmatory Typing Packages.

Significant enhancements during this grant period included:

- Added notification to search coordinator one day after collection allowing coordinator to "tag" donor with FDA Eligibility/Ineligibility status. This was done to comply with regulatory requirements.
- Added shipping information including tracking number to the CBU detail to improve communications between the TCs and the NMDP.
- Removed restrictions for requesting Cord CT packages to provide a la carte typing requests for all Cooperative Registry CBUs.
- Implemented search stage advancement for Coop CBUs to increase the growth of international cord blood activity without significantly increasing staff members in Search and Transplant Services.
- Added Donor's IDM Lab Status to the donor information report. IDM Lab Status includes: Clinical Laboratory Improvement Amendment (CLIA) Certified, Not Entirely CLIA Certified, and Unknown.
- Modified the donors' availability dates to align with the NMDP donation protocol to ensure donor safety.
- Developed the Search Coordinator Maintenance Tool (SCID), a new feature to improve operational efficiencies for reassigning EMDIS requests in Search and Transplant Services and Bioinformatics departments.
- Added "Request Date" to the Search Detail screens to improve operational efficiencies for the TCs and Search and Transplant Services department. Also added additional sort capabilities to the Search Detail screens to improve operational efficiencies in the Search and Transplant Services department.
- Added a new Qualification Status "Pre-Order Condition" to comply with FDA guidelines. This would indicate where additional testing is required by the CBB prior to transplantation. (CBU Inventory where a pooled testing method was utilized for NAT HIV/HCV testing will all be labeled with the Pre-Order Condition, until results are entered by the CBB).
- Reorganized several reports within the applications.
- Completed patches on systems and applications where required.

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STAR II

For STAR II, work has focused on the receipt of XML transactions from various parties. This was first implemented for the NMDP Web Scripts, which allow international centers to submit search-related data. Along with this enhancement, international centers now have the ability to submit Peripheral Blood Stem cell (PBSC) data electronically to the STAR system. In addition, IT:

- Converted messaging between STAR and STAR Link to XML. XML transactions will allow for greater flexibility and will help ensure that messaging between systems remains nimble as business processes evolve.
- Provided standard maintenance and upgrades to the existing architecture. Additionally, made changes to support HML, IDM, CORD Link and the Web Scripts.

SIP Database

The SIP database facilitates management of the search/transplant process through data exchange with DCs, CBBs, TCs, etc. During this grant period, a new project was started to investigate the ability to unify all databases that contain data involved in the search-tracking and invoicing process and migrate them to the SIP database. The goal was to unify various information systems under a single vision of the company's business processes. This was accomplished by modeling the corporate data in such a way that it would represent a cohesive data set, as well as eliminating unnecessary data redundancies, while also preserving efficient data access by various applications.

Aim B.4.2: Central Contingency Management

Development of Central Contingency Management (CCM) started with refinement and utilization of HLA tools to improve process efficiencies. This included the Search Assistance tools and the Repository Reporting Utility tools. Both of these provide internal search coordinators and, in part, external HLA search consultants with a centralized search management system capable of supporting and enhancing patient-donor matching processes at TCs. Due to this centralization of the data and access, these tools can be used in lieu of localized search processes at TCs if the need arises. These tools include the multi-cord selection tool, which was made available to the TC network. This program allows users to "blend" multiple CBUs together while considering degree of HLA match relative to patient and between CBUs. This program also allows for the manual addition of data from potentially matched units located on the BMDW listing. In this aspect, the application integrates best matched units from different search reports for transplants requiring multiple units.

In the first quarter, the central contingency management service was expanded from a Phase I pilot to a Phase II pilot. A cost-benefit analysis was performed and this project was modified to pilot the Phase II as fee-for-service. Additional staff were hired and trained in preparation for this expansion. The NMDP contracted with its first TC and continued to seek additional centers for this service. In following quarters, the team traveled to discuss this service with interested

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TCs to develop operational procedures customized to individual TC needs. Educational materials and presentations were developed for circulation to interested centers. In period five (5), two (2) new TCs started using the CCM service, one due to a TC coordinator leave of absence. Educational materials and presentations about the service were used at the ASHI and NMDP Council Meetings. In period six (6), two (2) additional TCs requested use of the service for select patients.

In total, 205 CCM HLA reviews were completed by the internal HLA search advisors as part of the Phase II pilot from March 01, 2006, to March 31, 2007. Each HLA search review was supplied to the TC within two (2) days of the request. This included HLA search reviews completed for patients at five (5) TCs with number of reviews per TC being 156, 45, 2, 1, and 1, respectively. The experiences and process improvements made during this time will help in expanding this service to additional TCs to help reduce the time and effort needed to identify closely matched donors for patients in need of HCT. In addition, the service builds a strong infrastructure that will be a foundation of high volume patient search management in the event of a contingency.

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II.C. Immunogenetic Studies – Hypothesis 1:

HLA mismatches may differ in their impact on transplant outcome, therefore, it is important to identify and quantify the influence of specific HLA mismatches. In contingency situations it will not be possible to delay transplant until a perfectly matched donor can be found.

Aim C.1.1: Donor Recipient Pair Project

A retrospective Donor/Recipient (D/R) Pair HLA typing project to perform HR class I (HLA-A, B and C) and class II (HLA-DRB, DQB1 and DQA1) typing of paired samples from the NMDP's Repository, was initiated in 1994. The primary objectives of the Donor/Recipient Pair Project are to:

- Determine the impact of DNA-based HLA matching on unrelated donor transplant outcome
- Develop strategies for optimal HLA matching
- Evaluate the impact of matching at alternative HLA loci on transplant outcome
- Promote the development of DNA-based HR HLA typing methodologies

Transplant pairs were chosen from stored samples at the NMDP Research Sample Repository and distributed to participating laboratories for HR HLA typing. Pairs were selected by the Center for International Blood and Marrow Transplant Research (CIBMTR) Statistical Center to ensure that the enrolled pairs support ongoing and future research needs. The cohorts tested during the project period consisted mainly of transplants that utilized PBSC as the cell source, reduced intensity or non-myeloablative preparative regimens, rare diseases and older patients reflecting the expanding indications for unrelated donor HCT. In addition, the project has added cord blood transplant pair samples to facilitate studies of HLA matching in this high growth field.

The project enrolled 1,893 transplant pairs during the project period bringing the total enrolled to over 11,000. Typing results were reported electronically to the NMDP and compared with previous TC results as a measure of quality control. During the project period data processing and report generation enhancements were performed to allow for result validation to occur within one-half hour of submission. The project laboratories also provide rapid turnaround time HR typing for the NMDP Contingency Plan. At the initiation of the project period seven laboratories participated in the project. Following a competitive bid process the laboratory network was reduced from seven to five due to decreased typing volumes and to achieve volume-based price breaks resulting in a 20% decrease in per sample typing cost, allowing enrollment of additional pairs.

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The HR HLA data generated through the project are routinely incorporated into all outcomes analyses performed by the NMDP/CIBMTR to provide the best HLA typing and matching information possible. The project has developed the largest fully validated pool of unrelated stem cell transplant donor-recipient HLA data in the world and is an unparalleled resource for transplant research. The data generated through the project have had a major impact on the evolution of the NMDP HLA matching requirements. A recent study by Lee et al.¹² utilized 3,857 donor recipient pairs typed through the project and found that HLA-A, B, C and DRB1 HR matching correlated with survival. It was also shown that the level of mismatch (antigen vs. HR) was inconsequential and that any degree of mismatch at HLA-A, B and DRB1 (HLA-C did not reach statistical significance for a HR mismatch) has an adverse effect on survival. Findings from the study have been incorporated into NMDP donor selection guidelines and driven educational efforts for Network TCs.

The project data are also used to assess genetic diversity within the NMDP transplant population and Registry. Genetic diversity analyses have focused on the evaluation of HLA haplotypes within the project data set and have generated two manuscripts and several abstracts. The analytical models developed for the project data were also incorporated into the development and enhancements of HapLogic matching algorithm. Seventy-three abstracts and forty-three manuscripts have been published using data generated from the HR Donor/Recipient Pair Project since the inception of the project. Fifteen abstracts^{5, 8, 13, 14, 18, 20-23, 25, 38,39, 41-43} and fifteen manuscripts^{1-9, 10-16} utilizing the data were supported by this grant (see Attachment B).

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II.C. Immunogenetic Studies – Hypothesis 2:

Even when patient and donor are HLA matched, GVHD occurs so other loci may play a role.

Aim C.2.1: Analysis of non-HLA Loci

Recent research has heightened interest in additional genetic polymorphisms which may modify the outcomes of transplantation. HLA genes other than the major histocompatibility complex (MHC) found on chromosome six (6) and non-HLA genetic factors may all influence the suitability and success of allogeneic stem cell transplants. The largest body of data with clear correlation to unrelated stem cell transplant outcome was surrounding the role of Natural Killer (NK) cells. These cells express inhibitory receptors (KIR) that specifically interact with MHC class I molecules. Genes encoding for these Ig-like ligands are found on chromosome 19. The regulatory mechanism mediated by these receptors is thought to protect normal cells from autologous NK attack, while rendering cells for which class I expression is compromised (e.g. by tumor transformation or viral infection) or incompatible (e.g. by stem cell transplant) susceptible to NK-mediated killing. This may be responsible for anti-leukemic effects and protection against GVHD following allogeneic HCT, particularly in two publications.^{13,14}

Based on this information, the NMDP developed a pilot study to perform KIR ligand typing utilizing selected donor and recipient pair samples. The project was launched in early 2005 with ongoing support provided through the project period. The NMDP selected three laboratories to participate in the project through a competitive bid process. The primary objectives of the study were to:

- Move technology forward from the current practice of locus level typing to HR typing
- Disseminate information and protocols in an open source mechanism
- Develop reference lines for use in individual laboratories. Additionally, the project will provide more fully characterized and highly quality controlled transplant pairs for use in research studies connecting these factors to clinical outcome data

During the period, the KIR Typing Pilot Project completed typing on 270 Caucasian donor samples for 14 KIR genes (2DL1-5, 2DS1-5, 3DL1-3 and 3DS1). Participating laboratories used a combination of molecular testing methodologies (SSOP, SSP and SBT) to obtain allele level typing results. The study has encountered a high degree of genetic polymorphism and allelic ambiguity in the KIR loci. Efforts are underway to resolve all discrepancies, analyze allelic ambiguities, characterize new alleles and analyze and assign KIR haplotypes. The preliminary results of the study were presented during an oral abstract session at the 2006 ASHI annual meeting.¹⁵ In addition, two of the participating laboratories have published the methods used in the project.^{16, 17} KIR testing data was also presented at the KIR Polymorphism Workshop. This analysis included an update on KIR gene-content haplotype estimation from a donor population,

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as well as the first-ever results of large-scale KIR allele level haplotype analysis. Results of the study will enhance the understanding of the role of the KIR loci in unrelated transplantation, characterize the diversity of the loci and optimize the development of testing systems.

Funding during the project period also supported initial development of a critical resource for the evaluation and integration of non-HLA immunobiological testing results into the NMDP research program, the IPR database. The IPR database will facilitate the capture and analysis of all HLA and non-HLA data generated through immunobiology projects that utilize the NMDP research samples. Immunobiological test results generated through NMDP/CIBMTR approved studies and reported to the NMDP are summarized in Table 2. These data will be used for testing, validation and population of the IPR database. During the project period database design, development and testing began. In addition, work began to define the requirements and technical specifications for software tools to allow interaction with the database and facilitate data analysis.

Table 2: Immunobiology typing projects utilizing NMDP samples and contributing data to the IPR database

Study Title	Investigator	Number of Samples	Genes of interest	Testing Method	Data Submitted
NK Cells, Their Receptors and Unrelated Donor Transplant	J. Miller	2300 pairs	KIR	RT-PCR, FACS, SSOP, MALDI-TOF	Yes
Survey of Diversity of Immune Response Genes in Unrelated Hematopoietic Stem Cell Transplantation	C. Hurley	40 Pairs	cytokine and KIR	SBT	Yes
Candidate Gene Study to Examine the Impact of Chemokine and Chemokine Receptor Gene Polymorphisms on the Incidence and Severity of Acute and Chronic GVHD	R. Abdi	1300 pairs	CCR5, CCR2, CX3CR1	Taqman PCR	In Process

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Study Title	Investigator	Number of Samples	Genes of interest	Testing Method	Data Submitted
Functional Significance of Killer Ig-like Receptor (KIR) Genes in HLA Matched and Mismatched Unrelated HCT	B. Dupont, K. Hsu	2000 pairs	KIR	SSP	In Process
Functional Significance of Cytokine Gene Polymorphism in Modulation Risk of Post-Transplant Complications	E. Petersdorf	2500 pairs	>30 Immune response genes	Taqman PCR	In Process
Identification of Functional SNPs in Unrelated HCT	E. Petersdorf	3500 pairs	Entire MHC region	Taqman PCR	In Process
Use of Female Donors with Pre-existing Antibody to H-Y Antigen will Result in Robust Serologic Response to H-Y Antigens in Male HSC transplantation Recipients	D. Miklos	288 pairs	H-Y Antigen	ELISA, protein array	Yes
Multiplexed Genotyping of Human Minor Histocompatibility Antigens (mHAg): Clinical Relevance of mHAg Disparity in Stem Cell Transplantation	T. Ellis	730 pairs	mHAg	Allele-specific Primer Extension	Yes
Genetic Polymorphisms in the Genes Encoding Human Interleukin-7 Receptor- α : Prognostic significance in Allogeneic Stem Cell Transplantation	K. Muller	851 pairs	IL-7	Taqman PCR	In process
The Effect of Non-Inherited Maternal Antigens in Cord Blood Transplantation	L. Baxter-Lowe	102 pairs	HLA	SBT	Yes

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Study Title	Investigator	Number of Samples	Genes of interest	Testing Method	Data Submitted
Detection of HLA Antibody in Single Antigen HLA-Mismatched Unrelated Donor Transplants	S. Arai, D. Miklos	200 pairs	Anti-body	ELISA, Protein array	Pending approval
Detection of Donor-Directed, HLA-Specific Alloantibodies in Recipients of Unrelated Stem Cell Transplantation and Their Relationship to Graft/Patient Outcome	R. Bray	111 pairs	Anti-bodies	Flow cytometry	Yes

Aim C.2.2: Related Pairs Research Repository

The research affiliation between the NMDP and the IBMTR, executed in July 2004 to form the CIBMTR, provided a previously unavailable source of statistical and scientific expertise that can help ensure that existing repository resources are fully utilized. The CIBMTR offered an unprecedented opportunity to expand the utility of the unrelated donor-recipient pair repository by including specimens from related pairs. Because of genetic identity for HLA haplotypes, related donor-recipient samples will greatly enhance the ability of researchers to conduct certain immunobiologic studies without the confounding effects of HLA disparity and will facilitate an organized approach to studying transplant biology across the full spectrum of allogeneic HCT.

During the project period, the NMDP initiated planning activities to begin collection of related pair research samples. Planning focused on identifying a network of TCs for participation in the project. The NMDP Core Contingency Transplant Center Network was determined to form an ideal group for initiation of the project. The development of sample collection and distribution procedures from related pairs through the Core Contingency Network was intended to establish a conduit for the handling of samples in support of family member HLA typing during contingency events.

However, midway through the project period, preparation for implementation of the repository with Navy grant support was placed on hold due to the receipt of the HRSA contract to operate the SCTOD that included funding to establish a related pairs repository. The results of the initial planning activity formed the basis for carrying the project forward under the HRSA contract. There was no overlap in components funded by HRSA and those funded by ONR.

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Navy funds were reallocated to other programs following the HRSA award with the exception of support for repository software enhancements. Requirements and analysis were completed on a set of software tools for managing the research repository database including sample selection, shipping requests and statistical reports. In addition, the modifications to repository inventory software and database required to facilitate the receipt, processing, storage and retrieval of the related samples was initiated. Related pair collection was scheduled to begin shortly after the end of the project period.

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II.D. Clinical Research in Transplantation – Hypothesis 1:

Clinical research in transplantation improves transplant outcomes and supports preparedness for a contingency response.

Aim D.1.1: Observational Research, Clinical Trials and NIH Transplant Center

During this grant, the NMDP moved forward its Prospective Research program. Within the CIBMTR affiliation, the NMDP established the RCI BMT. The goal of this program was an avenue for investigators to obtain statistical and data management support for prospective trials focusing on addressing various transplant issues. The following key elements were completed:

- Assembled a Clinical Trials Advisory Committee to provide scientific review and recommendations on clinical trial proposals. First meeting of this committee was February 2006.
- Developed a process for receiving, reviewing and approving proposals,
- Hired additional staff to ensure support.
- Established a Data Safety Monitoring Board for all trials facilitated through this program.
- Created a Manual of Operations for the program.
- Received a total of nine (9) proposals since the program was established.
- Initiated two trials with all appropriate approvals:
 - Unrelated allogeneic transplant for Renal Cell Carcinoma
 - Adult Double Cord in patients with hematologic malignancies
- Approved two trials for development:
 - A study to assess safety of donation in related donors
 - Lenalidomide after allogeneic HCT for Myeloma
- Selected a vendor for trial management system.
- Designed RCI BMT page on CIBMTR.org Web site.
- Produced RCI BMT brochure for distribution at scientific meetings (e.g., ASHI).

Staff continued to provide support to the BMT CTN PBSC vs Marrow trial. This support included managing the donor component of the study but also assisting the BMT CTN in the area of accrual initiatives on the recipient portion of the study. Activities included were:

- Facilitated multiple DC Coordinator meetings for training and information sharing.
- Developed training tools for DC Coordinators to assist them in the consenting process.

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- Created an email Snap Shot which is sent out monthly to participating sites. Provides accrual and news at a glance
- Facilitated the incorporation of Canadian Transplant Centers and DC onto the trial

In an effort to improve efficiency of studies going from proposal to publication, a training program was developed. This training consisted of 4 weeks over a 6 months period of hands-on activities. All new and current biostatisticians completed this training. In 2006, the CIBMTR published 20 peer reviewed manuscripts. In 2007, a total of 29 have been published with an additional 12 in press.

In collaboration with researchers at the University of Minnesota, a proposal was developed and accepted for a GCSF Genetics study. This study is designed to examine the potential epigenetic and genetic alterations of lymphocytes of normal donors of filgrastim mobilized PBSCs. During this grant the proposal and budget were established and approved.

The National Institutes of Health (NIH) was accepted as an NMDP transplant center in 2007. Prior to that time, the NIH, representing our Nation's premier medical research endeavor, was not applying their considerable problem-solving skills to issues surrounding unrelated donor transplantation. The NMDP, with ONR support, set out to remedy that deficiency by entering into collaboration with NIH. During the grant period, NIH developed five (5) research protocols with areas of research interest that included PBSC transplants for hematologic malignancies and immunodeficiencies and tandem cord transplants for severe combined and aplastic anemia. The NIH began enrolling patients during the grant period and has performed eleven unrelated transplants since the inception of the program. Navy funds allow NMDP to provide support for donor identification, selection and collection for the NIH intramural unrelated donor transplant program.

Aim D.1.2: Research with NMDP Donors

An infrastructure was created to support donor research for donor studies proposed by investigators outside the NMDP, the Office of Investigator Sponsored Research (OISR). The purpose of the OISR is to provide outside investigators with access to NMDP donors and resources in order to conduct studies that involve NMDP donors. During this grant period the OISR infrastructure was developed and the first study was implemented.

The first study implemented through the OISR was from Dr. Galen Switzer to examine the impact race and culture have on a donor's decision to proceed through the confirmatory testing and donation process. Dr. Switzer has a five year NIH grant through the University of Pittsburgh to conduct the study. The study was opened for enrollment in April 2007. A computer program was created by NMDP staff to identify donors who are eligible for the study. NMDP staff contact the eligible donors, give them a brief synopsis of the study and ask them if Dr. Switzer's study team at the University of Pittsburgh may contact them about joining the study.

Additional studies are now being developed through the OISR.

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Aim D.1.3: Expand Immunobiology Research

During the grant period, funds were used to support the research efforts of the CIBMTR IBWC and NMDP Scientific Services department. Research focused on the IBWC portfolio of studies and the Scientific Services department efforts to advance models for registry composition analyses, haplotype frequencies, predictive algorithms and automated donor selection algorithms.

To further stimulate completion of immunobiology studies within the CIBMTR, grant funds were used to provide monetary support to investigators whose studies require modest funding for completion. During the grant period the NMDP developed the Immunobiology Research grant request and award procedures for use by the IBWC and developed the IBWC Web site to post the information,

(http://www.cibmtr.org/COMMITTEES/Working_Committees/Immunobiology/index.html).

The Web site was the first for a CIBMTR working committee and includes up to date information on the IBWC mission statement, data tables, study list, annual meeting agendas and minutes, proposal submission guidelines and the Immunobiology Research grant application.

The IBWC awarded the following Immunobiology Research grants during the grant period:

- Support for a study investigating the role of chemokine and chemokine receptor polymorphisms in graft versus host disease.
- Support for HLA typing of maternal samples to evaluate the impact of non-inherited maternal alleles on cord blood transplant outcomes.
- Support for automated DNA extraction of 2,500 samples to facilitate MHC region single nucleotide polymorphism testing.

Grant funds also supported significant outreach efforts by the IBWC leadership to increase collaboration between the IBWC and basic scientists. The IBWC leadership developed a brochure and informational materials for distribution at basic science meetings and had a presence at the annual Federation of Clinical Immunology Societies (FOCIS), European Federation for Immunogenetics (EFI), and the Australasian and South East Asian Tissue Typing Association (ASEATTA) meetings. The IBWC continued work on the 33 active studies in the committee, accepted three (3) new proposals for analysis and submitted five (5) studies for publication^{1, 3, 4, 9, 15} (see Attachment B).

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Attachment B – Published Manuscripts and Abstracts
Supported with funding from this Grant

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