APHIA, Melbourne, November 2014

Improving HSCT outcomes by matching MHC genomic blocks

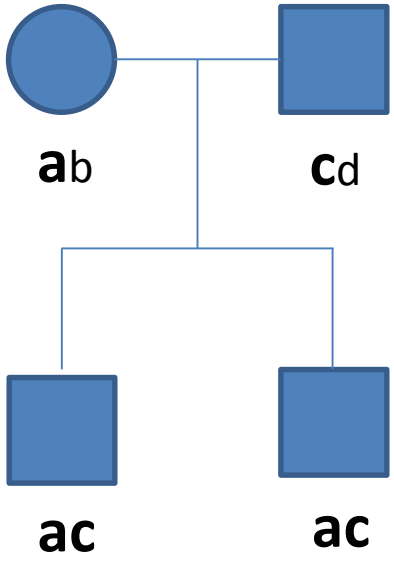
David Sayer
Conexio
Perth, Western Australia

MAR079 Version 1.0

Plan

- Matching for sequences in the C4 genes of the Gamma Block of the MHC reduces the risk of Severe aGvHD
- 4th Field HLA Class I alleles further characterise the beta block

Haplotype Matched siblings share identical MHC sequence….



…and are good HSCT donors…

Being HLA matched doesn't necessarily mean a haplotype match..

Patient: A*25,30; B*18,40; DRB1*03,13

Donor: A*25,30; B*18,40; DRB1*03,13

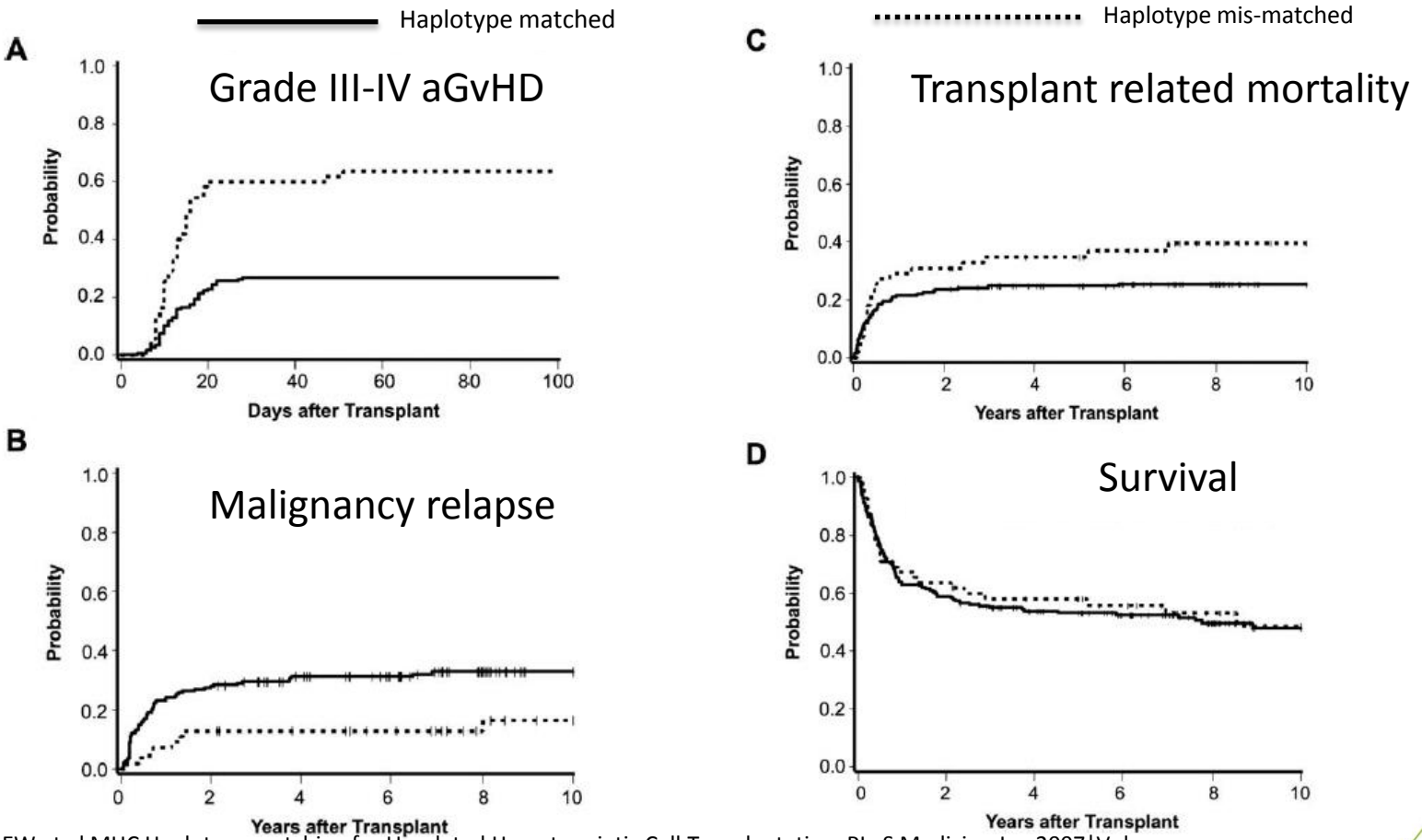
HLA matched

Patient
- a: **A*25; B*40; DRB1*03**
- d: A*30; B*18; DRB1*13

Donor
- a: **A*25; B*40;** DRB1*13
- d: A*30; B*18; **DRB1*03**

Haplotype mismatched

…and haplotype mismatch is associated with increased risk of severe aGvHD..



Petersdorf EW et al MHC Haplotype matching for Unrelated Hematopoietic Cell Tranplantation. PLoS Medicine Jan 2007|Vol 4|Issue 1. 59-68

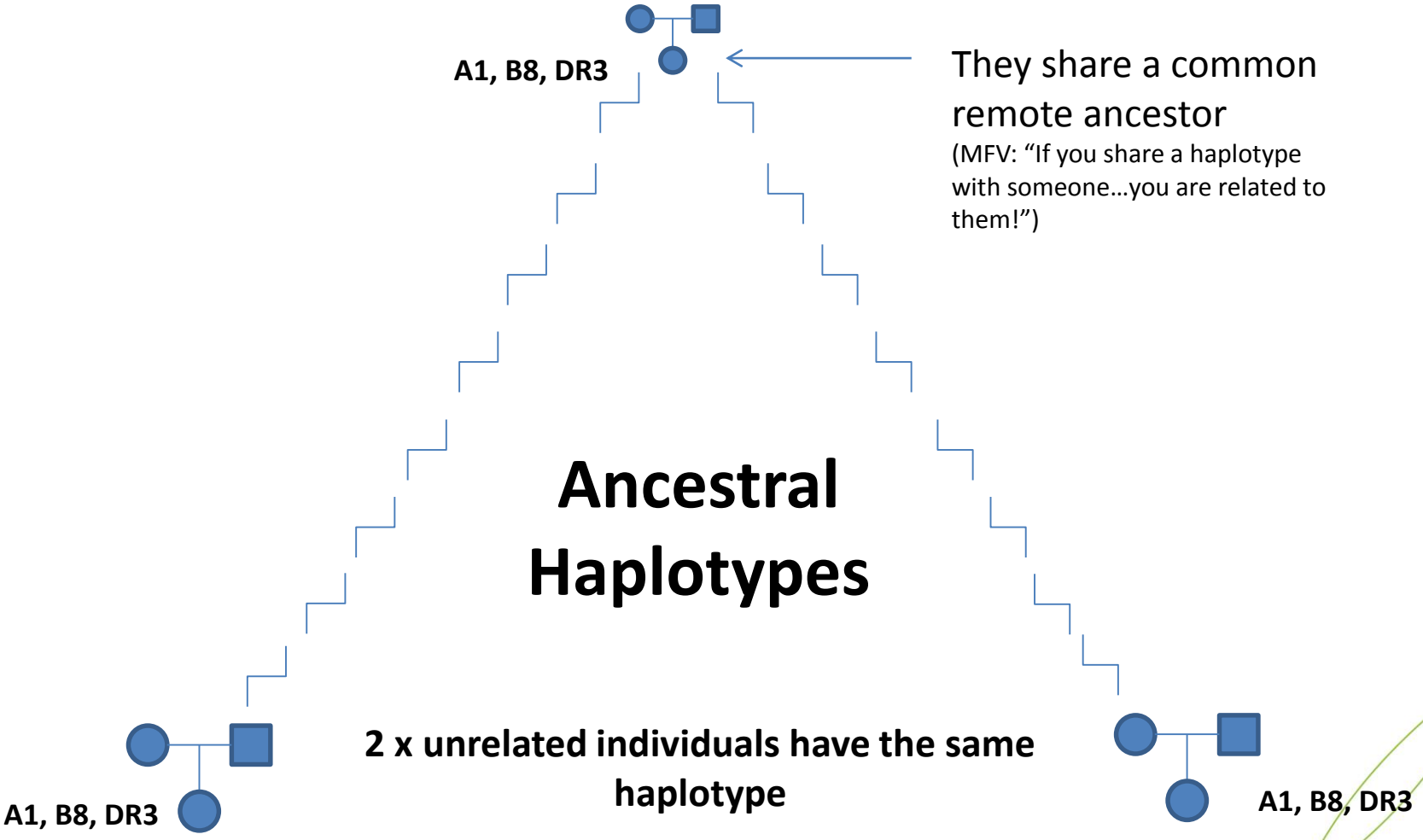
Matching for MHC haplotypes results in improved survival following unrelated bone marrow transplantation.

Bone Marrow Transplantation **(1995)** 15 381-385

Tay GK, Witt CS, Christiansen FT, Charron D, Baker D, Herrmann R, Smith LK, Diepeveen D, Mallal , S, McCluskey J et al,

- "If HLA and [MHC genomic] blocks are matched survival is the same as for HLA identical sibs".
  - HLA + non HLA MHC haplotype markers

…not really surprising that haplotype matched donors are good donors…



Ancestral Haplotypes

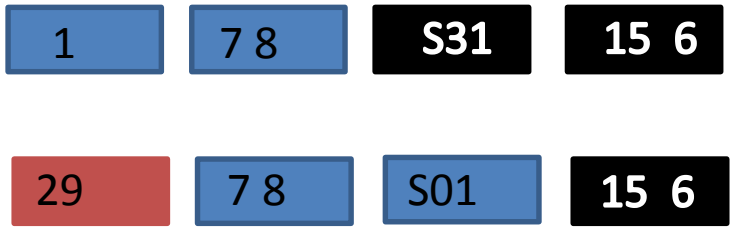
- **Dawkins / Christiansen group in Western Australia - 80's and 90's**
  - Significant contributors to the understanding of MHC structure/function and evolution
- **Conserved between unrelated individuals**
  - A1, B8, DR3 (8.1 AH) from 19 unrelated individuals
  - 3.8 SNPs per 2.6Mb from HLA-A to DQB1
    - Smith, WP; Genomics 2006
- **Some AH are found globally, some restricted to regions**
  - A1 B8 DR3 Northern European (recent…23.5K years +/- 10K)
  - A1 B57 DR7 All populations (much older…)
- **Significant differences between haplotypes**
  - Copy number variants
    - Zhang, WJ, J Exp Med 1990
  - SNP's, indels
  - Non HLA Haplotype differences explain increased risk of GvHD haplotype mismatching
    - How/where is not clear

AH, genomic blocks and recombinants in populations

Ancestral Haplotypes

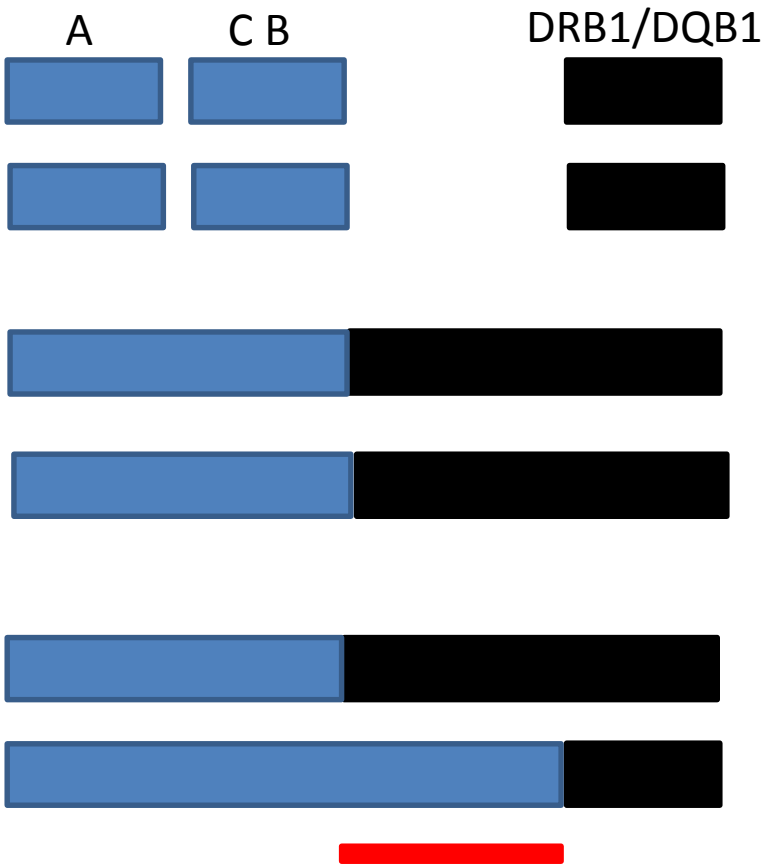
MHC genomic blocks

Recombinants

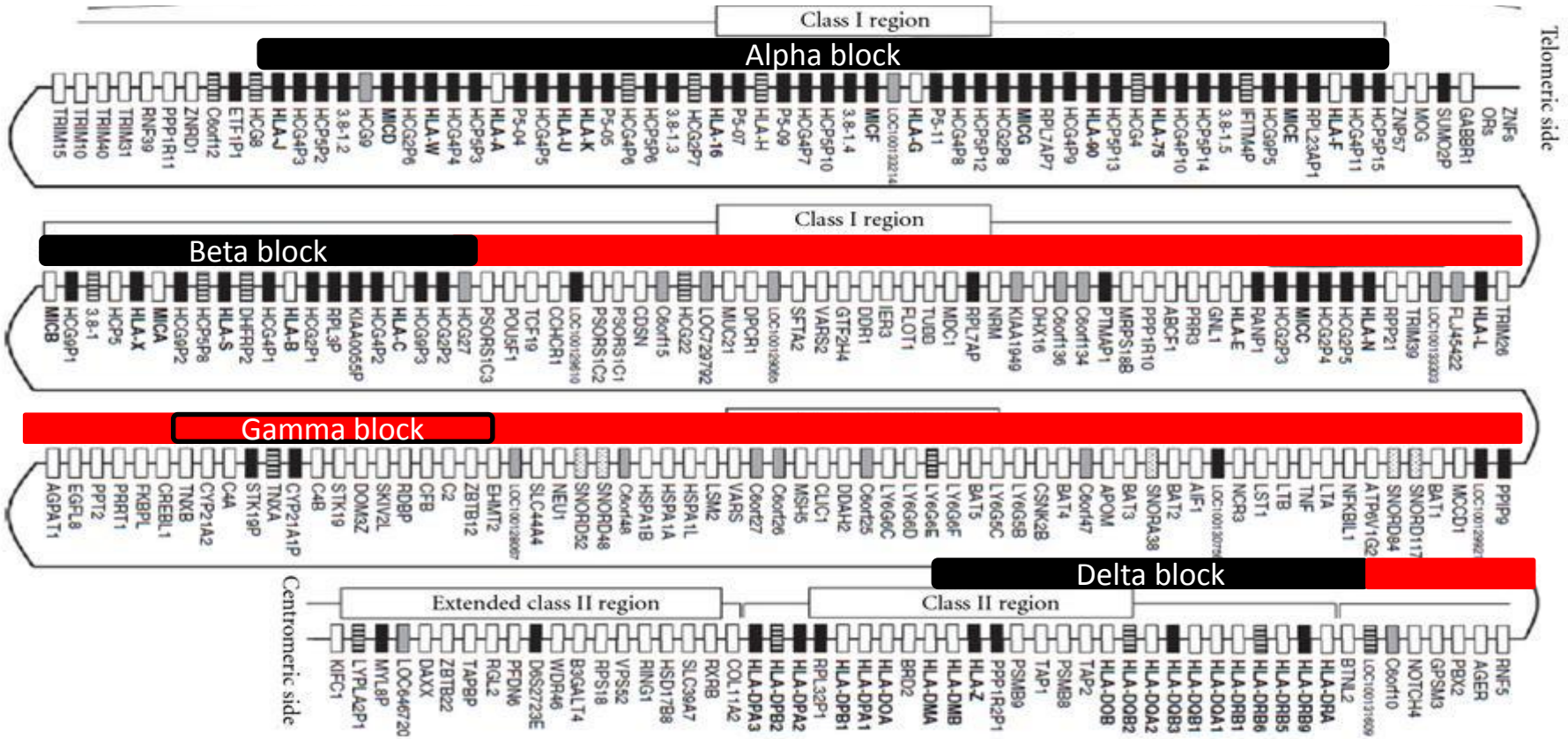


…plus individuals without recognisable AH

Limitations of current HLA typing approaches in unrelated HSCT



……some perspective….



How can we improve (Haplotype) matching in unrelated HSCT

- Complete MHC Sequencing **HARD**
- Chromosome separation **HARD**
- Match for markers in the 4 major MHC genomic blocks with conventional techniques! **SIMPLE**
  - Require haplotype specific markers in each block
    - High res for HLA (or greater…see later)

NEED GAMMA BLOCK MARKER

Defining Gamma Block Markers

- Contains genes for soluble complement proteins
- Polymorphisms defined by electrophoresis and immunofixation
- Not haplotype discriminating
- Complement allotyping NOT an option

AH	A	Cw	B	C2	Bf	C4A	C4B	DR	DQ
7.1	3	7	7	C	S	3	1	15	6
7.2	24	7	7	C	S	3+3	1	1	5
8.1	1	7	8	C	S	Q0	1	3	2
13.1	30	6	13	C	S	3	1	7	2
18.1	25		18	Q0	S	4	2	15	6
18.2	30	5	18	C	F1	3	Q0	2	2
18.3			18		S	3	1	11	7
35.2	11	4	35	C	F	3+2	Q0	1	5
35.2	11	4	35		S	3	Q0	1	5
35.4			35		S	3	1	5	
35.5			35		S	3	1	5	
37.1	1	6	37	C	F	3	1	10	
38.1	26		38		S	2	1	4	8
42.1		2	42	C	F	12+91	Q0	3	4
44.1	2	5	44	C	S	3+3	Q0	4	7
44.2	29		44	C	F	3	1	7	2
44.3	29		44		S	Q0	1	7	2
44.3	33		44	C	F	3	1	13	6

Degli-Esposti et al 1995

Gamma Block Marker Candidates: The C4 Genes

- Polymorphic
  - C4 allotyping protein assay
    - Difficult
    - No appropriate C4 typing molecular tests
- 1-3 CNV per haplotype / >99% similar
- Tandemly arranged / 10kb apart
- 41 exons, 14 or 20kb in length
- Sequenced the entire C4 genes (Sanger) on 24 diverse AH (except the HERV)

Co-amplification and sequencing of C4 genes

- Relative peak height reflects gene copy number
- 1:3 ratio's easily detected
- Good technique for SNP screening of homologous genes
- Sequencing as a technique for gene copy number comparisons

C4 Gene Sequencing Results

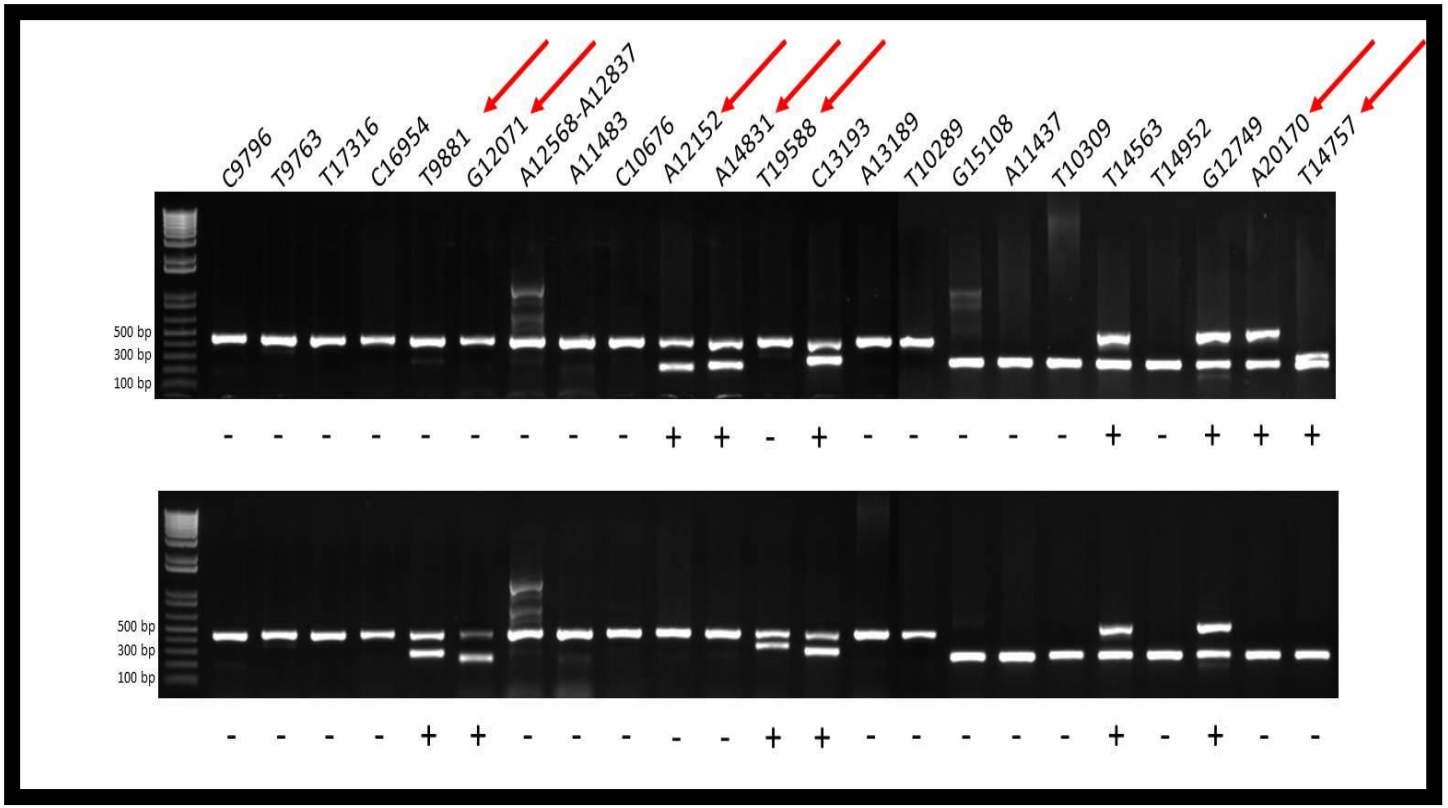
Reference sequence position		-165	495	2297	2321	9465	9763	9796	9819	9881	10289	10309	11437	11483	11627	11893	12051	12071	12152	12341	12568-12837	12837	12749	12877	12904	13189	13193	14563	14757	14831	14952	15108	16954	10676	17316	18997	19588	20170
Intron/Exon Position		UTR	I2	E9	E9	E11	E12	E12	E12	I12	I13	I13	E17	E17	I17	E19	I19	I19	I19	I20	E21	I21	I21	I21	I21	E22	I23	I23	I28	I28	E29	E29	I30	E33	E33	E34	E36	I38	E40
AH	C4																																						
Reference Sequence		A	TGTT	C	C	C	C	T	C	C	C	C	C	G	C	G	G	A	G	G	G	C	C	A	C	G	G	T	C	C	G	G	A	G	T	C	C	C	G
44.4	3-1	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	C	-	T	.	.	.	.	.	.	.	T	.
13.1	3-1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	G	.	.	.	C	-	.	.	.	.	.	C	.	.	T	.
44.2	3-1	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	G	.	A	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.
7.1	3-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	-	.	.	T	G	.	.	.	.	.	.
18.1	4-2	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	C	-	.	.	.	.	.	.	.	.	.	.
46.1	4-2	.	----	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	C	-	.	.	.	.	.	.	.	.	.	.
46.2	4-2	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	A	C	-	.	.	.	.	.	.	.	.	.	.
62.2	4-2	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	C	-	.	.	.	.	.	.	.	.	.	.
44.3	Q0-1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
8.1	Q0-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T	T	.	.	.	.	C	-	.	.	.	.	.	.	.	T	.
55.1	4-5	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	-	.	.	.	C	-	.	.	.	.	.	.	.	.	.	.
7.2	3+3-1	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	C	.	T	A	.	.	.	.	.	.	A	.
44.1	3+3-Q0	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	G	.	.	.	C	.	T	.	.	.	.	.	.	.	A	.
18.2	3-Q0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	T	.	.	.	.	.	.	T	.	.
58.1	3-Q0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	T	.	.	.	.	.	.	T	.	.
35.2	3+2-Q0	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	C	.	T	.	.	.	.	.	.	T	.	.
62.1	3-3	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	A	.	.	T	T	G	.	.	.	C	.	T	A	.	.	.	.	.	.	A	.
52.1	3+2-Q0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.
54.1	3-5	.	.	.	A	T	.	.	.	T	.	.	.	.	T	.	A	.	.	.	.	.	T	G	.	A	.	C	-	.	.	.	.	.	.	.	.	T	.
38.1	2-1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	T	G	.	.	.	C	-	T	A	.	.	.	.	.	.	.	.
65.1	2-1+2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	G	.	.	.	C	-	.	.	.	.	.	.	T	.	T	.
57.1	6-1	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	G	.	.	.	C	-	.	.	.	.	.	.	.	.	T	.
42.1	12+91-Q0	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	C	-	T	.	.	.	C	.	.	T	T	.
47.1	91-Q0	.	.	.	.	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.

23 Unique sequences / 24 AH (18.2 and 58.1 identical: both associated with T1D??)

Summary of Sequence Results

- Low level of polymorphism – but haplotypic!
- No correlation between allotype and sequence (Cant extend the allotype nomenclature)
  - Allotype a consequence of net charge of the protein
    - Different amino acids (sequence) have same charge!
- Combination of haplotype specific SNPs and SNPs shared between haplotypes
  - Provides unique Gamma Block SNP Profiles
    - Disease gene mapping
    - Transplant matching

Gamma Block SNP profile (GBSP) by PCR SSP



GBSP are highly polymorphic

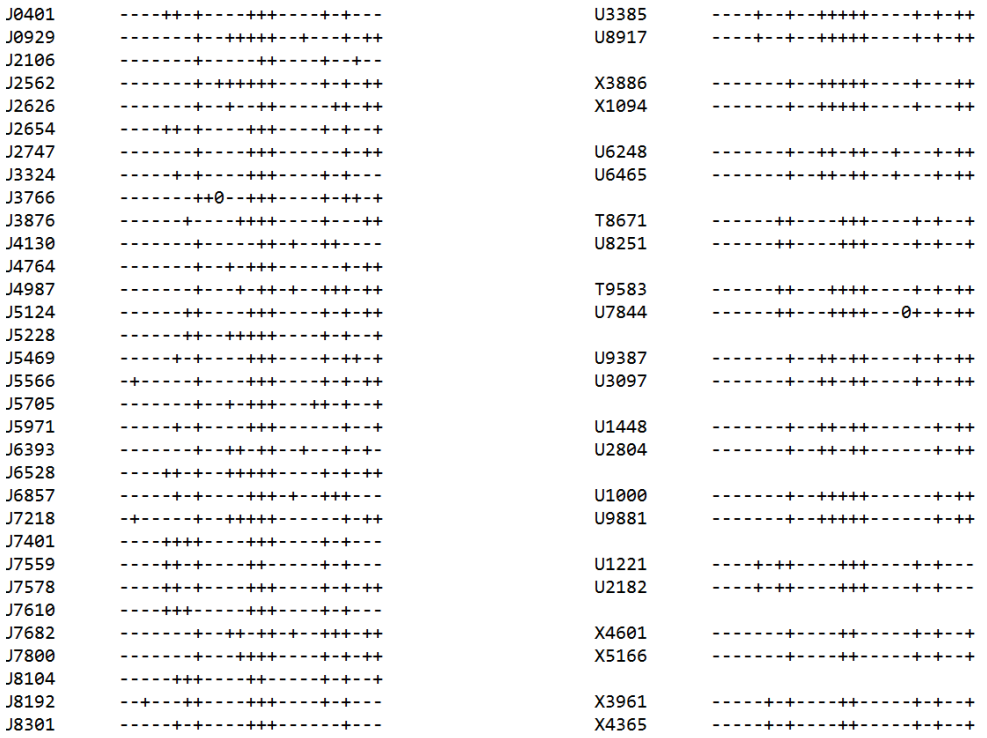
- Comparison of SNP profiles in **96** individuals

83 Unique profiles

22/23 SNPs pos
70 individuals
13 pairs

2/13 identical DR/DQ
3/13 share 1x B/C + 1xDR/DQ
4/13 share 1x B/C or 1xDR/DQ

4/13 share no alleles



GBSP matching and affect on outcomes in unrelated HSCT

Brazilian Study

- 225 unrelated HSCT pairs, transplanted from 1996-2005 in 3 Brazilian centres
- Luminex “4 digit” typed A,B,C,DRB1, DQB1
- 66% 10/10, 34% 9/10
- Gamma-Type – 23 SNPs, by PCR-SSP
- GT Match = complete Gamma-Type matched profile
- GT Mismatch = a difference of at least 1 SNP

- Probability of GVHD occurrence and overall survival were estimated from the time of transplant by Kaplan-Meier and the group differences compared by Log-rank

Getz et al, EFI abstract 2014

Results

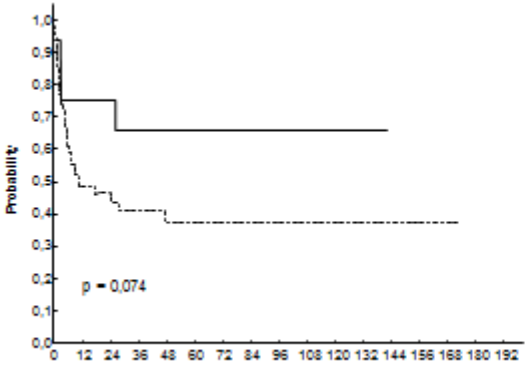
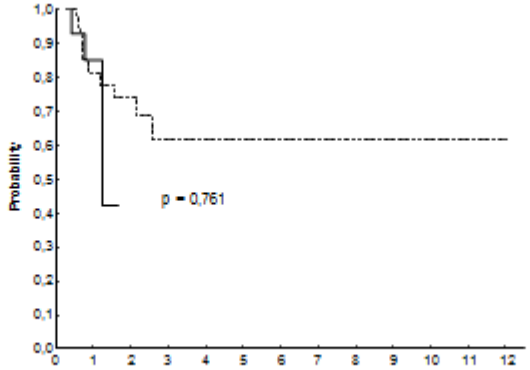
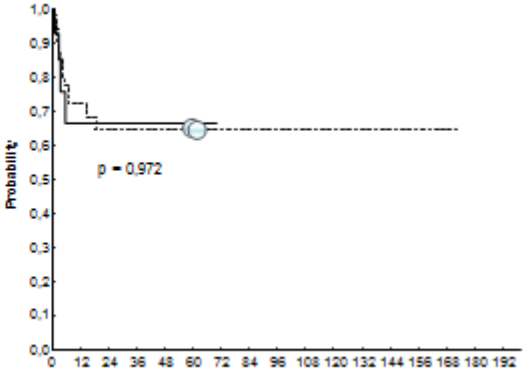
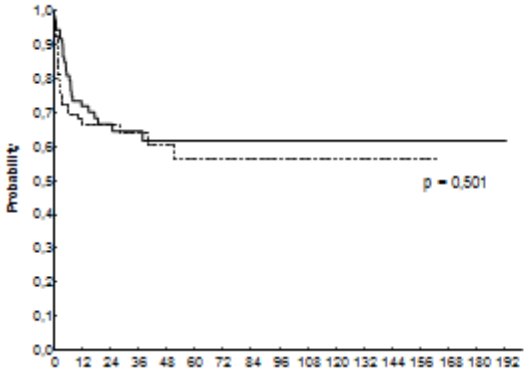
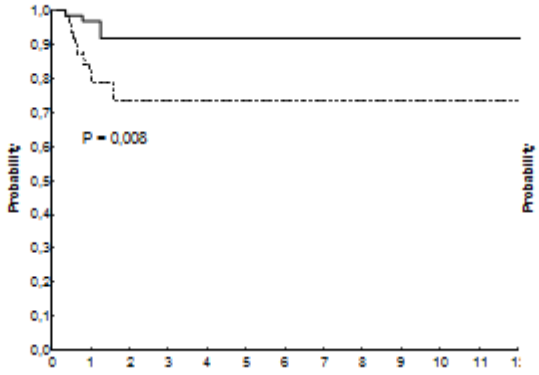
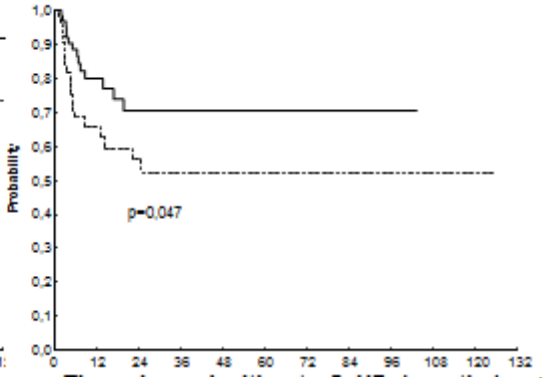
- HLA Matched pairs may be GBSP mismatched
- HLA mismatched pairs are more likely to be GBSP mismatched

	GT Matched	GT Mismatched
HLA 9/10 Matched	16 (21%)	61 (79%)
HLA 10/10 Matched	77 (52%)	71 (48%)

P<0.0001

Results

GT Match
GT MisMatch

	Survival	Grade III/IV aGvHD	cGvHD
9/10			
10/10			

Getz et al, EFI abstract 2014

Cleveland Clinic

- 236 HSCT recipient/unrelated donor pairs transplanted at the Cleveland Clinic (2000-2010)
- High resolution typing of HLA A, B, C, DRB1, DQB1, DPB1 loci and for MHC class I related chain A (MICA)

Results….to date..

"GT SNP mismatch was associated with increased risk of severe GVHD in univariate analysis (HR 2.43, 95% CI 1.32-4.47, P= 0.004)

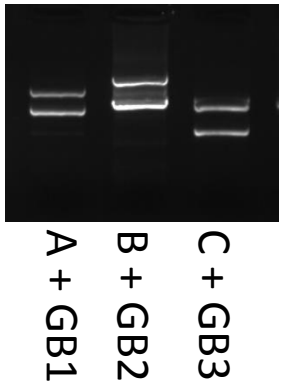
SNP mismatch remained significantly associated with severe GVHD in a multivariate analysis (HR 2.54, P= 0.002) after adjusting for graft source, HLA and MICA mismatch"

Medhat Askar (Cleveland Clinic)

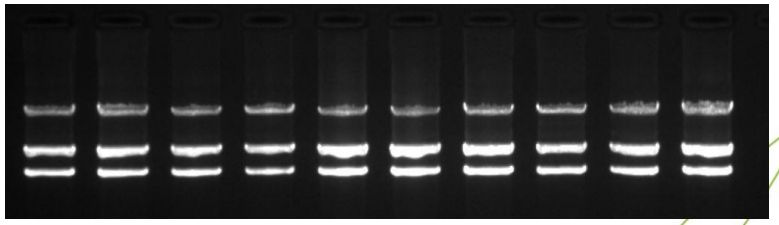
GBSP and Next Gen

The GB SNPS are amplified in 3 amplicons

- High Resolution HLA + GBSP
  - Each amplicon is multiplexed with A, B and C



- Standalone Testing
  High throughput
  1 x multiplexed PCR



GBSP analysis in Assign

Sample: **Patient**

GBSP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	24	25
Sequence	S	M	M	M	Y	Del	C	K	Y	S	W	S	Y	R	S	Y	K	S	S	hetDel	G	C	T	T

Sample: **Donor 1**

GBSP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	24	25
Sequence	S	M	M	M	Y	Del	C	K	Y	S	W	S	Y	R	S	Y	K	S	S	hetDel	G	C	T	T

Sample: **Donor 2**

GBSP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	24	25
Sequence	S	M	M	C	Y	Del	C	K	C	G	W	S	Y	R	S	Y	K	S	S	hetDel	G	C	T	T

GBSP sequence data easily analysed in Assign
Each SNP can be viewed to confirm the base call

Base calls at GBSP positions are reported and compared between donors and patients

Conclusion

- Avoiding GBSP mismatches can potentially reduce the risk of severe aGvHD in unrelated HSCT
  - Assist in donor selection
    - HLA Matched and mismatched
- The assay is a simple PCR-SSP that can easily be performed in most labs
  - Also include with HLA in Next Gen assays
- Studies are on going
  - 4 x Centres
    - Population diversity / haploidentical transplants

What about the other blocks?

One mans junk is another man's predictor of aGvHD??

Haplotype specific alleles in non-coding regions

- a new class of HLA alleles -

- B*18:01:01:01
  - Unique to A*30-C*05-B*18-DRB1*03-DQB1*02
- B*44:02:01:03
  - Unique to B*44-C*07:04-DRB1*11:01
- B*15:01:01A@444
  - Unique to a subset of B*15:01:01-C*04:01
- C*04:01:01:05
  - Unique to A*11-C*04-B*35-DRB1*01:03
    - A subset(10%) of C*04; B*35 haplotypes

Haplotype Specific Alleles

- Haplotype specific alleles (HSA) are a new class of HLA alleles identified by Conexio
- HSA described to date are characterised by non-coding polymorphisms.
- HSA alleles are markers for unique haplotypes and are important in HSCT and disease association studies
- More HSA are likely to be described as more non coding sequence is characterised in more genes (including class II)
- Conexio's SBT software (Sanger and Next Gen) enable characterisation of non coding polymorphisms

Acknowledgements

GB SNP characterisation

Mareike Puschendorf (MSc student)

Hayley Hogan (Hons student)

Assay Development

Luke Candy

Karolina Dimovski

Hayley Hogan

Lauren Glasson

Non Coding class I SNP study

Di De Santis (RPH)

Dmitri Monos (CHOP)

Curt Lind (CHOP)

David Senitzer (City of Hope)

GBSP Studies

Joselito Getz

Noemi Farah

Gordon Hill

Carla Wirtz

Marilyn Whetmore

Dawn Thomas

Medhat Askar

The rest of the amazing people at Conexio!