

## Advanced Search Strategy: Tips for a Smooth Journey

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# Disclosures

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

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# Map

- Itinerary for our journey
- 3 stops, exploring each section:
  - Less common patient HLA typing
  - Patient null allele
  - Patient antibody report
- References and resources



# Learning objectives

At the conclusion of this session, attendees will be able to:

- Develop approaches to improve identification of potential donors when patients have less common HLA typing
- Apply strategies for searching on null alleles
- Identify considerations for patient antibody reports in donor/cord blood selection

# Most Council slides available now or soon:

- On the Council Meeting mobile app
- And the Network web site



# Basics still apply with the complex

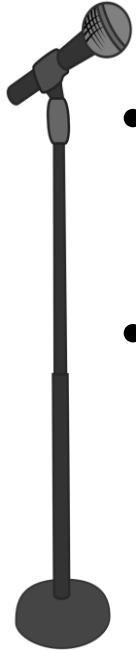
- As we travel through less common search strategy areas, we'll keep an eye on HLA basics, too

**Broads  
Splits**



**DRB1-DRB3/4/5**

# Consider this your tour bus



- Ask questions any time!
- Use a standing mic

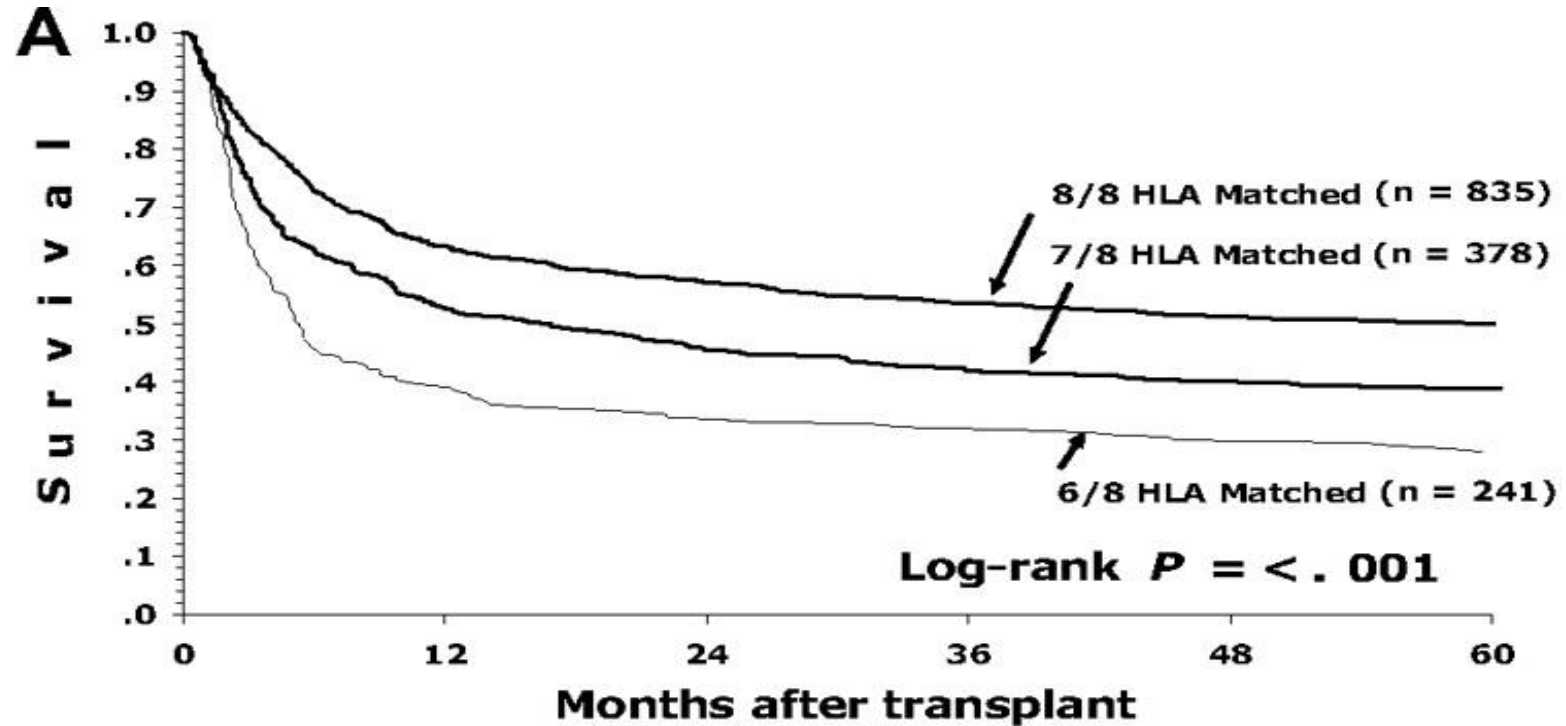


# Stop N°1: Less common patient HLA typing





# 8/8 remains gold standard for URD matching



Lee, S.J. et al. *Blood* 2007, 110

# Challenge: Identifying a matched URD from diverse global HLA



	A	B	C	DRB1	DQB1
Number of alleles	3,968	4,828	3,579	2,103	1,142

*Identification of  
matched  
unrelated donor*

# Example: Patient search and HLA information

HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
02:01	27:05	01:02	01:01	05:01
24:02	51:07	14:02	11:01	03:01

Patient #1
27 year old female with ALL
Patient ethnicity unknown
Patient has no siblings
<b>TC criteria: 10/10 matched URD</b>

# Traxis: HapLogic confirms B\*51:07 matching difficulty

MCat	Pr(n) of 10 (%)	Pr(n) of 8 (%)	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	P P 99	A D 99	P P 99	P A 99	A A 99	24:PDVJ 02:RGPK	51:07 27:EKN	14:CBF 02:ATZ	11:CTPB 01:01	03:01 05:01
10/10	10/10=8 9/10=86 8/10=99	8/8=8 7/8=86 6/8=99	P P 98	P D 8	A A 99	A A 99		s24 s2	s51 s27	14:02 02:02	11:01 01:01	

# No HapLogic predictions: BMDW potential 10/10s

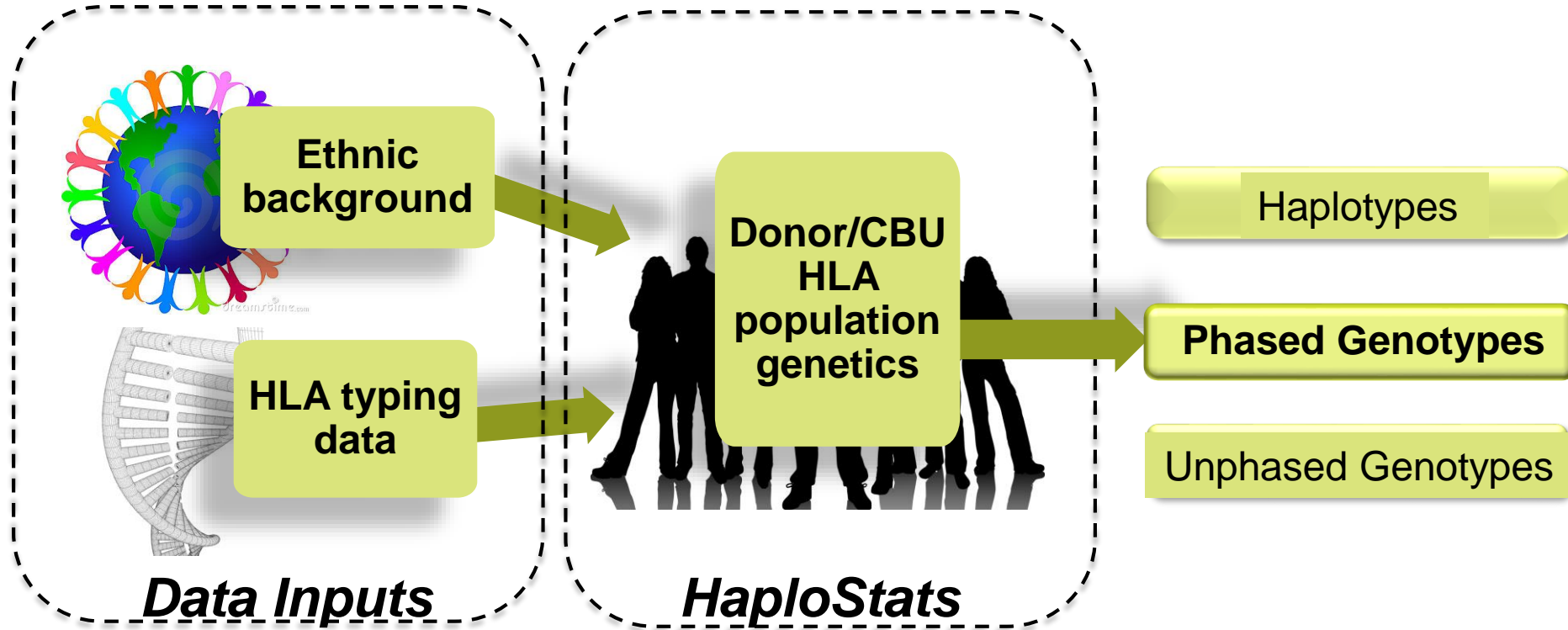
Demographics <a href="#">Add/Remove Data</a>	MCat	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
<a href="#">Italy</a> Donor Count: 1	10/10	<b>P</b> <b>A</b>	<b>P</b> <b>P</b>	<b>P</b> <b>P</b>	<b>A</b> <b>A</b>		24:XX 02:01	51:XX 27:XX	14:XX 02:XX	11:01 01:01	
<a href="#">Denmark-BMDC</a> Donor Count: 1	10/10	<b>P</b> <b>P</b>	<b>P</b> <b>P</b>		<b>P</b> <b>P</b>	<b>P</b> <b>P</b>	s24 s2	s51 s27		11:XX 01:XX	03:XX 05:XX
<a href="#">France</a> Donor Count: 1	10/10	<b>P</b> <b>P</b>	<b>P</b> <b>P</b>		<b>P</b> <b>P</b>	<b>P</b> <b>P</b>	s24 s2	s51 s27		11:XX 01:XX	03:XX 05:XX
<a href="#">Spain</a> Donor Count: 1	10/10	<b>P</b> <b>P</b>	<b>P</b> <b>P</b>		<b>P</b> <b>P</b>		24:XX 02:XX	51:XX 27:XX		11:XX 01:XX	
<a href="#">Austria</a> Donor Count: 1	10/10	<b>P</b> <b>P</b>	<b>P</b> <b>P</b>		<b>P</b> <b>P</b>		s24 s2	s51 s27		11:XX 01:XX	
<a href="#">Portugal</a> Donor Count: 2	10/10	<b>P</b> <b>P</b>	<b>P</b> <b>P</b>		<b>P</b> <b>P</b>		24:XX 02:XX	51:XX 27:XX		11:XX 01:XX	

# Develop a strategy for screening potential donors

- CT all potential 10/10 donors, NMDP and BMDW
- HR type all BMDW potential 10/10s
- Don't type any donors; select the best 9/10 as backup
- Use an HLA tool to predict which A/B/DRB1 typed donors may be most likely to match



# HaploStats



# Phased vs. unphased genotype data

- **Unphased**
  - Most commonly used in HaploStats
  - Displays likelihood of each possible HLA type, not haplotypes
  - **What is the likelihood a donor will match your patient's typing?**
- **Phased**
  - Used primarily to access global maps and A/B/DRB1 haplotype data from many donor registries
  - Displays haplotype information, frequency in ethnic backgrounds
  - **What ethnic backgrounds to focus on in your donor search?**



# What information is required?

Ethnic  
background

Populations

<input checked="" type="checkbox"/> <b>AFA - African American</b> <input type="checkbox"/> AAFA - African American <input type="checkbox"/> AFB - African <input type="checkbox"/> CARB - Caribbean Black	<input checked="" type="checkbox"/> <b>API - Asian or Pacific Islander</b> <input type="checkbox"/> AINDI - South Asian Indian <input type="checkbox"/> FILII - Filipino <input type="checkbox"/> HAWI - Hawaiian or other Pacific Islander <input type="checkbox"/> JAPI - Japanese	<input type="checkbox"/> KORI - Korean <input type="checkbox"/> NCHI - Chinese <input type="checkbox"/> SCSEAI - Southeast Asian <input type="checkbox"/> VIET - Vietnamese
<input checked="" type="checkbox"/> <b>CAU - Caucasian</b> <input type="checkbox"/> MENAFC - Middle Eastern or North Coast of Africa <input type="checkbox"/> EURCAU - European Caucasian	<input checked="" type="checkbox"/> <b>HIS - Hispanic</b> <input type="checkbox"/> CARHIS - Caribbean Hispanic <input type="checkbox"/> MSWHIS - Mexican or Chicano <input type="checkbox"/> SCAHIS - South or Central American Hispanic	<input type="checkbox"/> <b>NAM - Native American</b> <input type="checkbox"/> AMIND - North American Indian <input type="checkbox"/> CARIBI - Caribbean Indian

Select All Clear Populations

HLA loci of interest

Haplotype Loci

A~C~B~DRB1~DQB1

HLA typing

HLA type

Enter an HLA type:	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1	HLA-DRB3	HLA-DRB4	HLA-DRB5
Type 1	24:02	51:07	14:02	11:01	03:01			
Type 2	02:01	27:05	02:02	01:01	05:01			

SUBMIT QUERY

# HaploStats Results – Phased Genotypes

HaploStats



DISCLAIMER: The data available here are intended for research purposes only.

## HLA Typing

Dataset: NMDP full 2011 Populations: AFA, API, CAU, MENAF

A	B	C	DRB1	DQB1
24:02	51:07	14:02	11:01	03:01
02:01	27:05	02:02	01:01	05:01

▶ (A~C~B~DRB1~DQB1) Haplotypes

▶ (A~C~B~DRB1~DQB1) Phased Genotypes

▶ (A-C-B-DRB1-DQB1) Unphased Genotypes (HLA type)

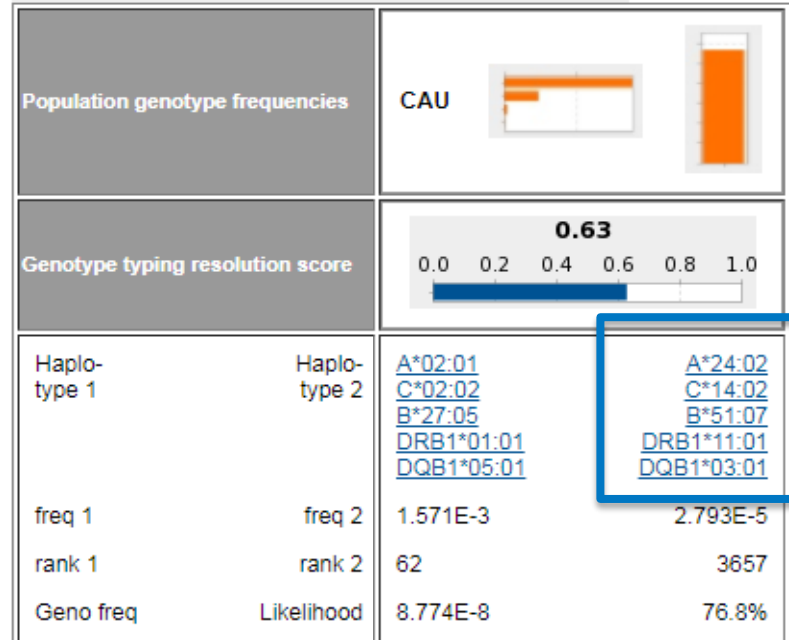
## Phased Genotypes:

- Displays *haplotype* information
- Focus on ethnic background in your donor search

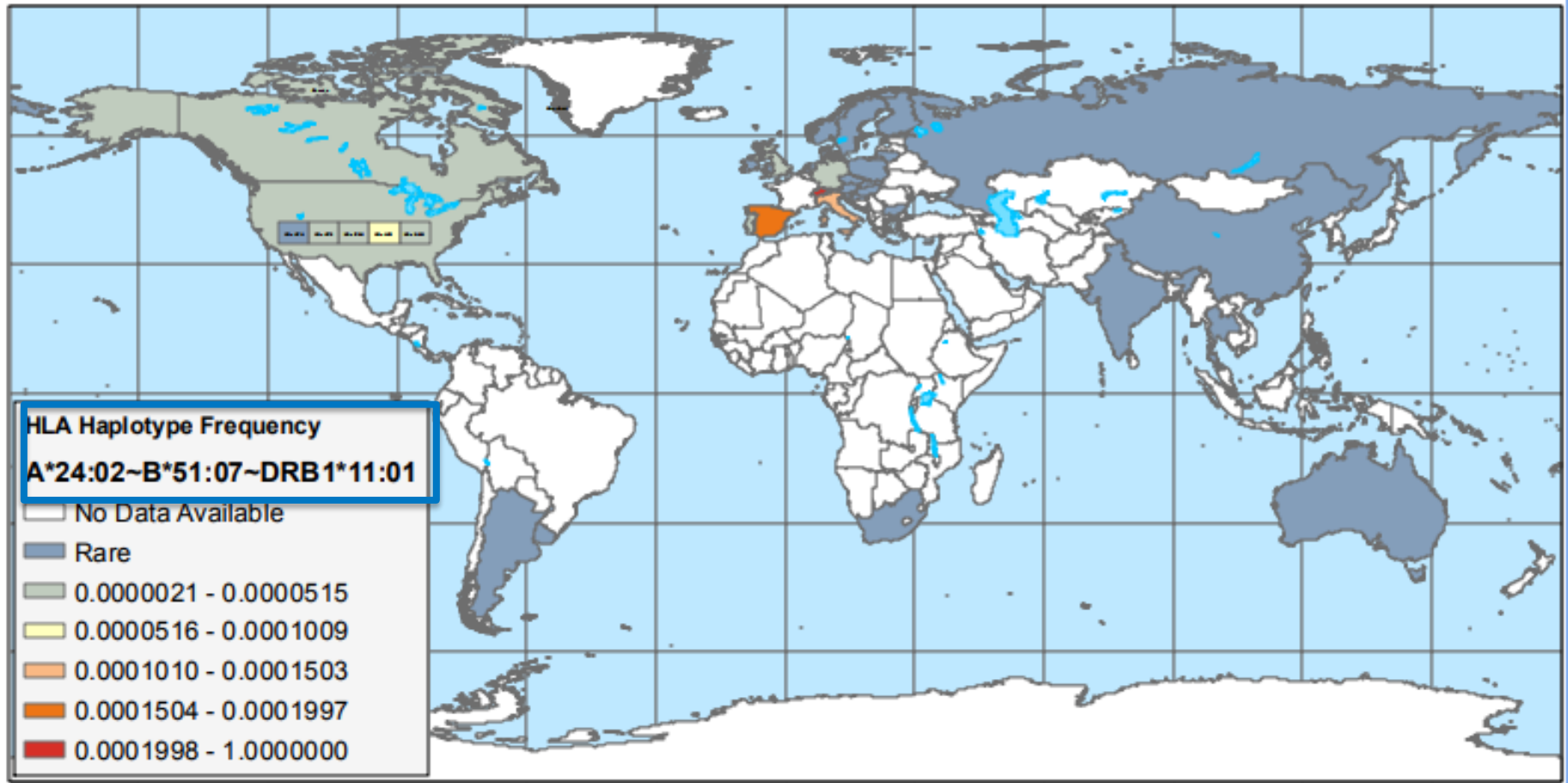
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# Phased genotypes predicted in each ethnic background



# Global HLA Haplotype Map



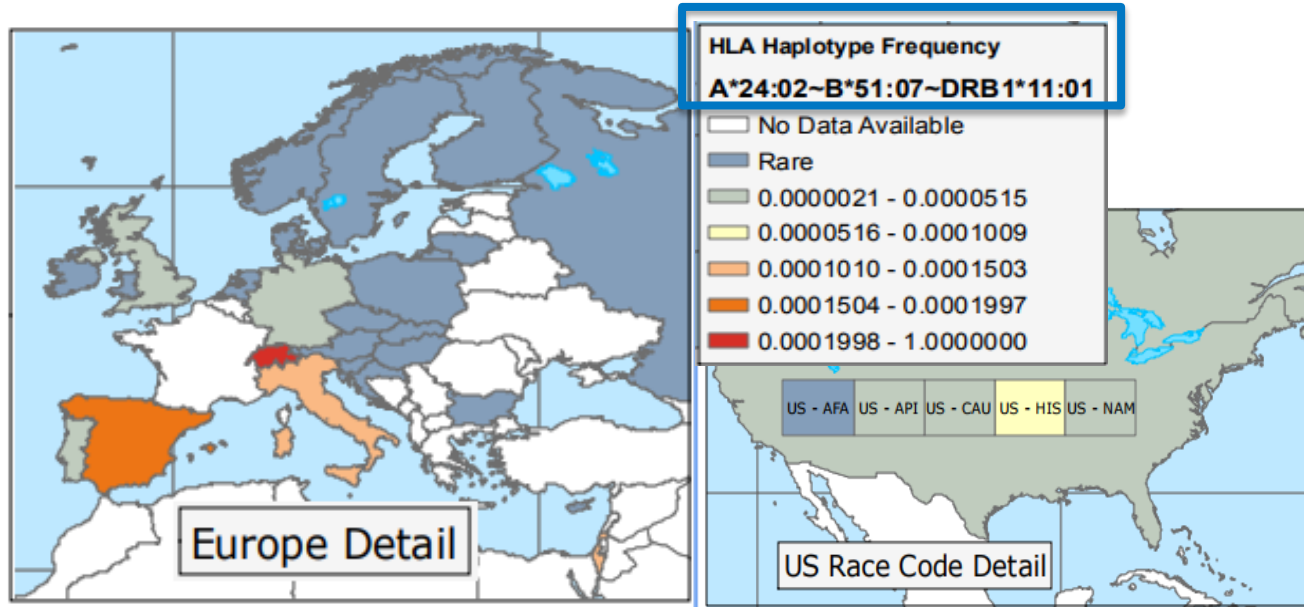
# Global Haplotype Maps : Information Source

- Global HLA haplotype maps became available for hyperlinked haplotypes in the Phased Genotypes section of HaploStats in April 2011
- Maps were developed in collaboration with consenting registries listing donors in BMDW
- Maps are a product of the **16<sup>th</sup> International HLA and Immunogenetics Workshop Registry Diversity Project**

# HaploStats phased genotype map caveats

- Maps reference only one haplotype
- **A-B-DRB1 haplotypes**; no help with uncommon C or DQ
- Not all countries submitted data; consult the paper for sample sizes which isn't evident via maps
- Not all haplotypes are hyperlinked: top 40,000 A-B-DRB1 haplotypes

# B\*51:07 haplotype: Switzerland, Spain, Italy, and Israel

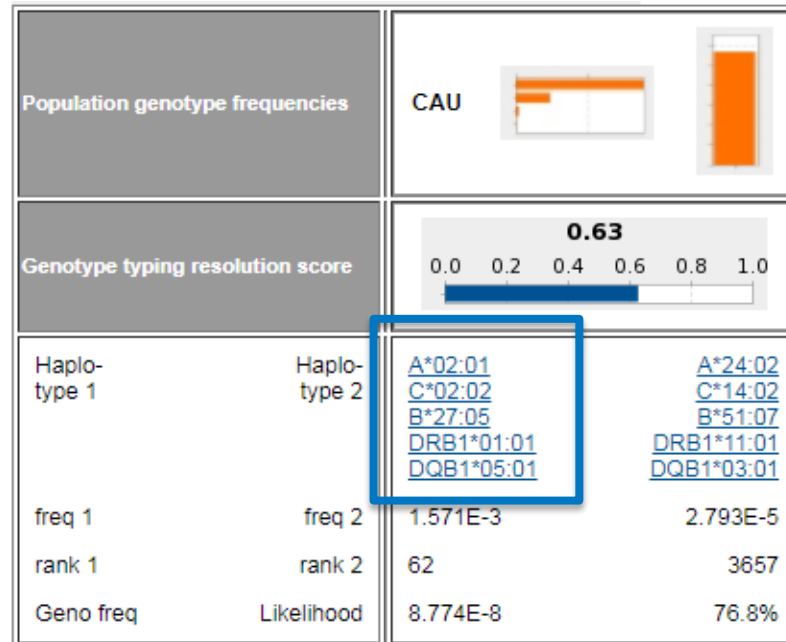


# Potentially more efficient screening

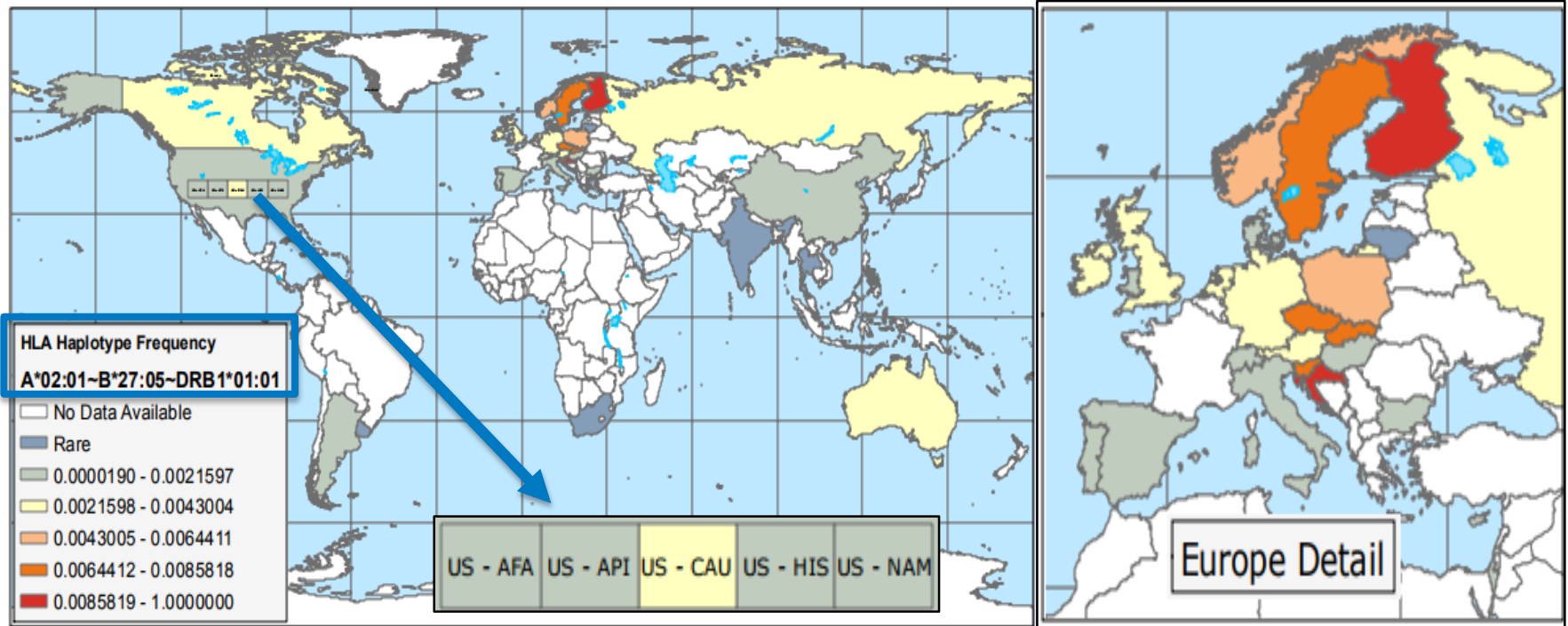
Demographics <small>Add/Remove Data</small>	MCat	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
<a href="#">Italy</a> Donor Count: 1	10/10	P A	P P	P P	A A		24:XX 02:01	51:XX 27:XX	14:XX 02:XX	11:01 01:01	
<a href="#">Denmark-BMDC</a> Donor Count: 1	10/10	P P	P P		P P	P P	s24 s2	s51 s27		11:XX 01:XX	03:XX 05:XX
<a href="#">France</a> Donor Count: 1	10/10	P P	P P		P P	P P	s24 s2	s51 s27		11:XX 01:XX	03:XX 05:XX
<a href="#">Spain</a> Donor Count: 1	10/10	P P	P P		P P		24:XX 02:XX	51:XX 27:XX		11:XX 01:XX	
<a href="#">Austria</a> Donor Count: 1	10/10	P P	P P		P P		s24 s2	s51 s27		11:XX 01:XX	
<a href="#">Portugal</a> Donor Count: 2	10/10	P P	P P		P P		24:XX 02:XX	51:XX 27:XX		11:XX 01:XX	



# What about that 2<sup>nd</sup> patient haplotype?



# More frequently seen globally



## 2<sup>nd</sup> example: Which BMDW donors to screen?

	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
Ethnicity: Hispanic	02:01	40:02	03:05	08:02	04:02
	31:01	39:05	07:02	04:07	04:02

Demographics <a href="#">Add/Remove Data</a>	MCat	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
<a href="#">Brazil</a> Donor Count: 1	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	
<a href="#">Uruguay</a> Donor Count: 1	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		s2 s31	s39 s61		s4 s8	
<a href="#">Argentina</a> Donor Count: 4	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	
<a href="#">Mexico</a> Donor Count: 2	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	

# Patient ethnic background known: Select 'splits'

Populations

<input checked="" type="checkbox"/> <b>AFA - African American</b> <input type="checkbox"/> AAFA - African American <input type="checkbox"/> AFB - African <input type="checkbox"/> CARB - Caribbean Black	<input checked="" type="checkbox"/> <b>API - Asian or Pacific Islander</b> <input type="checkbox"/> AINDI - South Asian Indian <input type="checkbox"/> FILII - Filipino <input type="checkbox"/> HAWI - Hawaiian or other Pacific Islander <input type="checkbox"/> JAPI - Japanese	<input type="checkbox"/> KORI - Korean <input type="checkbox"/> NCHI - Chinese <input type="checkbox"/> SCSEAI - Southeast Asian <input type="checkbox"/> VIET - Vietnamese
<input checked="" type="checkbox"/> <b>CAU - Caucasian</b> <input type="checkbox"/> MENAFC - Middle Eastern or North Coast of Africa <input type="checkbox"/> EURCAU - European Caucasian	<input checked="" type="checkbox"/> <b>HIS - Hispanic</b> <input checked="" type="checkbox"/> CARHIS - Caribbean Hispanic <input checked="" type="checkbox"/> MSWHIS - Mexican or Chicano <input checked="" type="checkbox"/> SCAHIS - South or Central American Hispanic	<input type="checkbox"/> <b>NAM - Native American</b> <input type="checkbox"/> AMIND - North American Indian <input type="checkbox"/> CARIBI - Caribbean Indian

Select All Clear Populations

Haplotype Loci  
A~C~B~DRB1~DQB1

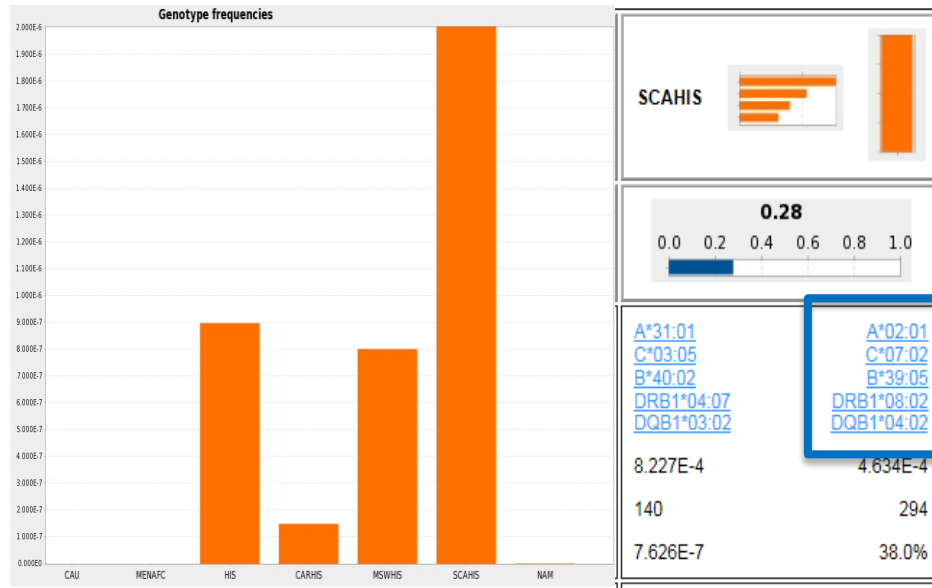
HLA type

Enter an HLA type:	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1	HLA-DRB3	HLA-DRB4	HLA-DRB5
Type 1	02:01	40:02	03:05	08:02	04:02			
Type 2	31:01	39:05	07:02	04:07	04:02			

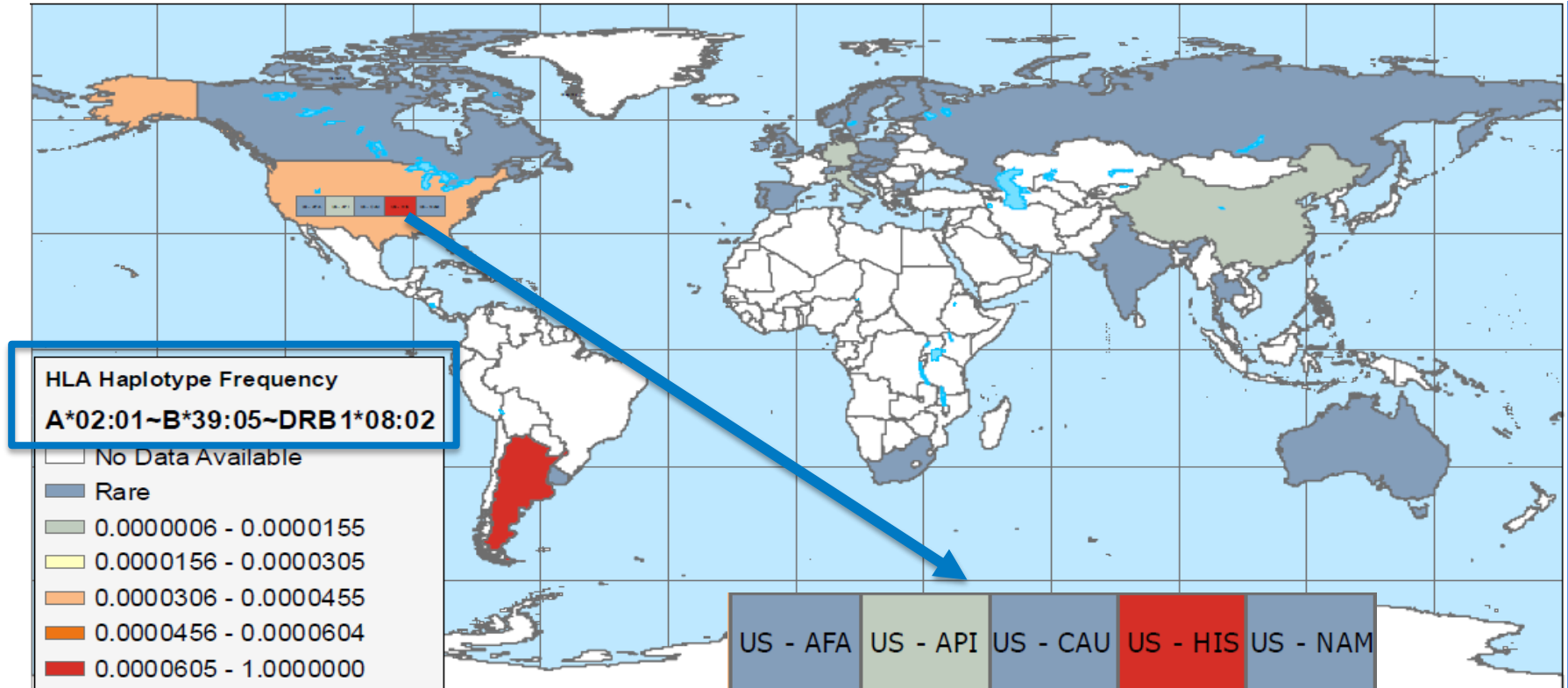
SUBMIT QUERY

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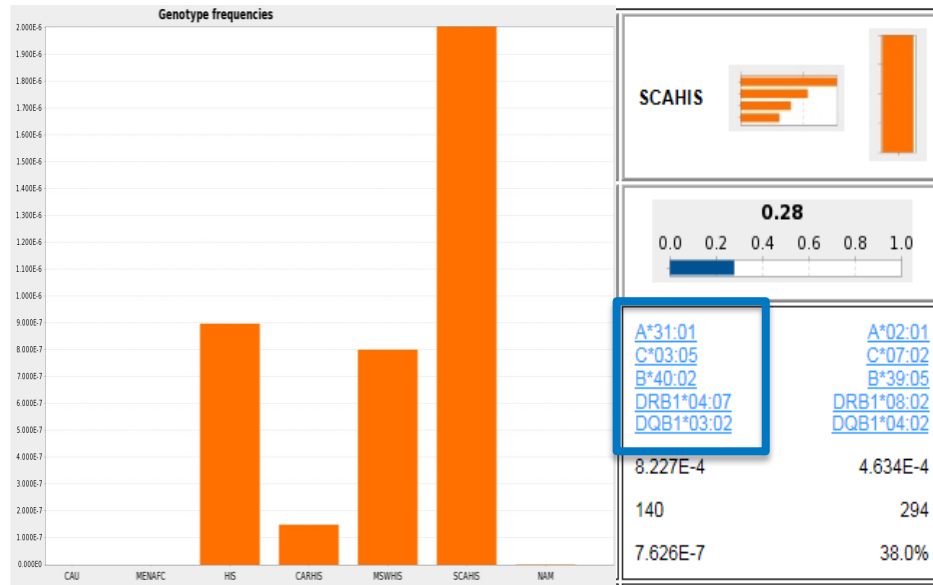
# Phased genotypes in South/Central American Hispanics (SCAHIS)



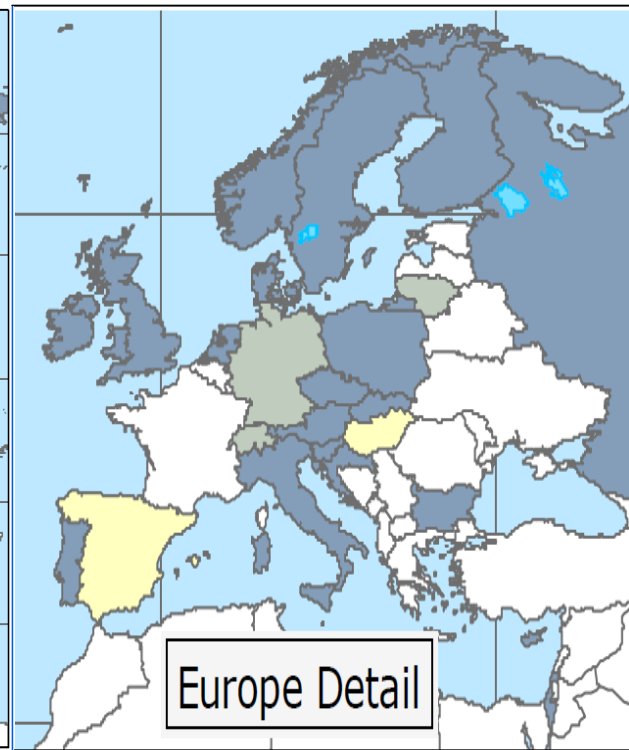
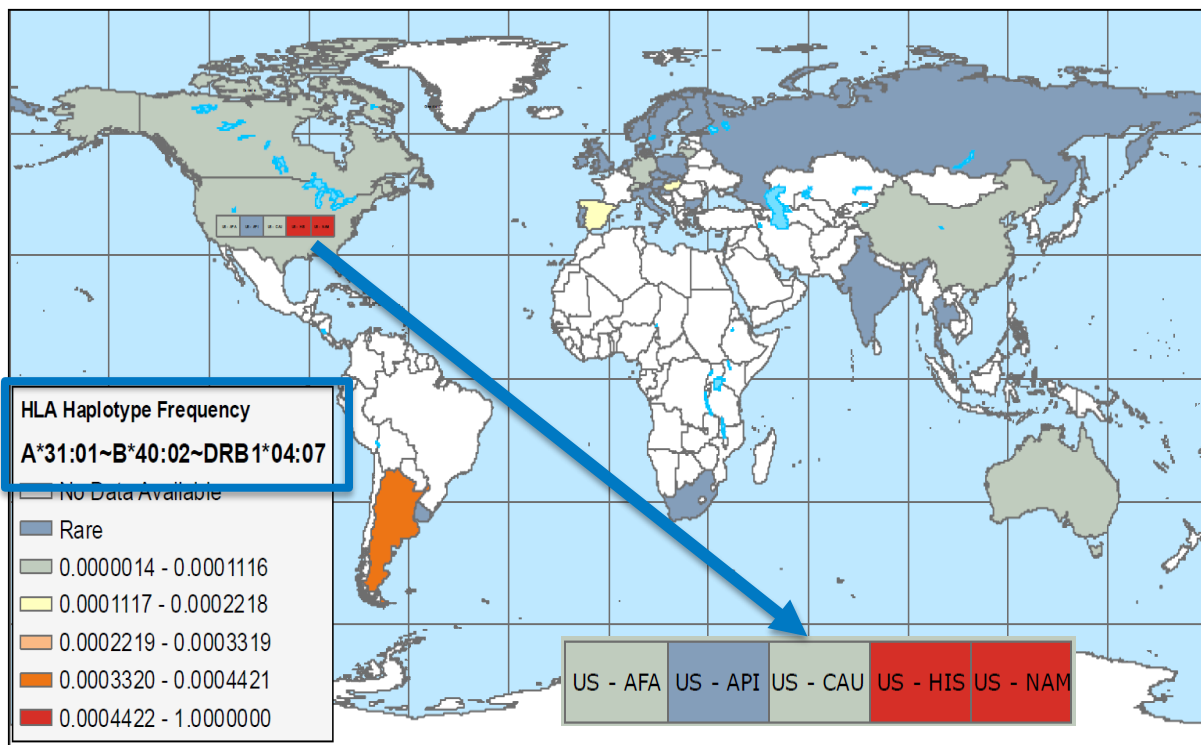
# Focus from global maps, B\*39:05 haplo: Argentina



# And the 2<sup>nd</sup> haplotype?



# 2<sup>nd</sup> A/B/DRB1 haplo map directs us to: Argentina, Spain

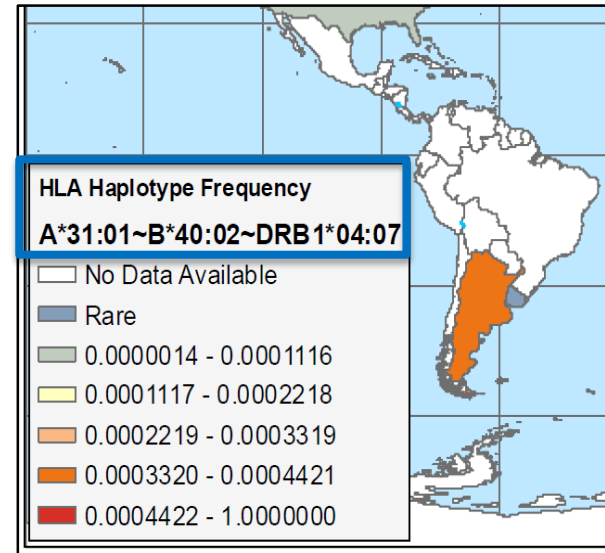
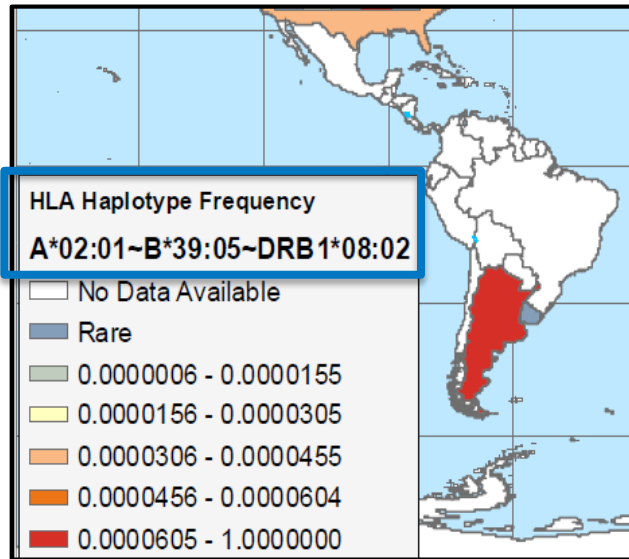




# Global maps directed us to Argentina

Demographics <a href="#">Add/Remove Data</a>	MCat	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
<a href="#">Brazil</a> Donor Count: 1	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	
<a href="#">Uruguay</a> Donor Count: 1	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		s2 s31	s39 s61		s4 s8	
<a href="#">Argentina</a> Donor Count: 4	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	
<a href="#">Mexico</a> Donor Count: 2	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	

# Rare in Uruguay, no data from Brazil or Mexico



# No registry data from Brazil or Mexico

Demographics <a href="#">Add/Remove Data</a>	MCat	A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1
<a href="#">Brazil</a> Donor Count: 1	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	
<a href="#">Uruguay</a> Donor Count: 1	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		s2 s31	s39 s61		s4 s8	
<a href="#">Argentina</a> Donor Count: 4	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	
<a href="#">Mexico</a> Donor Count: 2	10/10	<div>P</div> <div>P</div>	<div>P</div> <div>P</div>		<div>P</div> <div>P</div>		02:XX 31:XX	39:XX 40:XX		04:XX 08:XX	

# When are global haplotype maps useful?

- Limited NMDP search
  - Patient has common B-C and DRB1-DQB1 associations
- Low resolution typed BMDW donors
- You have the time and funds to screen for a 10/10 donor



# References - Less common patient alleles

- Lee, S.J. et al. *High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood* 2007, 110: 4576-4583

# Resources

- <https://bioinformatics.bethematchclinical.org/hla-resources/haplostats/>

# Stop N°2: Patient null allele



# N, S alleles: Cell surface level not expressed

Character	Example	Meaning
<b>N</b>	<b>C*04:09N</b>	<b>Protein not expressed on the cell surface</b>
L	A*24:21:01:02L	Protein expressed at low levels on cell surface
<b>S</b>	B*44:02:01:02S	Protein secreted, not on the cell surface
<b>Q</b>	A*24:02:03Q	Likely that the protein expression will not be “normal” but not tested

- Matching
  - L considered normal expression
  - N and S are consider null; some Q alleles may also be null



# C\*04:09N: Most likely haplotype

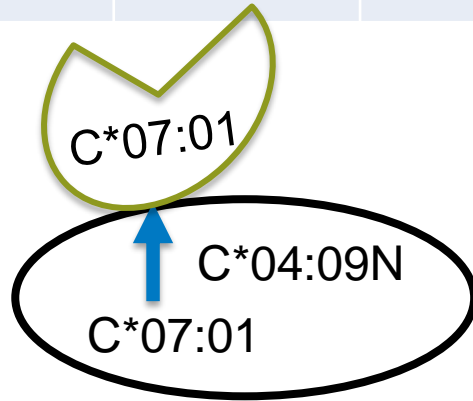
A	B	C	DRB1	DQB1
01:01	08:01	07:01	03:01	02:01
23:01	44:03	04:09N	07:01	02:02

- C\*04:09N: most often seen with B\*44:03; A\*23:01 is often present
- **A\*23:01 /B\*44:03 /DRB1\*07:01**

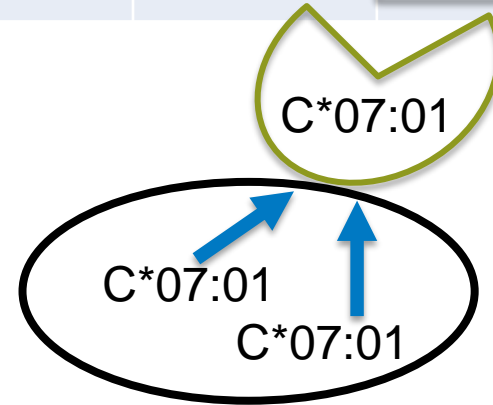
# Patient Typing

# vs What HapLogic “sees”

A	B	C
01:01	08:01	07:01
23:01	44:03	04:09N



A	B	C
02:01	40:01	07:01
23:01	44:03	07:01













# Let's look at nulls in Traxis

- How does HapLogic treat nulls for match grade assignments?

A P M

Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1 ▼	01:01 23:01	08:01 44:03	07:01 04:09N	03:01 07:01	02:01 02:02

07:01, 07:01

<a href="#">2014-1881-1</a> Age: 21 Sex: M CMV: Untested Race(Eth): White (NHIS)	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	 99	 99	 99	 99	 99	01:01 23:01	08:01 44:03	04:09N 07:01
<a href="#">2045-9863-5</a> Age: 20 Sex: F CMV: Untested Race(Eth): White (NHIS)	10/10	10/10= 99 9/10=99 8/10=99	8/8=0 7/8=99 6/8=99	 99	 99	 99	 99	 99	01:01 23:01	08:01 44:03	07:01 07:01

C locus match grade = **A** for donors typed as **C\*04:09N**, **C\*07:01** or **C\*07:01, 07:01**

Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1 ▼	01:01 23:01	08:01 44:03	07:01 04:09N	03:01 07:01	02:01 02:02

07:01, 07:01

<a href="#">1679-8671-0</a> Age: 27 Sex: F CMV: Untested Race(Eth): White (NHIS)	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	P P 99	A A 99	P P 99	A A 99	A A 99	01:BMMP 23:CJT	08:01 44:03	04:09N 07:AGCEU
<a href="#">1341-7330-1</a> Age: 34 Sex: F CMV: Untested Race(Eth): Unknown ()	10/10	10/10=99 9/10=99 8/10=99	8/8=99 7/8=99 6/8=99	P P 99	P A 99	P P 99	A A 99	P P 99	01:MNZC 23:FGSP	08:XKT 44:03	07:JECH 07:JECH

C locus match grade = **P** for donors typed as **C\*04:09N, 07:XX** or **07:allele code**; **homozygous C\*07:allele code**; **homozygous C\*07:XX**; or **serologic Cw7**

Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1 ▼	01:01 23:01	08:01 44:03	07:01 04:09N	03:01 07:01	02:01 02:02

07:01, 07:01

<a href="#">1433-1061-3</a>	9/10	10/10=0	8/8=0	P	P	P	A	P	01:RGPH	08:XKT	07:JECH
Age: 21 Sex: F CMV:		9/10=99	7/8=99	P	A	M	A	P	23:ENWE	44:03	04:NZEG
Race(Eth): White (NHIS)		8/10=99	6/8=99	99	99	0	99	99			

04:NZEG=**04:01/04:09N**/04:28/04:30/04:41/04:79/04:82/04:84/04:106

Match grade = **M** for donors typed **C\*07:01**, **04:XX** or **04:allele code** with C\*04:09N

**\*\*\*BUT** donors **could type as C\*07:01, 04:09N**; can consider C locus MG as **P\*\*\***

Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1 ▾	01:01 23:01	08:01 44:03	07:01 04:09N	03:01 07:01	02:01 02:02

07:01, 07:01

<a href="#">1442-2913-5</a>	9/10	10/10=0	8/8=0	A	P	P	P	A	01:01	08:WUHH	07:AJKDW
Age: 26 Sex: M CMV: Negative		9/10=99	7/8=99	P	A	M	P	A	23:CJT	44:03	04:AMEKR
Race(Eth): White (NHIS)		8/10=99	6/8=99	99	99	0	99	99			


04:AMEKR=04:01/04:30/04:82/04:226

A donor typed with a **C\*04:allele code** that **does not include C\*04:09N** (surprisingly) may or may not be a mismatch.

# Patients with C\*04:09N: Potential 10/10s in 2 categories

View Donor Selections

10 Allele		8 Allele	AB Only
Donor:10/10 ABCDRDQ		Total: 8	
Select	Row	Mismatch	Count
<input checked="" type="checkbox"/>	1	None	8
Donor:9/10 ABCDRDQ		Total: 1349	
<input type="checkbox"/>	2	HLA-A	85
<input type="checkbox"/>	3	HLA-B	292
<input checked="" type="checkbox"/>	4	HLA-C	30
<input type="checkbox"/>	5	HLA-DRB1	941
<input type="checkbox"/>	6	HLA-DQB1	1



Remember: **Potential 10/10 could also be in 9/10 C mismatch category**



Status	Phenotype	A	B	C	DRB1	DQB1
PRLM	Pheno 1 ▼	01:01 23:01	08:01 44:03	07:01 04:09N	03:01 07:01	02:01 02:02

07:01, 07:01

<a href="#">0141-9543-2</a>	9/10	10/10=0	8/8=0	P	P	P	A	P	s1	s8	s7
Age: 57 Sex: M CMV: Positive		9/10=92	7/8=92	P	P	M	A	P	s23	s44	s4
Race(Eth): White ()		8/10=99	6/8=99	99	99	0	99	99			

Any donor whose typing is **Cw4 serology** would be a **true mismatch**;  
(**Cw4 protein** vs. no protein expressed by C\*04:09N, serologic blank)

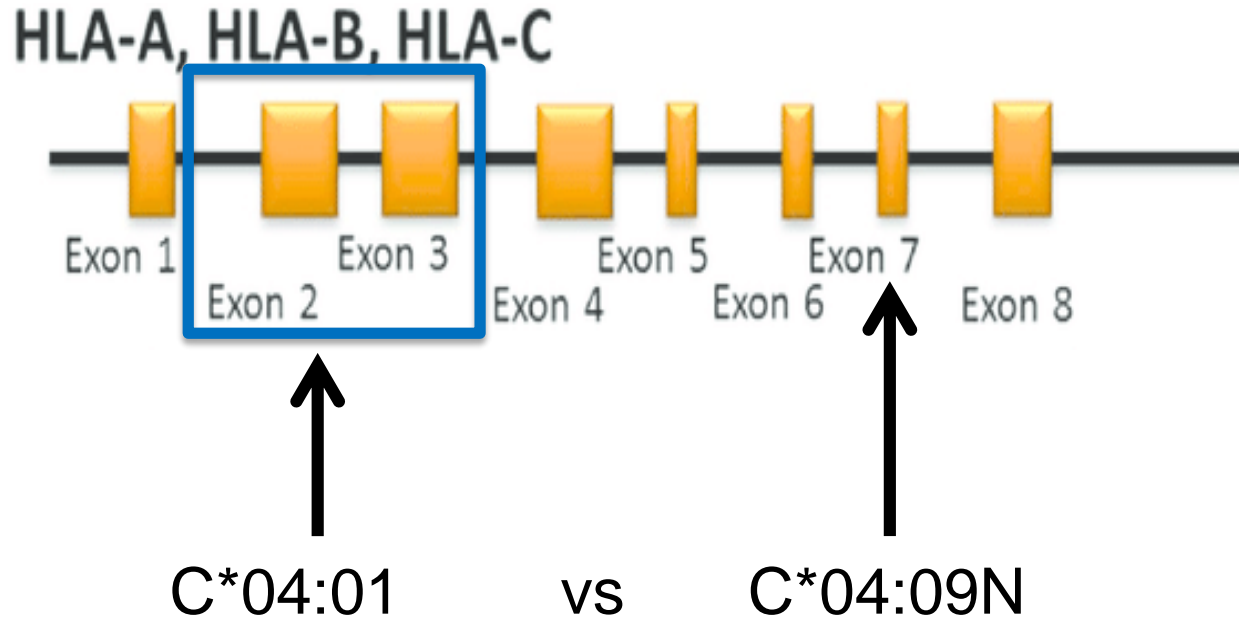
# Why should we be worried about the Nulls?

## G group (ARS) matching

- G group level matching is adequate in most cases
  - Exons 2 and 3 for Class I alleles
  - Exon 2 for Class II alleles
- Some G groups contain common null alleles with differences outside these exons



# Exons outside the ARS



## C\*04:01:01G

04:01:01:01/04:01:01:02/04:01:01:03/04:01:01:04/04:01:01:05/  
04:09N/04:28/04:30/04:41/04:79/04:82/04:84/04:106/04:144/...

- Both C\*04:01:01 and C\*04:09N are common in European ancestry
- Difference results in loss of HLA-C expression (**null** allele) and immune recognition; patient typed as C\*04:09N could develop anti-Cw4 if exposed to donor cells containing Cw4
- **Recommendation:** Test for common null alleles within a G group

# Common, well-documented (CWD) Null Alleles in G Groups

Null Allele	G Group
A*01:04N	A*01:01:01G
A*03:21N	A*03:01:01G
A*24:09N	A*24:02:01G
A*24:11N	A*24:02:01G
A*68:11N	A*68:01:02G
B*15:01:01:02N	B*15:01:01G
B*51:11N	B*51:01:01G
C*04:09N	C*04:01:01G

- These non-expressed alleles are thought to be common
- They are found within G groups
- Your lab should test for them when typing for match
- NMDP CT policy requires that these CWD null alleles in a G group be resolved

# References – Null alleles

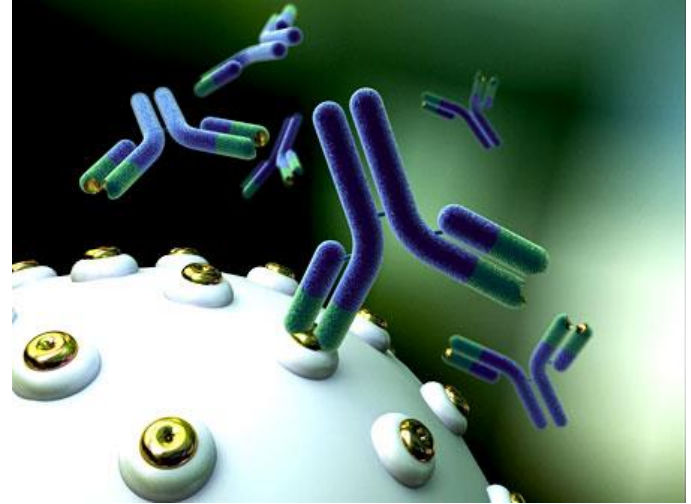
- Frequency of HLA-B\*44:03-C\*04:09N Bearing Haplotypes and Phenotypes in Leukemia Patients. ASHI 2014-Human Immunology Volume 75, Supplement, October 2014, Page 16
- HLA-Cw\*0409N is associated with HLA-A\*2301 and HLA-B\*4403-carrying haplotypes. Hum Immunol. 2004 Feb;65(2):181-7.
- Frequency of Class I Common or Well Documented Null Alleles in NMDP High Resolution Typing Programs. ASHI 2015- Human Immunology Volume 76, Supplement, October 2015, Page 133
- Limited Efficacy of Using Linkage to Identify Null Alleles in Germany- ASBMT 2016

# Stop N°3: Patient antibodies



# Patient antibodies

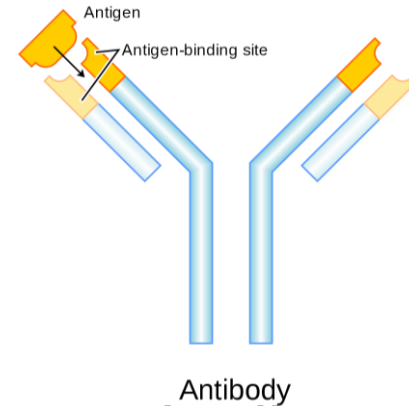
- Prevalence
- Importance
- Report timing/interpretation
- Sample dates
- Examples





# Formation/prevalence of HLA antibodies

- HLA antibodies form via:
  - Pregnancy
  - Transfusion of blood products
  - Prior stem cell transplant
- **Prevalence in patients** with hematologic diseases:  
~ 20 to 39%



Morin-Zorman, Sarah et al. *Donor-Specific Anti-HLA Antibodies in Allogeneic Hematopoietic Stem Cell Transplantation*. *Frontiers in Immunology* 7 (2016): 307.

# Patient HLA antibodies can be significant: **DSA**

- **Donor specific antibodies** (DSA) are directed against antigens carried by a prospective donor or cord blood unit
- Patient lab results are consulted to see if there are reported antibodies against antigens carried by the donors/cords being considered for activation/transplant

# Effects of Donor Specific Antibodies (DSA) on transplant

- Outcomes data:
  - **Increased risk of primary graft failure** (PGF) when patient carries DSA
  - PGF “**considerably increases...early non-relapse mortality** after allogeneic stem cell transplantation”

Morin-Zorman, Sarah et al. *Donor-Specific Anti-HLA Antibodies in Allogeneic Hematopoietic Stem Cell Transplantation*. *Frontiers in Immunology* 7 (2016): 307.

# Graft failure data: With or without DSA

Reference	Patients ( <i>n</i> )	Stem cell source	Conditioning	Anti- HLA%	DSA%	Graft failure with/without DSA
Spellman et al. (34)	115	Mismatched unrelated	RIC	ND	9	24 versus 1%
Ciurea et al. (36)	592	10/10 and 9/10 unrelated	MACorRIC	19.6	1.4	37.5 versus 2.7%
Yoshihara et al. (39)	79	Haplo-identical	RIC	20.2	14	27 versus 3%
Ciurea et al. (36)	24	Haplo-identical	RIC	ND	21	60 versus 5%
Chang et al. (40)	345	Haplo-identical	MAC	25.2	11.3	61% (MFI <sub>&gt;10,000</sub> ) versus 3.2%

Reference	Patients (n)	Stem cell source	Conditioning	Anti- HLA%	DSA%	Graft failure with/without DSA
Ciurea et al. (36)	122	Haplo-identical	Non- specified	ND	18	32 versus 4%
Takanashi et al. (41)	386	Single CBU	MAC	23.1	5	83 versus 32%
Cutler et al. (42)	73	Double CBU	MACorRIC	ND	24	57 versus 5.5%
Ruggeri et al. (43)	294	Single and double CBU	RIC	23	5	81 versus 44%
Yamamoto et al. (44)	175	Single CBU	MACorRIC	39.4	ND	50% if anti-HLA-C, DP, DQ, DRB1/2/3 versus 16%

# Timing of antibody report

**Prioritize:** Concurrent with HLA typing

- Before requesting an HLA review
- No donor/cord selection prior to pt. antibody report



# Most TCs avoid ~~all DSA~~

- This is the NMDP search strategy team's practice for HLA reviews
  - Need the patient antibody report **before** selecting or recommending donors or CBUs
- Note: There is some disagreement in the cord transplant community whether DSA need to be avoided in units

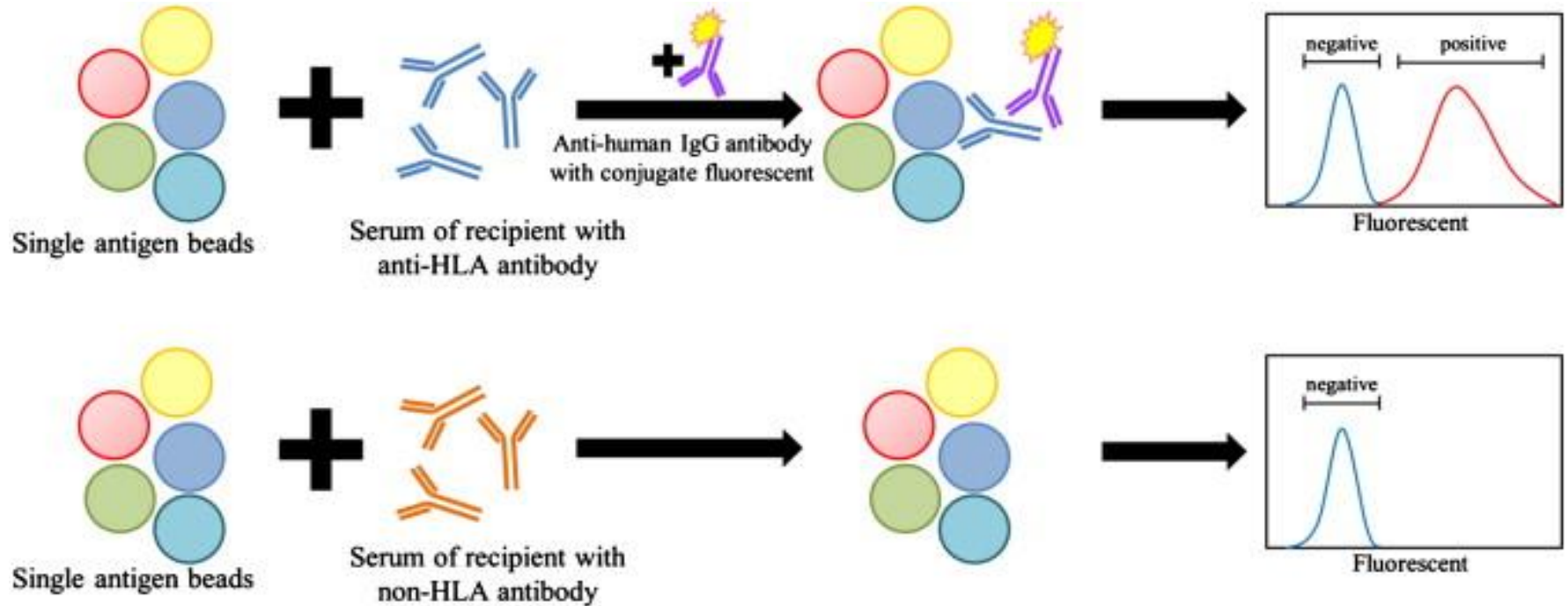
# Reading antibody reports

- Basics of antibody testing
- Strength of reactivity
- Sample date
  - Use most recent sample's report
  - 2 important reasons





# Mean fluorescence intensity (MFI) = Reactivity strength

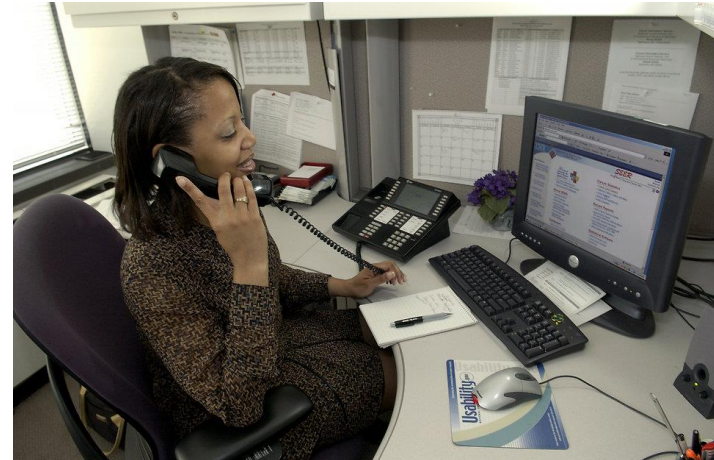


# MFI interpretation is tricky

- “Several studies have shown that higher MFI of DSA were associated with an increased rate of graft failure.
- However, there is **no consensus on a clear cut-off above which the DSA is likely to cause graft failure.**”

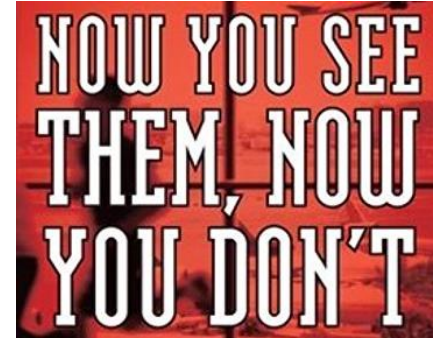
Morin-Zorman, Sarah et al. *Donor-Specific Anti-HLA Antibodies in Allogeneic Hematopoietic Stem Cell Transplantation*. *Frontiers in Immunology* 7 (2016): 307.

# Consult with your HLA lab director



# Antibody reactivity can be transient or false positives

- Causes of transient reactivity
  - Exposure to antigen (transfusion) without re-stimulation
  - Chemotherapy
  - Patient's immune system is compromised



# Initial sample report: 35 antibody specificities

Sample Date = 5/1/2017

## ANTI-HLA ANTIBODY SPECIFICITY:

A\*25:01 (MFI = 1686)

A\*32:01 (MFI = 1716.26)

B\*07:02 (MFI = 1337.88)

B\*08:01 (MFI = 4360.5)

B\*14:01 (MFI = 1583.3)

B\*14:02 (MFI = 1747.63)

B\*15:01 (MFI = 1846.15)

B\*15:02 (MFI = 2177.05)

B\*15:03 (MFI = 2371.8)

B\*15:10 (MFI = 3054.37)

B\*15:11 (MFI = 1606.11)

B\*15:12 (MFI = 1025.71)

B\*15:13 (MFI = 2146.7)

B\*15:16 (MFI = 2352.04)

B\*35:01 (MFI = 2066.22)

B\*38:01 (MFI = 2766.41)

B\*39:01 (MFI = 1137.76)

B\*42:01 (MFI = 1260.32)

B\*46:01 (MFI = 1161.24)

B\*48:01 (MFI = 3390.71)

B\*49:01 (MFI = 3196.86)

B\*50:01 (MFI = 1577.2)

B\*51:01 (MFI = 3061.06)

B\*51:02 (MFI = 3116.71)

B\*52:01 (MFI = 2347.19)

B\*53:01 (MFI = 2599.13)

B\*55:01 (MFI = 2351.39)

B\*56:01 (MFI = 2540.87)

B\*58:01 (MFI = 1068.77)

B\*59:01 (MFI = 4197.96)

B\*67:01 (MFI = 1323.84)

B\*73:01 (MFI = 1659.51)

B\*78:01 (MFI = 1486.3)

B\*81:01 (MFI = 1945)

B\*82:01 (MFI = 2454.25)

# 2<sup>nd</sup> sample report: 17 antibody specificities

AMENDMENT: Tests repeated on 6/12/2017 showing that some previously reported reactivity were false positive reactions. Specificity of antibodies are as reported here.

B\*07:02 (MFI = 1591.79)

B\*08:01 (MFI = 3234.12)

B\*14:01 (MFI = 2071.13)

B\*14:02 (MFI = 2502.68)

B\*15:03 (MFI = 1960.95)

B\*15:10 (MFI = 2259.9)

B\*38:01 (MFI = 2843.01)

B\*39:01 (MFI = 1263.97)

B\*42:01 (MFI = 1452)

B\*48:01 (MFI = 4435.86)

B\*55:01 (MFI = 2847.49)

B\*56:01 (MFI = 1289.06)

B\*59:01 (MFI = 3573.12)

B\*67:01 (MFI = 1690.26)

B\*73:01 (MFI = 2216.19)

B\*81:01 (MFI = 2976.34)

B\*82:01 (MFI = 2812.4)

Most recent sample report could reflect more antigen exposure,  
more antibody reactivity

<u>Test</u>	<u>Sample Date</u>	<u>Test Date</u>	<u>Specificity</u>
ANTIBODY ID I	01/05/2017	01/10/2017	B:82 45 44 Cw:1
ANTIBODY ID I	10/29/2012	10/31/2012	B:82

# Antibody reports & donor/cord selections

EXAMPLES Examples  
Examples EXAMPLES  
Examples Examples Examples  
Examples Examples  
Examples



# NMDP strategy team: **Current** cord selection algorithm\*

- **CD34+**
  - Single unit  $\geq 0.15 \times 10^6$  CD34+/kg; paired units  $\geq 0.10 \times 10^6$  CD34+/kg
- **TNC**
  - Single unit  $\geq 2.50 \times 10^7$  TNC/kg; paired units  $\geq 1.50 \times 10^7$  TNC/kg
- **Best matched x/8 units**
  - After minimum CD34+ and TNC doses met
- **RBC reduced**
  - Avoid RBC replete units
- **Total frozen volume ~ 25-30 ml**

\*Based on outcomes data and recommendations from cord blood consultants, 11/10/2017; may change

Would you consider requesting a CBU with this typing, given the patient's reported antibody reactivity?

## Patient antibody report

**A:** 29 43    **B:** 13 18 35 37 46 48 49 50 51 52 56 62 63

**Cw:** 10 9

**DR:** 1 16    **DR53**

A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4	DRB5	DQA1	DPB1	DPA1
  0	  0	  0	  0	  0	03:01 30:01	42:01 58:02	06:DDAR 17:MN	03:02 11:02	04:02 03:01	01:01 02:02			04:01 05:05	01:AETTA 29:01	

# Would you consider this cord blood unit for selection?

## Patient antibody report

**A:** 29 43    **B:** 13 18 35 37 46 48 49 50 51 52 56 62 63  
**Cw:** 10 9  
**DR:** 1 16    **DR53**

A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4	DRB5	DQA1	DPB1	DPA1
P	M		A		03:AAUAT	14:02		03:01							
P	P		M		33:AAAVJ	58:ADPDR		13:02							
99	0	1	0	1											

# Allele Reveal mode in Traxis

- ‘Reveals’ the most likely alleles at untyped loci

A		B		C		DRB1		DQB1		DRB3		DRB4		DRB5	
<b>P</b>	03:01g	<b>M</b>	14:02•		(03:02g)	<b>A</b>	03:01g•		(02:01g)		(02:02g)				
<b>P</b>	33:03g	<b>P</b>	58:01g		(08:02g)	<b>M</b>	13:02g•		(06:09g)		(03:01g)				
99	<u>info (d)</u>	0	<u>info (d)</u>	1	<u>info</u>	0	<u>info (d)</u>	1	<u>info</u>		<u>info</u>		<u>info</u>		<u>info</u>

# Predicted allele likelihoods in Allele Reveal

A	B	C	DRB1	DQB1	DRB3	DRB4	DRB5
<b>P</b> 03:01g <b>P</b> 33:03g 99 <u>info (d)</u>	<b>M</b> 14:02• <b>P</b> 58:01g 0 <u>info (d)</u>	(03:02g) (08:02g) 1 <u>info</u>	<b>A</b> 03:01g• <b>M</b> 13:02g• 0 <u>info (d)</u>	(02:01g) (06:09g) 1 <u>info</u>	(02:02g) (03:01g) <u>info</u>	<u>info</u>	<u>info</u>

Locus Information		
C Genotype Probabilities		
Genotype	Percent	MatchGrade
C*03:02+C*08:02	99%	M M
OK		

Locus Information		
DQB1 Genotype Probabilities		
Genotype	Percent	MatchGrade
DQB1*02:01+DQB1*06:09	94%	A M
DQB1*02:01+DQB1*05:01	3%	A M
DQB1*02:01+DQB1*06:04	3%	A M
OK		

# Broads and splits

## Patient antibody report

**A:** 29 43    **B:** 13 18 35 37 46 48 49 50 51 52 56 62 63

**Cw:** 10 9

**DR:** 1 16    **DR53**

A		B		C		DRB1		DQB1		DRB3		DRB4		DRB5	
<b>P</b>	03:01g	<b>M</b>	14:02•		(03:02g)	<b>A</b>	03:01g•		(02:01g)		(02:02g)				
<b>P</b>	33:03g	<b>P</b>	58:01g		(08:02g)	<b>M</b>	13:02g•		(06:09g)		(03:01g)				
99	<u>info (d)</u>	0	<u>info (d)</u>	1	<u>info</u>	0	<u>info (d)</u>	1	<u>info</u>		<u>info</u>		<u>info</u>		<u>info</u>

# How do these two units look for potential DSA issues?

## Patient antibody report

A: 29 43    B: 13 18 35 37 46 48 49 50 51 52 56 62 63

DR: 1 16

DR53



A		B		C	DRB1	DQB1	DRB3	DRB4	DRB5
P	03:01g	M	38:01	(03:02g)	A	03:01g	(02:01g)		
P	33:03g	P	58:01g	(12:03g)	M	07:01g	(02:01g)		
99	info (d)	0	info (d)	1 info	0	info (s)	1 info	info	info
P	03:01g	M	14:02	(03:02g)	P	03:01g	02:01g	01:01	
P	33:03g	P	58:01g	(08:02g)	M	07:01g	02:01g	02:02g	
90	info (d)	0	info (d)	1 info	0	info (d)	0 info (s)	info (d)	info

03	<b>DRB3</b>
05	
06	
11	
12	
13	
14	

<b>DNA</b>	<b>Serology</b>
DRB3	DR52
DRB4	DR53
DRB5	DR51

04	<b>DRB4</b>
07	
09	

02	<b>DRB5</b>
15	
16	

01	-
08	
10	



# Check antibody report for DRB3/4/5 serology

## Patient antibody report

A: 29 43    B: 13 18 35 37 46 48 49 50 51 52 56 62 63

Cw: 10 9

DR: 1 16 **DR53**

A		B		C	DRB1	DQB1	DRB3	DRB4	DRB5
P	03:01g	M	38:01	(03:02g) (12:03g)	A 03:01g• M 07:01g• 0 info (d)	(02:01g) (02:01g) 1 info	(02:02g) info	info	info
	33:03g		P 58:01g						
	99 info (d)		0 info (d)						
P	03:01g	M	14:02	(03:02g) (08:02g)	P 03:01g M 07:01g 0 info (d)	P 02:01g M 02:01g 0 info (s)	02:02g• info (d)	01:01 info (d)	info
	33:03g 90 info (d)		P 58:01g 0 info (d)						

**No Allele Reveal DRB4 locus info**

# Keep these loci in mind: Patient antibody reports



DPB1

DPA1

DQA1






DRB4

# Likely 10/10 donors, patients with HLA antibodies

## Patient antibody report

**A\*03:01 24:02 B\*35:03 C\*04:01 DRB1\*07:01**

**DRB3\*01:01 DPB1\*02:01**















A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4	DRB5	DPB1	DPB1 TCE
  99	  99	  99	  99	  99	02:DFKP 31:CGAJ	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03	01:AYG 02:UNV				
  99	  99	  99	  99	  99	02:AWFBC 31:AUKJP	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03					
  99	  99	  99	  99	  99	02:ACMGD 31:AAAVF	15:ACMGN 35:08	03:ABGFK 04:AAAWS	03:01 13:MJMR	02:DYCD 06:ZANE	02:RGPY 02:RGPY				
  99	  99	  99	  99	  99	02:AWFBC 31:AUKJP	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03					

# Likely 10/10 donors, patients with HLA antibodies

## Patient antibody report

A\*03:01 24:02 B\*35:03 C\*04:01 DRB1\*07:01

DRB3\*01:01 **DPB1\*02:01**

A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4	DRB5	DPB1	DPB1 TCE
					02:DFKP 31:CGAJ	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03	01:AYG 02:UNV				
					02:AWFBC 31:AUKJP	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03					
					02:ACMGD 31:AAAVF	15:ACMGN 35:08	03:ABGFK 04:AAAWS	03:01 13:MJMR	02:DYCD 06:ZANE	02:RGPY 02:RGPY				
					02:AWFBC 31:AUKJP	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03					

<b>DRB1</b>	<b>DRB3</b>	<b>DRB5</b>	
12:01	01:01 or 02:02		
12:02	03:01		
13:01	01:01 or 02:02		<i>Potential AFA 'rulebreakers'</i>
13:02	03:01		<i>Potential AFA 'rulebreakers'</i>
13:03	01:01		<i>Potential AFA 'rulebreakers'</i>
14:01	02:01		
14:02	01:01		
14:54	02:02		
15:01		01:01	<i>Potential API 'rulebreakers'</i>
15:02		01:02	<i>Potential API 'rulebreakers'</i>
15:03		01:01	<i>Potential API 'rulebreakers'</i>
16:01		02:02	
16:02		02:02	





















# Likely 10/10 donors, patients with HLA antibodies

## Patient antibody report

A\*03:01 24:02    B\*35:03    C\*04:01    DRB1\*07:01

**DRB3\*01:01**

**DPB1\*02:01**

A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4	DRB5	DPB1	DPB1 TCE
 99	 99	 99	 99	 99	02:DFKP 31:CGAJ	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03	01:AYG 02:UNV				
 99	 99	 99	 99	 99	02:AWFBC 31:AUKJP	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03					
 99	 99	 99	 99	 99	02:ACMGD 31:AAAVF	15:ACMGN 35:08	03:ABGFK 04:AAAWS	03:01 13:MMR	02:DYCD 06:ZANE	02:RGPY 02:RGPY				
 99	 99	 99	 99	 99	02:AWFBC 31:AUKJP	15:ACMGP 35:08	03:AWFCC 04:AWFCF	03:01 13:01	02:01 06:03					

# Patient antibody recap

- Prioritize patient antibody screening
  - Wait for patient antibody report before requesting an HLA review or activating donors/CBUs
- Consult with your HLA lab director
  - Antibody reactivity (MFI) interpretation
- Ensure you have the most recent sample's report
- Avoid selecting donors/CBUs with DSA issue
  - Remember DRB3/4/5, DPB1, DPA1, DQA1

# References - Patient antibodies

- Morin-Zorman, Sarah et al. *Donor-Specific Anti-HLA Antibodies in Allogeneic Hematopoietic Stem Cell Transplantation*. *Frontiers in Immunology* 7 (2016): 307. PMC. Web. 10 Oct. 2017.
- Barker, Juliet N et al. *Optimal Practices in Unrelated Donor Cord Blood Transplantation for Hematologic Malignancies*. *Biol Blood Marrow Transplant*. 2017 Jun;23(6):882-896. doi: 10.1016/j.bbmt.2017.03.006. Epub 2017 Mar 6.
- Hollenbach, Jill A et al. *A Combined DPA1-DPB1 Amino Acid Epitope Is the Primary Unit of Selection on the HLA-DP Heterodimer*. *Immunogenetics* 64.8 (2012): 559–569. PMC. Web. 2 Nov. 2017.



# Resources

- <https://www.haplostats.org/haplostats?execution=e1s1>
- [search-strategies@nmdp.org](mailto:search-strategies@nmdp.org)
  - HLA and/or search strategy questions
  - Request Allele Reveal
- [SSARequest@nmdp.org](mailto:SSARequest@nmdp.org)
  - Request an HLA review

# Thanks for traveling with me!



# Evaluation Reminder

- Please complete the Council Meeting 2017 evaluation in order to receive continuing education credits and to provide suggestions for future topics

We appreciate your feedback!

# You're invited!

