

COUNCIL MEETING*Sharing Our Passion for Life*

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

Miranda Bauer, PhD, Laboratory Services Team
NMDP Immunogenetics Operations and Research (IgOR)



BE THE MATCH

COUNCIL MEETING: *Sharing Our Passion For Life*

Disclosures

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

Name	Institution
Miranda Bauer	Be The Match



BE THE MATCH

COUNCIL MEETING: *Sharing Our Passion For Life*

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

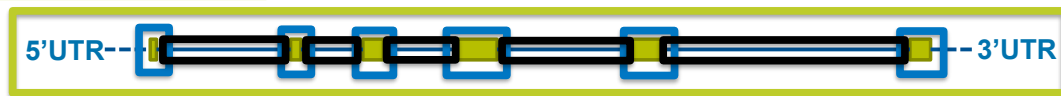
Road Map

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?

Welcome!

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 Δ 32 genotyping

NGS Typed Donors in Traxis

TC999 - National Marrow Donor Registry

Find Patient, Donor or Cord [Look-Up](#) [Advanced](#)

Mrinda Bauer (bawb2)

Home | New Patient | Allele Lookup

[212-929-3 PATIENT, NEW](#)

Local ID: Age: 3 Sex: F

Center: 500

Race(Eth): White - Unspecified (HIS)

Weight: 15kg

CMV: Negative

Disease: SCID

ABO: O+

Status: PRM

Phenotype: Pheno 1

02:ANGA 15:01 03:04 15:02 06:01 06:01 02:01

02:ANGA 52:01 12:02 04:01 03:02 04:01

Maintenance | Help | Logout

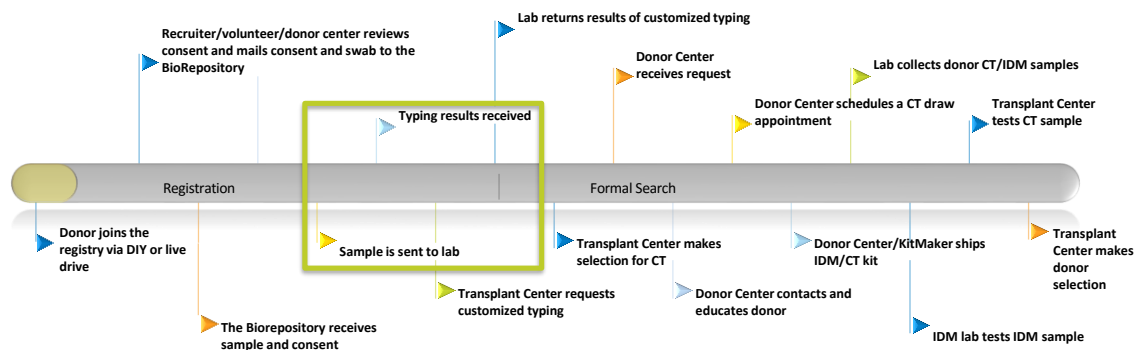
DRB1 DRB3 DRB4 DRB5 DPB1

Go to: 1 2 3 4 5 >>>> Find NMDP Donor List - Default Request

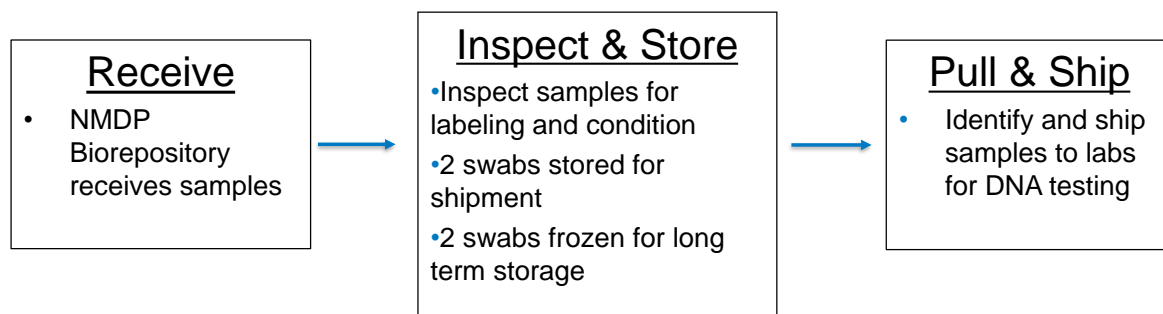
Donor List: 22,419

Ref	Demographics <small>Age/Sex/Donor Data</small>	CMV	Hcat	Pr(s) of 10 (%)	Pr(s) of 8 (%)	A	B	C	DRB1	DRB1 A	B	C	DRB1	DRB1	DRB3	DRB4	DRB5	DPB1	DPB1 TCE
AV 1	1944-2753-0 Age: 18 Sex: F CMV: Untested Race(Eth): White (HIS)	ABO: O+	1	10/10	10/10-99 8/8-99 7/8-99 6/8-99	P	A	A	A	A	02:ANGA 52:01	03:04 12:02	15:02 04:01	06:01 03:02				04:01 04:01	Permissive
AV 2	1473-0247-9 Age: 19 Sex: F CMV: Untested Race(Eth): White (HIS)	ABO: A-	1	10/10	10/10-99 8/8-99 7/8-99 6/8-99	P	A	A	A	A	02:ANGA 52:01	03:04 12:02	15:02 04:01	06:01 03:02				02:AFCK 03:FIND	Nonpermissive
AV 3	2019-8494-7 Age: 20 Sex: F CMV: Untested Race(Eth): White (HIS)	ABO: O+	1	10/10	10/10-99 8/8-99 7/8-99 6/8-99	P	A	A	A	A	02:ANGA 52:01	03:04 12:02	15:02 04:01	06:01 03:02				03:ACHK 04:JETT	Nonpermissive

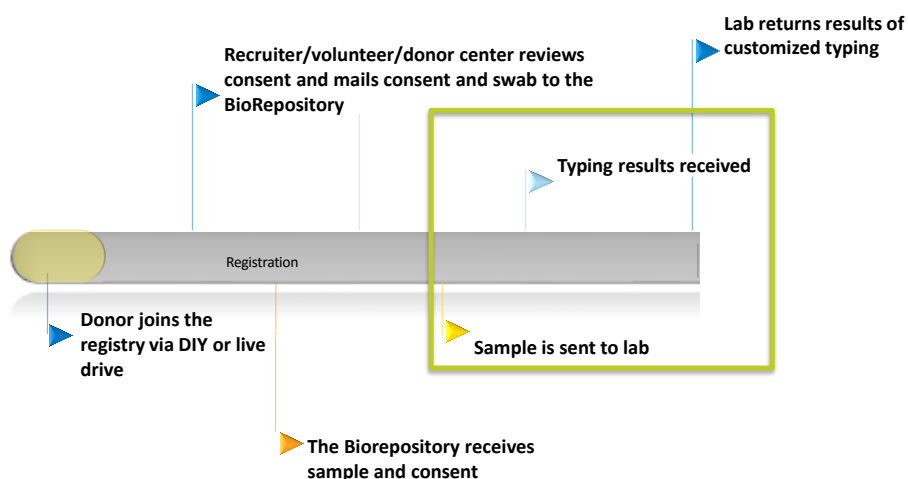
The Path to Testing (and Beyond)



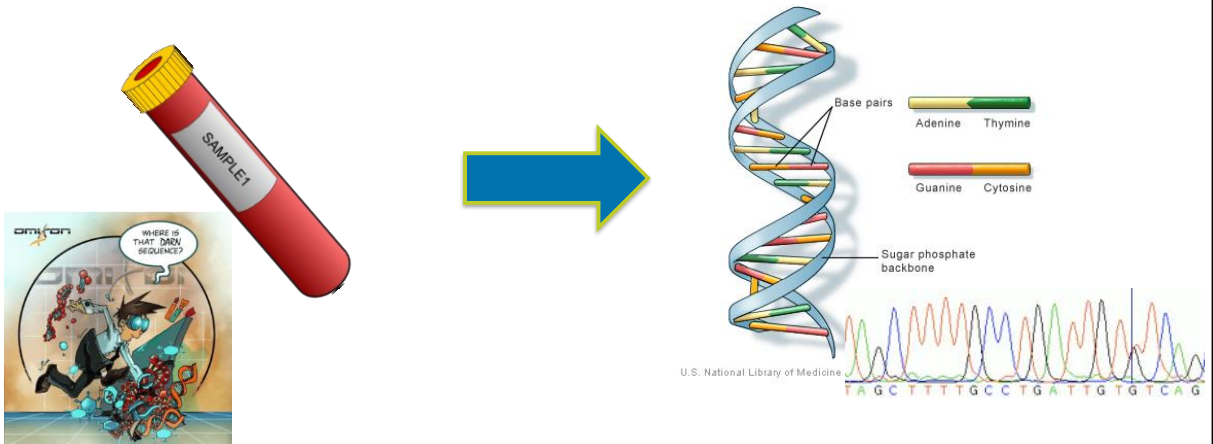
Before A Donor Sample Arrives at Lab



Where Does Testing Occur in the Path?

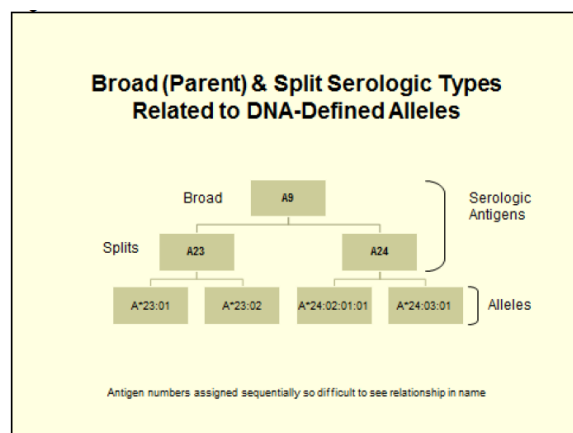


Chapter 2: HLA Typing History to Present



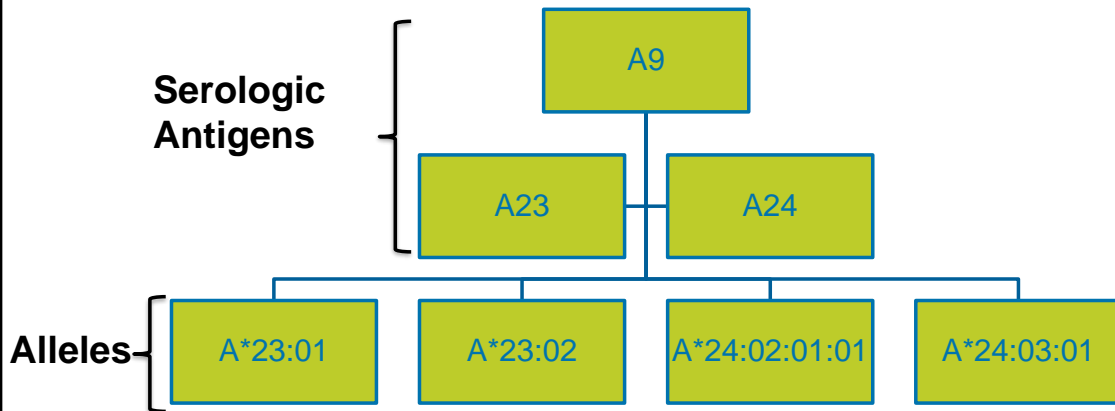
Serology: A Broad Sweep of HLA

- 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

TGGATTGGTCCATGTTGTGTGATTCAAGTGGTTTGTTCCT
GAGATTGCTCCATGTTGTGTGATTCAAGTGGTTTGTTCCT



NGS Sequencing

TGGATTGGTCCATGTTGTGTGATTCAAGTGGTTTGTTCCT

<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



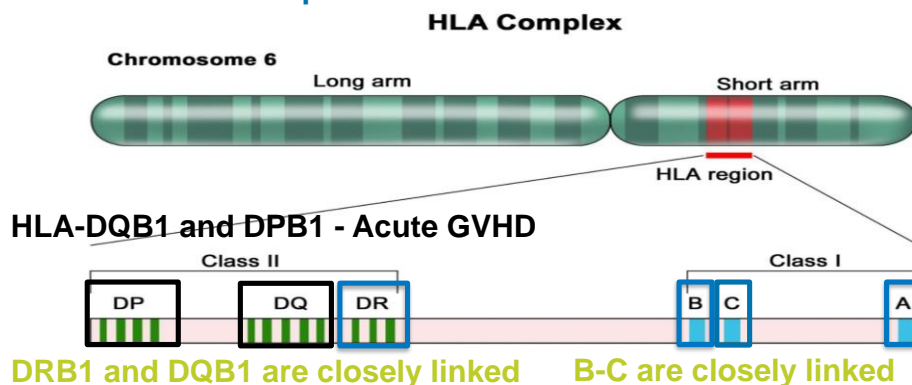
NMDP's Goal With Adoption of NGS

- 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

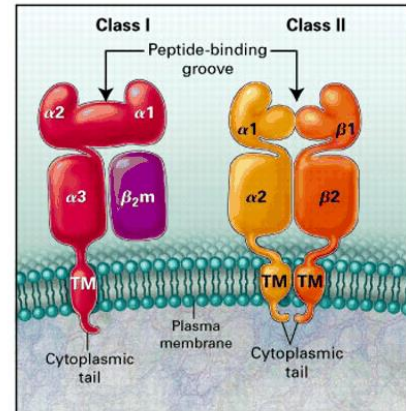
HLA-C also affects transplant outcome



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



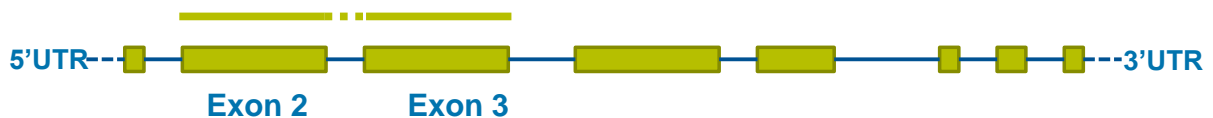
*Jan Klein, Ph.D. and Akie Sato, Ph.D.
Volume 343(10):702-709, Sep 7, 2000
Volume 343(11):782-786, Sep14, 2000*

A Deeper Dive Into the ARS

Class I: HLA-A ≈ 1 kb

$\approx 20\%$ of total gene

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

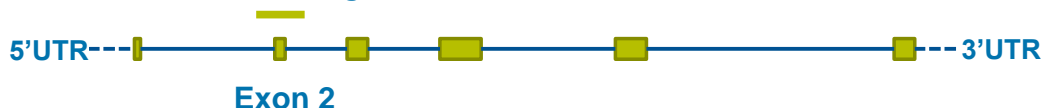


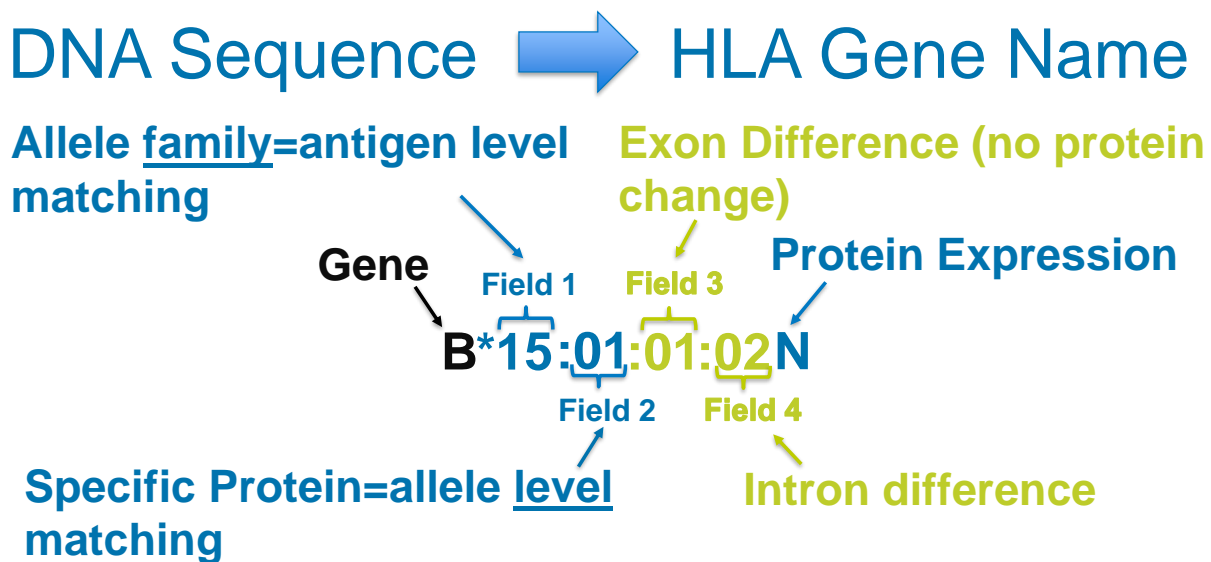
Class II: HLA-DRB1

≈ 300 bp

$\approx 2\%$ of total gene

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





Summary of HLA Typing History

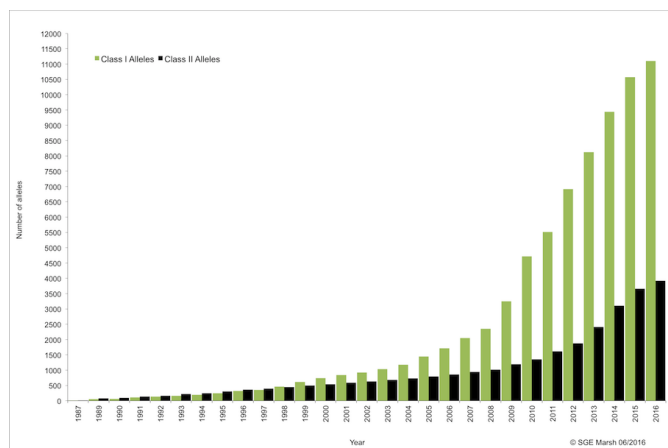
- Several HLA screening methodologies have been utilized over the years, each increasing in specificity in an effort to improve HSCT outcomes
- The number of HLA loci screened has also increased with the intent of improving HSCT outcomes
- Historically, the ARS region of the HLA molecule was primarily 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

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 in donors from Germany, the United States, and Poland.

Hernández-Frederick CJ¹, Careb 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 C¹, Ronce 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, of two new allelic variants: Cw*1801 and Cw*0706. The former allele, initially 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 structure 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 F

PMID: 9008313

[PubMed - indexed for MEDLINE]

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

© 2015 The Authors. HLA published by John Wiley & Sons Ltd

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

A possible new HLA-DR allele.

Rehuel H, Gebuhrer L, Lambert J, Freidel AC, Farre A

Abstract

It 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.12394.

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

Hernández-Frederick CJ¹, Giani AS, Careb 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.

© 2014 The Authors. *Tissue Antigens* published by John Wiley & Sons Ltd.

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



COUNCIL MEETING: Sharing Our Passion For Life

28

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



COUNCIL MEETING: Sharing Our Passion For Life

29

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

Phased
Allele 1

Phased
Allele 2



Unphased
Double Strand

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

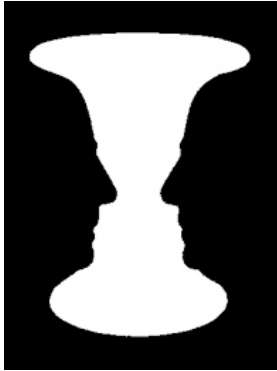
- Primary strategy for NMDP recruitment typing
- Longest, continuous reads in phase
 - 3-6kb on average
- Ability to accurately perform whole gene sequencing for HLA Class I genes in one read
- Short run time of .5-6hrs



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

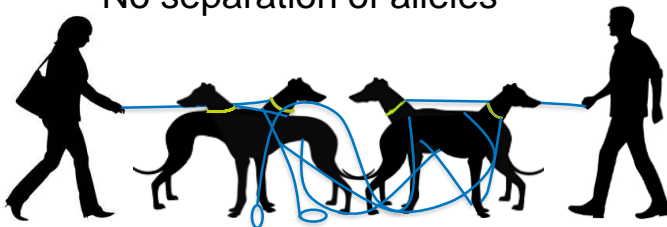
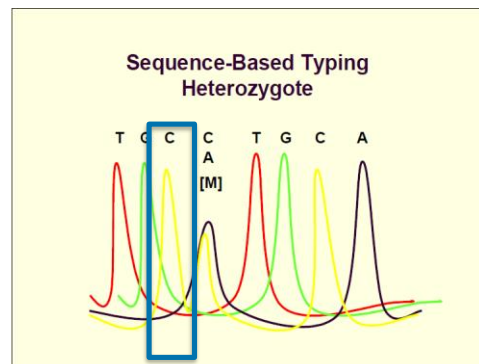
Chapter 4: Demystifying Ambiguous Pairs



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 <input type="button" value="v"/>	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

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*02:30 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
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

CMV: Negative	Status	Phenotype	A			B		C		DRB1		DQB1	
Disease: AML			PRLM	Pheno 1 ▾	02:AKBTP	07:AGUJE	07:ABXAM	13:AHPNG	06:YZWM				
ABO: B-					03:AKBT	02:01 02:30	02:43N 02:305N	15:ADPGK	06:03				
<input type="text"/>		<input type="button" value="Find"/>	<input type="text" value="NMDP Donor List - Default ▾"/>		<input type="button" value="Request"/>								
A	B	C	DRB1	DQB1	A	B	C	DRB1	DQB1	DRB3	DRB4		
<div>P</div>	<div>P</div>	<div>P</div>	<div>P</div>	<div>P</div>	02:01	07:TDVB	07:02	13:01	06:02	01:FX			
<div>P</div>	<div>A</div>	<div>P</div>	<div>P</div>	<div>A</div>	03:XKS	38:01	12:AFCTH	15:01	06:03				
95	99	99	99	99									

Patient Search with Ambiguous Alleles (cont.)

NATIONAL MARROW DONOR PROGRAM

Home | [About Us](#) | [Ala Linkup](#)

TC99 - National Marrow Donor Registry

[Basic](#)
[Advanced](#)

Wanda Burr (parent)

208-522-5 AMBIGUOUS PAT

Local ID: 800

Center: 300

Phenotype: White - Unspecified (NHIS)

Home | [About Us](#) | [Ala Linkup](#)

Donor ID: 97,274

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952

Patient Search with Ambiguous Alleles (cont.)

- Some donors also have ambiguous typing

P+	P+	P	P+
91	99	99	98
P	P	P	P
91	99	99	98

Patient Search with Ambiguous Alleles (cont.)

- Other donors are defined A*02:30

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

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/GBT 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.

NATIONAL MARROW DONOR PROGRAM **Traxis®** **TC500 - NMDP Alternative Phenotype** For Patient, Donor & Cord **Log On / Advanced** Utricle River (subset)

Home | New Patient | Allie Lookup

NEW-200-1 NULL ALLELE REGS **Weight: 55kg** **Age: 22** **CHV: Negative** **Status** **Phenotype** **A** **B** **C** **DRB1** **DQB1** **DRB3** **DRB4** **DRB5** **DRB6** **DRB7** **DRB8** **DRB9** **DRB10** **DRB11** **DRB12** **DRB13** **DRB14** **DRB15** **DRB16** **DRB17** **DRB18** **DRB19** **DRB20** **DRB21** **DRB22** **DRB23** **DRB24** **DRB25** **DRB26** **DRB27** **DRB28** **DRB29** **DRB30** **DRB31** **DRB32** **DRB33** **DRB34** **DRB35** **DRB36** **DRB37** **DRB38** **DRB39** **DRB40** **DRB41** **DRB42** **DRB43** **DRB44** **DRB45** **DRB46** **DRB47** **DRB48** **DRB49** **DRB50** **DRB51** **DRB52** **DRB53** **DRB54** **DRB55** **DRB56** **DRB57** **DRB58** **DRB59** **DRB60** **DRB61** **DRB62** **DRB63** **DRB64** **DRB65** **DRB66** **DRB67** **DRB68** **DRB69** **DRB70** **DRB71** **DRB72** **DRB73** **DRB74** **DRB75** **DRB76** **DRB77** **DRB78** **DRB79** **DRB80** **DRB81** **DRB82** **DRB83** **DRB84** **DRB85** **DRB86** **DRB87** **DRB88** **DRB89** **DRB90** **DRB91** **DRB92** **DRB93** **DRB94** **DRB95** **DRB96** **DRB97** **DRB98** **DRB99** **DRB100** **DRB101** **DRB102** **DRB103** **DRB104** **DRB105** **DRB106** **DRB107** **DRB108** **DRB109** **DRB110** **DRB111** **DRB112** **DRB113** **DRB114** **DRB115** **DRB116** **DRB117** **DRB118** **DRB119** **DRB120** **DRB121** **DRB122** **DRB123** **DRB124** **DRB125** **DRB126** **DRB127** **DRB128** **DRB129** **DRB130** **DRB131** **DRB132** **DRB133** **DRB134** **DRB135** **DRB136** **DRB137** **DRB138** **DRB139** **DRB140** **DRB141** **DRB142** **DRB143** **DRB144** **DRB145** **DRB146** **DRB147** **DRB148** **DRB149** **DRB150** **DRB151** **DRB152** **DRB153** **DRB154** **DRB155** **DRB156** **DRB157** **DRB158** **DRB159** **DRB160** **DRB161** **DRB162** **DRB163** **DRB164** **DRB165** **DRB166** **DRB167** **DRB168** **DRB169** **DRB170** **DRB171** **DRB172** **DRB173** **DRB174** **DRB175** **DRB176** **DRB177** **DRB178** **DRB179** **DRB180** **DRB181** **DRB182** **DRB183** **DRB184** **DRB185** **DRB186** **DRB187** **DRB188** **DRB189** **DRB190** **DRB191** **DRB192** **DRB193** **DRB194** **DRB195** **DRB196** **DRB197** **DRB198** **DRB199** **DRB200** **DRB201** **DRB202** **DRB203** **DRB204** **DRB205** **DRB206** **DRB207** **DRB208** **DRB209** **DRB210** **DRB211** **DRB212** **DRB213** **DRB214** **DRB215** **DRB216** **DRB217** **DRB218** **DRB219** **DRB220** **DRB221** **DRB222** **DRB223** **DRB224** **DRB225** **DRB226** **DRB227** **DRB228** **DRB229** **DRB230** **DRB231** **DRB232** **DRB233** **DRB234** **DRB235** **DRB236** **DRB237** **DRB238** **DRB239** **DRB240** **DRB241** **DRB242** **DRB243** **DRB244** **DRB245** **DRB246** **DRB247** **DRB248** **DRB249** **DRB250** **DRB251** **DRB252** **DRB253** **DRB254** **DRB255** **DRB256** **DRB257** **DRB258** **DRB259** **DRB260** **DRB261** **DRB262** **DRB263** **DRB264** **DRB265** **DRB266** **DRB267** **DRB268** **DRB269** **DRB270** **DRB271** **DRB272** **DRB273** **DRB274** **DRB275** **DRB276** **DRB277** **DRB278** **DRB279** **DRB280** **DRB281** **DRB282** **DRB283** **DRB284** **DRB285** **DRB286** **DRB287** **DRB288** **DRB289** **DRB290** **DRB291** **DRB292** **DRB293** **DRB294** **DRB295** **DRB296** **DRB297** **DRB298** **DRB299** **DRB300** **DRB301** **DRB302** **DRB303** **DRB304** **DRB305** **DRB306** **DRB307** **DRB308** **DRB309** **DRB310** **DRB311** **DRB312** **DRB313** **DRB314** **DRB315** **DRB316** **DRB317** **DRB318** **DRB319** **DRB320** **DRB321** **DRB322** **DRB323** **DRB324** **DRB325** **DRB326** **DRB327** **DRB328** **DRB329** **DRB330** **DRB331** **DRB332** **DRB333** **DRB334** **DRB335** **DRB336** **DRB337** **DRB338** **DRB339** **DRB340** **DRB341** **DRB342** **DRB343** **DRB344** **DRB345** **DRB346** **DRB347** **DRB348** **DRB349** **DRB350** **DRB351** **DRB352** **DRB353** **DRB354** **DRB355** **DRB356** **DRB357** **DRB358** **DRB359** **DRB360** **DRB361** **DRB362** **DRB363** **DRB364** **DRB365** **DRB366** **DRB367** **DRB368** **DRB369** **DRB370** **DRB371** **DRB372** **DRB373** **DRB374** **DRB375** **DRB376** **DRB377** **DRB378** **DRB379** **DRB380** **DRB381** **DRB382** **DRB383** **DRB384** **DRB385** **DRB386** **DRB387** **DRB388** **DRB389** **DRB390** **DRB391** **DRB392** **DRB393** **DRB394** **DRB395** **DRB396** **DRB397** **DRB398** **DRB399** **DRB400** **DRB401** **DRB402** **DRB403** **DRB404** **DRB405** **DRB406** **DRB407** **DRB408** **DRB409** **DRB410** **DRB411** **DRB412** **DRB413** **DRB414** **DRB415** **DRB416** **DRB417** **DRB418** **DRB419** **DRB420** **DRB421** **DRB422** **DRB423** **DRB424** **DRB425** **DRB426** **DRB427** **DRB428** **DRB429** **DRB430** **DRB431** **DRB432** **DRB433** **DRB434** **DRB435** **DRB436** **DRB437** **DRB438** **DRB439** **DRB440** **DRB441** **DRB442** **DRB443** **DRB444** **DRB445** **DRB446** **DRB447** **DRB448** **DRB449** **DRB450** **DRB451** **DRB452** **DRB453** **DRB454** **DRB455** **DRB456** **DRB457** **DRB458** **DRB459** **DRB460** **DRB461** **DRB462** **DRB463** **DRB464** **DRB465** **DRB466** **DRB467** **DRB468** **DRB469** **DRB470** **DRB471** **DRB472** **DRB473** **DRB474** **DRB475** **DRB476** **DRB477** **DRB478** **DRB479** **DRB480** **DRB481** **DRB482** **DRB483** **DRB484** **DRB485** **DRB486** **DRB487** **DRB488** **DRB489** **DRB490** **DRB491** **DRB492** **DRB493** **DRB494** **DRB495** **DRB496** **DRB497** **DRB498** **DRB499** **DRB500** **DRB501** **DRB502** **DRB503** **DRB504** **DRB505** **DRB506** **DRB507** **DRB508** **DRB509** **DRB510** **DRB511** **DRB512** **DRB513** **DRB514** **DRB515** **DRB516** **DRB517** **DRB518** **DRB519** **DRB520** **DRB521** **DRB522** **DRB523** **DRB524** **DRB525** **DRB526** **DRB527** **DRB528** **DRB529** **DRB530** **DRB531** **DRB532** **DRB533** **DRB534** **DRB535** **DRB536** **DRB537** **DRB538** **DRB539** **DRB540** **DRB541** **DRB542** **DRB543** **DRB544** **DRB545** **DRB546** **DRB547** **DRB548** **DRB549** **DRB550** **DRB551** **DRB552** **DRB553** **DRB554** **DRB555** **DRB556** **DRB557** **DRB558** **DRB559** **DRB560** **DRB561** **DRB562** **DRB563** **DRB564** **DRB565** **DRB566** **DRB567** **DRB568** **DRB569** **DRB570** **DRB571** **DRB572** **DRB573** **DRB574** **DRB575** **DRB576** **DRB577** **DRB578** **DRB579** **DRB580** **DRB581** **DRB582** **DRB583** **DRB584** **DRB585** **DRB586** **DRB587** **DRB588** **DRB589** **DRB590** **DRB591** **DRB592** **DRB593** **DRB594** **DRB595** **DRB596** **DRB597** **DRB598** **DRB599** **DRB600** **DRB601** **DRB602** **DRB603** **DRB604** **DRB605** **DRB606** **DRB607** **DRB608** **DRB609** **DRB610** **DRB611** **DRB612** **DRB613** **DRB614** **DRB615** **DRB616** **DRB617** **DRB618** **DRB619** **DRB620** **DRB621** **DRB622** **DRB623** **DRB624** **DRB625** **DRB626** **DRB627** **DRB628** **DRB629** **DRB630** **DRB631** **DRB632** **DRB633** **DRB634** **DRB635** **DRB636** **DRB637** **DRB638** **DRB639** **DRB640** **DRB641** **DRB642** **DRB643** **DRB644** **DRB645** **DRB646** **DRB647** **DRB648** **DRB649** **DRB650** **DRB651** **DRB652** **DRB653** **DRB654** **DRB655** **DRB656** **DRB657** **DRB658** **DRB659** **DRB660** **DRB661** **DRB662** **DRB663** **DRB664** **DRB665** **DRB666** **DRB667** **DRB668** **DRB669** **DRB670** **DRB671** **DRB672** **DRB673** **DRB674** **DRB675** **DRB676** **DRB677** **DRB678** **DRB679** **DRB680** **DRB681** **DRB682** **DRB683** **DRB684** **DRB685** **DRB686** **DRB687** **DRB688** **DRB689** **DRB690** **DRB691** **DRB692** **DRB693** **DRB694** **DRB695** **DRB696** **DRB697** **DRB698** **DRB699** **DRB700** **DRB701** **DRB702** **DRB703** **DRB704** **DRB705** **DRB706** **DRB707** **DRB708** **DRB709** **DRB710** **DRB711** **DRB712** **DRB713** **DRB714** **DRB715** **DRB716** **DRB717** **DRB718** **DRB719** **DRB720** **DRB721** **DRB722** **DRB723** **DRB724** **DRB725** **DRB726** **DRB727** **DRB728** **DRB729** **DRB730** **DRB731** **DRB732** **DRB733** **DRB734** **DRB735** **DRB736** **DRB737** **DRB738** **DRB739** **DRB740** **DRB741** **DRB742** **DRB743** **DRB744** **DRB745** **DRB746** **DRB747** **DRB748** **DRB749** **DRB750** **DRB751** **DRB752** **DRB753** **DRB754** **DRB755** **DRB756** **DRB757** **DRB758** **DRB759** **DRB760** **DRB761** **DRB762** **DRB763** **DRB764** **DRB765** **DRB766** **DRB767** **DRB768** **DRB769** **DRB770** **DRB771** **DRB772** **DRB773** **DRB774** **DRB775** **DRB776** **DRB777** **DRB778** **DRB779** **DRB780** **DRB781** **DRB782** **DRB783** **DRB784** **DRB785** **DRB786** **DRB787** **DRB788** **DRB789** **DRB790** **DRB791** **DRB792** **DRB793** **DRB794** **DRB795** **DRB796** **DRB797** **DRB798** **DRB799** **DRB800** **DRB801** **DRB802** **DRB803** **DRB804** **DRB805** **DRB806** **DRB807** **DRB808** **DRB809** **DRB810** **DRB811** **DRB812** **DRB813** **DRB814** **DRB815** **DRB816** **DRB817** **DRB818** **DRB819** **DRB820** **DRB821** **DRB822** **DRB823** **DRB824** **DRB825** **DRB826** **DRB827** **DRB828** **DRB829** **DRB830** **DRB831** **DRB832** **DRB833** **DRB834** **DRB835** **DRB836** **DRB837** **DRB838** **DRB839** **DRB840** **DRB841** **DRB842** **DRB843** **DRB844** **DRB845** **DRB846** **DRB847** **DRB848** **DRB849** **DRB850** **DRB851** **DRB852** **DRB853** **DRB854** **DRB855** **DRB856** **DRB857** **DRB858** **DRB859** **DRB860** **DRB861** **DRB862** **DRB863** **DRB864** **DRB865** **DRB866** **DRB867** **DRB868** **DRB869** **DRB870** **DRB871** **DRB872** **DRB873** **DRB874** **DRB875** **DRB876** **DRB877** **DRB878** **DRB879** **DRB880** **DRB881** **DRB882** **DRB883** **DRB884** **DRB885** **DRB886** **DRB887** **DRB888** **DRB889** **DRB890** **DRB891** **DRB892** **DRB893** **DRB894** **DRB895** **DRB896** **DRB897** **DRB898** **DRB899** **DRB900** **DRB901** **DRB902** **DRB903** **DRB904** **DRB905** **DRB906** **DRB907** **DRB908** **DRB909** **DRB910** **DRB911** **DRB912** **DRB913** **DRB914** **DRB915** **DRB916** **DRB917** **DRB918** **DRB919** **DRB920** **DRB921** **DRB922** **DRB923** **DRB924** **DRB925** **DRB926** **DRB927** **DRB928** **DRB929** **DRB930** **DRB931** **DRB932** **DRB933** **DRB934** **DRB935** **DRB936** **DRB937** **DRB938** **DRB939** **DRB940** **DRB941** **DRB942** **DRB943** **DRB944** **DRB945** **DRB946** **DRB947** **DRB948** **DRB949** **DRB950** **DRB951** **DRB952** **DRB953** **DRB954** **DRB955** **DRB956** **DRB957** **DRB958** **DRB959** **DRB960** **DRB961** **DRB962** **DRB963** **DRB964** **DRB965** **DRB966** **DRB967** **DRB968** **DRB969** **DRB970** **DRB971** **DRB972** **DRB973** **DRB974** **DRB975** **DRB976** **DRB977** **DRB978** **DRB979** **DRB980** **DRB981** **DRB982** **DRB983** **DRB984** **DRB985** **DRB986** **DRB987** **DRB988** **DRB989** **DRB990** **DRB991** **DRB992** **DRB993** **DRB994** **DRB995** **DRB996** **DRB997** **DRB998** **DRB999** **DRB1000** **DRB1001** **DRB1002** **DRB1003** **DRB1004** **DRB1005** **DRB1006** **DRB1007** **DRB1008** **DRB1009** **DRB1010** **DRB1011** **DRB1012** **DRB1013** **DRB1014** **DRB1015** **DRB1016** **DRB1017** **DRB1018** **DRB1019** **DRB1020** **DRB1021** **DRB1022** **DRB1023** **DRB1024** **DRB1025** **DRB1026** **DRB1027** **DRB1028** **DRB1029** **DRB1030** **DRB1031** **DRB1032** **DRB1033** **DRB1034** **DRB1035** **DRB1036** **DRB1037** **DRB1038** **DRB1039** **DRB1040** **DRB1041** **DRB1042** **DRB1043** **DRB1044** **DRB1045** **DRB1046** **DRB1047** **DRB1048** **DRB1049** **DRB1050** **DRB1051** **DRB1052** **DRB1053** **DRB1054** **DRB1055** **DRB1056** **DRB1057** **DRB1058** **DRB1059** **DRB1060** **DRB1061** **DRB1062** **DRB1063** **DRB1064**





















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

10/10=0	8/8=0						25:AH	07:AMFTK	03:AHTPB	04:04	03:02					04:01
9/10=99	7/8=99						24:ABWMU	40:ANAGY	07:ANAHR	15:01	06:02					06:01
8/10=99	6/8=99	0	99	99	99	99		24:02 24:09N	24:11N 24:40N 24:76 24:79 24:83N 24:144 24:150 24:153 24:154 24:155N 24:163N 24:183N 24:231							
10/10=0	8/8=0						25:AH	07:AMFTK	03:AHTPB	04:04	03:02					04:01
9/10=99	7/8=99						24:ABWMU	40:ANAGY	07:ANAHR	15:01	06:02					06:01
8/10=99	6/8=99	0	99	99	99	99		24:02 24:09N	24:11N 24:40N 24:76 24:79 24:83N 24:144 24:150 24:153 24:154 24:155N 24:163N 24:183N 24:231							

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	8/8=99	A	P	A	A	A	24:09N 25:01	07:TDVB 40:01	03:04 07:02	04:04 15:01	03:02 06:02
9/10=99	7/8=99	A	A	A	A	A					
8/10=99	6/8=99	99	99	99	99	99					

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 collect 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

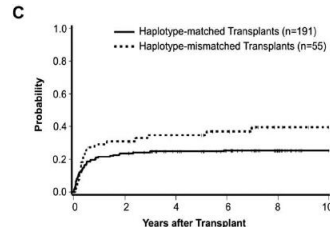
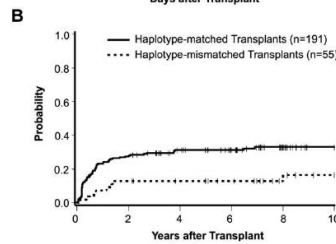
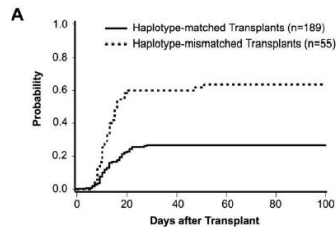
Effie W. Petersdorf,¹ Mari Malkki,¹ Mary M. Horowitz,² Stephen R. Spellman,³ Michael D. Haagenson,³ and Tao Wang⁴

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

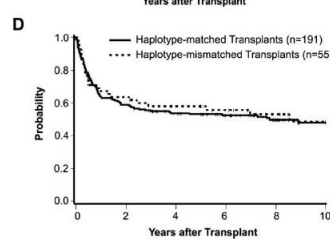
Haplotype Matching Outcomes Charts

**Grade III-IV
GvHD**

**Recurrent
Malignancy**



TRM

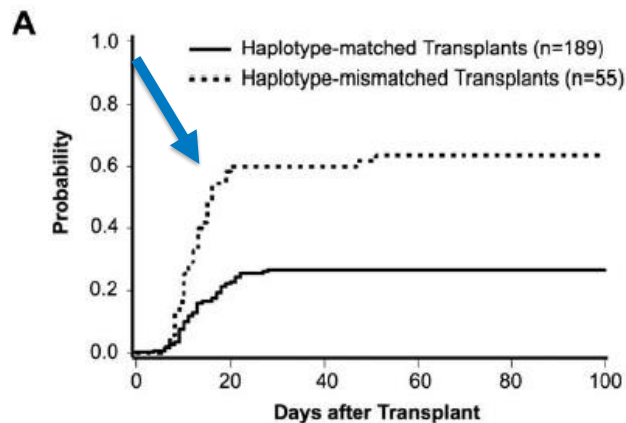


**Overall
Survival**

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/variants associated with HSCT outcome
- Could influence future search strategy but more studies needed

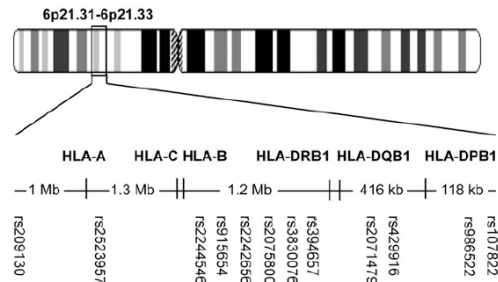


Figure 2. Twelve SNPs of clinical significance in HLA-mismatched unrelated donor transplantation. Each of the 12 SNPs having an association with grades II-IV or III-IV acute GVHD, chronic GVHD, relapse, transplant-related mortality, disease-free survival, or survival are shown on a map of the MHC on 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 EW¹, 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 EW¹, Gooley TA², Malkki M², Bacigalupo AP³, Cesbron A⁴, Du Toit E⁵, Ehninger G⁶, Egeland T⁷, Fischer GF⁸, Gervais T⁹, Haagenson MD¹⁰, Horowitz MM¹¹, Hsu K¹², Jindra P¹³, Madrigal A¹⁴, Oudshoorn M¹⁵, Ringden O¹⁶, Schroeder ML¹⁷, Spellman SR¹⁰, Tiercy JM¹⁸, Velardi A¹⁹, Witt CS²⁰, O'Huigin C²¹, Apps R²², Carrington M²²; 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.

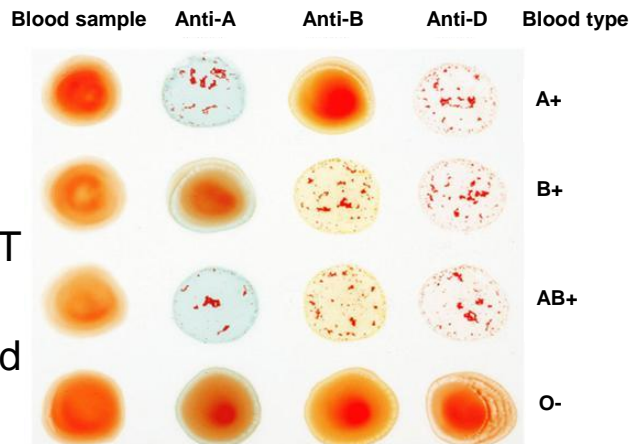
* Pediatric patients.

† 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 myelogenous 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

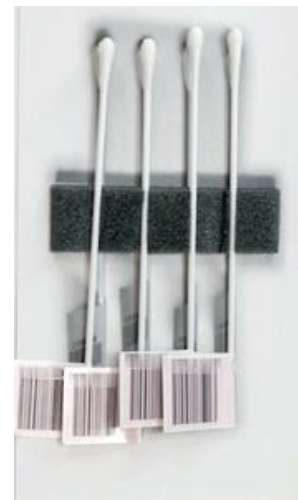
- Agglutination tests subject's ABO antibodies with serum
 - Need a blood sample
- Confirming ABO using serology is still needed at CT or at the time of IDMs
- Standardized, FDA approved screening method



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

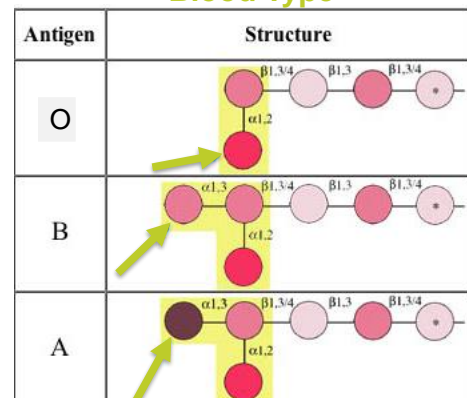
- 12 samples corrected ABO from serology
- 31 samples corrected RhD from serology

Nezh 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

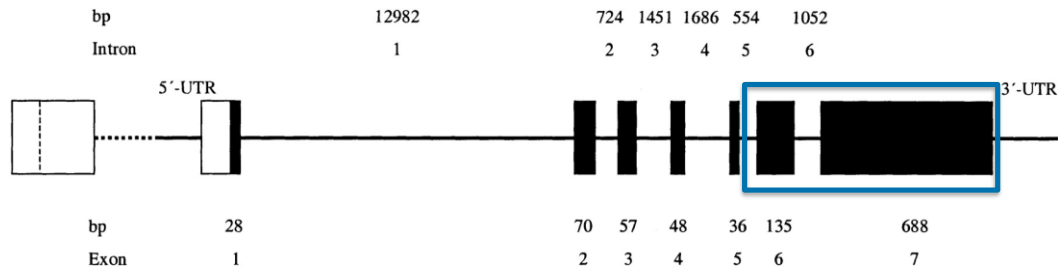
RhD From a Genetics Perspective

- 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
<input type="checkbox"/>	1	1719-9771-1 AV 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
<input type="checkbox"/>	2	1675-5998-8 AV 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
<input type="checkbox"/>	3	1829-2762-4 AV 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	A A 99	A A 99
<input type="checkbox"/>	4	0927-4584-3 AV 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

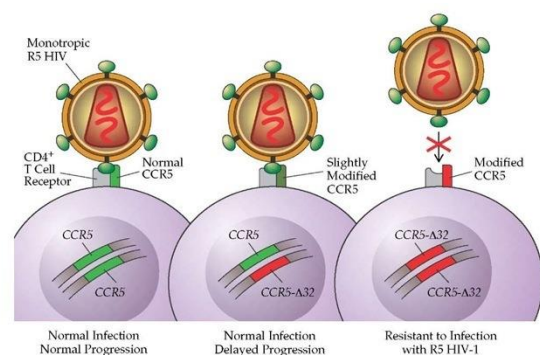
Demographics	Infectious Disease Markers	Search History	HLA Detail
Form Id: 24.0 Version: 14.0 Blood Collection Date: Sep 01 2015 ABO: A Rh: Positive Lab Status: CMS certified laboratory Donor Weight (kg): 63.63 Transfusion: No Pregnancies: Required Tests for Donor Infectious Disease Markers			

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