1ST INTERNATIONAL CONFERENCE ON
Ph+Leukemias

Bologna, Royal Hotel Carlton
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Beyond the Ph+ cell: the role of microenvironment

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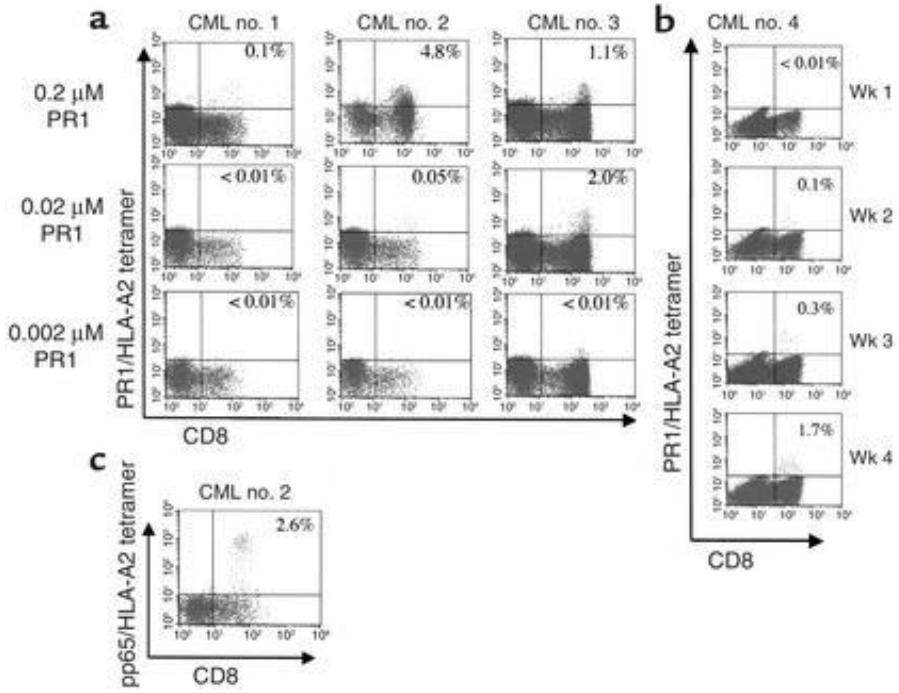
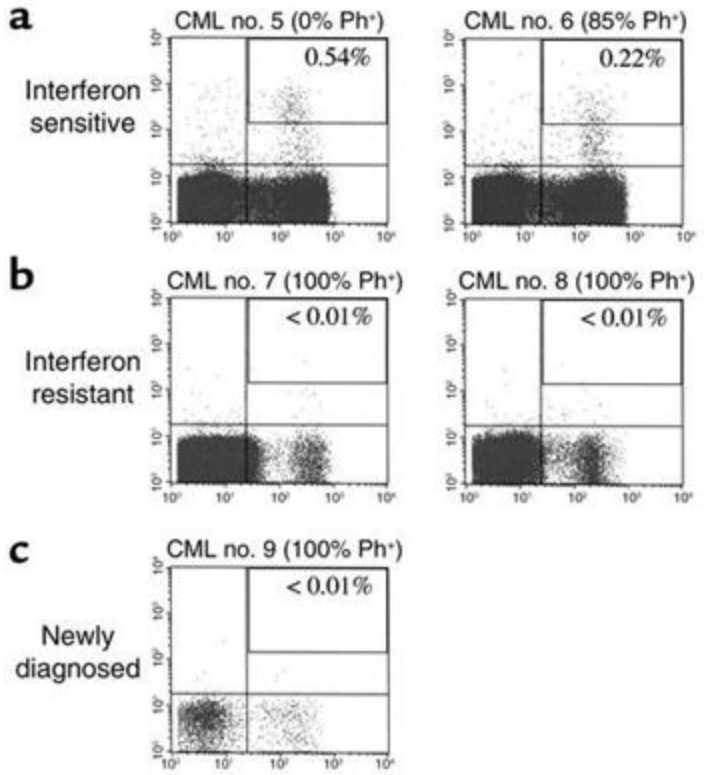
Disclosures Antonio Curti

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

Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia-specific T cells

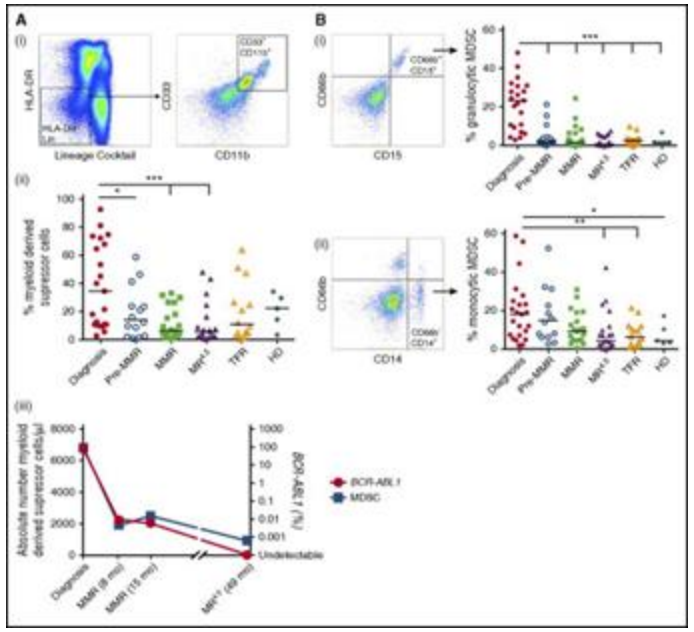




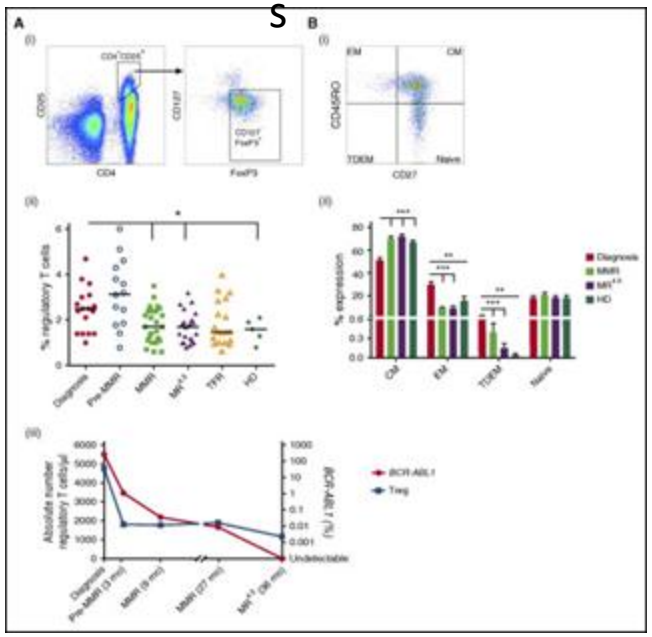
Molldrem JJ et al, J Clin Invest. 2003 Mar 1;111(5):639–647.

CML patients with deep molecular responses to TKI have restored immune effectors and decreased PD-1 and immune suppressors

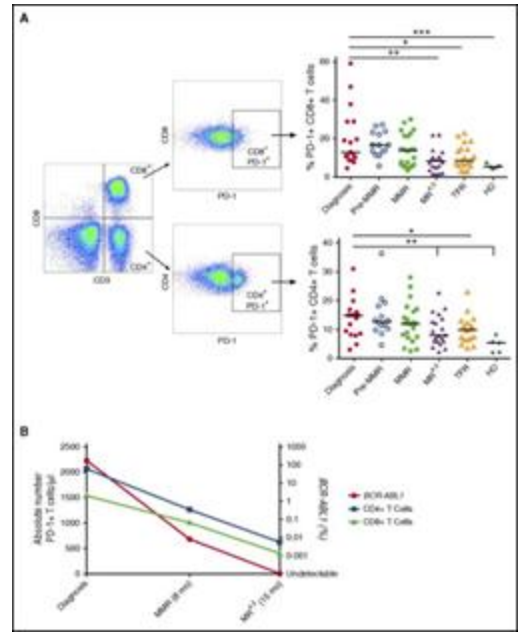
MDSC



Tregs



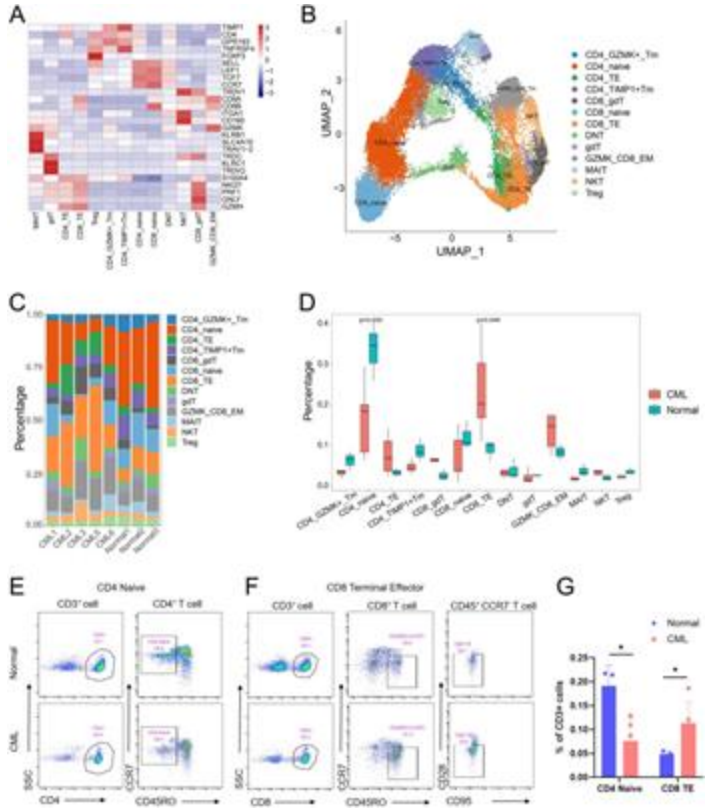
PD-1-expressing T cells



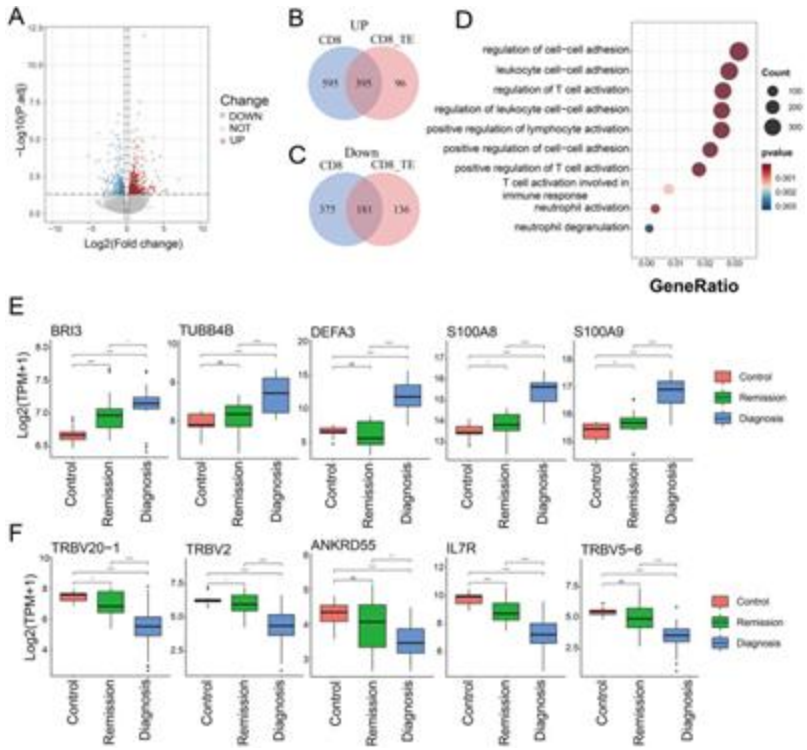
Hughes A et al, Volume 129, Issue 9, 2 March 2017, Pages 1166-1176

Single-cell sequencing reveals the expansion and diversity of T cell subsets in the bone marrow microenvironment of chronic myeloid leukemia

Differential T cell subpopulations in CML and healthy controls

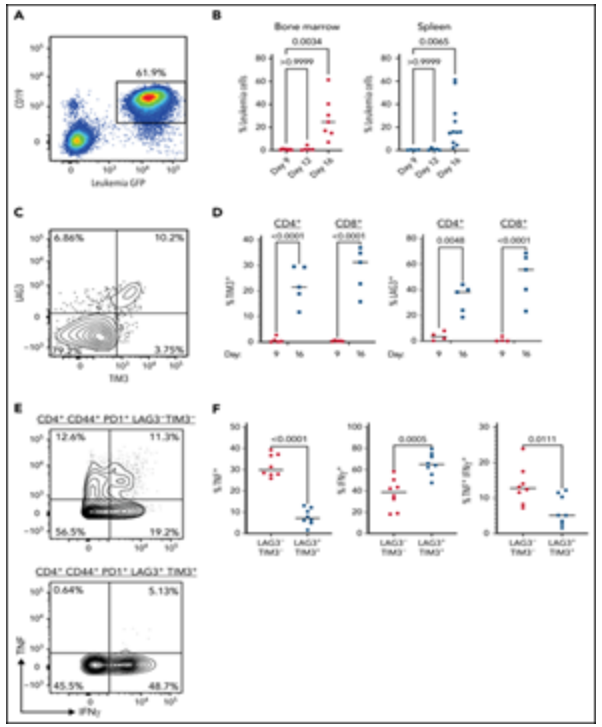


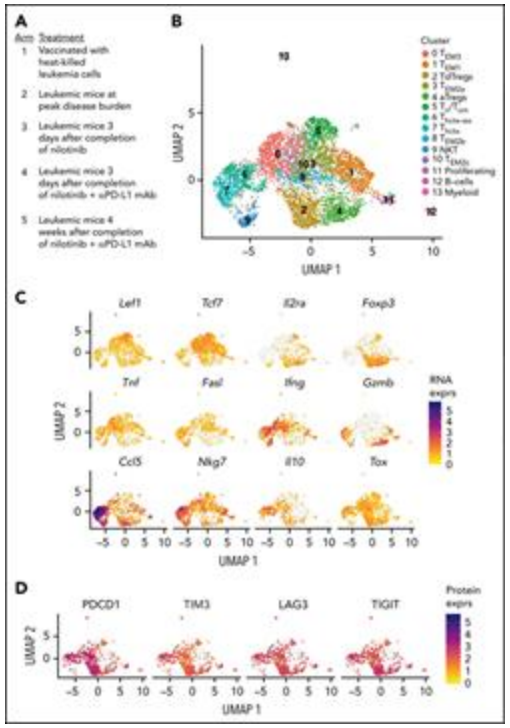
CD8 TE cell demonstrates CML-specific gene expression

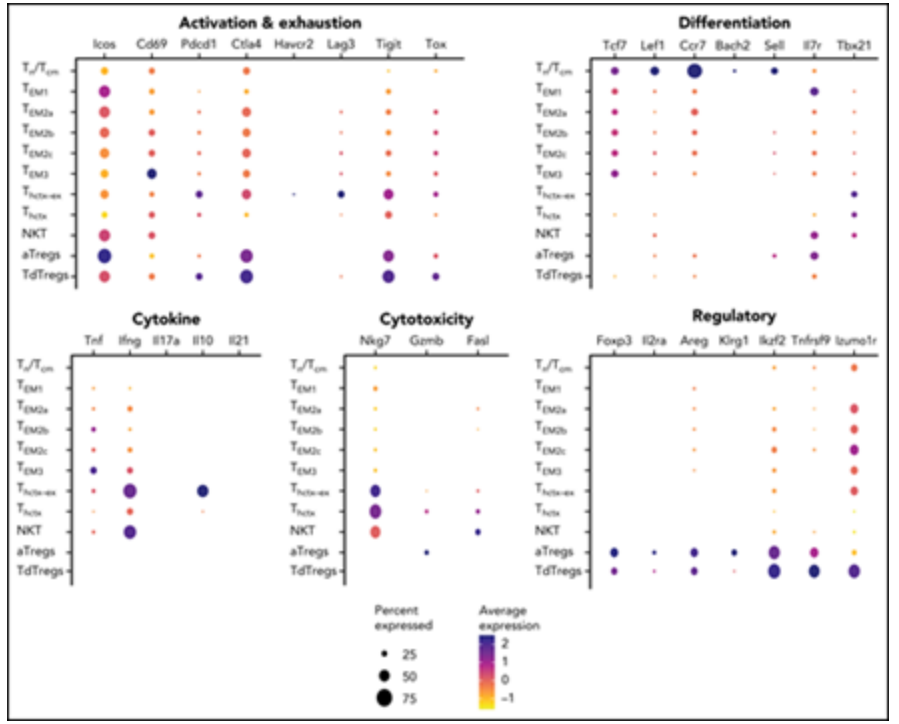


Zhuo C et al, Genes and Disease, Volume 12, September 2025

Ph+ leukemia induces an exhaustion phenotype in activated T cells, especially CD4+ T cells, which is associated with leukemia progression



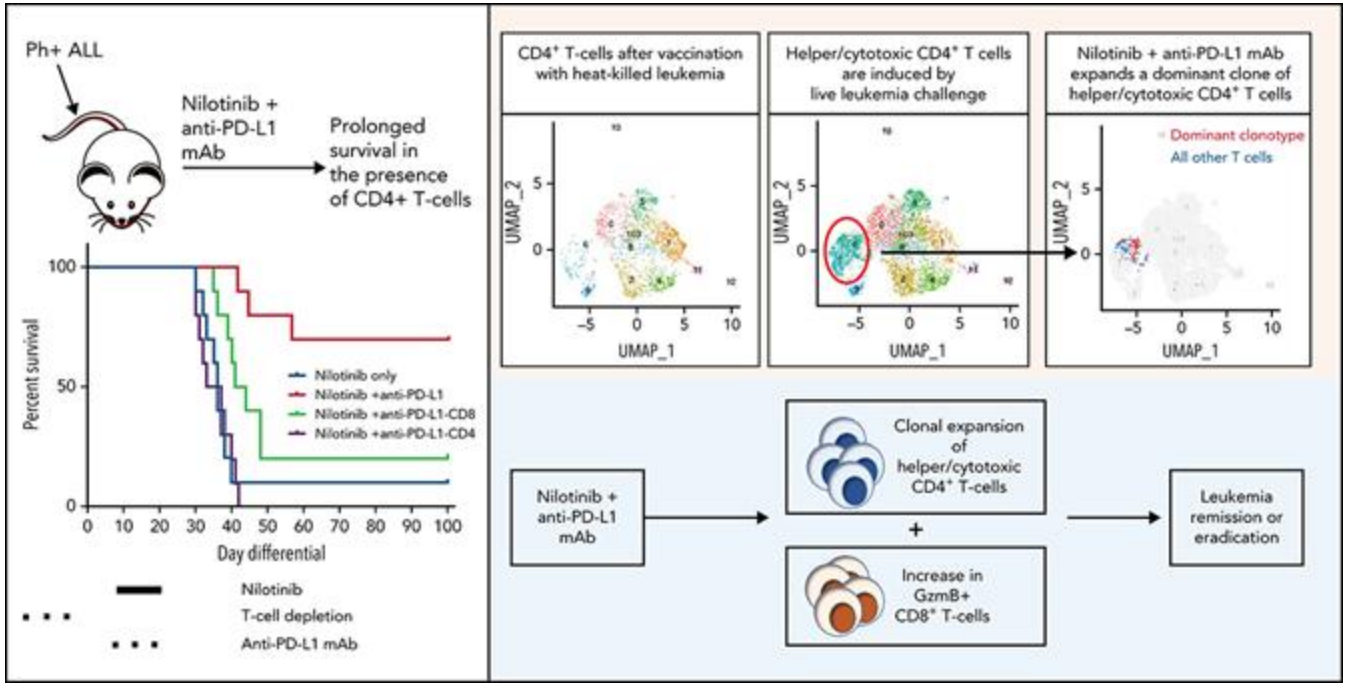




Experimental model: murine BCR-ABL^{+} leukemia cell line (LM138)

Tracy SI *et al*, Blood (2022) 140 (4): 335–348.

Combining nilotinib and PD-L1 blockade reverses CD4⁺ T-cell dysfunction, expands a unique T-helper/cytotoxic subset and prevents relapse in acute B-cell leukemia



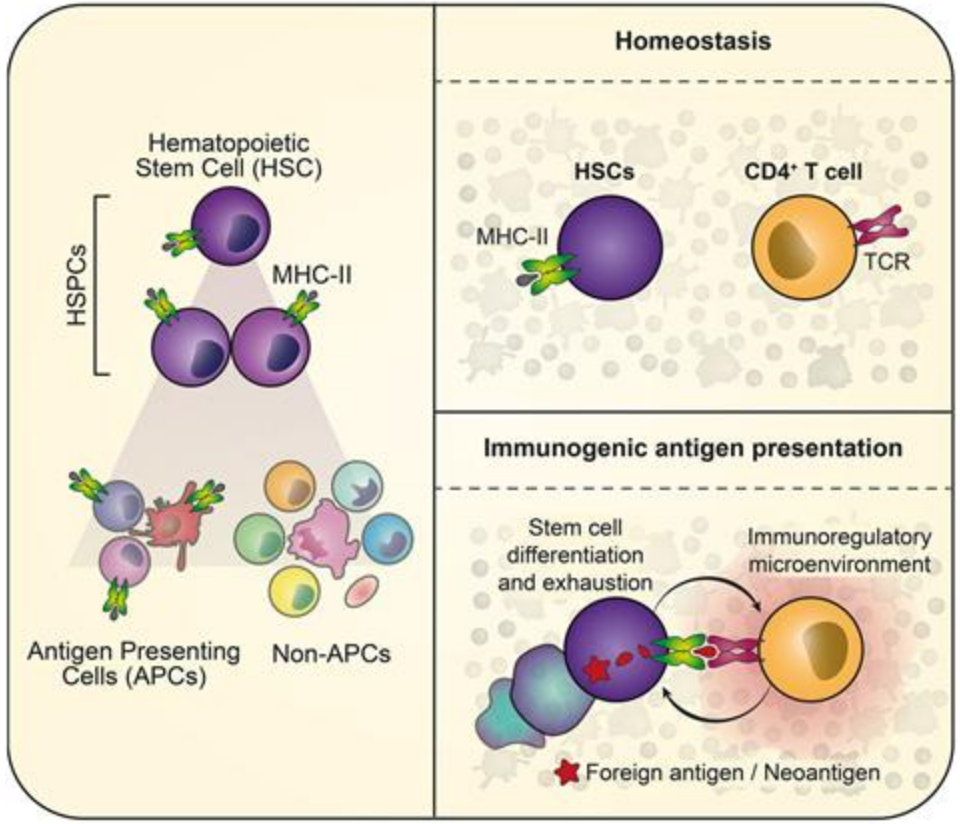
Key Points

- Anti–PD-L1 blockade significantly improves the efficacy of nilotinib against BCR-ABL⁺ B-ALL in a CD4⁺ T-cell–dependent manner.

- Anti–PD-L1 clonally expands leukemia-specific CD4⁺ T cells with a helper/cytotoxic phenotype and reduced expression of exhaustion markers.

Tracy SI *et al*, Blood (2022) 140 (4): 335–348.

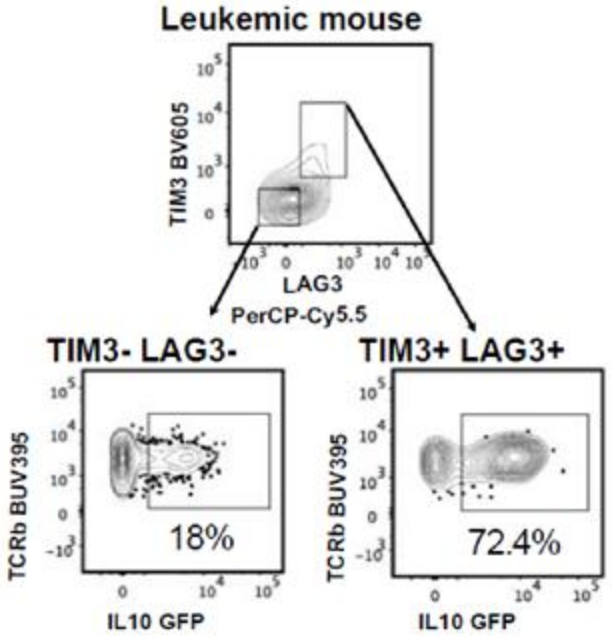
Antigen presentation safeguards the integrity of the hematopoietic stem cell pool

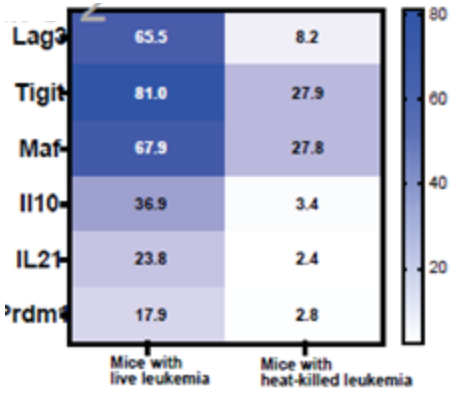


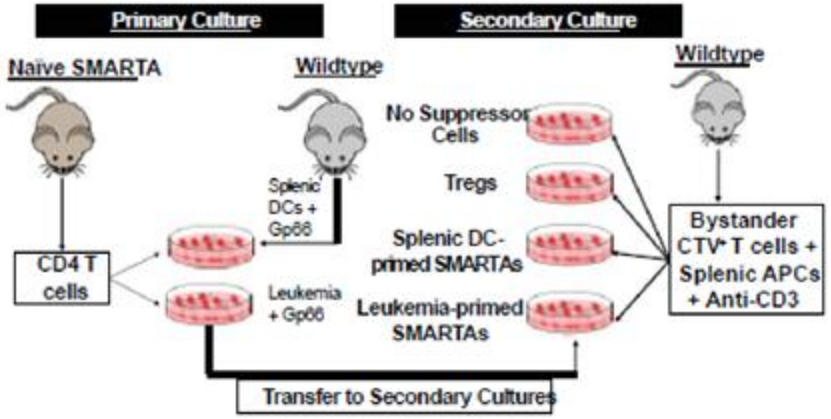
- HSPCs constitutively present antigens via MHC-II
- Presentation of immunogenic antigens results in the activation of $CD4^+$ T cells
- Antigen presentation causes differentiation and depletion of immunogenic HSPCs
- This prohibits the onset of HSC-derived leukemias presenting neoantigens via MHC-II
- $CD4^+$ 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

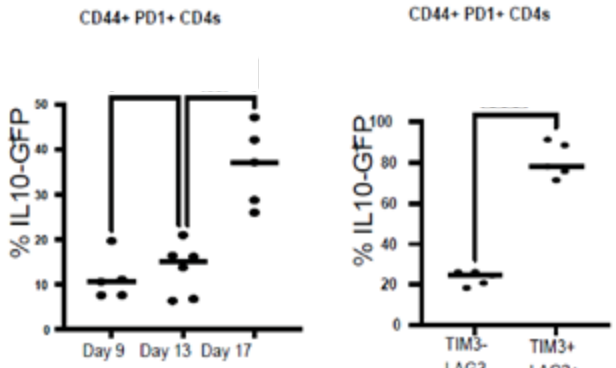
B-ALL induces dysfunctional T cells with a Tr1 phenotype that resemble HSC-induced suppressive CD4+ T cells

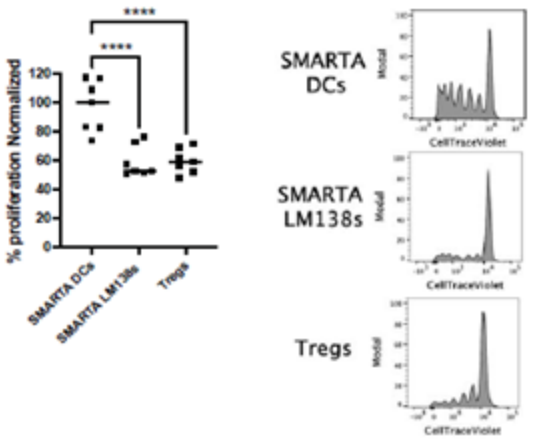






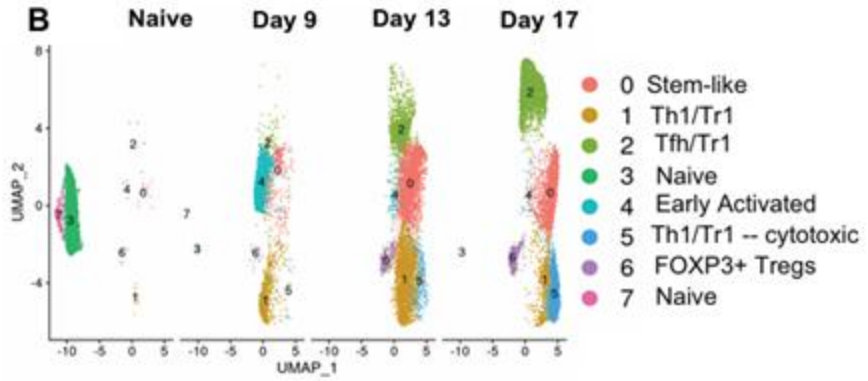
A similar pattern was observed by using a TCR knock-in mouse (HV1) that produces leukemia antigen-specific CD4+ T cells

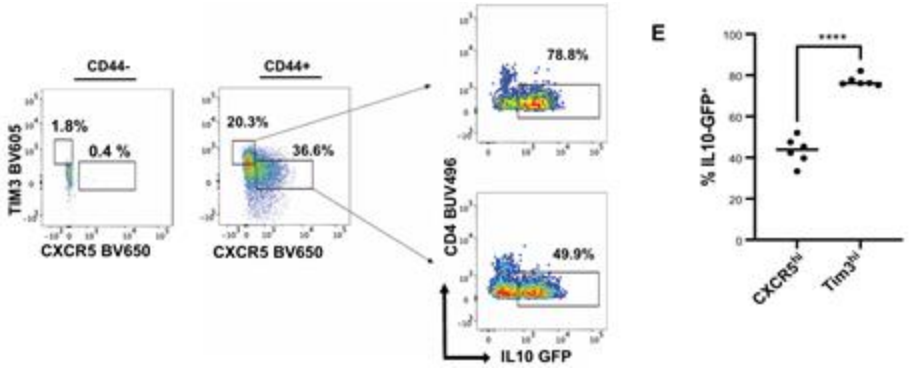


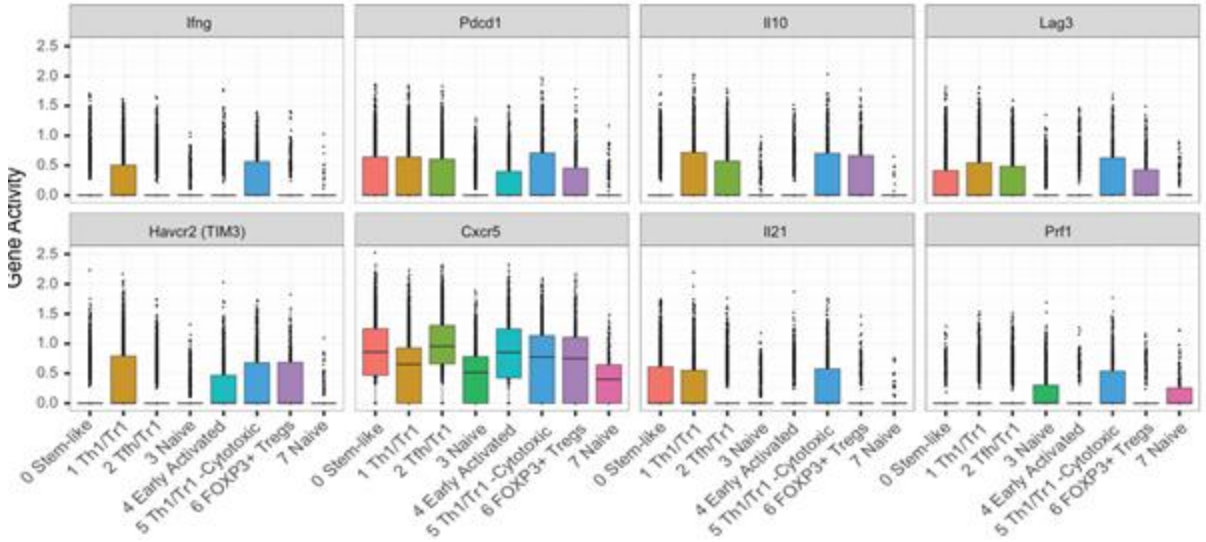


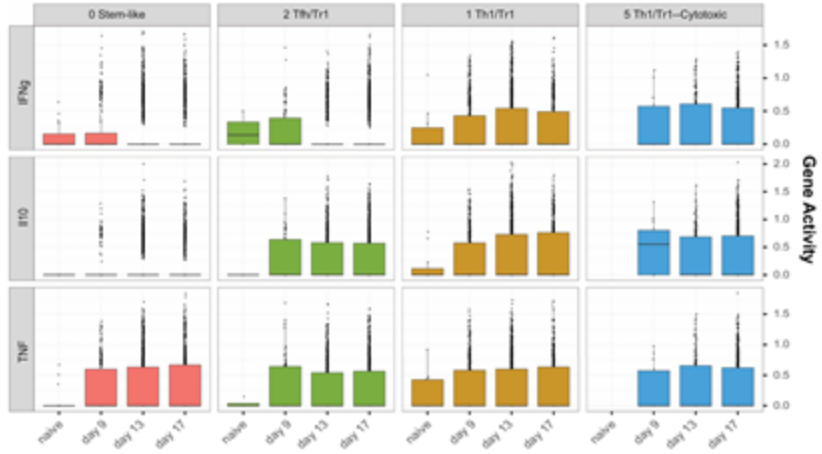
Venkatesh H et al, Blood Sept 4 2025

Tr1 cells maintain accessible loci within Ifng or Tnf suggesting a functional state distinct from terminal exhaustion



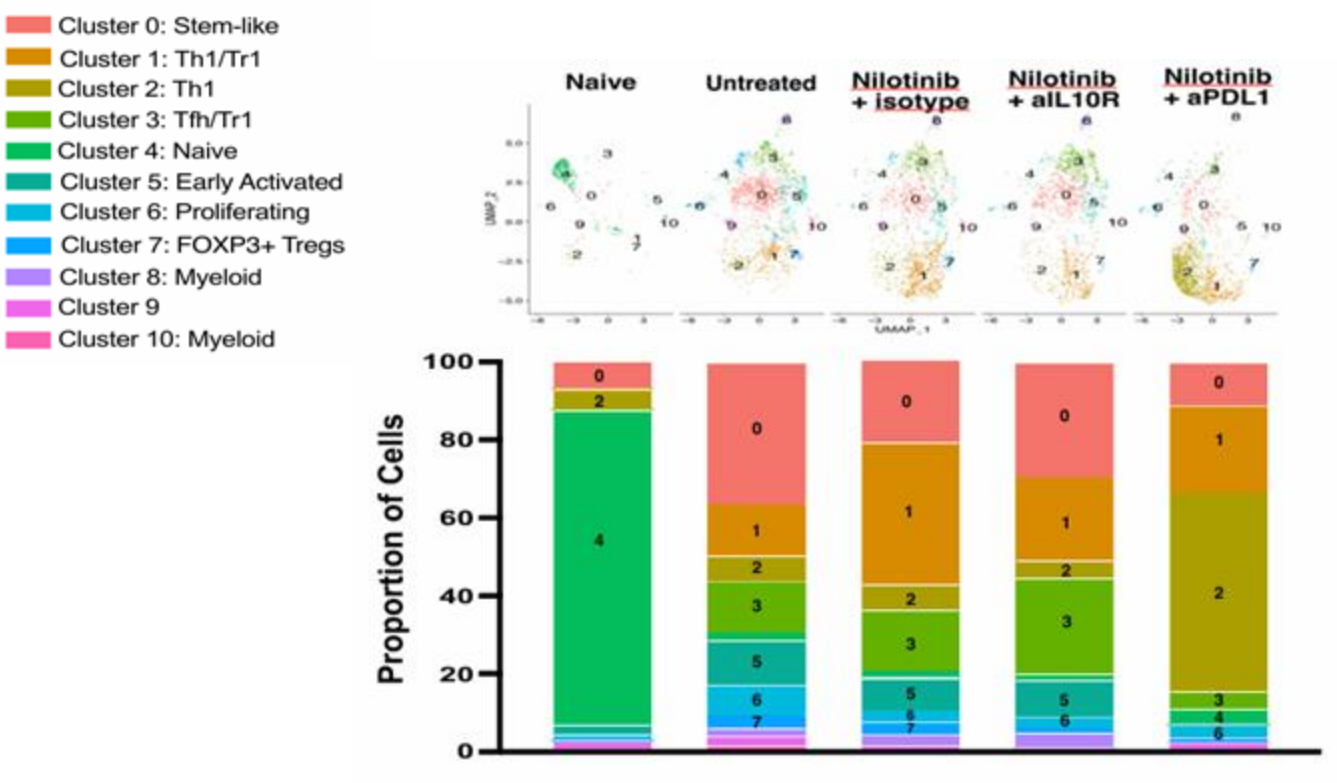


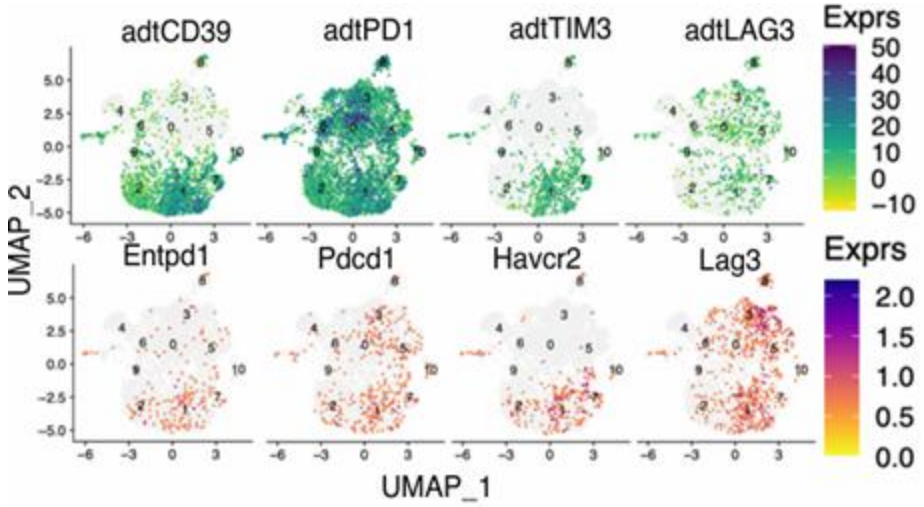




Venkatesh H et al, Blood Sept 4 2025

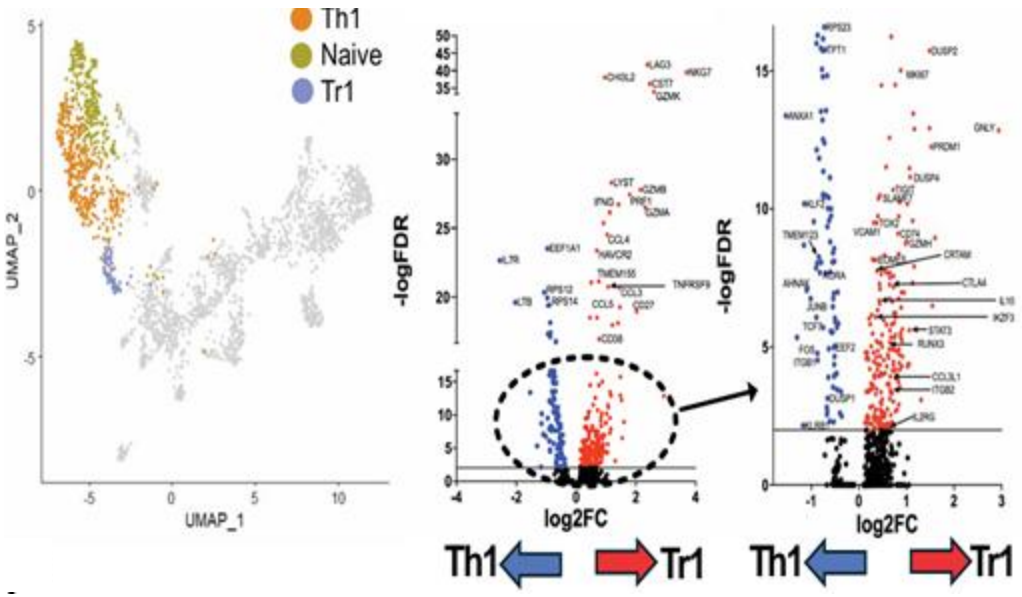
IL10R blockade alters Tr1 fates, while PDL1 blockade directs CD4+ T cells towards Th1 differentiation

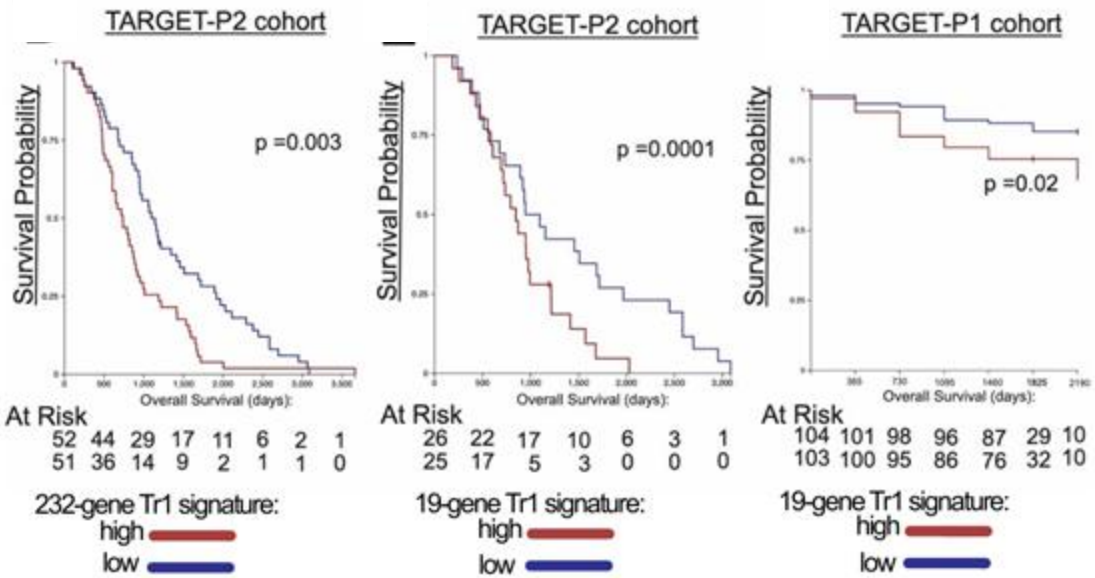




Venkatesh H et al, Blood Sept 4 2025

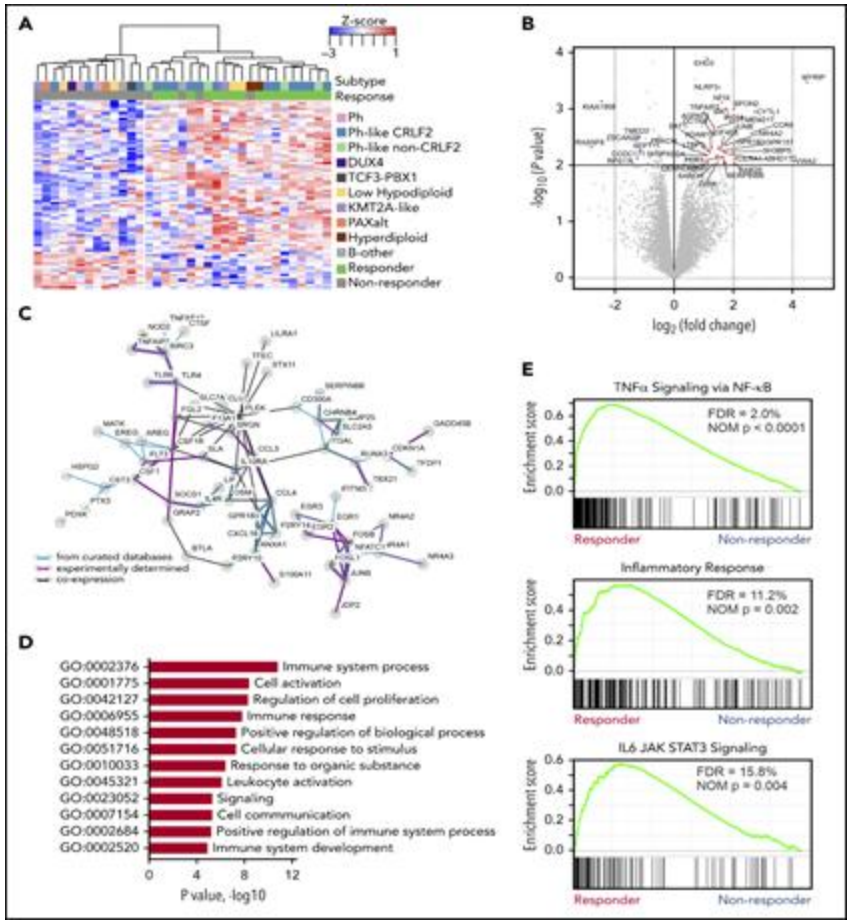
Type 1 regulatory signatures correlate with inferior overall survival among pediatric patients with B-ALL





Venkatesh H et al, Blood Sept 4 2025

Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL

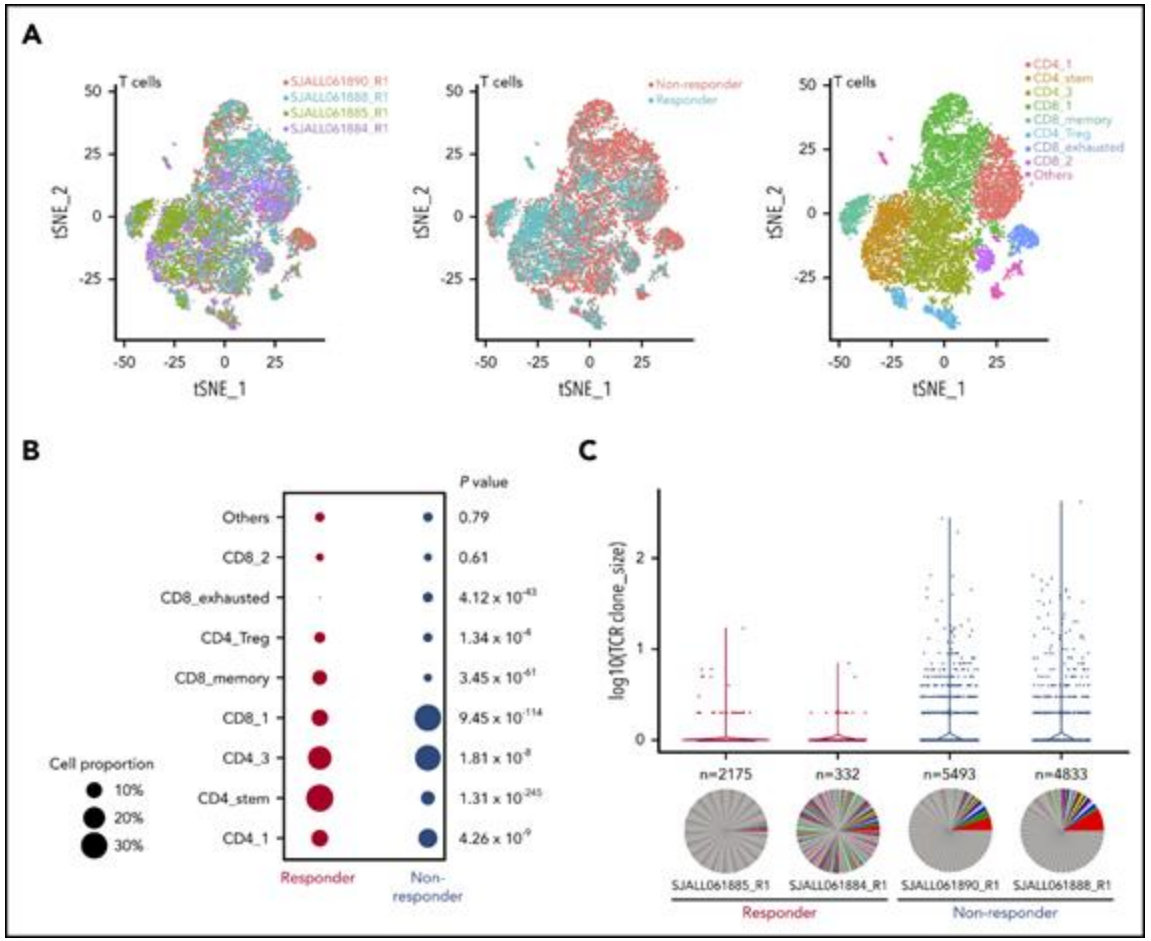


Heightened immune response signature in blinatumomab responders

- IL-6–JAK–STAT3 enrichment is a hallmark of response irrespective of somatic tumor cell genetics.
- Additional hallmark pathways with significant enrichment in responders included tumor necrosis factor α signaling via NF-κB and the inflammatory response characterized by IL genes
- Single-cell analysis (RNA seq) identifies immune signature in tumor cells

Zhao Y et al, Blood (2021) 137 (4): 471–484

Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL



Single-cell analysis of T cells

- High expression of immune checkpoint receptors, such as *LAG3*, *TIM3*. These cells are increased *in* non-responders over responders
- A superior response to blinatumomab is associated with an increase in naïve and central memory T cells
- Response to blinatumomab is associated with a restricted TCR clonal expansion and increased diversity. Thus, the presence of clonally expanded T cells may abrogate an effective immune response

Zhao Y et al, Blood (2021) 137 (4): 471–484

Blinatumomab Maintenance After Allogeneic Hematopoietic Cell Transplantation for B-Acute Lymphoblastic Leukemia

A single center phase II study evaluating the feasibility of 4 cycles of blinatumomab administered every 3 months during the first year after HCT in high-risk ALL patients. With a median follow-up of 14.3 months, the 1-year overall survival, progression-free survival, and nonrelapse mortality rates were 85%, 71%, and 0%, respectively.

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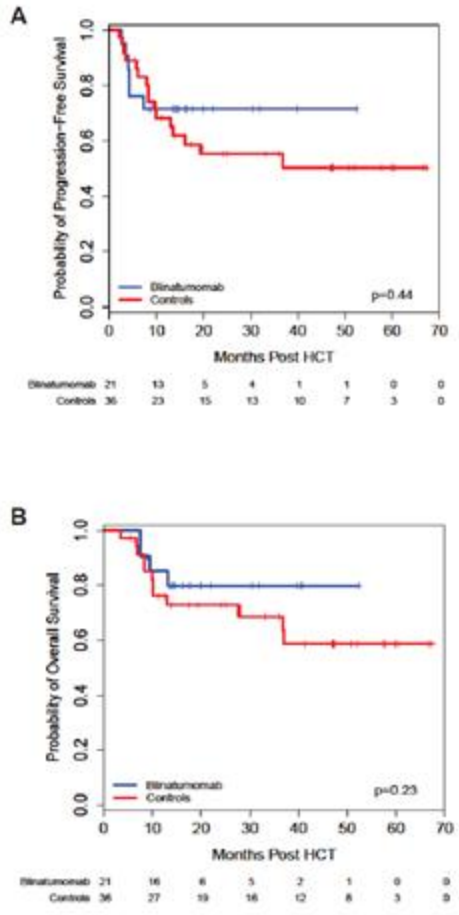
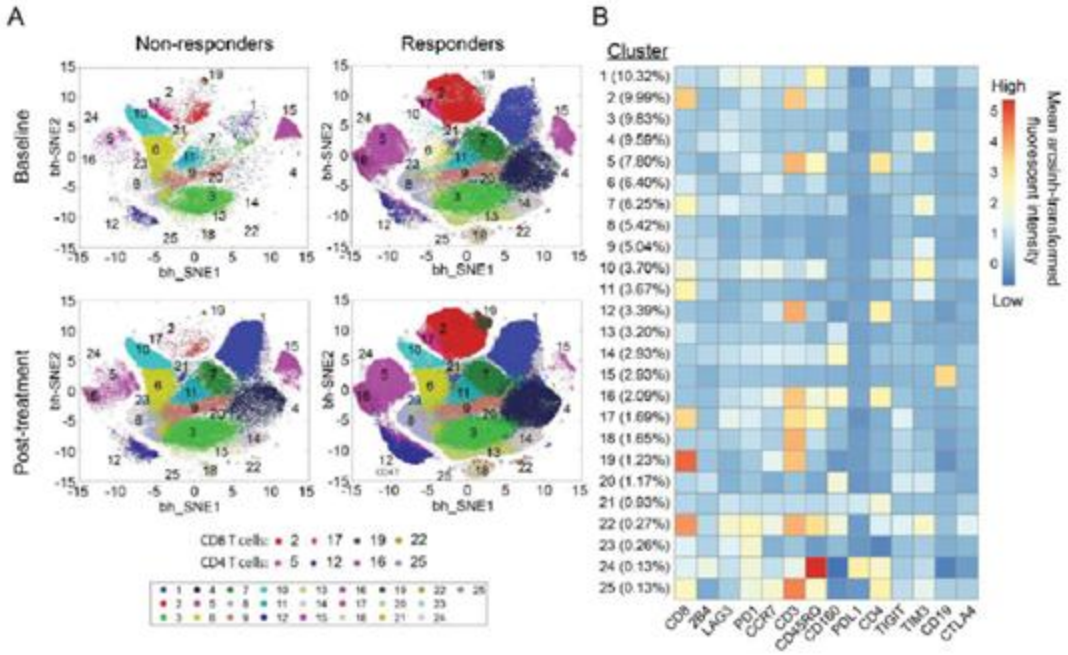
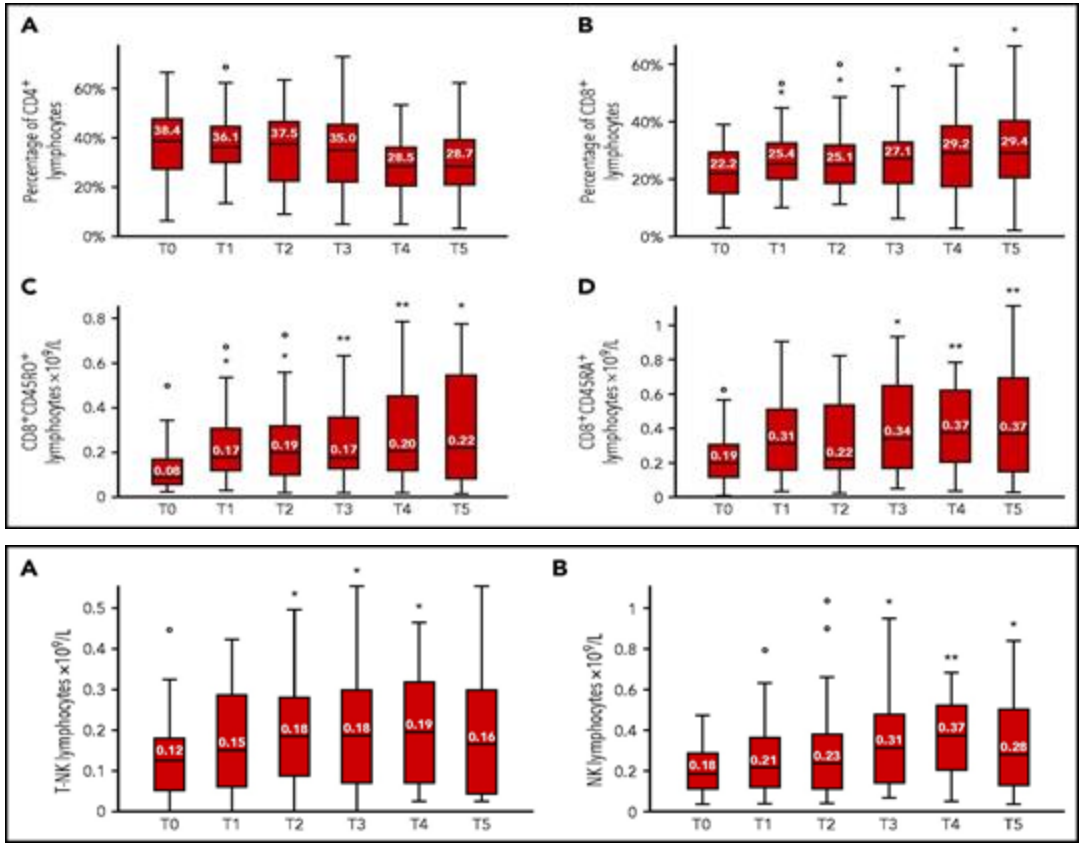


Responders had higher proportions of effector memory CD8 T-cell subsets



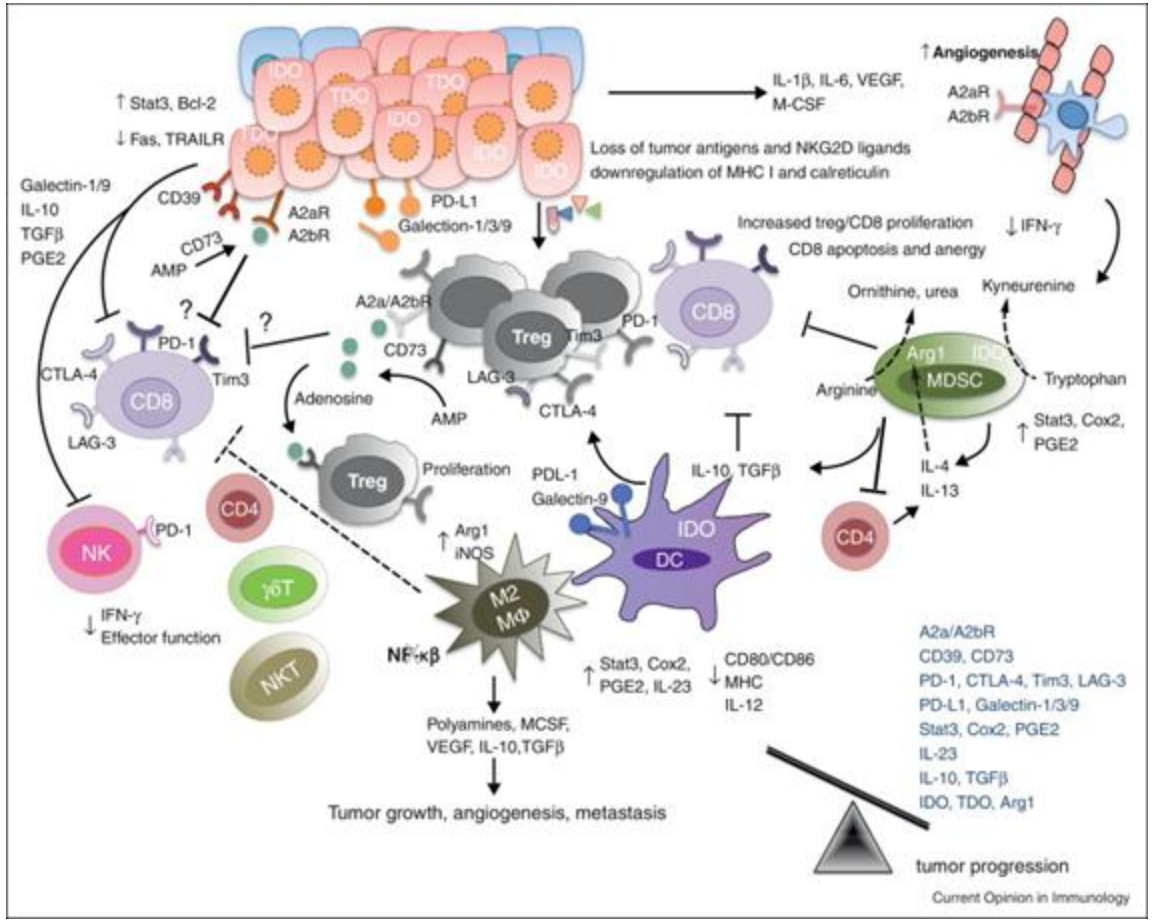
Gaballa et al, Blood, Dec 2021

Host immune system modulation in Ph+ acute lymphoblastic leukemia patients treated with dasatinib and blinatumomab (D-ALBA Study)



Puzzolo et al, Blood (2021) 138 (22): 2290–2293.

Response to immunotherapy approaches: the role of tolerogenic pathways



Deepak Mittal et al , Current Opinion in Immunology, 2014, 16 - 25

Treg number correlates with response in R/R ALL patients treated with blinatumomab (N=42)

Table 2. Analysis of potential prediction markers

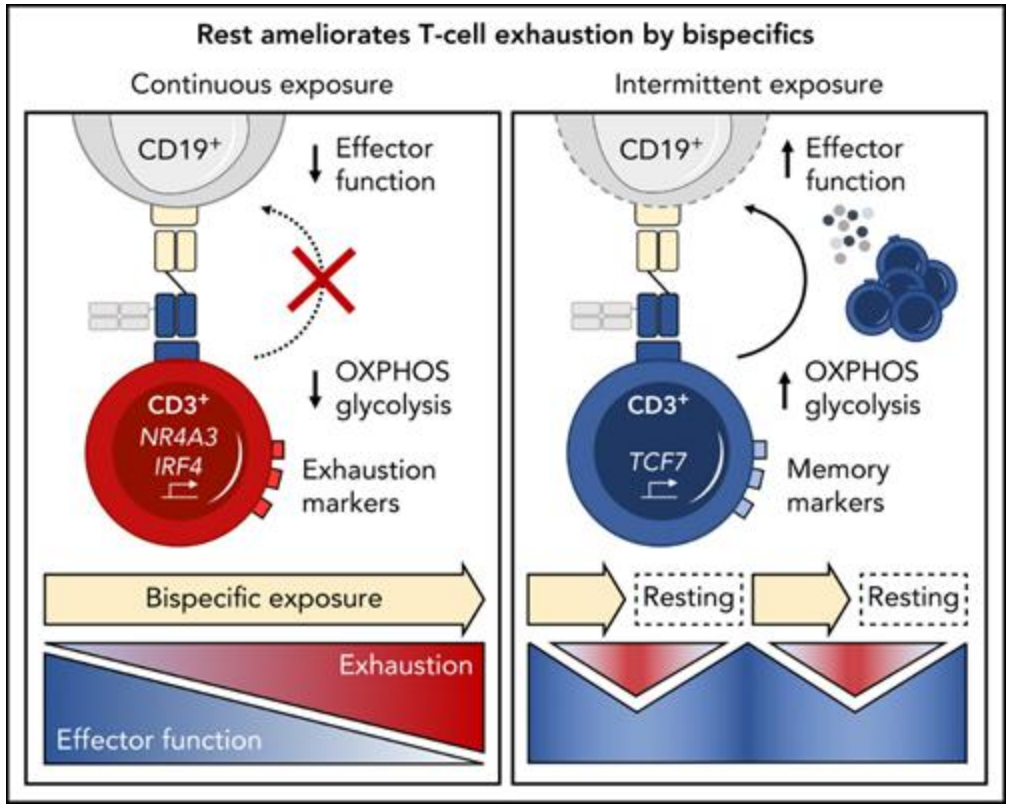
Variable	*Non-responder*			*Responder*			*P-value*
	Median	*Min*	*Max*	*Median*	*Min*	*Max*	
Age	34	20	69	38	21	77	0.47241
LDH	402.5	182	1860	211.5	119	904	0.00321
Blast cells local	0.78	0.2	0.95	0.4	0.04	1	0.00934
CD3 abs. numbers	0.0845	0	1.077	0.569	0.001	1.4	0.06976
Treg Perc.FoxP3	14.25	5.65	73	8.75	3.2	14.2	0.00024
Treg Perc.CD25/FOXP3	10.25	3.36	65.9	4.82	1.79	8.34	0.00010
Ratio Treg/CD3 abs. numbers	0.077	0.023	1.985	0.045	0.018	0.112	0.01261

	Non-responder	*Responder*	*P-value*
Sex			
Male	17	11	0.023
Female	3	11	
AlloTx before therapy			
Yes	8	7	1.000
No	12	15	

Abbreviations: AlloTx, allogeneic stem cell transplantation; LDH, lactate dehydrogenase.

Duell et al Leukemia 2017

T-cell exhaustion induced by continuous bispecific molecule exposure is ameliorated by treatment-free intervals (TFIs)

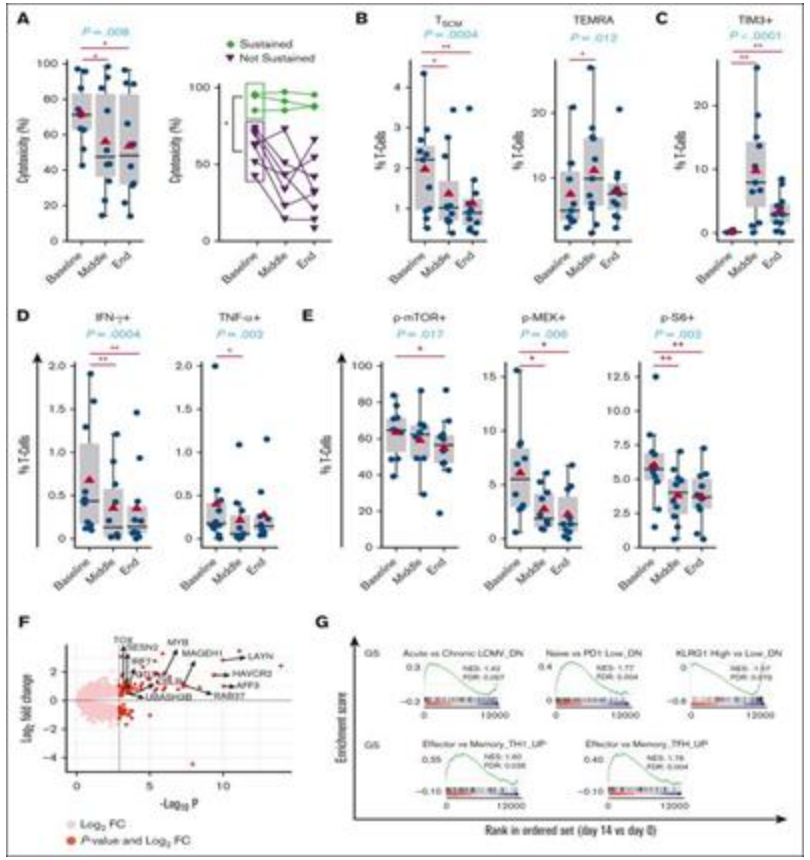


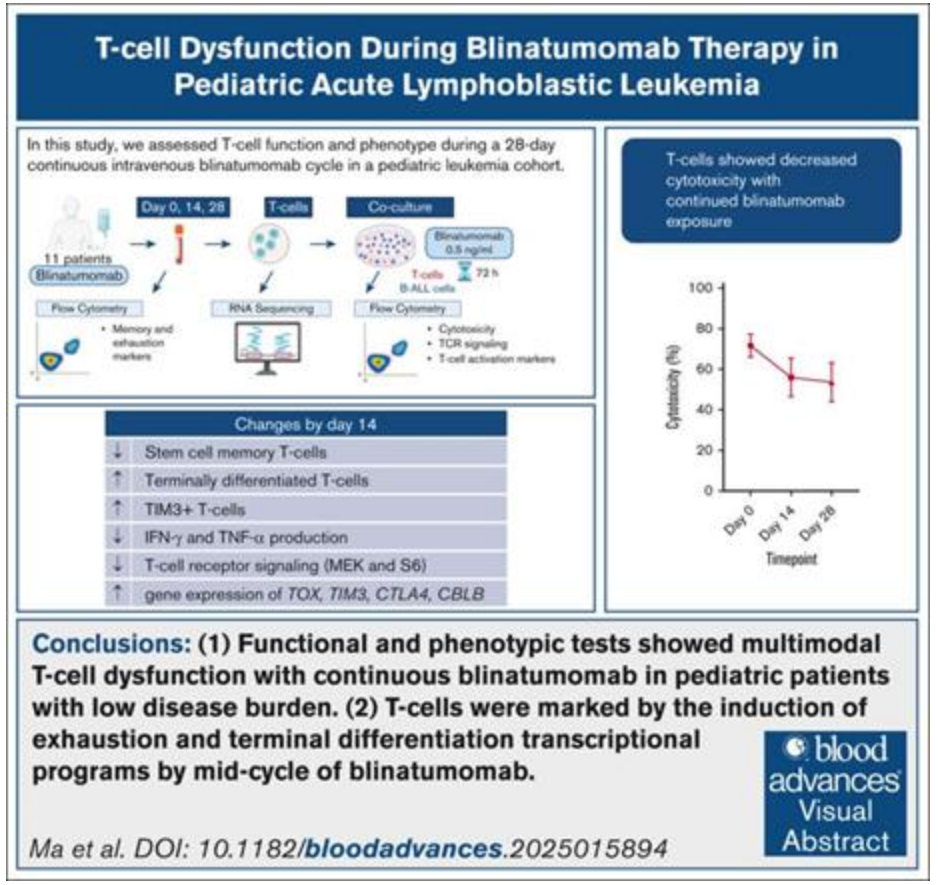
Key Points

- Continuous exposure to a CD19xCD3 bispecific molecule induces T-cell exhaustion.
- Treatment-free intervals transcriptionally reprogram and functionally reinvigorate T cells.

Philipp N et al, Sep 8;140(10):1104-1118. Blood, 2022

T-cell dysfunction during blinatumomab therapy in pediatric acute lymphoblastic leukemia





Ma J et al, Blood Adv. 2025 Aug 12;9(15):3689-3693

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BRITISH JOURNAL OF HAEMATOLOGY
British Society for Haematology

ORIGINAL PAPER | Open Access

Blinatumomab differentially modulates peripheral blood and bone marrow immune cell repertoire: A Campus ALL study

Darina Ocadlikova, Federico Lussana, Nicola Fracchiolla, Massimiliano Bonifacio, Lidia Santoro, Mario Delia, Sabina Chiaretti, Crescenza Pasciolla, Alessandro Cignetti, Fabio Forghieri, Francesco Grimaldi, Giulia Corradi, Letizia Zannoni, Stefania De Propris, Gian Maria Borleri, Ilaria Tanasi, Jayakumar Vadakekolathu, Sergio Rutella, Anna Rita Guarini, Robin Foà, Antonio Curti ✉ the Campus ALL

First published: 12 September 2023 | https://doi.org/10.1111/bjh.19104 | Citations: 6

After first treatment cycle:

PB

- no changes in immune cell composition
- up-regulation of cytotoxic NK cells
- up-regulation of Tim-3 on naive CD8 T cells

BM

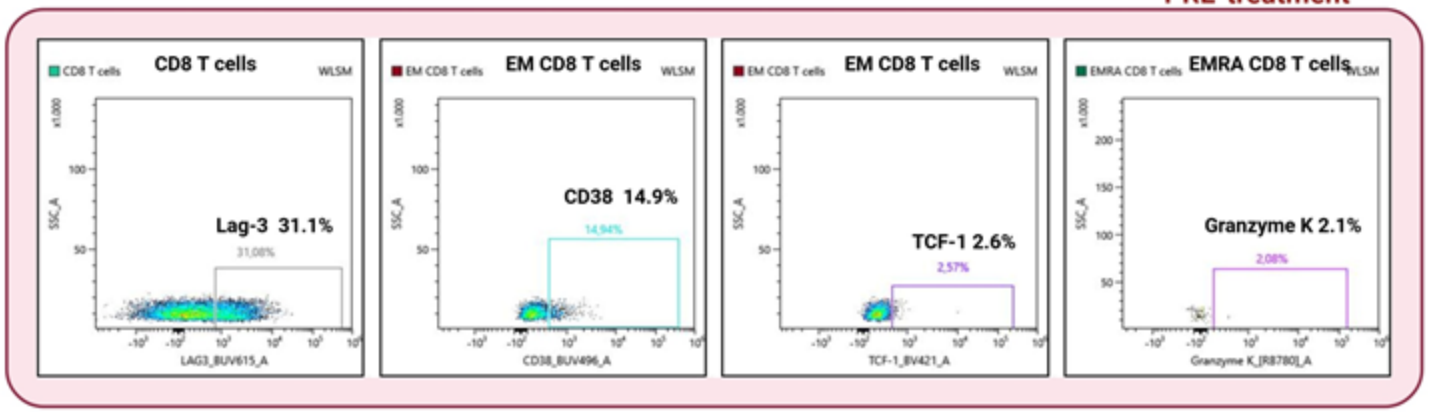
- up-regulation of CD3, CD8 and CD8 EM T cells
- no changes in cytotoxic NK cells
- up-regulation of PD-1 on CM CD4, Tim-3 on naive CD4, CTLA-4 on CM CD8, Lag-3 on EM and EMRA CD8 T cells
- up-regulation of CD39 on naive Tregs

Ocadlikova D et al, B J Haematol, Sep 12, 2023

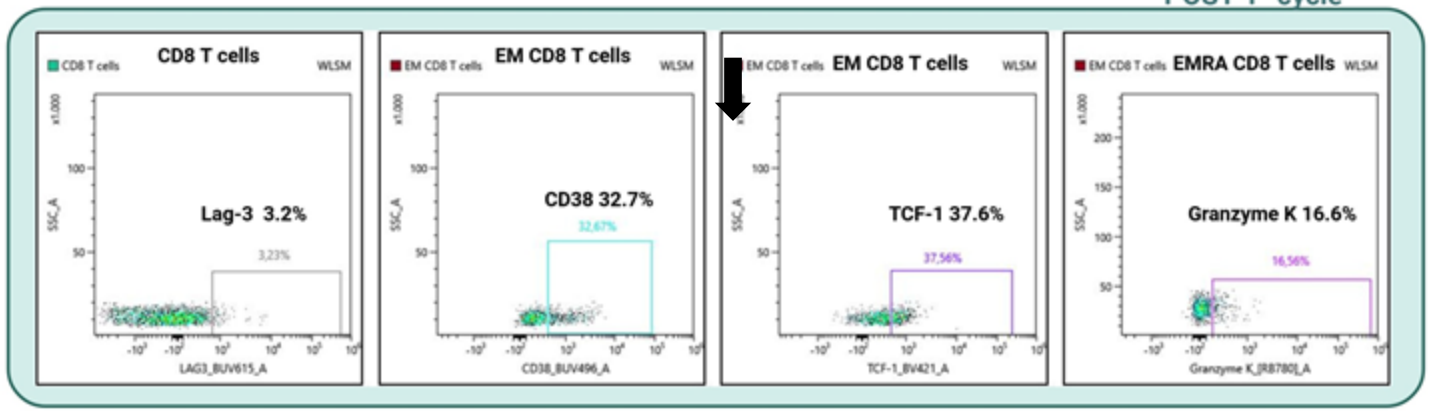
In Vivo Evaluation of Exhaustion by spectral flow cytometry: Preliminary Data

T cells

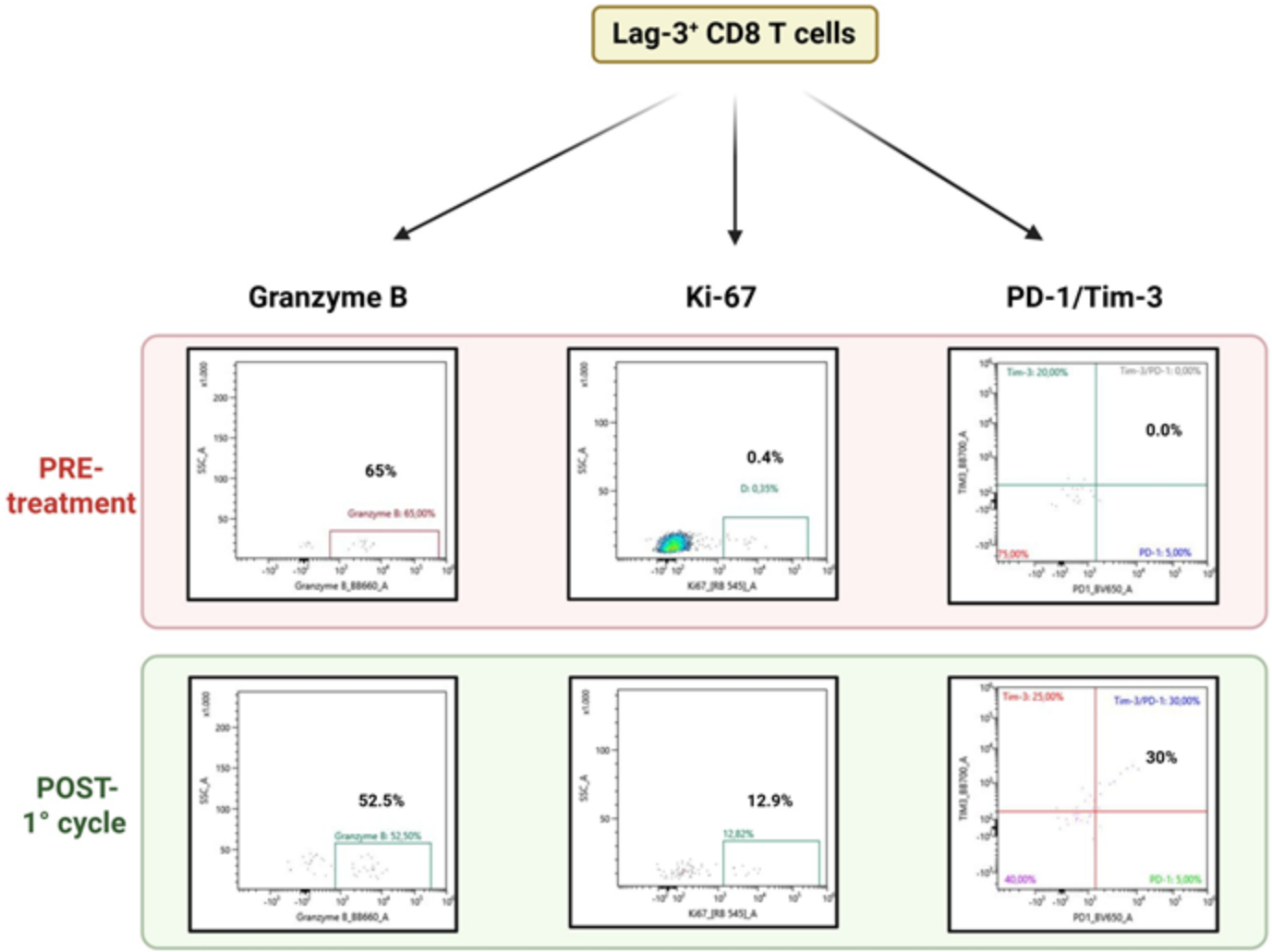
PRE-treatment



POST-1° cycle



Unpublished



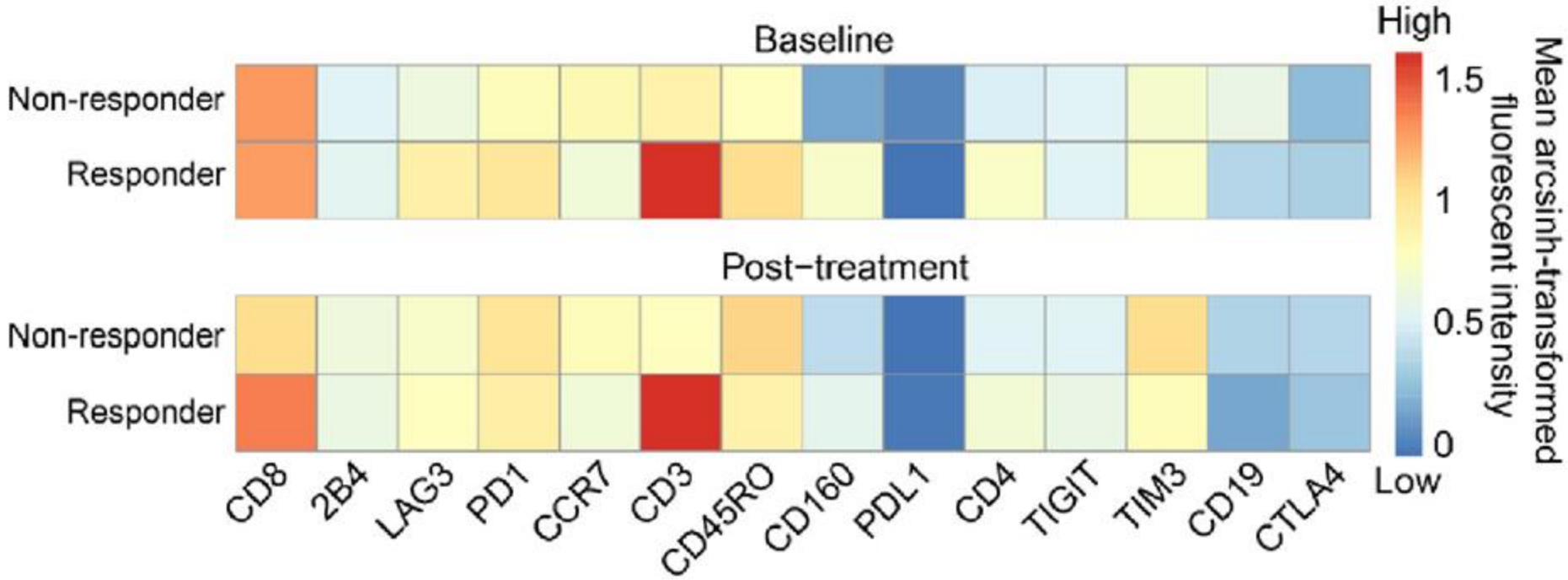
Lag-3+ CD8+ T cells have:

- Reduced Granzyme B
- Increased Ki-67
- Increased co-espression of PD-1 and TIM-3

Exhausted phenotype

Unpublished

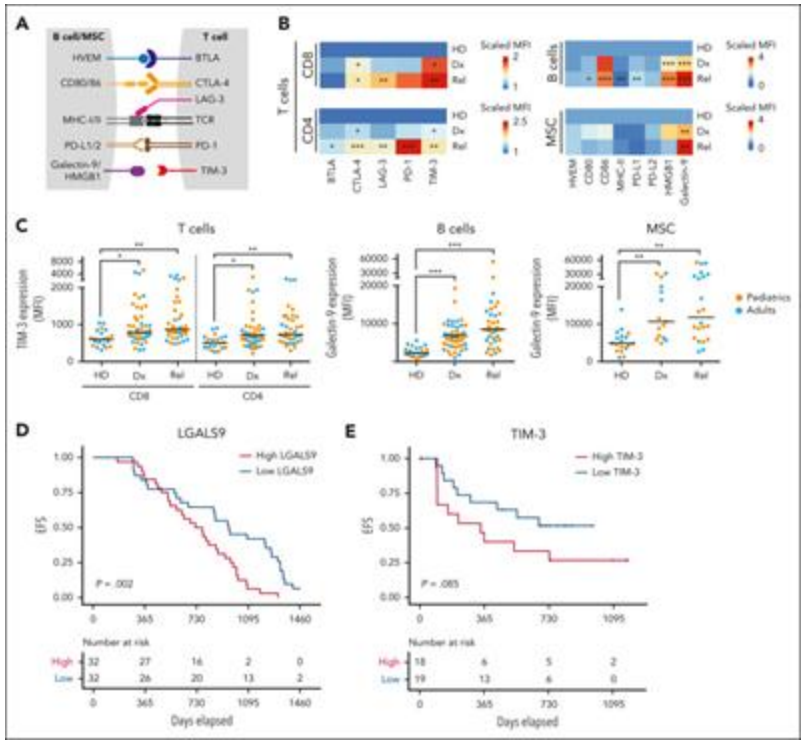
Checkpoint molecules LAG3, PD1, TIGIT and TIM3 are expressed at high levels both at baseline and after treatment

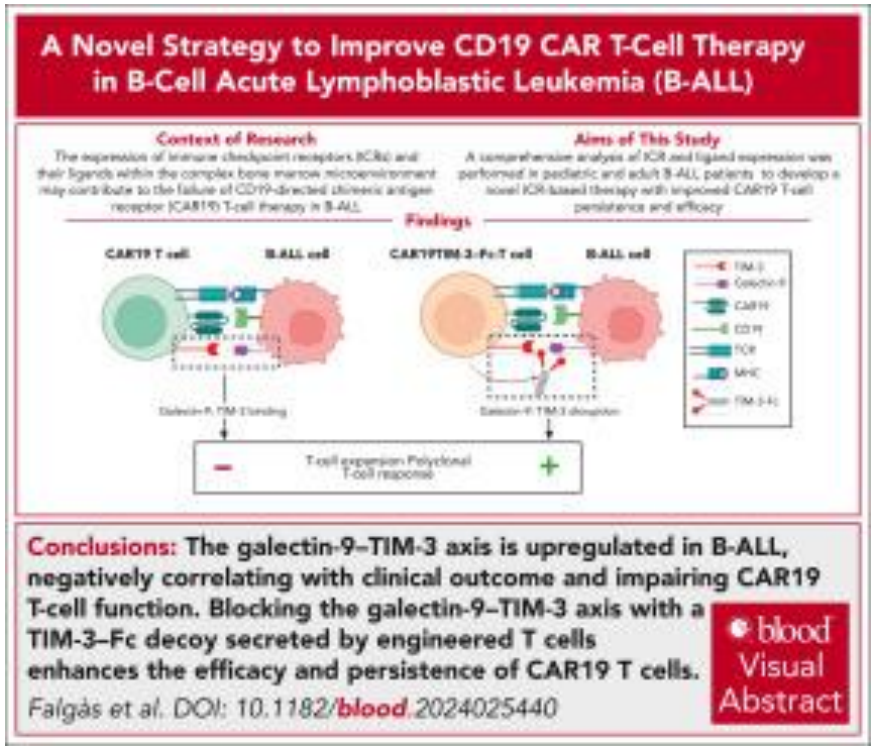


TIM3 was the only checkpoint that was expressed at statistically higher levels in non-responders compared to responders after blinatumomab treatment (P=0.04)

Gaballa et al, Blood, Dec 2021

Increased expression of the TIM-3–galectin-9 axis throughout disease progression in B-ALL





Key Points

- Galectin-9–TIM-3 axis is upregulated in B-ALL, negatively correlating with clinical outcome, and galectin-9 impairs CAR19 T-cell function.
- Blocking galectin-9–TIM-3 axis with a TIM-3–Fc decoy secreted by engineered T cells enhances the efficacy and persistence of CAR19 T cells.

A TIM-3–Fc decoy secreted by engineered T cells improves CD19 CAR T-cell therapy in B-ALL

Falgas A et al, Volume 145, Issue 22, 29 May 2025, Pages 2599-2613

Summary

Ph+ leukemias, including B-ALL, escape immunity by imposing a type 1 regulatory program on neoantigen-specific CD4+ T cells

These findings indicate a similar and common module of antigen presentation through HLA-class II molecules by normal and leukemic HSPCs, thus revealing potential points of vulnerability for immune modulation and immunotherapies

In that, the combination of molecular target therapies with strategies aimed at concomitantly activating effector T cells and inhibiting immunosuppressive pathways is likely to impact on clinical response and outcome

The translational studies from the utilization of immunotherapies, such as T-cell engagers and CAR-T cells, offer the unique opportunity to understand the mechanisms underlying the intricate and complex interplay between leukemic cells and immune microenvironment

Seragnoli Hematology Institute
Acute Leukemia and MDS Group

Laboratory	***Clinical Team***	***Study coordinators***
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