Meccanismi di escape immunologico della recidiva post trapianto allogenico

Fabio CICERI

Hematology and Bone Marrow Transplantation Unit

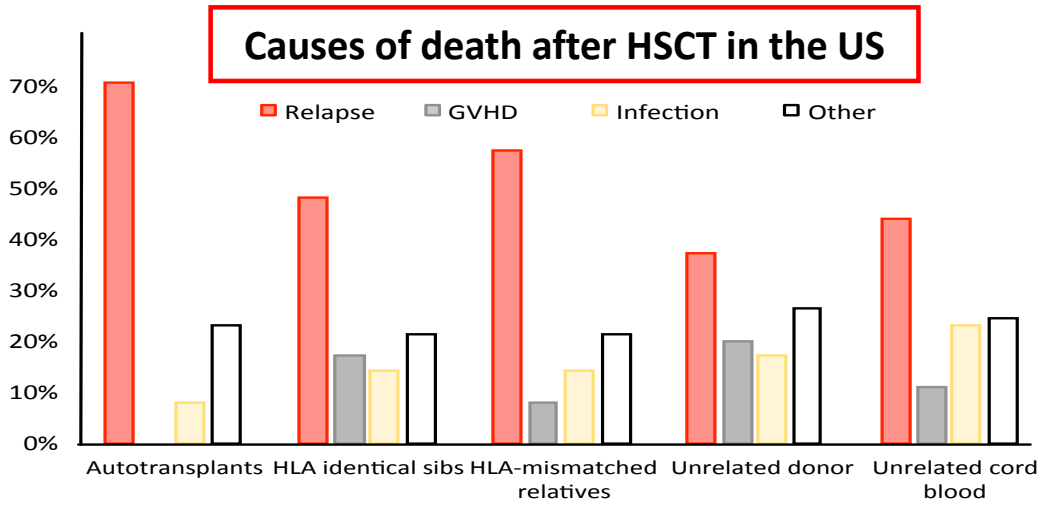
San Raffaele Scientific Institute, Milano

Disclosures

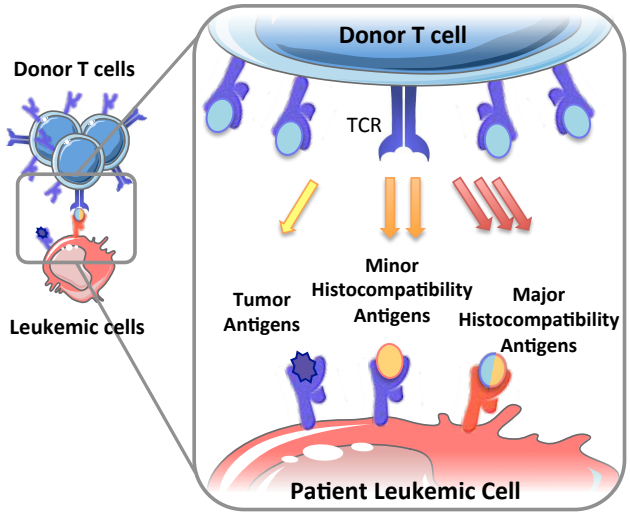
- I have NO following relevant financial relationships to disclose

Background: HSCT and Relapse

In allogeneic HSCT immune cells from the donor recognize and eliminate residual malignant cells ("**Graft versus Leukemia effect**")



Adapted from Horowitz, Bone Marrow Transplant, 2018



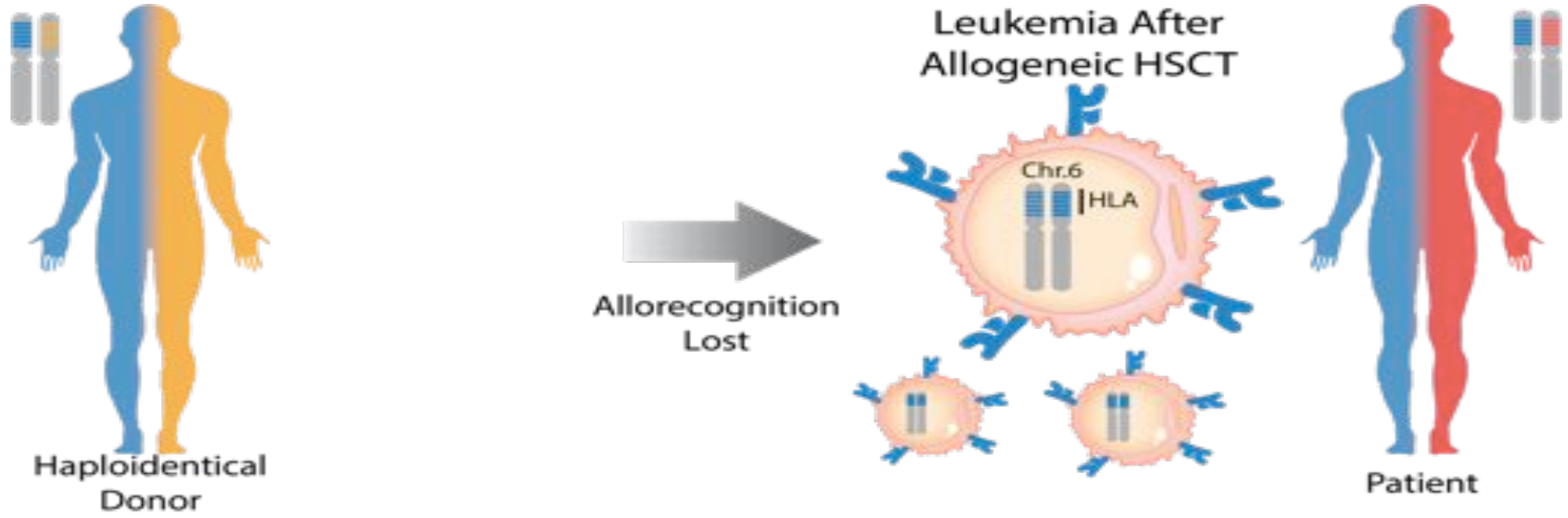
Reappearance of disease after initial remission (**relapse**) represents the most frequent cause of post-transplantation mortality

Outline of the Talk

1. Genomic HLA loss (10 years later)

2. Novel modalities of relapse (not all in genomics...)

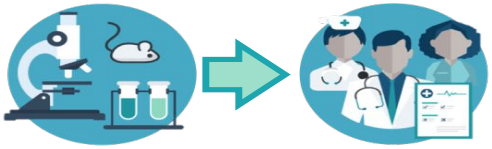
Molecular Mechanism and Immunological Consequences of HLA Loss



- Loss of the entire HLA complex (both class I and class II)
- Genomic mechanism (irreversible)
- Occurs only in leukemia cells, and rapidly becomes clonally prevalent
- Loss is counterbalanced by duplication of the other haplotype (expression level unchanged)

Vago, N Engl J Med, 2009; Toffalori, Blood, 2012
Crucitti, Leukemia, 2015; Ahci and Toffalori, Blood, 2017

The HLA loss Timeline

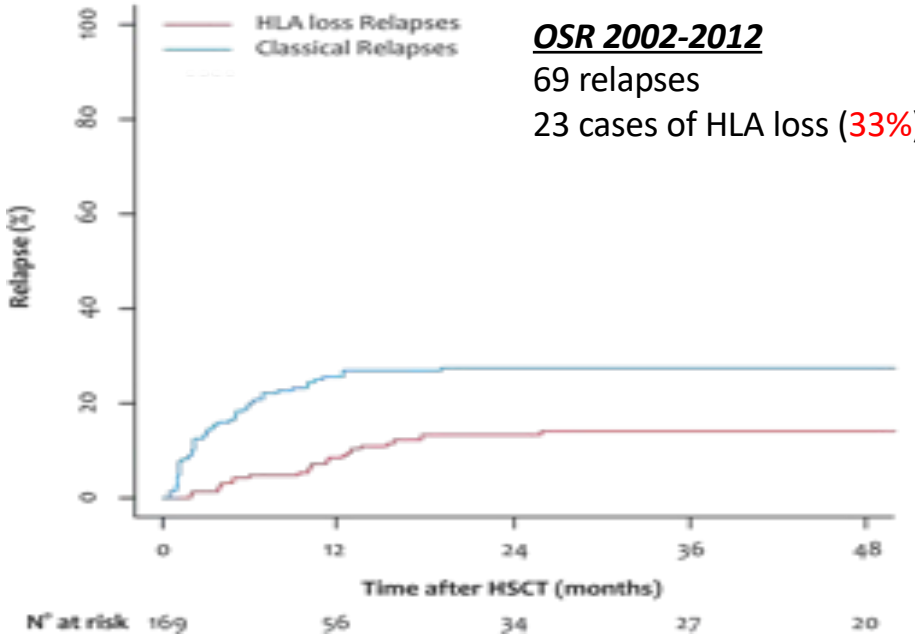


2009 2019

Confirmation/Epidemiology

Studies confirming HLA loss relapses in other HSCT settings and addressing the frequency of the event

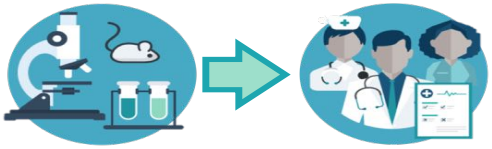
Villalobos, Blood, 2010
Waterhouse, Biol Blood Marrow Transplant, 2011
Toffalori, Blood, 2012
McCurdy, Leukemia, 2016



OSR 2002-2012
69 relapses
23 cases of HLA loss (33%)

Crucitti, Leukemia, 2015

The HLA loss Timeline

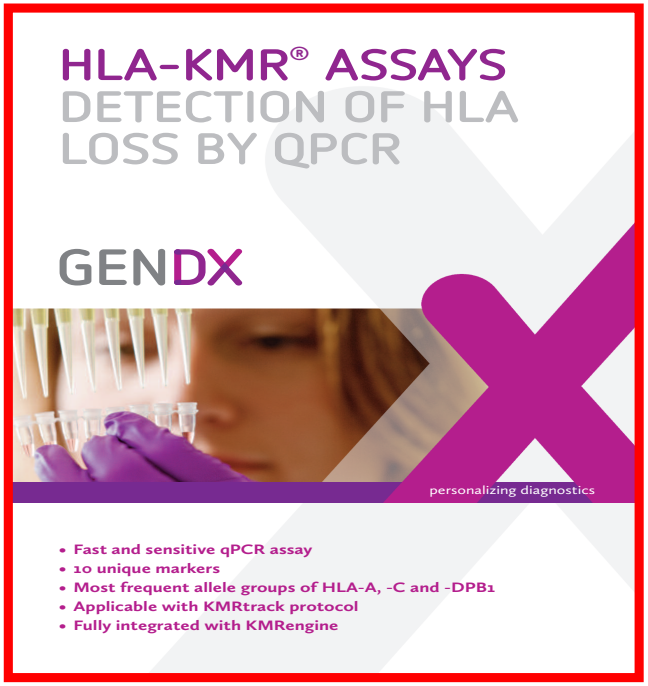


2009 2019

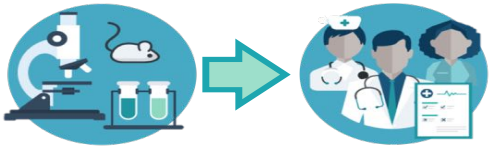
Routine Diagnosis

Development and industrial validation of fast and and reliable molecular assays amenable to routine laboratory practice

Ahci and Toffalori, Blood, 2018



The HLA loss Timeline



2009 → 2019

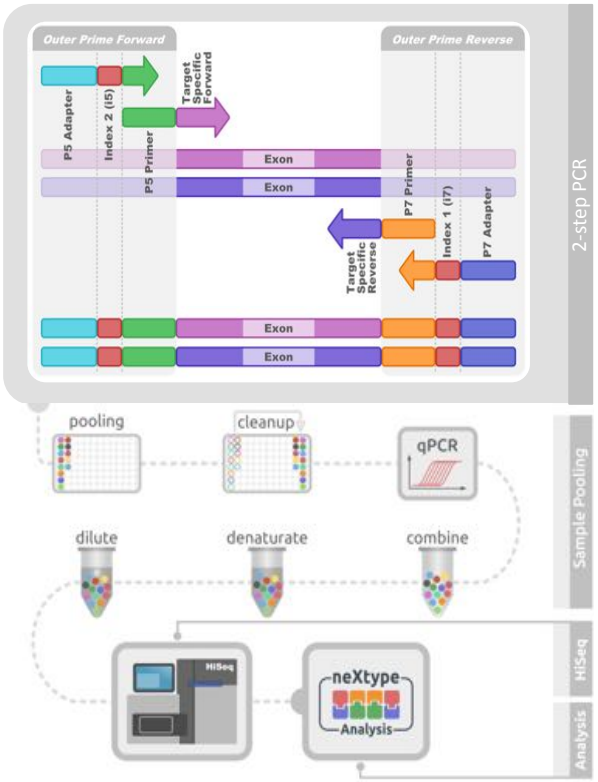
Routine Diagnosis

Development and industrial validation of fast and and reliable molecular assays amenable to routine laboratory practice

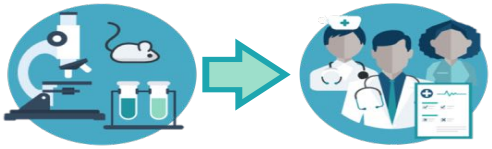
Ahci and Toffalori, Blood, 2018

Adaptation of an HLA NGS typing pipeline to the analysis of HLA loss in up to 48 samples in a single run

with DKMS



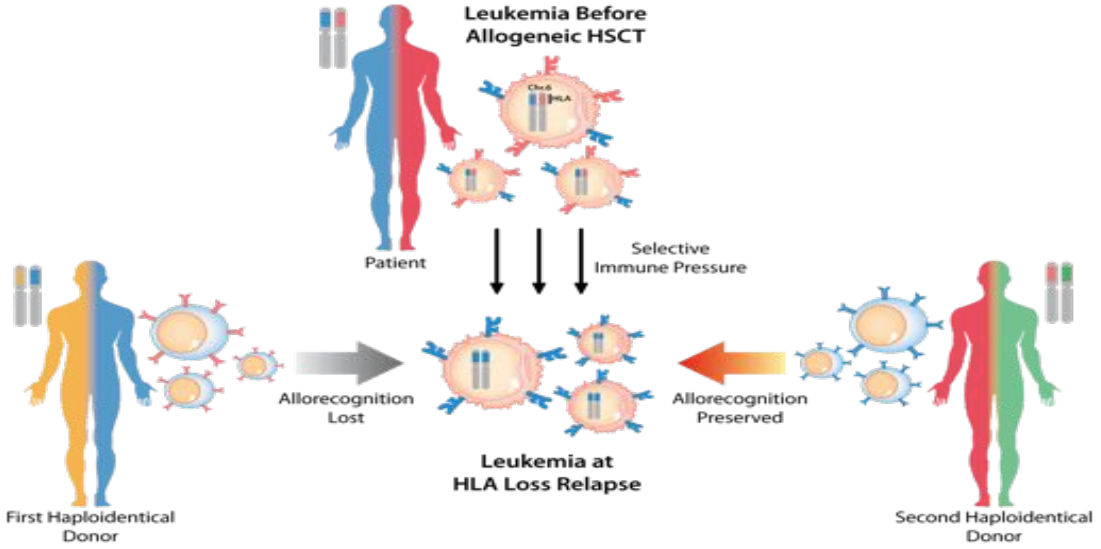
The HLA loss Timeline

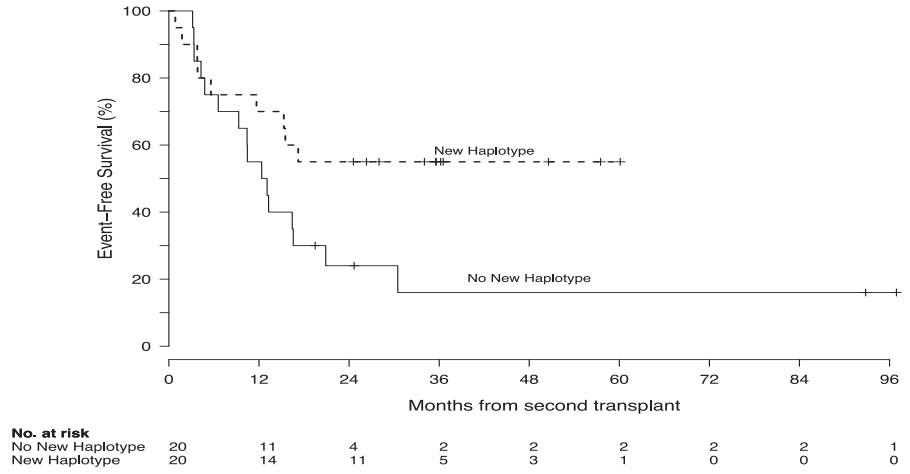


2009 2019

Therapy

Criterion for AVOIDING DLI and selection of a second haplo donor

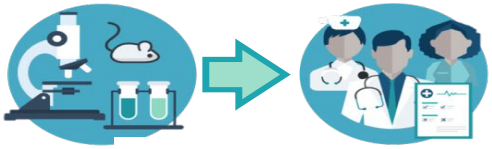




Vago and Ciceri, BBMT, 2017

Imus, BBMT, 2017

The HLA loss Timeline

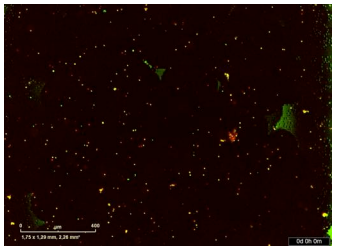


2009 2019

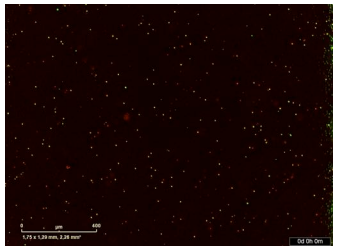
Therapy

Rationale for non HLA-restricted cancer immunotherapy approaches
(bispecific antibodies, CAR T cells)

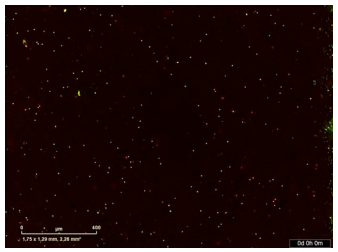
vs Diagnosis

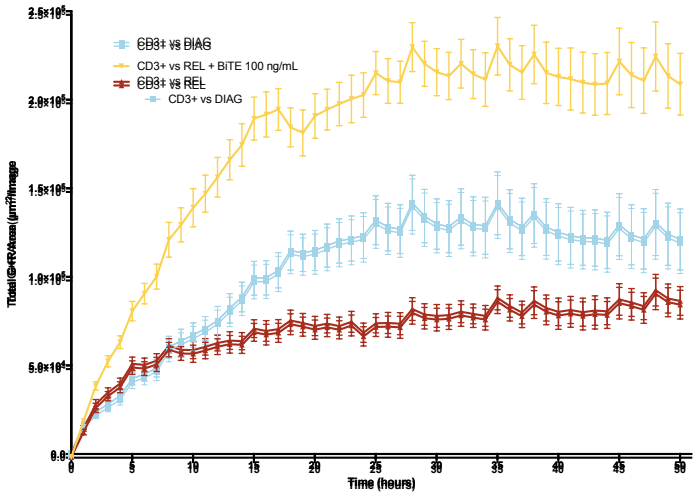


vs HLA loss relapse



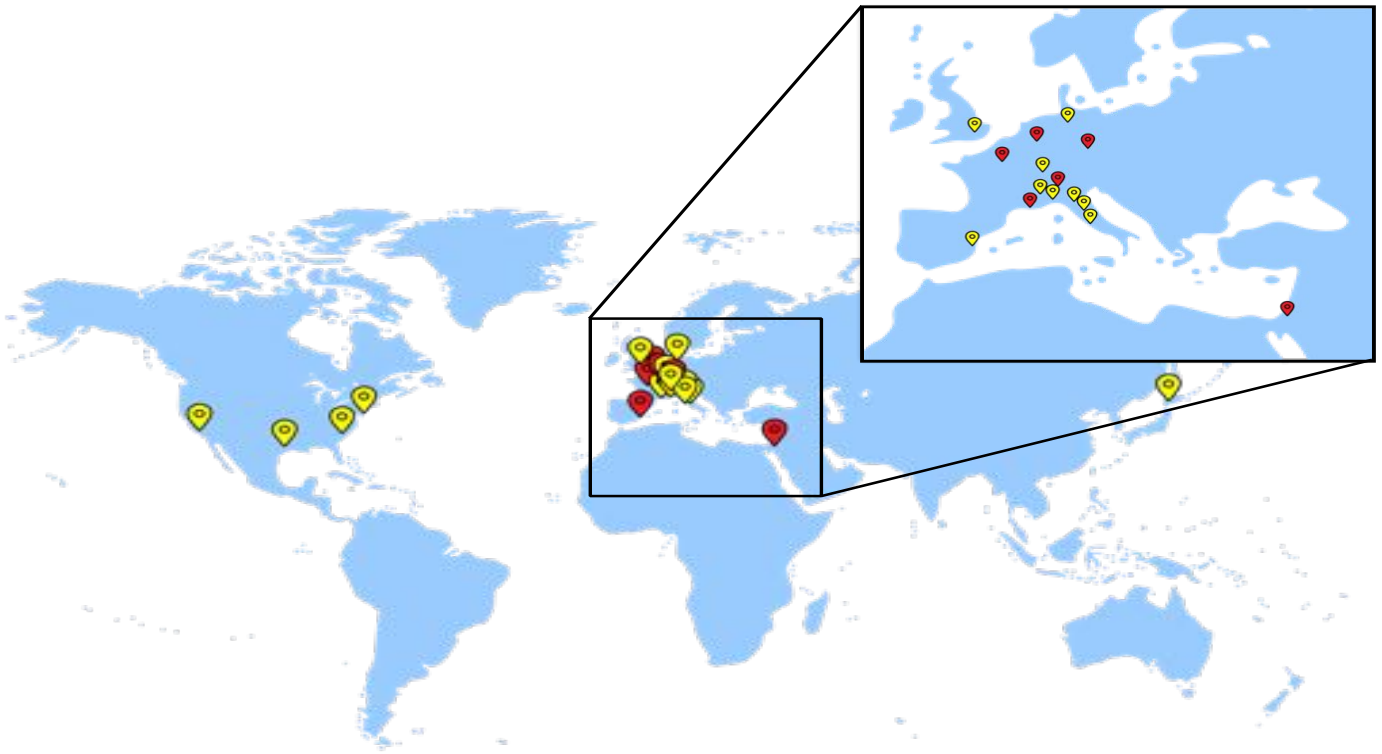
vs HLA loss relapse + antiCD3/CD33 BiTE





With M. Subklewe

The HLALOSS Transcan Project



Aim: addressing the frequency and risk factors of HLA loss in different HSCT settings

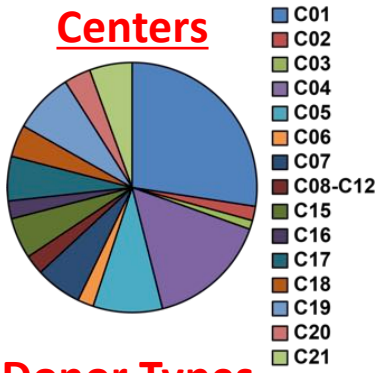
More than 20 centers from Europe, US, Japan

More than 1000 patient and donor samples (>600 relapses) characterized by an ad hoc optimized NGS pipeline

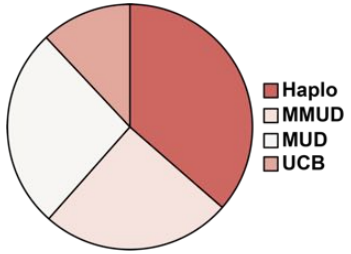
Patient and Transplant Characteristics

	Total (n=425)	Haplo (n=155)	MMUD (n=110)	MUD (n=111)	UCB (n=49)	P
Male/Female Ratio	1.14	0.96	1.15	1.31	1.45	0.5
Median Age (range)	51 (16-77)	49 (16-72)	56 (17-77)	55 (22-75)	34 (16-67)	<0.001
Diagnosis						<0.001
AML	317	122	74	93	28	
ALL	47	9	12	5	21	
MDS or MPN	61	24	24	13	0	
Disease status						<0.001
CR	192	50	56	51	35	
Active Disease	233	105	54	60	14	
Cytogenetic risk						0.41
Favourable	16	4	7	5	0	
Intermediate	209	80	55	59	15	
Adverse	160	60	39	43	18	
Chemorefractory Disease						<0.001
Yes	164	85	24	30	25	
No	257	70	82	81	24	
Hyperleukocytosis at Diagnosis						0.057
Yes	74	36	14	12	12	
No	268	96	61	79	32	
Previous Allogeneic HSCT						<0.001
Yes	47	37	4	4	2	
No	378	118	106	107	47	
Stem Cell Source						0.008
PB	331	127	103	101	/	
BM	45	28	7	10	/	
Conditioning Intensity						0.005
MAC	259	75	70	74	40	
RIC	136	61	32	34	9	
ATG Use						<0.001
Yes	295	88	70	93	44	
No	117	65	31	17	4	
Median infused CD3+ (x10e5/Kg), range	2350 (3-9995)	2560 (20-9995)	2550 (3-7950)	2000 (21-6300)	53 (40-70)	0.09
Number of GvL mismatches						<0.001
1	71	5	27	38	1	
2	52	3	31	14	4	
3	38	9	22	0	7	
4	36	27	3	0	6	
5	69	55	3	0	11	
6	47	42	1	0	4	
>6	6	1	0	0	5	

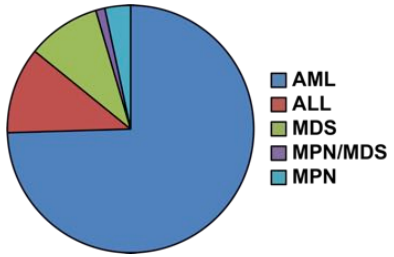
Centers



Donor Types



Diseases



Results: Incidence of HLA Loss

Haplo
5-6 HLA mm
n=155

HLA loss
n=38 (**24%**)

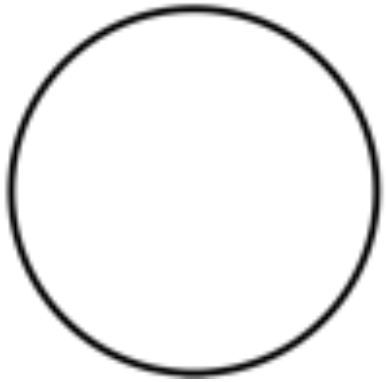
MMUD
3-4 HLA mm
n=110

HLA loss
n=12 (**11%**)

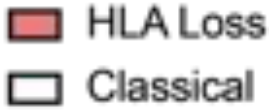
MUD
1-2 HLA mm
n=111

HLA loss
n=7 (**6%**)

Cord Blood
4-6 HLA mm
n=49

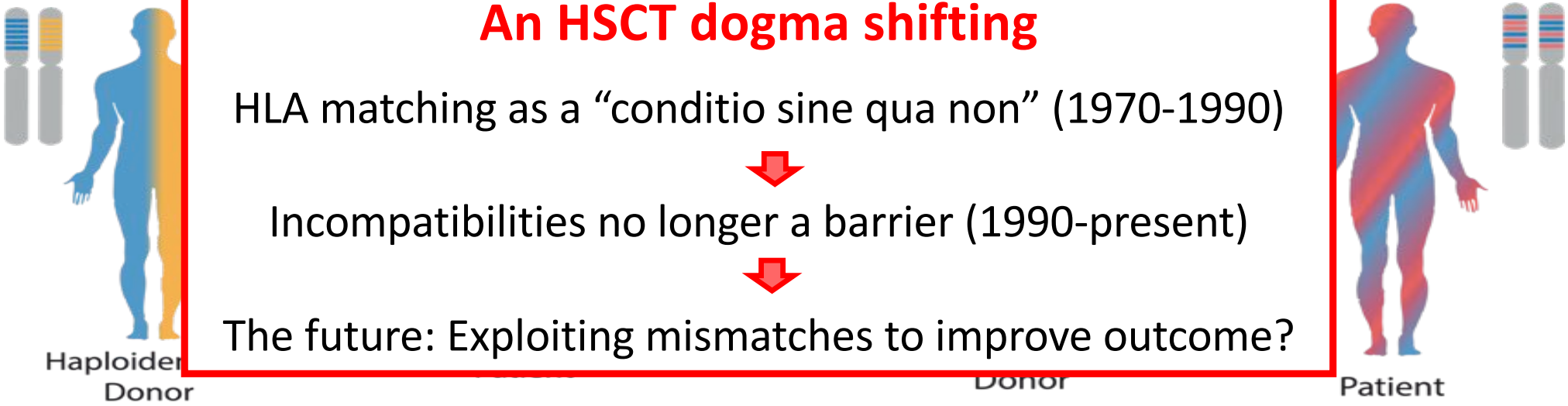


HLA loss
n=0 (**0%**)



An Immunogenetic Perspective

Haploidentical HSCT **Unrelated Cord Blood HSCT**



An HSCT dogma shifting

HLA matching as a “conditio sine qua non” (1970-1990)

↓

Incompatibilities no longer a barrier (1990-present)

↓

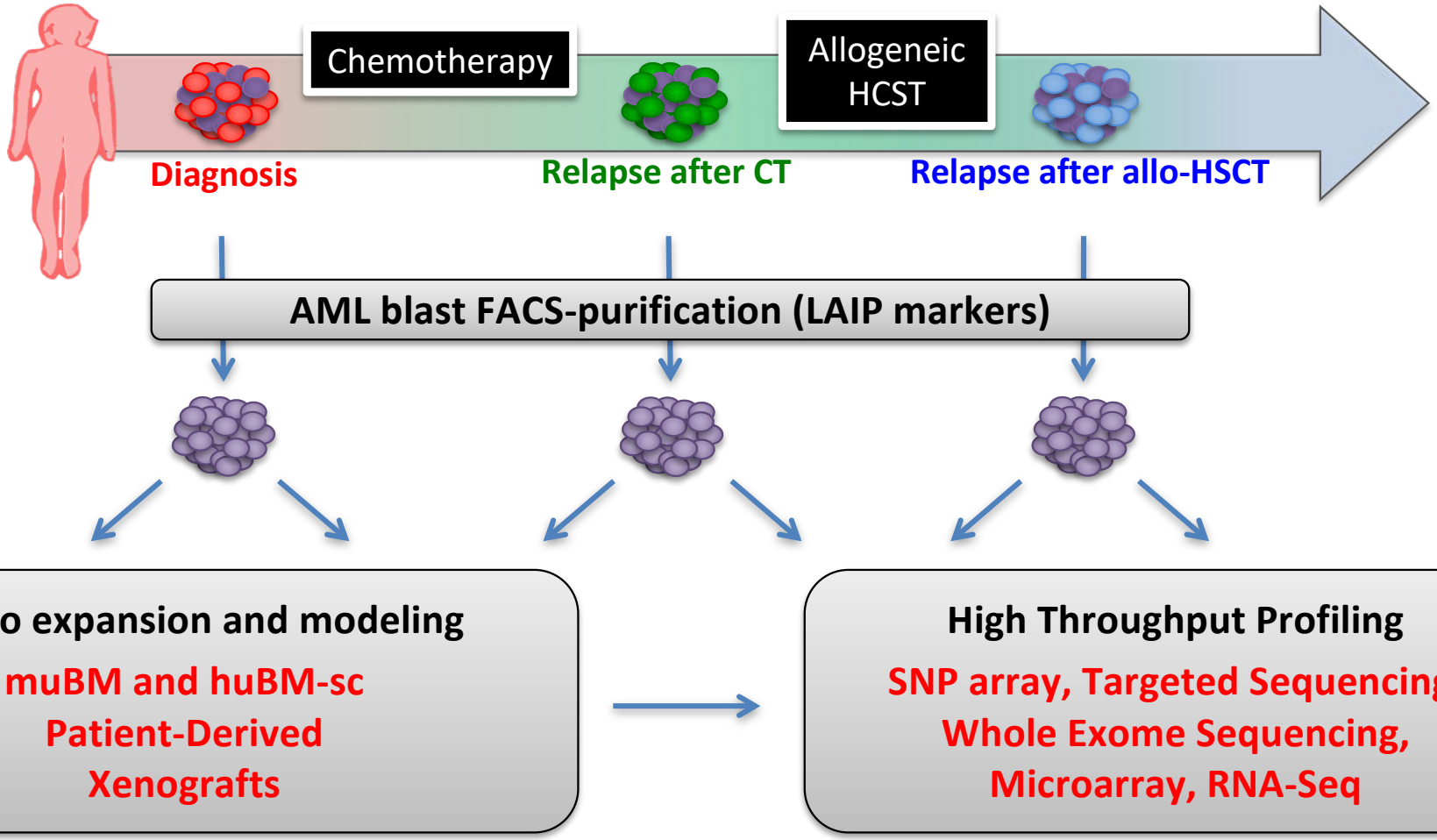
The future: Exploiting mismatches to improve outcome?

Outline of the Talk

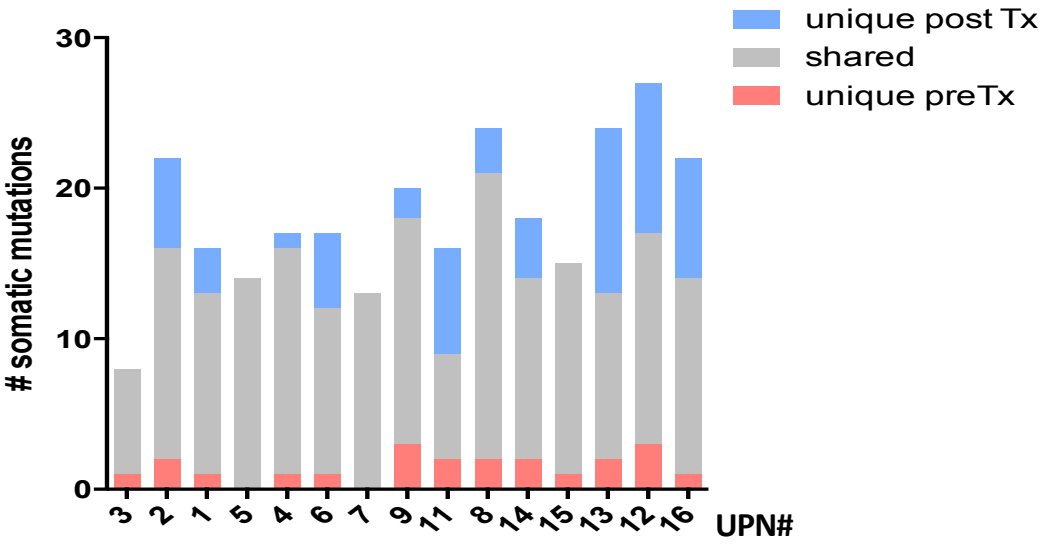
1. Genomic HLA loss (10 years later, becoming an evergreen?)

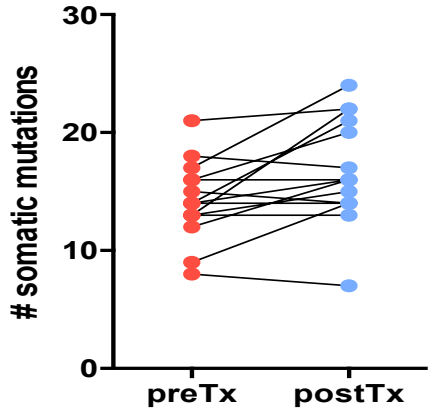
2. **Novel modalities of relapse (it's not all in genomics...)**

Seeking New Mechanisms of AML Immune Evasion



Mutational Landscape of Relapsed AML





Wilcoxon matched-pairs Test p= 0.0093

preTx: average n=14.2, range 8-21

postTx: average n=16.7, range 7-24

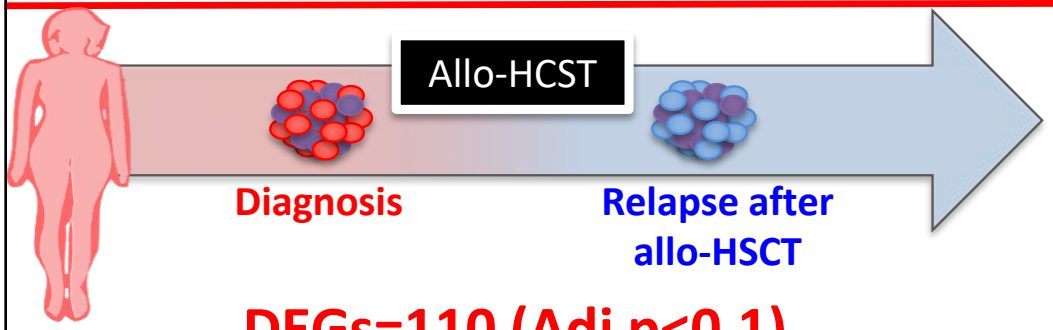
Average coverage for tumor sample: 120X

		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

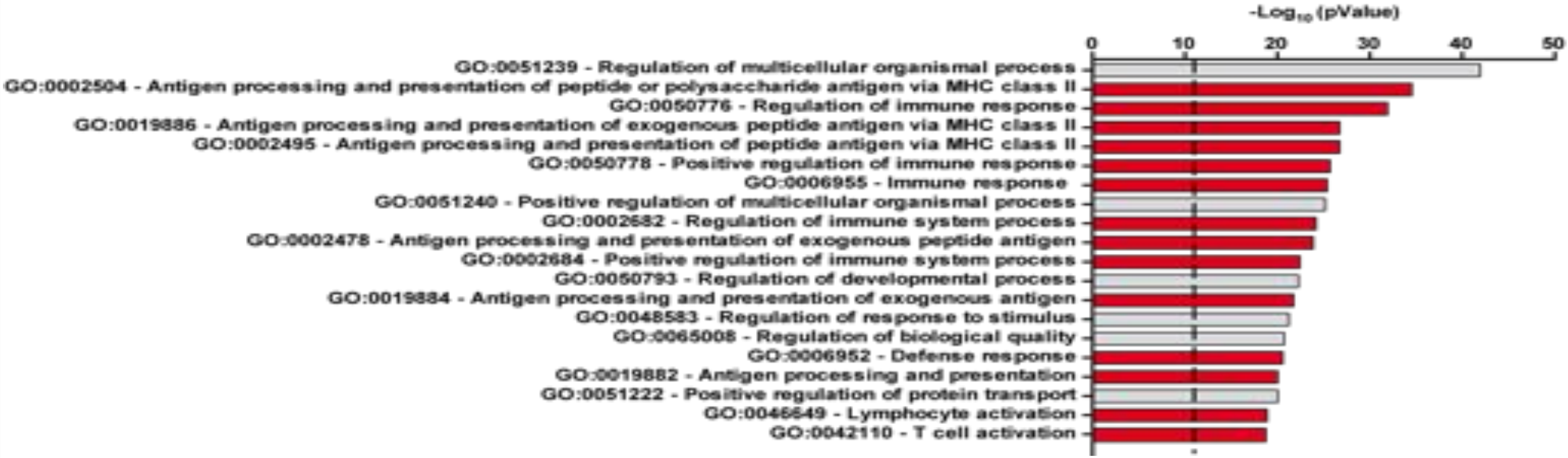
Zanotti, EBMT Presidential Symposium 2018 (ASH 2018 update)

Deregulation of Immune Processes after Allo-HSCT



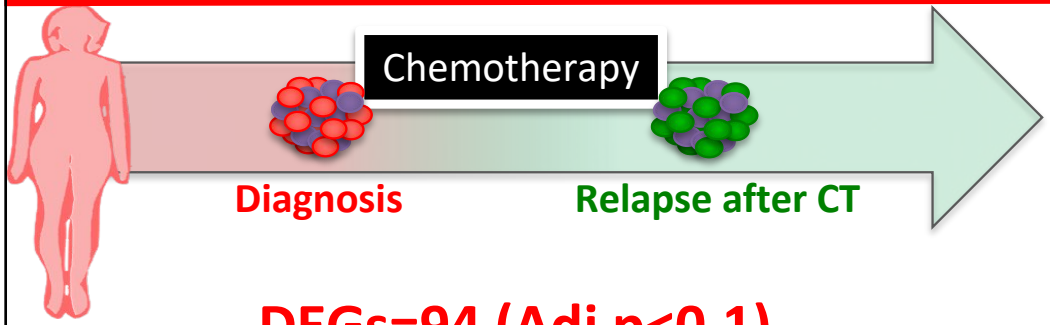
DEGs=110 (Adj p<0.1)

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



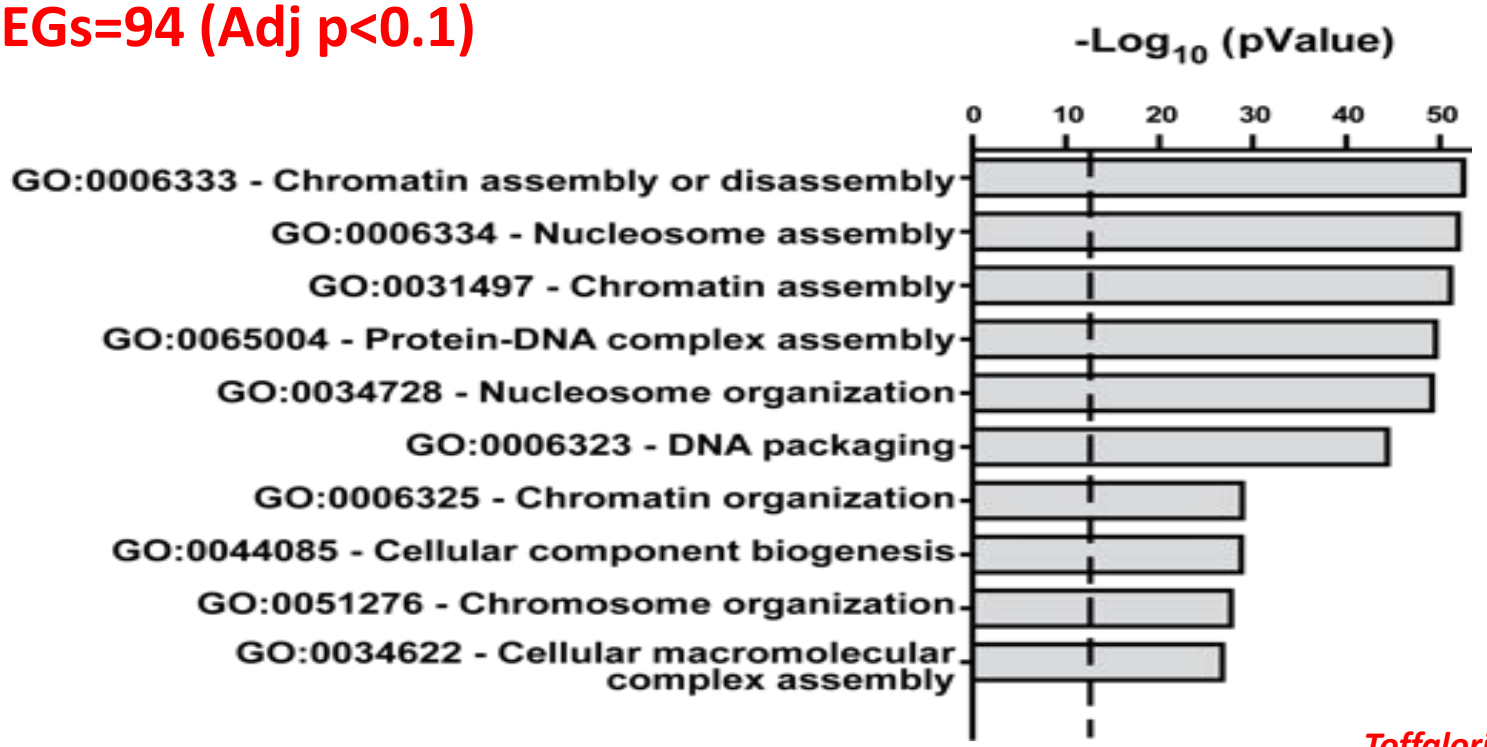
Toffalori et al, Nat Medicine, 2019

Deregulation of Immune Processes after Allo-HSCT



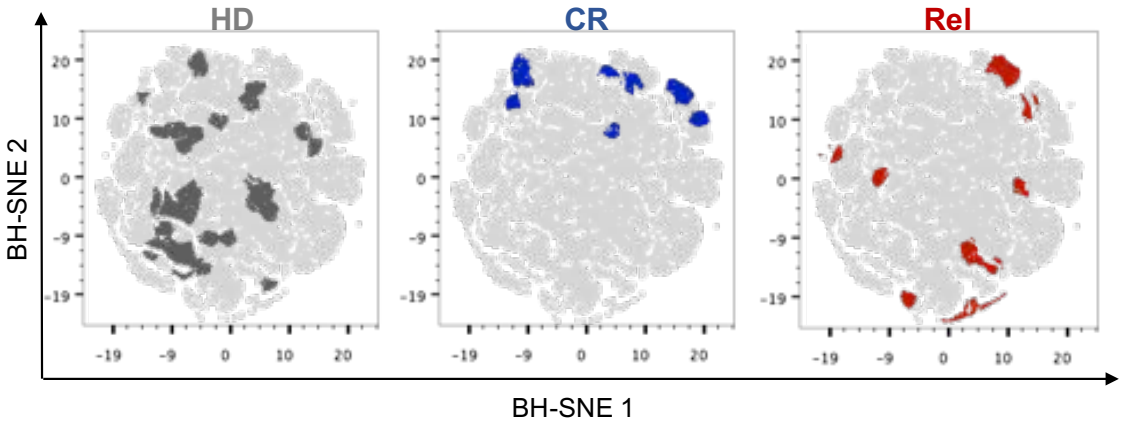
No significant enrichment for immune-related processes

DEGs=94 (Adj p<0.1)



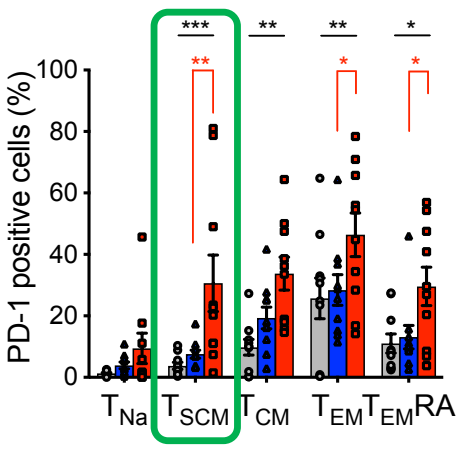
Toffalori et al, Nat Medicine, 2019

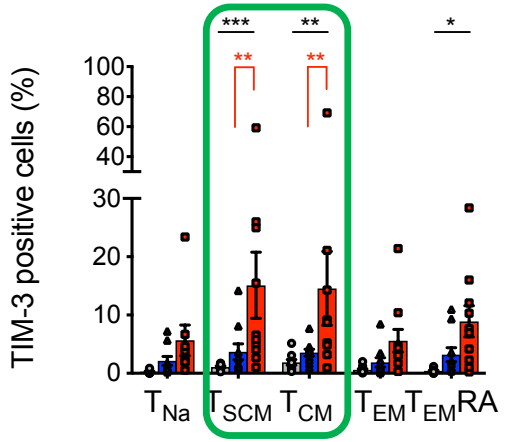
Exhaustion of Early-Differentiated Tumor-Specific T Cells in the Bone Marrow Anticipates Relapse



Phenotypic features of T cells infiltrating the bone marrow of relapsing patients are unique

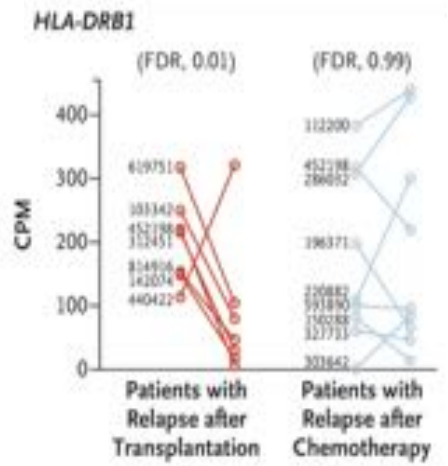
In relapsing patients, T cell exhaustion encompasses not only effectors, but also early differentiated T_{SCM} and T_{CM}

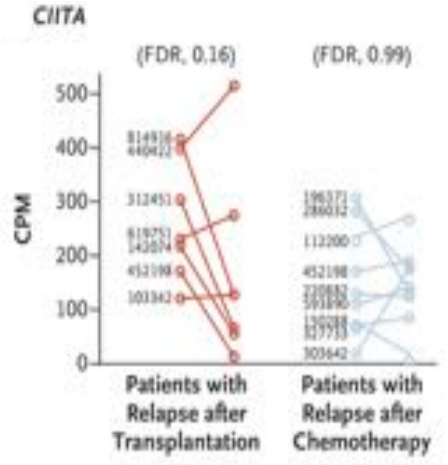


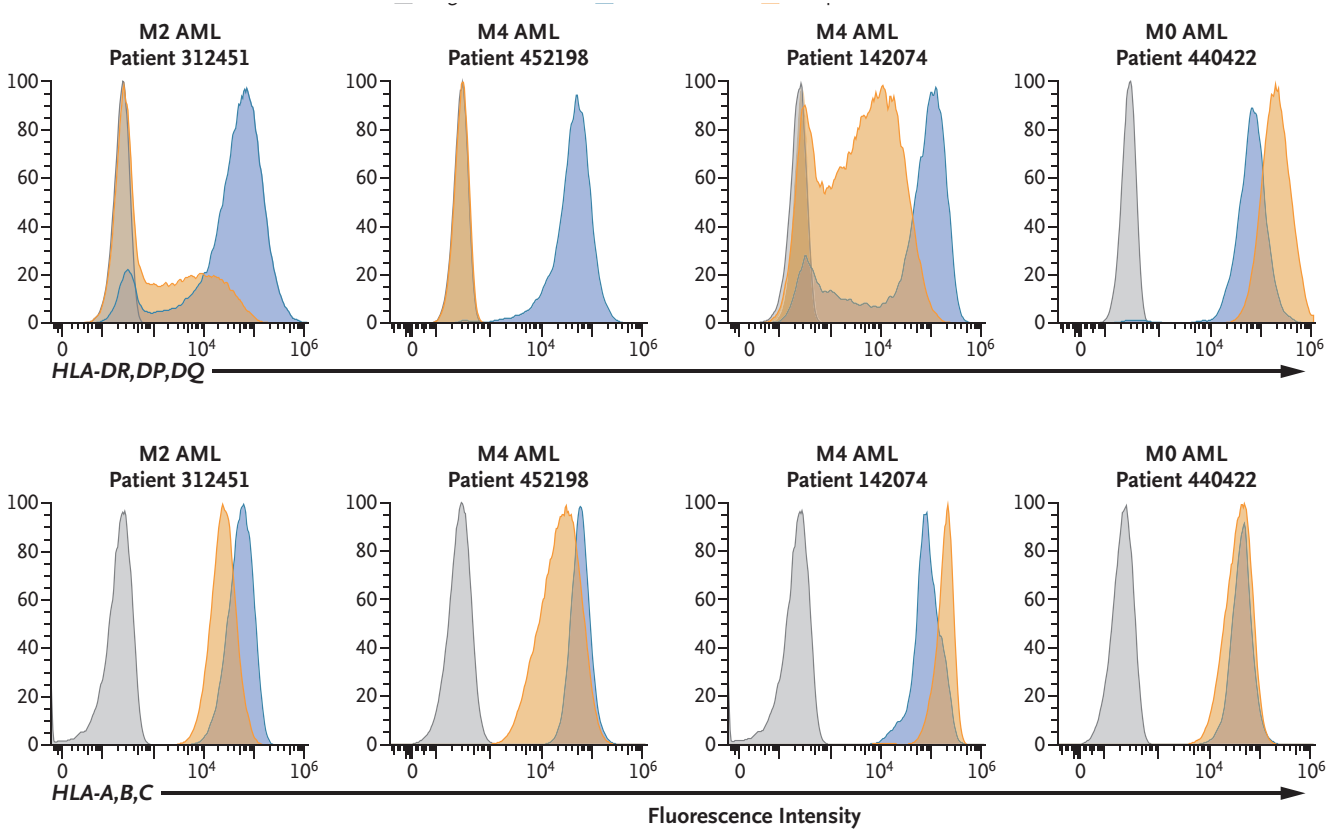


Noviello and Manfredi, Nat Communications, 2019

Downregulation of Molecules Involved in HLA class II Presentation

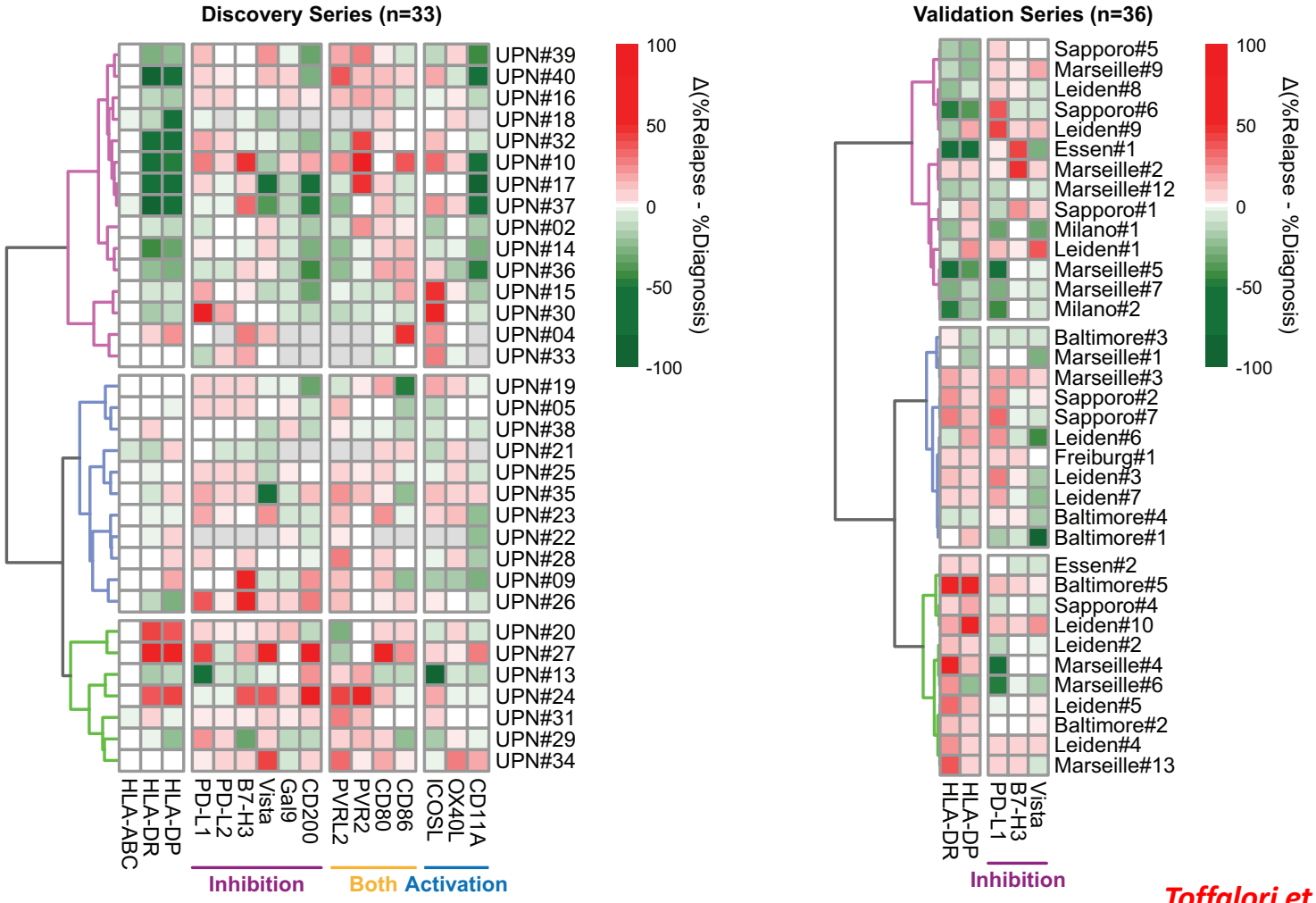






Cristopher, N Engl J Med, 2018

Deregulation of Cohinibitory Ligands and of HLA class II Molecules Are Largely Non-Overlapping



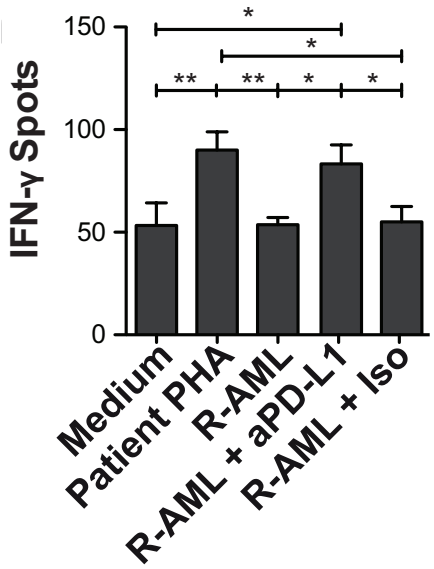
Toffalori et al, Nat Medicine, 2019

Deregulation of Cohinibitory Ligands and of HLA Class II Molecules: Therapeutic Implications

Upregulation of Inhibitory Ligands

(mostly IFN-regulated genes)

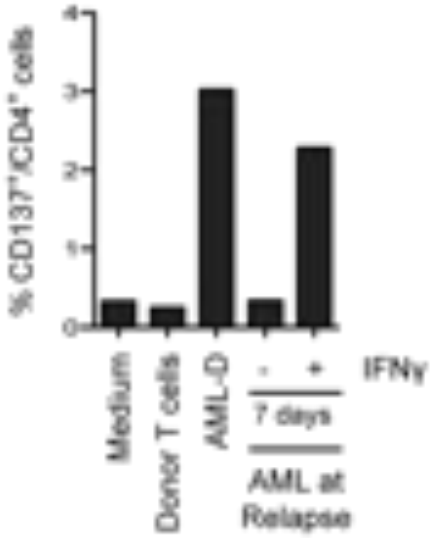
Worsened by inflammatory signals, bypassed through checkpoint blockade



Downregulation of HLA class II molecules

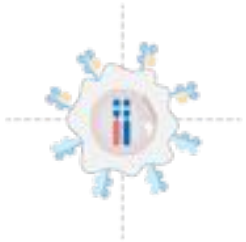
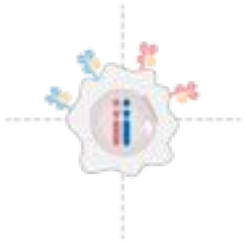
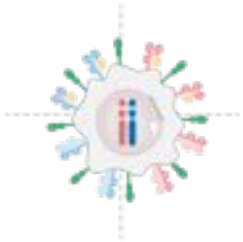
(also IFN-regulated genes)

Recovered though inflammatory signals



Toffalori et al, Nat Medicine, 2019

Post-Transplantation Relapse Modalities: A Recap

	Genomic HLA haplotype loss ("HLA loss")	Downregulation of HLA class II molecules	Upregulation of inhibitory molecules
			
Molecules involved	Incompatible HLAs (both class I and class II)	Compatible and incompatible class II HLAs	PD-L1, B7-H3, PVR, PVRL2
Molecular mechanism	Genomic (CN-LOH)	Epigenetic	Epigenetic
Type of transplants	Mainly HLA-incompatible (haplo>unrelated>cord)	HLA-matched and incompatible	HLA-matched and incompatible
Frequency	~30% in haploidentical, 5-15% in unrelated	30-40% overall	~20% (difficult to address due to complex pattern)
Detection method	HLA typing of sorted blasts, HLA-KMR, HLA-NGS	Flow cytometry, RNA-seq of sorted blasts	Flow cytometry, RNA-seq of sorted blasts
Therapy	No donor lymphocyte infusions, re-transplant or non HLA-restricted immunotherapies	Induction of IFN-γ release through leukemia cross-recognition or inflammatory microenvironment (GvHD?)	Immune checkpoint blockade (combinatorial?)

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Relapse therapy should be **personalized** and **guided by immunobiology**

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Pier Edoardo Rovatti

Unit of Immunogenetics, Leukemia Genomics and Immunobiology

LUCA VAGO

Massimo Bernardi
Jacopo Peccatori

Hematology and BMT Unit

CHIARA BONINI

Experimental Hematology Unit

Davide Cittaro
Giovanni Tonon

Centre for Translational Genomics and Bioinformatics

Alessandro Rambaldi

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Arnon Nagler

Chaim Sheba M.C., Tel Hashomer

Miguel Waterhouse

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Universitätsklinikum Freiburg, Freiburg

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Jaimie Sanz

Jose Luis Pinana

Guillermo Sanz

Hospital Universitario La Fe, Valencia

Masahiro Onozawa

Takanori Teshima

Hokkaido University, Sapporo

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Monzr Al Malki

City Of Hope Hospital C.C.C., Duarte

Stefan Ciurea

MD Anderson C.C., Houston

Mi Kwon

Carolina Martínez Laperche

Hospital Gregorio Marañon, Madrid

Ivana Gojo

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