The DISCOVeRY-BMT Project

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Lara Sucheston-Campbell, PhD, The Ohio State University

Disclosures

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

Name	Institution
Lara Sucheston-Campbell, PhD	The Ohio State University
Stephen Spellman, MBS	CIBMTR

Disclosures

The following faculty and planning committee staff have the following financial disclosures:

Name	Institution	Disclosure
Theresa Hahn, PhD	RPCI	Novartis (stock)

Learning objectives

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

- Assess genome wide association studies (GWAS) and use in hematopoietic stem cell transplantation research
- Explore the development of the DISCOVeRY-BMT GWAS project
- Evaluate the results of the DISCOVeRY-BMT GWAS project

A population level perspective on the role of common and rare genetics in survival outcomes after transplant: some lessons from the DISCOVeRY-BMT study

Theresa Hahn, PhD

Lara E Sucheston-Campbell, PhD

THE OHIO STATE UNIVERSITY

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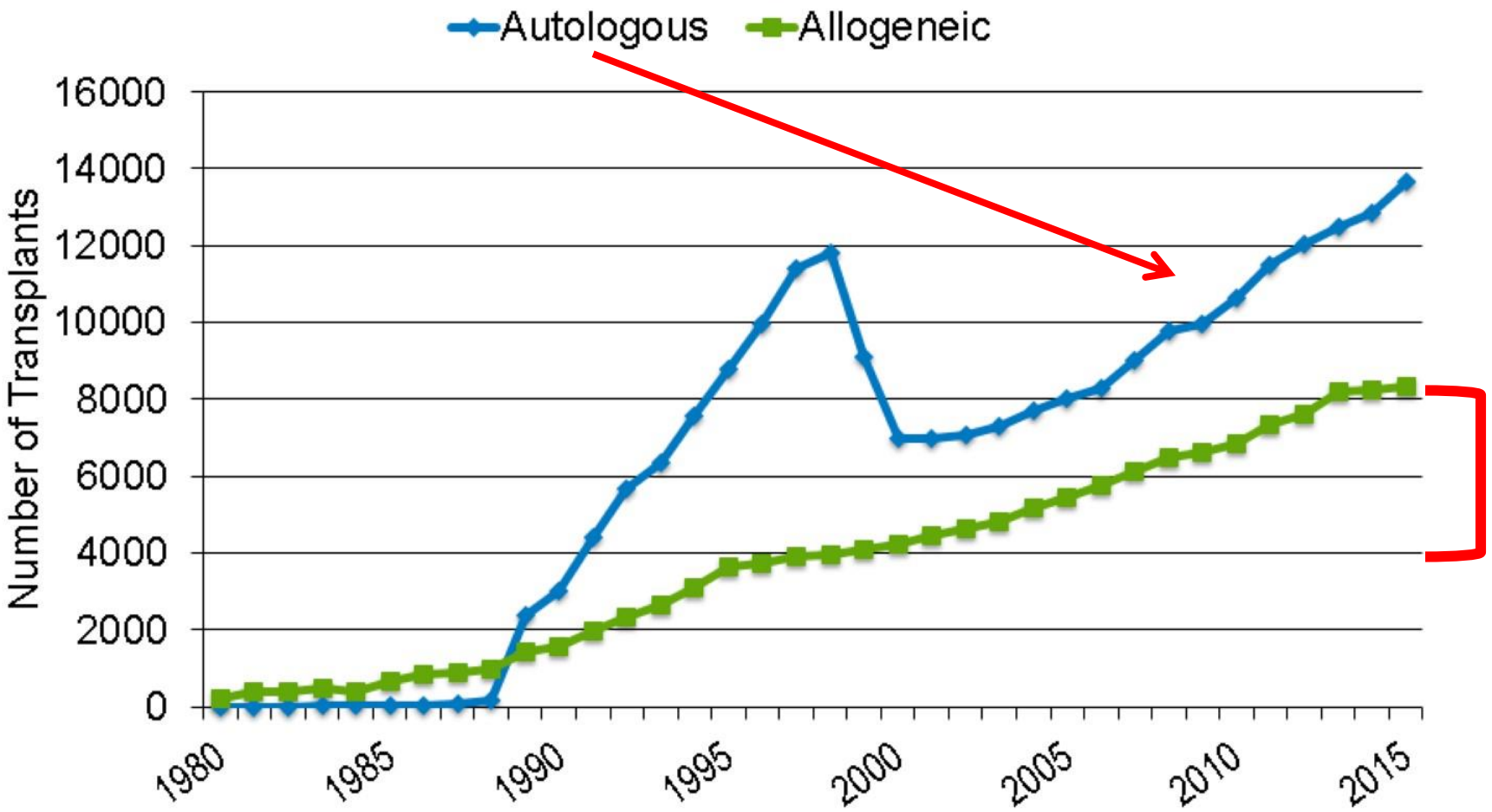
A research collaboration between the National Marrow Donor Program (NMDP)/Be The Match and the Medical College of Wisconsin

Annual Number of HCT Recipients in the US by Transplant Type



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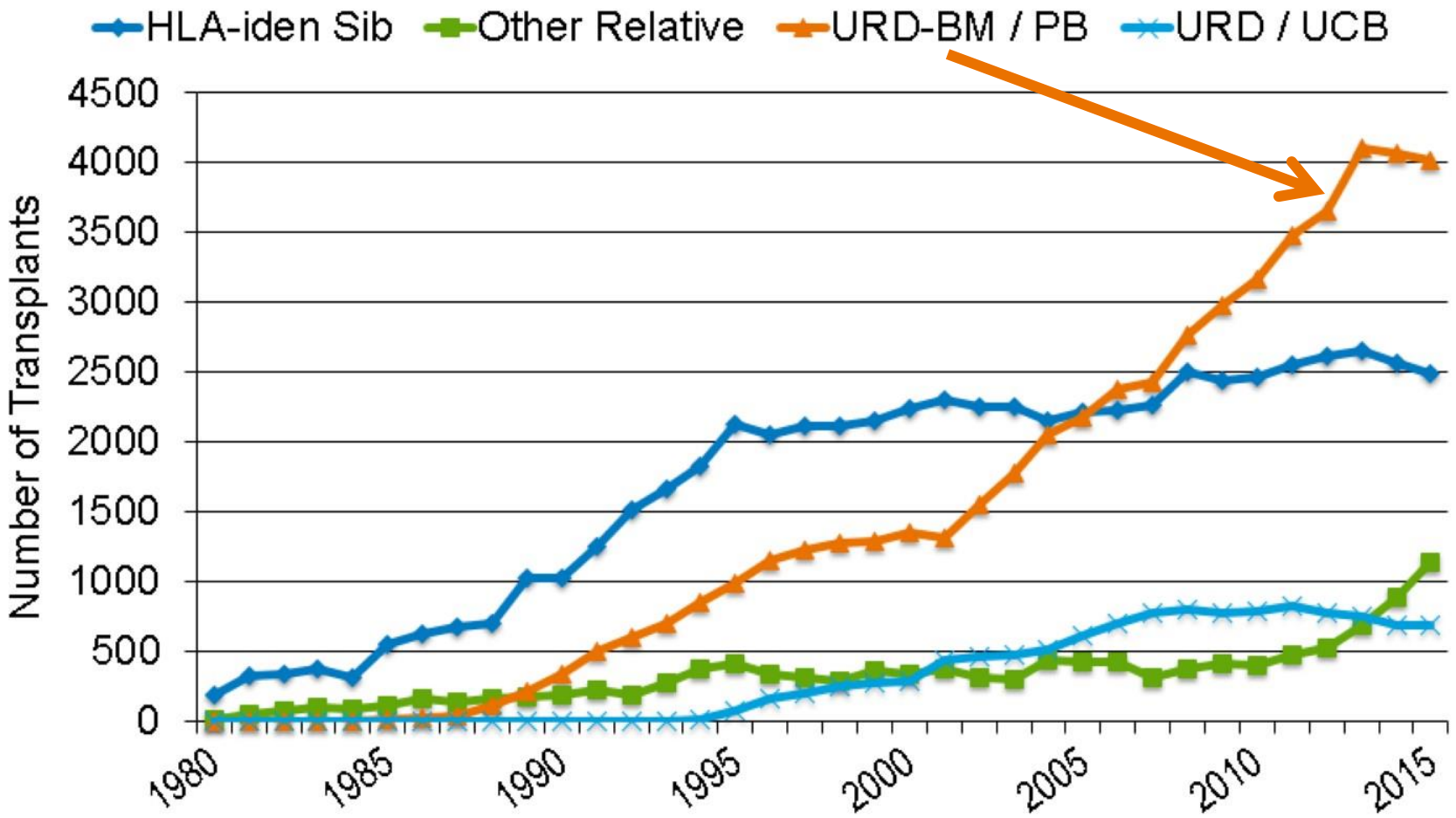
D'Souza A, Zhu X. Current Uses and Outcomes of Hematopoietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2016. Available at: http://www.cibmtr.org

Allogeneic HCT Recipients in the US, by Donor Type



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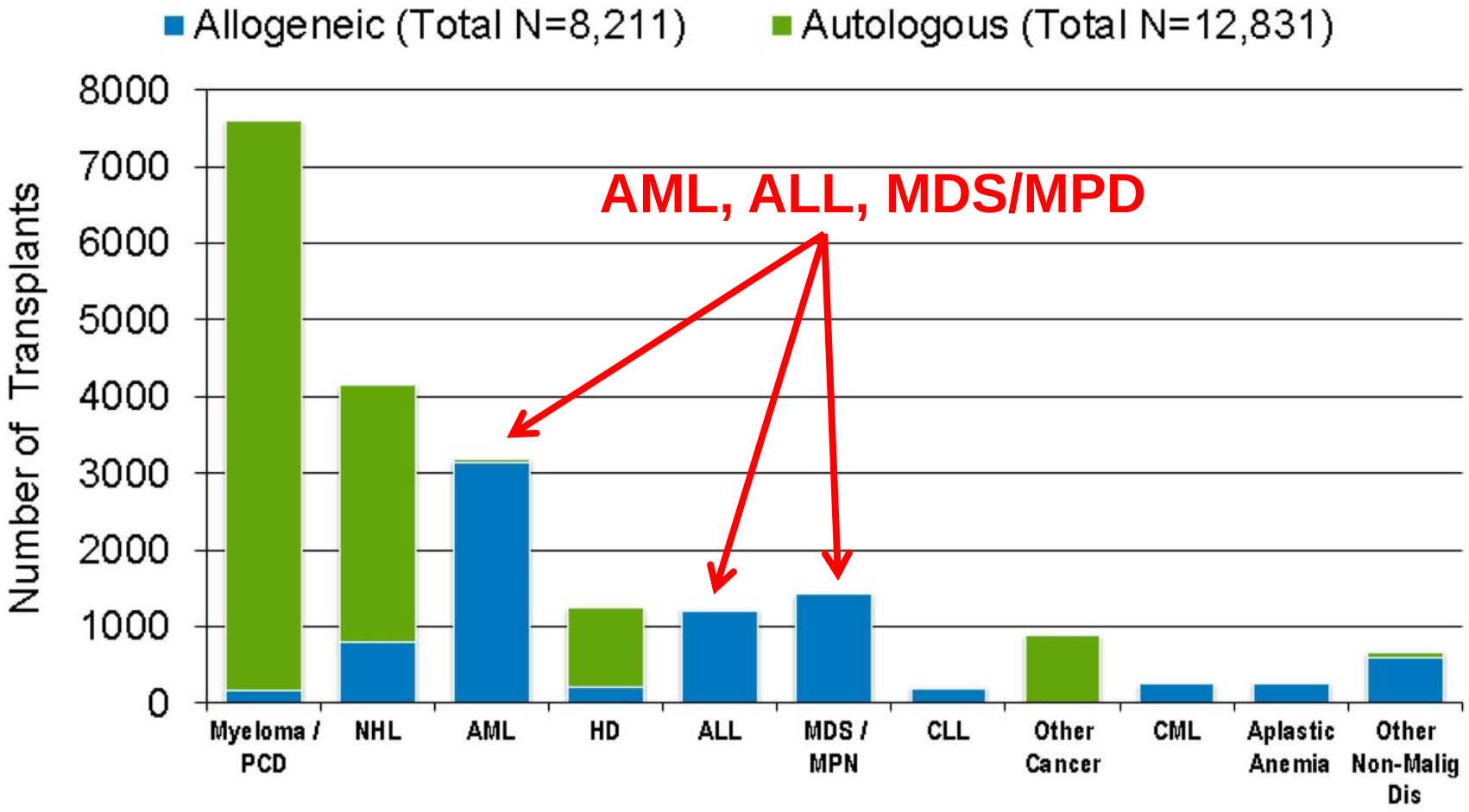
D'Souza A, Zhu X. Current Uses and Outcomes of Hematopoietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2016. Available at: http://www.cibmtr.org

Indications for Hematopoietic Cell Transplant in the US, 2014



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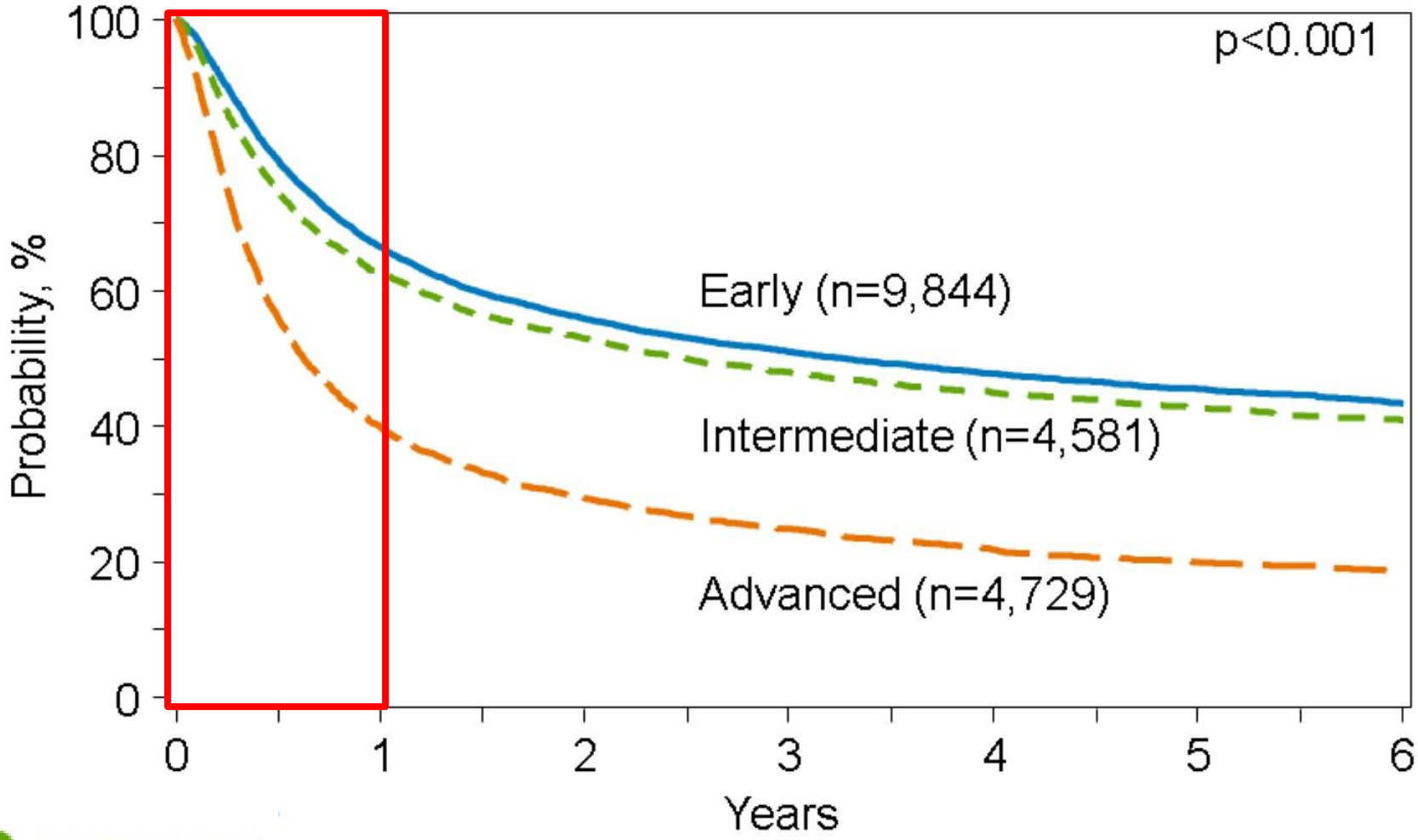
D'Souza A, Zhu X. Current Uses and Outcomes of Hematopoietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2016. Available at: http://www.cibmtr.org

Survival after Unrelated Donor HCT for AML, 2004-2014



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D'Souza A, Zhu X. Current Uses and Outcomes of Hematopoietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2016. Available at: http://www.cibmtr.org

Determining the **I**nfluence of **S**usceptibility **CO**nveying **V**ariants **R**elated to 1-**Y**ear mortality after **BMT** =>

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Data Management
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Why a GWAS?

- Agnostic approach to interrogate the genome for variants associated with an outcome
- Acknowledges limitations in current knowledge of biology of outcomes and functions of DNA in health & disease
- First step in identifying novel genomic regions of potential interest for further studies

Study Population

- Cohort 1
  - 2,609 AML, ALL or MDS patients treated with an 10/10 HLA-matched unrelated donor allogeneic BMT from 2000-2008, reported to the CIBMTR with an available sample from both the recipient and donor
- Cohort 2
  - 572 patients with identical criteria as Cohort 1 except treated 2009-2011
  - 351 AML, ALL or MDS patients with an 8/8 HLA-matched (DQB1 mismatched) unrelated donor allogeneic BMT from 2000-2011, reported to CIBMTR with an available sample from both the recipient and donor

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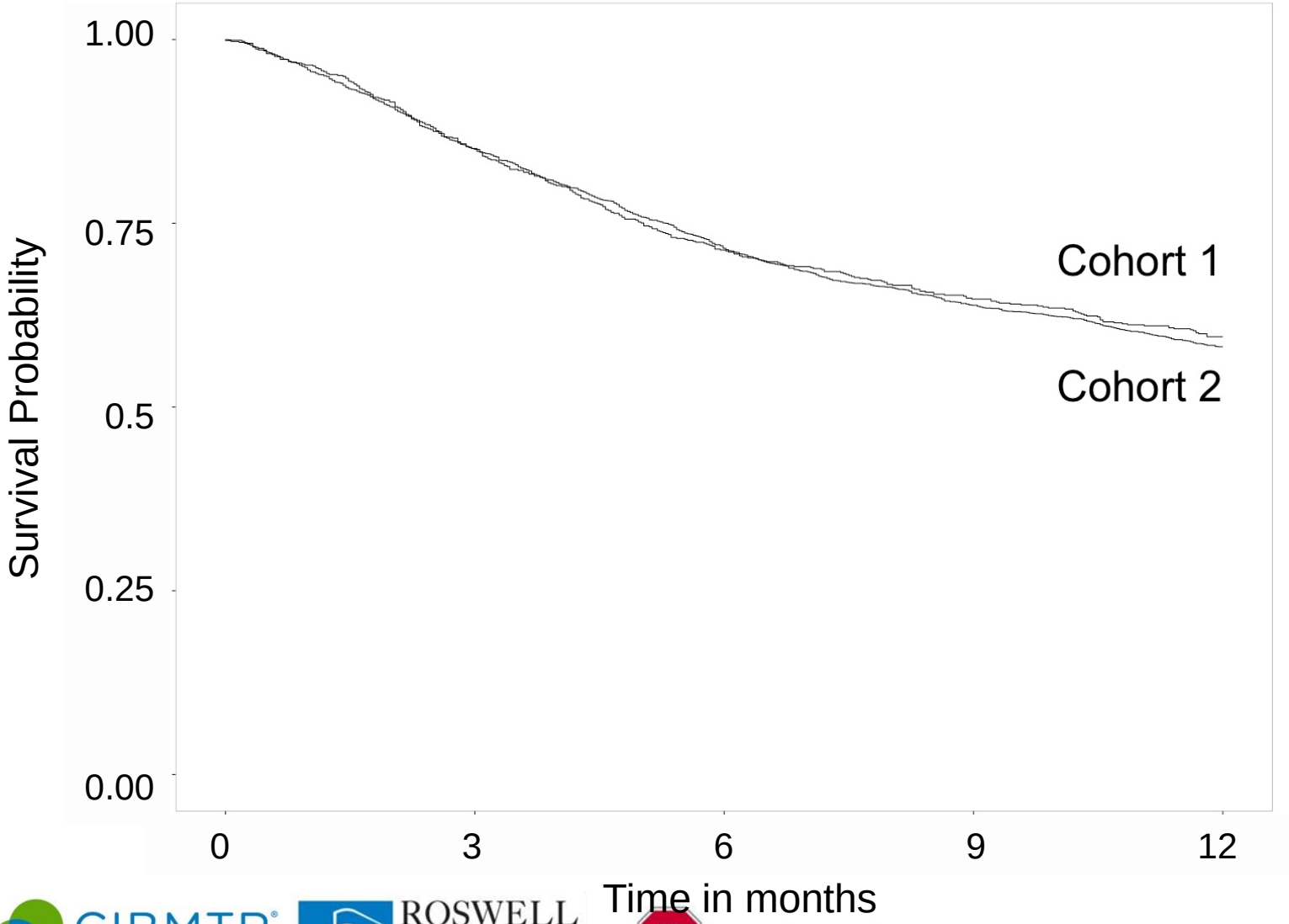
Overall Survival by Cohort



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Genotyping Methods

- Illumina HumanOmniExpress-24 Bead Chip contains ~ 732,000 SNPs
- Population specific quality control included assessment of duplicate concordance, unplanned duplicates/related samples, sample contamination, call rate, missingness, HWE
- Imputation to 1000 Genomes Project (phase 1) yielded ~ 9 million SNPs for analysis
- Imputation statistically infers untyped or missing SNPs using known haplotypes from a reference panel

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Analysis

- Cox proportional hazards models adjusted for significant covariates:
  - Recipient and donor age, disease (AML, ALL, MDS), disease status, year of BMT, graft source (PB, BM)
- Standard genome-wide significance set at $P < 5 \times 10^{-8}$
- $P<0.00000005$
- Meta *P*-value calculated from the *P*-value in 2 cohorts with weights proportional to the square root of the # of cases

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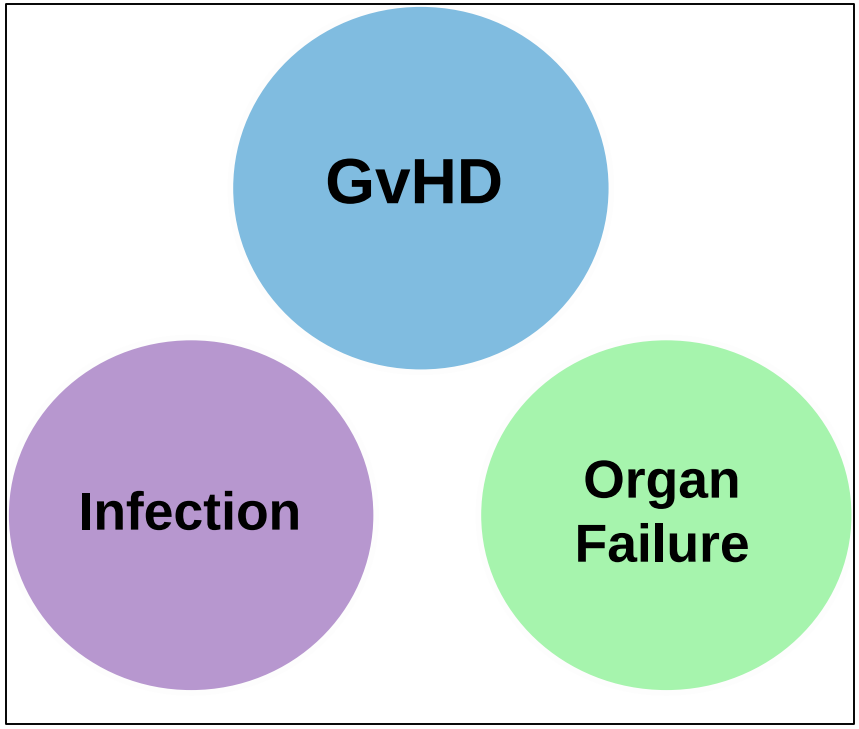
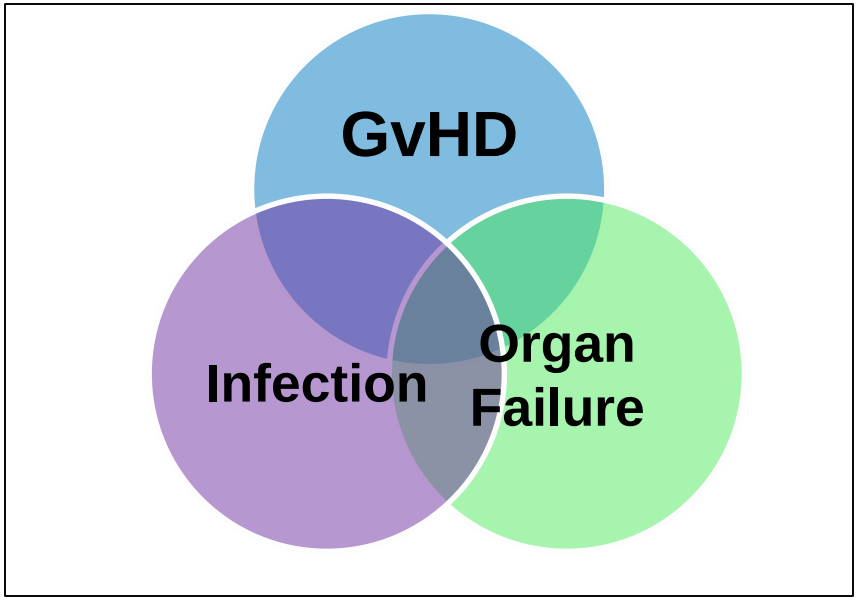
Genetic Variants Associated with Specific Causes of Death

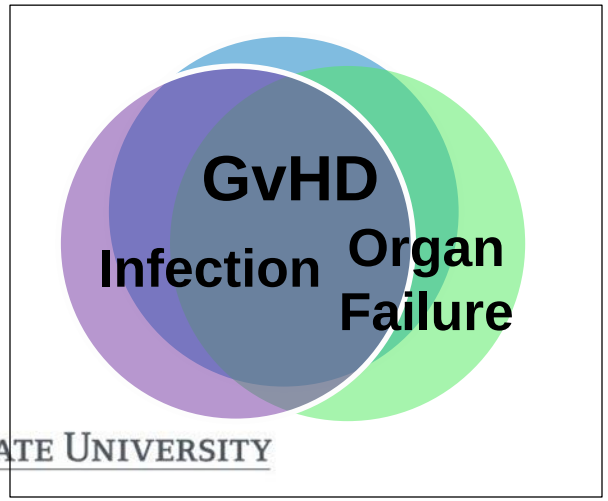
Unique, No Overlap



Unique & Some Overlap



Major Overlap



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Causes of death

- All deaths before 1 year post BMT were adjudicated by a panel
- Patients with 2 or more adjudicated causes of death were analyzed in each analysis, eg death due to GvHD and Infection are included in analyses of both GvHD and Infection
- Sensitivity analysis of single vs multiple causes of death (eg GvHD alone and in combination with other causes)

Hahn T et al BBMT 21:1679, 2015

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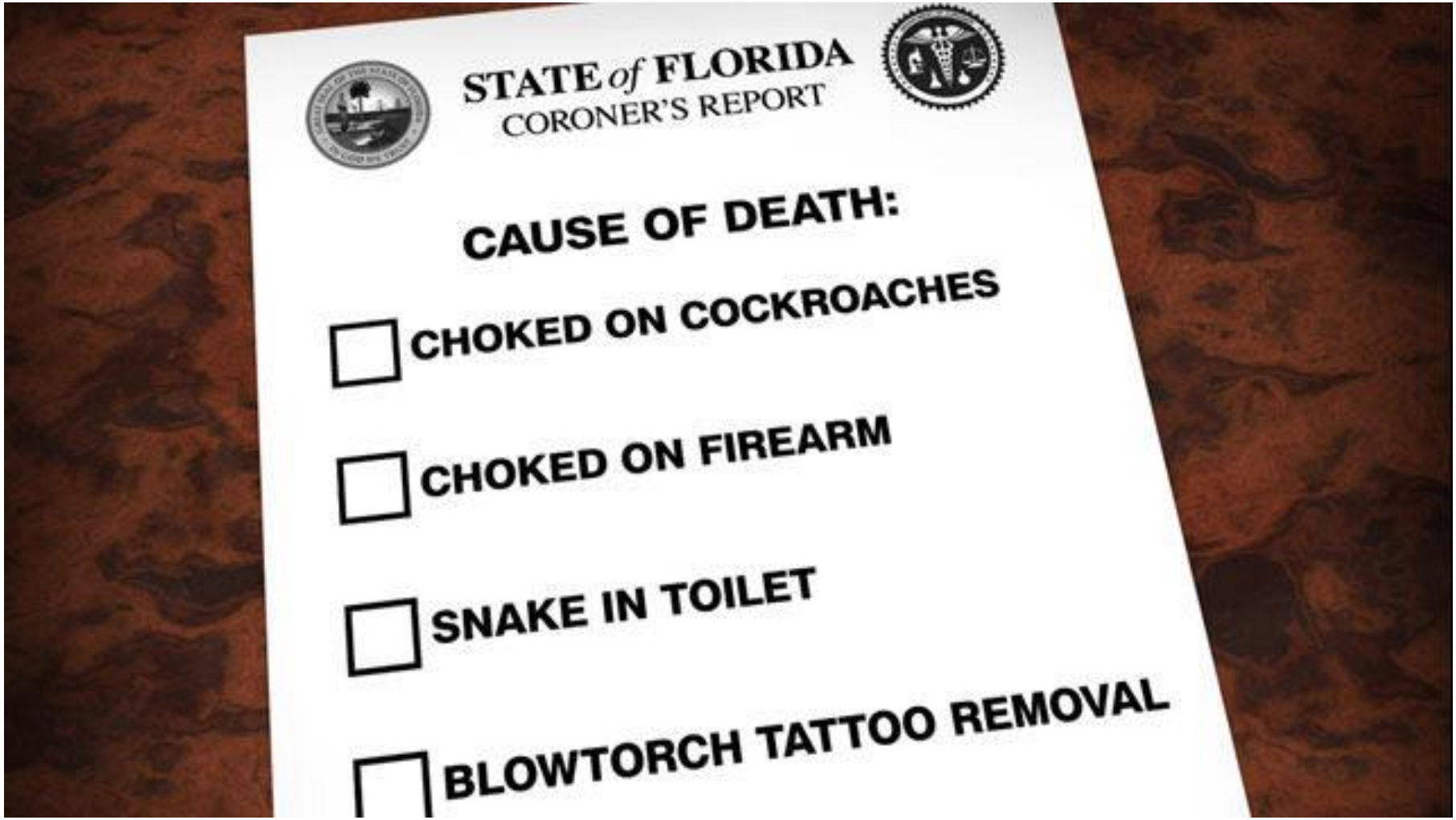

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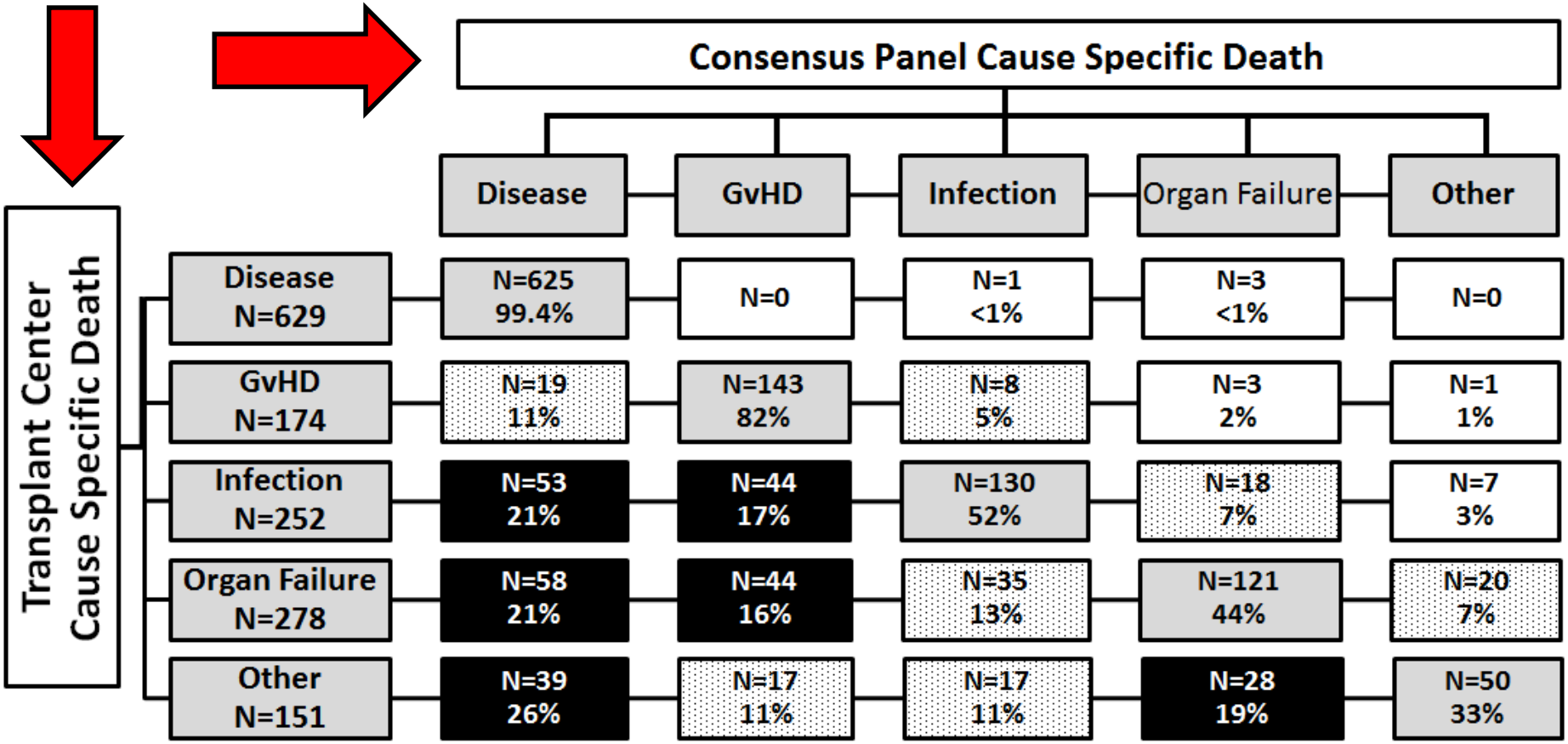
Cause of death adjudication



Hahn T et al BBMT 21:1679, 2015

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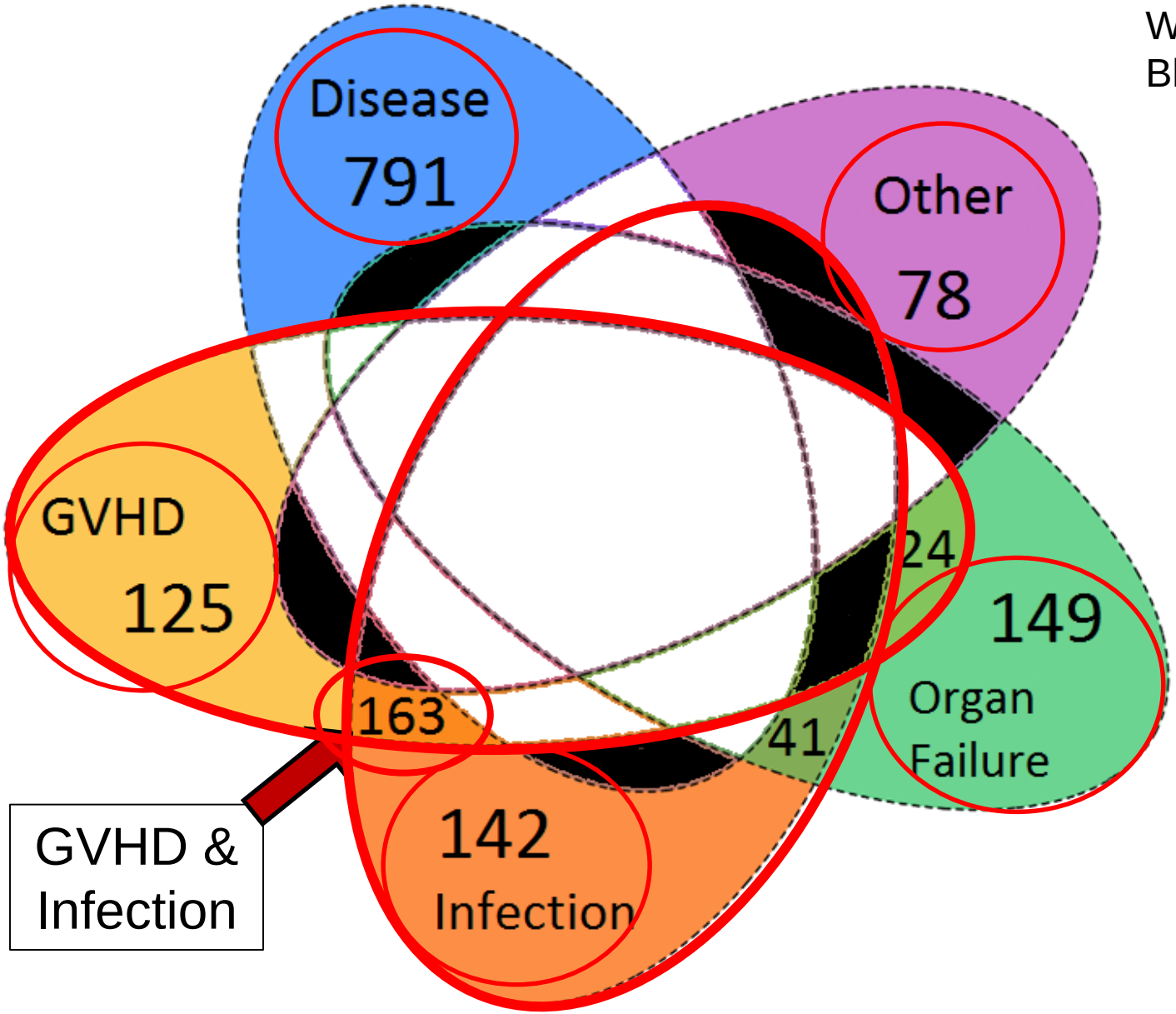
N recipients by cause-specific death

White = 0 pts
Black = 2-8 pts



Aim: Map the effects of recipient and donor genetic variation (non-HLA) associated with survival outcomes after HLA-matched allogeneic BMT.

Candidate gene approach

- Variation in genes with known mechanism
- Driven by known biological knowledge

Genome-wide approach

- Agnostic
- **D**etermining the **I**nfluence of **S**usceptibility **CO**nveying **V**ariants **R**elated to 1-**Y**ear mortality after **BMT** (**DISCOVeRY-BMT**)

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Published candidate gene studies of post-BMT survival

An exhaustive PubMed search using MeSH terms revealed 70 publications:

- 458 SNPs and 2 multi-allelic variants have been tested for associations with survival outcomes in the past two decades
- 45 SNPs in 36 genes were significantly associated with a survival outcome.

Karaesmen E, Rizvi AA et al., *Blood,* May 2017

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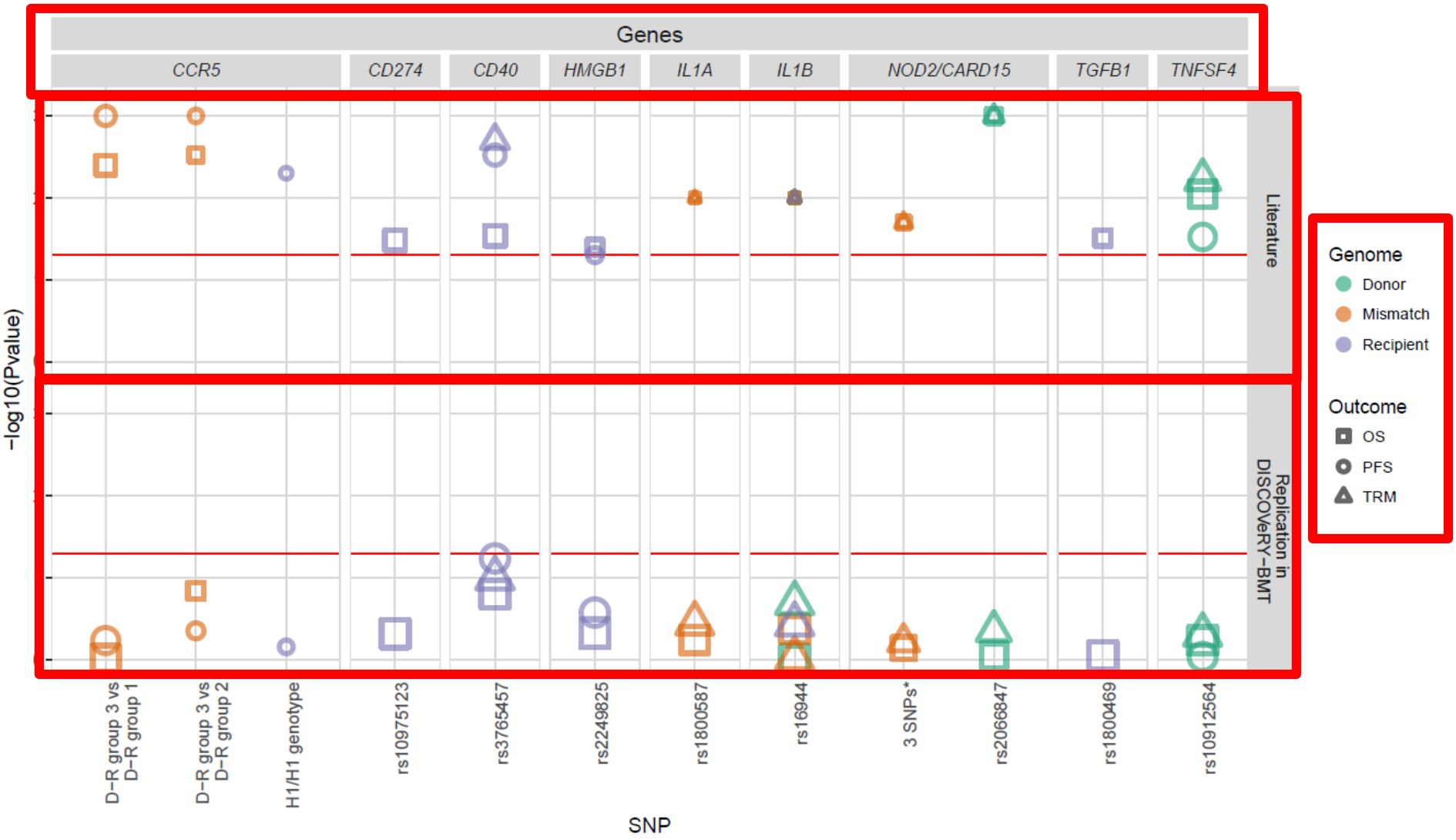
Results of candidate SNP analyses in DISCOVeRY-BMT



Karaesmen E, Rizvi AA et al., *Blood,* May 2017

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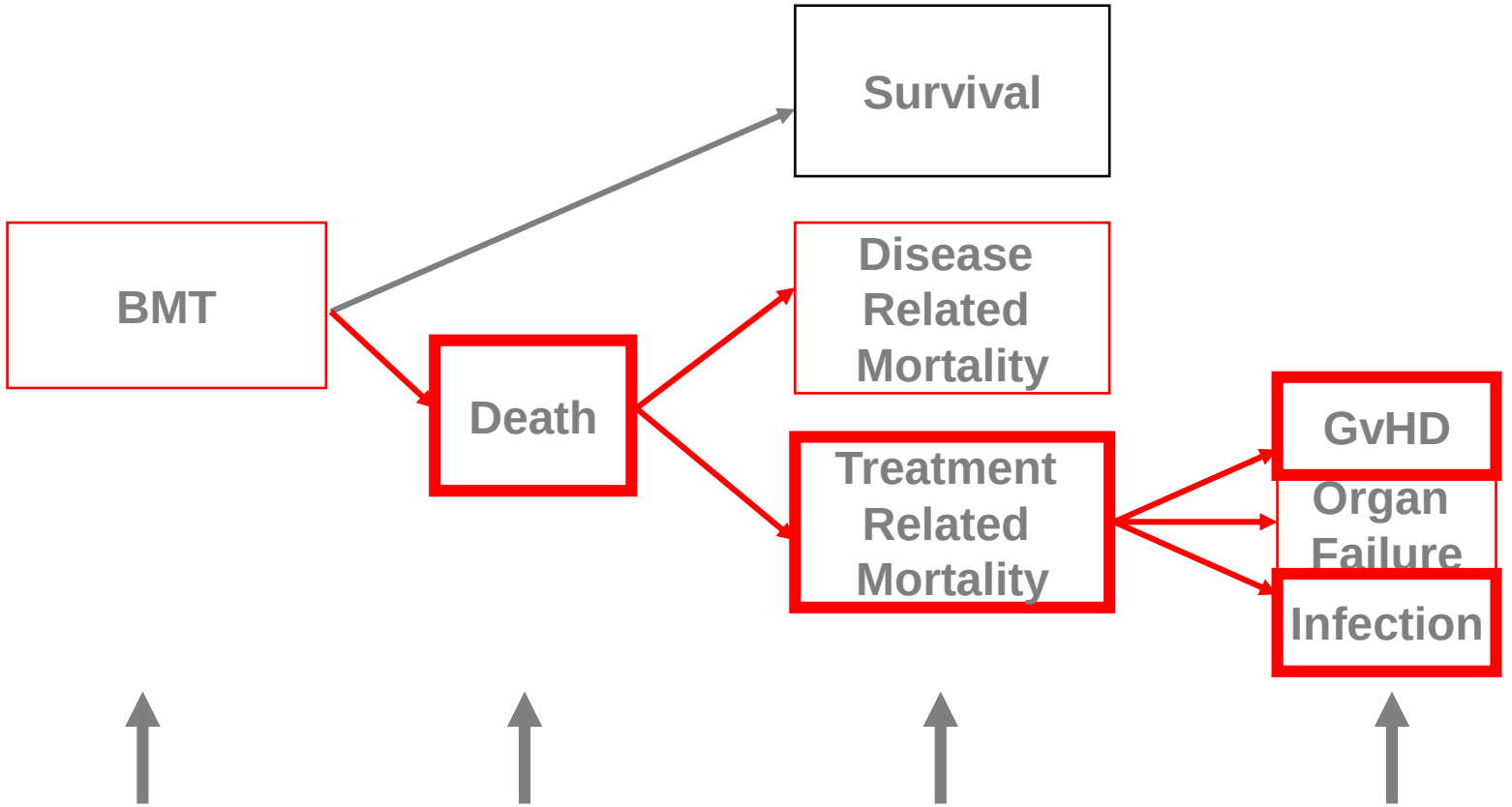
DISCOVeRY-BMT genome-wide analyses of SNPs related to outcomes 1 year after BMT



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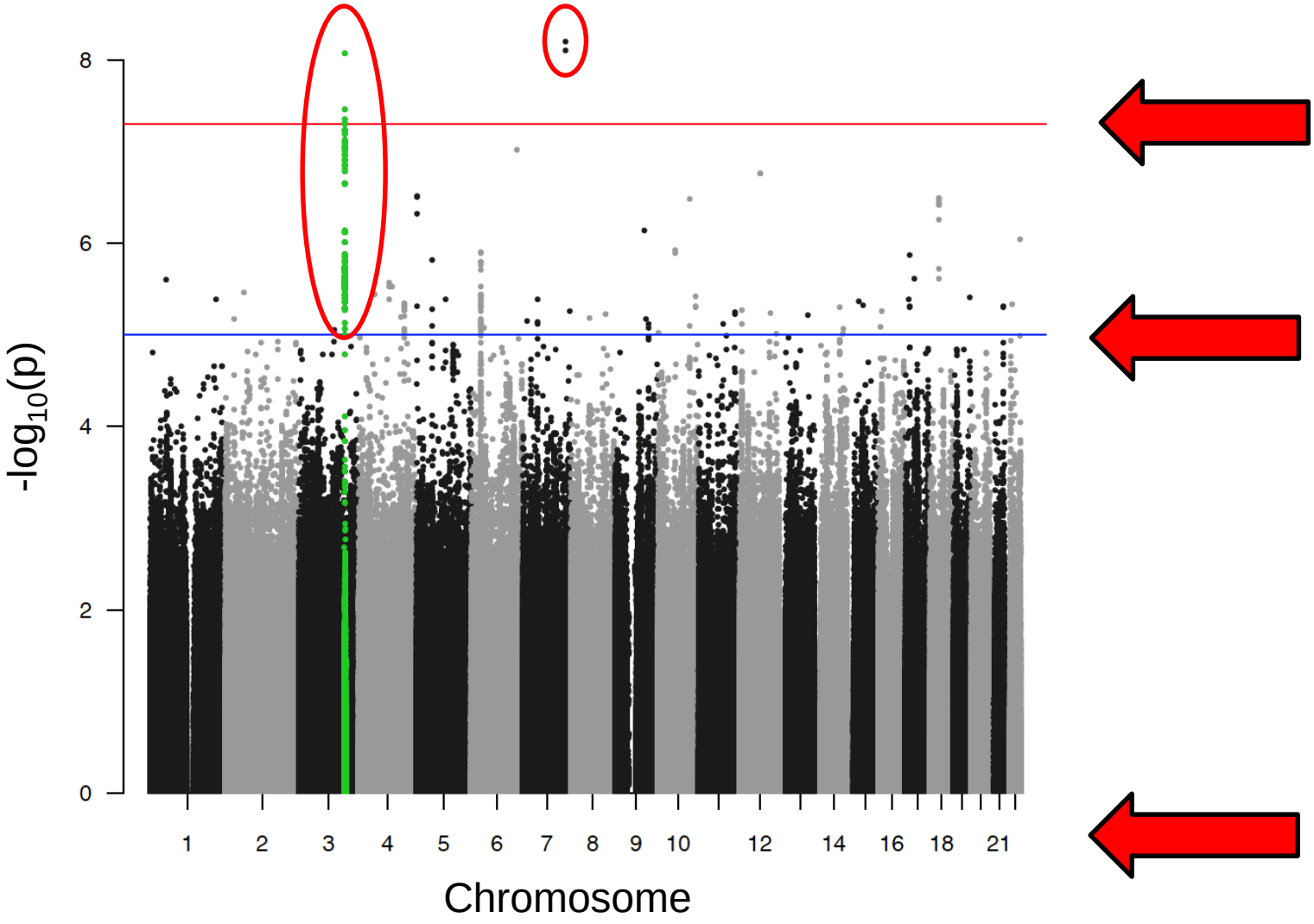
Recipient SNPs outside HLA region correlate with overall survival

Manhattan Plot – Recipient Genotypes



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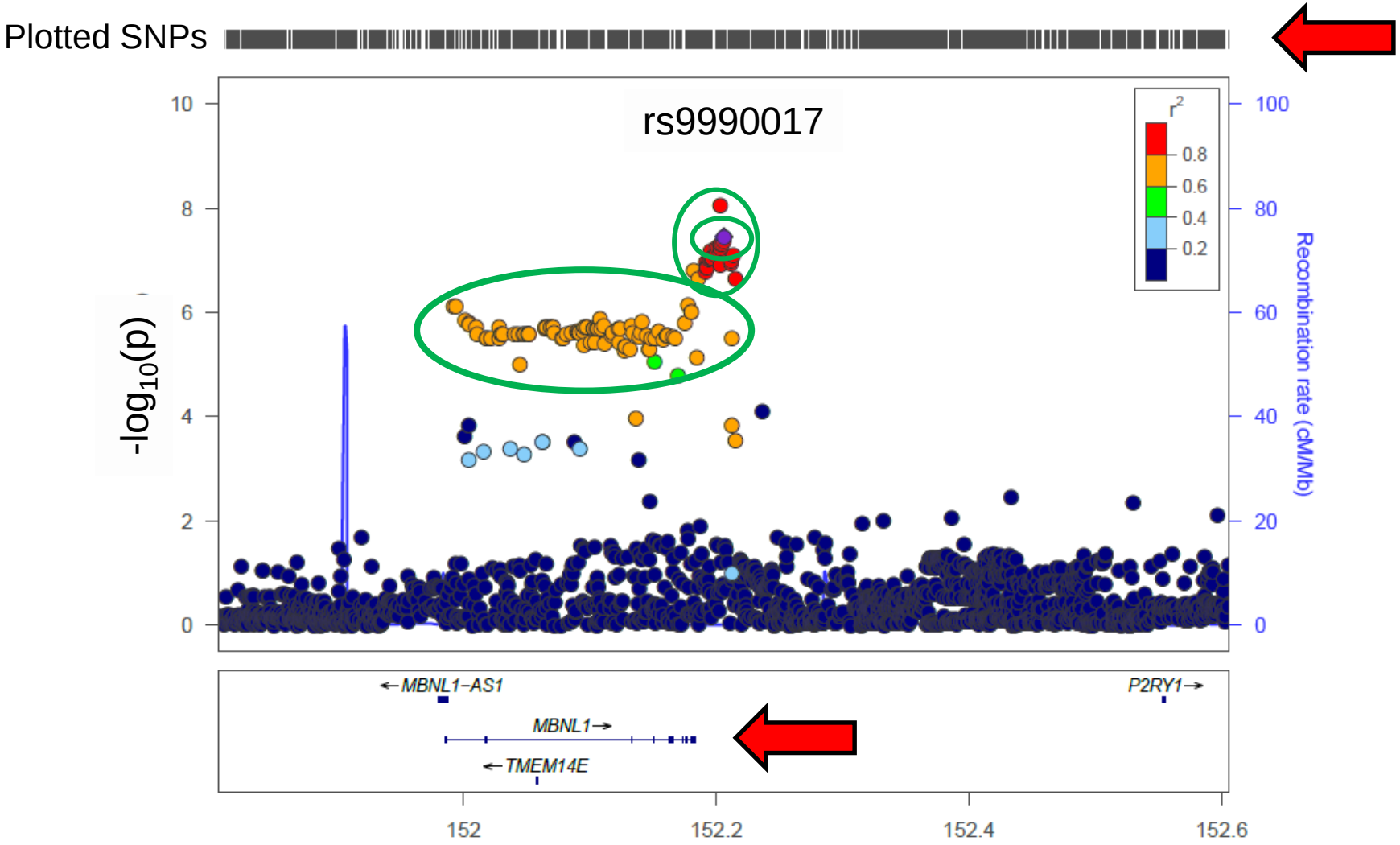
Turner, S.D. qqman: an R package, 2014

MBNL1 LocusZoom Plot



Pruim RJ, et al., *Bioinformatics* 2010

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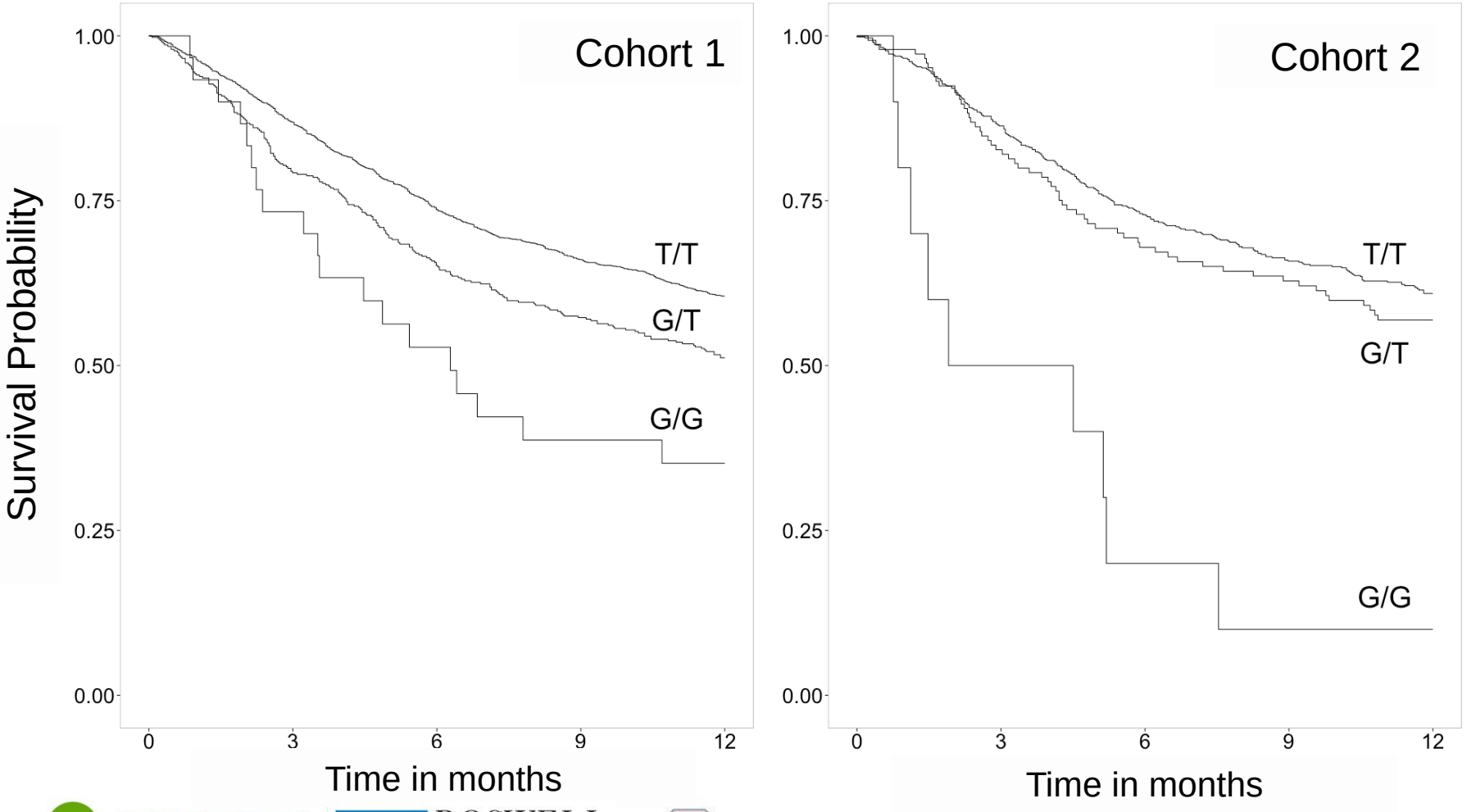
Overall Survival by Genotype rs9990017



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Cause of Death by Genotype at rs9990017

	GG	GT	TT	Total
Disease	4 (14%)	**155 (56%)**	**477 (54%)**	636
GVHD	**6 (21%)**	43 (16%)	142 (16%)	191
Infection	**7 (25%)**	32 (12%)	119 (14%)	158
Organ Failure	**6 (21%)**	32 (12%)	92 (10%)	130
Other	5 (18%)	11 (4%)	48 (5%)	64
Total	28	273	878	1179

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MBNL1 – Muscleblind-like protein 1

- Highly conserved gene across species
- Primary function is to regulate pre-mRNA alternative splicing
- MBNL1 protein is highly expressed in blood cells (monocytes, T, NK, DC)
- MBNL1 is overexpressed in MLL-fusion gene acute leukemias in children

Ross et al, *Blood* 104:3679, 2004

SNPs outside HLA region correlate with TRM when the donor-recipients are mismatched

Definition of allele mismatch between unrelated donor (D) and recipient (R)

		Recipient Genotype		
		AA	Aa	aa
Donor Genotype	AA	0	1	2*
	Aa	1	0	1
	aa	2*	1	0

0= No allele mismatch between D & R

1= One allele mismatch between D & R

2= Two allele mismatch between D &R

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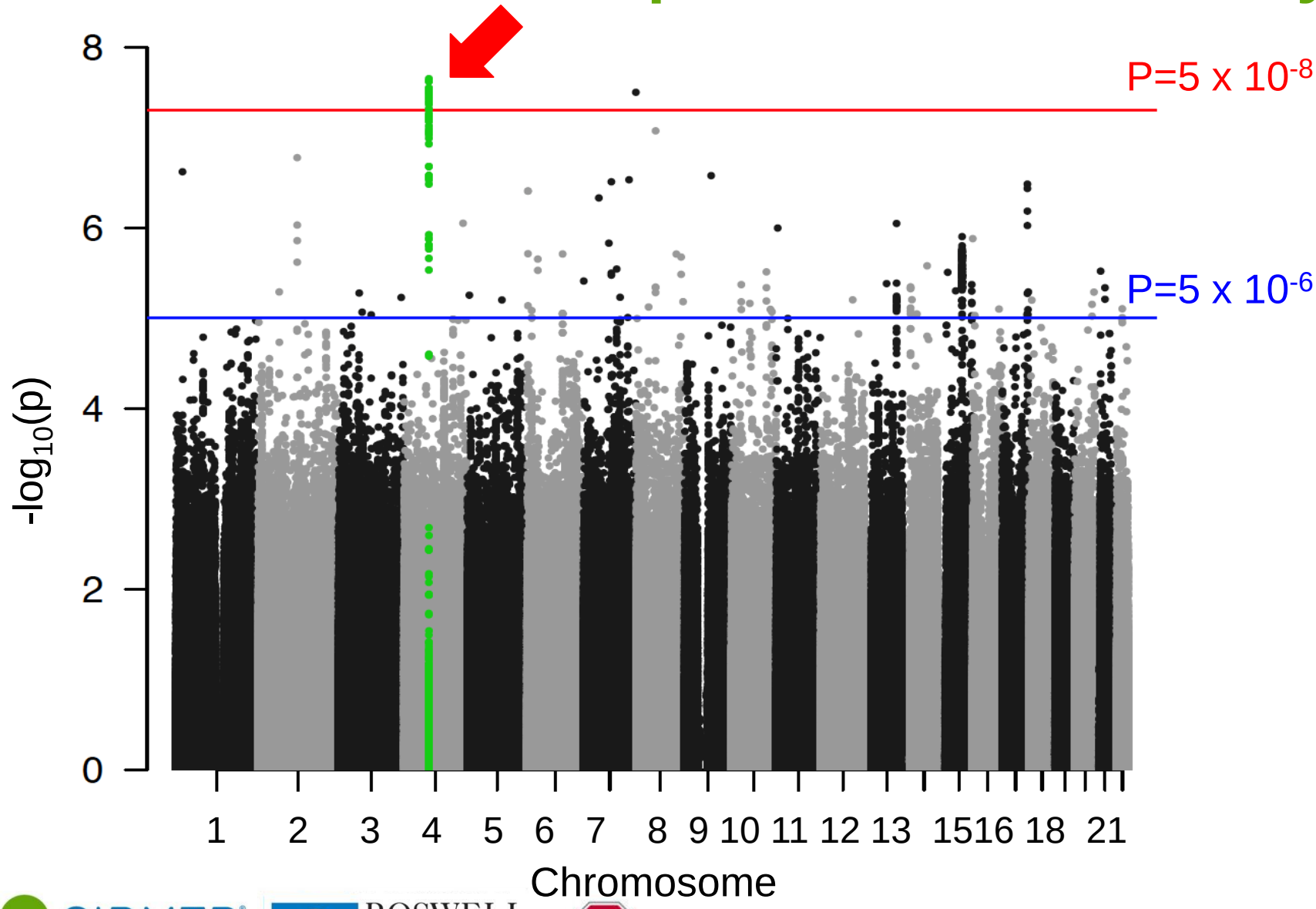
Manhattan Plot: Transplant related mortality



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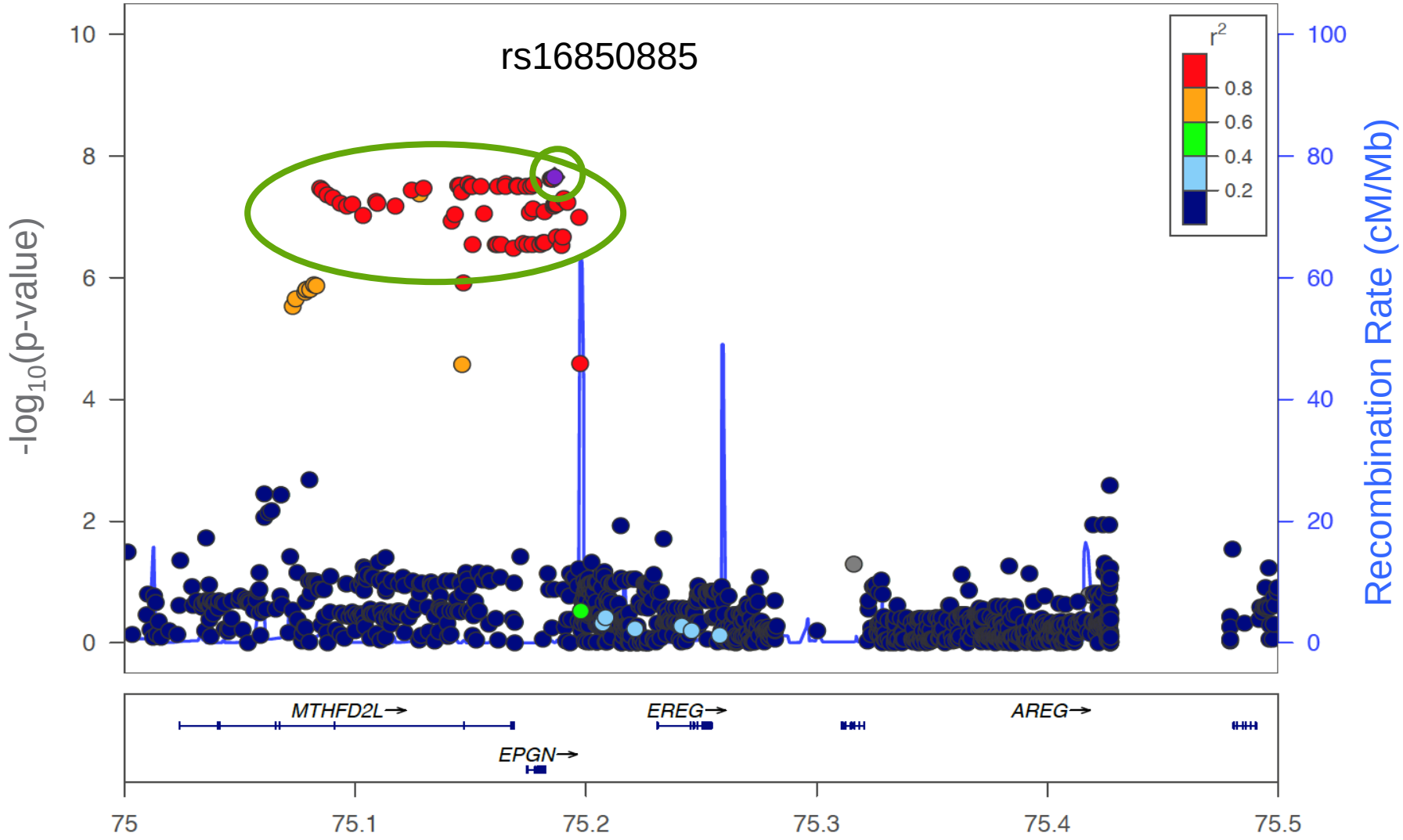
Regional Association Plot of Chromosome 4



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Pruim RJ, Welch RP et al., Bioinformatics 2010

TRM association with rs16850885

Donor / Recipient	HR (95% CI)	Transplant-Related Mortality			
	C1/C2	Alleles	Cohort 1 P	Cohort 2 P	P meta
D-R mismatch	2.6 (2.1, 3.1) 2.6 (1.7, 3.5)	--	7.6×10^{-7}	6.0×10^{-3}	1.8×10^{-8}
Donor	2.3 (1.7, 2.9) 4.5 (1.9, 6.6)	*A/G*	1.6×10^{-3}	4.0×10^{-4}	4.7×10^{-6}
Recipient	2.6 (1.9, 3.2) 1.2 (0.5, 1.9)	*A/G*	4.0×10^{-4}	0.8	2.0×10^{-3}

Sucheston-Campbell, et al, Tandem BMT meeting Feb 2016

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Summary

- Prior GWAS showed genome-wide significant association of this SNP with inflammatory response to smallpox vaccine
- Individuals with at least 1 A allele have significantly lower levels of secreted IL-1β after immune stimulatory response
- IL-1β is a pro-inflammatory cytokine secreted early in the inflammatory response and has been previously associated with risk of GVHD and infection in BMT patients
- Several other SNPs in strong linkage disequilibrium with rs16850885 are predicted to likely affect transcription factor binding

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Different SNPs contribute to different cause-specific deaths

Top Recipient SNPs and Infection Death

SNP	MAF/ effect allele	Chr	Nearest Gene	HR (95% CI) Cohort 1 / Cohort 2	P Cohort 1 / Cohort 2	P meta
Infection as sole cause of death						
rs1791577 typed	13% / T	11	*GUCY1A2*	1.5 (1.2, 1.8) 2.3 (1.8, 2.8)	1.6×10^{-3} 0.02	2.8×10^{-4}
rs2511141 imputed	13% / T	11	*GUCY1A2*	1.5 (1.2, 1.8) 2.3 (1.8, 2.8)	1.2×10^{-3} 0.01	1.2×10^{-4}
Infection with or without other causes of death						
rs1791577 typed	13% / T	11	*GUCY1A2*	1.7 (1.5, 1.9) / 1.9 (1.5, 2.1)	5.7×10^{-6} / 8×10^{-4}	2.0×10^{-8}
rs2511141 imputed	13% / T	11	*GUCY1A2*	1.7 (1.5, 2.0) / 1.9 (1.5, 2.2)	2.4×10^{-6} / 9×10^{-4}	6.9×10^{-9}

Hahn, et al, Tandem BMT meeting Feb 2016

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Top Donor SNP and GvHD Death

SNP	MAF/ effect allele	Chr	Nearest Gene	HR (95% CI) Cohort 1 / Cohort 2	P Cohort 1 / Cohort 2	P meta
GvHD as the sole cause of death						
rs115483549 imputed	7% / G	6	*BTNL2*	2.5 (2.2, 2.9) / 2.3 (1.7, 2.9)	6.5×10^{-7} / 0.007	1.7×10^{-8}
GvHD with or without other causes of death						
rs115483549 inputed	7% / G	6	*BTNL2*	2.0 (1.6, 2.3) / 2.3 (1.7, 2.9)	4.3×10^{-5} / 0.004	1.9×10^{-8}

Hahn, et al, Tandem BMT meeting Feb 2016

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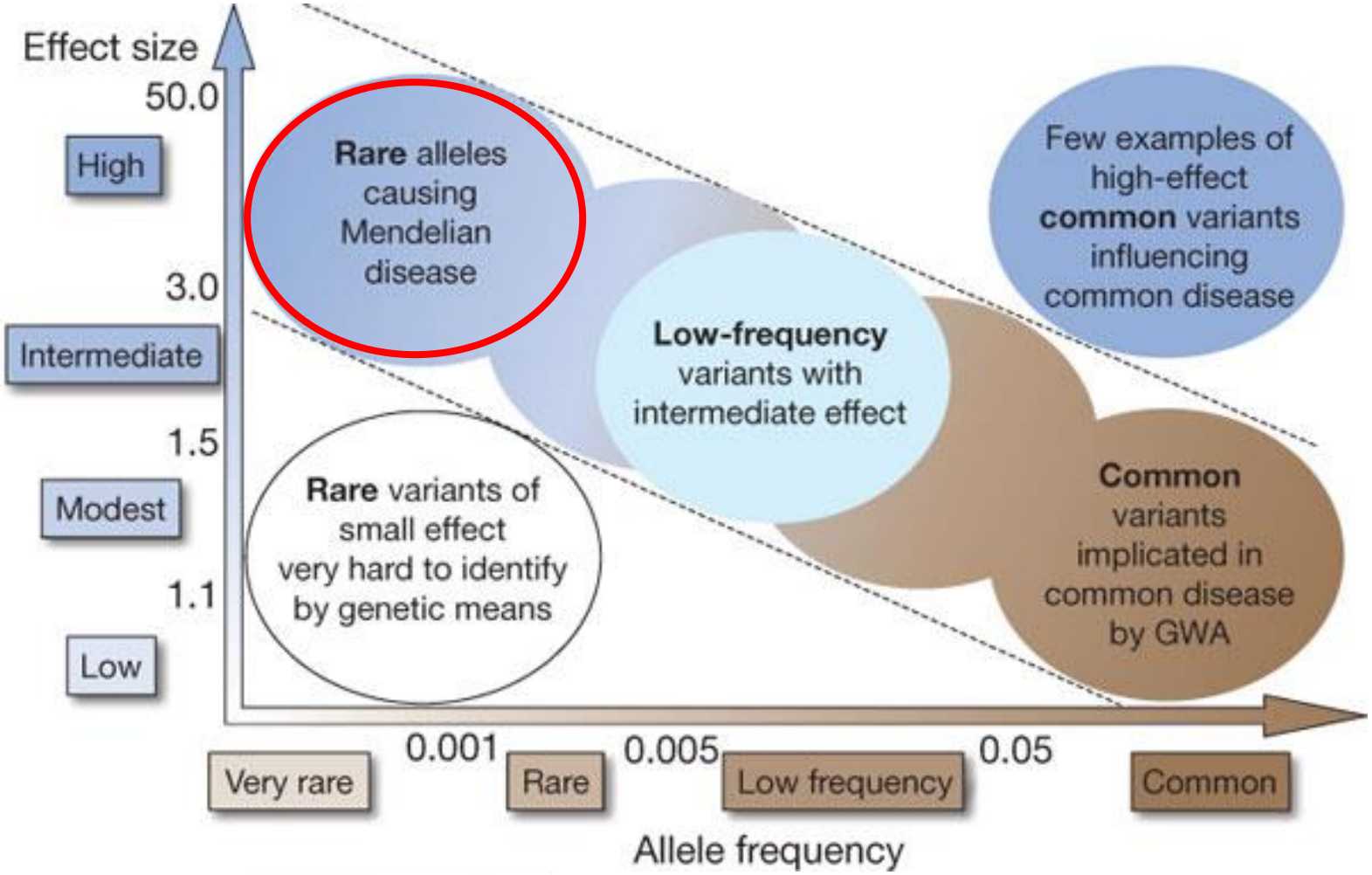
The role of rare and less common variants in BMT survival

Motivation for a genomic study of transplant outcomes



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Manolio *et al.* (2009) *Nature* 461:747-753

Illumina HumanExome BeadChip

- Putative functional exonic variants selected from over 12,000 individual exome and whole-genome sequences

Marker Categories	Number of Markers
Total markers	> 240,000
Number of unique RefSeq entries covered by at least 1 probe	> 20,000
Nonsynonymous SNPs (NCBI)	219,621
SNPs in splice sites	10,675
Stop variants	5,637
SNPs in promoter regions	7,012
SNPs in extended MHC region	5,158
GWAS tag markers[a]	4,761
HLA tags	2,061
Ancestry informative markers	3,468
Identity by descent markers	3,369
X / Y / mitochondrial markers	470 / 101 / 177
Indels	180

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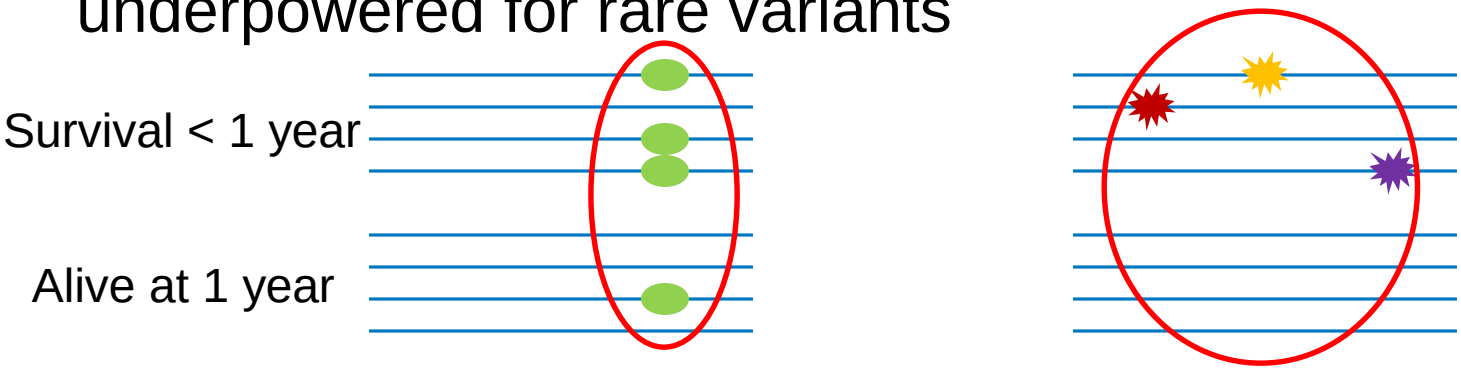
Gene-based statistical test

- Standard methods for single variant test can be underpowered for rare variants



- Collapse the effect of rare variants within a gene
- The unified **O**ptimal **S**equence **K**ernel **A**ssociation **T**est (**SKAT-O**)
  - Allow individual variants to have different directions and magnitude of effect
  - permit covariate adjustment

Ionita-Laza, *et al.* (2013) *AJHG* 92:841–853

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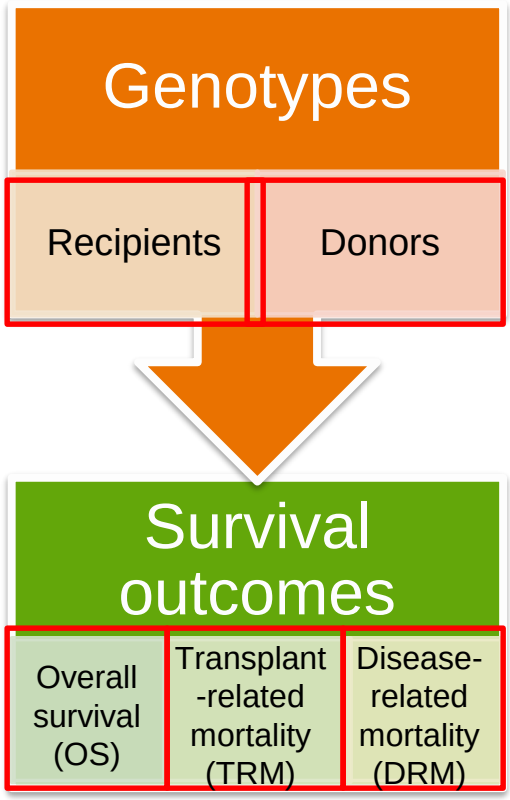
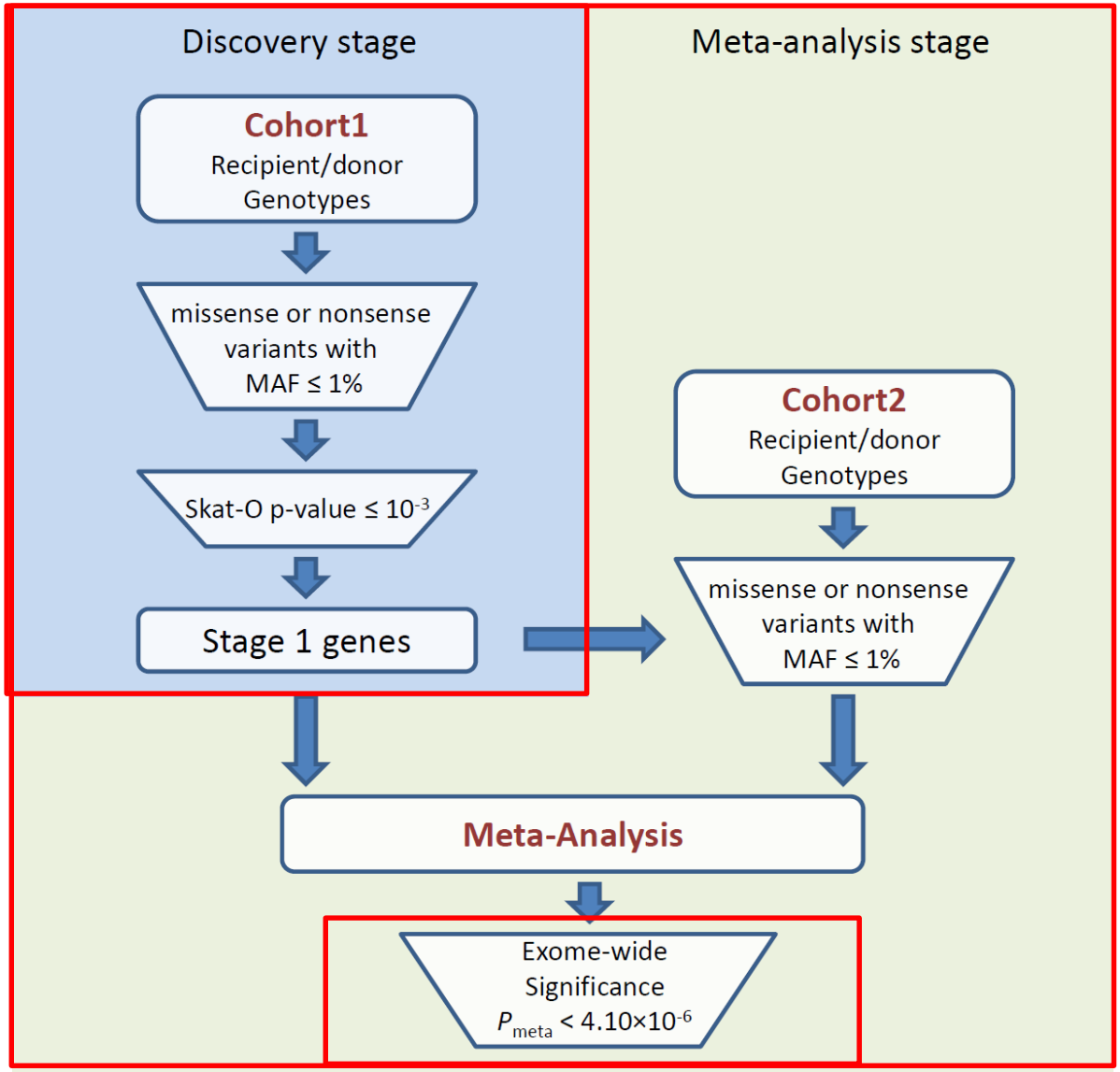
Analyses



Covariates include age, disease status pre-BMT, ALL status, MDS status, population stratification.



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Donor gene associations with recipient survival outcomes

Overall Survival		
Gene	Chr	P_{meta}
ALPP	2	1.05×10^{-6}
EMID1	22	1.05×10^{-6}
SLC44A5	1	1.05×10^{-6}
LRP1	12	2.86×10^{-6}

Transplant Related Mortality		
Gene	Chr	P_{meta}
HHAT	1	9.34×10^{-7}

Disease Related Mortality		
Gene	Chr	P_{meta}
LYZL4	3	1.05×10^{-6}
NT5E	6	1.05×10^{-6}

NT5E: 5'-nucleotidase, ecto (CD73)

- catalyzes the conversion of extracellular nucleotides to membrane-permeable nucleosides
- the encoded protein is used as a determinant of lymphocyte differentiation
- a key metabolic regulator of several drugs used for BMT including Fludarabine and mycophenolate mofetil
- overexpression has been found in various cancer types and is associated with poor prognosis

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Donor *NT5E* variant associations with recipient survival outcomes

Variant	Chr:BP	Alleles (Ref/Alt)	AA change	Impact*	MAF in DISCOVeRY-BMT
rs200250022	6:86,160,041	G/T	A62S	D	.000574
rs200369370	6:86,176,895	C/G	Q153E	T	.000287
rs41271617	**6:86,195,033**	**G/A**	**V278I**	**D**	**.00052**
rs200648774	6:86,197,163	C/T	R354C	D	.000278
rs144719925	6:86,199,308	C/T	R401C	D	.000287
rs145505137	6:86,201,876	A/C	E514D	T	.000287

*Amino acid change is predicted to be deleterious and probably/possibly damaging

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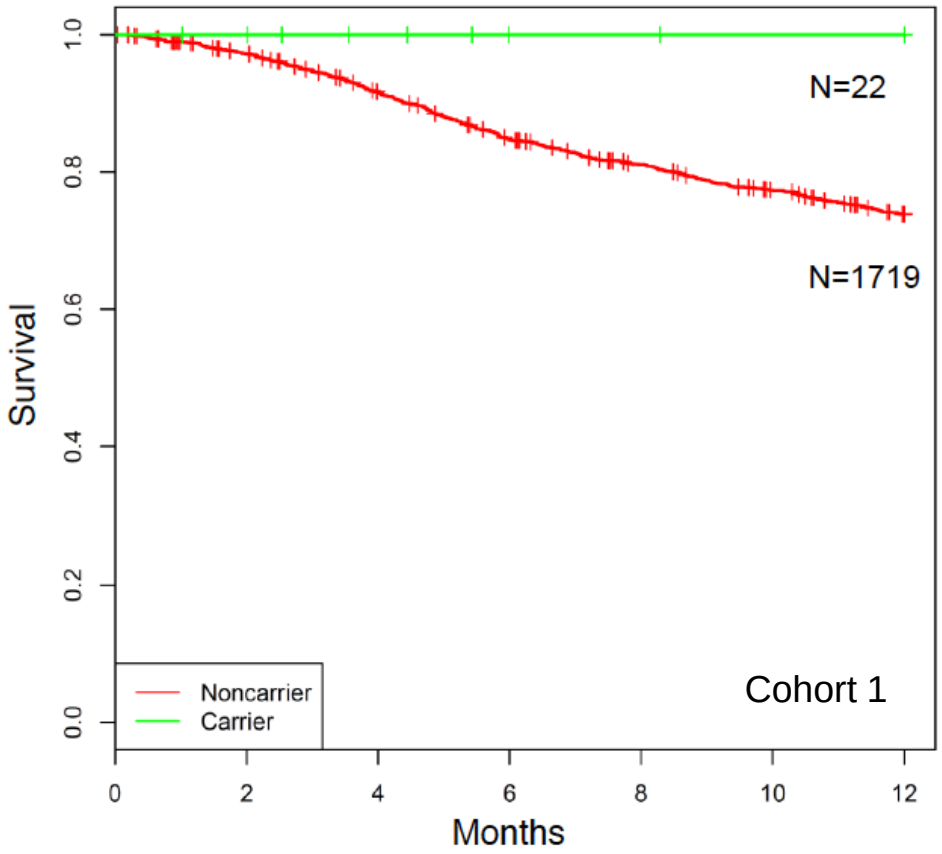
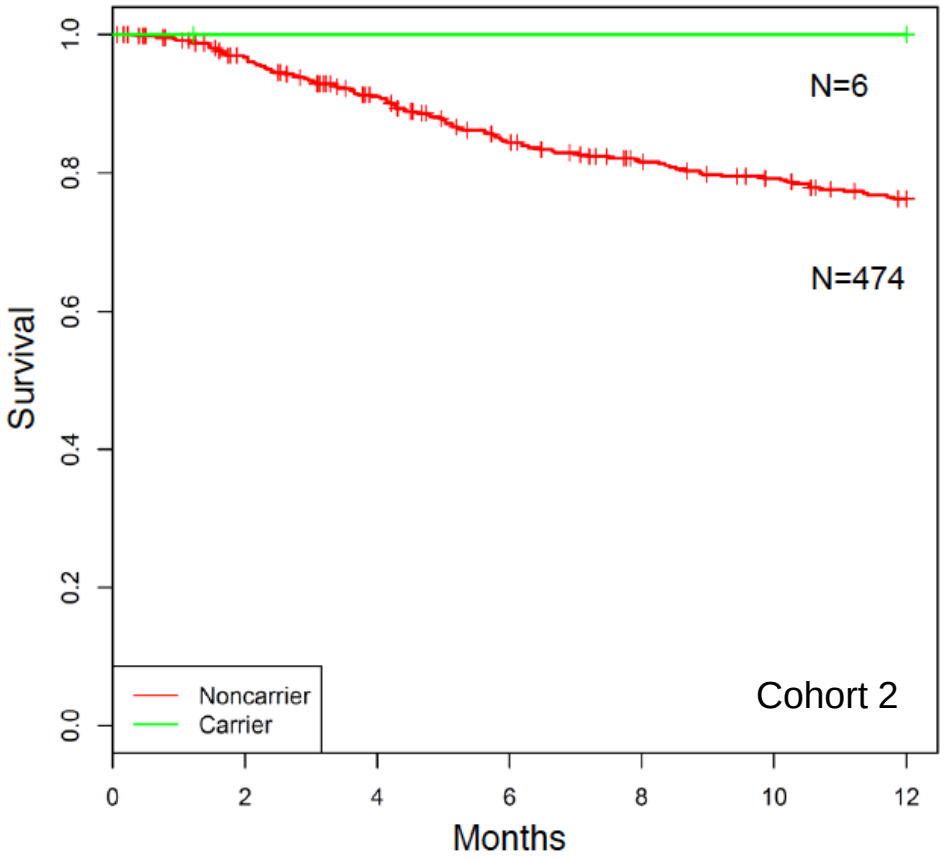
NT5E gene association with disease death





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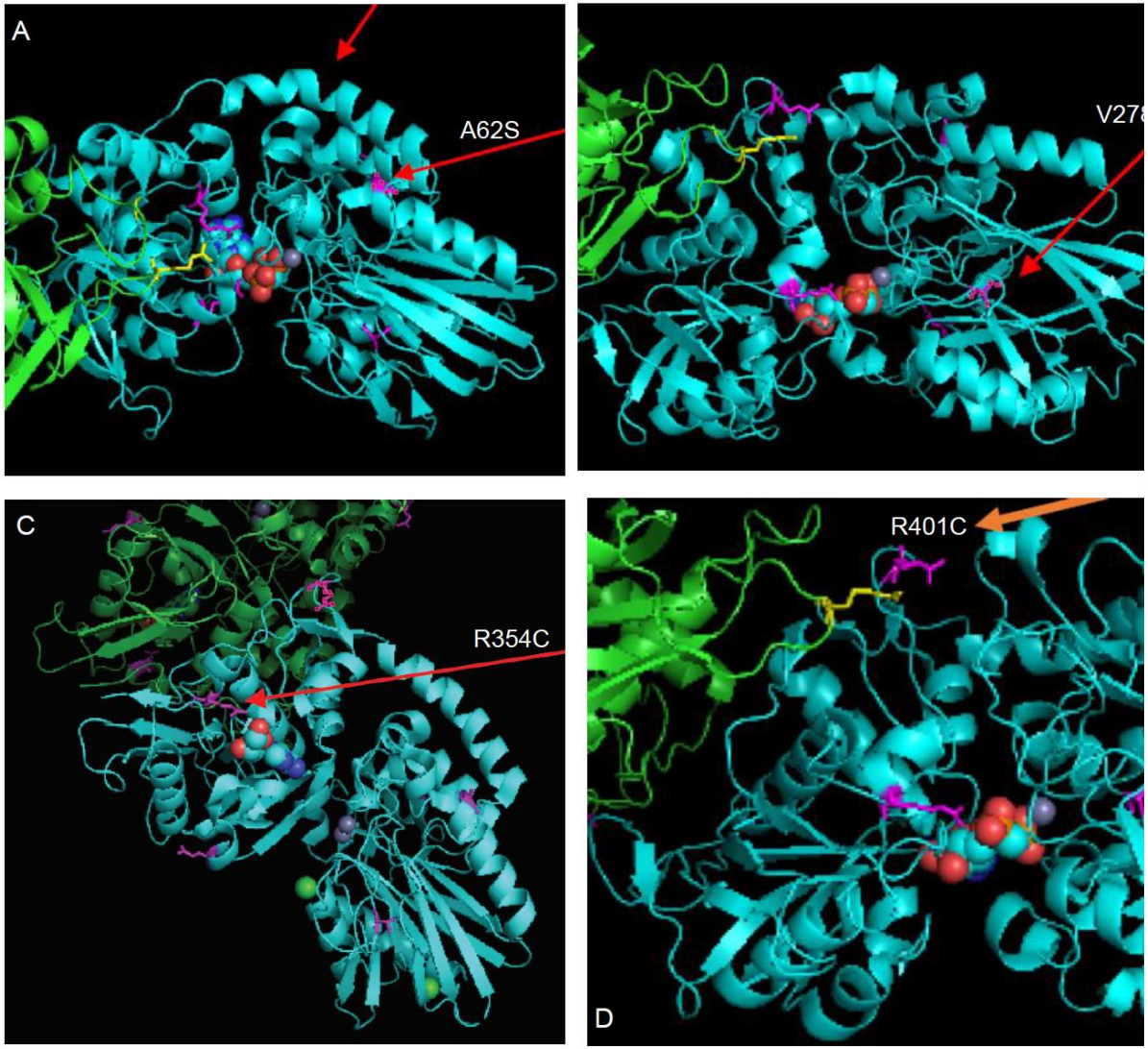

The *NT5E* variants in the crystal structure of the enzyme.

(A) A62S variant directly interacts with the α-helix, which connects the two protein domains and works as a hinge for the conformational change between open and closed states. A62S is predicted to impede the enzyme's conformational change.

(B)V278 is buried in the center of the N-terminal domain. V278I is predicted to impact the folding of the domain and the size of the catalytic pocket.

(C)The side chain of R354 forms a hydrogen bond with adenosine. R354C is predicted to disrupt the binding of adenosine.

(D)R401 interacts with R480 from the other chain (yellow) in the closed state. R401C is predicted to impede the binding of the two chains and NT5E dimerization.

Rare *NT5E* mutations present in donors can impact various aspects of the enzyme activity, including protein conformation change, dimerization, and binding with adenosine.

Summary

- *NT5E*- donors carrying mutations may have lower efficiency of generating adenosine, and hence the recipients whose donors carried the mutation experience what could be described as NT5E blockage.
  - Preclinical studies have demonstrated that targeted blockade of *NT5E* can effectively inhibit tumor growth. This observation may provide clinical evidence supporting anti-NT5E therapy for cancer patients.

A tool for exploring our results with collaborators

Gene Viewer | mary | Manhattan Plot | Gene Info

Show 10 entries Search:

gene	rsID	chr	BP	impute	Pvalue	cohort	disease	genome	outcome	geneBasedPvalue	topSNP	topSNPpVal	BPfromtopSNP
ABCB1	rs78753990	chr7	87123227	typed	0.01192	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	6880
ABCB1	rs998671	chr7	87123275	typed	0.66270	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	6832
ABCB1	rs12538707	chr7	87124268	typed	0.83030	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	5839
ABCB1	rs117503479	chr7	87124294	typed	0.54980	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	5813
ABCB1	rs17209837	chr7	87124822	typed	0.48010	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	5285
ABCB1	rs79246013	chr7	87124959	typed	0.01350	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	5148
ABCB1	rs73198343	chr7	87125565	typed	0.87970	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	4542
ABCB1	rs73198345	chr7	87125643	typed	0.56810	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	4464
ABCB1	rs12672720	chr7	87126438	typed	0.81670	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	3669
ABCB1	rs55747905	chr7	87127099	typed	0.34700	M	mixed	R	TRM	0.8361638	rs73383422	0.01111	3008
gene	rsID	chr	BP	impute	Pvalue	cohort	disease	genome	outcome	geneBasedPvalue	topSNP	topSNPpVal	BPfromtopSNP

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