Be The Match Donors and Immunogenetic Testing at Time of Recruitment
Council Meeting 2016

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Disclosures

The following faculty and planning committee staff have no financial disclosures:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Miranda Bauer</td>
<td>Be The Match</td>
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</tbody>
</table>
Learning Objectives

At the conclusion of this session, attendees will be able to:
• Describe NMDP’s current immunogenetic testing strategy at the time of recruitment
• Explain how this typing strategy affects patient searches and donor selection
• Define the difference between genetic ABO/RhD at recruitment and serological ABO/RhD at subsequent search stages

Road Map

1. How Does NMDP Immunogenetic Testing Set a Donor Up at Recruitment For Patients in Need?
2. HLA Typing History to Present
3. Difficulties With HLA Typing
4. Demystifying Ambiguous Pairs
5. Resolving Null Alleles
6. Future Directions of NGS for HSCT
7. Determining Blood Type: Comparing Molecular ABO/RhD Typing at Recruitment vs Serologic ABO/RhD
8. CCR5 Typing at Recruitment
Critical Genetic Vocabulary

- **Gene** = locus - encodes a protein
- **Allele** - encodes alternate forms of a gene (2 alleles per locus)
- **Exon** - DNA sequence that encodes a protein
- **Intron** - DNA sequence that does not encode a protein

Gene Diagram

Chapter 1: How Does NMDP Immunogenetic Testing Set a Donor Up at Recruitment For Patients in Need?
Current Unrelated Donor Selection Practices

Gold standard: 8/8 or 10/10 allele matched (HLA-A, B, C, DRB1, DQB1)

Other Considerations:
- Age/sex
- DPB1
- CMV status
- ABO/Rh
- KIR
- PBSC or BM
- More...

Current NMDP Donor Recruitment Package

- 6 locus (12 allele) HLA typing + DRB3/4/5 via NGS
  - Whole gene HLA-A, B, C
  - Long range DRB1, DQB1, DPB1, DRB3/4/5
  - Resolution to the 3rd and 4th fields
- Molecular ABO/RhD
- CCR5 Δ32 genotyping
NGS Typed Donors in Traxis

The Path to Testing (and Beyond)

- **Recruiter/volunteer/donor center reviews consent and mails consent and swab to the BioRepository**
- **Lab returns results of customized typing**
- **Donor Center receives request**
- **Lab collects donor CT/IDM samples**
- **Donor Center schedules a CT draw appointment**
- **Transplant Center tests CT sample**
- **Donor joins the registry via DIY or live drive**
- **Sample is sent to lab**
- **The Biorepository receives sample and consent**
- **Transplant Center requests customized typing**
- **Donor Center contacts and educates donor**
- **KitMaker ships IDM/CT kit**
- **Transplant Center makes donor selection**
- **IDM lab tests IDM sample**
Before A Donor Sample Arrives at Lab

- **Receive**
  - NMDP Biorepository receives samples

- **Inspect & Store**
  - Inspect samples for labeling and condition
  - 2 swabs stored for shipment
  - 2 swabs frozen for long term storage

- **Pull & Ship**
  - Identify and ship samples to labs for DNA testing

Where Does Testing Occur in the Path?

- **Registration**
  - Recruiter/volunteer/donor center reviews consent and mails consent and swab to the Biorepository
  - Typing results received

- **Sample is sent to lab**
  - Lab returns results of customized typing

- **Donor joins the registry via DIY or live drive**
  - The Biorepository receives sample and consent
Chapter 2: HLA Typing History to Present

Serology: A Broad Sweep of HLA

- At the start of HLA determination, serology was all that was available
- Antibodies used to assign HLA

Serology Pyramid

**Serologic Antigens**
- A9
- A23
- A24

**Alleles**
- A*23:01
- A*23:02
- A*24:02:01:01
- A*24:03:01

SSOP: Alphabet Soup of HLA Testing

- The Sequence Specific Oligonucleotide Probe (SSOP) method is a “fishing expedition” for known HLA sequences
  - Looking for what’s already there
  - Numerous probes used to identify and assign HLA
DNA Sequencing: Getting to the Point

- **Sequenced Based Typing**
  - Sanger sequencing thought of as the “Gold Standard” of SBT
  - Read length average: 700-900 bp
  - Sequence the gene

- **NGS**
  - Takes gold standard one step higher
  - Longer sequence reads possible
  - Sequence each allele

---

**Sanger Sequencing**

```
TGGATTGGTCCATGGTGTTGATCAGTGTTTTTGCCT
GAGATTCGTCATGGTGATCAGTGTTTTTGCCT
```

**NGS Sequencing**

```
TGGATTGGTCCATGGTGTTGATCAGTGTTTTTGCCT
```

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[Link: http://histogenetics.com/research/advances-in-dna-sequencing-technologies-for-high-resolution-hla-typing]

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**NGS Typing Accuracy vs Sanger**

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<tr>
<th>LOCUS</th>
<th>NGS</th>
<th>SANGER</th>
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<tbody>
<tr>
<td>HLA-A</td>
<td>99.94</td>
<td>75.6</td>
</tr>
<tr>
<td>HLA-B</td>
<td>99.75</td>
<td>80</td>
</tr>
<tr>
<td>HLA-C</td>
<td>97.75</td>
<td>60</td>
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<tr>
<td>HLA-DRB1</td>
<td>100.00</td>
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<td>96.1</td>
</tr>
<tr>
<td>HLA-DPB1</td>
<td>100.00</td>
<td>98</td>
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**Gold Standard**
### History of HLA Typing as Seen By Traxis

#### SBT/NGS
- DNA-low resolution
- Serology

#### SBT or Full Gene/Long Range NGS
<table>
<thead>
<tr>
<th>Date</th>
<th>Patient</th>
<th>HLA Typing</th>
<th>SBT/NGS</th>
<th>Serology</th>
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<tbody>
<tr>
<td>10/10</td>
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<td>A</td>
<td>A</td>
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#### SSOP
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<tr>
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<td>10/10</td>
<td>P</td>
<td>P</td>
<td>P</td>
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<tr>
<td>10/10</td>
<td>10/10</td>
<td>P</td>
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#### Low-res DNA
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<tbody>
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<td>10/10</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<tr>
<td>10/10</td>
<td>10/10</td>
<td>A</td>
<td>A</td>
<td>A</td>
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#### Serology
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<th>Date</th>
<th>Patient</th>
<th>HLA Typing</th>
<th>SBT/NGS</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/10</td>
<td>10/10</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>10/10</td>
<td>10/10</td>
<td>A</td>
<td>A</td>
<td>A</td>
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</tbody>
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**THE MATCH**

**COUNCIL MEETING: Sharing Our Passion For Life**
NMDP’s Goal With Adoption of NGS

- Meet current and future HLA matching requirements of transplant centers and their patients to enable the best possible outcomes
- Reduce time to transplant by providing the highest resolution donor typing available
- Anticipate future matching algorithms
- Reduce cost by leveraging high throughput efficiencies
- Provide best-in-class registry

Evolution of HLA Genes Tested for HSCT

HLA-A, B and DRB1 = Original Transplantation Antigens
HLA-C also affects transplant outcome

![Diagram showing HLA Complex and genes affecting acute GVHD](image)

- DRB1 and DQB1 are closely linked
- B-C are closely linked
ARS: The Target For HLA Locus Screening

- Antigen recognition site (ARS) encodes for the peptide binding groove in the HLA molecule
- Aids in self vs non-self discrimination
- Presents antigens to T cells
- Results in immunological response

Goal is to minimize mismatches in this region when selecting a donor for HSCT transplant

A Deeper Dive Into the ARS

Class I: HLA-A
- ≈1 kb
- ≈20% of total gene

Whole gene length: 4.6-5.5 kb
Total exons: 8

Class I: HLA-DRB1
- ≈300 bp
- ≈2% of total gene

Whole gene length: 10.8-13.7 kb
Total exons: 5-6
Summary of HLA Typing History

- Several HLA screening methodologies have been utilized over the years, each increasing in specificity in an effort to improve HSCT outcomes
- The number of HLA loci screened has also increased with the intent of improving HSCT outcomes
- Historically, the ARS region of the HLA molecule was primarily targeted for sequencing
  - It is responsible for triggering an immune response to “non-self”
  - Patient/donor HLA matching focuses on this region
Chapter 3: Why is HLA Typing Problematic and How Does NMDP’s NGS Typing Strategy Address These Issues?

Growth of Described HLA Alleles

• The number of named HLA alleles has grown since 1987 (linearly starting in 2011)

• This growth could continue as NGS typing methodologies reveal more comprehensive sequence information
HLA Allele Growth by Publications

**Abstract**

Nucleotide sequence analysis of the HLA-C alleles of the GB52 cell line, a two new allele variants: Cw*1801 and Cw*0706. The former allele, Cw*1801, sharing sequence motifs with Cw*0702.1 and 2, and with Cw*46. Both alleles were recognized by some cell lines. Cw*0706 shows a primary structure; carriers have new sequence motifs at the 3'-end. Preliminary data indicate that Cw*0706 could account for a part of the Cw7, B44 haplotypes observed in Africans.

**IMGT Ambiguous Allele Combinations**

| B*35:01:01G + B*49:01:01G | B*35:11:01 + B*49:11 | B*35:280 + B*49:10 | B*50:01:01G + B*53:01:01G |


**HLA Typing Difficulties Result From:**

- Polymorphic genes
- Ambiguous alleles
  - Heterozygous combinations can have similar sequences/hybridization

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
</tr>
</thead>
<tbody>
<tr>
<td># of alleles</td>
<td>3,492</td>
<td>4,358</td>
<td>3,111</td>
<td>1,929</td>
</tr>
<tr>
<td># of nulls</td>
<td>158</td>
<td>137</td>
<td>115</td>
<td>48</td>
</tr>
</tbody>
</table>
More On HLA Difficulties-Null Alleles

- Null alleles often resolved within sequence outside of ARS

<table>
<thead>
<tr>
<th>Null Allele</th>
<th>HLA G Group</th>
<th>Location of Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*01:04N</td>
<td>A*01:01:01G</td>
<td>Exon 4</td>
</tr>
<tr>
<td>A*03:21N</td>
<td>A*03:01:01G</td>
<td>Exon 4</td>
</tr>
<tr>
<td>A*24:09N</td>
<td>A*24:02:01G</td>
<td>Exon 4</td>
</tr>
<tr>
<td>A*24:11N</td>
<td>A*24:02:01G</td>
<td>Exon 4</td>
</tr>
<tr>
<td>A*68:11N</td>
<td>A*68:01:02G</td>
<td>Exon 1</td>
</tr>
<tr>
<td>B*15:01:02N</td>
<td>B*15:01:01G</td>
<td>Intron 1</td>
</tr>
<tr>
<td>B*51:11N</td>
<td>B*51:01:01G</td>
<td>Exon 4</td>
</tr>
<tr>
<td>C*04:09N</td>
<td>C*04:01:01G</td>
<td>Exon 7</td>
</tr>
</tbody>
</table>

NGS Benefits: Refining the Process

- Phase information
  - Separate each chromosome/allele
  - Resolve ambiguous pairs
  - Rapid resolution of new alleles

- Longer sequence reads
  - Identify null alleles without additional typing
  - More information across genes
  - Higher resolution

Added bonus
- High throughput and cost efficient
NGS Recruitment Typing vs CT Typing

- Donor typing after CT may be lower resolution than NGS recruitment typing

### Recruitment Typing

<table>
<thead>
<tr>
<th>Typing Date</th>
<th>Reporting Date</th>
<th>Method</th>
<th>Ctr</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DB1</th>
<th>DOB1</th>
<th>DRB1</th>
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<tbody>
<tr>
<td>Jul 27 2016</td>
<td>Jul 27 2016</td>
<td>LAB</td>
<td>744</td>
<td>01:01</td>
<td>06:01</td>
<td>07:01</td>
<td>03:01</td>
<td>02:01</td>
<td>01:01</td>
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</table>

### CT Typing

<table>
<thead>
<tr>
<th>Typing Date</th>
<th>Reporting Date</th>
<th>Method</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DB1</th>
<th>DOB1</th>
<th>DRB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep 09 2016</td>
<td>Sep 19 2016</td>
<td>22</td>
<td>*01:AMUBK</td>
<td>*06:AM2U</td>
<td>*07:AM2W</td>
<td>*03:AMCN</td>
<td>*02:AM2B</td>
<td>*02:AM2K</td>
</tr>
</tbody>
</table>

Recruitment Donor Typing: Introducing MiSeq

- Low error rate
- Can do full gene testing but lose phasing
- Shorter reads
  - 250-300bp
- Minimum 4 hour run time
- Used as a back-up methodology or in parallel to ensure accuracy
Recruitment Donor Typing: Introducing PacBio

- **Primary strategy** for NMDP recruitment typing
- Longest, continuous reads in phase
  - 3-6kb on average
- Ability to accurately perform whole gene sequencing for HLA Class I genes in one read
- Short run time of .5-6hrs

Summary of HLA Typing Difficulties

- HLA typing poses many difficulties
  - A plethora of different HLA alleles have been described
  - SSOP/SBT strategies may have difficulty resolving ambiguities in heterozygous patients when they occur
  - Additional typing is generally needed to resolve null alleles

- NGS can alleviate these typing difficulties
  - Each allele is sequenced “in phase”
  - Long sequence reads resolve null alleles and provide 3rd and 4th field resolution

- Because of the resolution achieved using NGS, donor typing may appear as high resolution codes after CT compared to the allele level typing initially seen on the search
Chapter 4: Demystifying Ambiguous Pairs

SBT and SSOP May Show Ambiguous Pairs

- Mixed bases
  - Which allele goes where?
- SSOP/SBT may not be able to determine
  - No separation of alleles
**Ambiguous Pairs and Common Alleles**

- Patient typing submitted for a donor search
- B typing revealed
  
  - 35:ANZDP = B*35:01/04/10/20/28/34/42
  
  - 40:ANZDR = B*40:01/07/25/38/52/106

- Problem if patients carry two different alleles at one locus where multiple common/well documented (CWD) alleles are present in the code(s)

<table>
<thead>
<tr>
<th>Status</th>
<th>Phenotype</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRML</td>
<td></td>
<td>03:AMTF</td>
<td>35:ANZDP</td>
<td>03:HUYYK</td>
<td>08:01</td>
<td>04:02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32:CJT</td>
<td>40:ANZDR</td>
<td>04:XHDF</td>
<td>13:02</td>
<td>06:04</td>
</tr>
</tbody>
</table>

**Ambiguous Pairs May Interfere With Matching**

- CWD alleles are defined as “alleles for which the frequencies are well known or which have been identified multiple times through the use of sequence-based typing methods”
- Patient typing via SBT revealed the following possibilities
- Is additional resolution required?

- **CWD + CWD**
  - B*35:01:01G+B*40:01:01G

- **CWD + Non-CWD**
  - B*35:04:01+B*40:52

- **CWD + CWD**
  - B*35:10+B*40:25

- **CWD + CWD**
  - B*35:20:01+B*40:07

- **Non-CWD + Non-CWD**
  - B*35:28+B*40:106

- **CWD + Non-CWD**
  - B*35:34+B*40:38

- CWD pairs still need to be resolved
Case Study: Ambiguous Pairs and Donor Selection

- Patient typing submitted as A*02:AKBTP, A*03:AKBTR
- Multiple SBT/SSOP Methods showed the following HLA-A options:
  - One kit showed stronger A*02:01 but did not rule out A*02:30
  - One kit did not differentiate between A*02:01 or A*02:30
  - One kit showed strong A*02:30

  **CWD + CWD**
  A*02:01+A*03:01

  **CWD + CWD**
  A*02:30+A*03:01

- Further testing definitively revealed A*02:30 as the patient’s A*02 allele

Ambiguous Alleles Affect Patient Searches

- Patient code contains A*02:01 and A*02:30 (both CWD)
- Further resolution with SSOP/SSP kits problematic due to A*03
Patient Search with Ambiguous Alleles (cont.)

- Matching predictions favor the more common A*02:01 allele
- Some donors also have ambiguous typing
Patient Search with Ambiguous Alleles (cont.)

- Other donors are defined A*02:30

<table>
<thead>
<tr>
<th>10/10=5</th>
<th>9/10=99</th>
<th>8/8=5</th>
<th>7/8=99</th>
<th>6/8=99</th>
</tr>
</thead>
</table>

NGS Resolves Ambiguous Combinations

- Each allele independently amplified “in phase”
- Long Range Sequencing
  - Completely phased alleles
  - Clear base pair assignment
Ambiguous Alleles Summary

- SSOP/SBT methods may have difficulties resolving ambiguous combinations when a patient is heterozygous at a locus.

- If more than one CWD combination is present in donor or patient allele codes, additional resolution is required to ensure an appropriate match is identified.

- NGS resolves ambiguous combinations due to the phasing of alleles during the sequencing process and the long range methodology employed.

Chapter 5: Resolving Null Alleles
What is a Null Allele?

- It means the HLA protein is not expressed
- Misidentifying a null allele significantly impacts transplant outcome
  - Considered a mismatch to its expressed counterpart
- May be more commonly associated with specific haplotypes

A*23:01-B*44:03-C*04:09N-DRB1*07:01-DQB1*02:02

Case Study: Patient Search With Null Allele

- Null alleles affect patient searches.
Patient Search With Null Allele (cont.)

• Patient carries well-documented allele, A*24:09N

<table>
<thead>
<tr>
<th>Status</th>
<th>Phenotype</th>
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<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
</tr>
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<tbody>
<tr>
<td>PRLM</td>
<td>Pheno 1</td>
<td>24:09N</td>
<td>07:TDVB</td>
<td>03:04</td>
<td>04:04</td>
<td>03:02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25:01</td>
<td>40:01</td>
<td>07:02</td>
<td>15:01</td>
<td>06:02</td>
</tr>
</tbody>
</table>

Case Study: Patient Search With Null Allele

• 24:09N is in the A*24:02:01G group
  – Mutation located in exon 4
  – May not have been resolved at the time of donor recruitment
Case Study: Patient Search With Null Allele

- Donor #1 on this patient’s search is a known match
  - Full gene NGS testing at the A locus means no additional screening is needed to identify the null allele

<table>
<thead>
<tr>
<th>Pr(α) of 10 (%)</th>
<th>Pr(α) of 8 (%)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
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<td>8/8=99</td>
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<td>A99</td>
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<td>A99</td>
<td>A99</td>
<td>24:09N</td>
<td>07:1DVB</td>
<td>03:04</td>
<td>04:04</td>
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<tr>
<td>9/10=99</td>
<td>7/8=99</td>
<td>A99</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>25:01</td>
<td>40:01</td>
<td>07:02</td>
<td>15:01</td>
<td>06:02</td>
</tr>
</tbody>
</table>

Resolving Null Alleles Summary

- NGS will resolve null alleles at recruitment due to the full gene/long range sequencing strategy
- Regions outside of the ARS site will be resolved at recruitment before donors ever show up on a patient search
Chapter 6: Beyond HLA Matching: Future Directions of NGS Sequencing for HSCT

HLA Haplotype Matching May Decrease GvHD in Fully Matched Donors

- A haplotype is a DNA sequence inherited together on a particular chromosome
- NGS can currently collects this information at the whole gene level
- With longer reads, NGS could report haplotype data across multiple genes
- This information is currently NOT displayed in Traxis

Mapping MHC haplotype effects in unrelated donor hematopoietic cell transplantation

Effie W. Petersdorf,1 Mari Malkki,1 Mary M. Horowitz,2 Stephen R. Spellman,3 Michael D. Haagenson,3 and Tao Wang4

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**Haplotype Matching Outcomes Charts**

**Grade III-IV GvHD**

**Recurrent Malignancy**

**TRM**

**Overall Survival**

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**Study Results Suggest Affect on GvHD Risk**

Lower GvHD risk for haplotype matched transplants

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Haplotype Matching in Mismatched Pairs

- Recipient/donor pairs mismatched at 1 HLA locus
- Identified 12 SNPs/variations associated with HSCT outcome
- Could influence future search strategy but more studies needed

![Diagram showing HLA loci and SNPs](image.png)

Figure 2. Twelve SNPs of clinical significance in HLA-mismatched unrelated donor transplantation. Each of the 12 SNPs having an association with grades II-IV or III-IV acute GVHD, chronic GVHD, relapse, transplant-related mortality, disease-free survival, or survival are shown on a map of the MHC on chromosome 6p21.3 (not to scale). SNPs are identified by their rs numbers. Chromosome 6 drawing modified from the National Library of Medicine, the National Center for Biotechnology Information public website.\(^2^)\n

HLA Gene Expression Level May Also Impact HSCT Outcome

- Gene expression is affected by variations in the intron or UTR
  - Data in some of these regions are currently captured by NGS
  - May be valuable for cases where a mismatched donor is the best option
  - Current studies report effect of DPB1 and C expression on HSCT transplant outcomes
  - More studies needed to determine search strategy influence

Class I: HLA-C

![Diagram of HLA-C gene expression](image.png)

5'UTR --- 50bp 150bp 200bp 50bp 100bp 300bp --- 3'UTR
DPB1 Expression May Affect HSCT Outcomes

- Mutation found in the 3’ untranslated region of DPB1 affects allele expression level
  - High and low expression variants described
- Patients carrying a DPB1 allele linked with the low expression variant may experience increased aGvHD if donor is mismatched at DPB1, where allele linked with the high expression variant

HLA-C Expression Data May Also Affect Mismatched Donor Transplant Outcomes

For mismatched donor transplants

- C mismatches between low expression alleles (i.e. C*03:03/03:04) may result in a better outcome than mismatches between high expression alleles
- Positive impact of low expression mismatches on non-relapse mortality per this study
Future Matching and NGS Summary

- Various research groups have begun looking at HLA factors that may influence HSCT transplant outcomes beyond those currently utilized for matching strategies.
- NGS currently captures sequence information within some of these published regions and is poised to anticipate future matching strategies that arise from the reported data.

Chapter 7: Determining Blood Type: Comparing Molecular Typing of ABO/RhD at Recruitment vs Serologic ABO/RhD
Why Consider ABO in HSCT?

Mismatch may lead to a variety of post-transplant complications

- Red cell hemolysis
- Delayed red cell engraftment
- Pure red cell aplasia

Some studies show an effect on

- Non-relapse mortality
- Overall survival
- GvHD


Outcomes Data for HSCT Show Inconsistent Associations with Overall Survival and GvHD

<table>
<thead>
<tr>
<th>Study Authors</th>
<th>Year</th>
<th>Survival after ABO-Incompatible HCT Transplantation</th>
<th>Risk of Graft-versus-Host Disease</th>
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<tr>
<td></td>
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<td>Kimura et al. [3]</td>
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<td>Helming et al. [13]</td>
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<td>Erler et al. [15]</td>
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<td>Kim JG et al. [12]</td>
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<td>Stussi et al. [14]</td>
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<td>Benjamin et al. [18]</td>
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<td>Baciagalupo et al. [19]</td>
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<td>Benninger et al. [41]</td>
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<td>Buckner et al. [17]</td>
<td>1978</td>
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RR indicates relative risk.

- Pediatric patients.
- Only in patients being treated for acute myeloid leukemia or myelodysplastic syndrome. A difference was not observed in a larger subset of patients who were treated for chronic myelogenous leukemia.

Gold Standard: Serologic ABO/RhD Testing

- Agglutination tests subject’s ABO antibodies with serum
  - Need a blood sample
- Confirming ABO using serology is still needed at CT or at the time of IDMs
- Standardized, FDA approved screening method

<table>
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<th>Blood sample</th>
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<th>Anti-D</th>
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</tr>
<tr>
<td>AB+</td>
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</tr>
<tr>
<td>O-</td>
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</table>

Serology and Buccal Swabs Don’t Mix

- High throughput NGS typing of ABO/RhD genes is possible on saliva and buccal swab samples
- Cannot perform serology on this type of sample
Is Molecular Typing Concordant?

- ABO/RhD is currently being screened on NMDP donors at recruitment using DNA sequencing
- Targeted exon sequence data obtained for both ABO gene (glycosyltransferase) and RhD gene
- In preliminary tests, 1376 samples of diverse ethnic origin on Illumina MiSeq to determine accuracy

Agreement with serology
- 12 samples corrected ABO from serology
- 31 samples corrected RhD from serology

What’s in a Blood Type, Genetically?

- More complex than first meets the eye
- The ABO gene encodes for an enzyme that adds a sugar residue to an antigen on a red blood cell
- Different from HLA, which is an expressed protein
- The RhD gene encodes for the RhD protein expressed on RBCs
- ABO and RhD are highly immunogenic
More ABO From a Genetics Perspective

- Complex and not fully understood
- Extensive heterogeneity in ABO alleles, subgroups and noncoding regions
- Variation in exons 6 and 7 important in determining blood type
- Limited studies describe mutations outside of exons 6 and 7 that may affect ABO determination
- Some ABO subgroups may be missed by serologic methods

RhD From a Genetics Perspective

- Also highly complex
- Part of a larger Rh blood group system
- Presence or absence detected through testing
- Many genetic variants resulting from deletions, mutations and recombination events
- Known variations in expression level (weak D)
NMDP ABO/RhD Testing at Recruitment

- DNA sequencing at key genetic regions of the glycosyltransferase and RhD antigen genes, which are known to affect the donor’s ABO/RhD phenotype (observed blood type)


Serologic Method Still Required to Confirm HSCT Donor’s ABO/RhD Phenotype

- For determining transplant compatibility and donor/recipient transfusion, molecular ABO/RhD testing is **not** a substitute for serologic testing
  - DNA sequencing is predictive of actual phenotype
  - At this time, it is acceptable for screening donors at recruitment **only**

- Current testing standards still apply when a donor’s ABO/RhD has been determined genetically

- An FDA approved serologic method must be used on two independent samples to confirm ABO/RhD at CT and subsequent search stages
How Does Molecular ABO/RhD Data Look in Traxis?

- On the Potential Donor List in Traxis, it is identical to ABO/RhD data captured by serology at the CT stage

<table>
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<th>B</th>
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</table>

How Are Clinical Serologic ABO/RhD Results Displayed in Traxis?

- If the donor has been previously requested for CT, ABO/RhD may have been determined/confirmed by serology
- Click on donor ID to find IDM tab
ABO/RhD Typing Summary

- DNA screening gives us blood type information that is predictive of actual phenotype
- Serologic ABO/RhD determination is still required at subsequent search stages to determine patient/donor compatibility for HSCT
- Using NGS, we can find and document genetic information within the ABO/RhD genes that no one else is investigating yet
- Future research may offer new ways of looking at this marker

Chapter 8: CCR 5 Δ 32 Mutation and HIV Resistance

- CCR5 is a cytokine receptor expressed on WBCs
- CCR5 Δ 32 mutation is associated with HIV resistance
- Homozygous mutation needed to confer resistant
  - Occurs in about 1% of populations of European descent
CCR5Δ32 Screening At Recruitment

• Strategy is to determine presence or absence of mutation in donors at recruitment
  – If present, differentiate between homozygous vs heterozygous donors
• Contact NMDP Case Manager to determine if a donor of interest has been screened

In Summary

• NMDP now offers full gene HLA Class I and long range (exons 2 through 4) HLA Class II typing on donors at recruitment using Next Generation Sequencing technology
• NGS technology results in resolution of ambiguous pairs and identification of new and null alleles at the time of recruitment
• Donor’s ABO/RhD genes are screened at recruitment using molecular typing methods and this information is displayed in Traxis
• Serologic methods are still required to confirm donor’s ABO/RhD phenotype
• Donors are being screened for the CCR5 Δ32 mutation at recruitment
• NGS provides exciting opportunities for advancement in the world of HSCT matching and research