Leukemia Immune Escape after Transplantation: Personalized Therapy and New Biological Insights

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Immunotherapy in Hematological Malignancies 2023

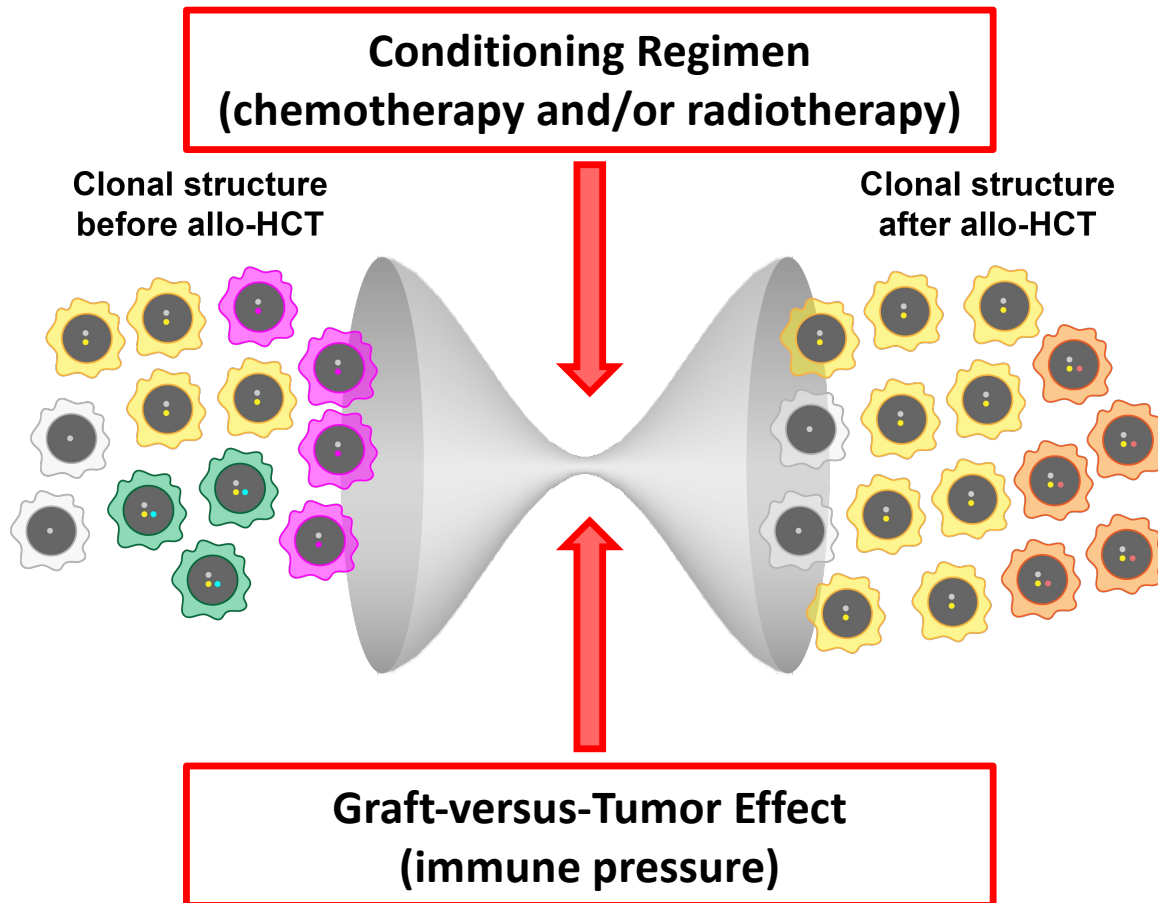*3rd Cuneo City ImmunoTherapy Conference (CCITC)*
Cuneo, May 18-20, 2023

Allogeneic Hematopoietic Cell Transplantation and Relapse

Despite the constant improvement in the outcome of **allogeneic Hematopoietic Cell Transplants (allo-HCT)**, reappearance of the original malignant disease (**relapse**) remains frequent and largely uncurable
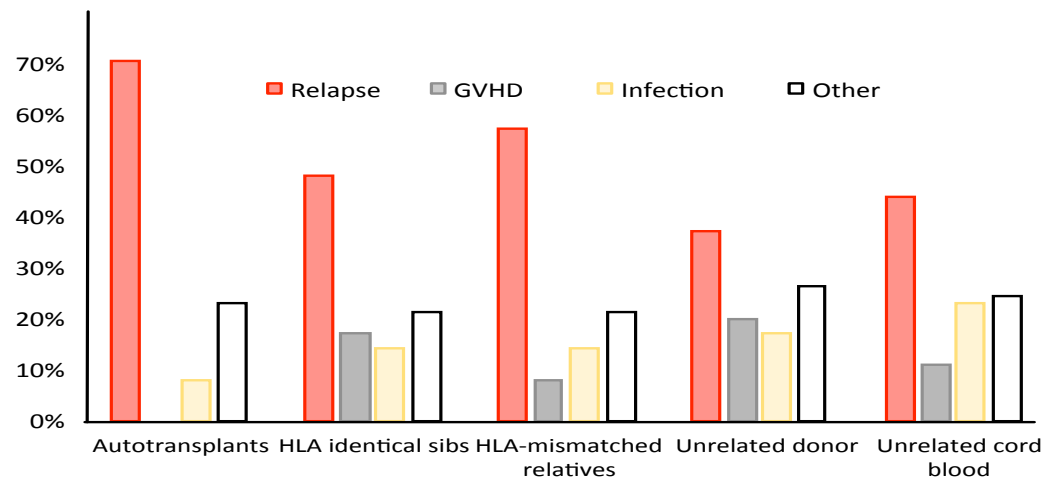
1980-2014 CIBMTR data, adapted from Horowitz, Bone Marrow Transplant, 2018

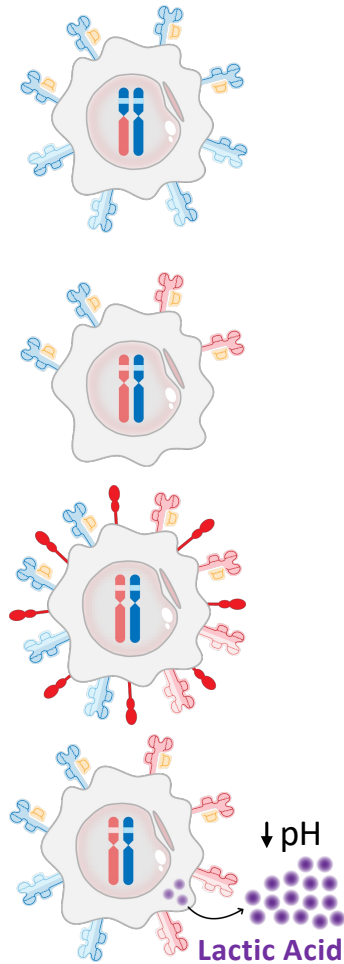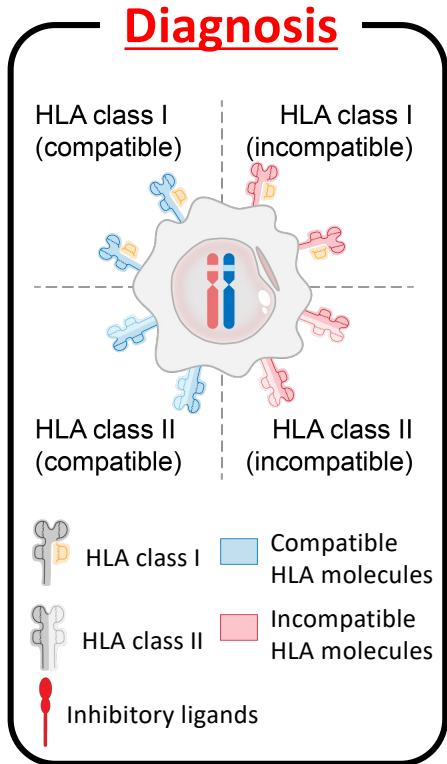# Relapse Through the Scope of an Immunologist

**Conditioning Regimen
(chemotherapy and/or radiotherapy)**

Clonal structure
before allo-HCT

Clonal structure
after allo-HCT

**Graft-versus-Tumor Effect
(immune pressure)**

**Post-transplantation relapses may
be driven by:**

- Disappearance of the most
  immunogenic clones

- Gain of additional mutations
  and epimutations conferring
  immune resistance

Vago, ASH Educational Program Book 2019

Mechanisms of Post-Transplantation Immune Escape

Relapse

Diagnosis

HLA class I (compatible) | HLA class I (incompatible)

HLA class II (compatible) | HLA class II (incompatible)

HLA class I | Compatible HLA molecules
HLA class II | Incompatible HLA molecules
Inhibitory ligands

↓pH

Lactic Acid

Genomic

HLA haplotype loss

Vago, NEJM, 2009; Crucitti, Leukemia, 2015; Ahci and Toffalori, Blood, 2017

Non-Genomic

Downregulation of HLA Class II molecules

Christopher, NEJM, 2018; Toffalori, Nat Med, 2019
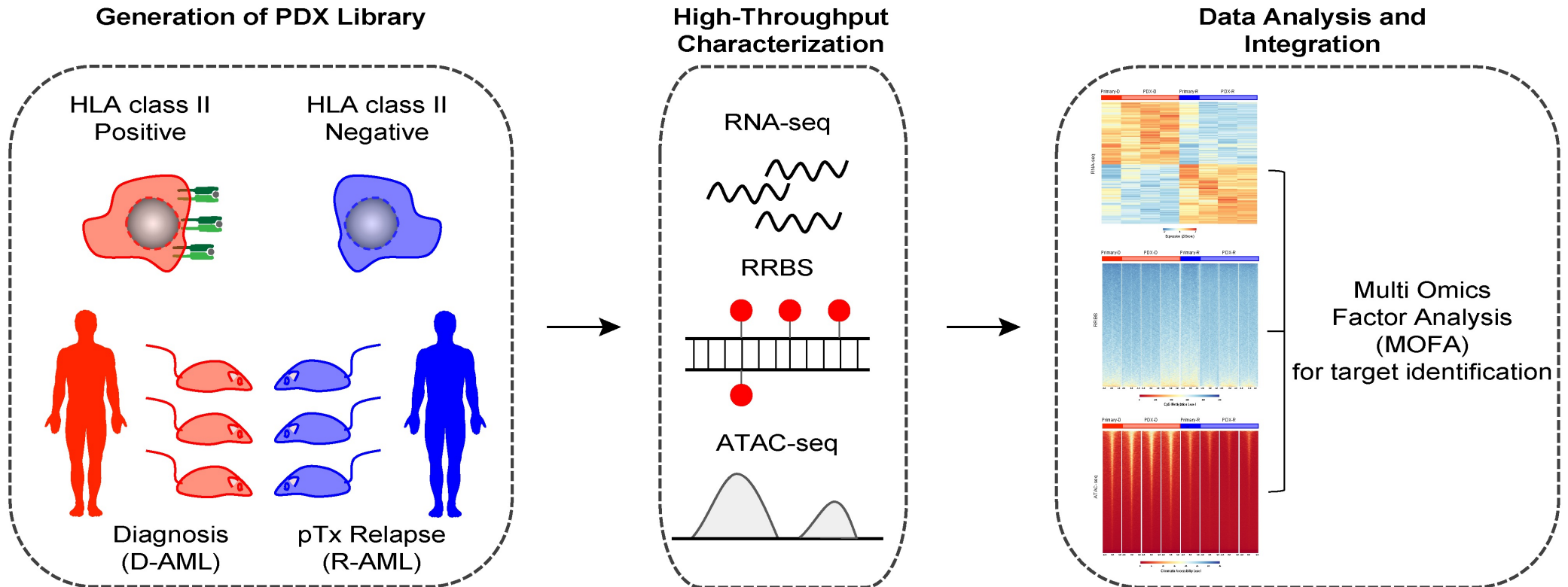
Upregulation of T cell inhibitory ligands

Toffalori, Nat Med, 2019; Noviello and Manfredi, Nat Comm, 2019

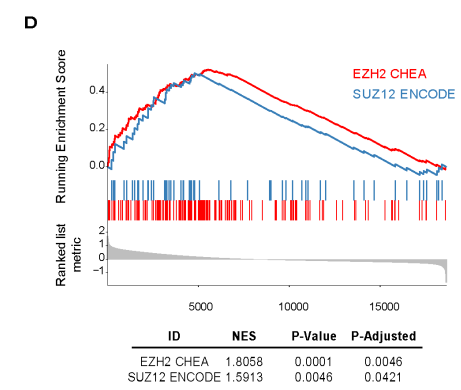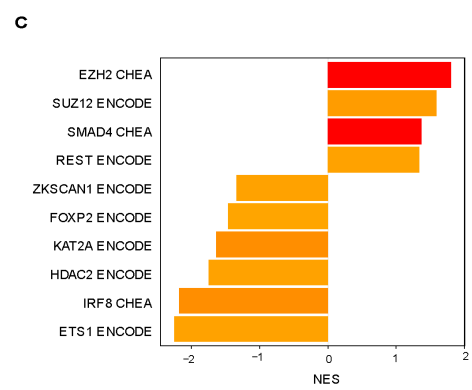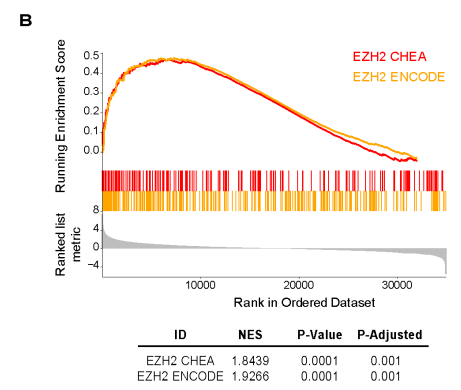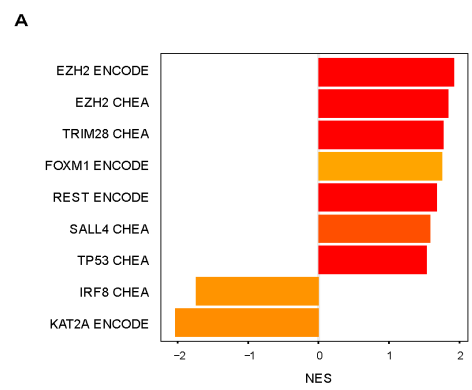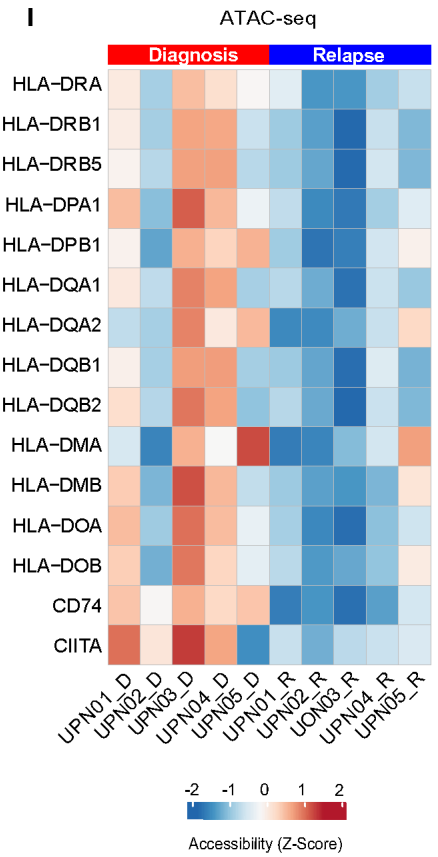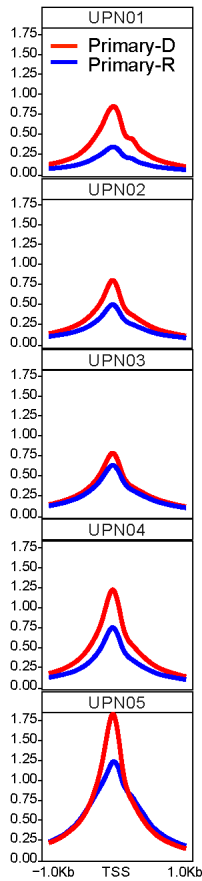Impairment of T cell metabolic fitness

Uhl, Sci Transl Med, 2020

Searching for Epigenetic Drivers of HLA Class II Downregulation: Experimental Outline

Generation of PDX Library

HLA class II Positive

HLA class II Negative

Diagnosis (D-AML)

pTx Relapse (R-AML)

High-Throughput Characterization

RNA-seq

RRBS

ATAC-seq

Data Analysis and Integration

Multi Omics Factor Analysis (MOFA) for target identification

Gambacorta et al, Cancer Discovery, 2022

Reduced Chromatin Accessibility Genomewide and at HLA Genes at Post-Transplantation Relapse

PRC2 Inhibitors Recover HLA Class II Expression in Relapsed Leukemia
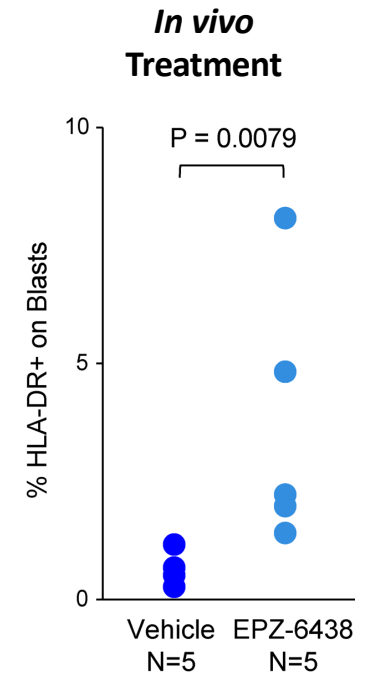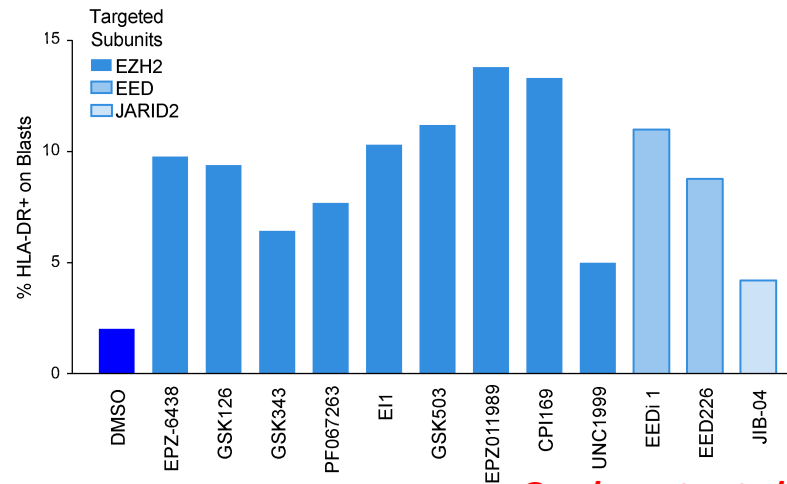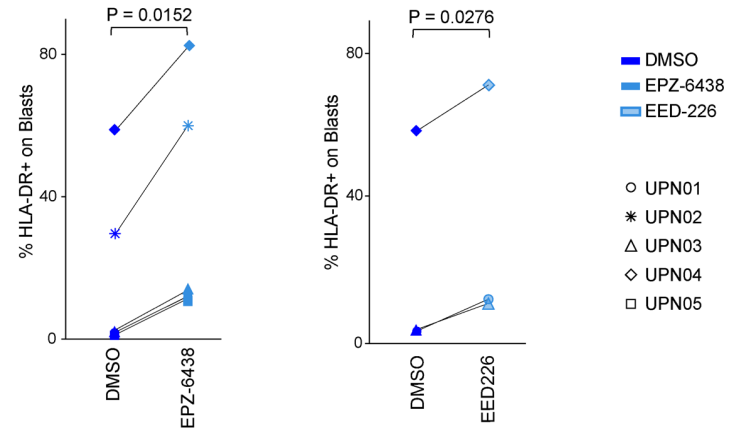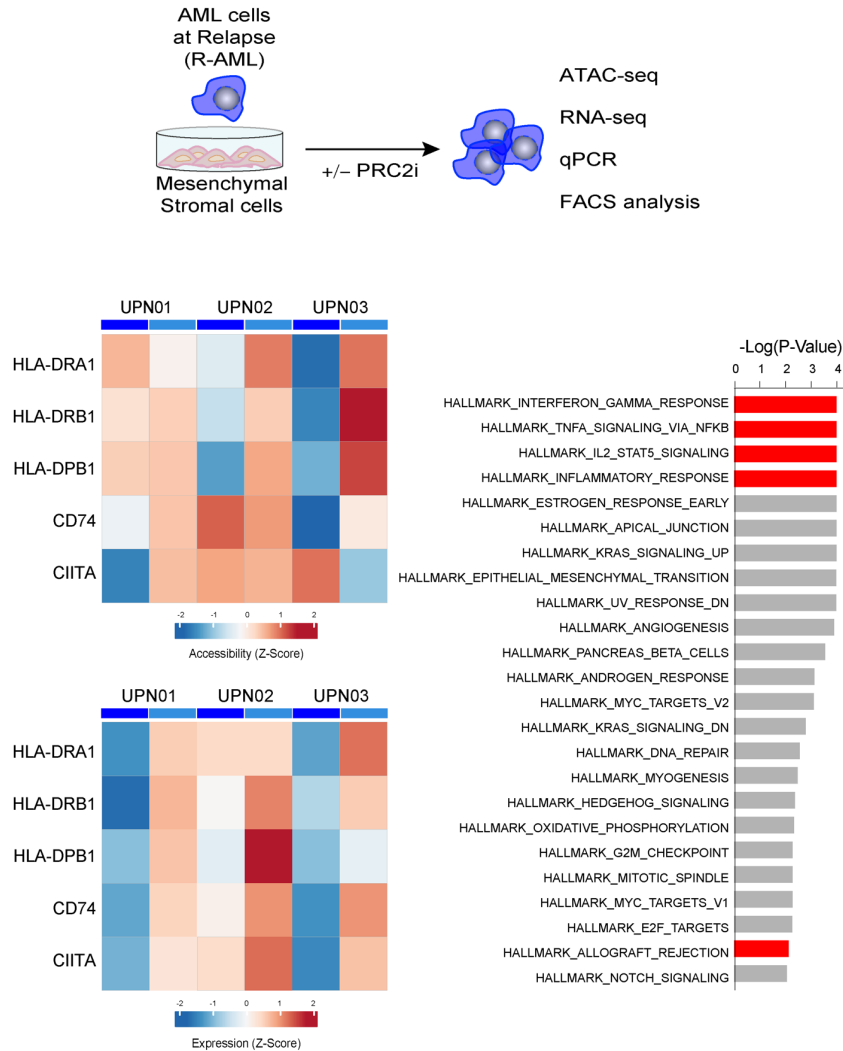
Gambacorta et al, Cancer Discovery, 2022

Tailoring the Therapy of Relapse
on its Immunobiology

Mechanism

HLA haplotype loss

Downregulation of HLA
Class II molecules

Upregulation of T cell
inhibitory ligands

Impairment of T cell
metabolic fitness

Approach

Second allo-HCT,
Bispecific antibodies

Vago and Ciceri, BBMT, 2017; Imus, BBMT, 2017;
Rovatti, in preparation

Delivery of IFN-γ,
Epigenetic drugs (PRC2i)

Rimando, Blood, 2023; Ito, TCT, 2023;
Gambacorta, Cancer Discovery, 2022

Immune Checkpoint Blockade
(+ Hypomethylating agents?)

Davids, NEJM, 2016; Penter, Blood, 2023;
Apostolova, under review

Rewiring BM metabolome
with NaBi

Uhl, Sci Transl Med, 2020

Studying Mechanisms of Relapse Can...
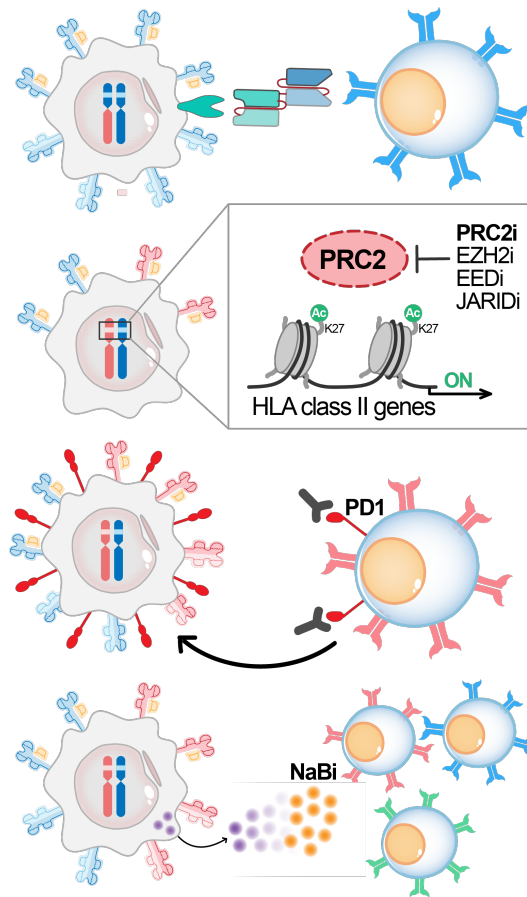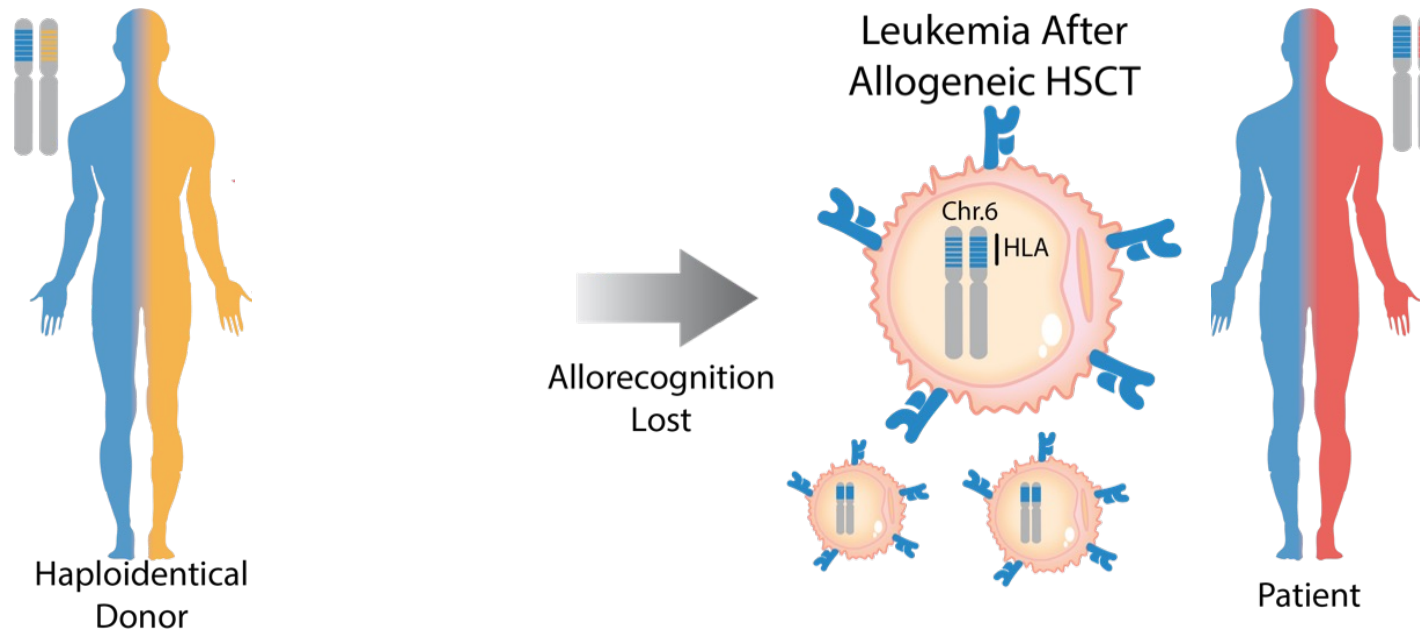
- Provide rationales for the implementation of new therapeutics

- Improve our understanding of fundamental biological questions

Molecular Mechanism and Immunological Consequences of HLA Loss

- Copy Neutral Loss of Heterozygosity of the entire HLA complex (both class I and class II)
- Loss is counterbalanced by duplication of the other haplotype (expression level unchanged)

Vago et al, N Engl J Med, 2009; Toffalori et al, Blood, 2012
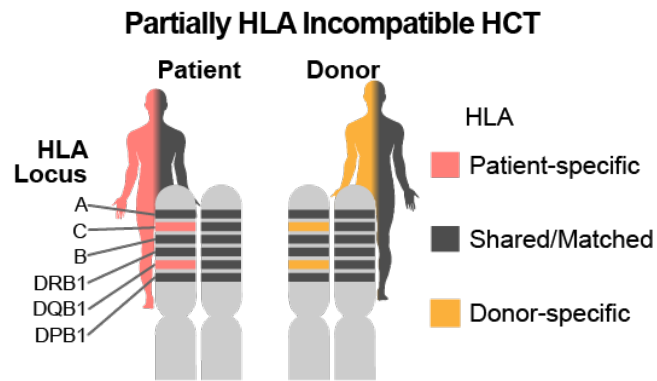*Crucitti et al, Leukemia, 2015; Ahci and Toffalori et al, Blood, 2017*
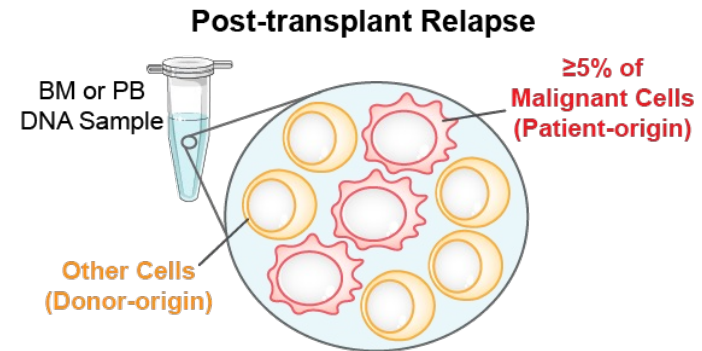
HLA Loss Frequency after Haploidentical HCT

Reference	N° of relapse cases	HLA loss frequency	Additional relevant observations
Vago et. al., 2009	17	29%	ATG
Villalobos et al., 2010	3	66.6%	ATG, Pediatric cases
Crucitti et al., 2015	69	33%	Mixed PTCy and ATG, risk factor analysis
McCurdy et al., 2016	2	Case report	PTCy
Grosso et al., 2017	12	50%	PTCy
Muñiz et al., 2021	22	27%	PTCy
Wu et al., 2022	106	50.9%	ATG, includes ALL, risk factor analysis

Rizzello, in preparation

Outline of the HLALOSS Study

Transplant Selection

Partially HLA Incompatible HCT

Patient   Donor

HLA Locus
A
C
B
DRB1
DQB1
DPB1

HLA
- Patient-specific
- Shared/Matched
- Donor-specific

Sample Criteria

Post-transplant Relapse

BM or PB DNA Sample

≥5% of Malignant Cells (Patient-origin)

Other Cells (Donor-origin)

Sample and Data Processing

Next-generation Sequencing of HLA

MiSeq   NexType   Analysis

Counting of Patient-specific Reads

Locus A   C   B   DRB1   DQB1   DPB1

Data Interpretation

Discrimination Based on Patient-specific HLA

Patient HLA signal **coherent** with the disease percentage

Patient HLA signal **non coherent** with the disease percentage

Classical Relapse

HLA loss Relapse
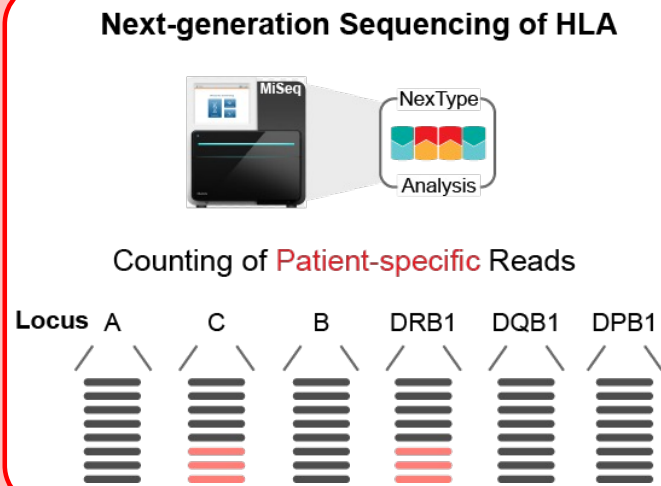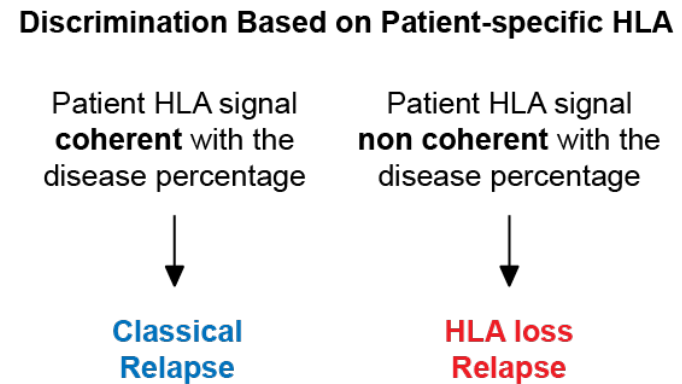
A Global Effort

Supplementary Table 1. Centers participating in the study.			
Center Code	**Center**	**City**	**Country**
C01	I.R.C.C.S. San Raffaele Scientific Institute	Milano	Italy
C02	Hospital Saint Antoine	Paris	France
C03	Chaim Sheba Medical Centre	Tel-Hashomer	Israel
C04	Universitätsklinikum Carl Gustav Carus	Dresden	Germany
C05	Universitätsklinikum Essen	Essen	Germany
C06	Institut Paoli-Calmettes	Marseille	France
C07	Universitätsklinikum Freiburg	Freiburg	Germany
C08	A.O. SS. Antonio e Biagio e Cesare Arrigo	Alessandria	Italy
C09	Hematology Institute "Lorenzo ed Ariosto Seragnoli"	Bologna	Italy
C10	I.R.C.C.S. Policlininco Gemelli	Roma	Italy
C11	I.R.C.C.S. Arcispedale Santa Maria Nuova	Reggio Emilia	Italy
C12	A.O.U. Careggi	Firenze	Italy
C15	City Of Hope Comprehensive Cancer Center	Duarte	CA, USA
C16	Hokkaido University	Sapporo	Japan
C17	A.O.U. Citta della Salute e della Scienza di Torino	Torino	Italy
C18	Tor Vergata University	Roma	Italy
C19	Hospital Universitari i Politècnic La Fe	Valencia	Spain
C20	Universitätsklinikum Eppendorf	Hamburg	Germany
C21	Universitätsklinikum Düsseldorf, Heinrich-Heine University	Dusseldorf	Germany
C22	University of Perugia	Perugia	Italy
C23	Hospital General Universitario Gregorio Marañón	Madrid	Spain
C24	Dana-Farber Cancer Institute	Boston	MA, USA
C25	I.R.C.C.S. Humanitas Cancer Center	Milano	Italy
C26	Thomas Jefferson University	Philadelphia	PA, USA
C27	Fred Hutchinson Cancer Center	Seattle	WA, USA
C28	Kyoto University	Kyoto	Japan
C29	The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins	Baltimore	MD, USA

Total number of relapses = 533

C01 C06 C15 C20 C25
C02 C07 C16 C21 C26
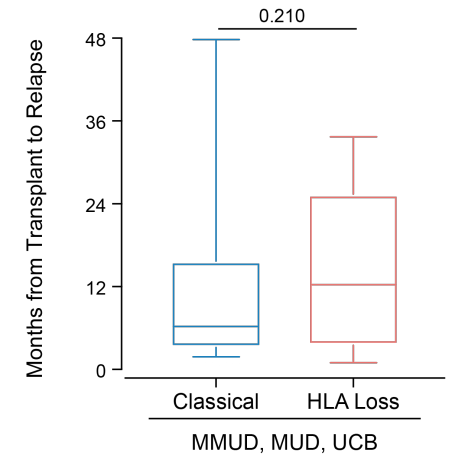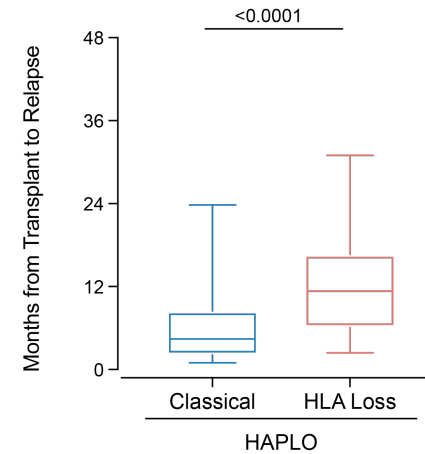C03 C08 C17 C22 C27
C04 C11 C18 C23 C28
C05 C12 C19 C24 Others
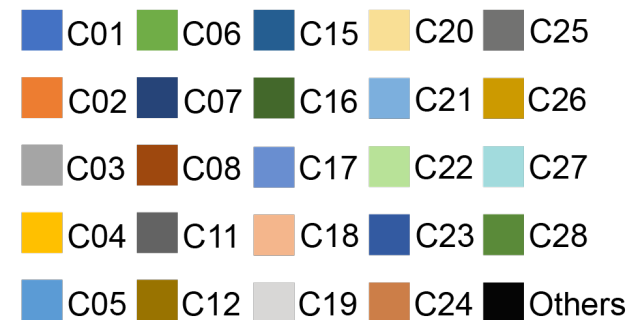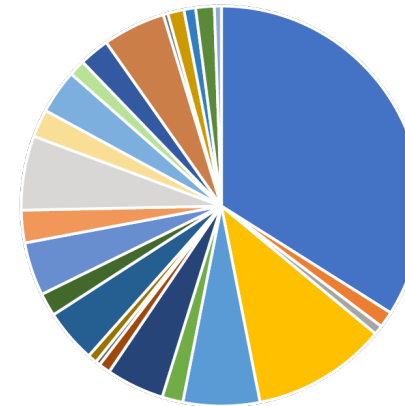
Fleischhauer et al, under submission

Different Donor Types
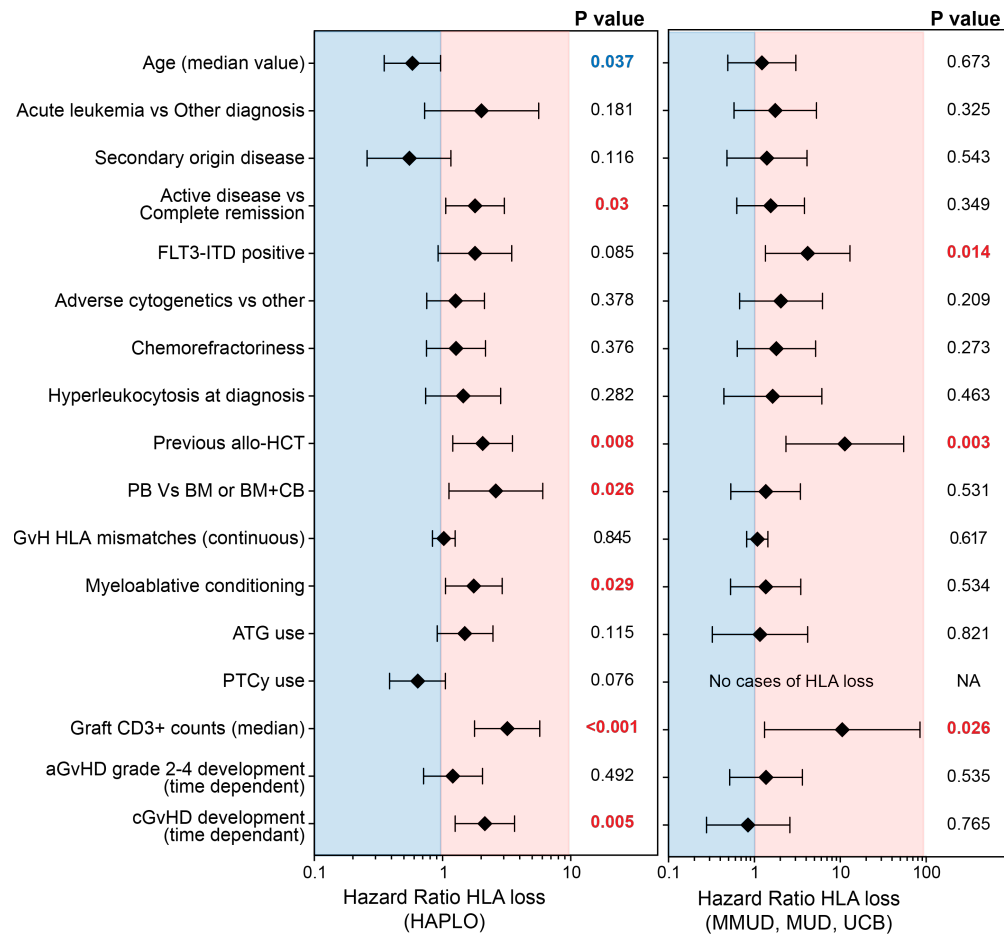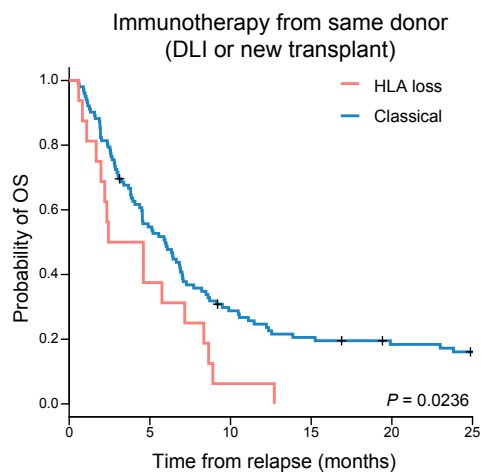

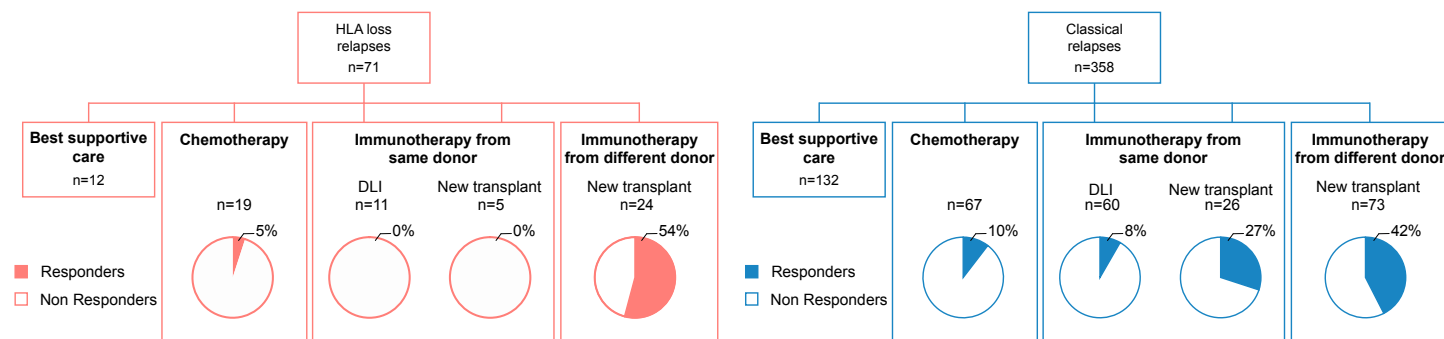Fleischhauer et al, under submission
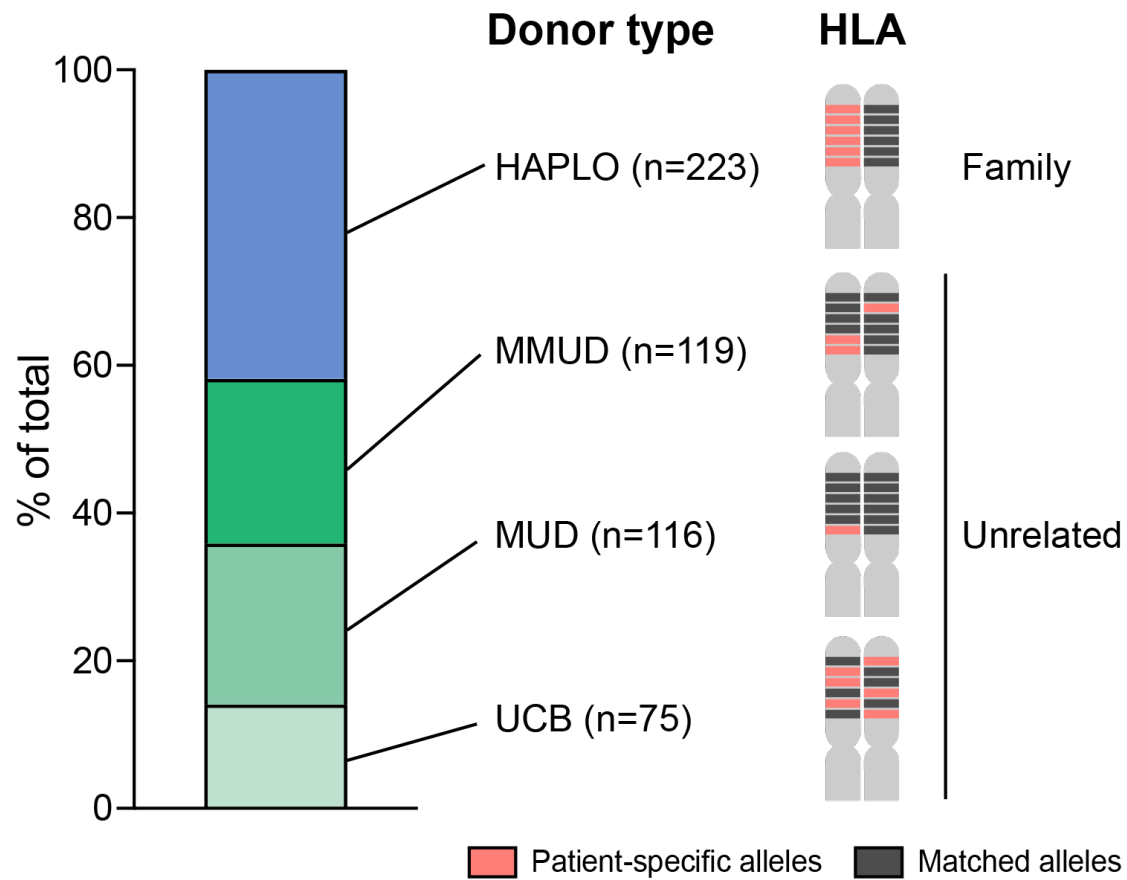
Clinical Variables Associated to HLA loss

Overall Incidence of HLA loss: 83/533 (15.6%)

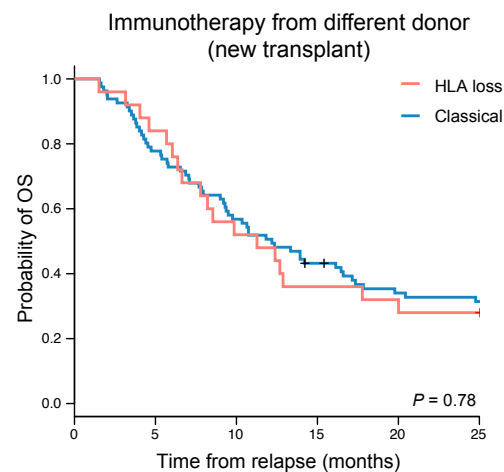*Fleischhauer et al, under submission*

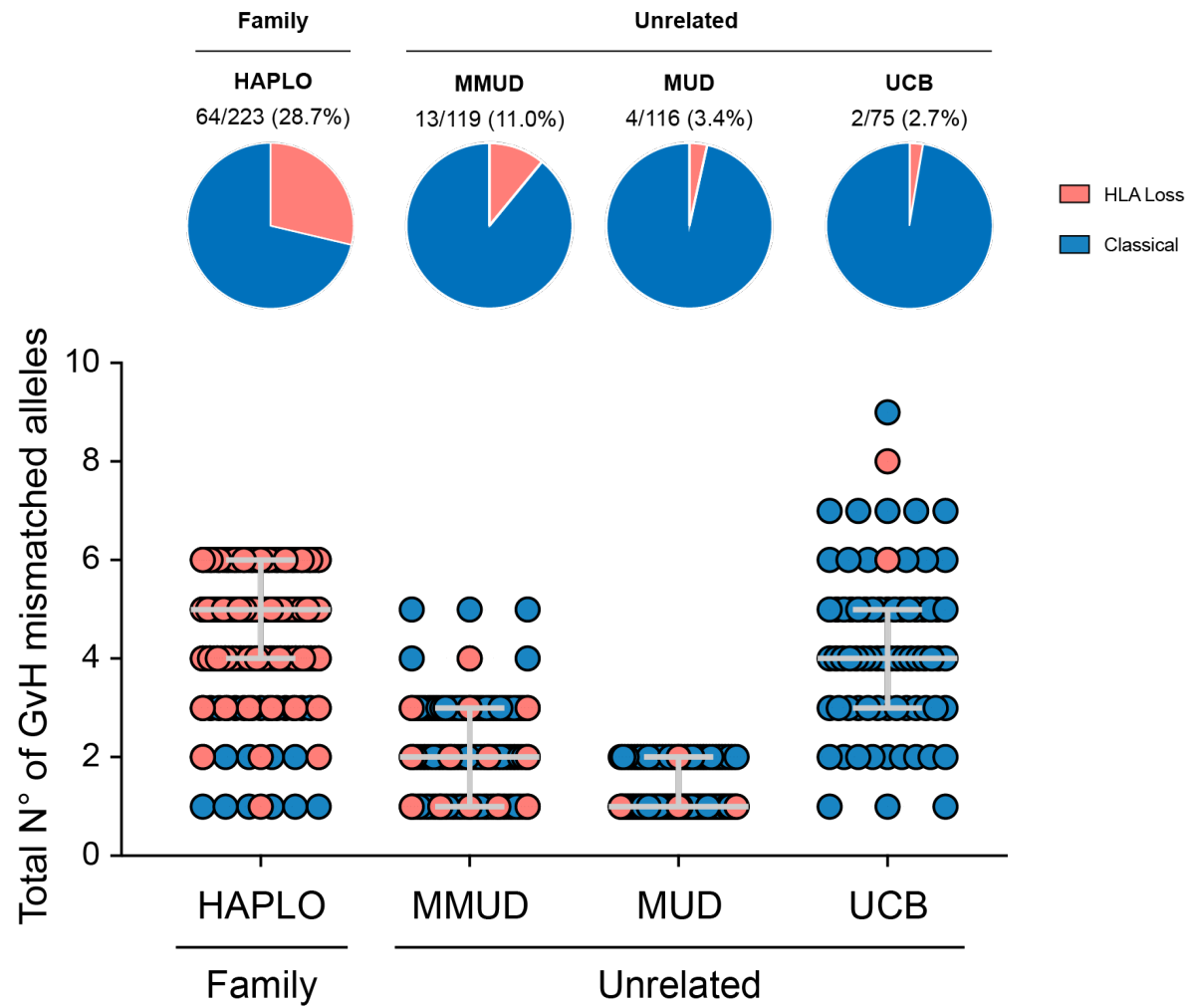# Response to Treatments and Outcome of HLA loss Relapses

Fleischhauer et al, under submission

HLA loss and Donor Type

Fleischhauer et al, under submission
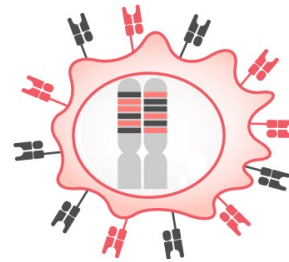
Why HLA loss Is So Rare After UCB-HCT?
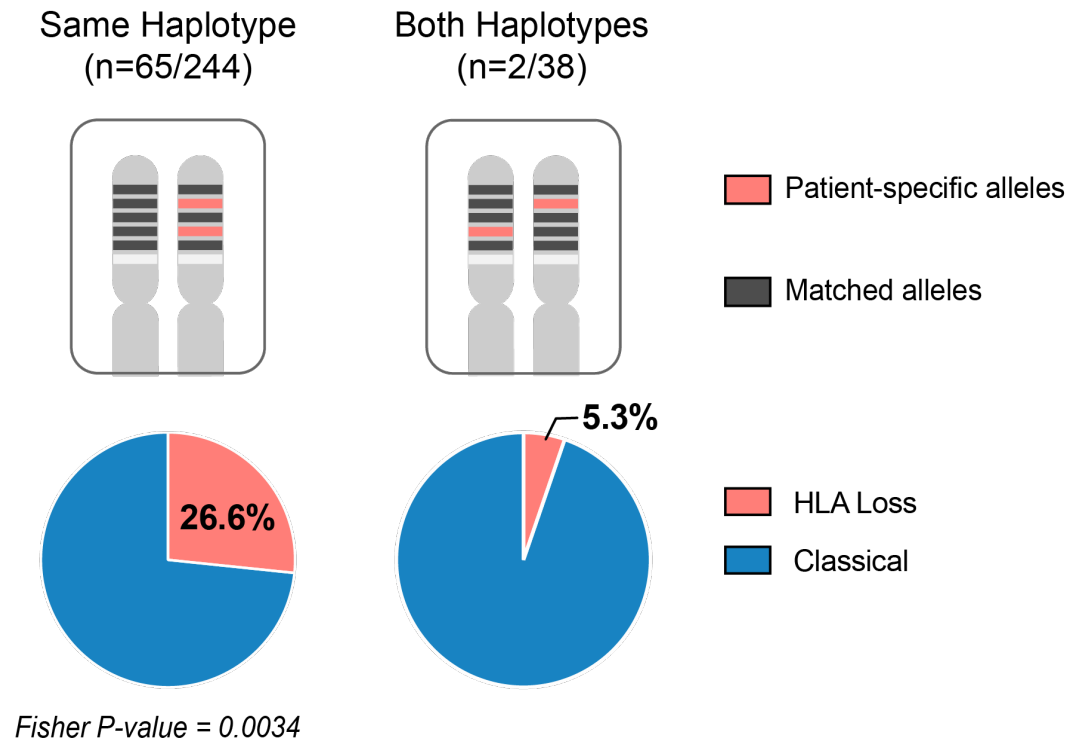
Haploidentical HCT
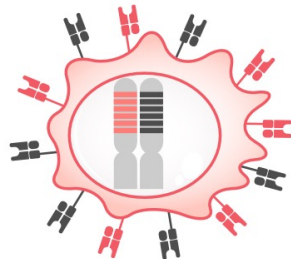
Unrelated Cord Blood HCT

Patient-specific alleles   Matched alleles

Fleischhauer et al, under submission

Haplotype Distribution of Mismatched HLAs

Same Haplotype
(n=65/244)

Both Haplotypes
(n=2/38)

Patient-specific alleles

Matched alleles

26.6%

5.3%

HLA Loss

Classical

Fisher P-value = 0.0034

Fleischhauer et al, under submission

New rationale for unrelated UCB and MMUD selection, prioritizing donors with incompatibilities on both haplotypes?

Conclusions and Future Challenges

- Allo-HCT is an incredibly **complex system**, and a multitude of variables impact on its immunobiology, also in unexpected ways (type of donor, drugs?)

- This knowledge should impact on the **design of clinical trials** (aim at a biologically meaningful subset of patients rather than wide indications, selecting the appropriate time of intervention)

- More real-life data and AIs might allow to better define drivers/risk factors for the different relapse modalities, anticipating intervention and **preventing clinical recurrence** (GITMO-RELAPSE study, PI Fabio Ciceri)

- Lessons from allo-HCT will be key to fully exploit the potential of **targeted cell therapies** and possibly to generate synergistic combinations

Acknowledgements