

Leukemia Epigenetic Remodelling: the Lesson of Allogeneic Stem Cell Transplantation

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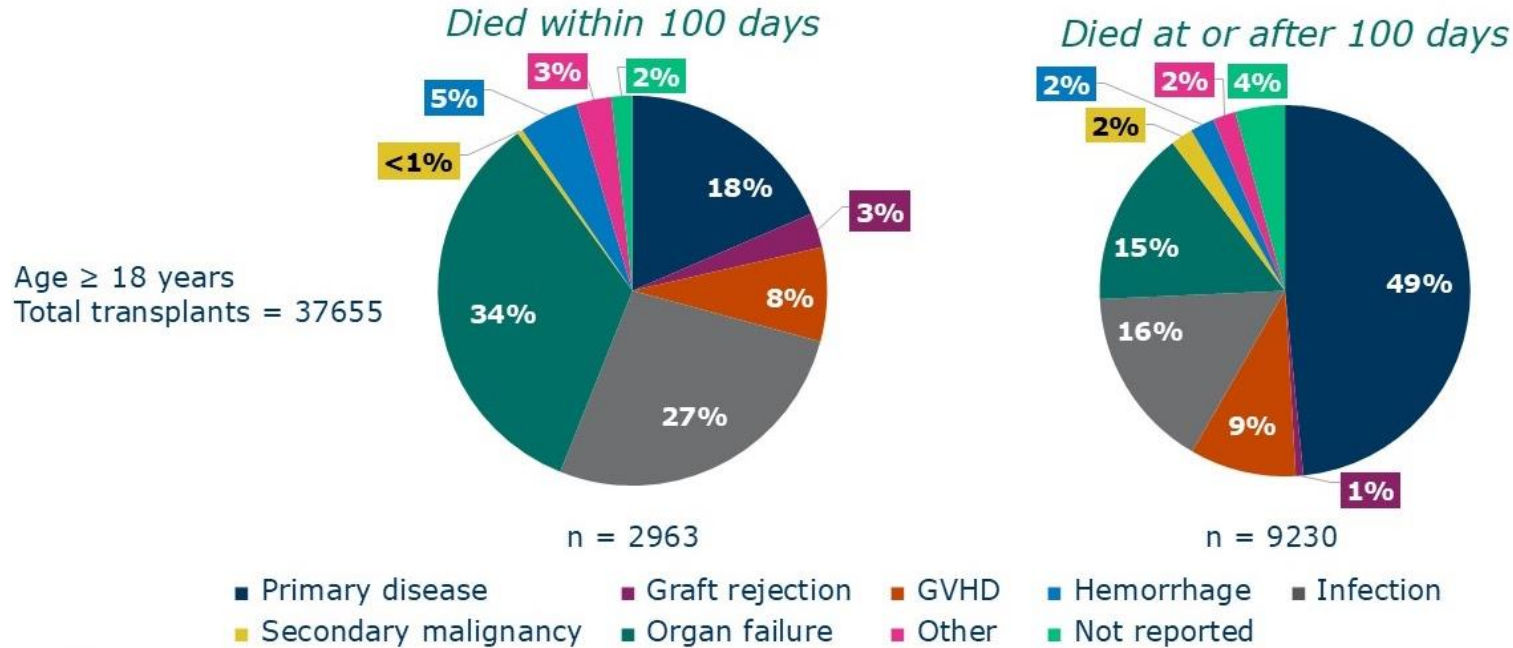
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2nd BOLOGNA WORKSHOP on **IIMMUNOTHERAPY FOR AML**
Bologna, September 29-30, 2026

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Allo-HCT in 2026: Where We Still Fail



**Mortality is mostly late (>100 days 3x)
and due to disease recurrence**

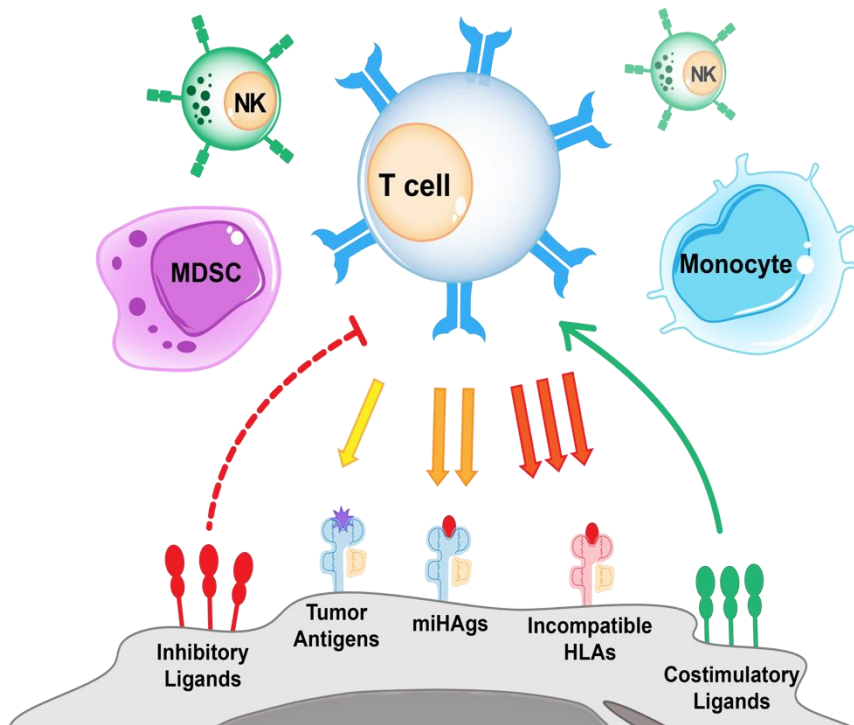
Tumor Resistance: A Change In Perspective

- Historical (naive) explanation of relapse was: therapy was not powerful/effective/on spot enough -> need to increase doses (and toxicities)
- New understanding: tumors are smart moving targets, changing in response to therapies -> evolutionary perspective

Relapse mechanisms are specific for the modality of initial treatment, and mostly intrinsic to the main mechanism of action of that therapy

Allo-HCT MoA: a Paradigmatic Form of Immunotherapy

Allo-HCT represents one of the first, and by large the most widely clinically implemented, demonstration that **adoptive immunotherapy of cancer** can be curative



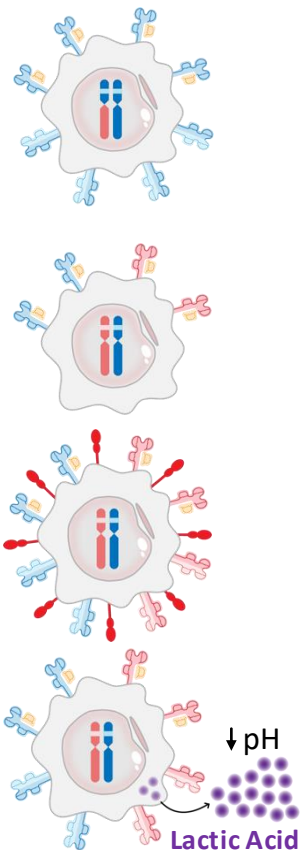
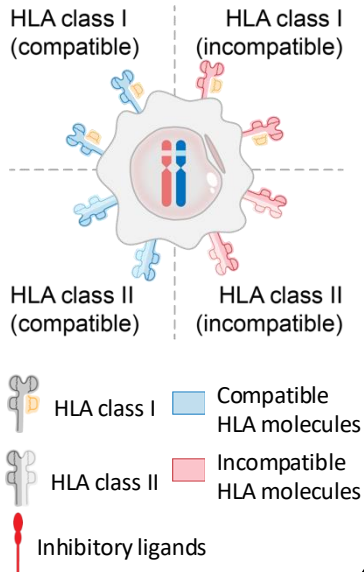
The Graft-versus-Tumor effect of Allo-HCT is:

- largely directed against minor and major **histocompatibility antigens** mismatched between donor and recipient
- highly dependent on the **balance of signals** received by T cells from tumor cells and the bone marrow immune microenvironment

Mechanisms of Post-Transplantation Immune Escape

Relapse

Diagnosis



Genomic

HLA haplotype loss

Vago, NEJM, 2009; Crucitti, Leukemia, 2015; Fleischhauer, J Clin Oncol, 2026

Non-Genomic

Downregulation of HLA Class II molecules

Christopher, NEJM, 2018; Toffalori, Nat Med, 2019; Gambacorta, Cancer Disc, 2022

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

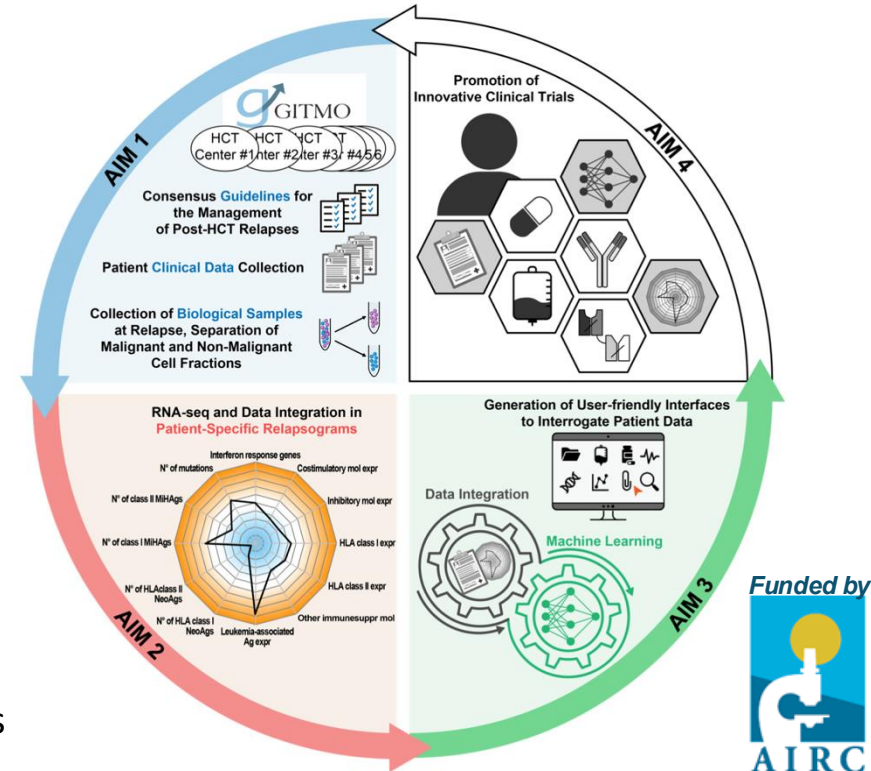
The GITMO-RELAPSE Study – Rationale and Aims

In the last decade, considerable progress has been achieved in understanding the biology that underlies leukemia **relapse after allogeneic HCT**, but most of this knowledge remains confined to a **research scope**

The **GITMO-RELAPSE Study** aims to demonstrate that an in-depth biological profiling of relapses is **feasible in clinical routine** and can inform the selection of effective treatments, improving allo-HCT outcome

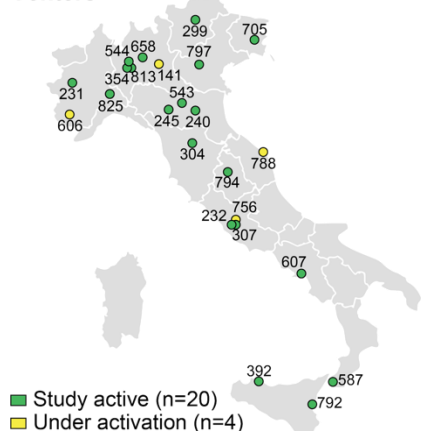
Study Goals

1. Generation of consensus procedures for characterization and treatment of post-transplantation relapses
2. Cutting-edge RNA-seq analysis to map features of relapses, integrating them in a comprehensive matrix (relapsogram)
3. Machine-learning algorithms and generation of user-friendly interfaces to interrogate patient data
4. Promotion of innovative clinical trials in relapsed patients

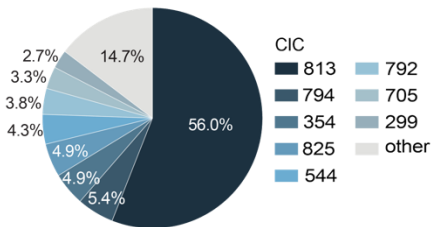


The GITMO-RELAPSE Study – Data Generation

Centers and Patients enrollment

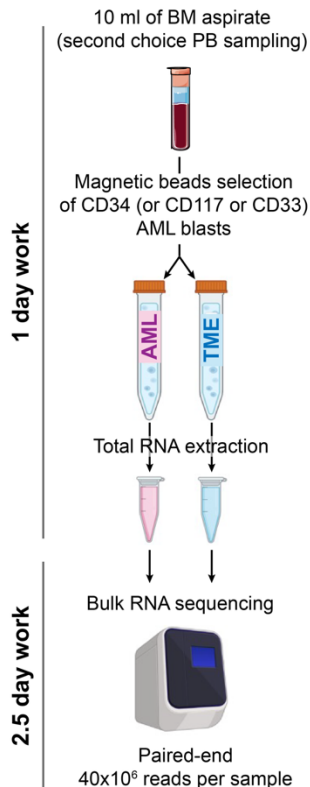


Post-transplant leukemia samples



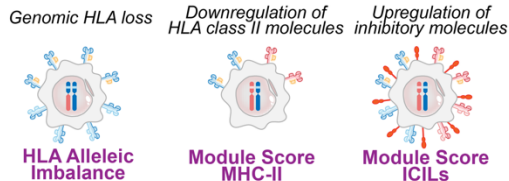
From April 2023, **184 patients** have been recruited across **20 active centers**

Sample Processing

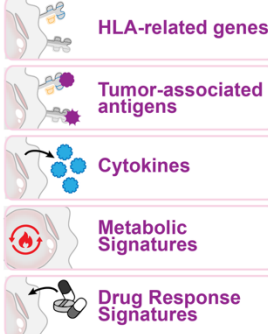


Analyses on AML

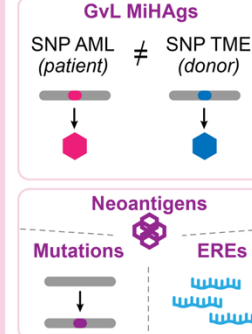
Definition of the Immune Escape Modality



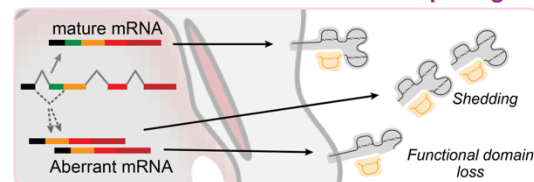
Expression Analyses



Immunogenic Antigens

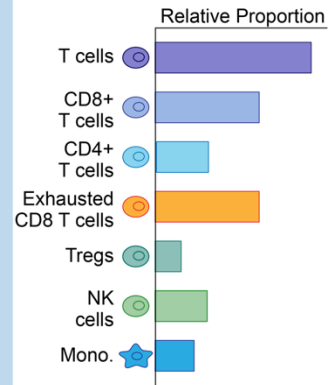


HLA Mutations and Alternative Splicing

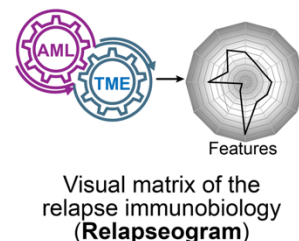


Analyses on TME

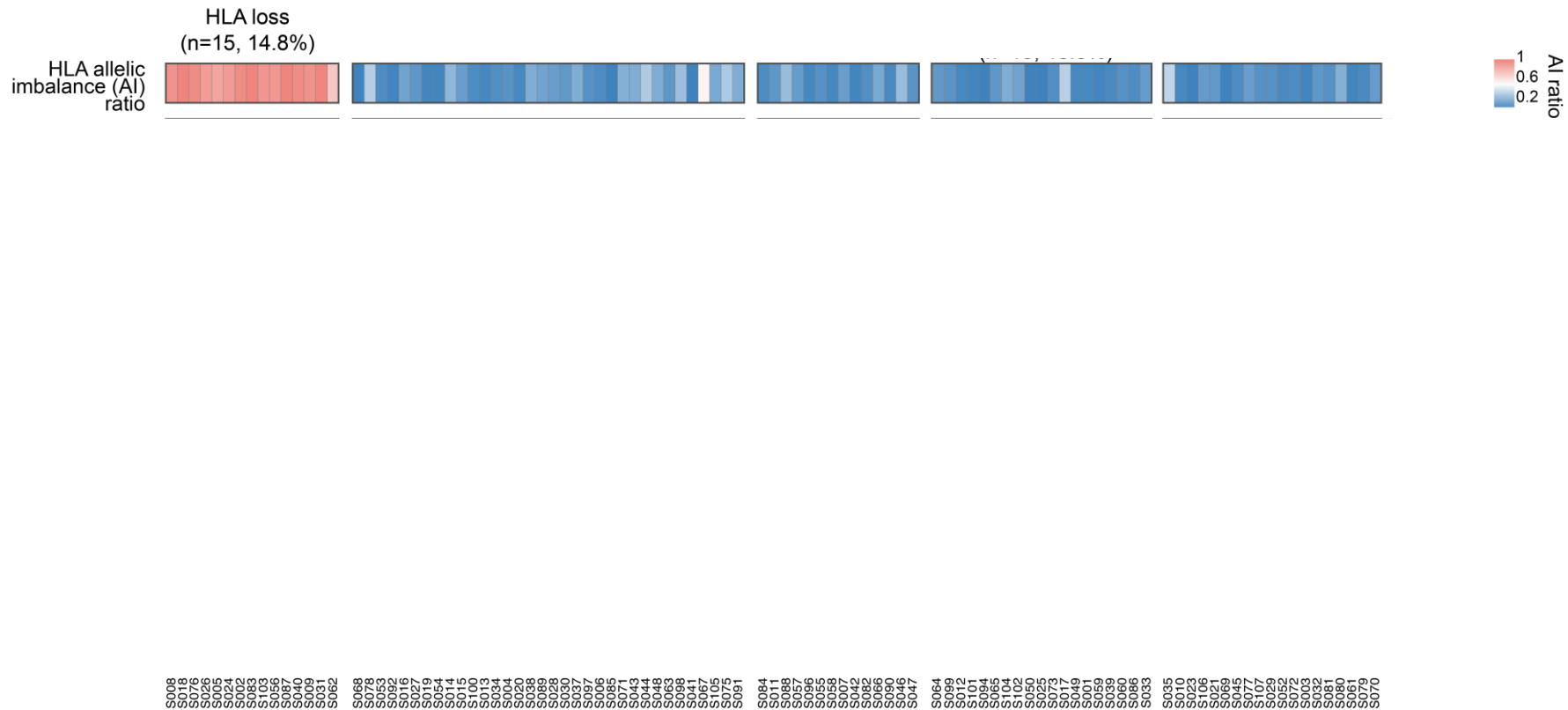
Deconvolution Algorithms



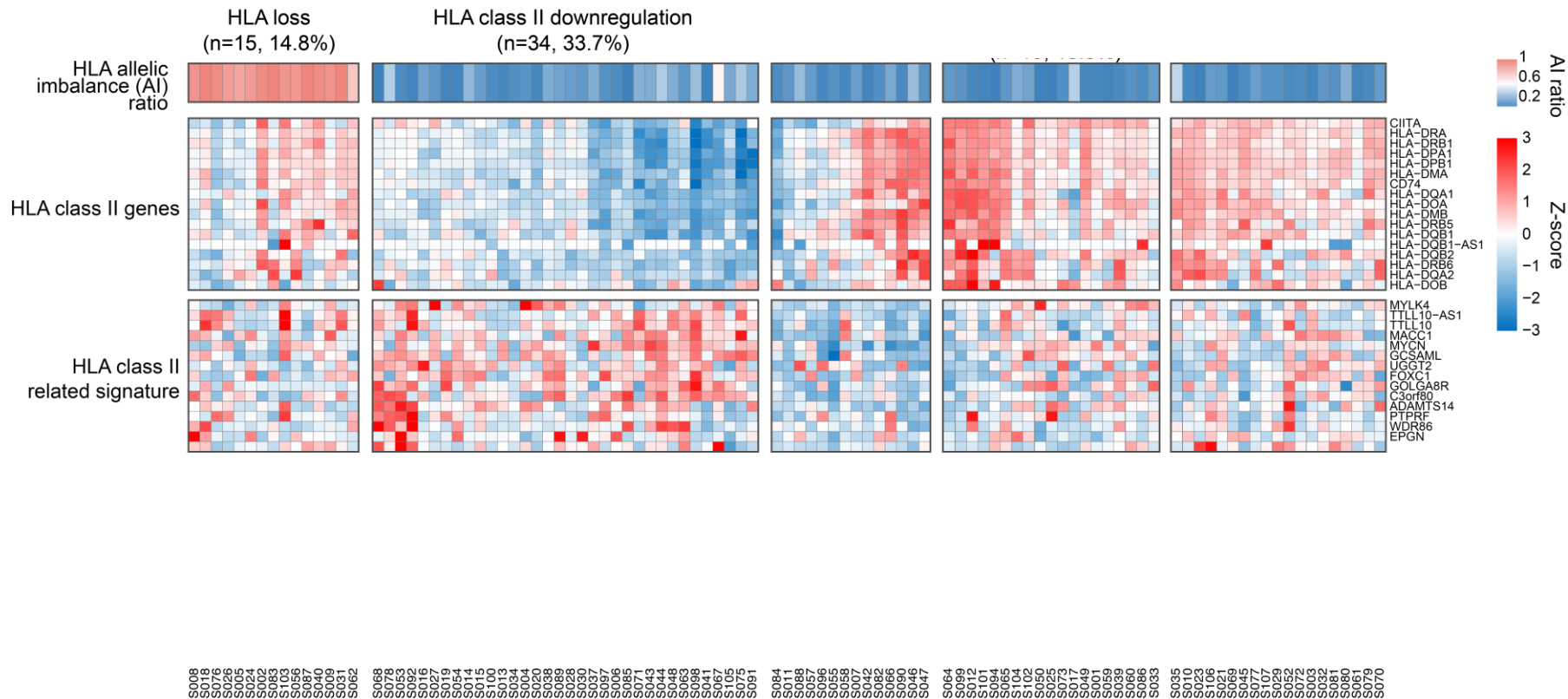
Data Integration



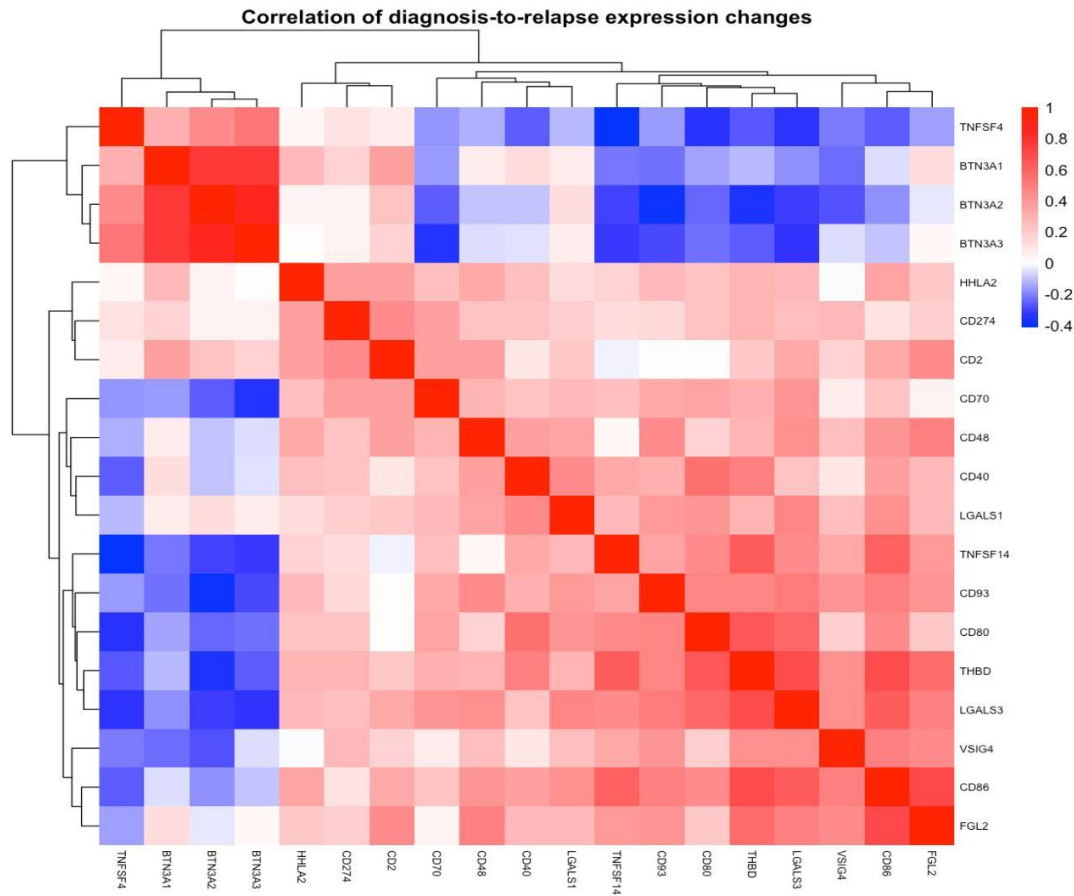
Defining Relapse Modalities from RNA-Seq Data



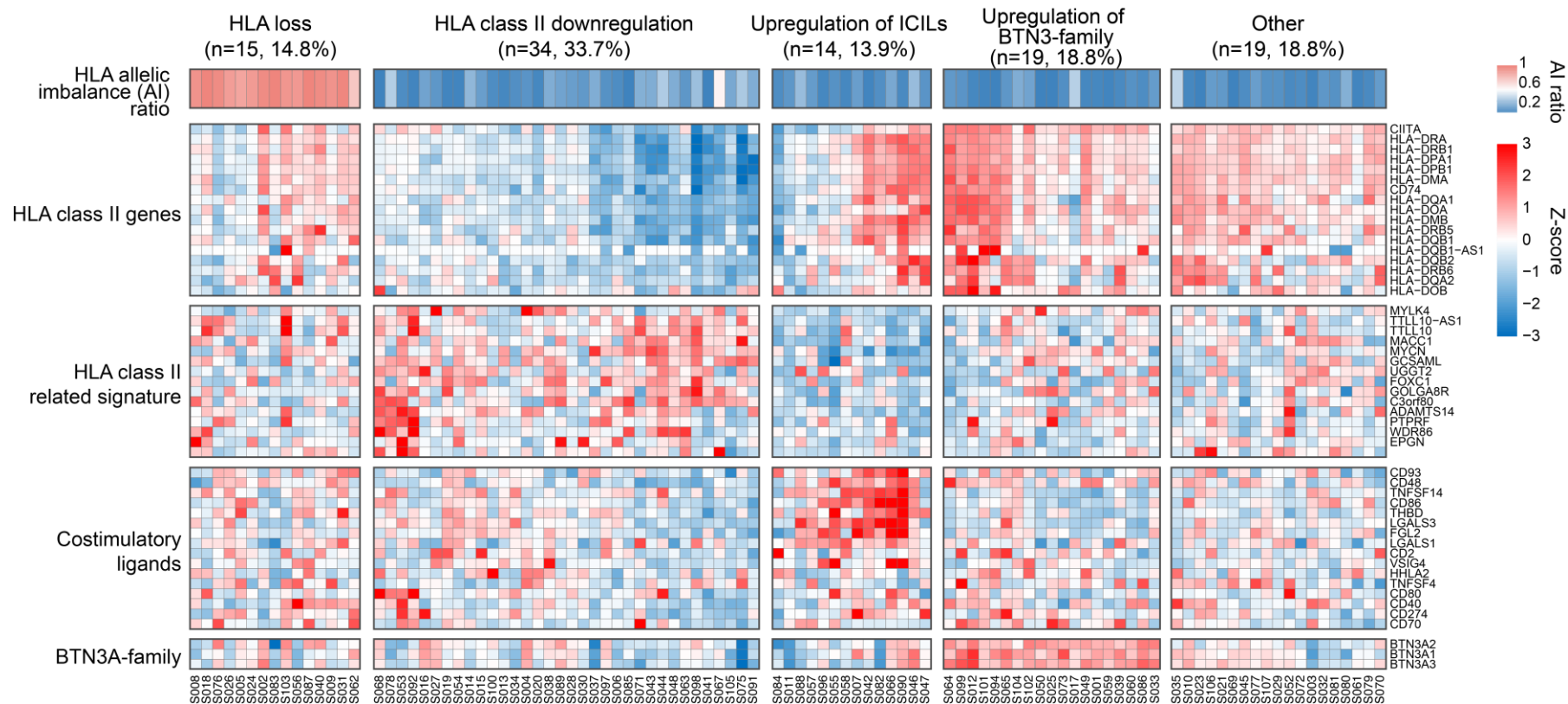
Defining Relapse Modalities from RNA-Seq Data



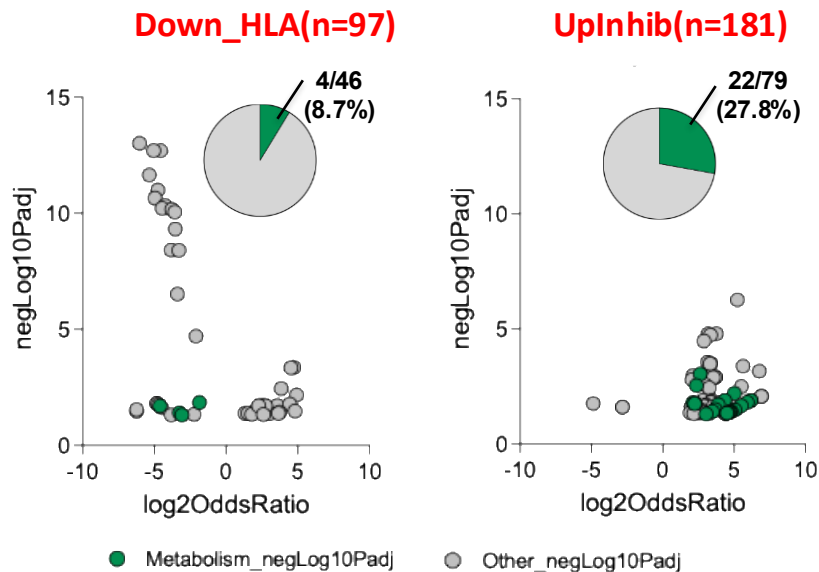
Co-Regulated Inhibitory Ligands Upon allo-HCT



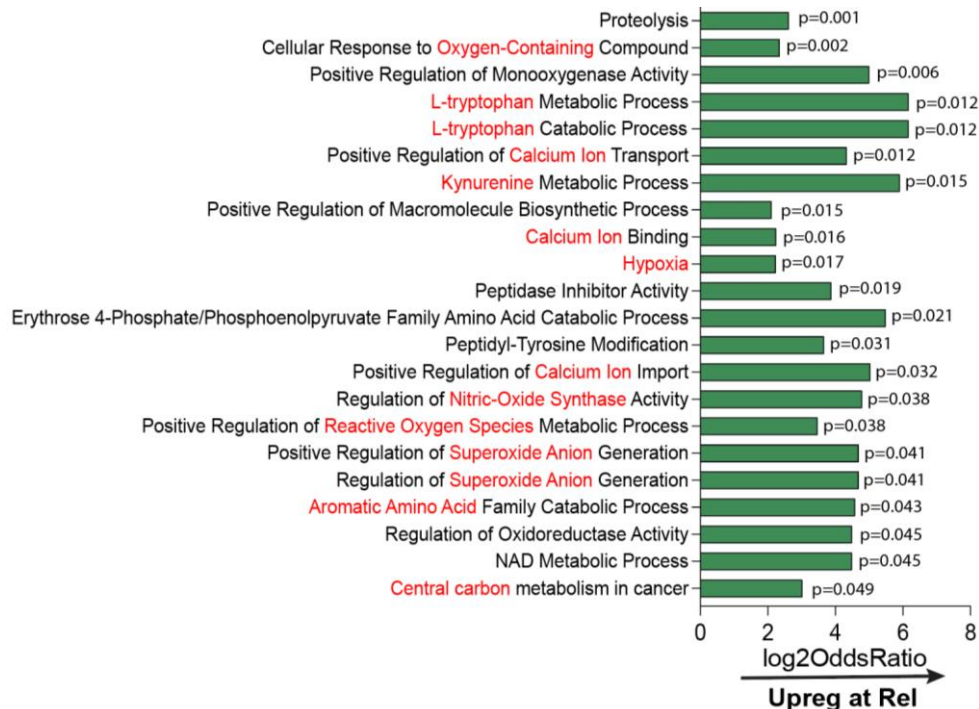
Defining Relapse Modalities from RNA-Seq Data



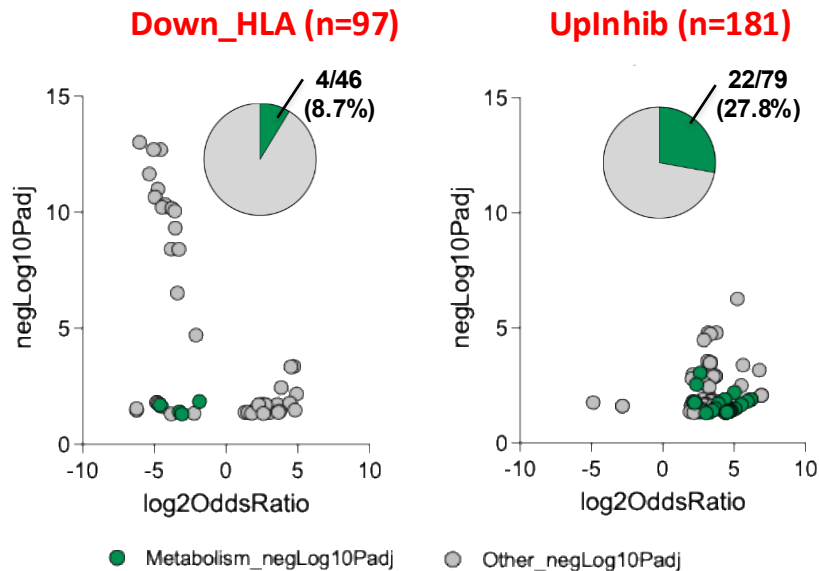
UpInhib Relapses are Characterized by Metabolic Changes



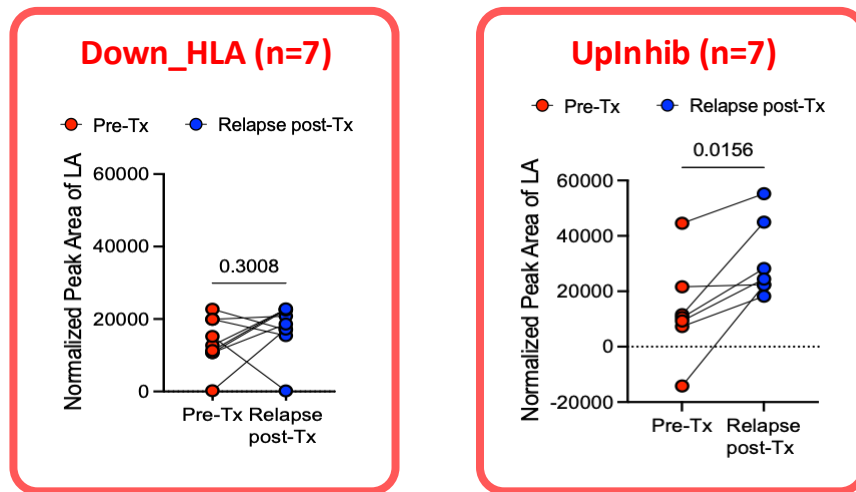
UpInhib Relapses



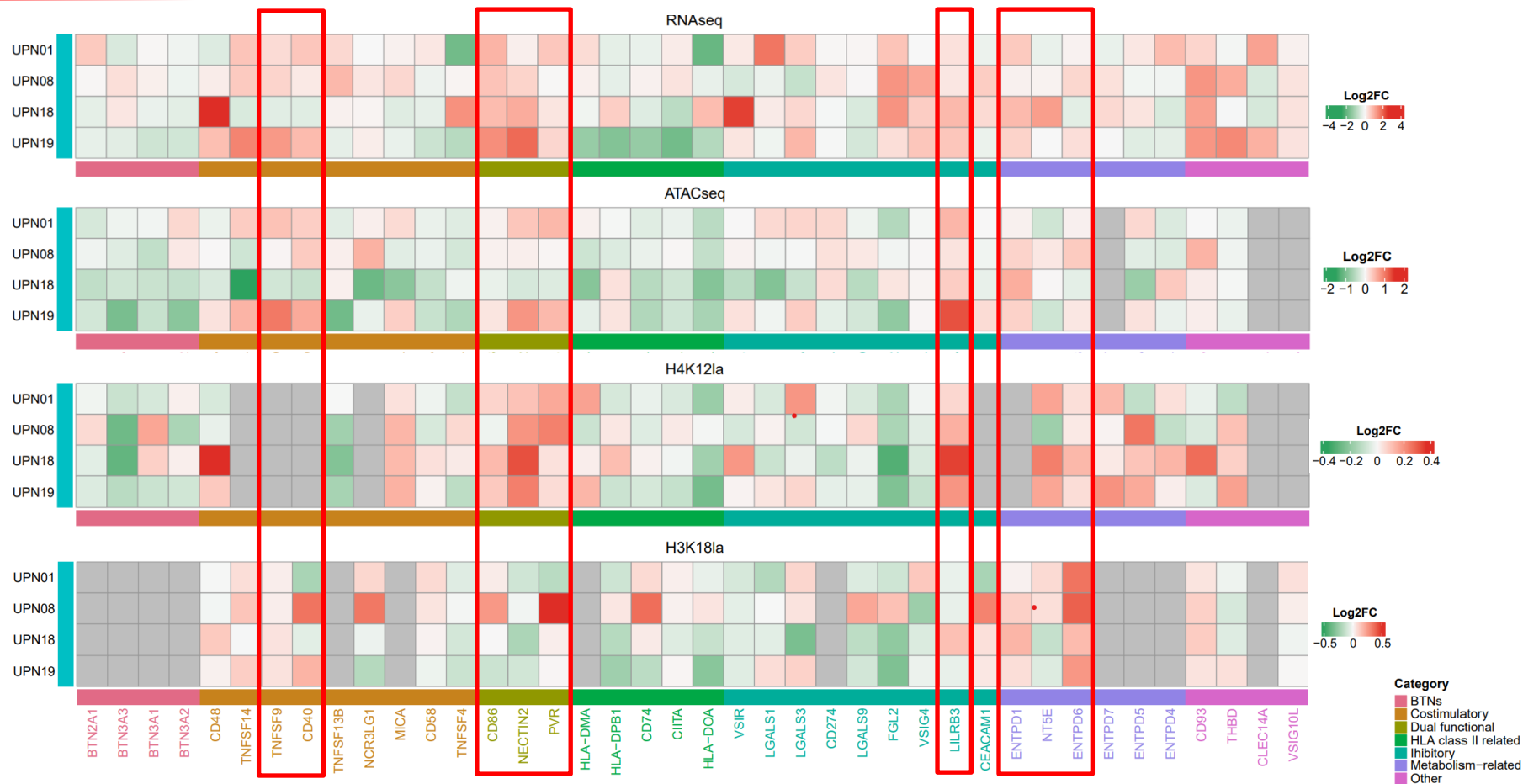
UpInhib Relapses are Characterized by Metabolic Changes



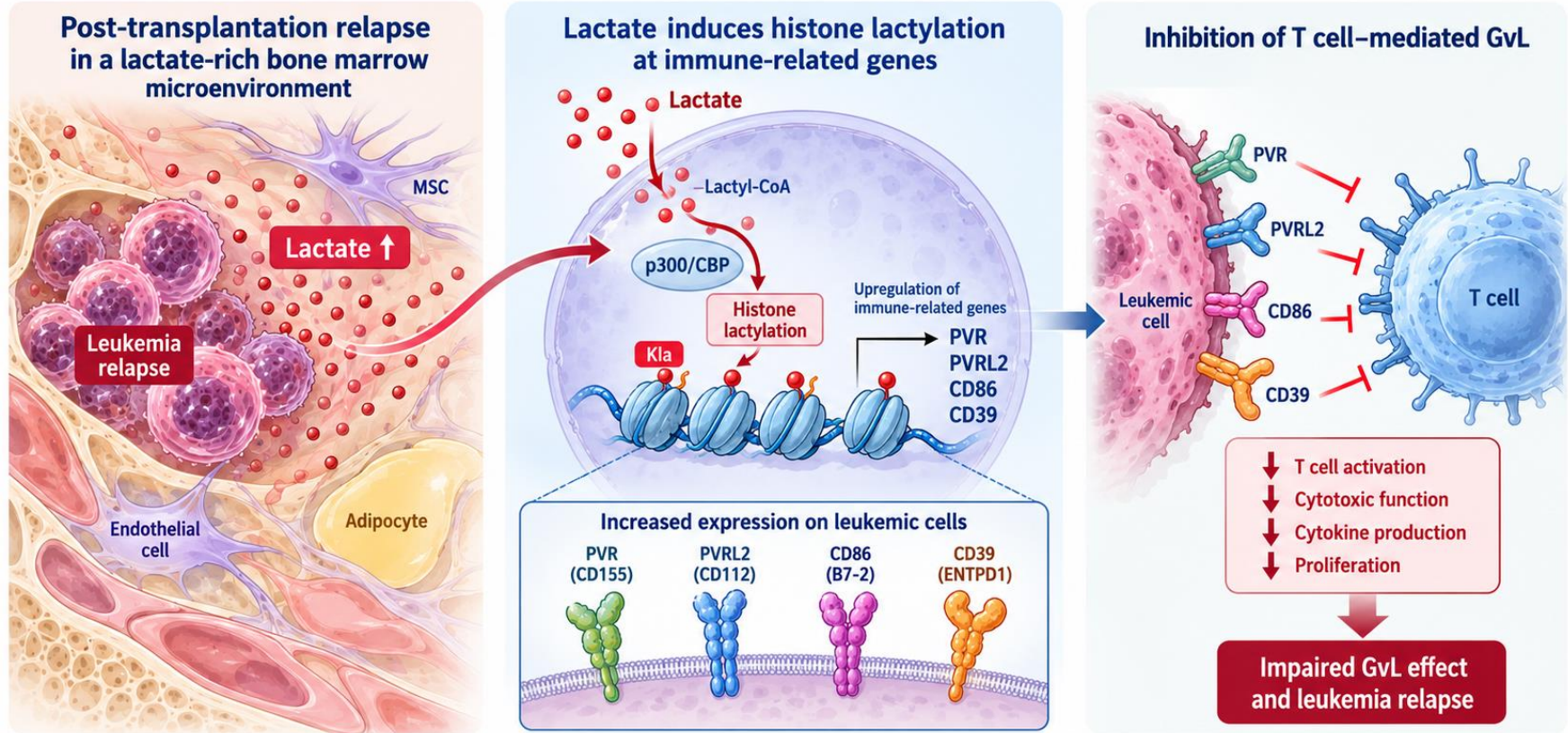
Lactic Acid



Histone Lactylation Regulates Expression of Inhibitory Ligands



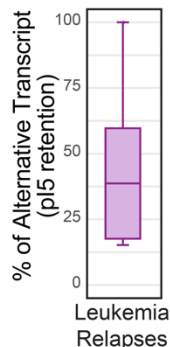
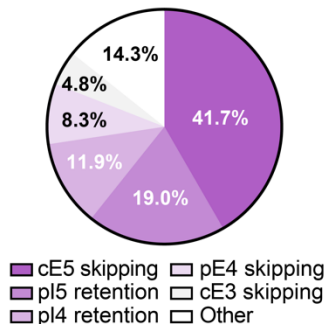
Histone Lactylation Regulates Expression of Inhibitory Ligands



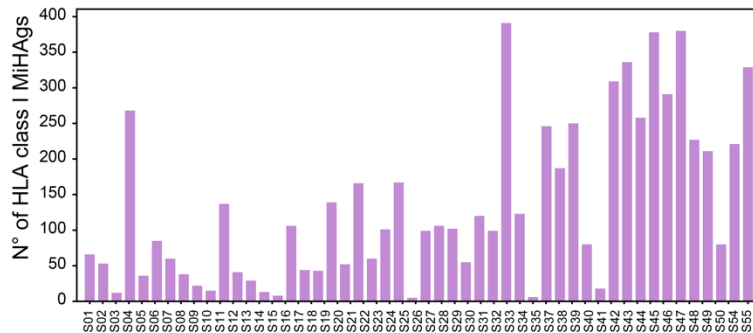
Other Immune Features Derived from the AML Fraction

HLA Alternative Splicing

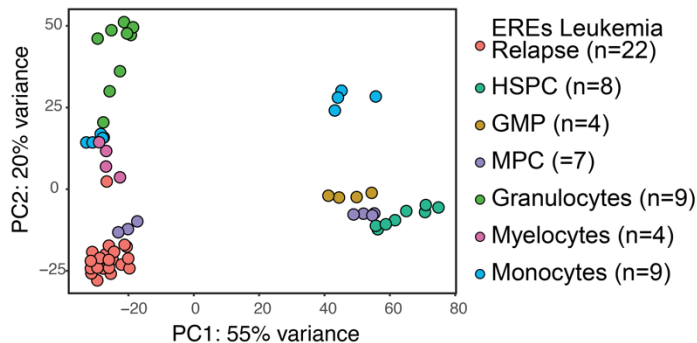
Observed in 39/50 (78%) of leukemia relapses



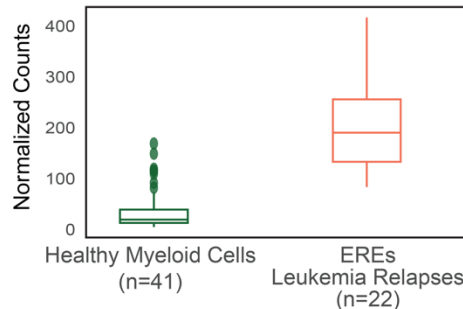
GvL MiHAGs



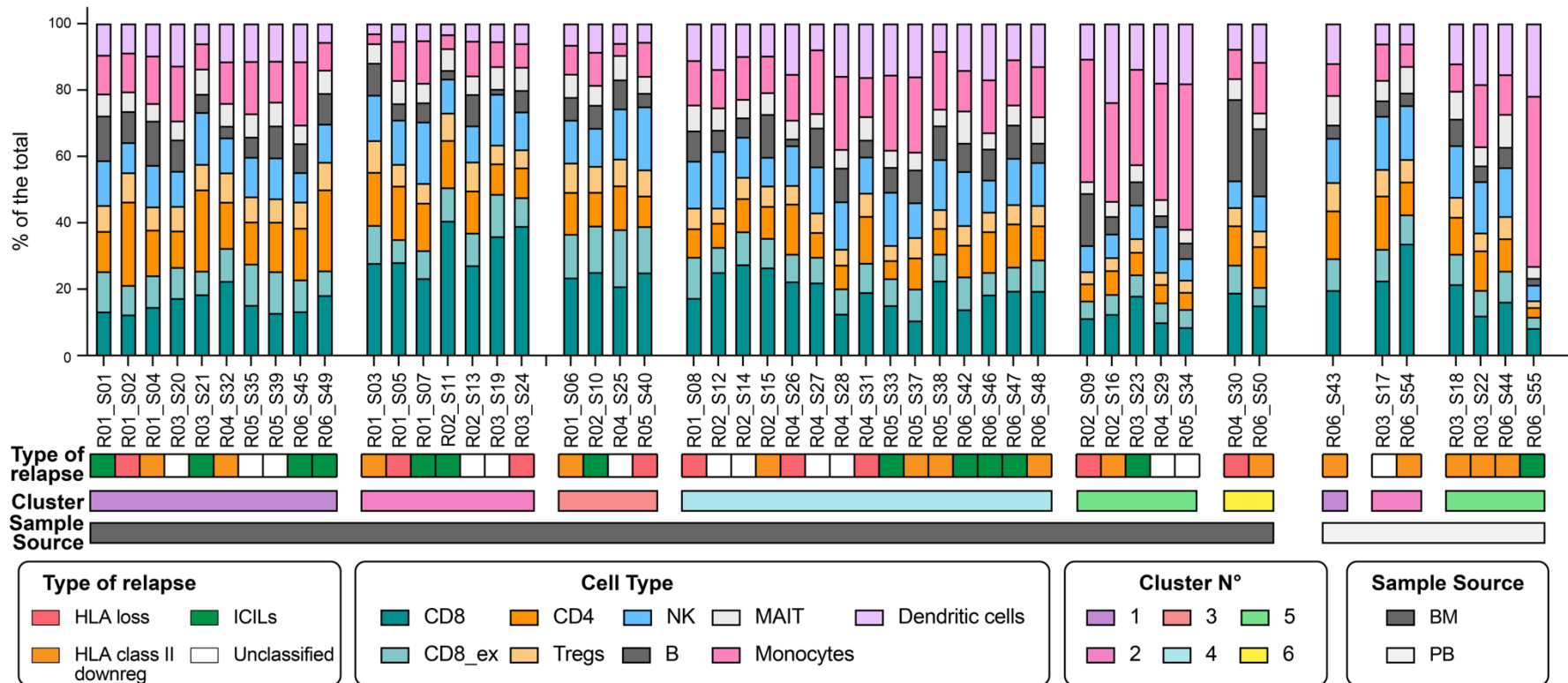
Leukemia Retroelements



Differentially expressed EREs between AML and healthy myeloid cells



Analyses on the TME Fraction



Extramedullary Relapses: Where the TME Matters Most?

ORBIT — periorbital cutaneous EM-AML

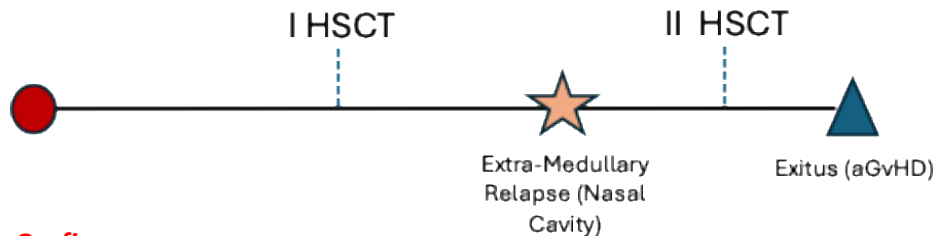
Clinical history - OSR-EMR008

Female – 46 yo at diagnosis

Diagnosis: sAML (previous Breast Cancer)

Normal Cytogenetics – FLT3-TKD mutation

Donor: MUD M - Isolated EM relapse (nasal cavity – orbit)



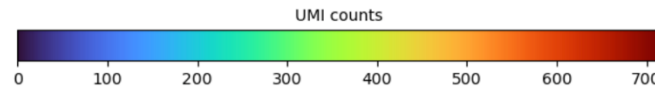
G. Orofino

ERA-NET PIXEL

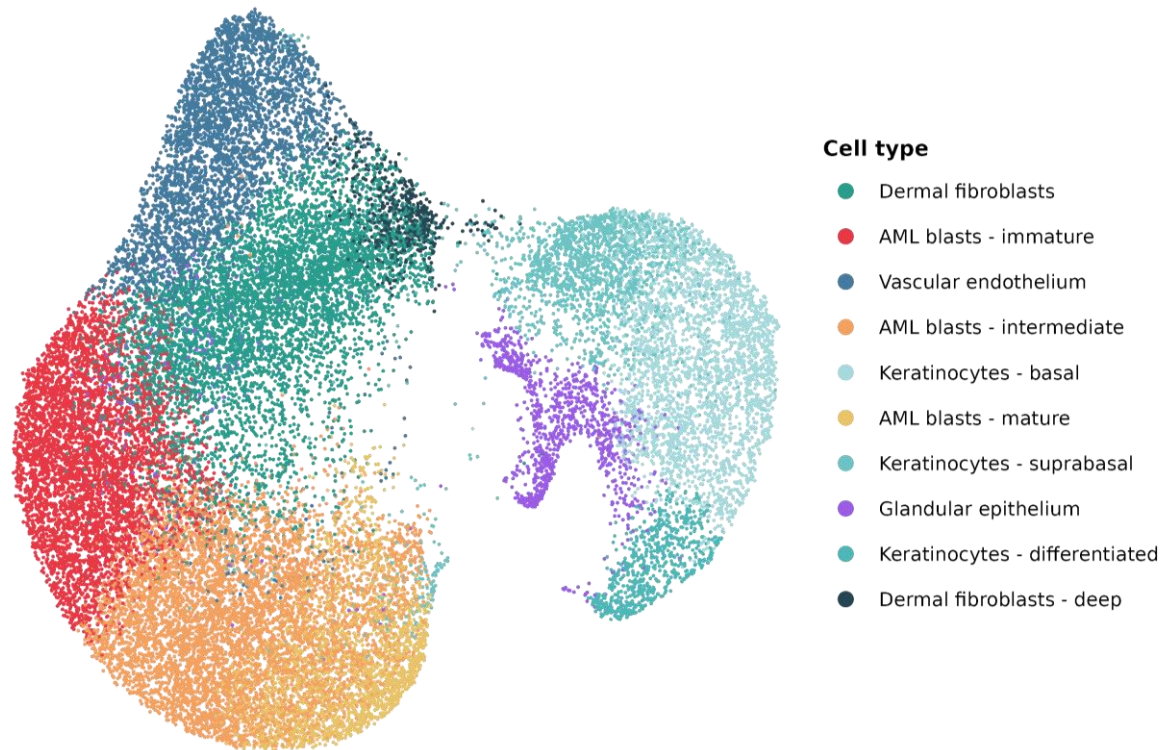
In collaboration with N. Cieri



135,004
analyzed
8- μ m bins
416 median
UMI / bin ·
368 median
genes / bin



Cellular Landscape in an Extramedullary Orbital Relapse

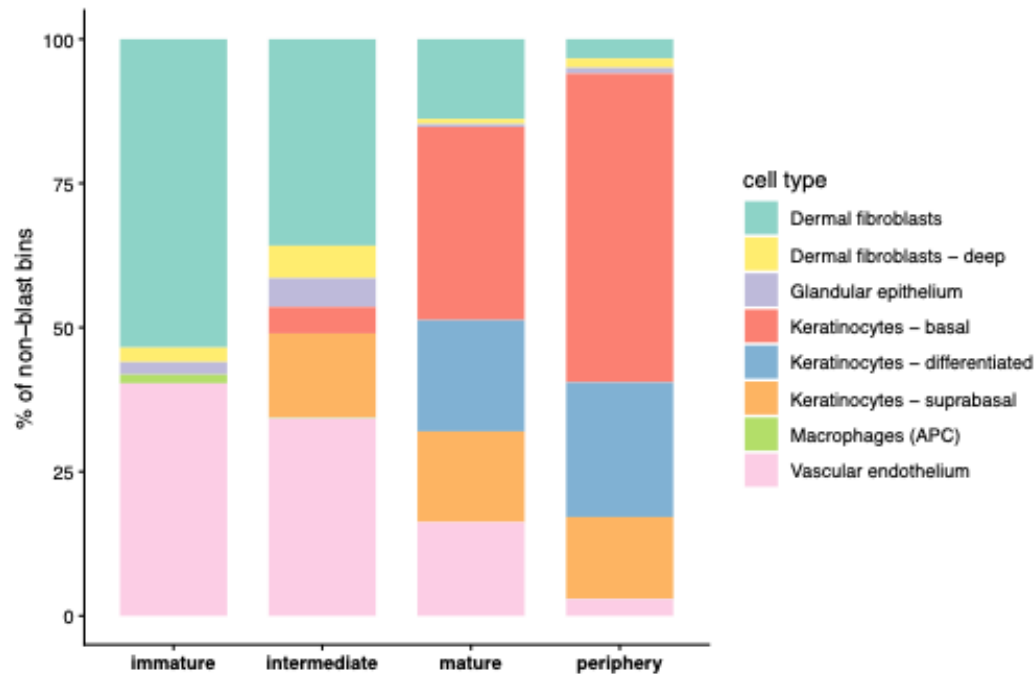


G. Orofino

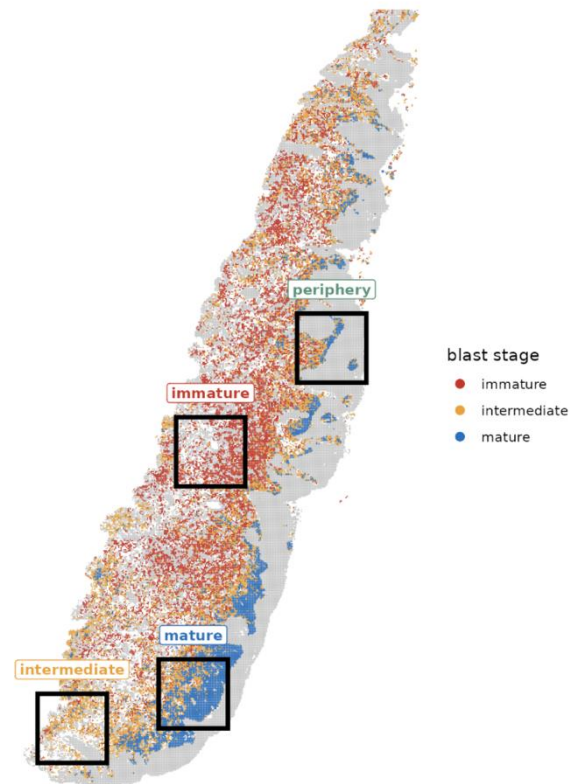
ERA-NET PIXEL

In collaboration with N. Cieri

Niche Composition Correlates with AML Maturation

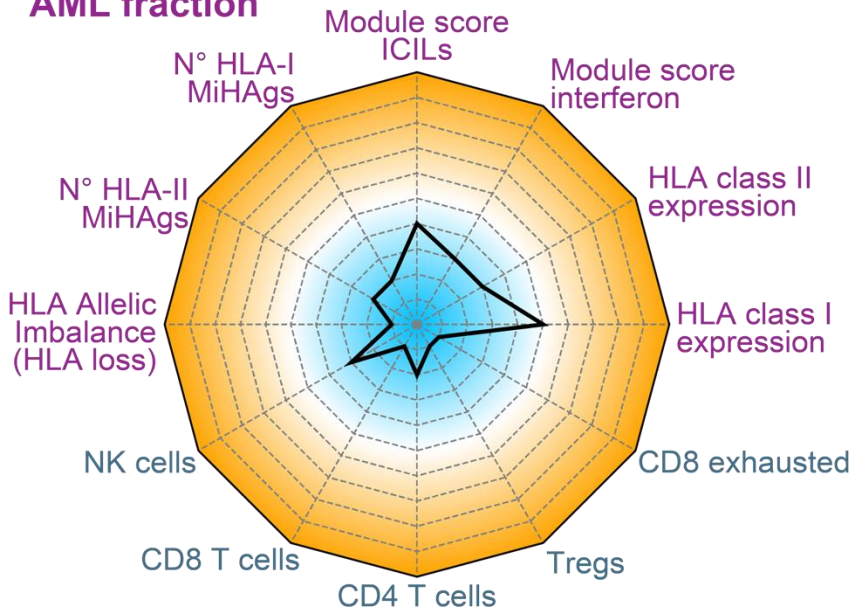


Selected regions on the maturation map



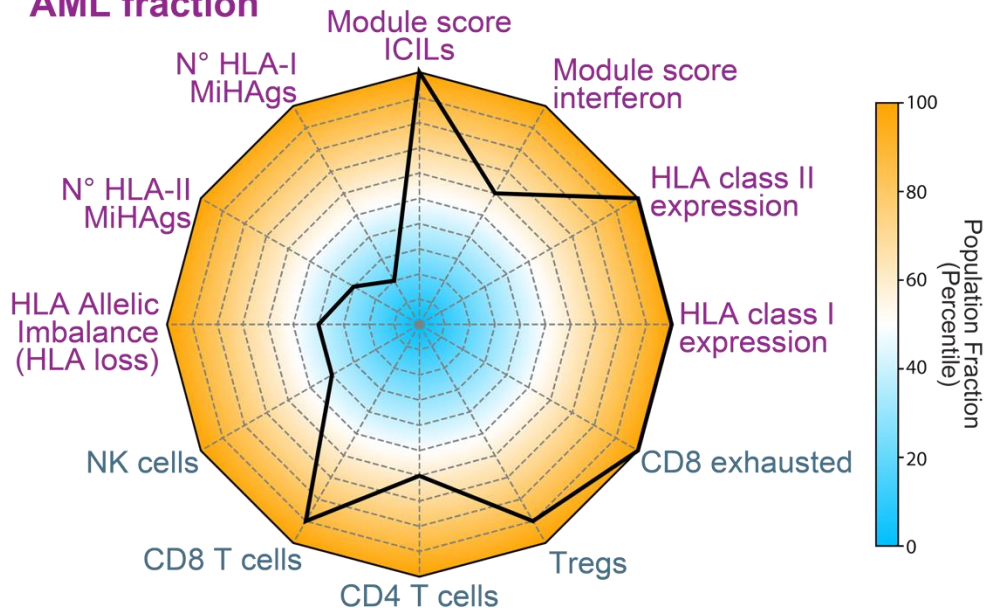
Integrating Data in Patient-Specific Relapsograms

AML fraction

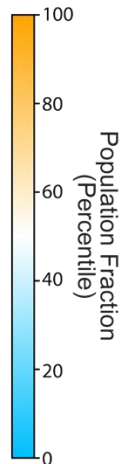


TME fraction

AML fraction



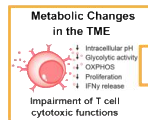
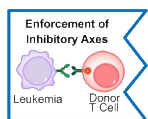
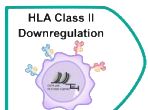
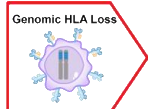
TME fraction



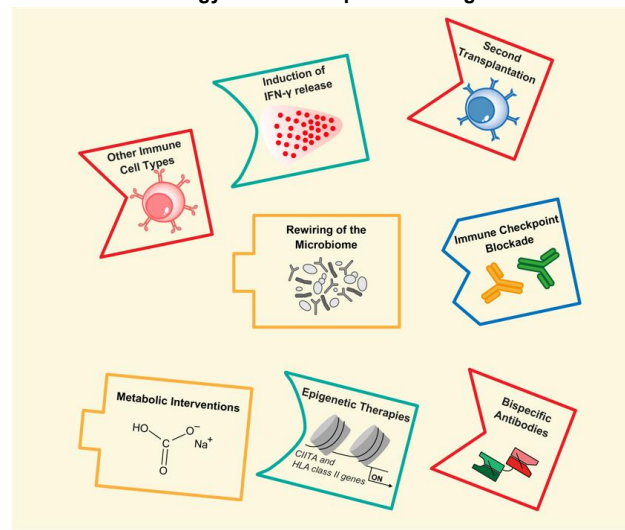
Our Final Goal: Tricking the Trickster

Precision Medicine Approaches To Counteract Leukemia Immune Escape After Allogeneic Hematopoietic Cell Transplantation (HCT)

Mechanisms of leukemia relapse after allogeneic HCT



Biology-driven therapeutic strategies



Conclusions: Leukemia relapse after allogeneic HCT can follow different patterns, often reflecting immune escape. Characterizing the mechanisms of relapse can provide a rationale for new therapies and help select the most appropriate treatment for each relapsing patient.

Tameni et al. DOI: 10.1182/*blood*.2023019962

 blood
Visual
Abstract

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**Blood Cancer
United**