

Investigating Mechanisms of Leukemia Immune Evasion and Relapse after Hematopoietic Stem Cell Transplantation

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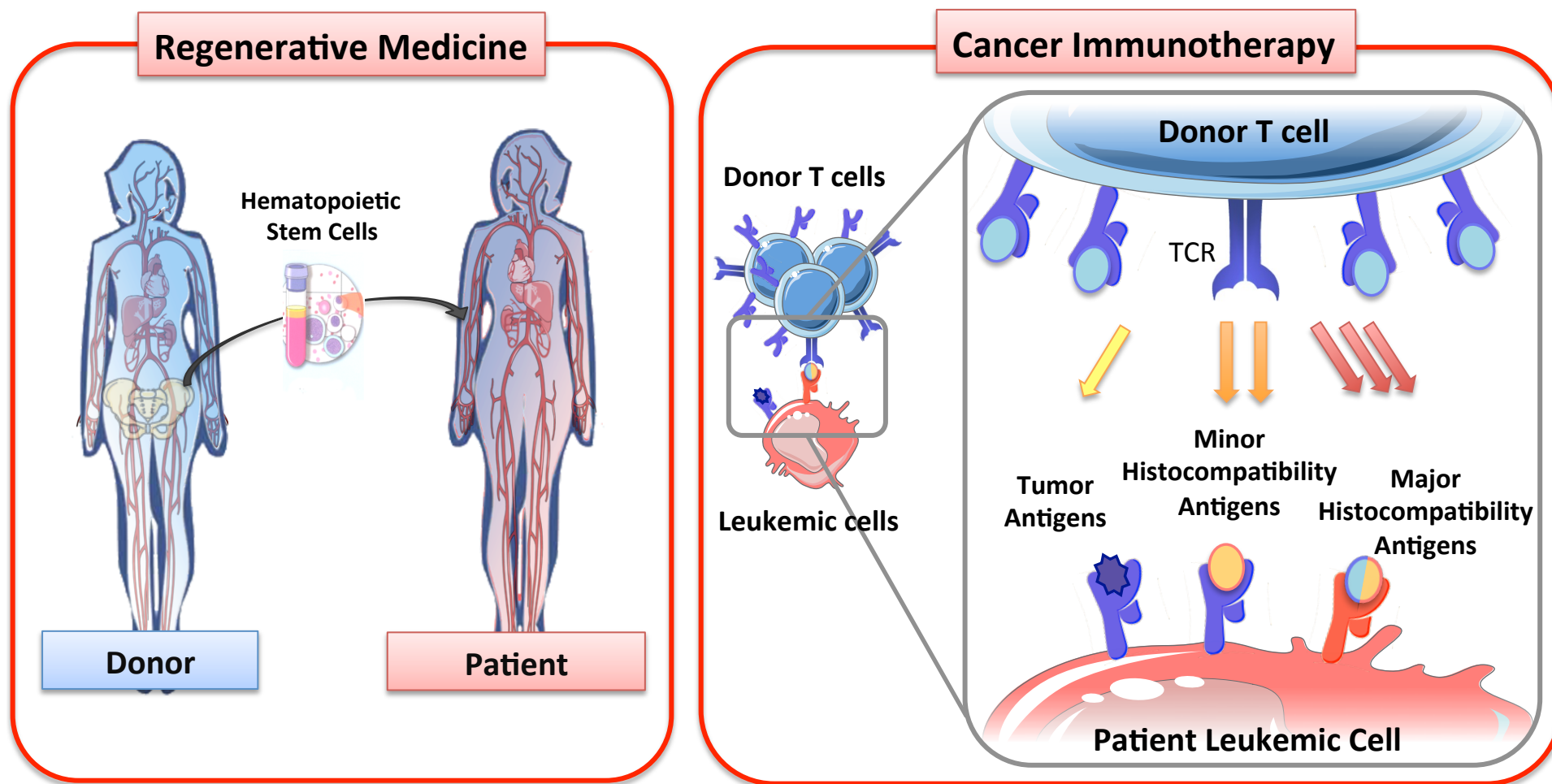


OSPEDALE SAN RAFFAELE

**1st Cuneo City Immunotherapy Conference (CCITC)
May 18th, 2018**

Rationale of Allogeneic HSCT

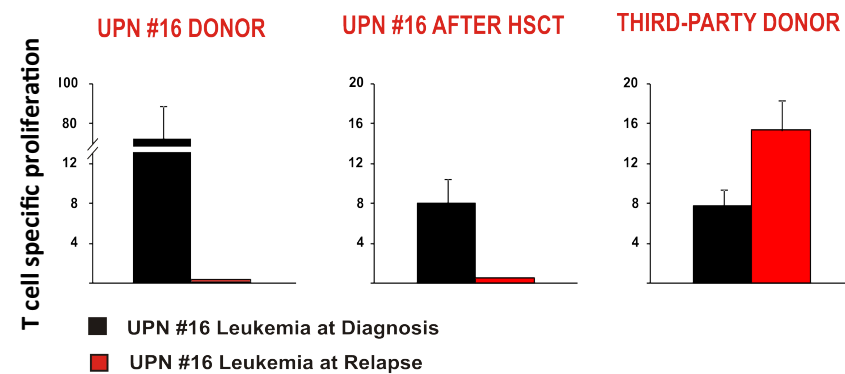
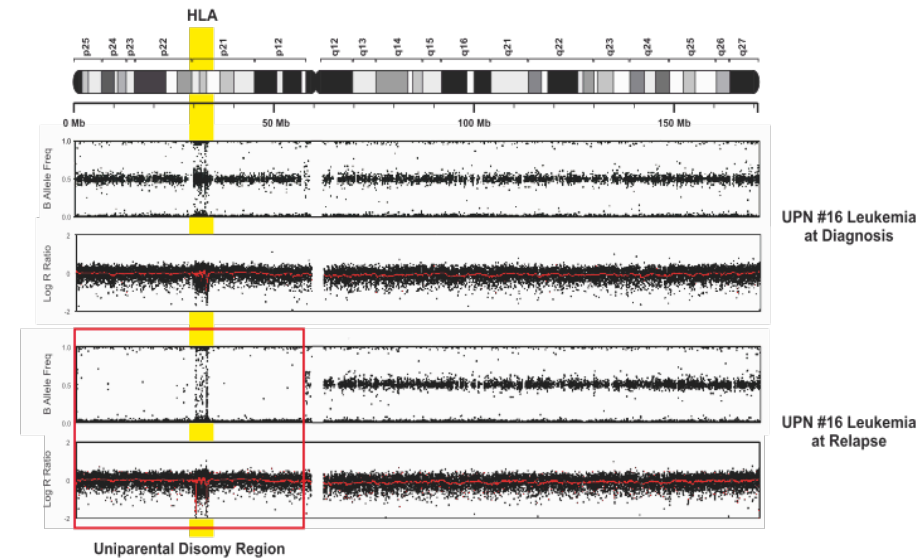
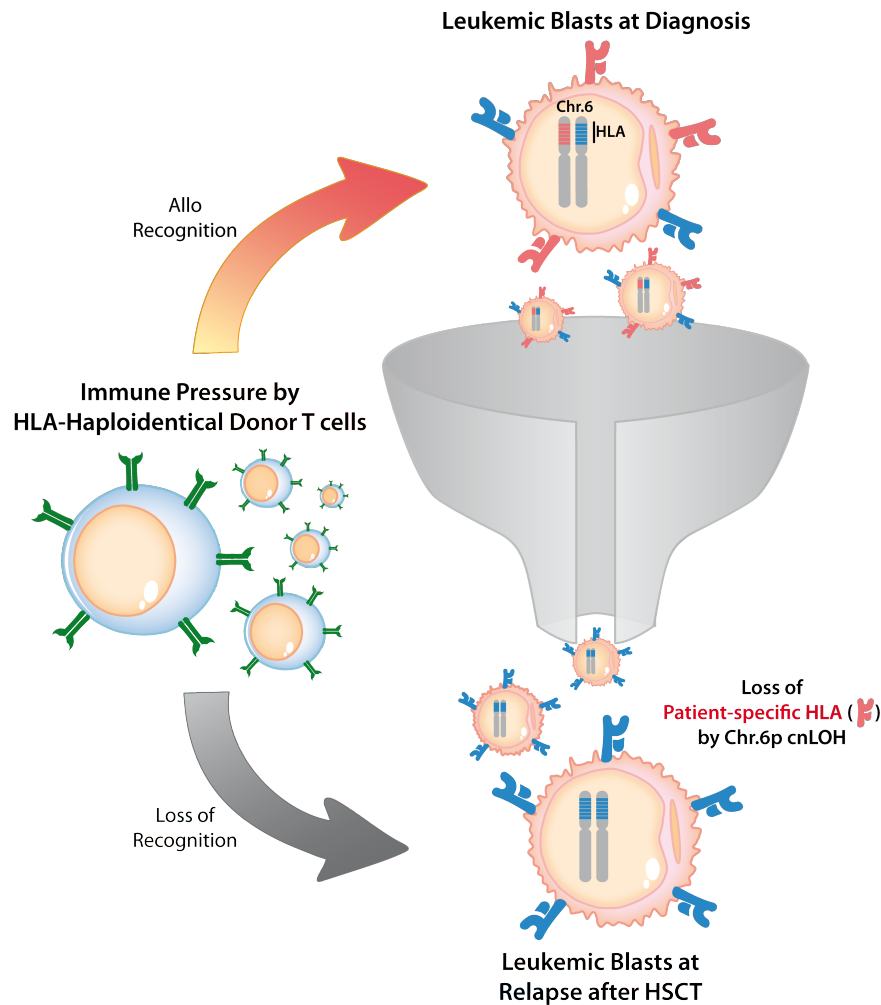
Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT) represents the most successful and widely available form of **adoptive immunotherapy** for leukemia



Outline of the Talk

1. **HLA loss:** going back and forth from bedside to bench
2. **Beyond HLA loss:** deciphering novel mechanisms of leukemia immunoediting

Background: Molecular Mechanism and Immunological Consequences of HLA Loss

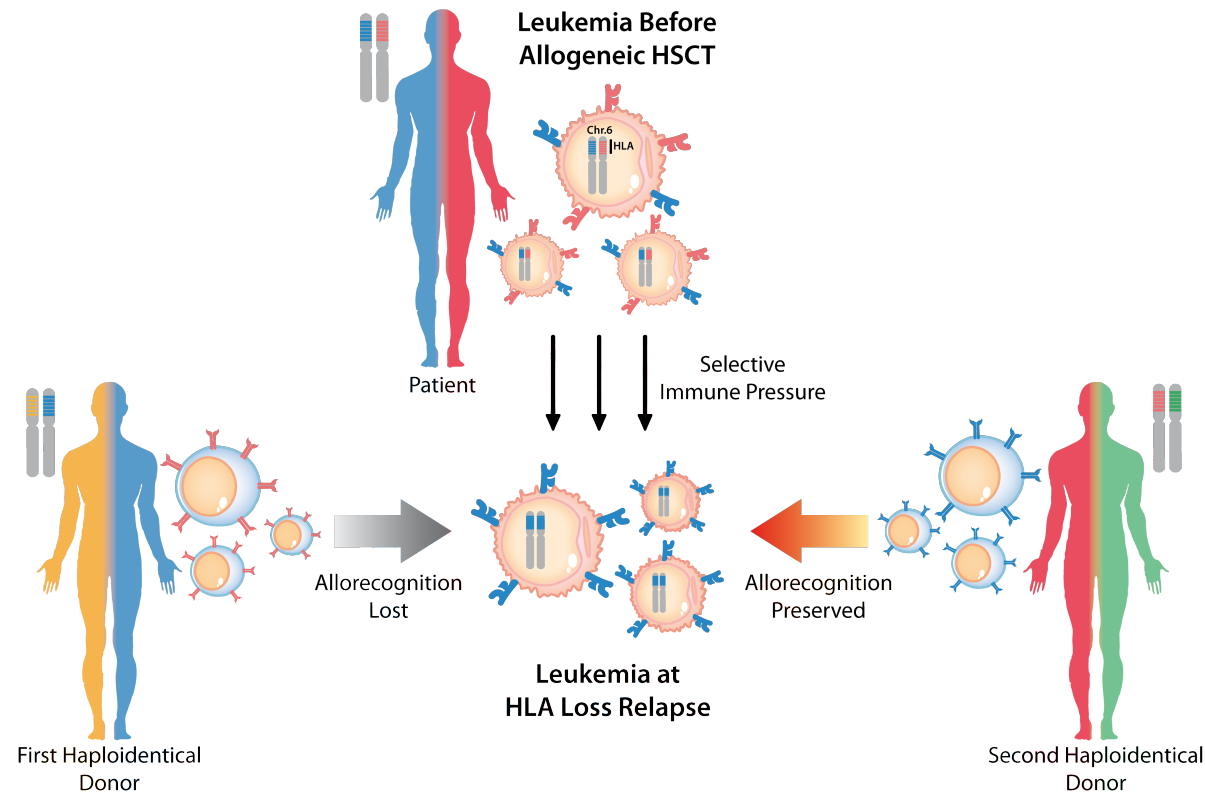


Horowitz, Bone Marrow Transplant, 2018

Vago, N Engl J Med, 2009

Background: Clinical Implications of HLA Loss Relapses

- Reduced therapeutic potential of DLIs from the original donor
- Specific rationale for immunotherapy with bispecific antibodies or CAR T cells
- Criterion for selection of a second haploidentical donor

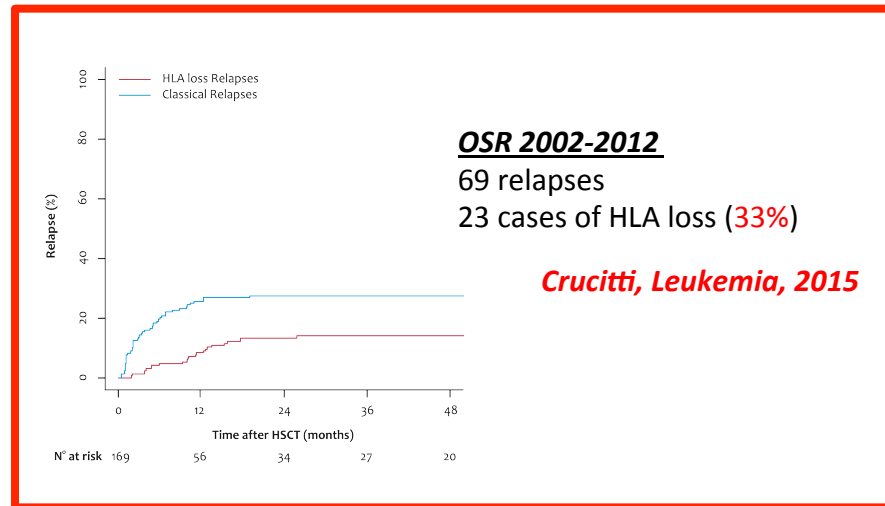


Frequency of HLA loss in Different HSCT Settings

Haploidentical HSCT

Several relatively small studies in different settings and with different platforms, all confirming high frequency of HLA loss

Villalobos, Blood, 2010
Peccatori, Leukemia, 2014
McCurdy, Leukemia, 2016
Grosso, Bone Marrow Transplant, 2017



Unrelated Donor HSCT

Even smaller series, demonstrating that HLA loss relapses can occur in UD-HSCT, but the actual incidence (possibly lower) still remain to be assessed

Waterhouse, Biol Blood Marrow Transplant, 2011
Toffalori, Blood, 2012
Hamdi, Bone Marrow Transplant, 2015

Hypothesis and Objectives of the Project

Hypothesis

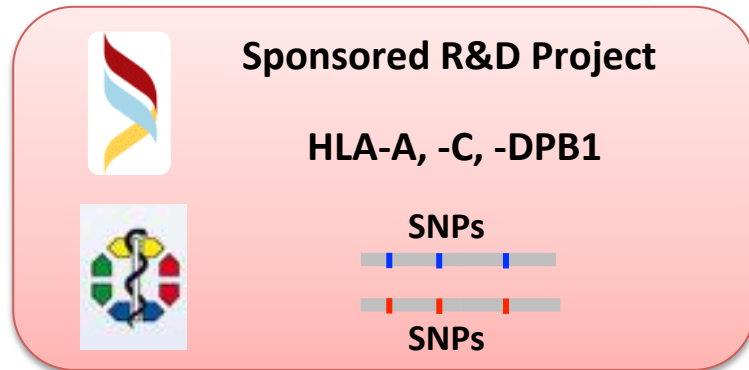
Developing innovative tools for the detection of HLA loss relapses may improve current knowledge on the immunobiology of relapse, allow informed choice of treatment, and ultimately improve the clinical outcome of allo-HSCT

Objectives

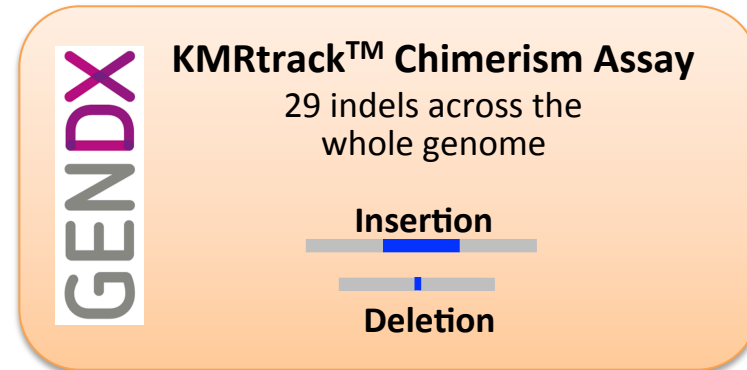
- Setup and validation of molecular assays for the detection of HLA loss variants (Partners 1 and 5)
- Analysis of the frequency and risk factors for these variants in a large multi-centric trial (All Partners)
- Dissemination of know-how to implement HLA loss analysis in routine post-transplantation diagnostics (All Partners)

HLA-KMR: A rapid and sensitive qPCR-based assay

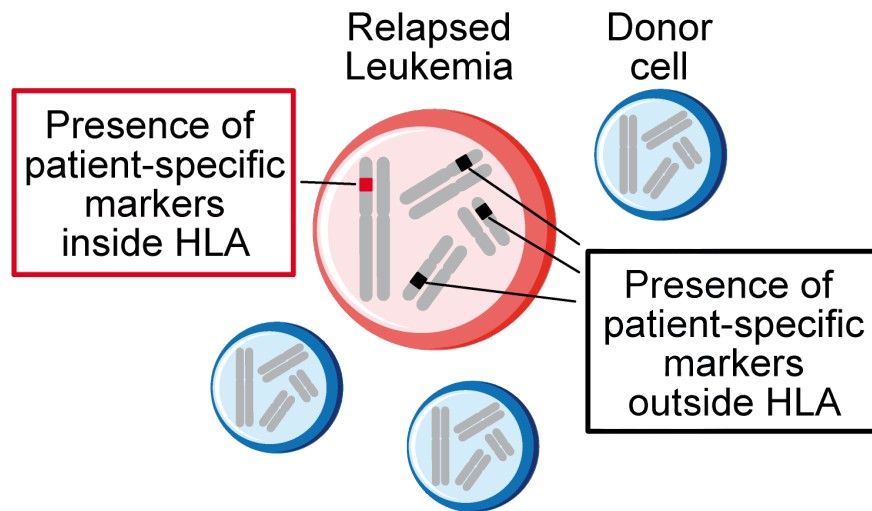
“Inside HLA” host chimerism (%)



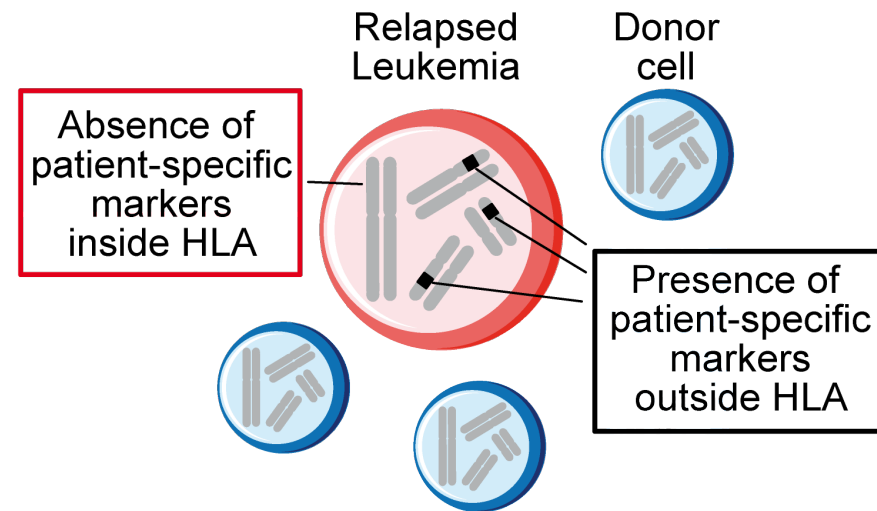
“Outside HLA” host chimerism (%)



“Classical” relapse



HLA loss relapse



HLA-KMR: A rapid and sensitive qPCR-based assay

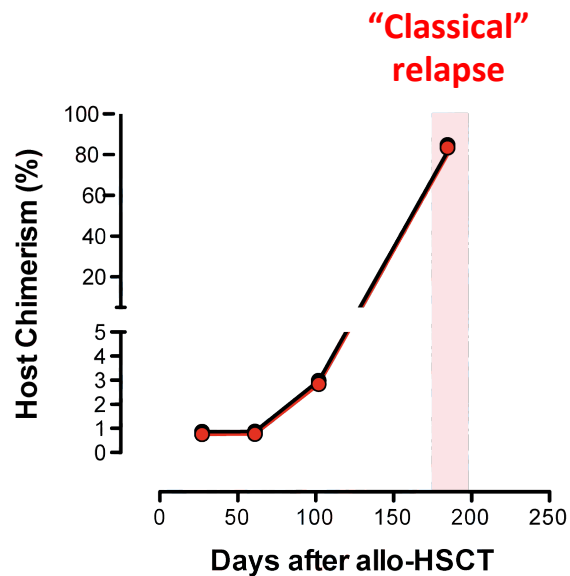
10 reactions validated for prototype kit:

- 5 reactions for HLA-A alleles
- 2 reactions for HLA-C alleles
- 3 reactions for HLA-DPB1 alleles

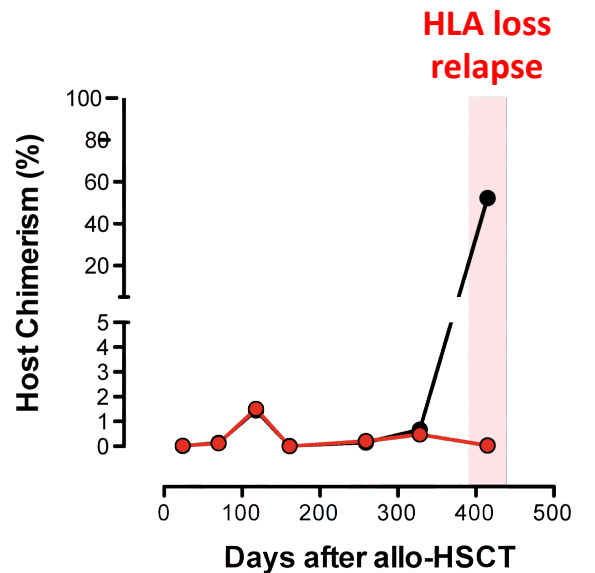
Informative in:

71.6% of 454 Haploidentical HSCTs (OSR)

66.4% of 113 UD HSCTs (UK-Essen)



- Patient-specific marker inside HLA (KMR521-DPB1)
- Patient-specific marker outside HLA (KMR033)



- Patient-specific marker inside HLA (KMR522-DPB1)
- Patient-specific marker outside HLA (KMR017)

HLA-KMR® ASSAYS
DETECTION OF HLA
LOSS BY QPCR

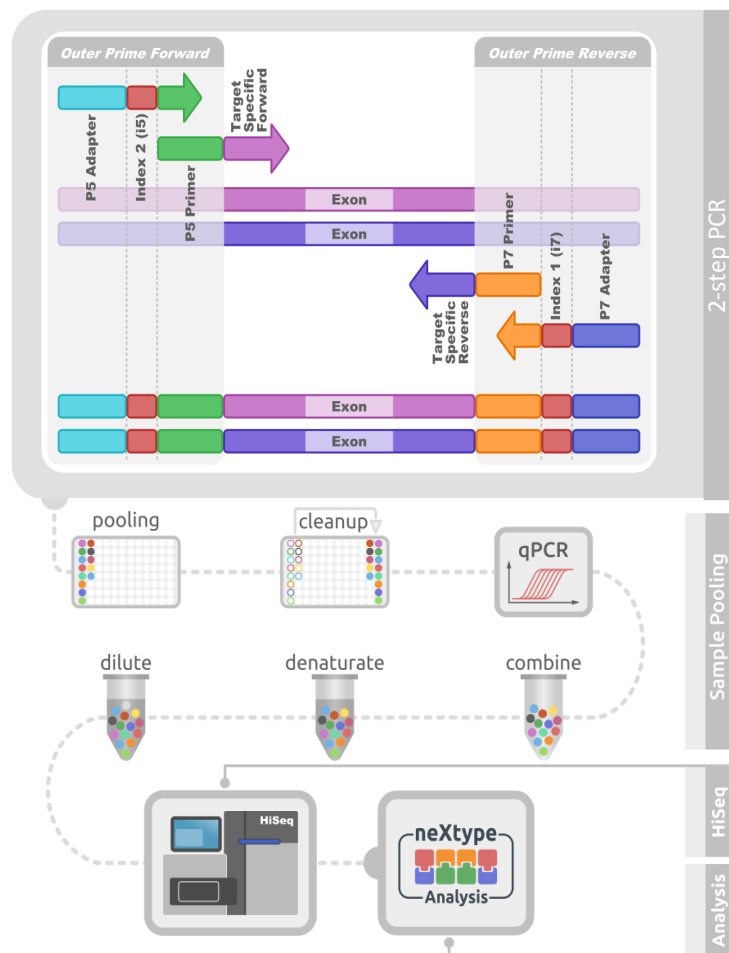
GENDX

personalizing diagnostics

- Fast and sensitive qPCR assay
- 10 unique markers
- Most frequent allele groups of HLA-A, -C and -DPB1
- Applicable with KMRtrack protocol
- Fully integrated with KMRengine

HLA Sequencing to Detect HLA Loss Relapses

HLAseq Workflow



Advantages

- Informative in the totality of partially HLA-incompatible HSCTs
- Allows to analyse a large number of relapses in a single MiSeq run

Disadvantages

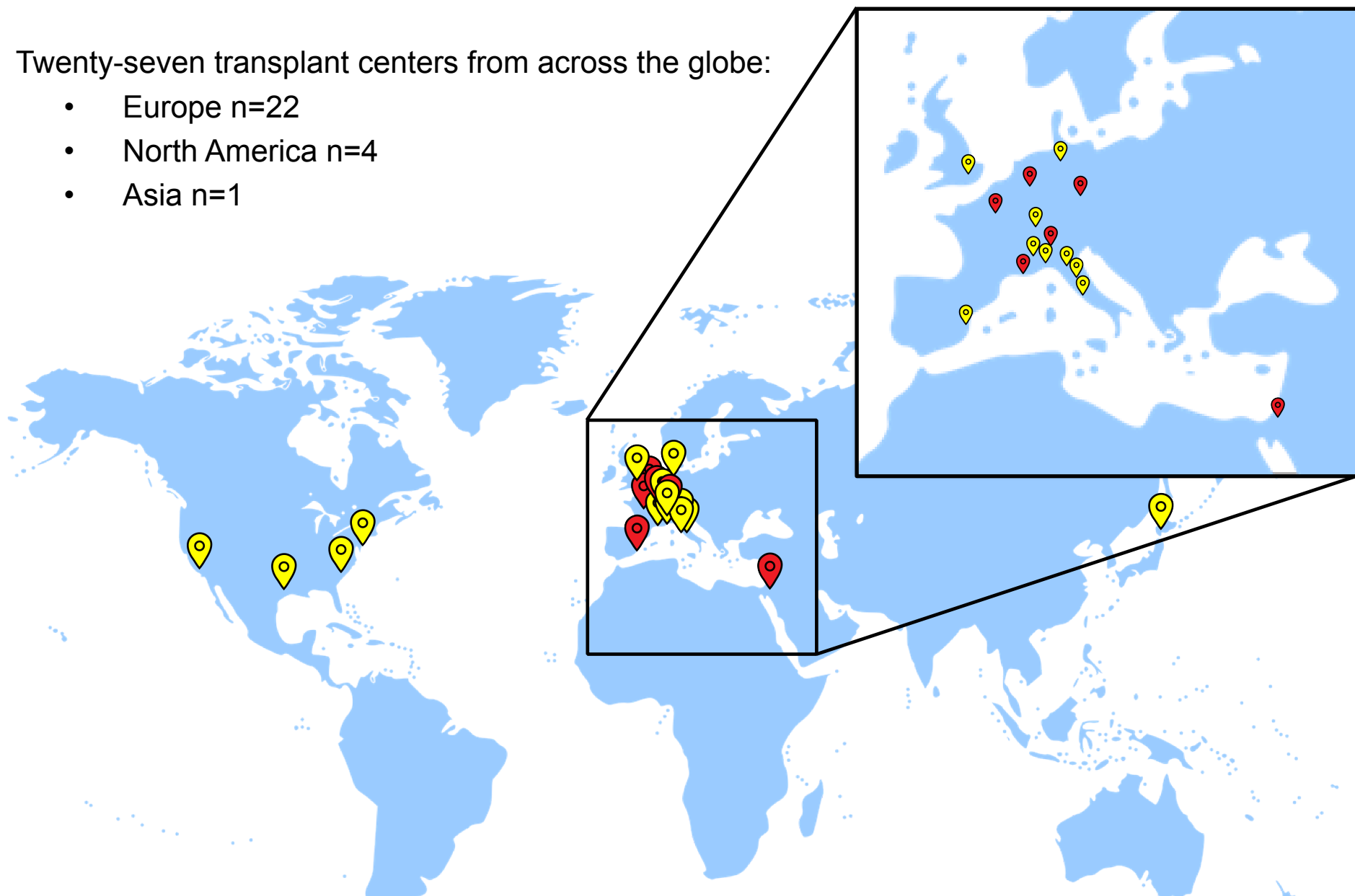
- Technology not currently in use in most HSCT diagnostic labs

In collaboration with

Expansion of the HLALOSS Transcan Consortium

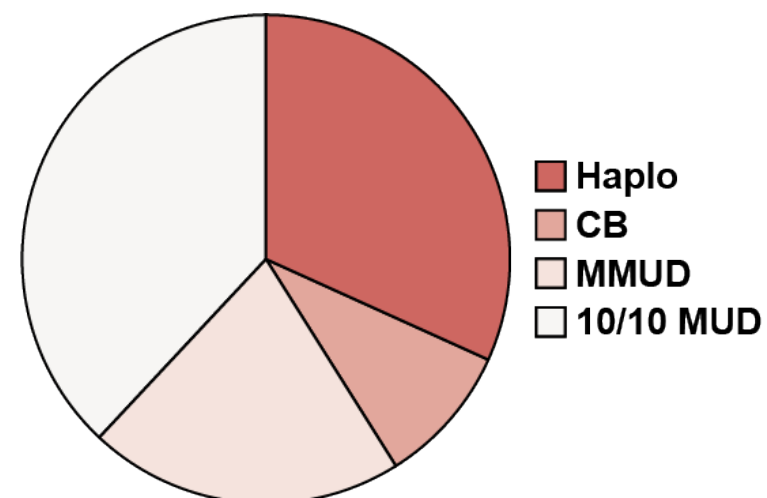
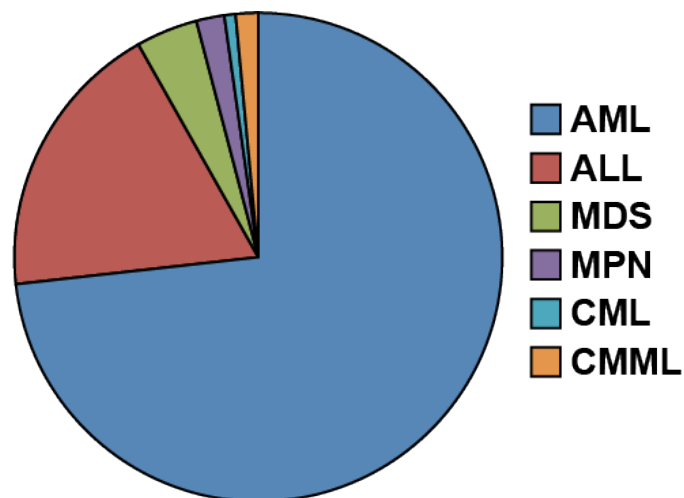
Twenty-seven transplant centers from across the globe:

- Europe n=22
- North America n=4
- Asia n=1



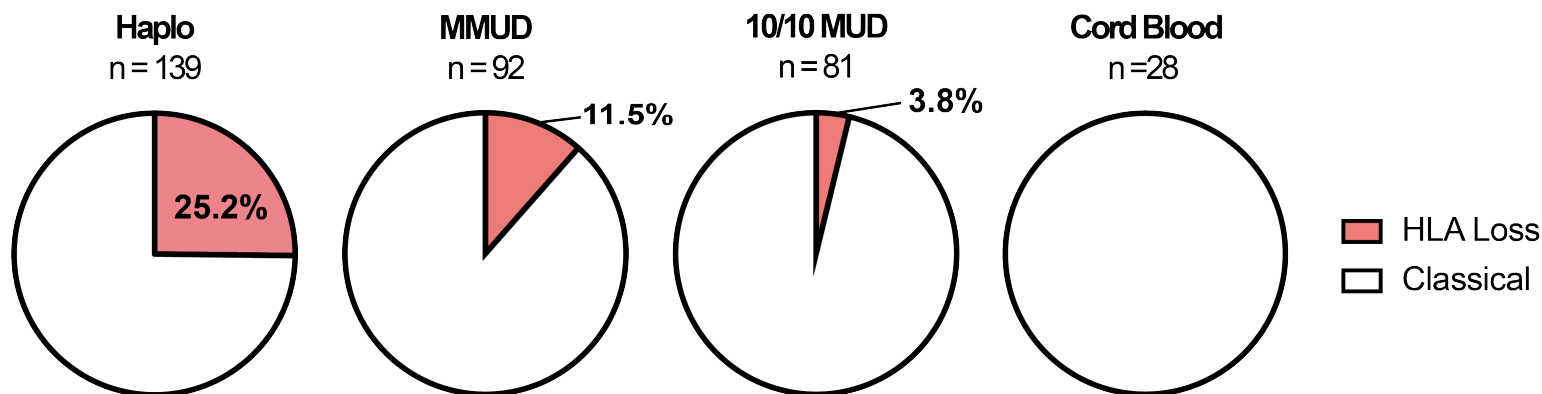
Current Status of Sample Accrual

	Total Relapses
Milano	35 (+84)
Essen	91
Paris	12
Marseille	9
Dresden	101
Tel Hashomer	10
Torino	39
Valencia	37
Hamburg	40
Freiburg	34
Roma	24
Duarte	24
Sapporo	14
Others	11
Total	565

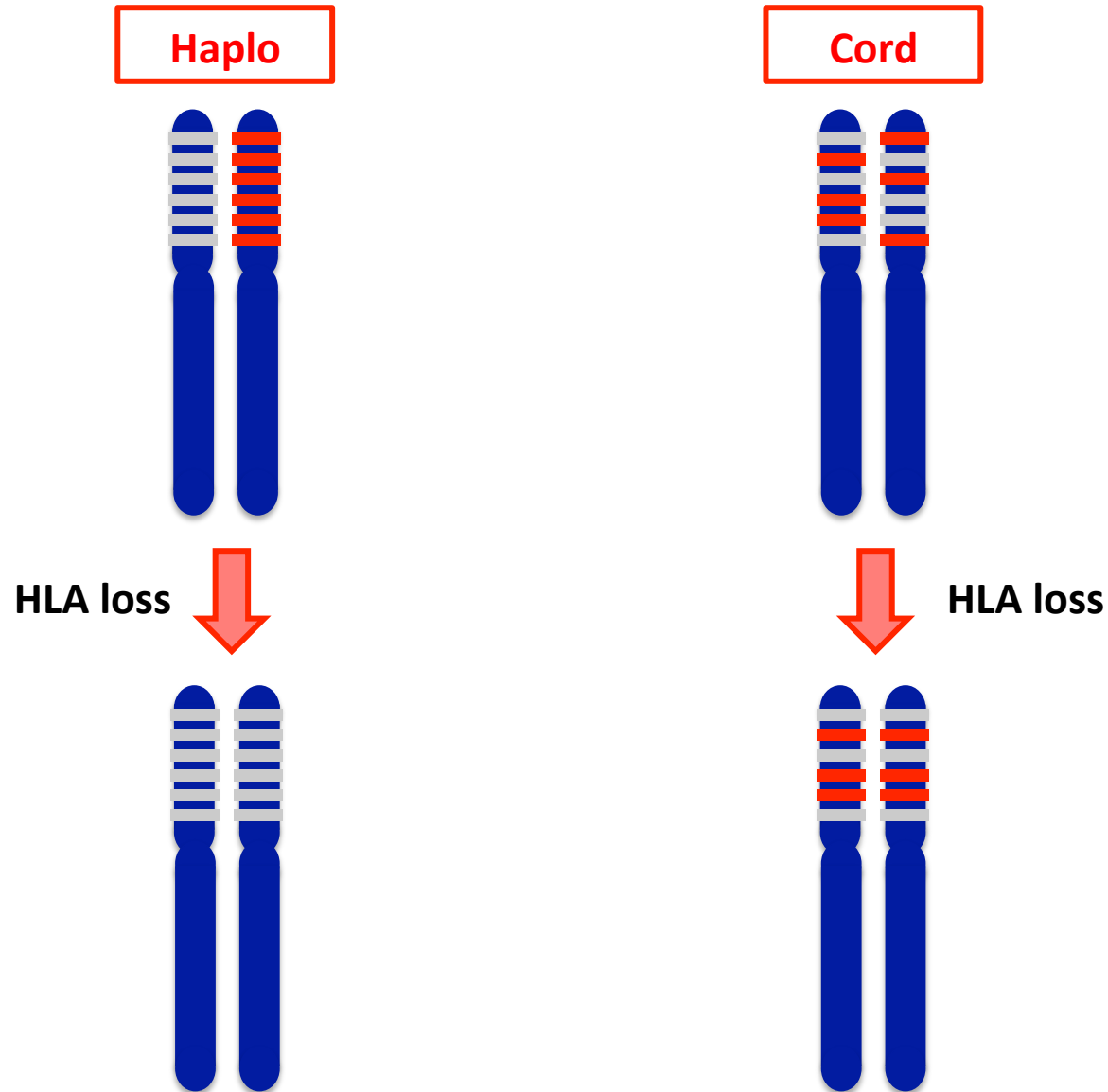


Preliminary Study Results

Total Relapses		AML	ALL	MDS	MPN	CML	CMML	10/10 MUD	MMUD	UCB	Haplo	HLA Loss
Milano	35 (+84)	34 (+75)	0 (+2)	1 (+4)	0 (+1)	0 (+2)	0	6 (+8)	3 (+4)	2 (+3)	24 (+69)	✓
Essen	38	0	0	0	0	0	0	24	14	0	0	✓
Paris	10	4	4	1	1	0	0	4	4	0	2	✓
Marseille	9	7	0	0	0	0	0	0	0	4	5	✓
Dresden	77	NA	NA	NA	NA	NA	NA	11	51	2	13	✓
Tel Hashomer	5	2	2	1	0	0	0	1	2	1	1	✗
Valencia	16	5	9	0	0	0	0	0	0	15	1	✗
Freiburg	26	0	0	0	0	0	0	14	12	0	0	✗
Duarte	20	14	3	3	0	0	0	9	0	0	11	✓
Sapporo	9	7	2	0	0	0	0	1	0	0	8	✗
Others	11	6	2	0	0	0	0	3	2	1	5	✓
Total	340	154	24	10	2	2	0	81	92	28	139	



Immunogenetic Differences Between Haplo and Cord

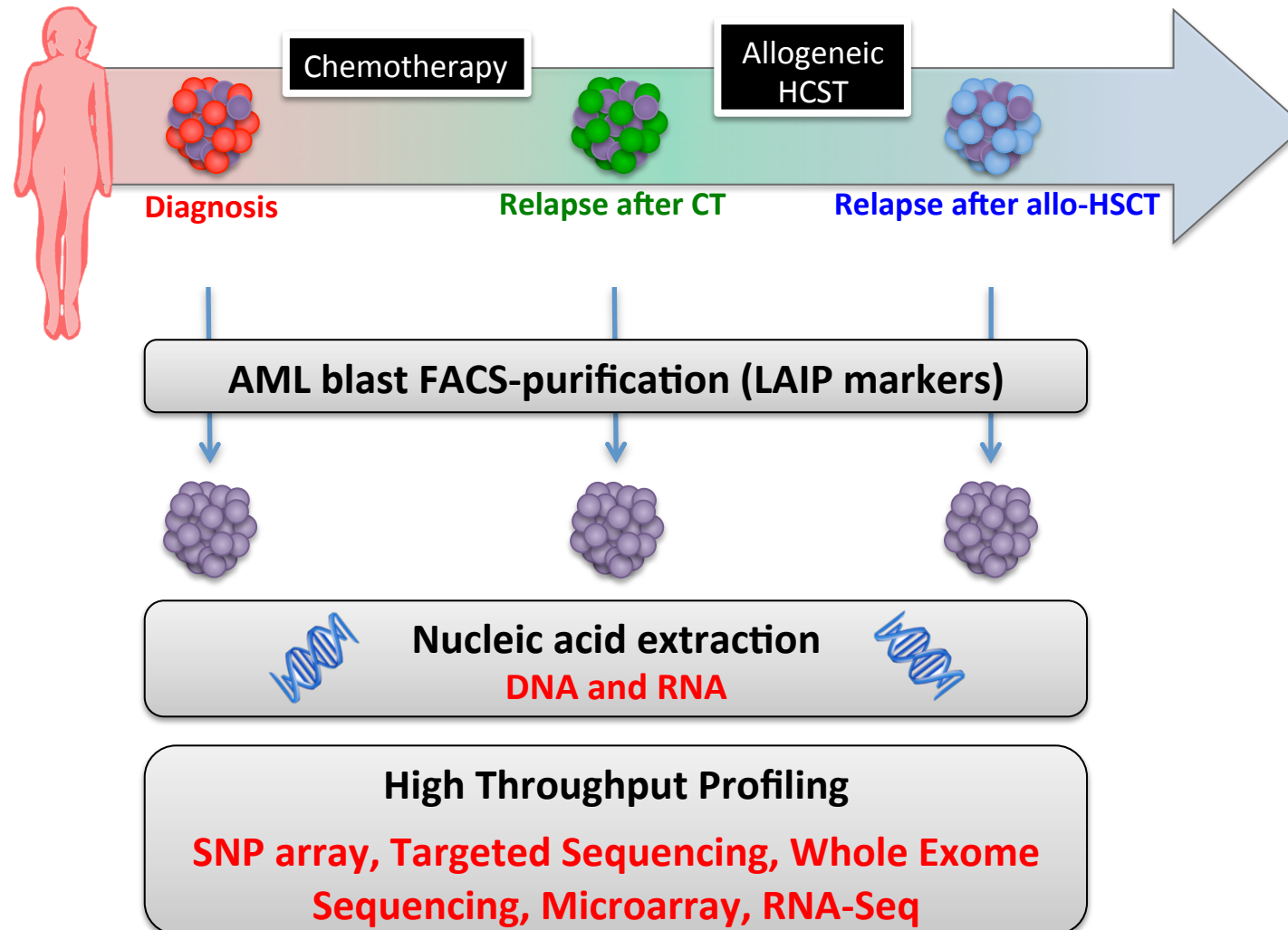


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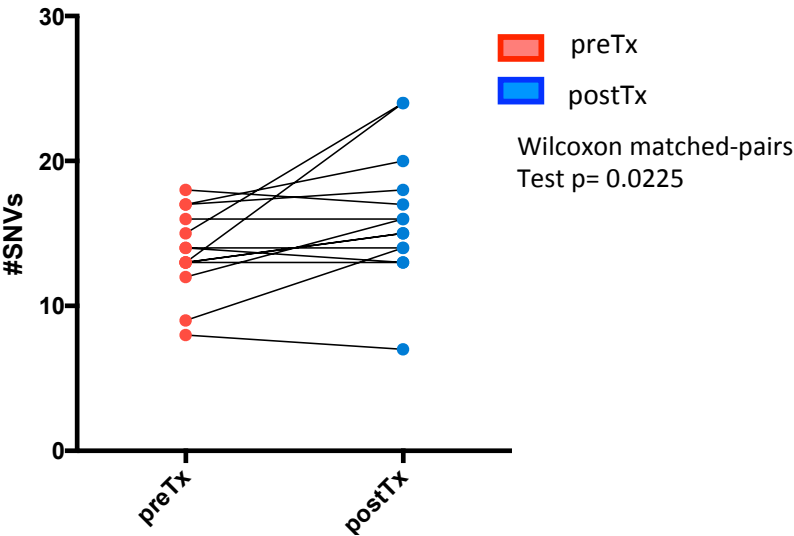
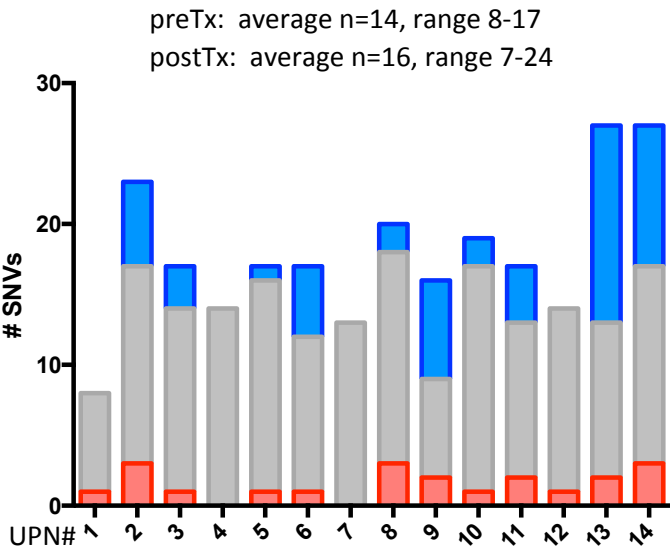
Seeking New Mechanisms of AML Immune Evasion

Experimental Layout



Seeking New Mechanisms of AML Immune Evasion

Whole Exome Sequencing



unique postTx
shared
unique preTx

Recurrence of mutated genes in postTx

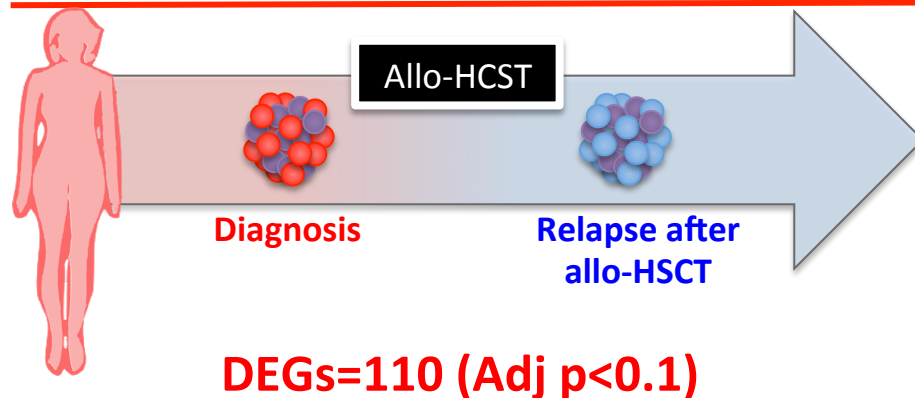
		UPN#7	UPN#11	UPN#6	UPN#5	UPN#13	UPN#4	UPN#3	UPN#15	UPN#14	UPN#2	UPN#12	UPN#1	UPN#9	UPN#8
tumor suppressor	WT1														
	BRCA1														
myeloid TFs	CBFA2T3														
other TFs or repressor	GFI1B														
	UBTF														
	TRIM13														
	TRIM15														
	ZHX3														
	ZBTB42														
	FOXB1														
RAS pathway	KRAS														
	RIT2														
Ser-Thre kinase pathway	CCND3														
protein phosphatase	PTPN9														
ion channel or transporter	GRIN3B														
	STXBP1														
	SCN3A														
ubiquitin pathway	UBE3B														
RNA splicing	SUGP1														

+ other 30 SNV unclassified

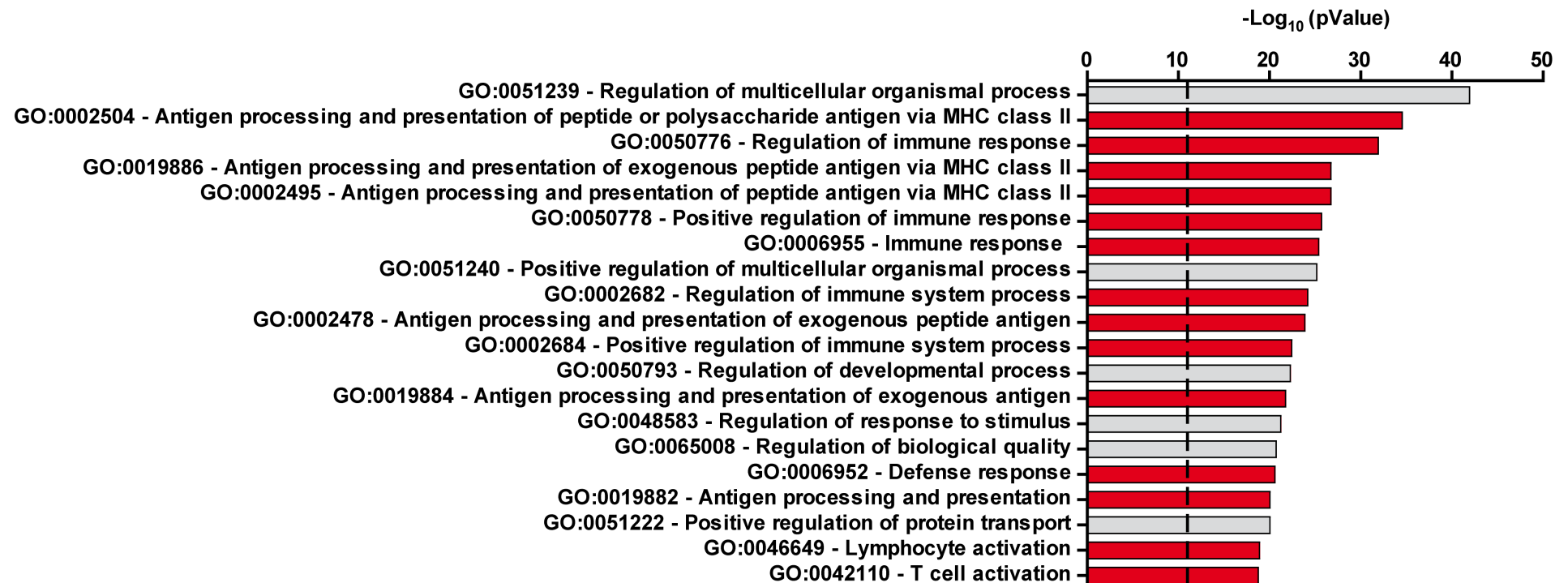
Average coverage for tumor sample: 120X

Seeking New Mechanisms of AML Immune Evasion

Experimental Layout



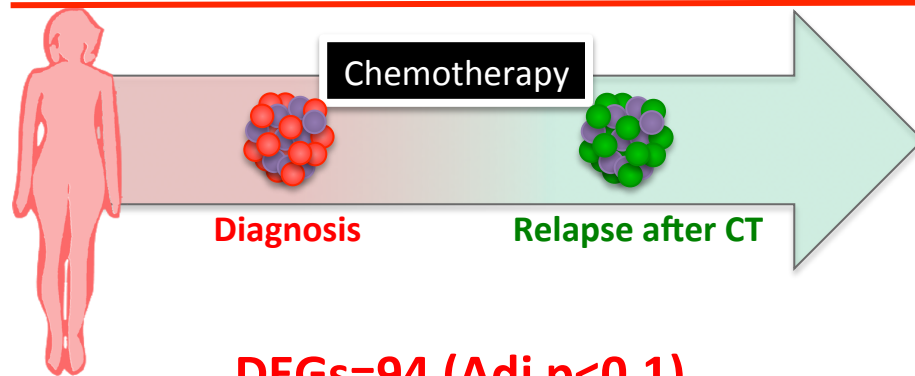
62% (18/29) of the BP
(pValue<0.05) are involved in
immune related processes



Toffalori et al, under review

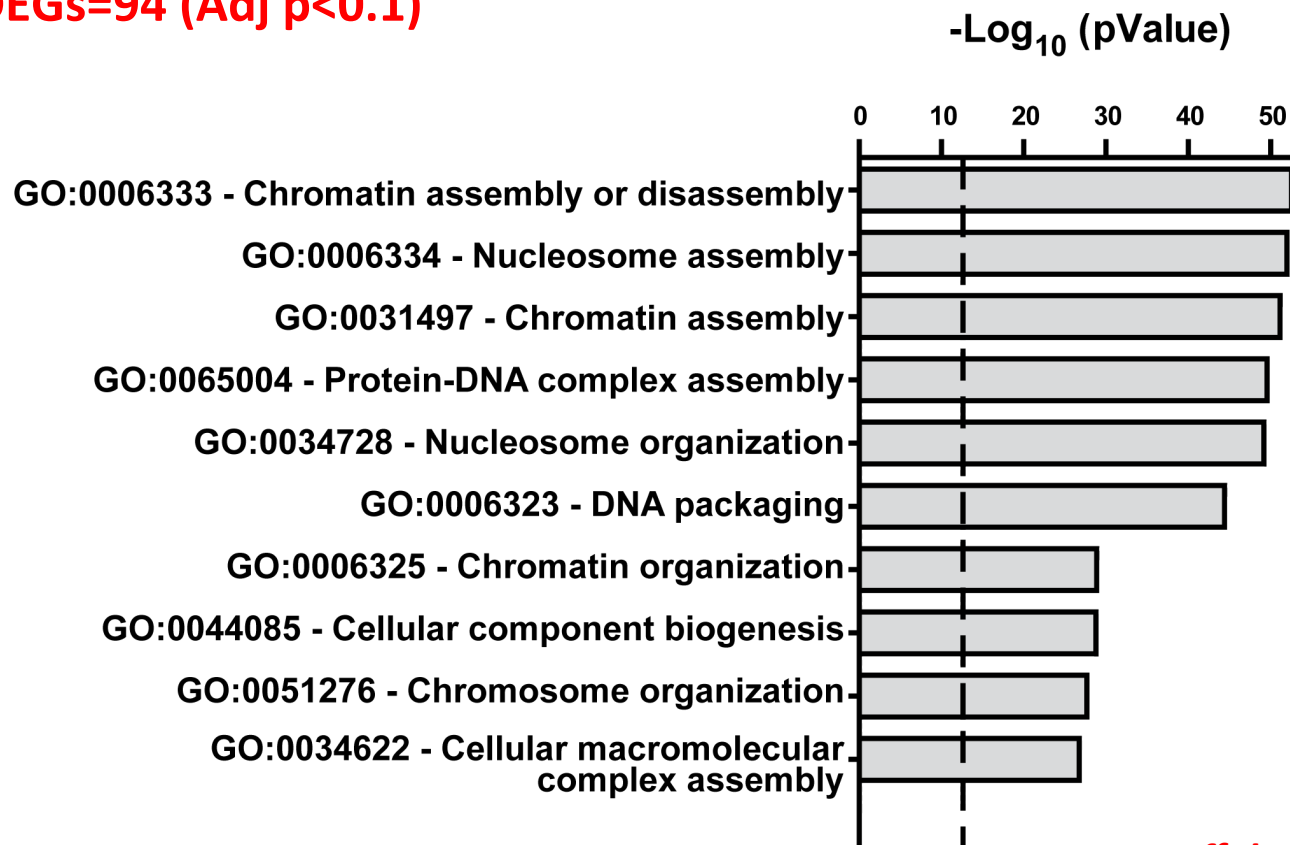
Deregulation of Immune Processes after Allo-HSCT

Gene Ontology Analysis for Biological Processes



No significant enrichment for immune-related processes

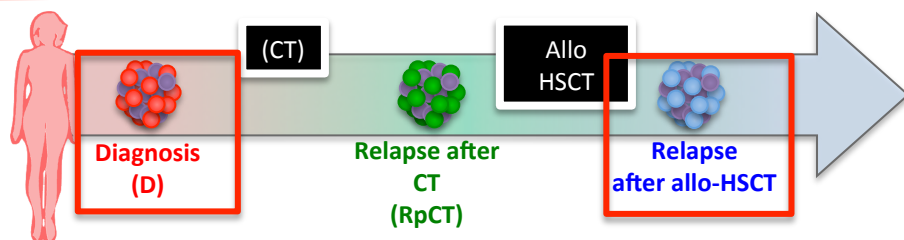
DEGs=94 (Adj $p < 0.1$)



Toffalori et al, under review

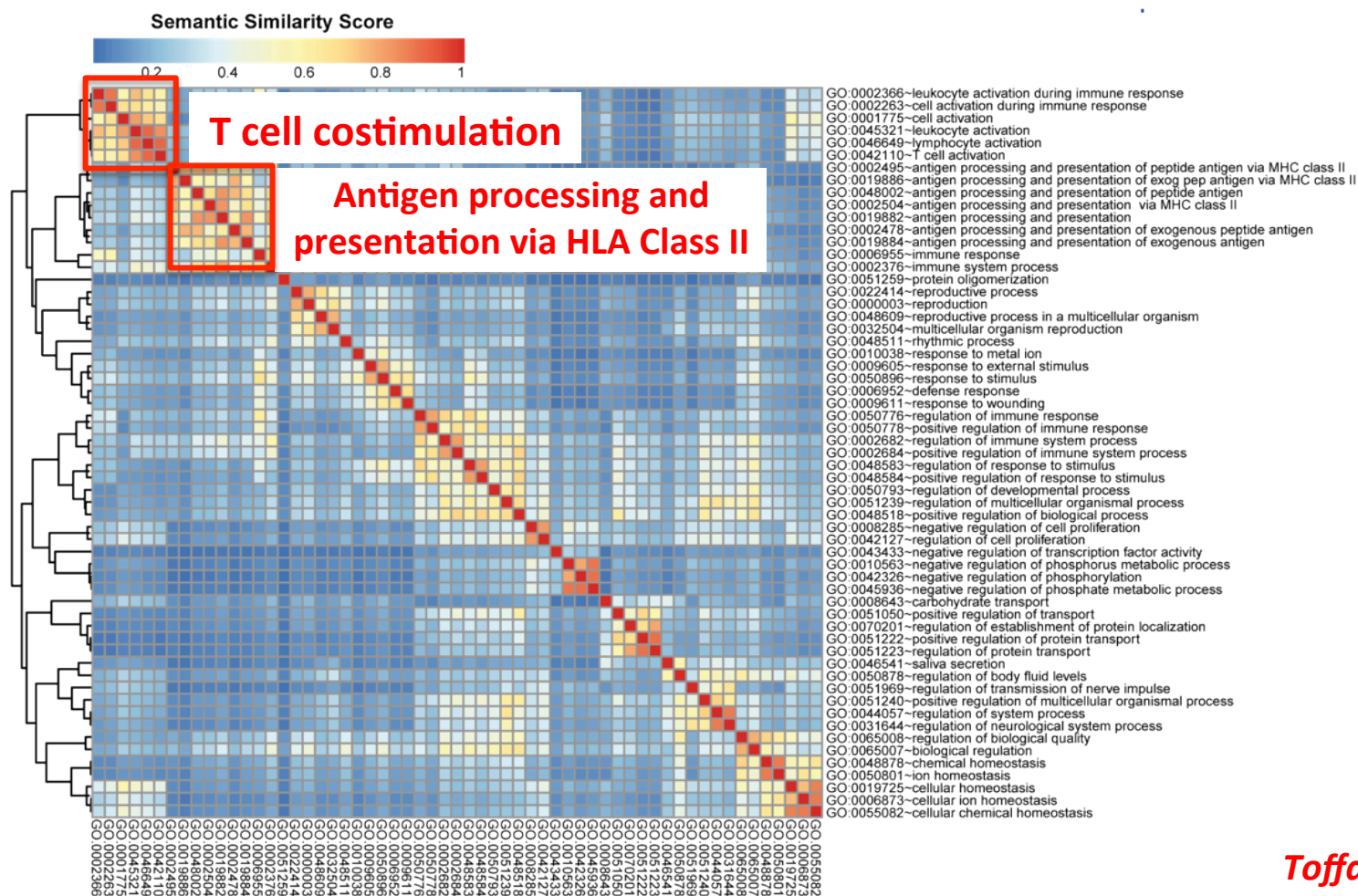
Deregulation of Immune Processes after Allo-HSCT

GO Semantic Similarity Analysis



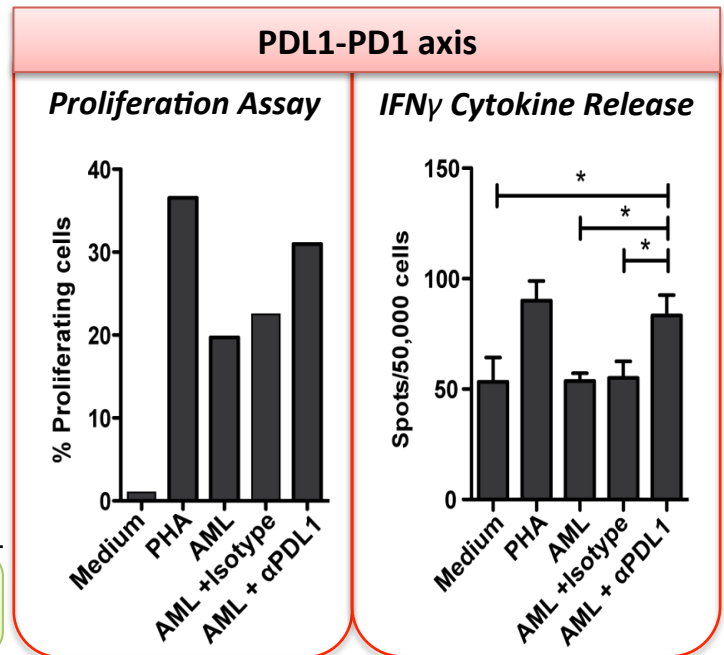
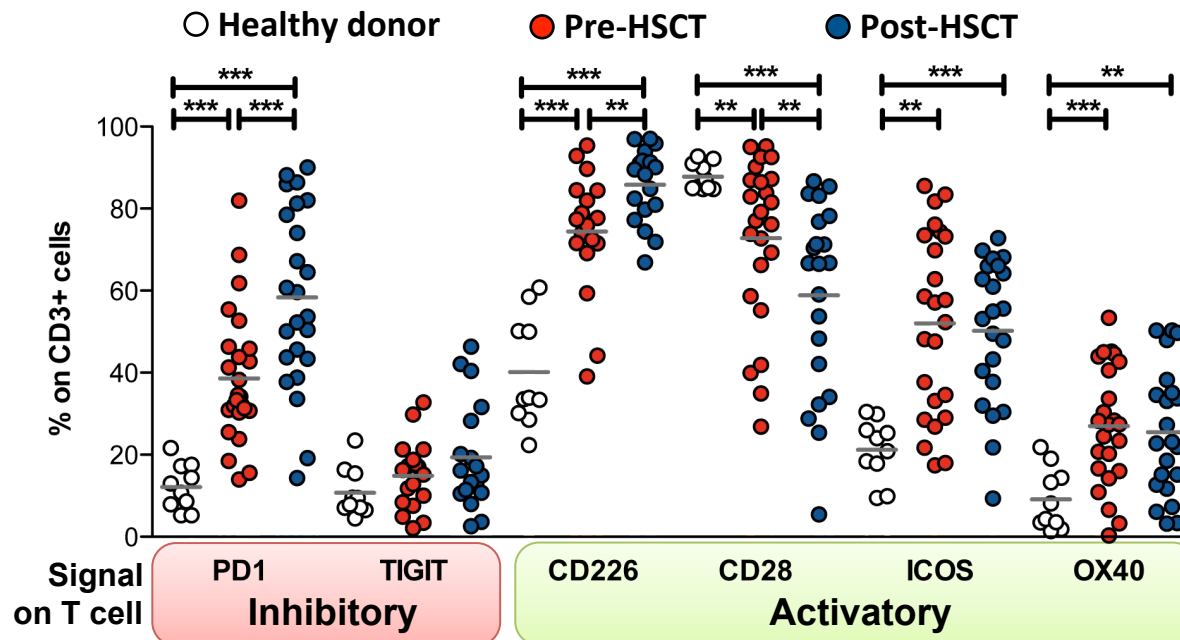
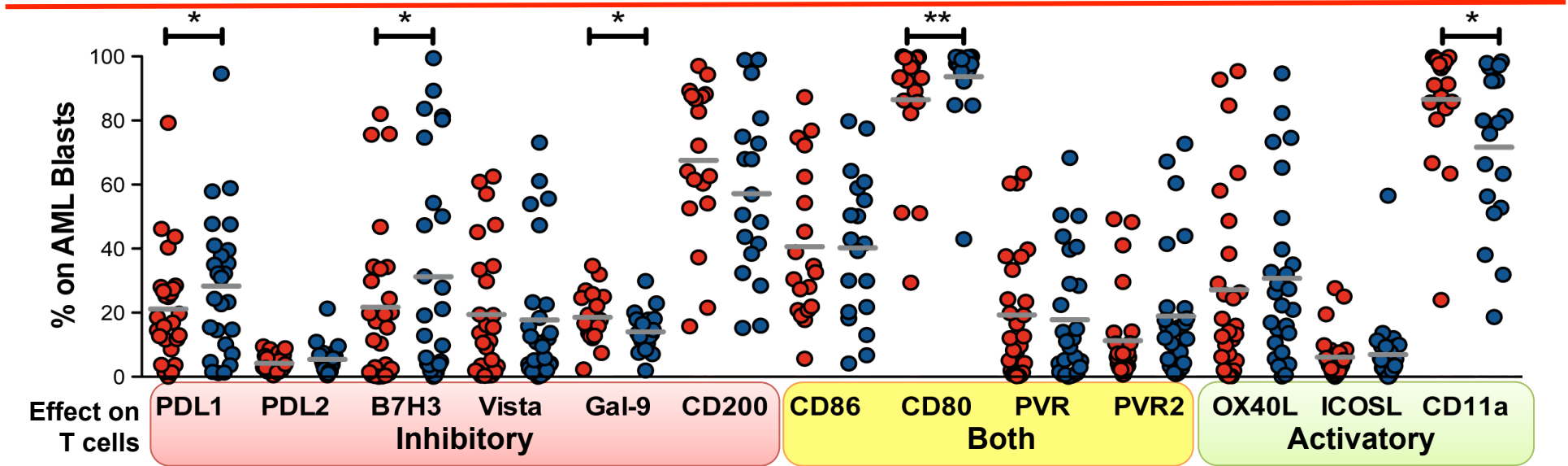
Similarity amongst Biological Process identified:

- **Red** -> **high** similarity
- **Blue** -> **low** similarity

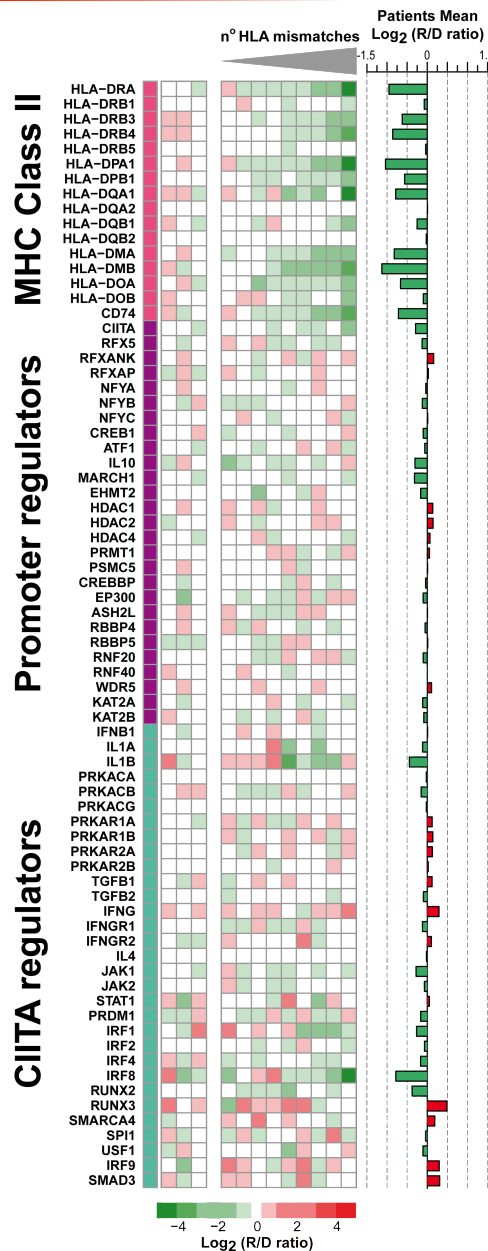


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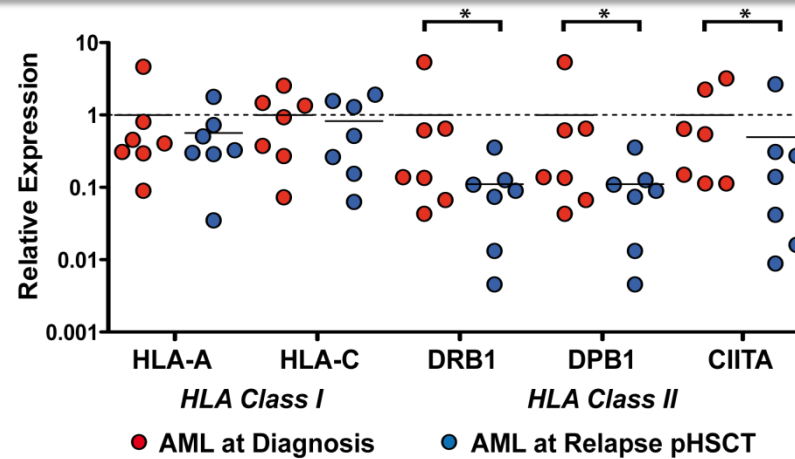
Deregulation of T Cell Costimulation at Relapse after Allo-HSCT



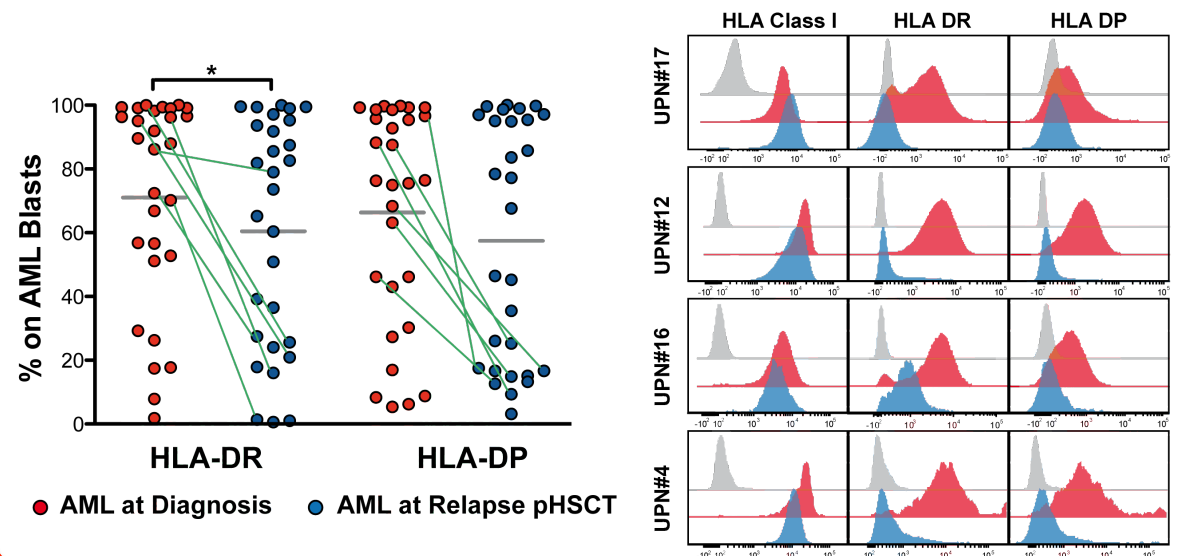
Downregulation of Molecules Involved in HLA Class II Presentation



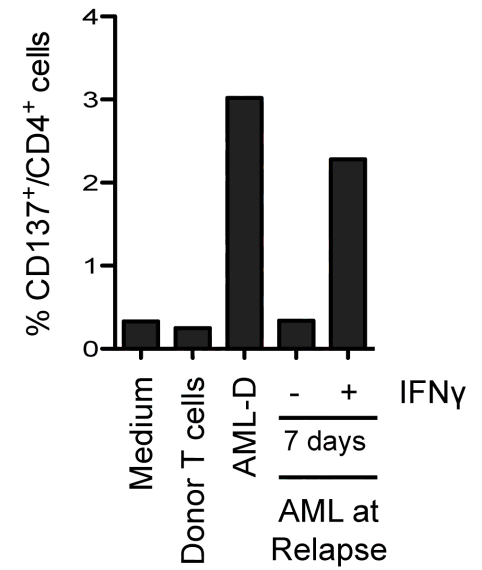
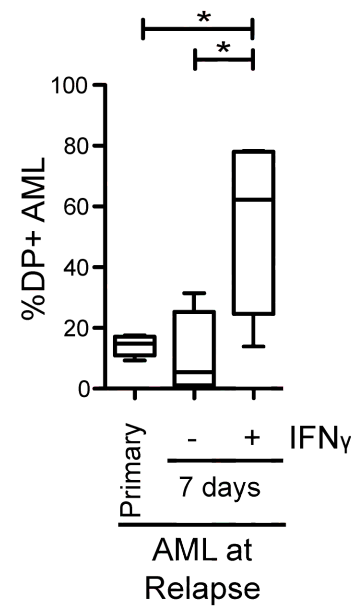
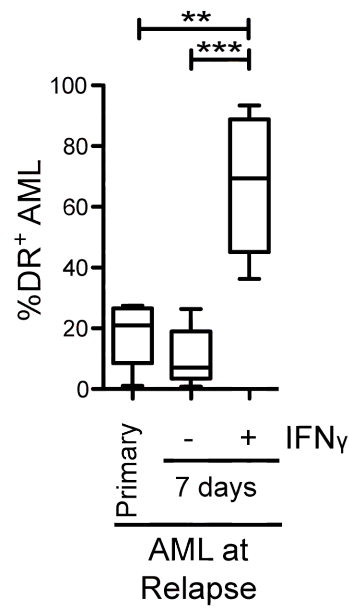
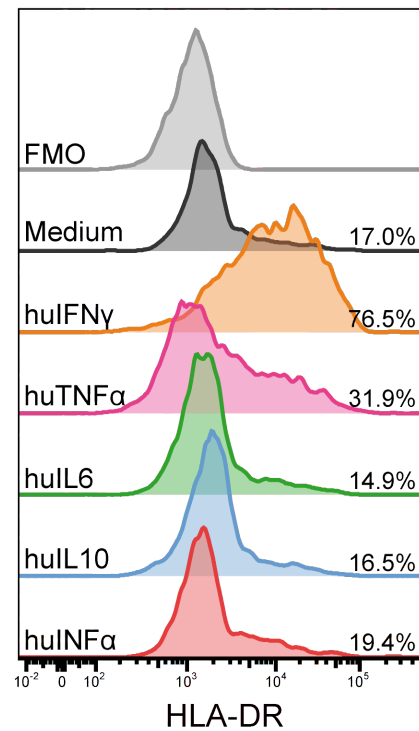
Locus Specific Expression (*RT-qPCR*)



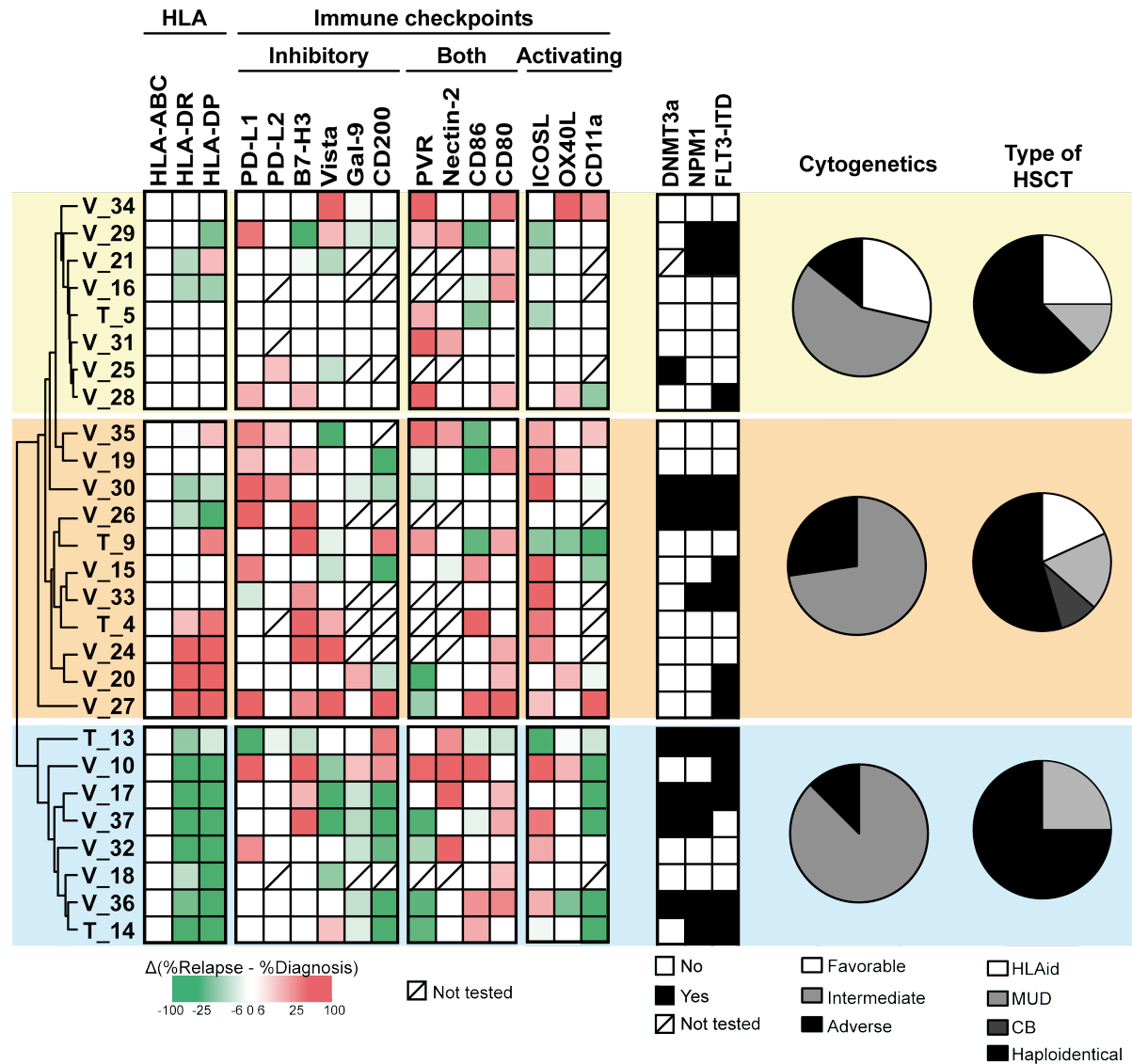
Surface Expression



HLA Class II Downregulation is Reversible

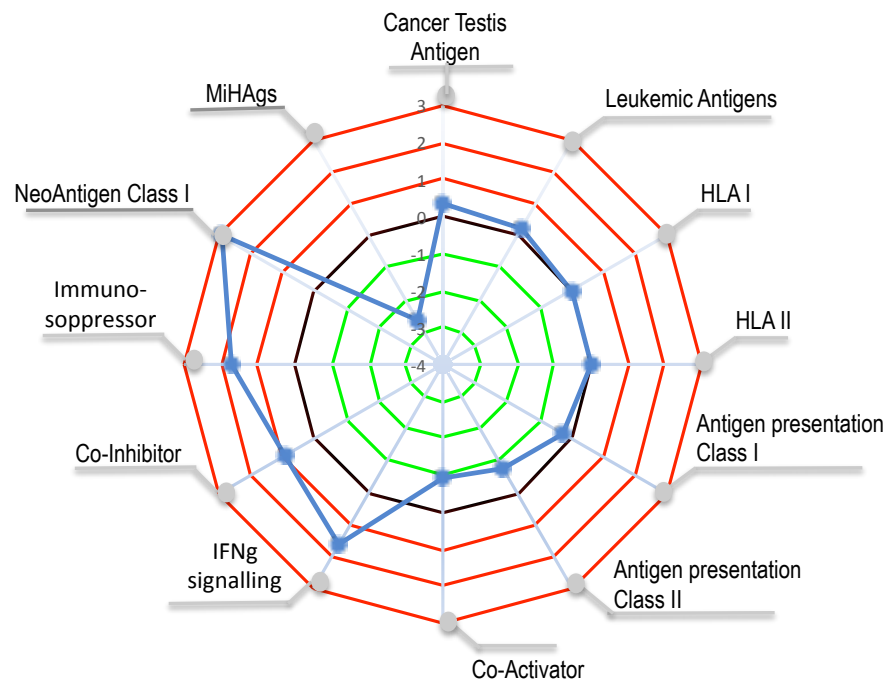


Deregulation of Cohinibitory Ligands and of HLA Class II Molecules Are Mutually Exclusive



Raising the Bar: Combining Whole Exome Sequencing and Rna-Seq to Profile Sorted Leukemic blasts

- Analysis of relapse-specific mutations, signatures, and clonal dynamics
- Development of pipelines for prediction of leukemia-expressed neoantigens and minor histocompatibility antigens
- Analysis of HLA somatic mutations



Zanotti, Bucci, Santaniello
EBMT 2018 Presidential Symposium

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