4th Cuneo City ImmunoTherapy Conference (CCITC)

Immunotherapy in Hematological Malignancies 2024

CUNEO
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Spazio Incontri Fondazione CRC

Immune dysregulation and driver mutations in myeloid malignancies

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Organized by Prof. Massimo Massaia, SC Ematologia AO S.Croce e Carle, Cuneo - Italy and
Centro Interdipartimentale di Biotecnologie Molecolari "Guido Tarone" (MBC), Torino - Italy

Disclosures of Antonio Curti

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
AbbVie	x					x	x
Pfizer	x					x	x
Jazz Pharma						x	x
Menarini-Stemline						x	x
Servier						x	x
Novartis						x	x

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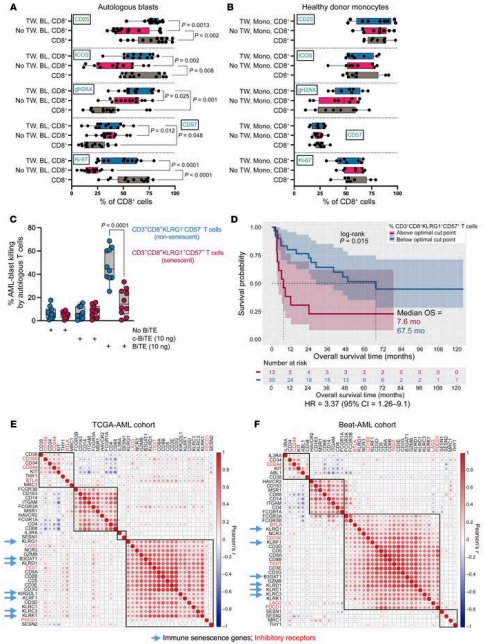
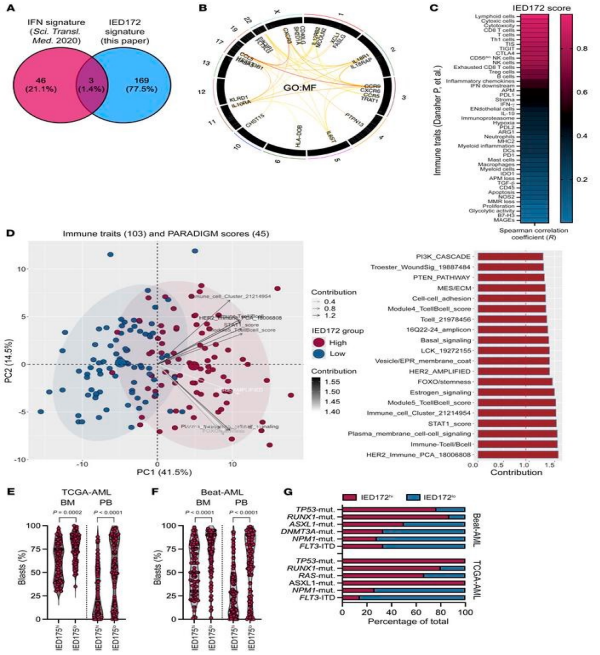
Markers of T cell exhaustion and senescence correlate with impaired T cell killing and poor clinical outcomes

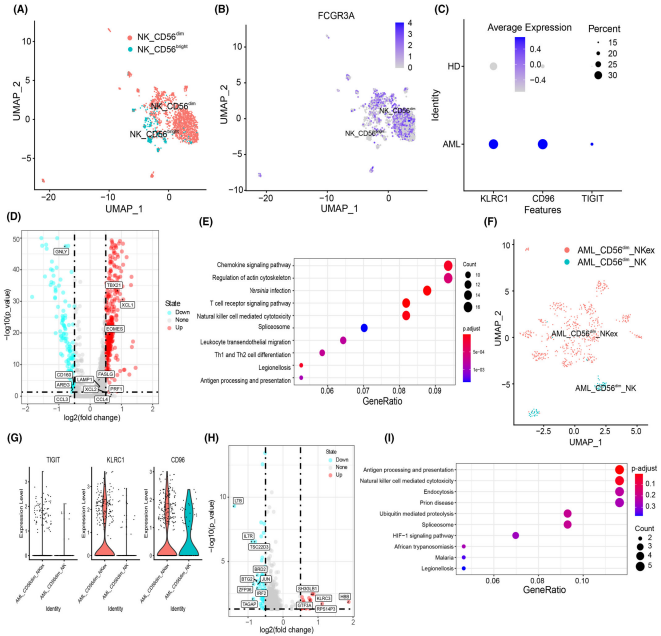


Rutella S et al, J Clin Invest, sept 13, 2022

Immune effector dysfunction correlates with immune infiltration and with adverse-risk molecular features



Single-cell RNA-seq reveals a microenvironment and an exhaustion state of NK cells in AML



Tumor-infiltrating NK cells display an exhaustion signature, as a consequence of triggered NK cytotoxicity.

This “activation-dependent exhaustion expression program” is very similar to the one reported for the induction of T-cell exhaustion

Zhang et al, Cancer Science, August 2023

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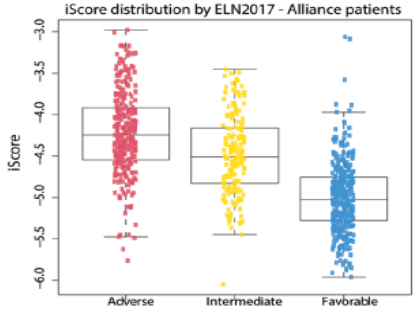
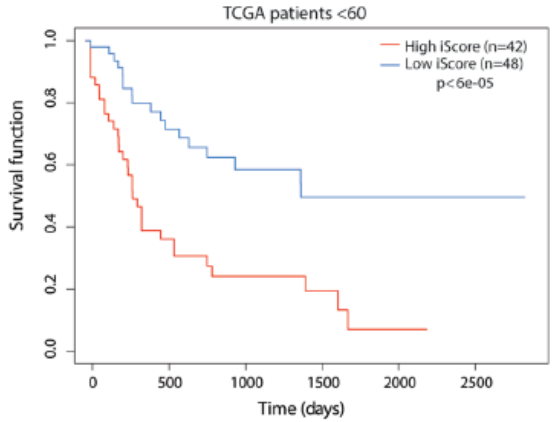
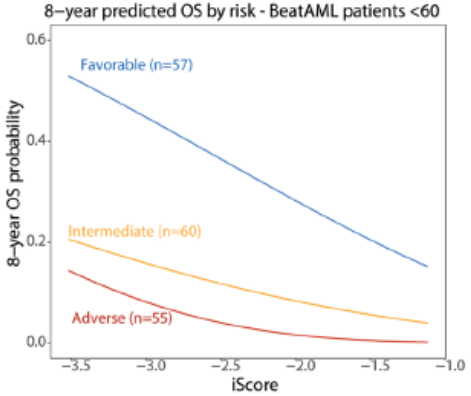
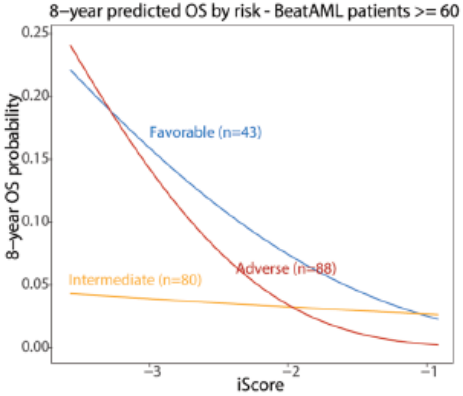


High inflammatory score is associated with adverse ELN risk group

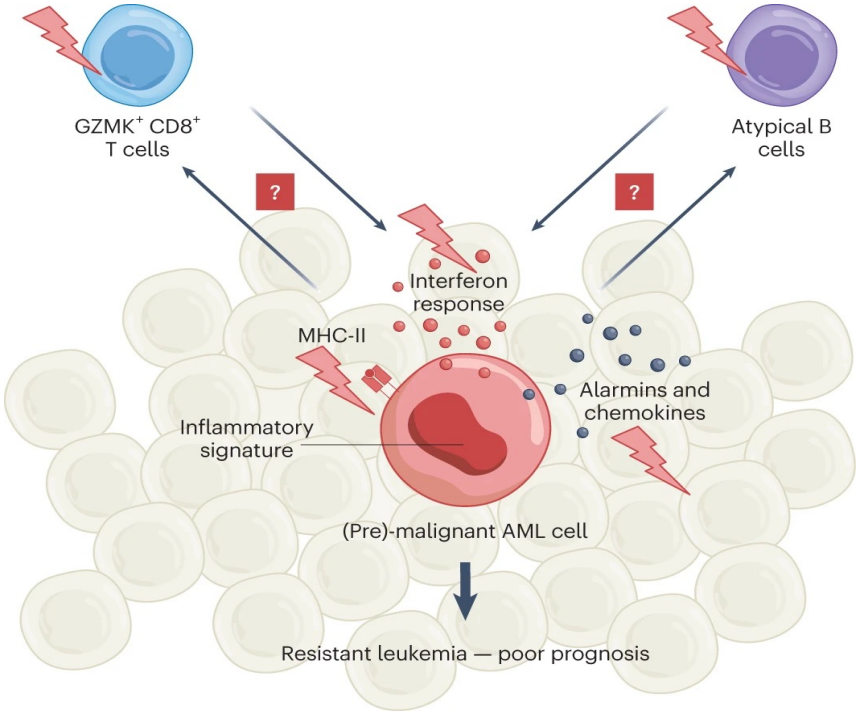
High inflammatory score prognostically stratifies AML patients





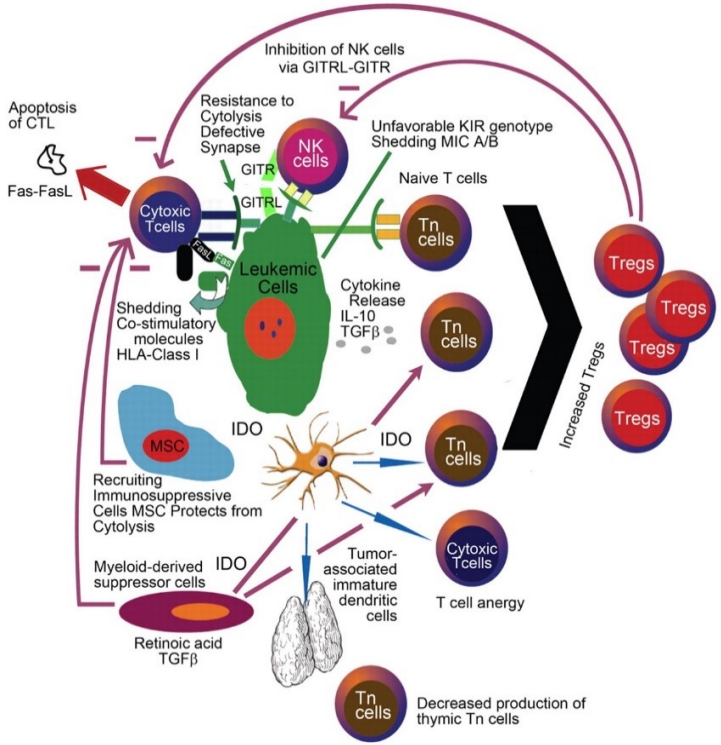


Lasry A et al, Nature Cancer, January 2023, 27-42



Atypical B cells and exhausted GZMK+ CD8 T cells are expanded in highly inflamed AML microenvironment

Asaf D. Yanir & Shai Izraeli Nature Cancer, 2023, pages 3–4

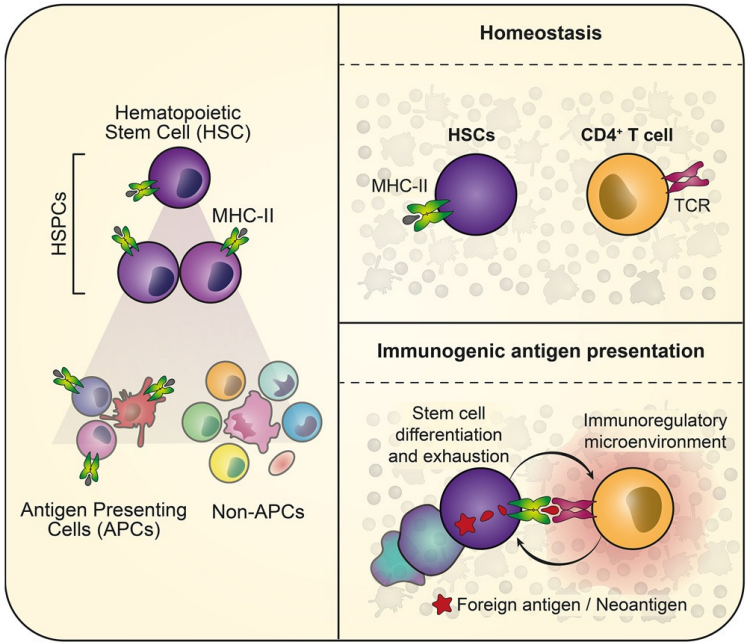


Tregs in AML: is it time for immunomodulation?

Although the notion that Tregs immunosuppression represents a crucial point in AML immune microenvironment, the mechanisms underlying Tregs induction are still poorly elucidated and largely unknown.

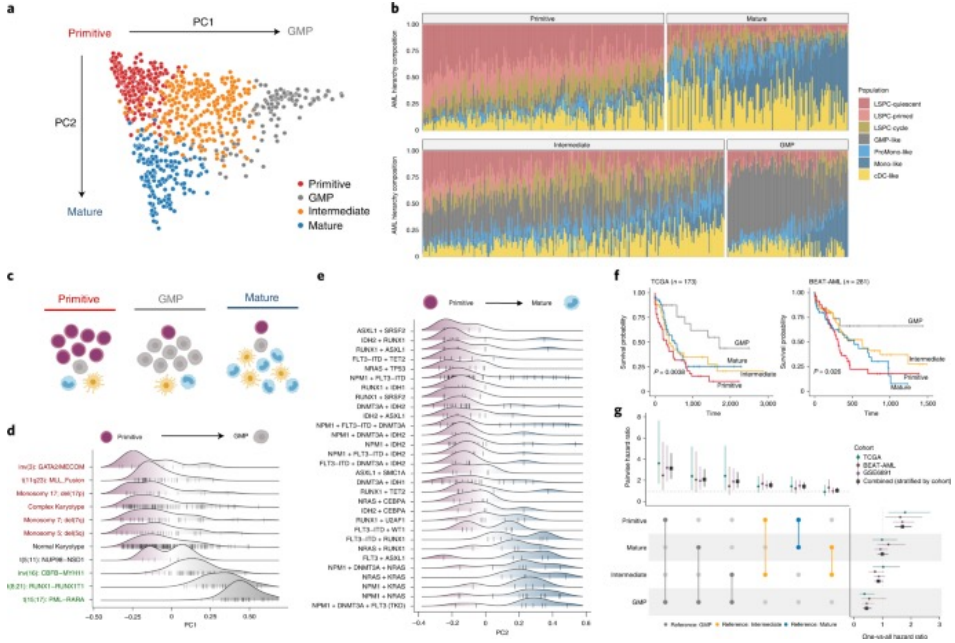
Ustun C et al. Blood 2011;118:5084-5095

Antigen presentation safeguards the integrity of the hematopoietic stem cell pool



- HSPCs constitutively present antigens via MHC-II
- Presentation of immunogenic antigens results in the activation of CD4⁺ T cells
- Antigen presentation causes differentiation and depletion of immunogenic HSPCs
- This prohibits the onset of HSC-derived leukemias presenting neoantigens via MHC-II
- CD4⁺ T cells activated by HSPCs confirmed that they acquired an immunoregulatory and anti-inflammatory phenotype

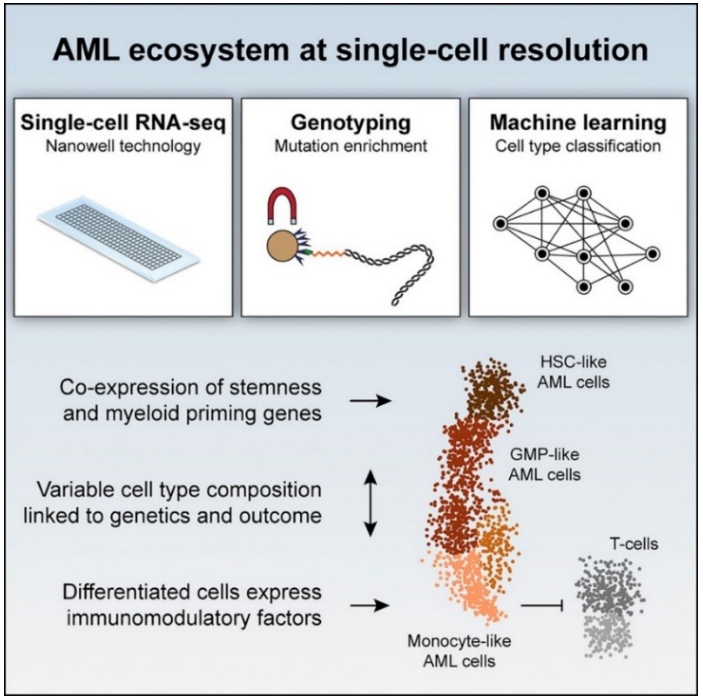
Hernandez-Malmierca et al, Cell Stem Cell, 29, 2022, Pages 760-775



Hierarchy composition is associated with AML genomics

- Leukemia hierarchy composition is associated with functional, genomic and clinical properties and converged into four classes, spanning Primitive, Mature, GMP and Intermediate.
- Variation in hierarchy composition along the Primitive versus GMP or Primitive versus Mature axes were associated with response to chemotherapy or drug sensitivity profiles of targeted therapies.

Zeng et al, Nat. Med. 2022



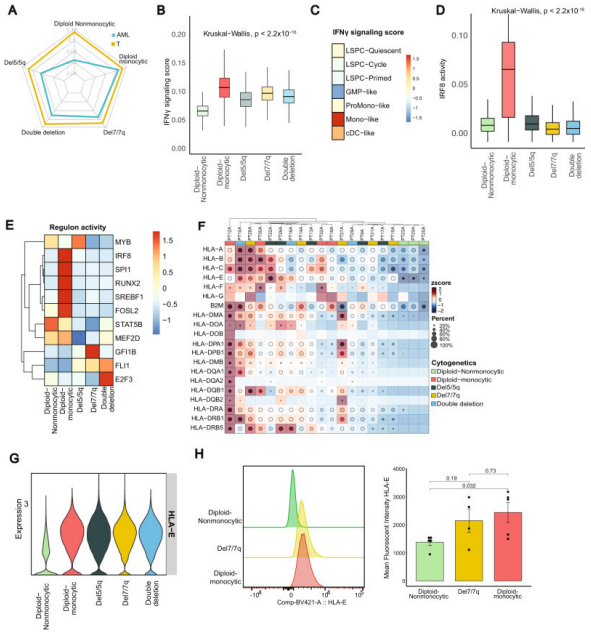
Single-Cell RNA-seq reveals AML hierarchies relevant to disease progression and immunity

Seminal studies using single cell sequencing have revealed the clonal diversity and phenotypic heterogeneity in AML with greater precision.

Cell ontogeny and function of leukemic cells may impact T cell responses, as single-cell sequencing revealed that monocytic AML cells are associated with more suppressive T cell landscapes.

Van Galen P, Cell, Volume 176, 2019, Pages 1265-1281

Comprehensive characterization of IFNγ signaling in AML reveals prognostic and therapeutic strategies



IFNγ signaling in AML blasts is dependent on phenotypic and cytogenetic groups.

- AML cells in patients with diploid monocytic AML had the highest expression of IFNγ signaling scores, correlating with higher expression of HLA-E, a non-classical class 1 HLA with regulatory functions
- Among nondiploid cytogenetic groups, IFNγ signaling activation was highest among those with del7/7q

Wang B. et al, Nat Commun. 2024; 15: 1821.

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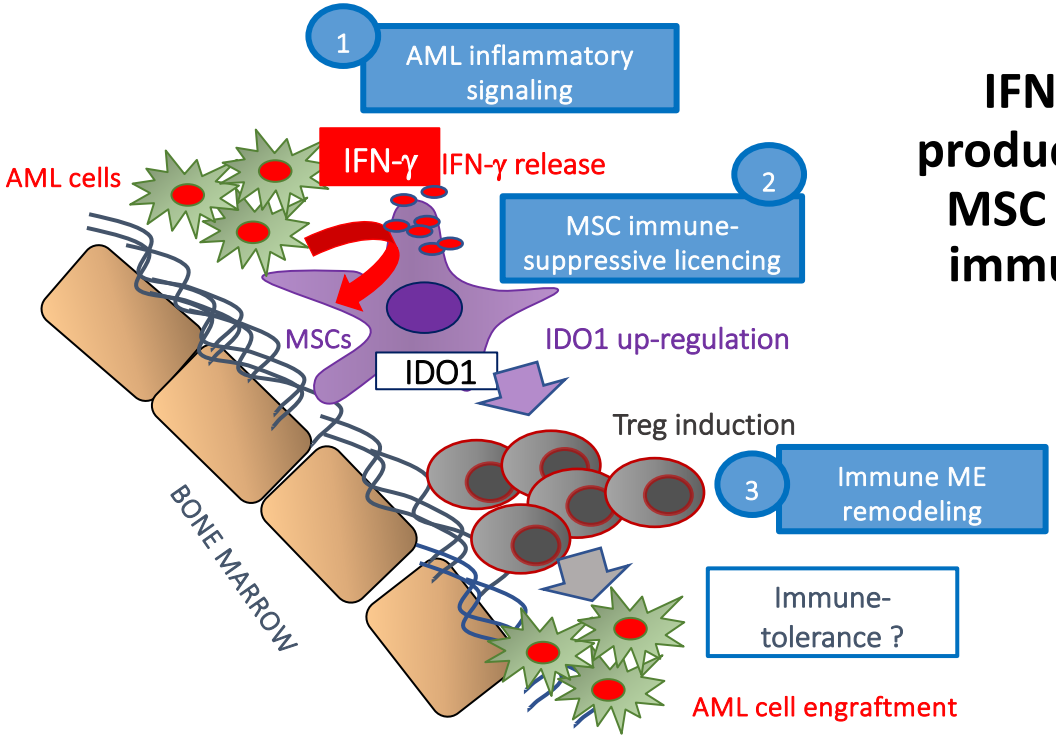
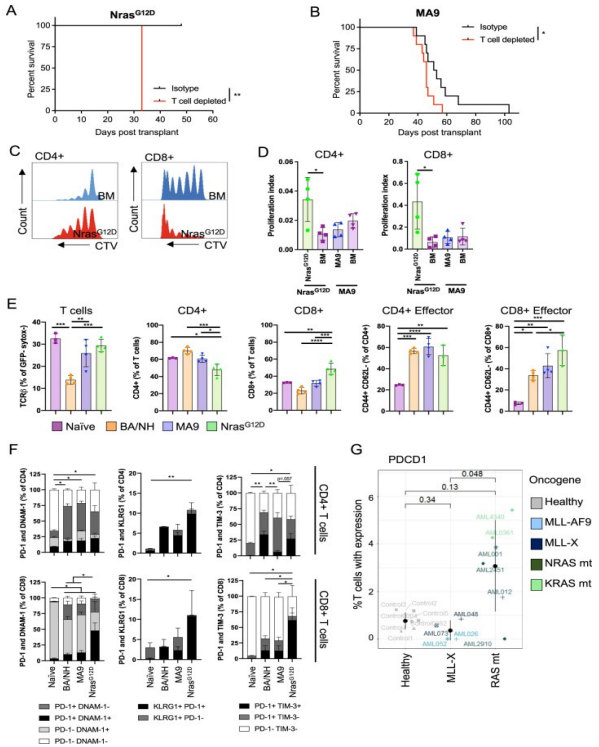


IFN-γ-dependent signals produced by AML cells modify MSC functions and favor an immune-modulating milieu

Corradi et al, Clin Cancer Res. 2022 Jul 15;28(14):3141-3155



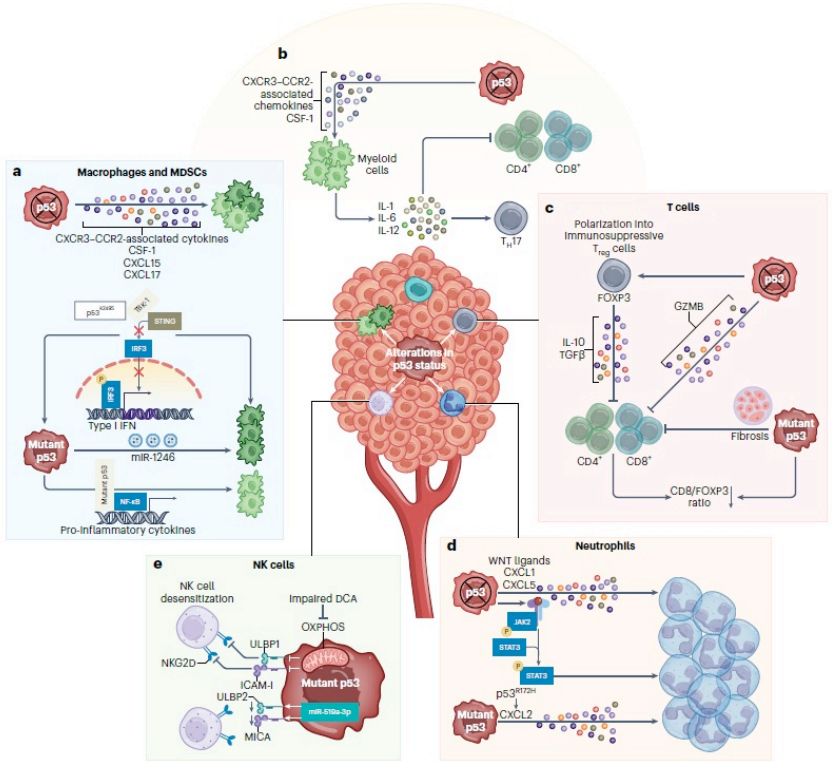
Austin RJ et al, Nat Commun. 2023; 14: 2155.

Oncogene specificity influences the type of immune response to AML cells

$Nras^{G12D}$ recipients having a greater frequency of CD8+ T effector memory compared to recipients harbouring other mutations

Co-expression of PD-1 and TIM-3, indicating a T cell exhaustion phenotype, was increased on CD4+ and CD8+ T cells from $Nras^{G12D}$ recipients

These data indicate expansion and dysfunction of the effector T cell compartment as a distinguishing feature of the immune microenvironment of immunogenic AML

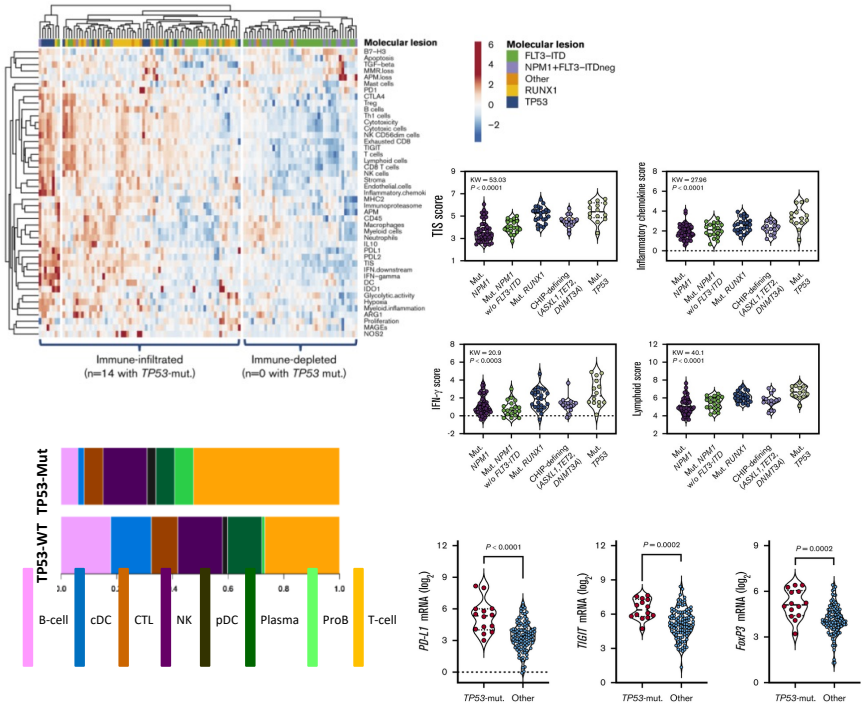


Alterations of tumor p53 status modulate the tumor immune microenvironment

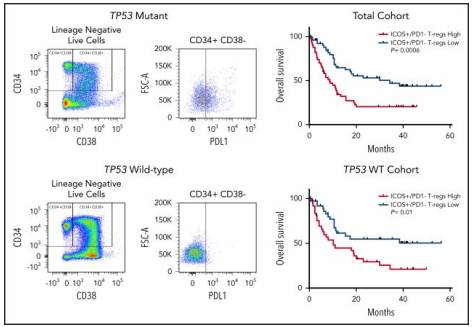
1) T cells: effects on differentiation and functions
2) NK cells: desensitization and impaired functionality
3) Neutrophils: pro-inflammatory effects through recruiting cytokines and chemokines
4) Macrophages and MDSCs; promotion of macrophage immunosuppressive polarization and cell expansion

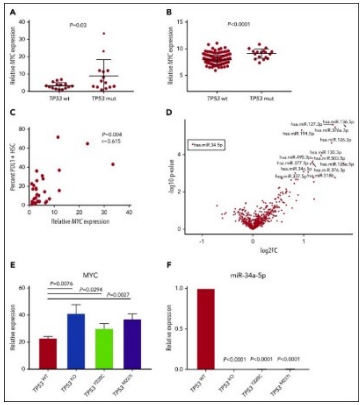
Efe G et al, Nat Cancer. 2024 Jul;5(7):983-995

*TP53*mut AML patients show an inflammatory and immunosuppressive microenvironment



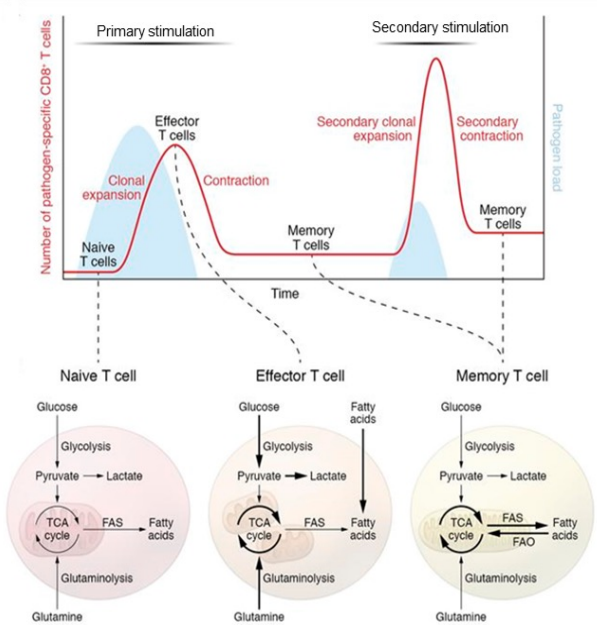
Vadakekolathu J . Blood Adv. 2020





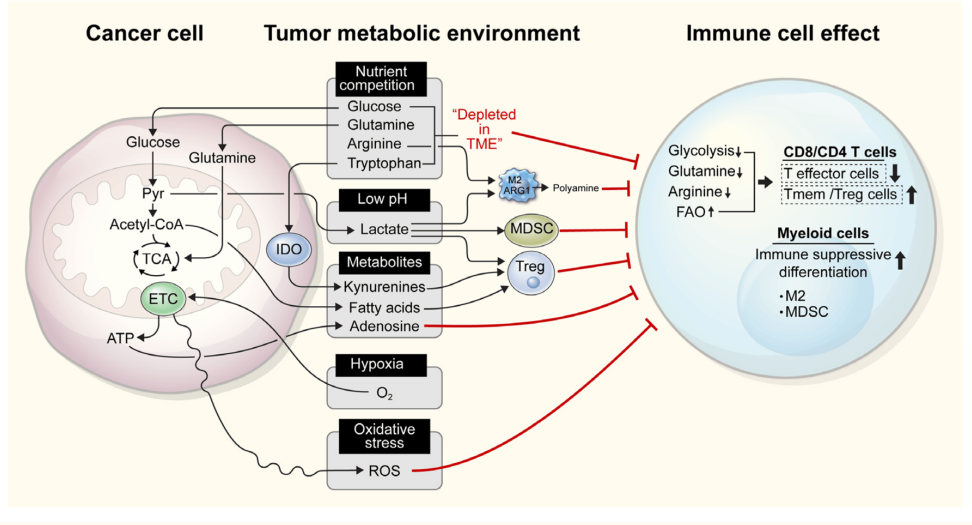
Sallman D et al,
Blood. 2020
10;136(24):2812-2823

Bioenergetic metabolism regulates T cell plasticity



Corrado M J Clin Invest. 2022

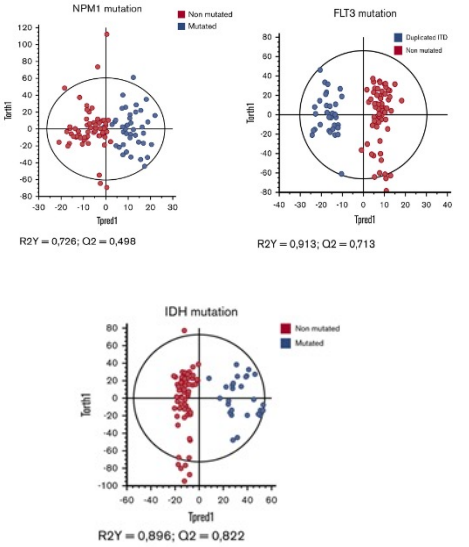
The altered metabolic activity of cancer cells affects the energetic rewiring of immune cells



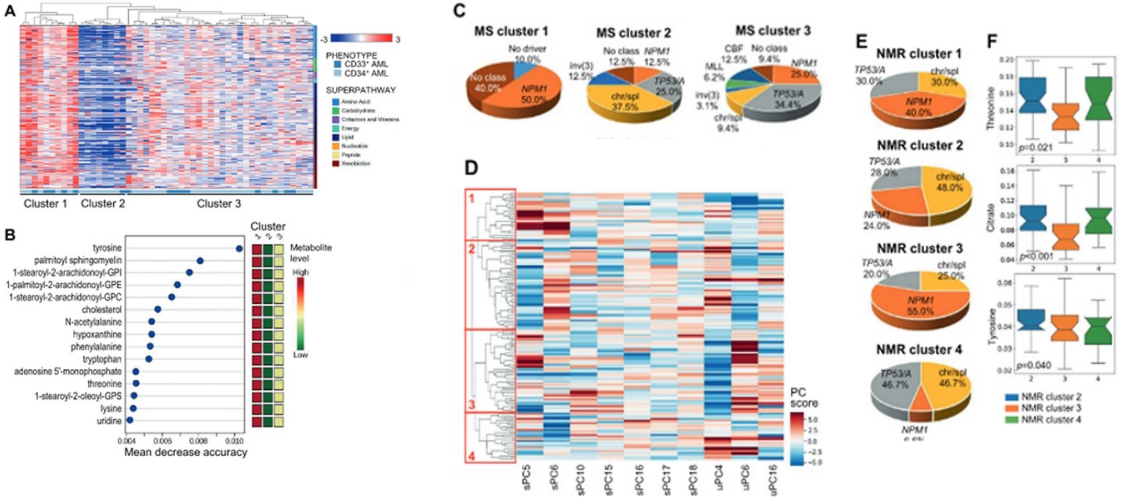
Sung JY Cells 2022

Different metabolic pathways could be activated in leukemic cells according to their genomics

Genome induced-metabolic phenotype



Intracellular and biofluid metabolomics show association with AML molecular classification



Lo Presti C, Blood Adv 2021; Simonetti G et al Leukemia. 2021

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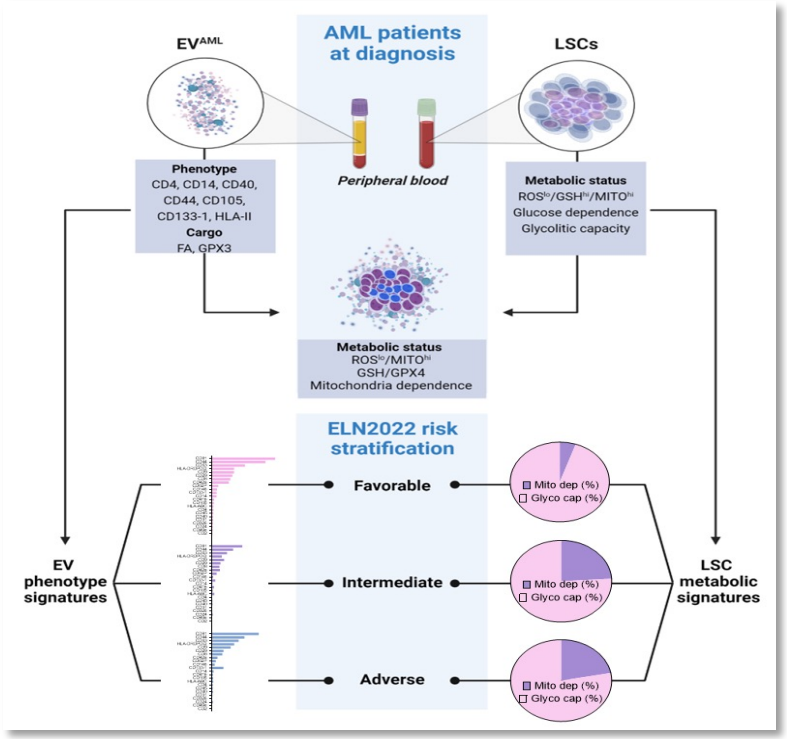
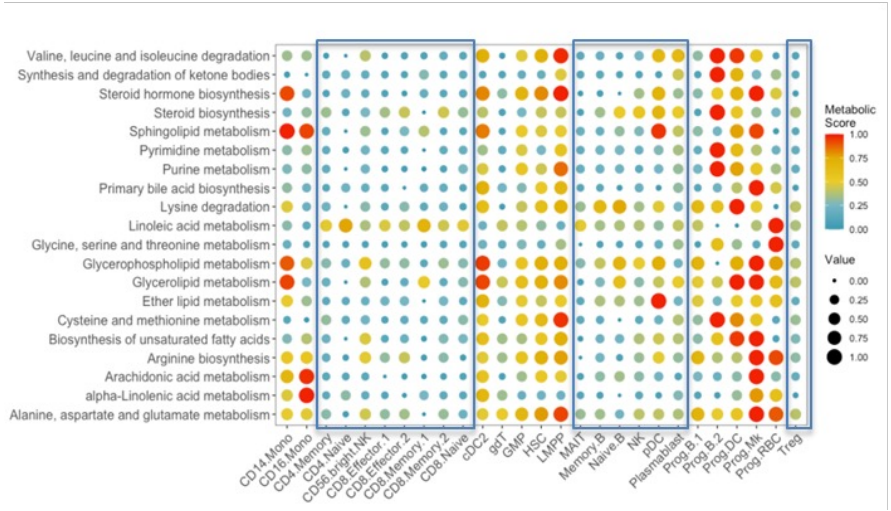


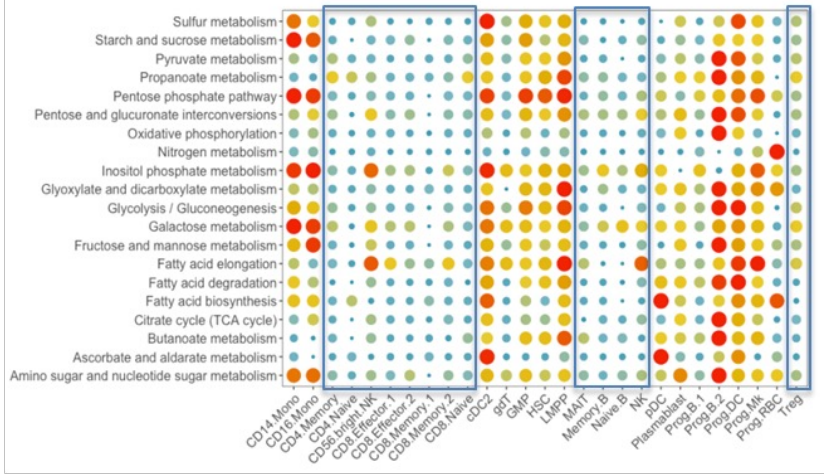
Parallel single-cell metabolic analysis and extracellular vesicle profiling reveal novel vulnerabilities with prognostic significance in AML

- ✓ AML CD34+ cells displayed low ROS levels with both high glutathione (GSH) levels and mitochondrial functionality
- ✓ AML CD34+ cells at diagnosis are highly dependent on glucose oxidation (contrary to immune cell subsets) and prone to exploit glycolysis for energy.
- ✓ The phenotype of circulating EVs from AML patients shows high expression for stem cell markers such as CD44 and CD133-1
- ✓ EVAML partially modulates the redox metabolism of CD34+ LSC-like cells through GSH/GPX4 axis
- ✓ Quantitative lipidomic analysis of EVs may support risk stratification for AML
- ✓ EVAML improve the engraftment of human cell line MOLM-13

Forte et al, under revision

The lymphoid compartment is “metabolically off" in TP53 mutant AML patients



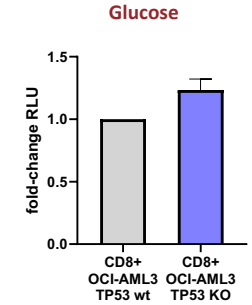


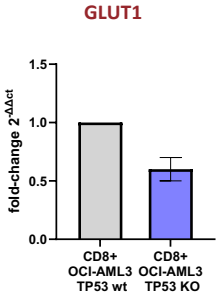
Salvestrini et al. manuscript in preparation.

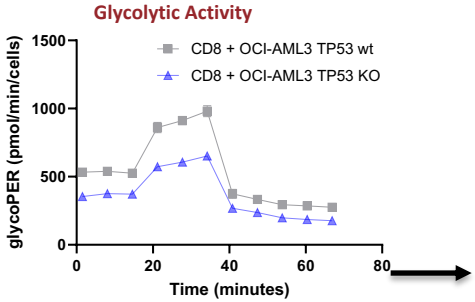
TP53 KO AML cells induce metabolic reprogramming of CD8 cells leading to reduced activation and promotion of an exhausted phenotype

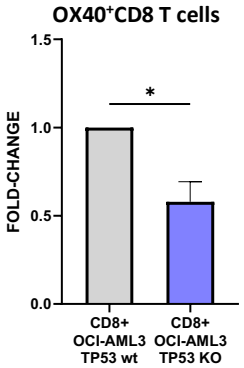
Microenvironment

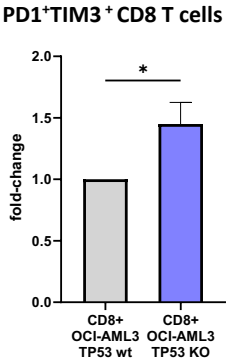
CD8 T cells

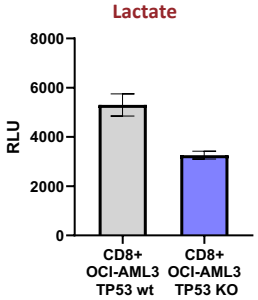


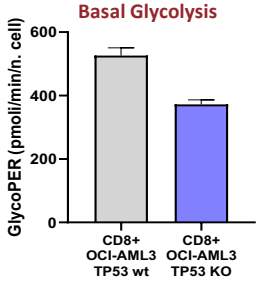


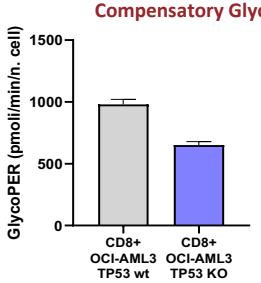








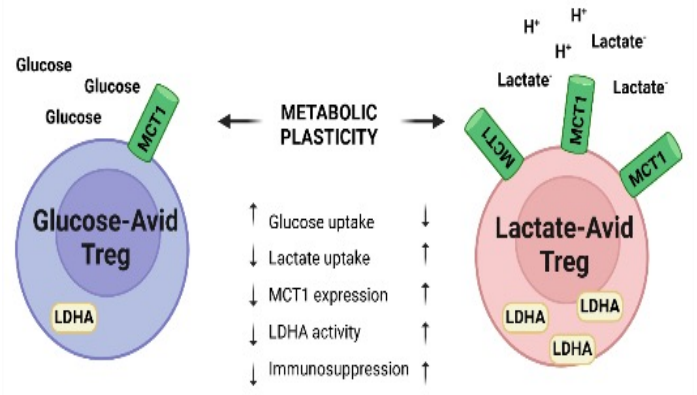


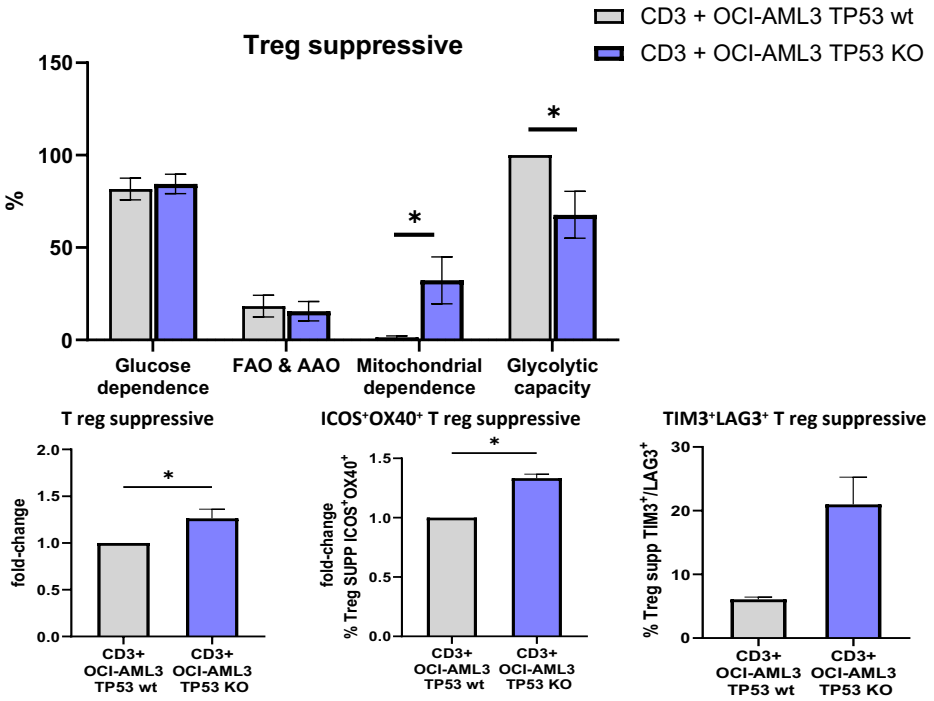


Salvestrini et al. manuscript in preparation.

Unpublished data

TP53 KO AML cells induce a metabolic reprogramming of Treg cells promoting a more immunosuppressive phenotype

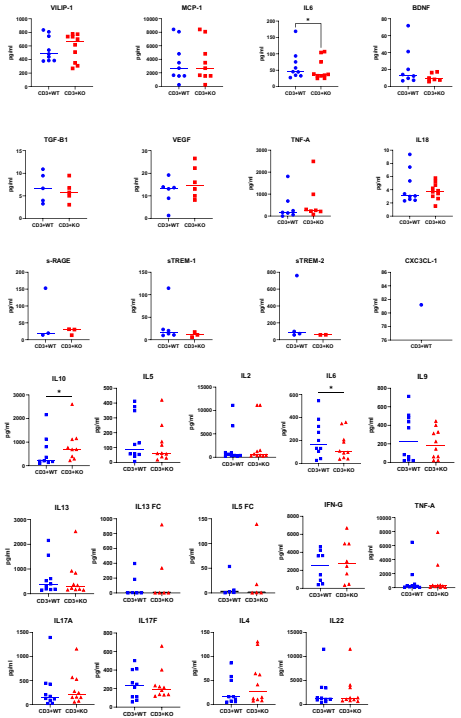


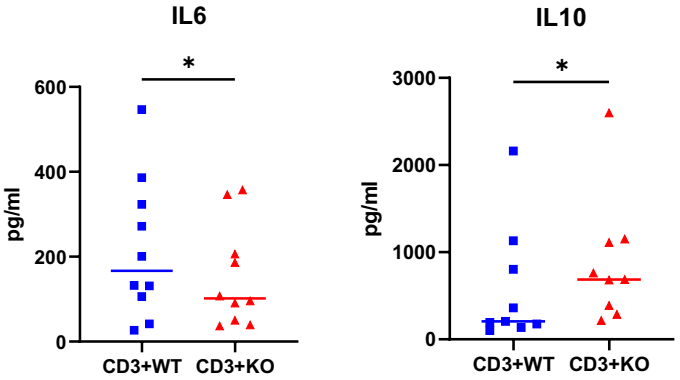


Salvestrini et al. manuscript in preparation.

Unpublished data

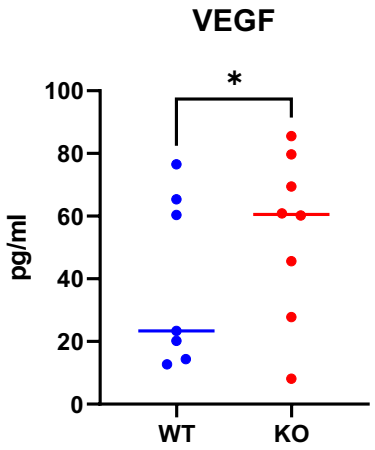
Cytokines secreted by T cells co-cultured with TP53 KO AML cells support inflammation and tolerance

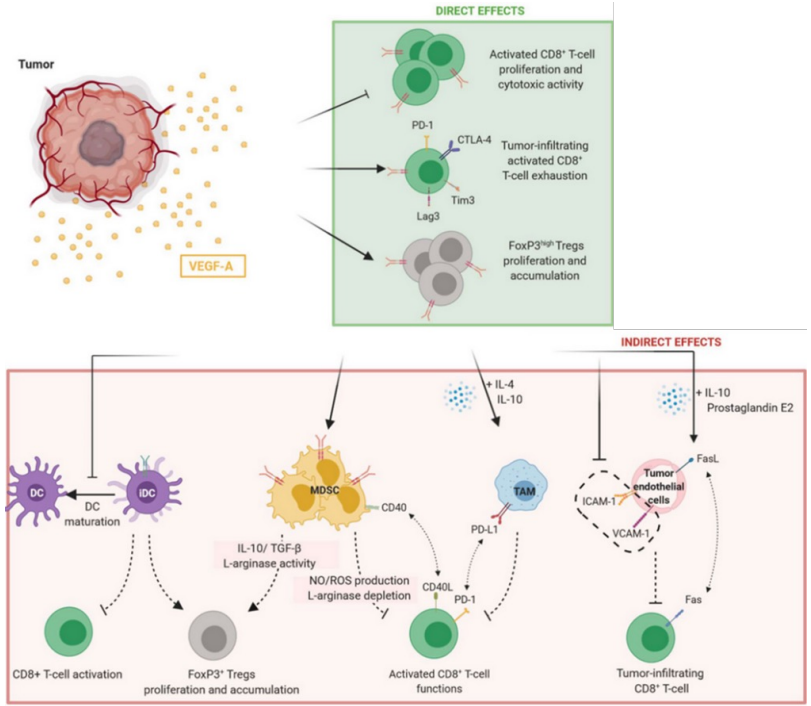




Salvestrini et al. manuscript in preparation.

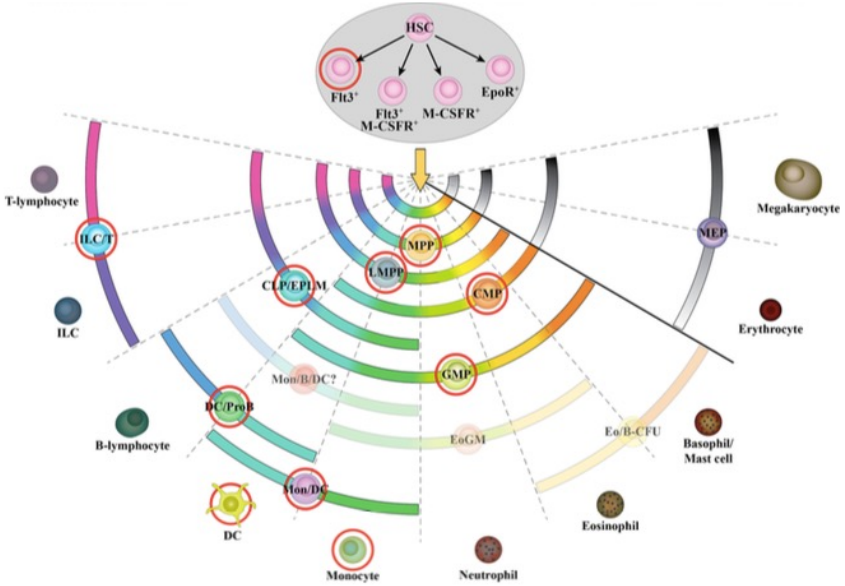
TP53 loss results in increased VEGF secretion in AML cell line





Salvestrini et al. Manuscript in preparation.

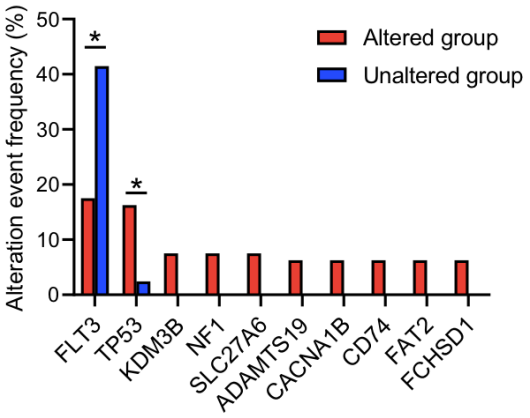
FLT3-FL signaling in normal hematopoieisis



Panagiotis T. et al, Int. J. Mol. Sci. 2017, 18, 1115

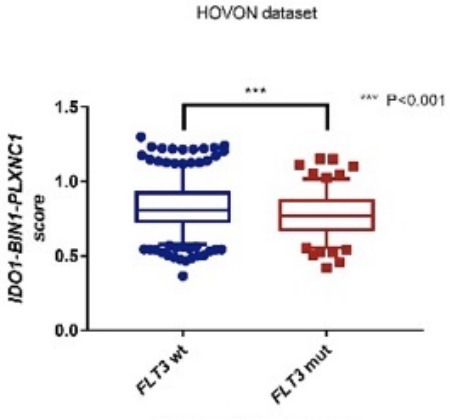
FLT3 mutational status frequently associates with immune dysregulation pathways

Correlation between DEGs in IFNG^{high} vs IFNG^{low} cases and *FLT3* mutational status (P*<0.001)**

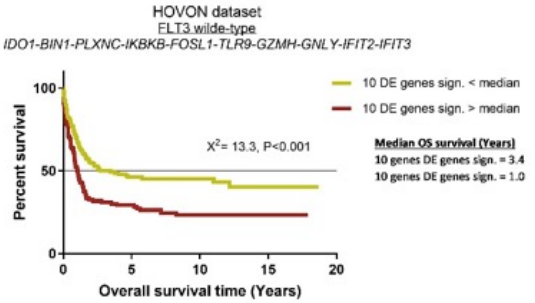


Corradi G et al. Clin Cancer Res, March 29, 2022

Score values significantly different according to FLT3 mutational status



Among FLT3 wt: the score remained statistically significant

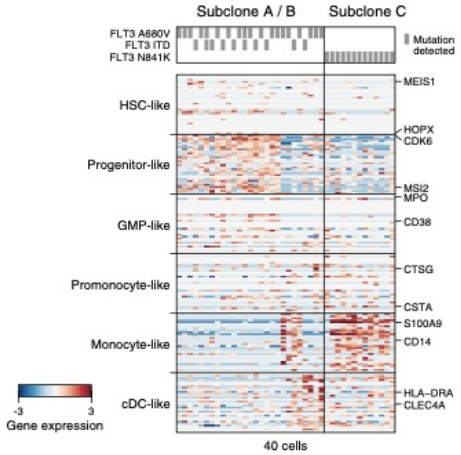


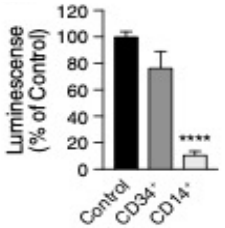
Ragaini et al, Blood Adv. 2022 Jan 11;6(1):87-99

AML Driver Lesions and Transcriptomics

A **Single-cell RNA-seq** study for the first time associated **AML-cell-type compositions/differentiation state** with the **Genetic Driver lesions (**single-cell transcriptomics and genotyping):
FLT3- ITD mutation resulted linked with abundant **progenitor-like** cells.

AML-cell-type composition was linked with consequent **immunological properties**:
Differentiated **monocyte-like AML cells** express diverse **immunomodulatory** genes and **suppressed T-cell activity** in vitro.



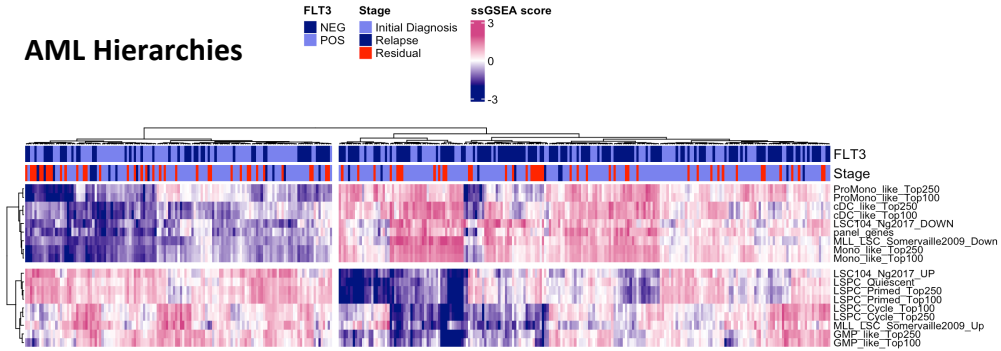


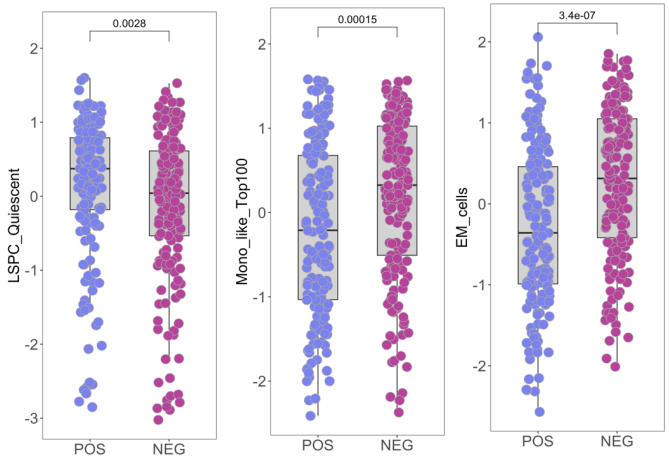
A complex interaction between AML driver lesion, differentiation state and immune stimulation

Van Galen P. et al, Cell, 2019

FLT3-pos cases had a higher score in signatures associated with stemness and an undifferentiated state, whereas FLT3-neg cases with a more differentiated state

AML Hierarchies





FLT3-mut

Stemness

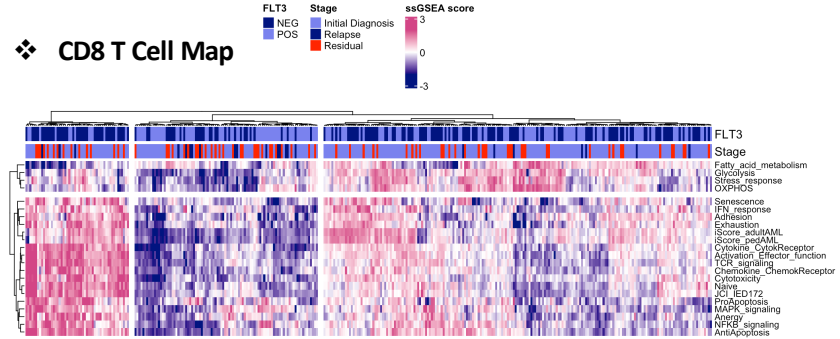
FLT3-wt

Mature

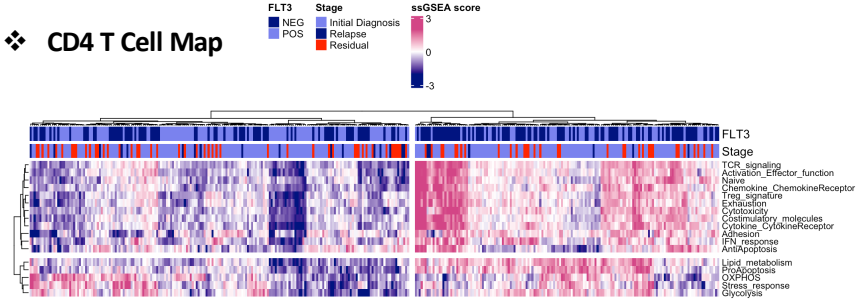
Nanni J et al. manuscript in preparation

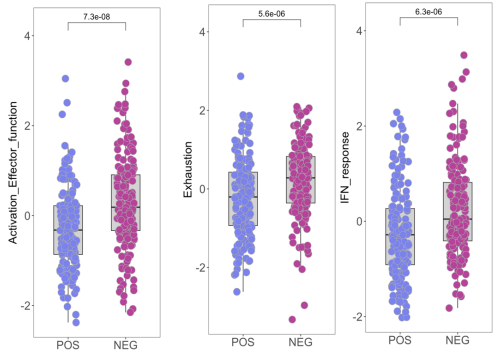
FLT3-mutated AML has a lower immune score for CD8+ and CD4+ T-Cell function signatures: T-cell Activation/Effector function, Exhaustion and Interferon Response signatures

❖ CD8 T Cell Map



❖ CD4 T Cell Map





FLT3-mut

Cold

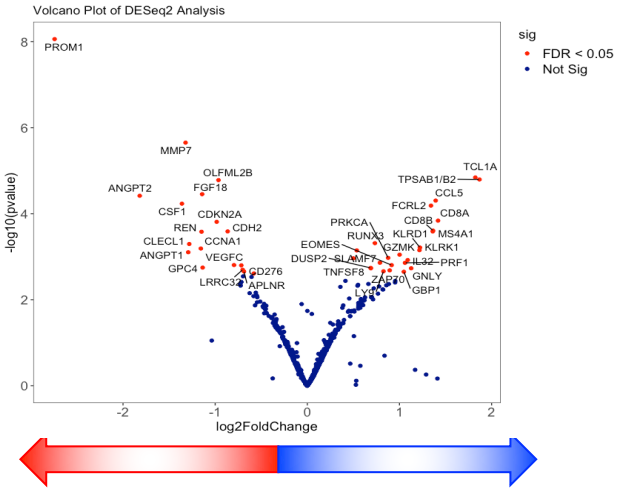
FLT3-wt

Hot but dysfunctional

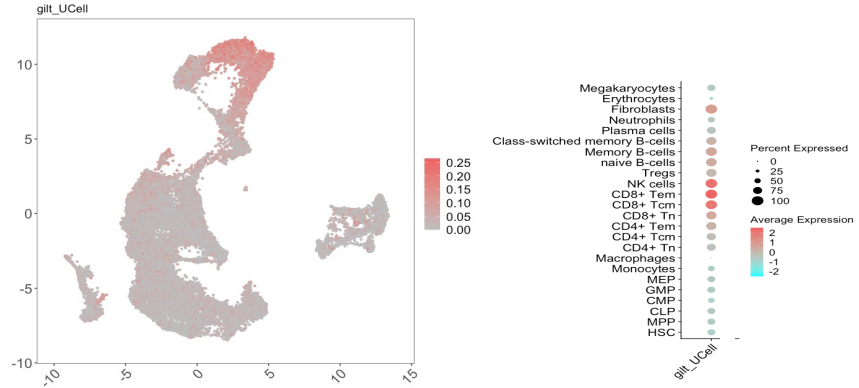
Nanni J et al. manuscript in preparation

Response to Gilteritinib is associated with cell-extrinsic pathways involving T/NK immunity

Volcano Plot from DEG analysis
***G Cohort*: Sensitive versus Resistant**



A higher expression of genes involved in T/NK function in Gilteritinib-Responders.

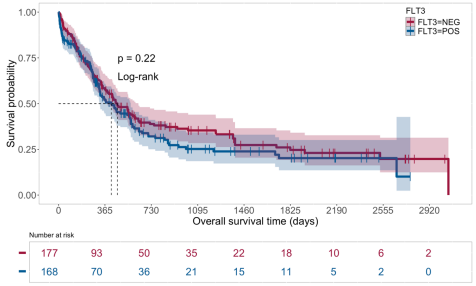


***Mapping** of the **top 30 DEGs (R versus NR)** onto the **sc-RNA-seq AML** dataset* (Dufva *et al*. Cancer Cell 2020): we found that they were primarily expressed by CD8$^+$ T_{EM}, CD8$^+$ T_{CM} and NK cells.

Nanni J et al. manuscript in preparation

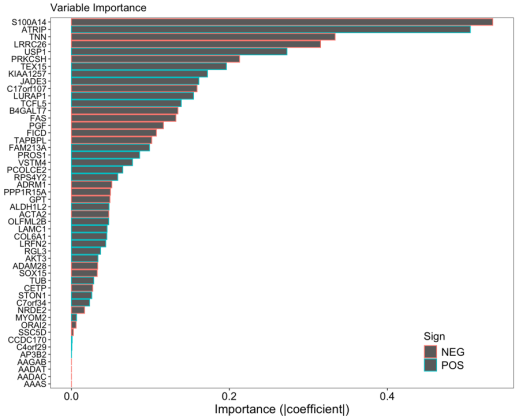
Prognostic Impact of DEGs between FLT3^{mut} and FLT3^{wt}

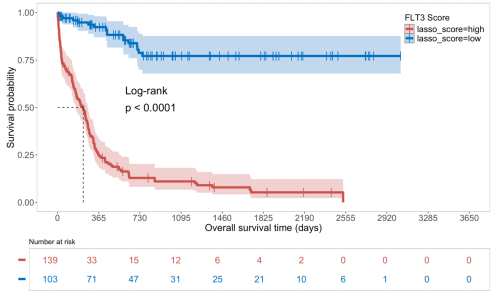
FLT3 mutational status per se does not **stratify prognosis** (considering the group of FLT3-pos and FLT3-neg samples from BEAT-AML2 cohort which entered previous analyses);



Using *LASSO-penalized regression for feature selection* and mitigating data collinearity on **DEGs** between **FLT3^{mut} vs FLT3^{neg}**:

identified **46 genes** with non-zero coefficients

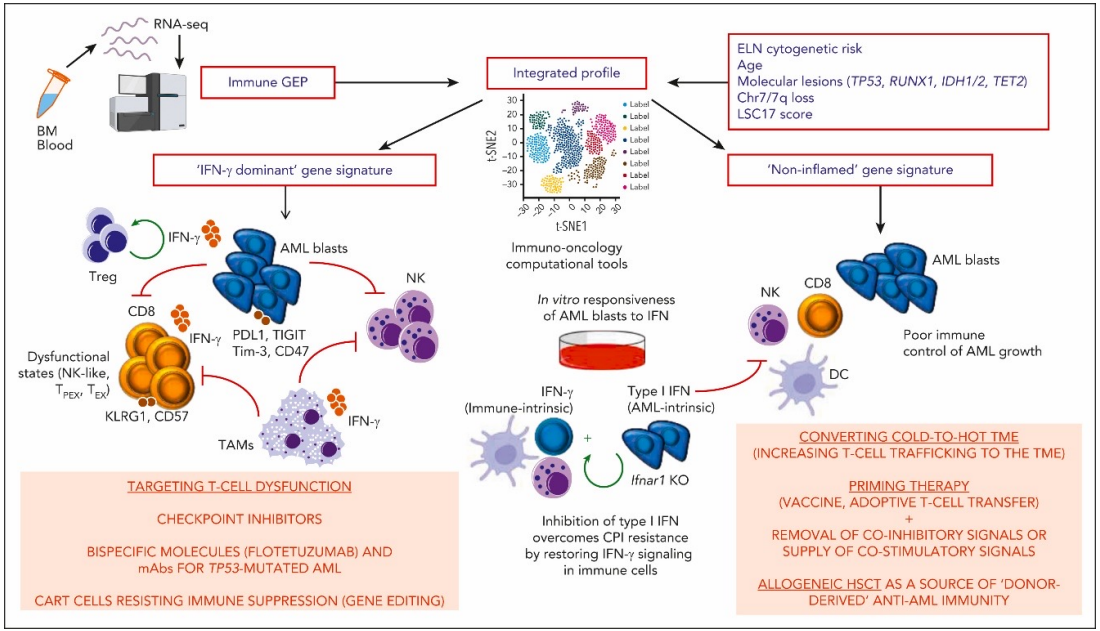




FLT3-PS: transcriptomic prognostic score

Nanni J et al. manuscript in preparation

Escape from T-cell–targeting immunotherapies in AML



Vadakekolathu J and Rutella S. *Blood* (2024) 143 (26): 2689–2700.

Immunotherapy in Hematological Malignancies 2024

Seragnoli Institute - *Acute Leukemia and MDS Group*

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ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

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