COUNCIL MEETING
Sharing Our Passion for Life

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:

Name	Institution
Miranda Bauer	Be The Match

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

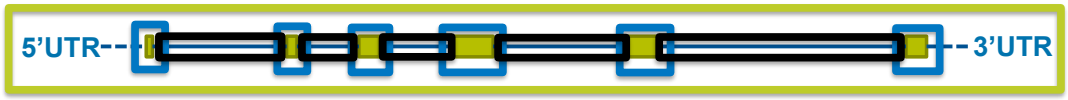
Road Map

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

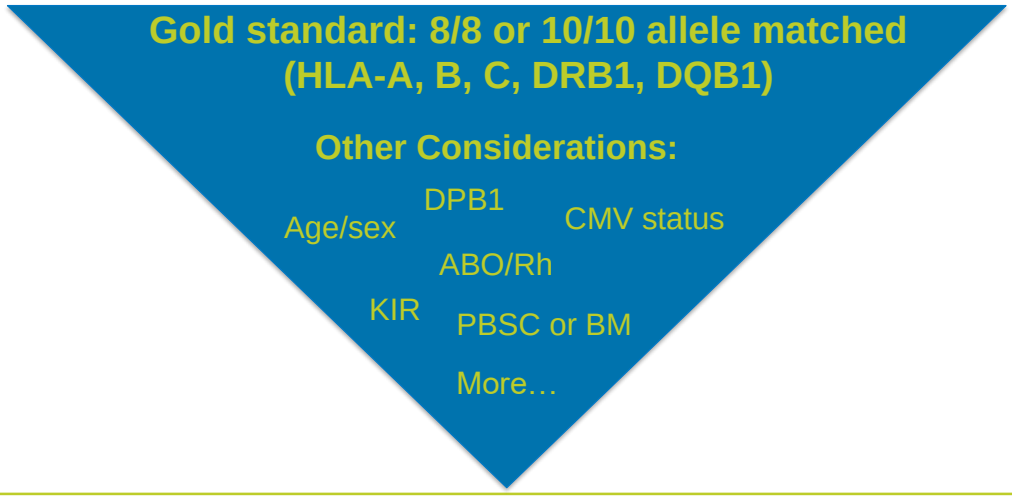
- **Gene**=**locus**-encodes a protein
- **Allele**-encodes alternate forms of a gene (2 alleles per locus)
- **Exon**-DNA sequence that encodes a protein
- **Intron**-DNA sequence that does not encode a protein

Gene Diagram



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

Current Unrelated Donor Selection Practices

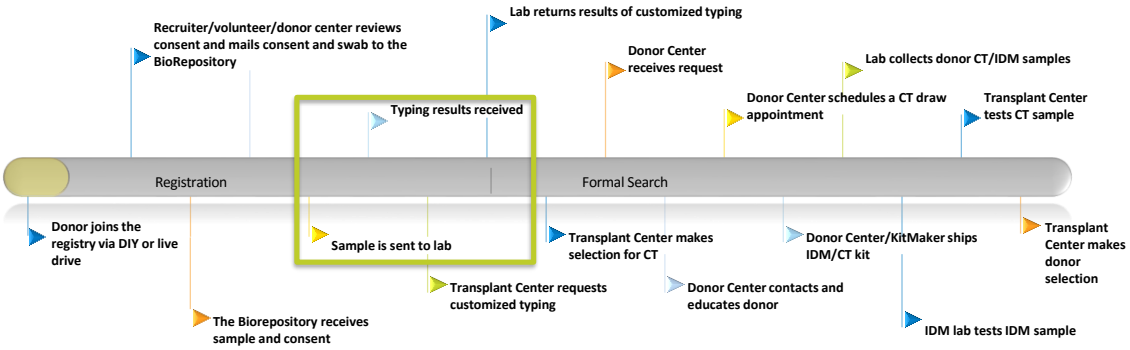


Current NMDP Donor Recruitment Package

- 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 Δ 32 genotyping

NGS Typed Donors in Traxis

The Path to Testing (and Beyond)



Before A Donor Sample Arrives at Lab

Receive

- NMDP Biorepository receives samples

→

Inspect & Store

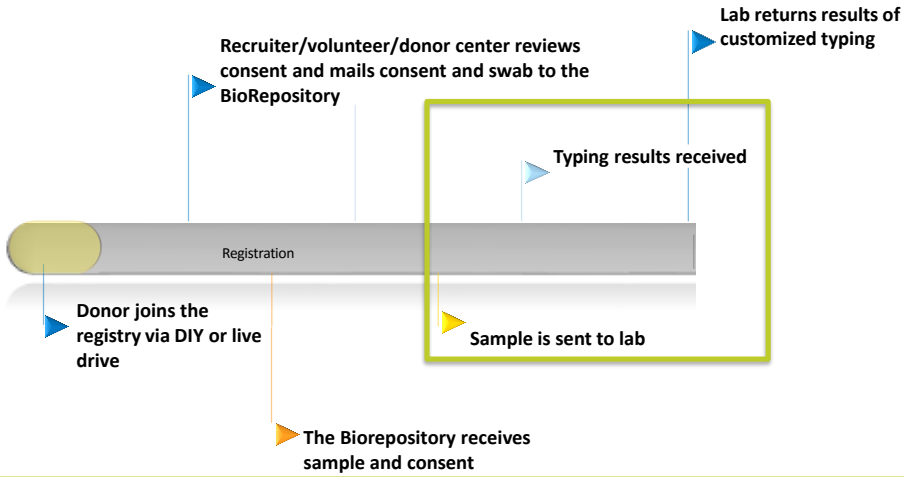
- Inspect samples for labeling and condition
- 2 swabs stored for shipment
- 2 swabs frozen for long term storage

→

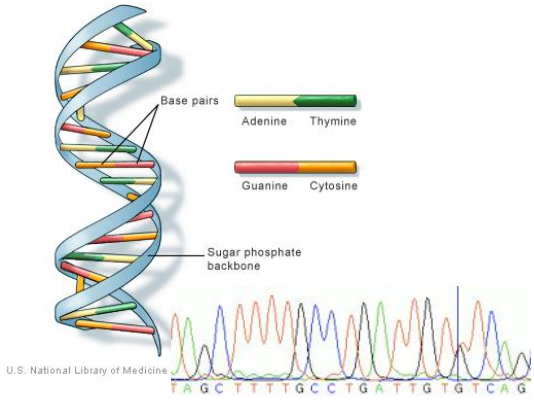
Pull & Ship

- Identify and ship samples to labs for DNA testing

Where Does Testing Occur in the Path?

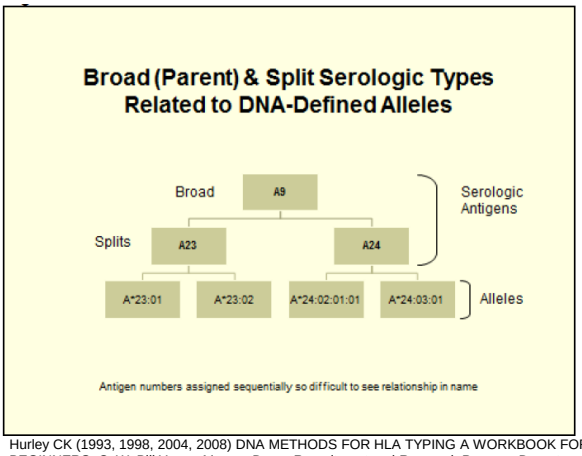


Chapter 2: HLA Typing History to Present



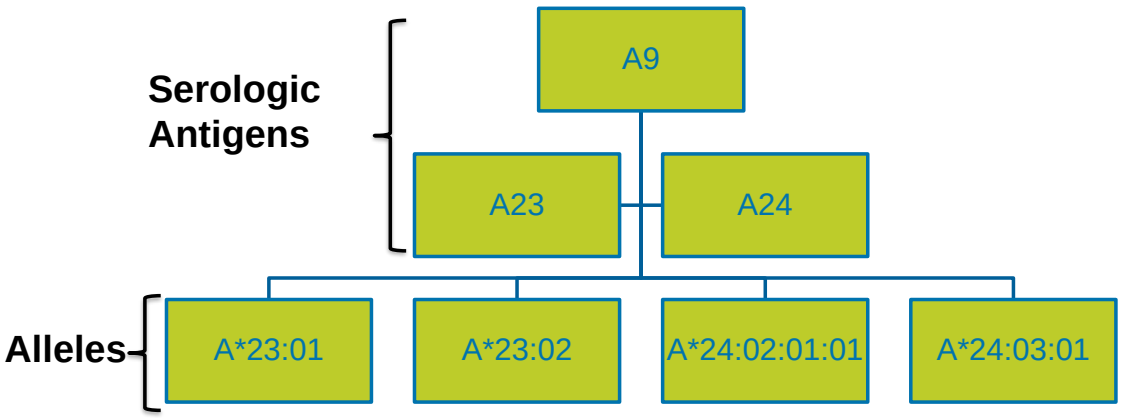
Serology: A Broad Sweep of HLA

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



Hurley CK (1993, 1998, 2004, 2008) DNA METHODS FOR HLA TYPING A WORKBOOK FOR BEGINNERS. C. W. Bill Young Marrow Donor Recruitment and Research Program Department of Oncology, Georgetown University School of Medicine, Washington, DC 2005: 41

Serology Pyramid



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

TGGATTGGTCCATGTTGTGTGATTCAGTGGTTTGTTCCCT
GAGATTCGTCCATGTTGTGTGATTCAGTGGTTTGTTCCCT

NGS Sequencing

TGGATTGGTCCATGTTGTGTGATTCAGTGGTTTGTTCCCT

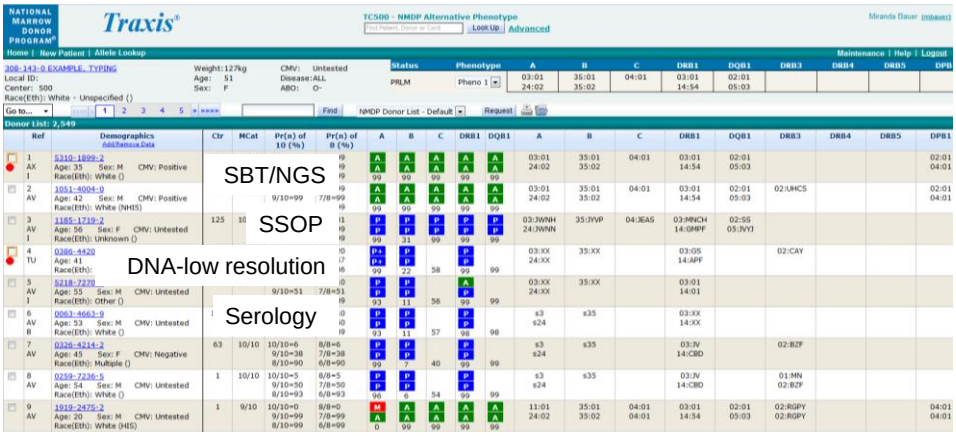
http://histogenetics.com/research/advances-in-dna-sequencing-technologies-for-high-resolution-hla-typing

NGS Typing Accuracy vs Sanger

High resolution result percentage

LOCUS	NGS	SANGER
HLA-A	99.94	75.6
HLA-B	99.75	80
HLA-C	97.75	60
HLA-DRB1	100.00	95.5
HLA-DQB1	100.00	96.1
HLA-DPB1	100.00	98

History of HLA Typing as Seen By Traxis



SBT or Full Gene/Long Range NGS

10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	A A 99	A A 99	A A 99	A A 99	A A 99	03:01 24:02	35:01 35:02	04:01	03:01 14:54	02:01 05:03				02:01 04:01
10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	A A 99	A A 99	A A 99	A A 99	A A 99	03:01 24:02	35:01 35:02	04:01	03:01 14:54	02:01 05:03	02:UHCS			02:01 04:01

SSOP

10/10	10/10=31 9/10=99 8/10=99	8/8=31 7/8=99 6/8=99	P P 99	P P 31	P P 99	P P 99	P P 99	03:JWNH 24:JWNN	35:JYVP	04:JEAS	03:MNCH 14:GMPF	02:SS 05:JVYJ				

Low-res DNA

10/10	10/10=20 9/10=57 8/10=86	8/8=20 7/8=57 6/8=86	P+ P+ 99	P P 22	58	P P 99	99	03:XX 24:XX	35:XX		03:GS 14:APF		02:CAY			
10/10	10/10=10 9/10=51 8/10=89	8/8=10 7/8=51 6/8=89	P P 93	P P 11	56	A P 99	99	03:XX 24:XX	35:XX		03:01 14:01					

Serology

10/10	10/10=10 9/10=50 8/10=88	8/8=10 7/8=50 6/8=89	P P 93	P P 11	57	P P 98	98	s3 s24	s35		03:XX 14:XX					
10/10	10/10=6 9/10=38 8/10=90	8/8=6 7/8=38 6/8=90	P P 99	P P 7	40	P P 99	99	s3 s24	s35		03:JV 14:CBD		02:BZF			
10/10	10/10=5 9/10=50 8/10=93	8/8=5 7/8=50 6/8=93	P P 96	P P 6	54	P P 99	99	s3 s24	s35		03:JV 14:CBD		01:MN 02:BZF			

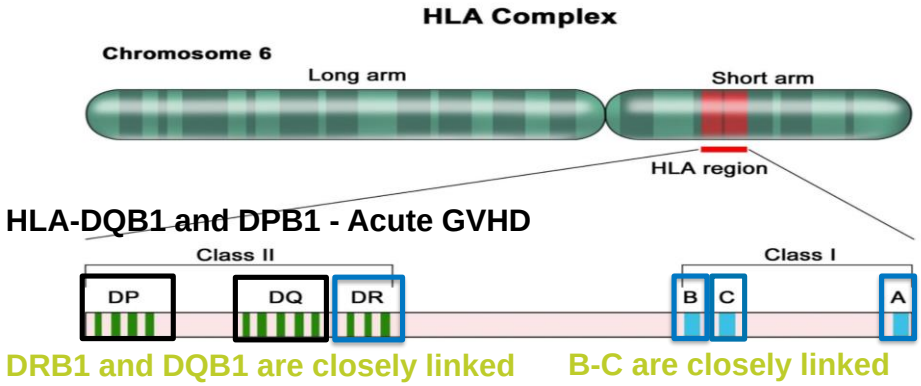
NMDP's Goal With Adoption of NGS

- 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

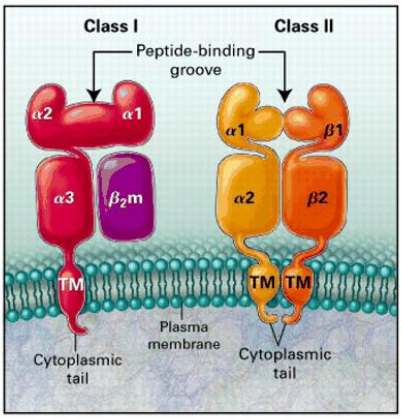
HLA-C also affects transplant outcome



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



Jan Klein, Ph.D. and Akie Sato, Ph.D.

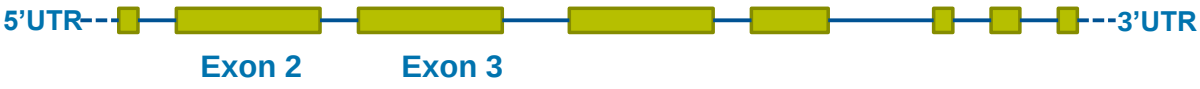
Volume 343(10):702-709, Sep 7, 2000
Volume 343(11):782-786, Sep14, 2000

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



Exon 2 **Exon 3**

Class II: HLA-DRB1

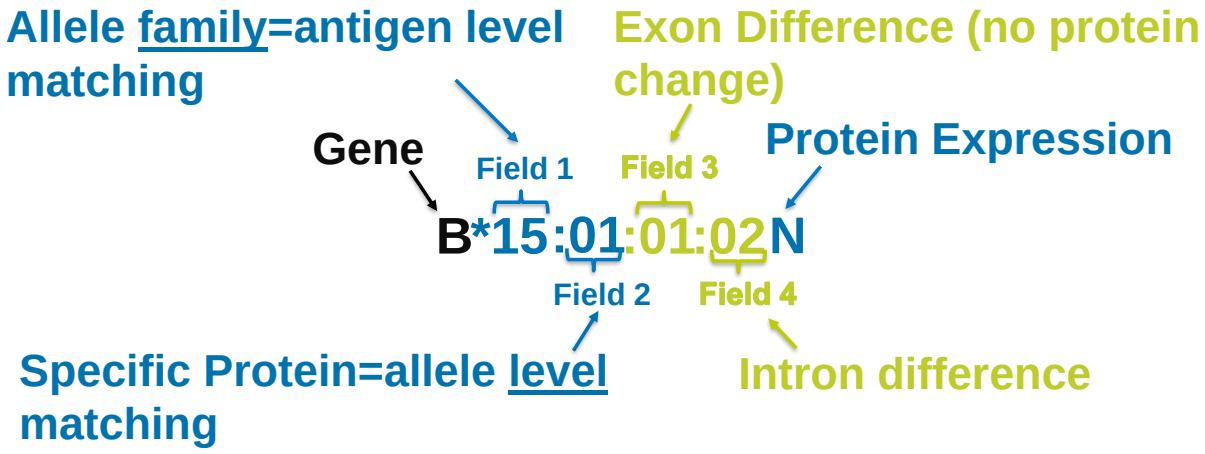
≈300 bp

≈2% of total gene

Whole gene length: 10.8-13.7 kb
Total exons: 5-6

Exon 2

DNA Sequence → HLA Gene Name



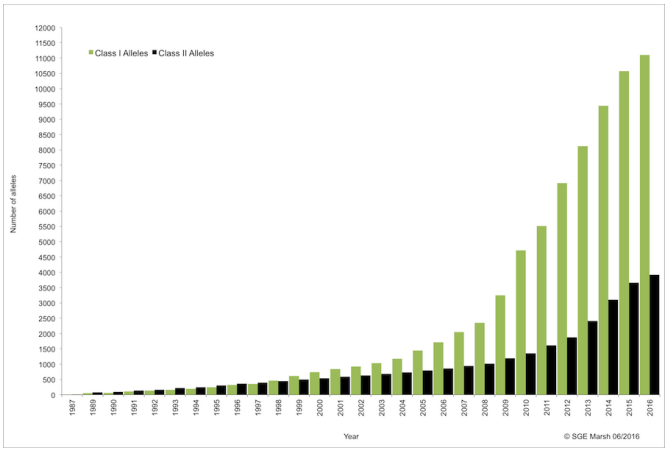
Summary of HLA Typing History

- 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

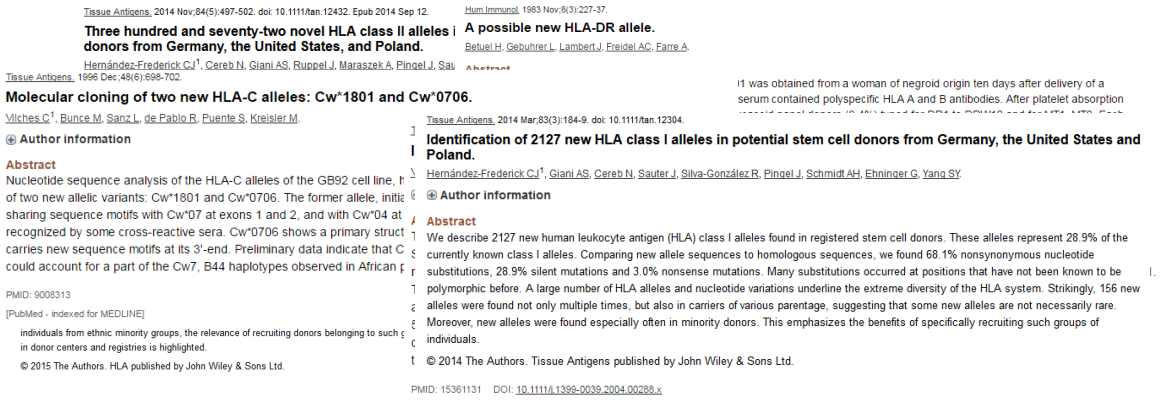
Chapter 3: Why is HLA Typing Problematic and How Does NMDP's NGS Typing Strategy Address These Issues?

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



Tissue Antigens. 2014 Nov;84(5):497-502. doi: 10.1111/tan.12432. Epub 2014 Sep 12.

Three hundred and seventy-two novel HLA class II alleles i donors from Germany, the United States, and Poland.

Hernández-Frederick CJ[1], Cereb N, Giani AS, Ruppel J, Maraszek A, Pingel J, Sau

Tissue Antigens. 1996 Dec;48(6):698-702.

Molecular cloning of two new HLA-C alleles: Cw*1801 and Cw*0706.

Vilches C[1], Bunce M, Sanz L, de Pablo R, Puente S, Kreisler M.

Author information

Abstract

Nucleotide sequence analysis of the HLA-C alleles of the GB92 cell line, h of two new allelic variants: Cw*1801 and Cw*0706. The former allele, initia sharing sequence motifs with Cw*07 at exons 1 and 2, and with Cw*04 at recognized by some cross-reactive sera. Cw*0706 shows a primary struct carries new sequence motifs at its 3'-end. Preliminary data indicate that C could account for a part of the Cw7, B44 haplotypes observed in African p

PMID: 9008313

[PubMed - indexed for MEDLINE]

individuals from ethnic minority groups, the relevance of recruiting donors belonging to such g in donor centers and registries is highlighted.

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Hum Immunol. 1983 Nov;8(3):227-37.

A possible new HLA-DR allele.

Betuel H, Gebuhrer L, Lambert J, Freidel AC, Farre A.

Abstract

l1 was obtained from a woman of negroid origin ten days after delivery of a serum contained polyspecific HLA A and B antibodies. After platelet absorption

Tissue Antigens. 2014 Mar;83(3):184-9. doi: 10.1111/tan.12304.

Identification of 2127 new HLA class I alleles in potential stem cell donors from Germany, the United States and Poland.

Hernández-Frederick CJ[1], Giani AS, Cereb N, Sauter J, Silva-González R, Pingel J, Schmidt AH, Ehninger G, Yang SY.

Author information

Abstract

We describe 2127 new human leukocyte antigen (HLA) class I alleles found in registered stem cell donors. These alleles represent 28.9% of the currently known class I alleles. Comparing new allele sequences to homologous sequences, we found 68.1% nonsynonymous nucleotide substitutions, 28.9% silent mutations and 3.0% nonsense mutations. Many substitutions occurred at positions that have not been known to be polymorphic before. A large number of HLA alleles and nucleotide variations underline the extreme diversity of the HLA system. Strikingly, 156 new alleles were found not only multiple times, but also in carriers of various parentage, suggesting that some new alleles are not necessarily rare. Moreover, new alleles were found especially often in minority donors. This emphasizes the benefits of specifically recruiting such groups of individuals.

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PMID: 15361131 DOI: 10.1111/j.1399-0039.2004.00288.x

HLA Typing Difficulties Result From:

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

	A	B	C	DRB1	DQB1
# of alleles	3,492	4,358	3,111	1,929	940
# of nulls	158	137	115	48	25

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

Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P and Marsh SGE. The IPD and IPD-IMGT/HLA Database: allele variant databases. Nucleic Acids Research (2015), **43**:D423-31

More On HLA Difficulties-Null Alleles

- Null alleles often resolved within sequence outside of ARS

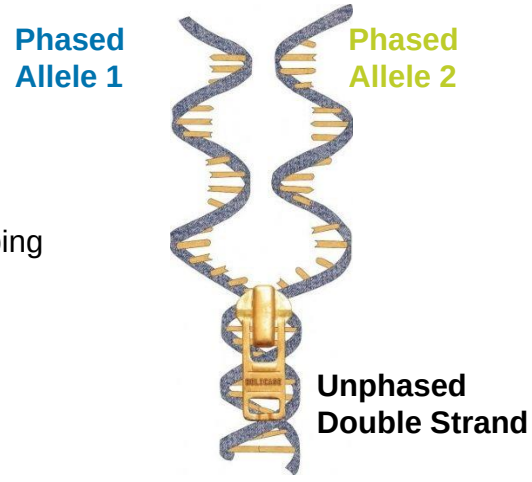
Null Allele	HLA G Group	Location of Polymorphism
A*01:04N	A*01:01:01G	Exon 4
A*03:21N	A*03:01:01G	Exon 4
A*24:09N	A*24:02:01G	Exon 4
A*24:11N	A*24:02:01G	Exon 4
A*68:11N	A*68:01:02G	Exon 1
B*15:01:01:02N	B*15:01:01G	Intron 1
B*51:11N	B*51:01:01G	Exon 4
C*04:09N	C*04:01:01G	Exon 7

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

Typing Date	Reporting Date	Method	Ctr	A	B	C	DRB1	DQB1	DRB3
Jul 27 2016	Jul 27 2016	LAB	744	01:01 01:01	08:01 08:01	07:01 07:01	03:01 03:01	02:01 02:01	01:FX 01:FX

CT Typing

Typing Date	Reporting Date	Method	A	B	C	DRB1	DQB1	DRB3
Sep 09 2016	Sep 19 2016	22	*01:AMUKB *	*08:AHPZU *	*07:ANVBW *	*03:ANVCN *	*02:AFMZB *	*02:ANKJK *

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

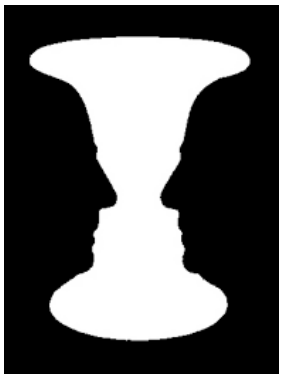
Recruitment Donor Typing: Introducing PacBio

- 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

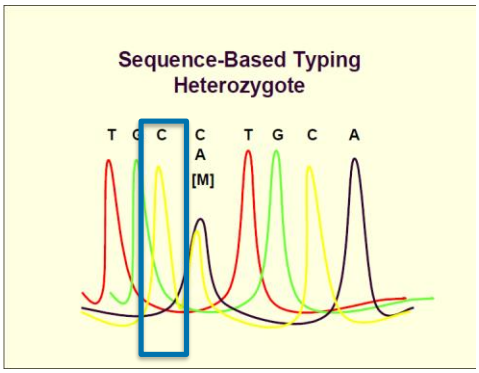
Chapter 4: Demystifying Ambiguous Pairs

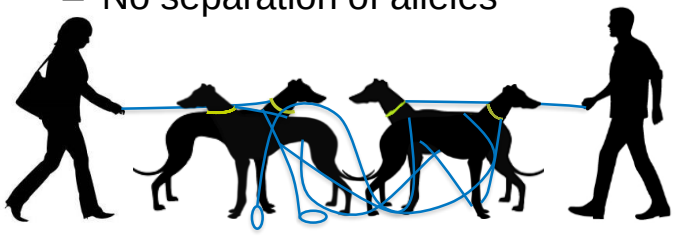


SBT and SSOP May Show Ambiguous Pairs

- Mixed bases
  - Which allele goes where?
- SSOP/SBT may not be able to determine
  - No separation of alleles

Hurley CK (1993, 1998, 2004, 2008) DNA METHODS FOR HLA TYPING A WORKBOOK FOR BEGINNERS. C. W. Bill Young Marrow Donor Recruitment and Research Program Department of Oncology, Georgetown University School of Medicine, Washington, DC 2005: 41





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)

Status	Phenotype	A	B	C	DRB1	DQB1
FRML	Pheno 1	03:AMTF 32:CJT	35:ANZDP 40:ANZDR	03:HUYK 04:XHDF	08:01 13:02	04:02 06:04

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	CWD + Non-CWD	CWD + CWD
B*35:01:01G+B*40:01:01G	B*35:04:01+B*40:52	B*35:10+B*40:25
CWD + CWD	**Non-CWD + Non-CWD**	**CWD + Non-CWD**
B*35:20:01+B*40:07	B*35:28+B*40:106	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*02:30 but did not rule out A*02:01
  - One kit did not differentiate between A*02:01 or A*02:30
  - One kit showed strong A*02:30

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

CMV: Negative
Disease: AML
ABO: B-

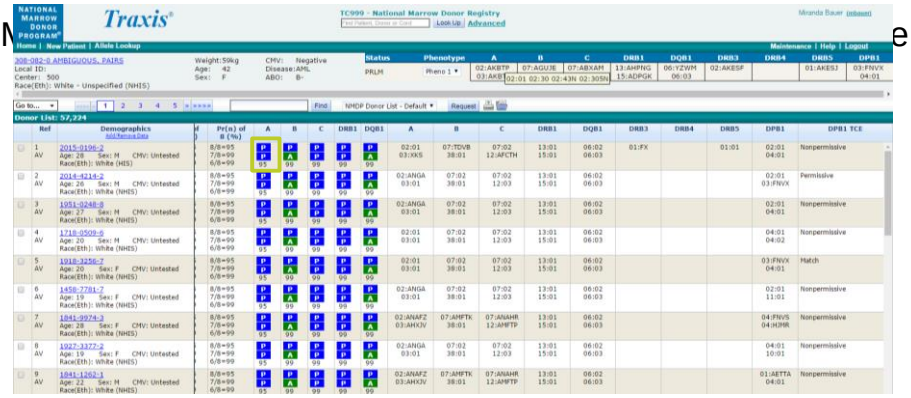
Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1	02:AKBTP 03:AKBT	07:AGUJE 02:01 02:30	07:ABXAM 02:43N 02:305N	13:AHPNG 15:ADPGK	06:YZWM 06:03

Find | NMDP Donor List - Default | Request

A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4
P P 95	P A 99	P P 99	P P 99	P A 99	02:01 03:XKS	07:TDVB 38:01	07:02 12:AFCTH	13:01 15:01	06:02 06:03	01:FX	

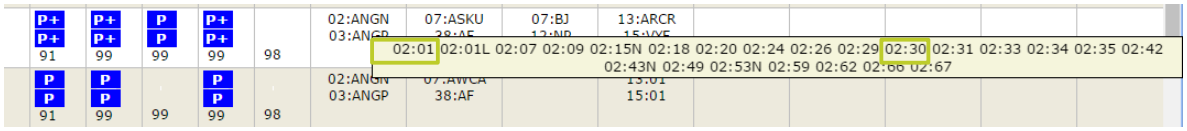
Patient Search with Ambiguous Alleles (cont.)

- M e



Patient Search with Ambiguous Alleles (cont.)

- Some donors also have ambiguous typing



Patient Search with Ambiguous Alleles (cont.)

- Other donors are defined A*02:30

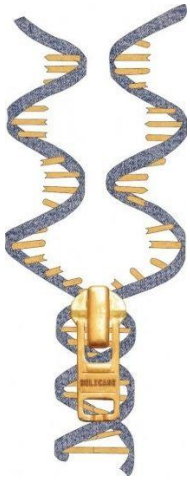
10/10=5 9/10=99 8/10=99	8/8=5 7/8=99 6/8=99	P P 5	P A 99	P P 99	P P 99	P A 99	02:30 03:AAAUY	07:TXXS 38:01	07:ABGFN 12:AETSZ	13:01 15:01	06:02 06:03	

NGS Resolves Ambiguous Combinations

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

Phased Haplotype 1

Phased Haplotype 2



Unphased Double Strand

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.

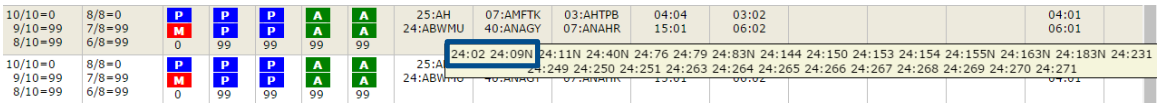
Patient Search With Null Allele (cont.)

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

Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1 ▼	24:09N 25:01	07:TDVB 40:01	03:04 07:02	04:04 15:01	03:02 06:02

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



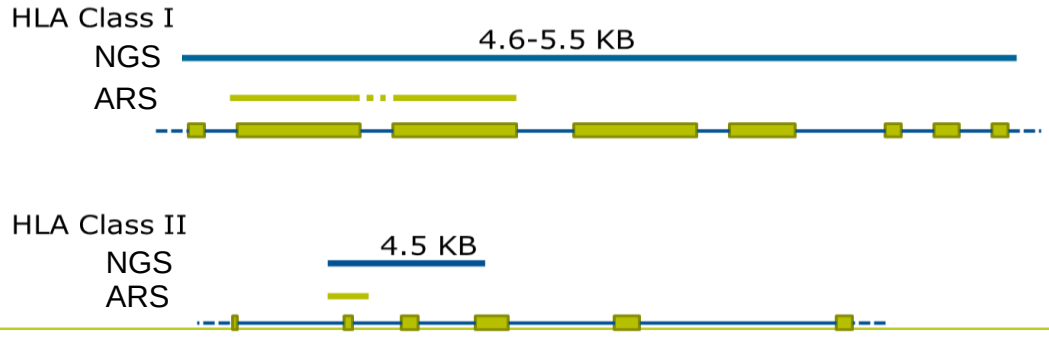
Case Study: Patient Search With Null Allele

- 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

Pr(n) of 10 (%)	Pr(n) of 8 (%)	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	A A 99	P A 99	A A 99	A A 99	A A 99	24:09N 25:01	07:TDVB 40:01	03:04 07:02	04:04 15:01	03:02 06:02

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

TRANSPLANTATION

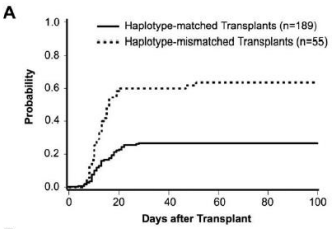
Mapping MHC haplotype effects in unrelated donor hematopoietic cell transplantation

Effie W. Petersdorf,[1] Mari Malkki,[1] Mary M. Horowitz,[2] Stephen R. Spellman,[3] Michael D. Haagenson,[3] and Tao Wang[4]

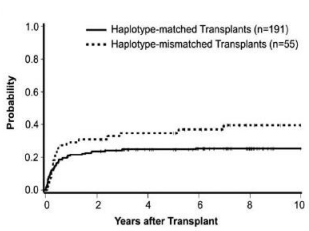
[1]Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA; [2]Center for International Blood and Marrow Transplant Research and the Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI; [3]Center for International Blood and Marrow Transplant Research, Minneapolis, MN; and [4]Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI

Haplotype Matching Outcomes Charts

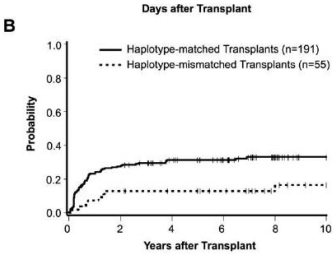
Grade III-IV GvHD



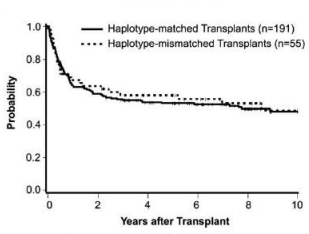
TRM



Recurrent Malignancy



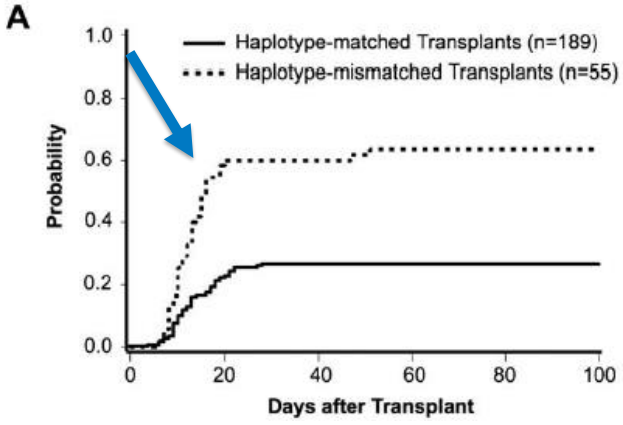
Overall Survival



Petersdorf, Effie W et al. "MHC Haplotype Matching for Unrelated Hematopoietic Cell Transplantation." Ed. John Trowsdale. *PLoS Medicine* 4.1 (2007): e8. *PMC*. Web. 13 Oct. 2016.

Study Results Suggest Affect on GvHD Risk

Lower GvHD risk for haplotype matched transplants



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

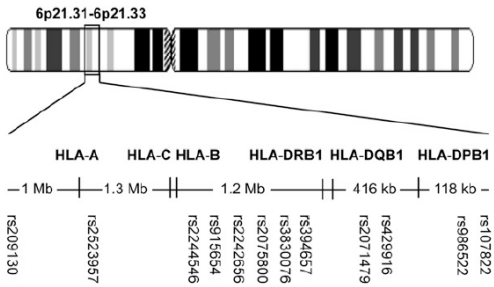


Figure 2. Twelve SNPs of clinical significance in HLA-mismatched unrelated donor transplantation. Each of the 12 SNPs having an association with grades II-IV or III-IV acute GVHD, chronic GVHD, relapse, transplant-related mortality, disease-free survival, or survival are shown on a map of the MHC on 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.[21]

Petersdorf, Effie W. et al. "Mapping MHC Haplotype Effects in Unrelated Donor Hematopoietic Cell Transplantation." *Blood* 121.10 (2013): 1896–1905. *PMC*. Web. 13 Oct. 2016.

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

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

N Engl J Med. 2015 Aug 13;373(7):599-609. doi: 10.1056/NEJMoa1500140.

High HLA-DP Expression and Graft-versus-Host Disease.

Petersdorf EW[1], Malkki M, O'hUigin C, Carrington M, Gooley T, Haagenson MD, Horowitz MM, Spellman SR, Wang T, Stevenson P.

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

Blood. 2014 Dec 18;124(26):3996-4003. doi: 10.1182/blood-2014-09-599969. Epub 2014 Oct 16.

HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation.

Petersdorf EW[1], Gooley TA[2], Malkki M[2], Bacigalupo AP[3], Cesbron A[4], Du Toit E[5], Ehninger G[6], Egeland T[7], Fischer GF[8], Gervais T[9], Haagenson MD[10], Horowitz MM[11], Hsu K[12], Jindra P[13], Madrigal A[14], Oudshoorn M[15], Ringdén O[16], Schroeder ML[17], Spellman SR[10], Tiercy JM[18], Velardi A[19], Witt CS[20], O'Huigin C[21], Apps R[22], Carrington M[22]; International Histocompatibility Working Group in Hematopoietic Cell Transplantation.

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

Logan, Aaron C. et al. "ABO Mismatch Is Associated with Increased Non-Relapse Mortality after Allogeneic Hematopoietic Cell Transplantation." *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 21.4 (2015): 746–754. *PMC*. Web. 13 Oct. 2016.

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

Table 2
Effect of ABO Incompatibility on Recipient Survival and Incidence of Graft-versus-Host Disease

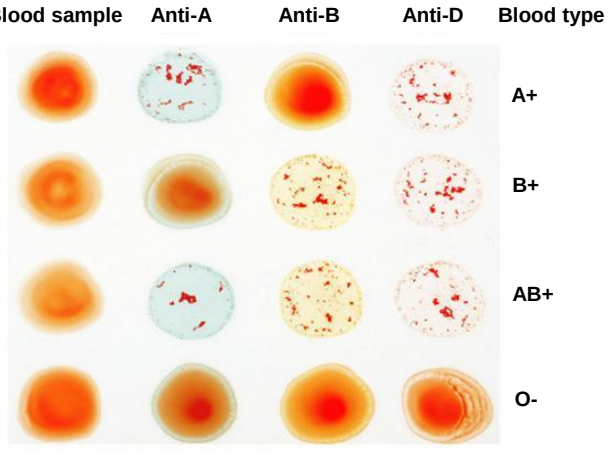
Study Authors	Year	Survival after ABO-Incompatible HCT Transplantation			Risk of Graft-versus-Host Disease
		Major	Minor	Bidirectional	
Kimura et al. [3]	2008	Decreased	Decreased	No difference	Increased with minor or major ABO mismatch
Helming et al. [13]	2007	No difference	No difference*	No difference*	No difference
Erker et al. [15]	2005	No difference	Decreased	Decreased	No difference
Kim JG et al. [12]	2005	No difference	No difference	No difference	No difference
Stussi et al. [14]	2002	Decreased	No difference	No difference	Increased with minor ABO mismatch
Benjamin et al. [18]	1999	Decreased†	Decreased†	No difference	No difference with minor or major mismatch
Bacigalupo et al. [19]	1988	–	–	–	Increased with minor ABO mismatch
Benisnger et al. [41]	1982	No difference	–	–	No difference with major ABO mismatch
Buckner et al. [17]	1978	–	No difference	–	No difference with minor ABO mismatch

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 myelogeneous leukemia.

Booth GS, Gehrie EA, Bolan CD, Savani BN. **Clinical guide to ABO-incompatible allogeneic stem cell transplantation.** Biol Blood Marrow Transplant 2013 Aug;19(8):1152-8. doi: 10.1016/j.bbmt.2013.03.018.

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



Textbook of Medical Physiology // A.C.Guyton, J.E.Hall. – Eleventh edition, 2005.

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

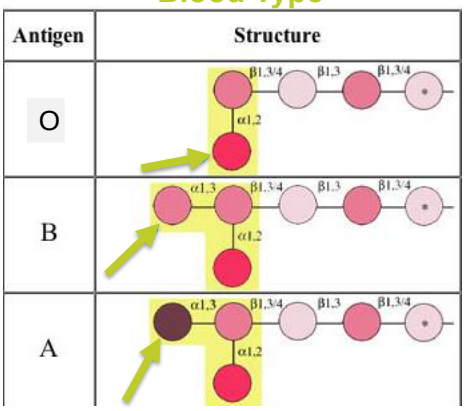
- 12 samples corrected ABO from serology
- 31 samples corrected RhD from serology

Nezih Cereb,Sang Yeol Seo,Amaralingeswara Rao,Gail Flickinger,Jangyoung Kwon,JeongOk Jeon,DongYong Kim,HwaRan Kim,Romy Kronstein,Torsten Tonn,Soo Young Yang. OR29 Prediction of abo rh serotypes by molecular typing of abo rh genes is highly concordant with serological typing: experience with typing 1000,000 samples. Hum Immunol. 09/2016.

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

Sugar Residue Determines Blood Type



More ABO From a Genetics Perspective

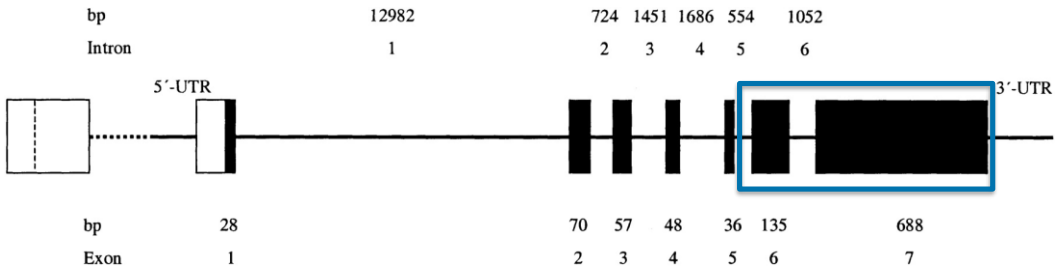
- Complex and not fully understood
- Extensive heterogeneity in ABO alleles, subgroups and noncoding regions
- Variation in exons 6 and 7 important in determining blood type
- Limited studies describe mutations outside of exons 6 and 7 that may affect ABO determination
- Some ABO subgroups may be missed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



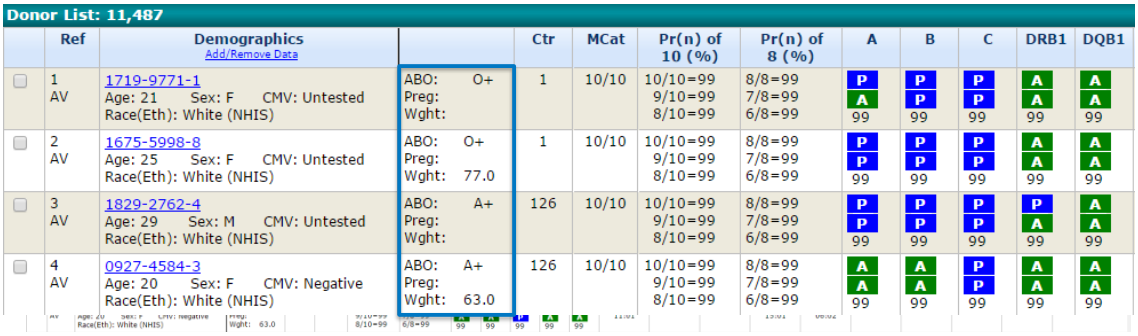
Seltsam, A., Hallensleben, M., Kollmann, A., & Blasczyk, R. (2003). The nature of diversity and diversification at the *ABO* locus. *Blood,* 102(8), 3035-3042.

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

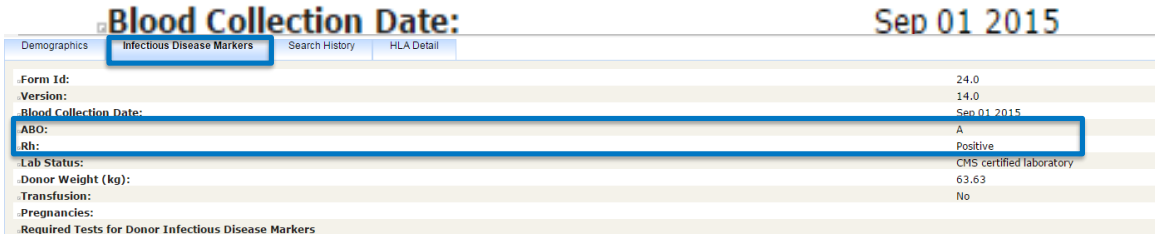


Donor List: 11,487

	Ref	Demographics (Add/Remove Data)		Ctr	MCat	Pr(n) of 10 (%)	Pr(n) of 8 (%)	A	B	C	DRB1	DQB1
☐	1 AV	1719-9771-1 Age: 21 Sex: F CMV: Untested Race(Eth): White (NHIS)	ABO: O+ Preg: Wght:	1	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	P A 99	P P 99	P P 99	A A 99	A A 99
☐	2 AV	1675-5998-8 Age: 25 Sex: F CMV: Untested Race(Eth): White (NHIS)	ABO: O+ Preg: Wght: 77.0	1	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	P P 99	P P 99	P P 99	A A 99	A A 99
☐	3 AV	1829-2762-4 Age: 29 Sex: M CMV: Untested Race(Eth): White (NHIS)	ABO: A+ Preg: Wght:	126	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	P P 99	P P 99	P P 99	P A 99	A A 99
☐	4 AV	0927-4584-3 Age: 20 Sex: F CMV: Negative Race(Eth): White (NHIS)	ABO: A+ Preg: Wght: 63.0	126	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	A A 99	A A 99	P P 99	A A 99	A A 99

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



Blood Collection Date: Sep 01 2015

Demographics | Infectious Disease Markers | Search History | HLA Detail

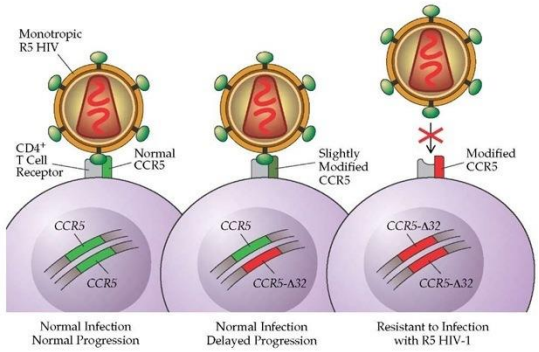
Form Id:	24.0
Version:	14.0
Blood Collection Date:	Sep 01 2015
ABO:	A
Rh:	Positive
Lab Status:	CMS certified laboratory
Donor Weight (kg):	63.63
Transfusion:	No
Pregnancies:	
Required Tests for Donor Infectious Disease Markers	

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 Δ 32 mutation at recruitment
- NGS provides exciting opportunities for advancement in the world of HSCT matching and research