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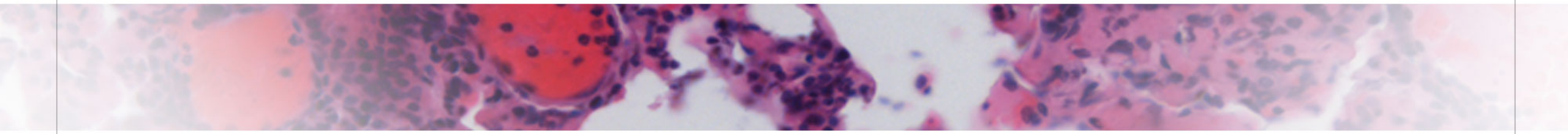


An Immune Senescence and Exhaustion-Related RNA Profile Predicts Clinical Outcomes in Acute Myeloid Leukemia

Jayakumar Vadakekolathu[1], Tung On Yau[1], Heidi Altmann[2], Sarah E. Church[3], Hanna Knaus[4], Mark D. Minden[5], Jan K. Davidson-Moncada[6], Sarah K. Tasian[7], Martin Bornhäuser[2], Ivana Gojo[8], Leo Luznik[8], Sergio Rutella[1,9]

[1]John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK; [2]Department of Internal Medicine I, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [3]NanoString Technologies, Inc., Seattle, WA; [4]Internal Medicine I, Medizinische Universität Wien, Austria; [5]Princess Margaret Cancer Centre, Toronto, Canada; [6]MacroGenics, Inc., Rockville, MD; [7]Division of Oncology and Centre for Childhood Cancer Research, Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, PA; [8]Department of Oncology, Johns Hopkins University, Baltimore, MD; [9]Centre for Health, Ageing and Understanding Disease (CHAUD), School of Science and Technology, Nottingham Trent University, Nottingham, UK

Background

- Chemotherapy refractoriness and disease relapse continue to be significant obstacles to therapeutic success in AML
- We have recently identified bone marrow (BM) IFN-γ-related transcriptional profiles that stratify patients with AML into an immune-infiltrated and an immune-depleted subtype, and that refine the accuracy of survival prediction in response to conventional "3+7" chemotherapy beyond that afforded by the ELN risk category (Vadakekolathu J, *et al*. *Sci. Transl. Med.* 2020; 12: eaaz0463)
- Senescent, natural killer (NK)-like T cells and exhausted T cells in the tumor microenvironment (TME) have defective effector functions (Liu X, et al. *J. Clin. Invest.* 2020; 130: 1073-83)

Senescence and Exhaustion in Anti-Tumor Immunity

- In healthy donors, terminal effector memory $CD8^+$ T cells upregulate NK receptors, but decrease T-cell receptor (TCR) components, and acquire a senescent-like phenotype (Pereira BI, et al. *Nat. Immunol.* 2020, 21: 684–94)
- $CD8^+$ T cells in AML exhibit features of immune exhaustion and senescence (IES), including the upregulation of NK cell-associated transcripts, which persist only in chemotherapy non-responders (Knaus HA, *et al*. *JCI Insight* 2018; 3: e120974)
- The aim of the current study was to determine whether IES correlate with immune infiltration, with prognostic molecular lesions and with clinical outcomes in treatment-naïve AML

Identification of an Immune Senescence and Exhaustion (IES) Gene Signature

7-gene expression score
(NK-related markers; knowledge, prior work)
TCGA-AML and Beat-AML Master Trial Cohorts

Gene name	Full Name
B3GAT1 (CD57)	Beta-1,3-Glucuronyltransferase 1
KIR2DL1 (CD158a)	Killer Cell Immunoglobulin-like Receptor 2DL1
KLRC1	Killer Cell Lectin Like Receptor C1
KLRC3	Killer Cell Lectin Like Receptor C3
KLRD1 (CD94)	Killer Cell Lectin Like Receptor D1
KLRF1	Killer Cell Lectin Like Receptor F1
KLRG1	Killer Cell Lectin Like Receptor G1

Top 25th and bottom 25th quartile

"Immunologic signature" gene sets
(C7; n=4,872) (MSigDB)

Gene Set Enrichment Analysis
(GSEA) – NES > 1.7 and FDR < 0.05

Leading-edge analysis

"Core" gene set accounting for the enrichment signal in top quartile

68 genes

Wet-lab AML cohorts
(NanoString Profiling)

Manually annotated (Knaus HA, et al. JCI Insight 2018; Kallies A, et al. Nat. Rev. Immunol. 2020; SeneQuest Portal, Gene-to-Senescence Associations)

Patients and Methods

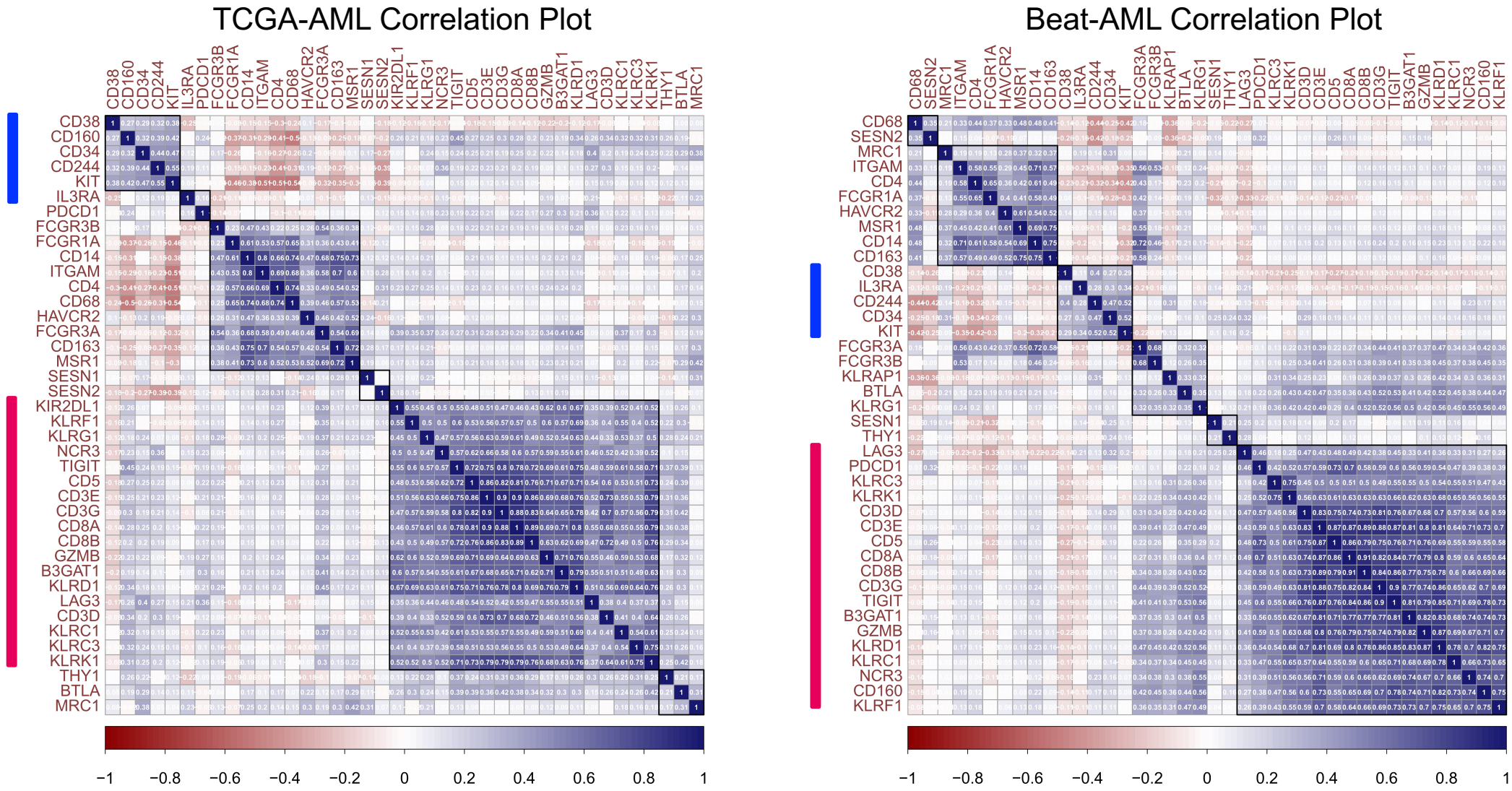
Wet-laboratory cohorts | *In silico* cohorts

	PMCC*	CHOP^	SAL^^		Beat AML Master Trial	TCGA	TARGET
Nr of patients	290	40	46		267	147	145
Age (y)	52 (18-81)	10 (0.1-20)	52.5 (23-75)		Adult	Adult	Pediatric
Disease status	Onset	Onset/CR	Onset/CR/Relapse		Onset	Onset	Onset/Relapse

- Wet-laboratory AML cohorts included a total of 376 BM samples from children and adults with AML treated with curative intent (PMCC, SAL and CHOP series). BMs were collected longitudinally at time of diagnosis, complete remission (CR) and relapse
- RNAs were analyzed on the nCounter® platform using the PanCancer Immune Profiling Panel® (NanoString Technologies, Seattle, WA)
- Immune signature scores and biological activity scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets (Vadakekolathu J, et al. *Sci. Transl. Med.* 2020)

*PMCC = Princess Margaret Cancer Centre, Toronto, Canada – Mark Minden
^CHOP = Children's Hospital of Philadelphia, Philadelphia, PA – Sarah K. Tasian
^^SAL = Studienallianz Leukämie and Technische Universität Dresden, Dresden, Germany – Martin Bornhäuser

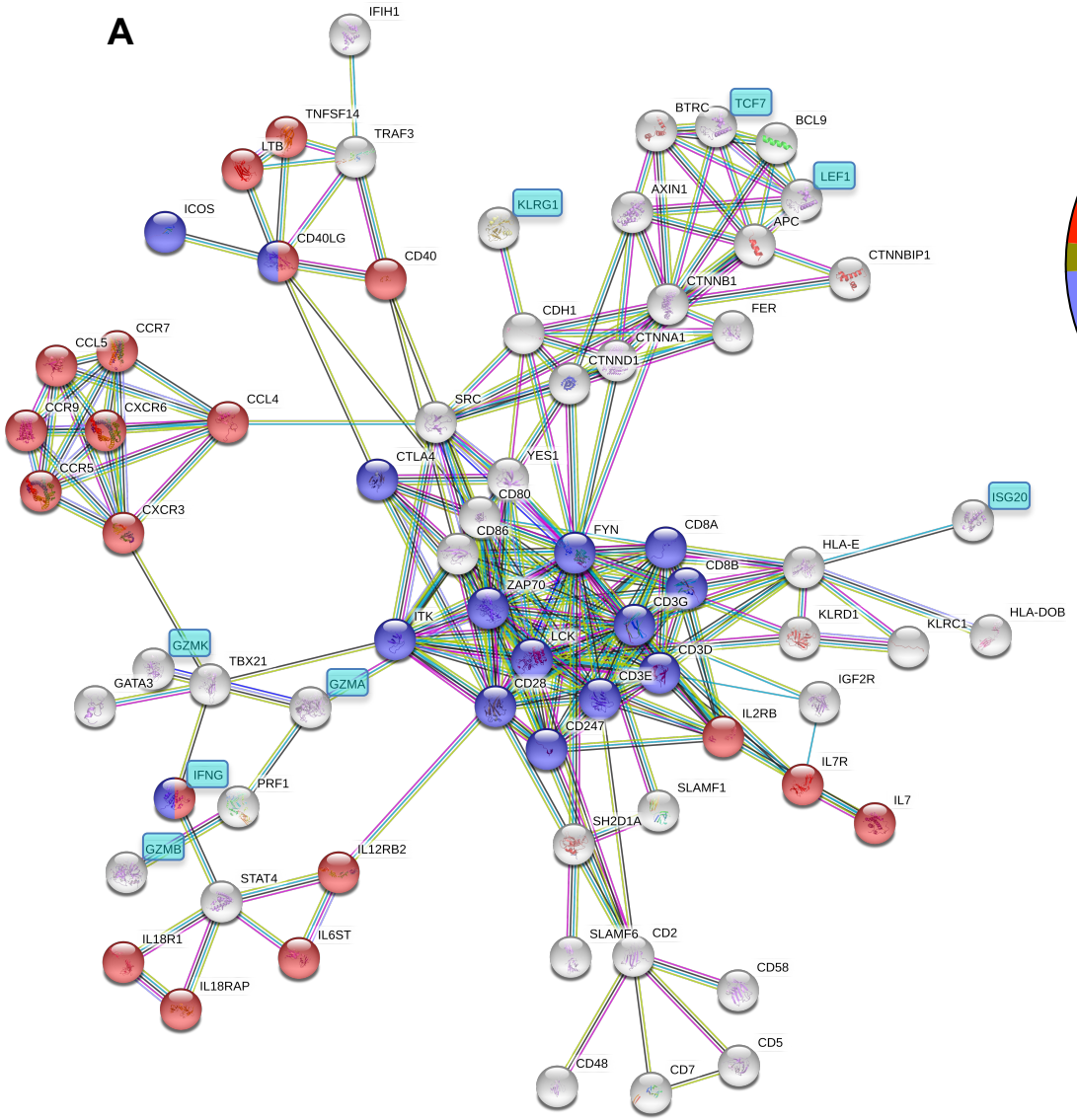
NK Cell-Associated Markers Are Largely Expressed by CD3^{+}CD8^{+} T Cells



IES Signature Genes — Functional Enrichment and Pathway Analysis

STRING Functional Protein Association Network

A



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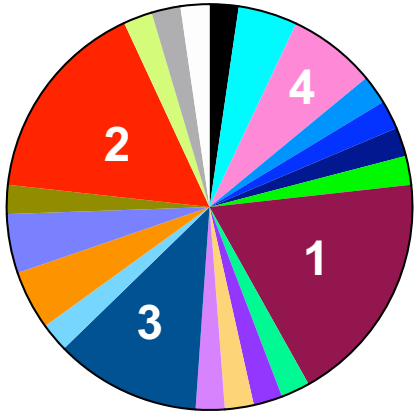


- Axon guidance mediated by semaphorins (P00007)
- Alzheimer disease-presenilin pathway (P00004)
- Angiogenesis (P00005)
- Apoptosis signaling pathway (P00006)
- Parkinson disease (P00049)
- Ras Pathway (P04393)
- T cell activation (P00053)
- Toll receptor signaling pathway (P00054)
- VEGF signaling pathway (P00056)
- Wnt signaling pathway (P00057)
- Cadherin signaling pathway (P00012)
- EGF receptor signaling pathway (P00018)
- Gonadotropin-releasing hormone receptor pathway (P06664)
- Integrin signalling pathway (P00034)
- Interferon-gamma signaling pathway (P00035)
- Interleukin signaling pathway (P00036)
- JAK/STAT signaling pathway (P00038)
- PDGF signaling pathway (P00047)
- Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
- Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade
- Insulin/IGF pathway-protein kinase B signaling cascade (P00033)

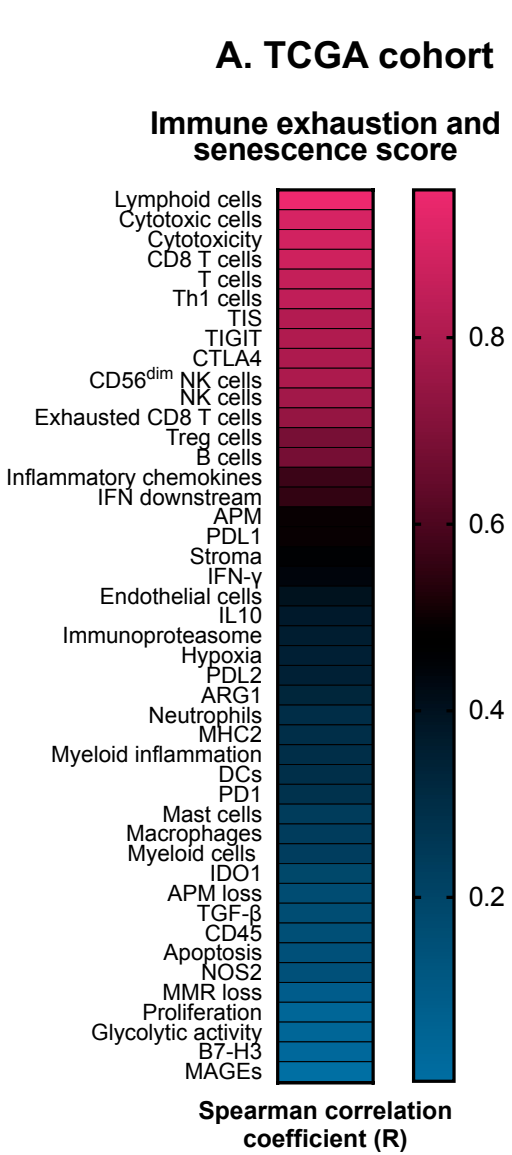
Top pathways (Panther):

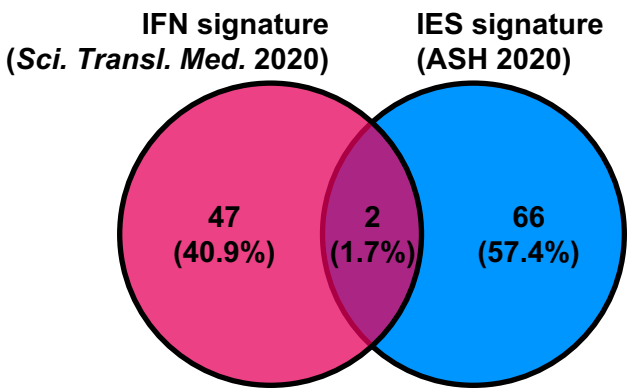
1. Inflammation mediated by cytokine/chemokine signaling
2. T cell activation
3. Interleukin signaling
4. IFN-γ signaling

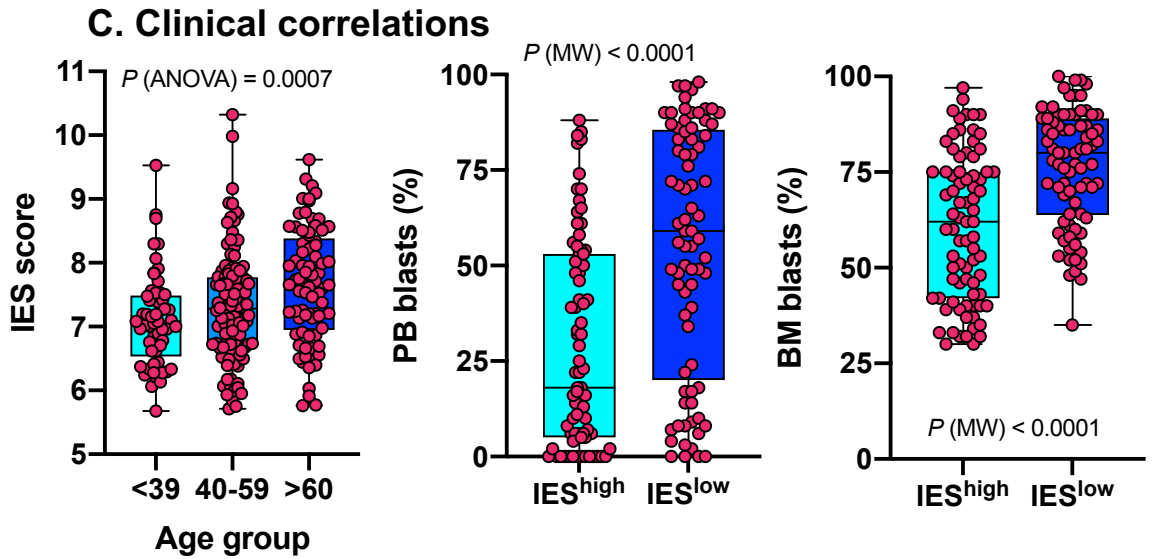
C Selected genes of interest:

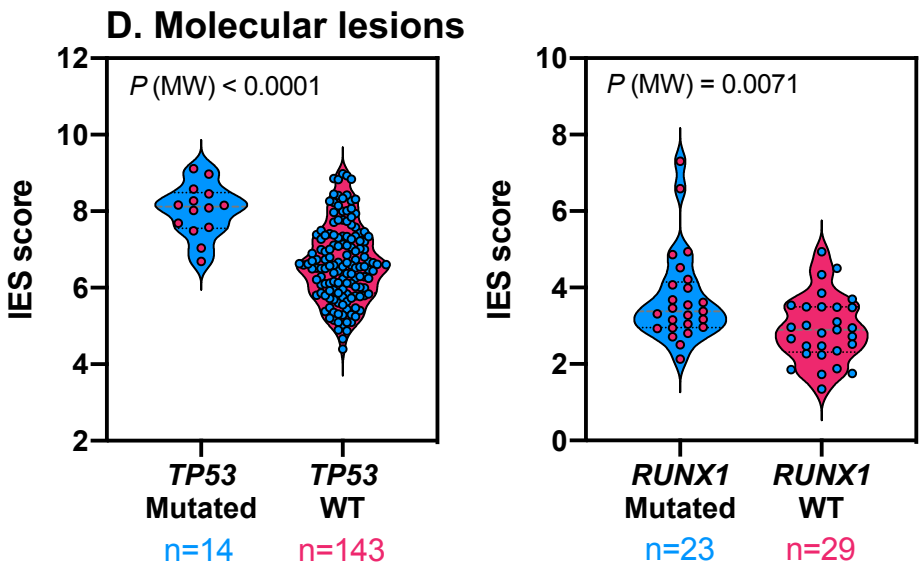
TCF1, *LEF1* (stem cell memory phenotype)
NK cell markers (GZMs, *KLRG1*, *KLRD1*, *KLRC1*)
Type I and II IFN response genes

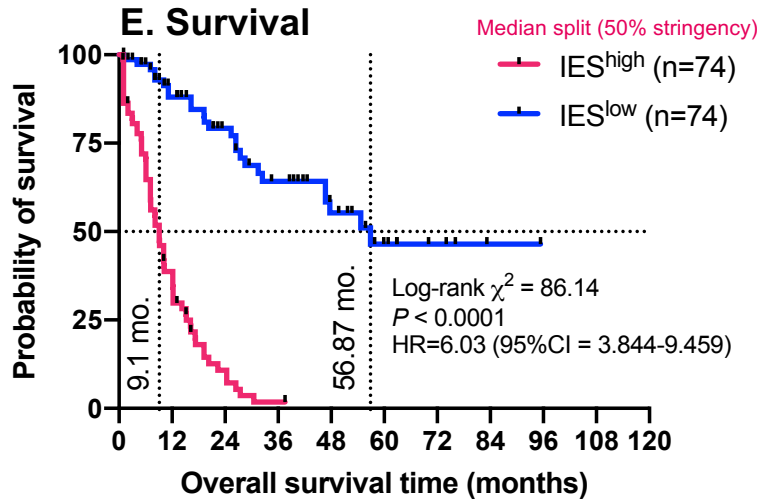
The IES Score Correlates with Immune Infiltration, *TP53* and *RUNX1* Mutations, and Prognosis











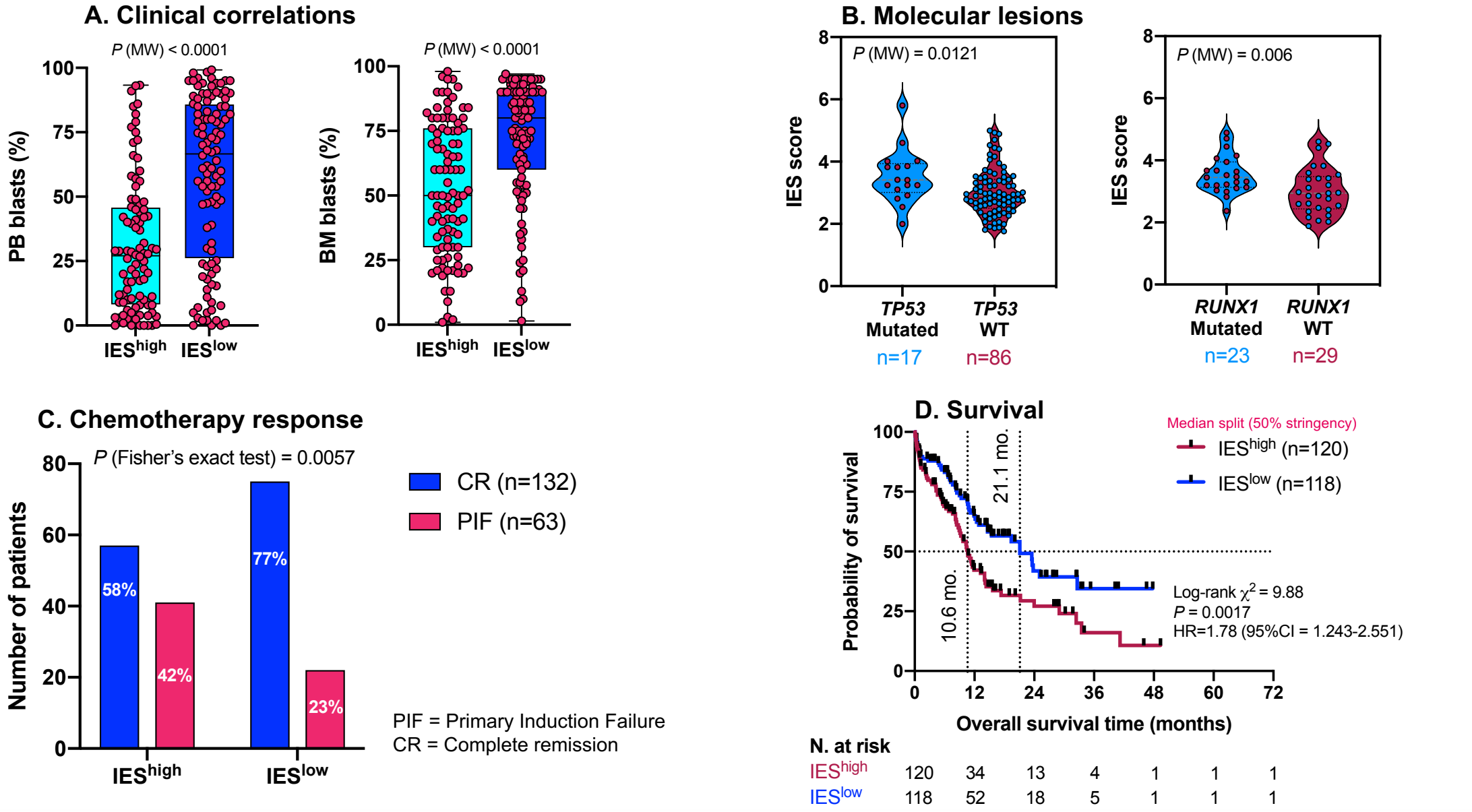
N. at risk

IES^{high}	74	26	6	1	1	1	1	1	1	1	1
IES^{low}	75	56	41	29	19	9	5	2	1	1	1

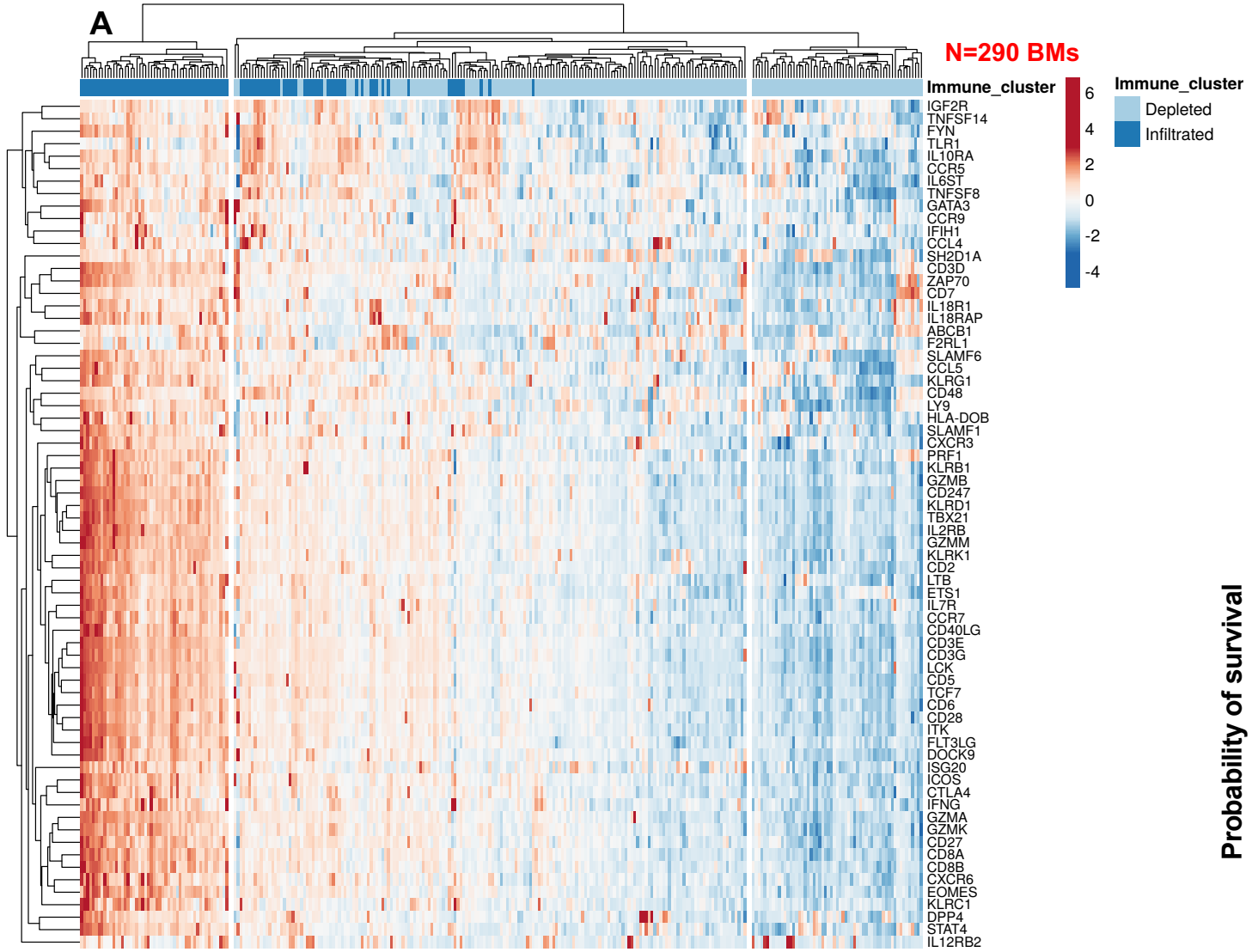
DO NOT POST

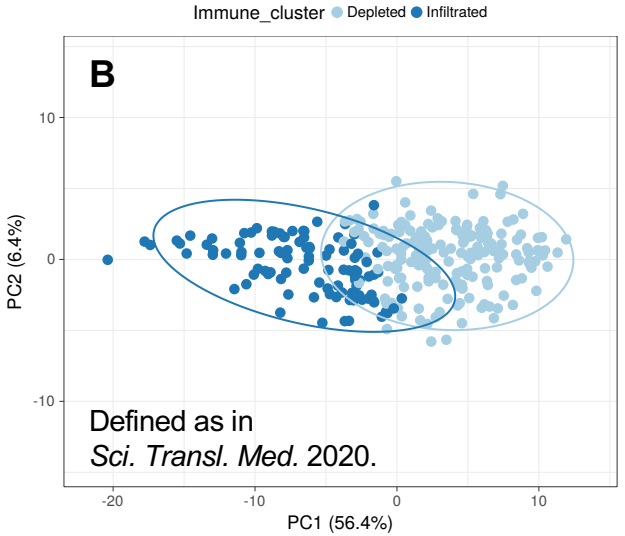
Vadakekolathu J, et al. ASH Presentation #2001, December 6, 2020

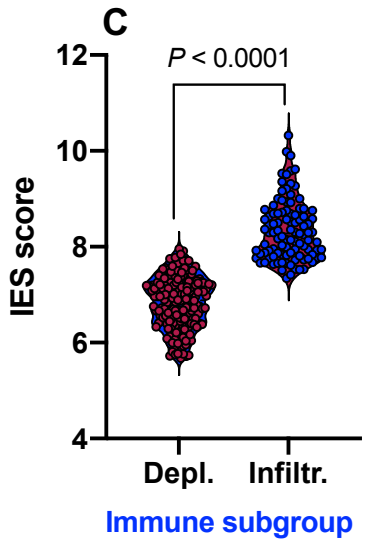
Independent Validation Series — Beat AML Master Trial

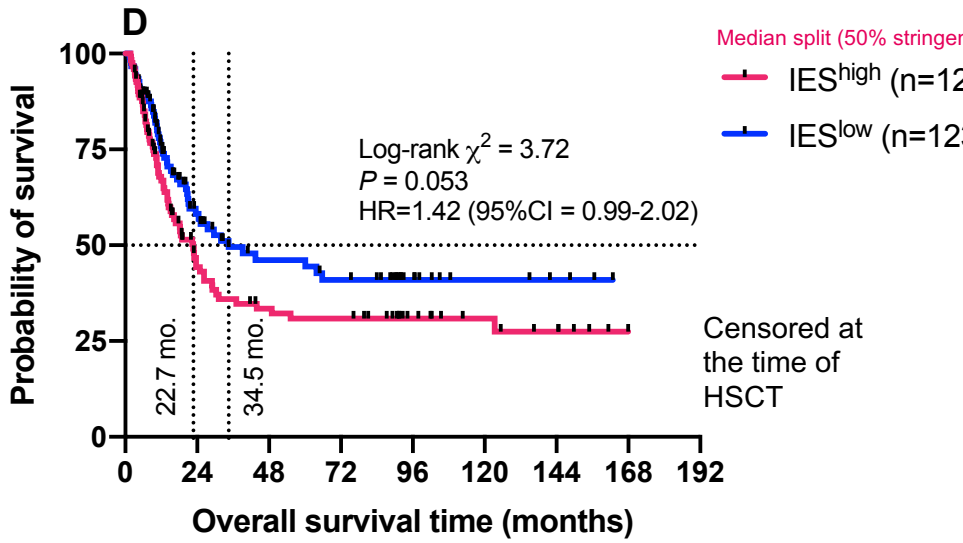


The IES Score Correlates with Immune Infiltration and Prognosis — PMCC Cohort



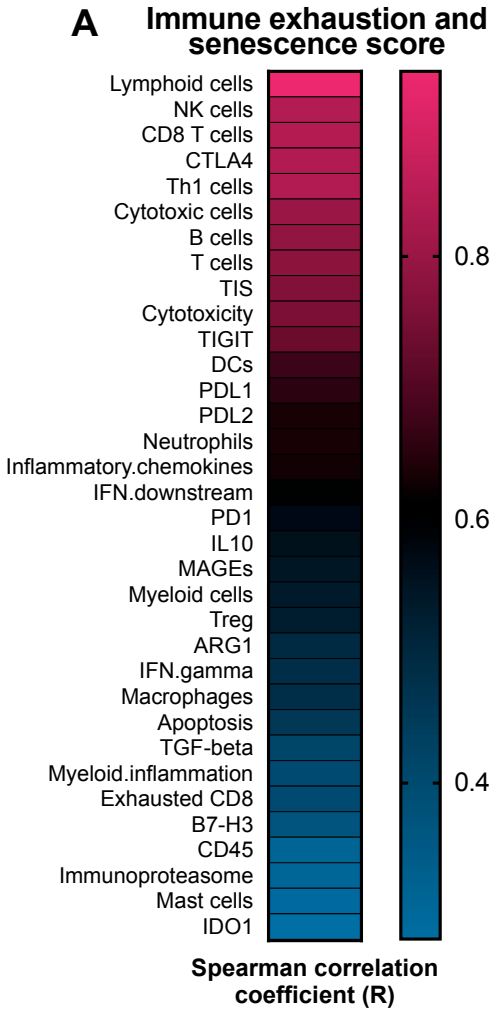


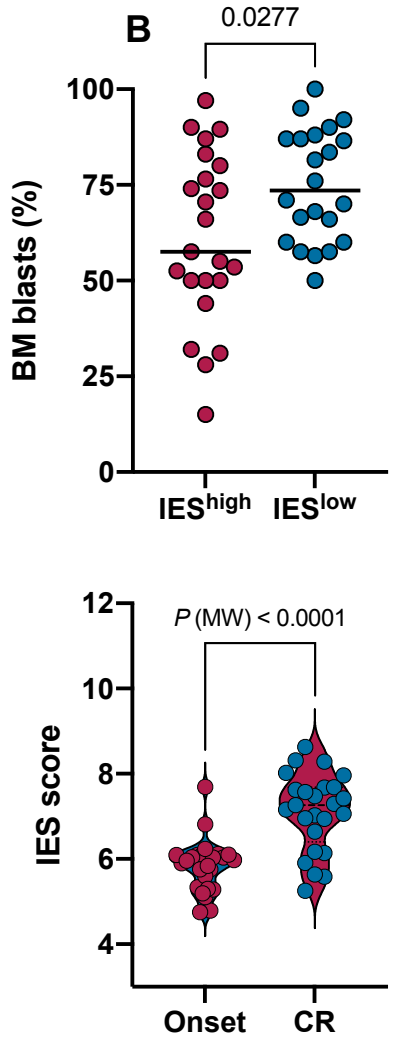


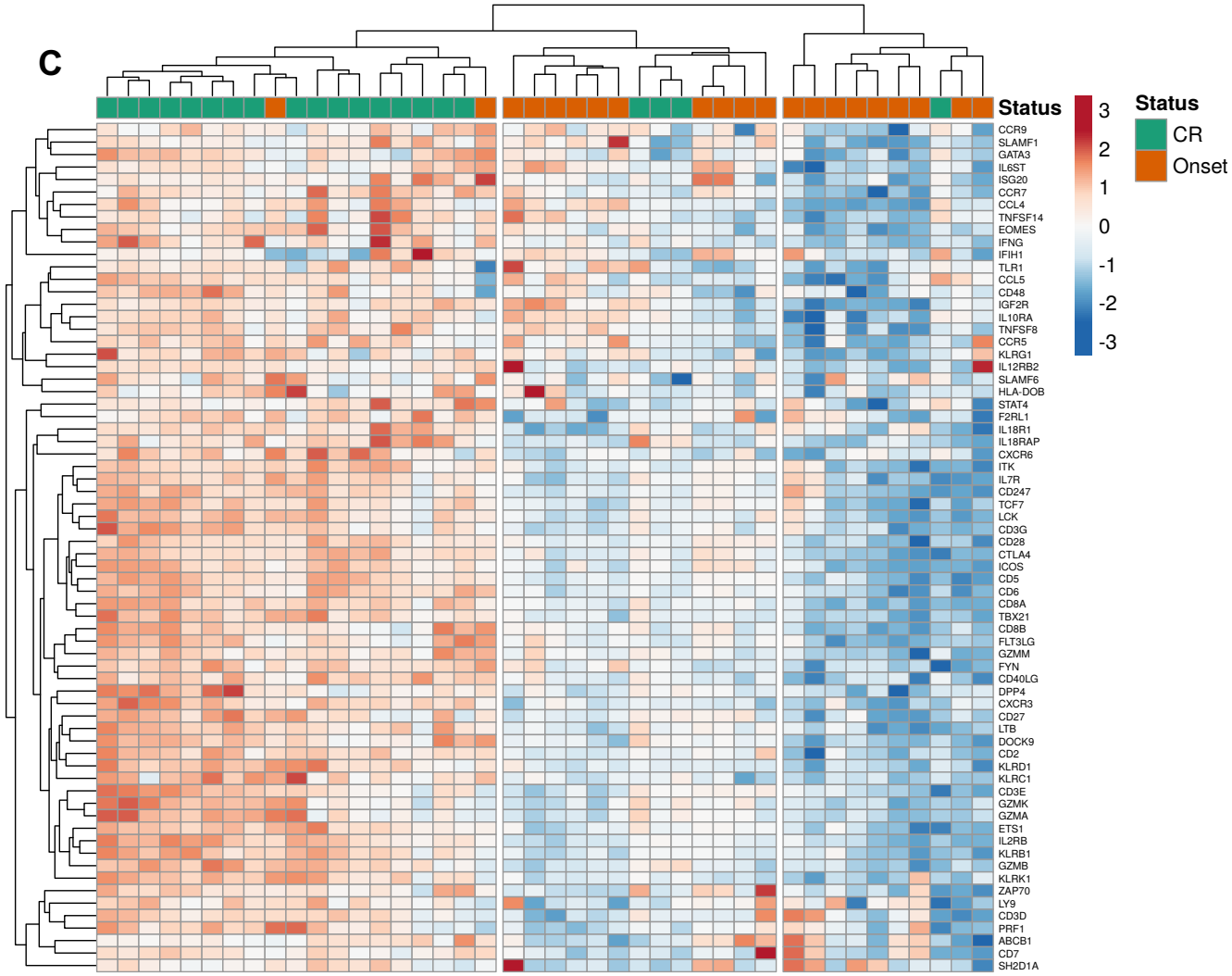


The IES Score Increases at Complete Remission — SAL Cohort

N=46 (onset)

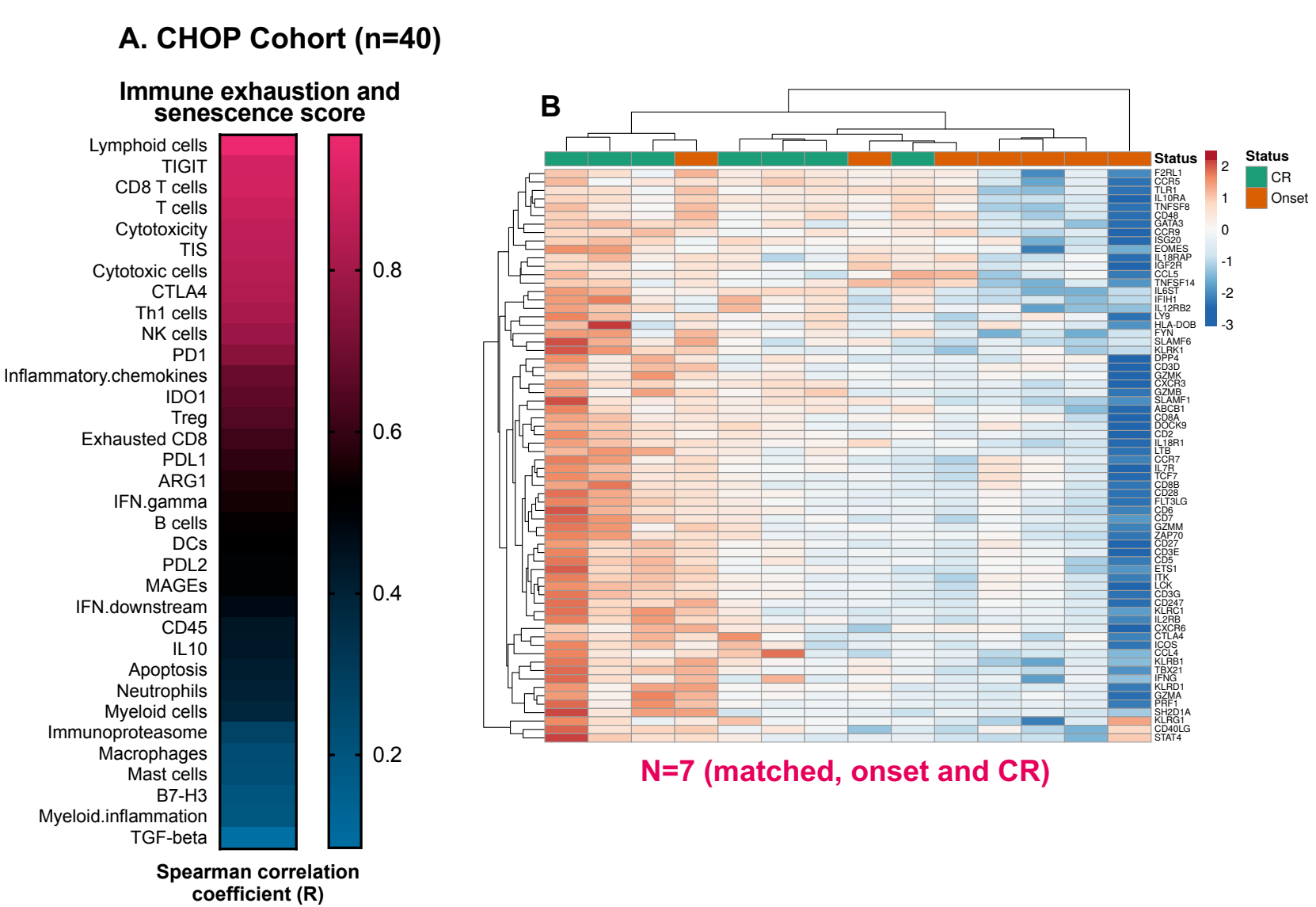






N=21 (matched, onset and CR)

The IES Score in Pediatric AML (CHOP Cohort)



Conclusions

- Patients with immune-infiltrated AML exhibit transcriptomic features of IES, including the up-regulation of NK-like cytotoxicity/effector markers, immune checkpoints and type I/II IFN response genes
- IES correlate with adverse-risk molecular lesions (*TP53* and *RUNX1* mutational status), and with chemotherapy resistance and shorter patient survival
- Senescent/exhausted T cells could be functionally rejuvenated by novel immunotherapies being investigated in AML
  - Microenvironmental Immune Senescence and Exhaustion in Acute Myeloid Leukemia Associate with Response to Flotetuzumab, an Investigational CD123×CD3 Bispecific DART® Molecule (Vadakekolathu J, *et a*l. ASH Presentation #2878; December 7, 2020)

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Co-authors and Collaborators

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Tasleema Patel
Sarah K. Tasian
Philadelphia, PA

Johns Hopkins University

Ivana Gojo
Leo Luznik
Sidney Kimmel Comprehensive Cancer Centre
Baltimore, MD

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Martin Bornhäuser
Marc Schmitz
SAL Studienallianz Leukämie
Dresden, Germany

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Michael Bailey
Joseph M. Beechem
Sarah E. Church
Sarah E. Warren
Seattle, WA

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