NOTTINGHAM TRENT UNIVERSITY

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Immune Landscapes Predict Chemotherapy Resistance and Anti-Leukemic Activity of Flotetuzumab, an Investigational CD123 × CD3 Bispecific DART® Molecule, in Patients with Relapsed/Refractory Acute Myeloid Leukemia

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Background

- Cytotoxic chemotherapy remains the standard-of-care for most patients with acute myeloid leukemia (AML), despite the recent approval of novel agents
- Immunotherapies such as monoclonal antibodies, bispecific molecules, immune checkpoint blockade (ICB) and CD123-CAR T cells are currently under investigation in AML
- There is an urgent need to identify predictive biomarkers in the tumor immunological microenvironment (TME)
  - IFN-γ-related mRNA profiles ("T cell-inflamed" GEP or "Tumor Inflammation Signature", TIS) predict response to pembrolizumab in multiple solid tumor types (Ayers M, et al. *JCI* 2017; Ott PA, et al. *JCO* 2019)
- Flotetuzumab, a CD123 × CD3 bispecific DART® molecule, is being tested in a phase 1 clinical trial of relapsed/refractory (R/R) AML (NCT#02152956)
- See also presentation #733. Monday, December 9, 2019: 2:45PM
  - Dr. Geoffrey Uy, Session #613. *Acute Myeloid Leukemia: Clinical Studies: Treatment of Relapsed/Refractory Disease*. Tangerine 3 (Orange County Convention Center)

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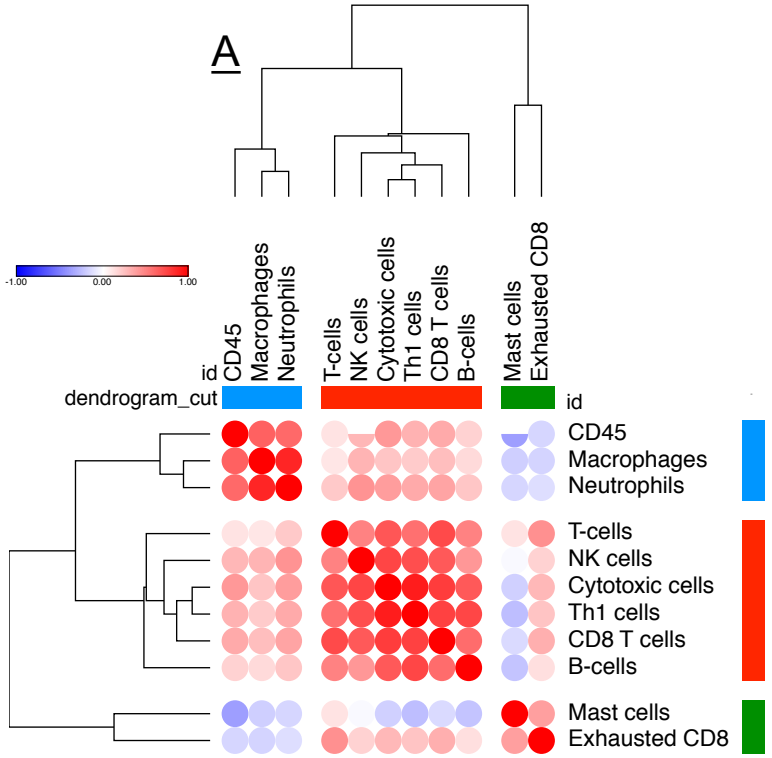
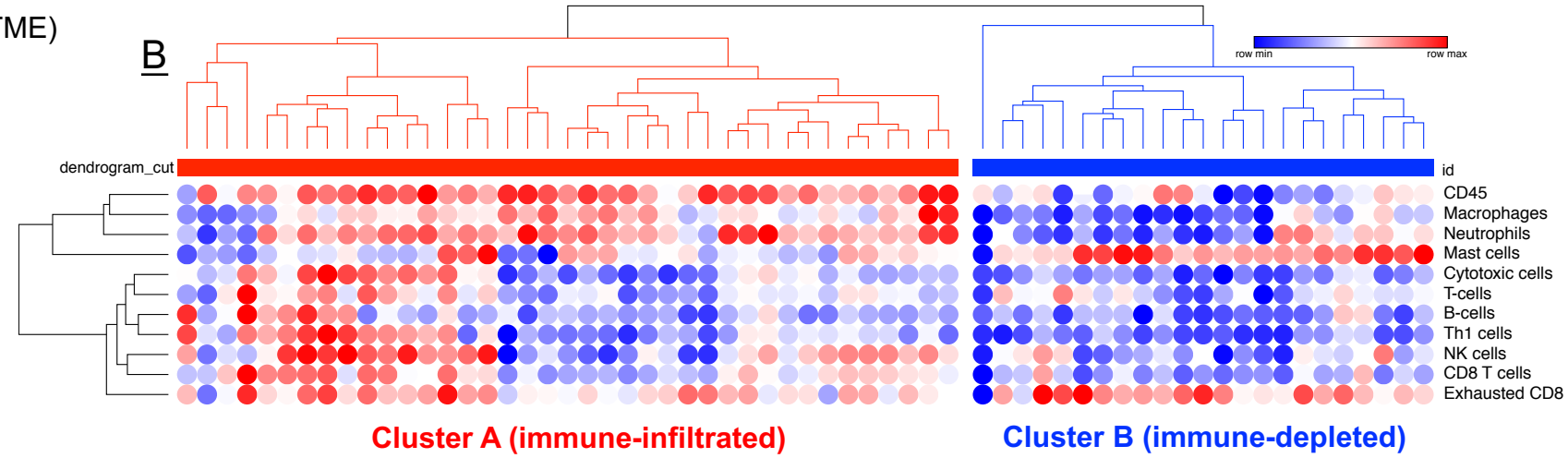
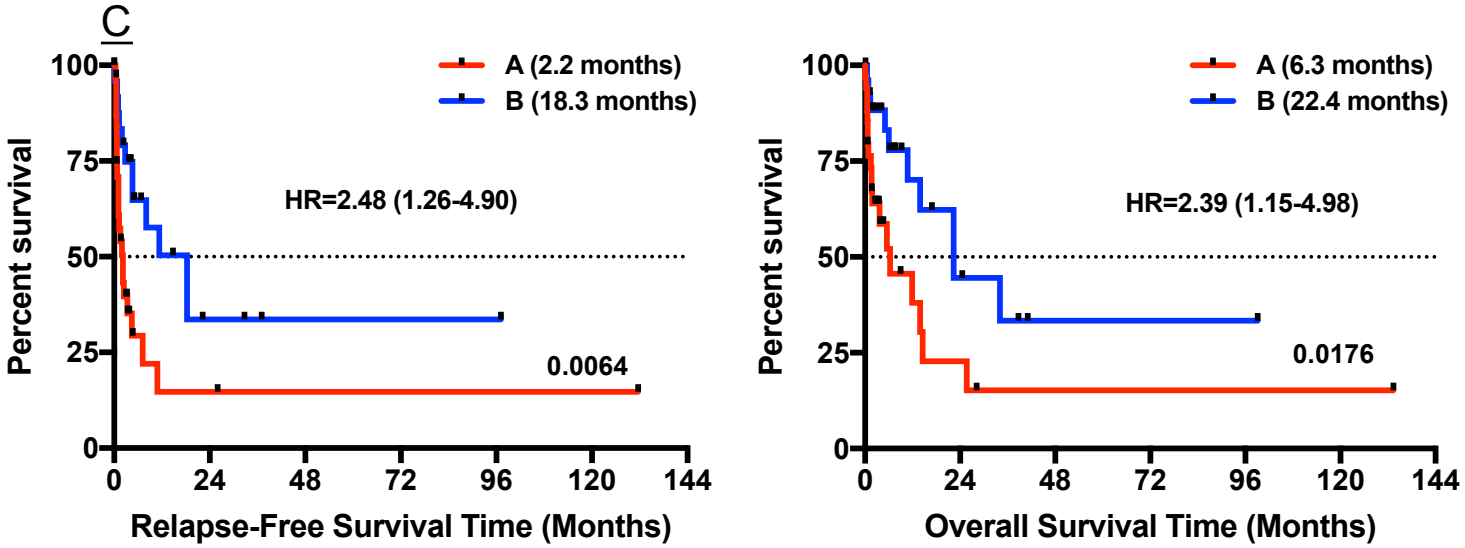
Diversity of immune landscapes in AML

The AML tumour immunological microenvironment (TME)

1. Innate (PMN, macrophages)
2. Adaptive (T, B, NK, CTL)
3. Mast cells, exhausted CD8$^+$ T cells







Discovery cohort (n=62)

34 non-promyelocytic *de novo* childhood AML
(Sarah K. Tasian, Children's Hospital of Philadelphia, USA)
28 non-promyelocytic *de novo* adult AML
(Martin Bornhäuser, Dresden, Germany)

Vadakekolathu J, Patel T, Reeder S, et al. Blood 2017; 130: 3942A.

Patient series and methods

Wet-lab cohorts

	PMCC*	CHOP^	SAL^^
Nr of patients	290	39	38
Age (y)	52 (18-81)	10 (0.1-20)	52.5 (23-75)
Disease status	Onset	Onset	Onset/CR/Relapse

In silico cohorts

HOVON	Beat AML Master Trial	TCGA
618	267	147
Adult	Adult	Adult
Onset	Onset	Onset

- The PanCancer Immune Profiling Panel (NanoString Technologies, Seattle, WA) was used to measure mRNA expression in bulk BM samples (n=770 immune-related genes)
- Immune signature scores and biological activity scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets
- Gene expression data have been deposited in NCBI's Gene Expression Omnibus and will be accessible through GEO Series accession number GSE134589

*PMCC = Princess Margaret Cancer Centre, Toronto, Canada – **Discovery cohort**
^CHOP = Children's Hospital of Philadelphia, Philadelphia, PA
^^SAL = Studienallianz Leukämie, Dresden, Germany

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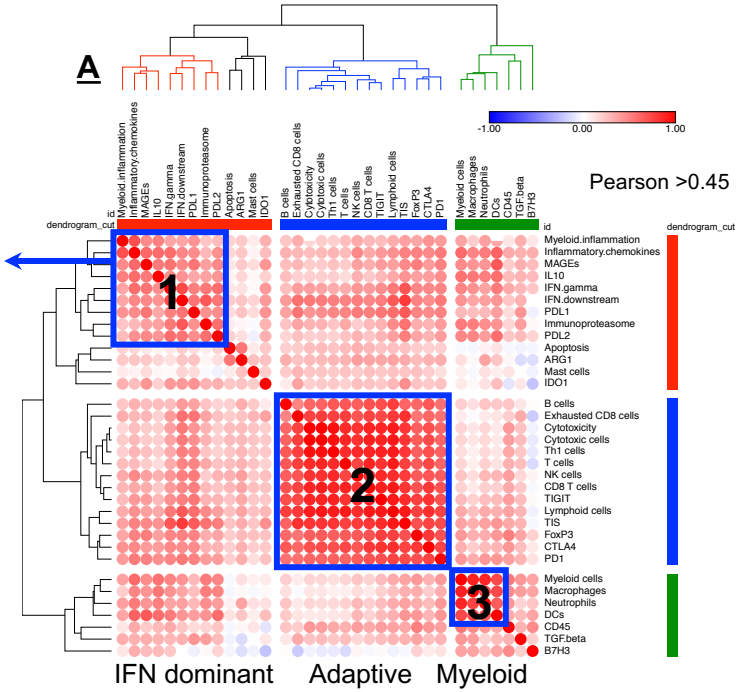
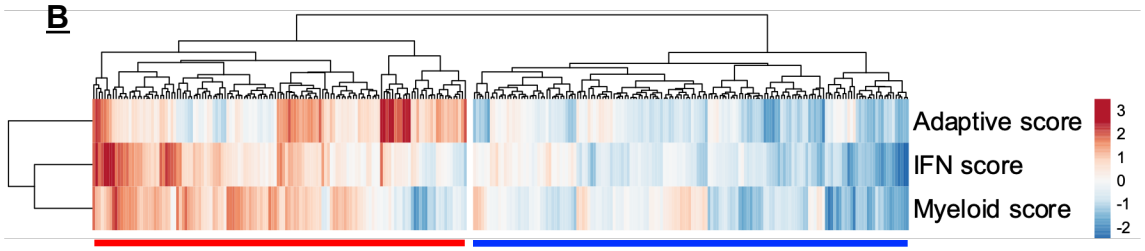
Immune landscapes assist stratification

Discovery cohort (n=290 patients)

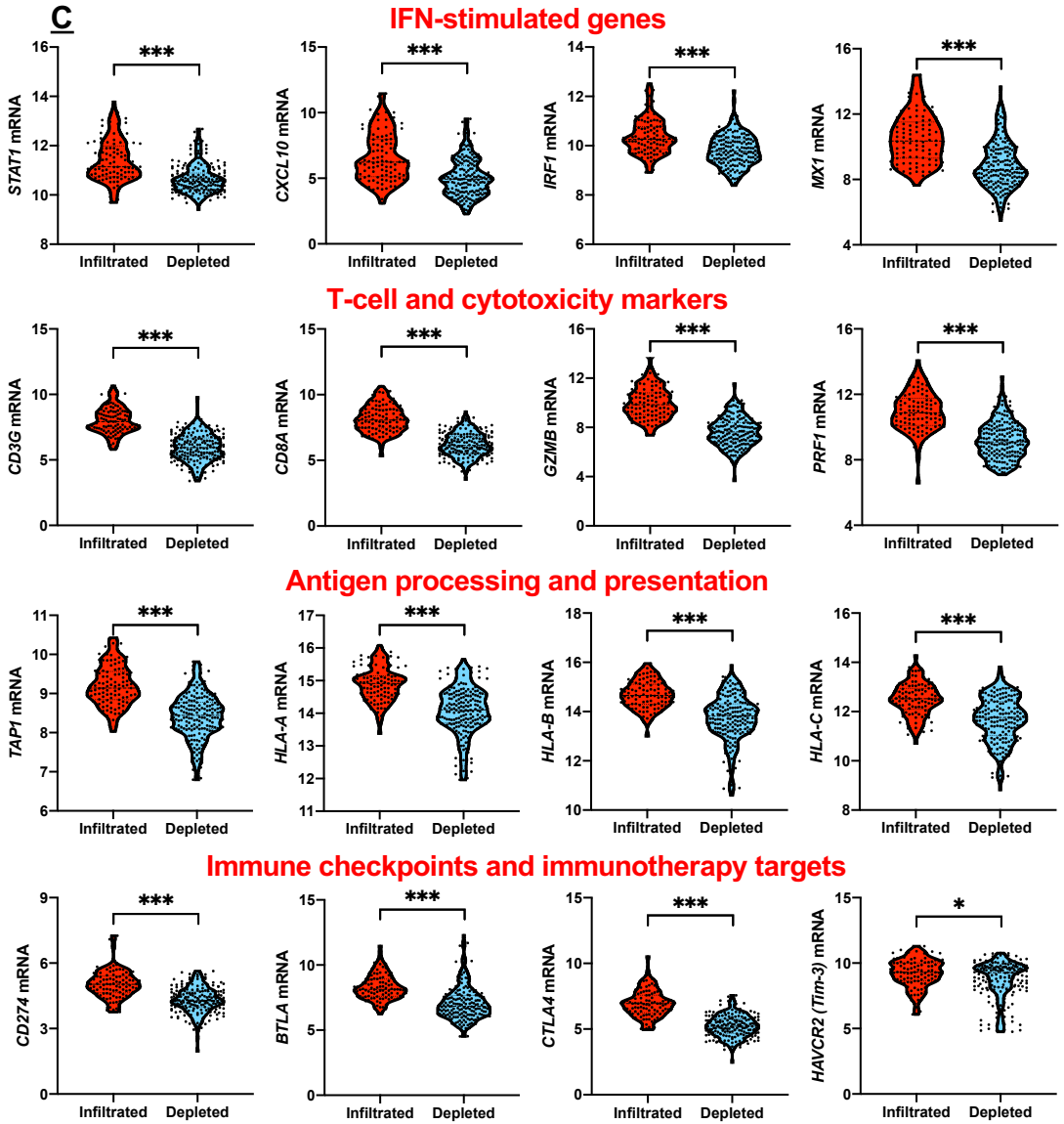
"IFN module" gene score
Myeloid inflammation
Inflammatory chemokines
Downstream IFN signaling
IFN-γ
PDL1
PDL2
MAGEs
IL10
Immunoproteasome





Immune-infiltrated (n=136)
(IFN + Adaptive + Myeloid)

Immune-depleted (n=154)



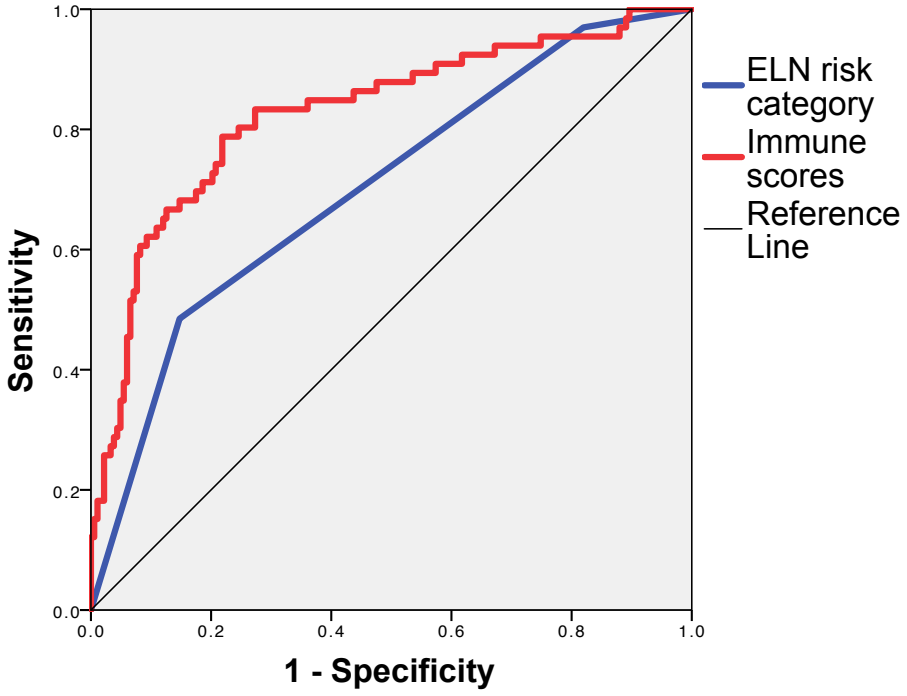
Vadakekolathu J, et al. bioRxiv 2019; DOI: 10.1101/702001. Under revision.

*P<0.05; ***P<0.001

Prediction of chemotherapy response

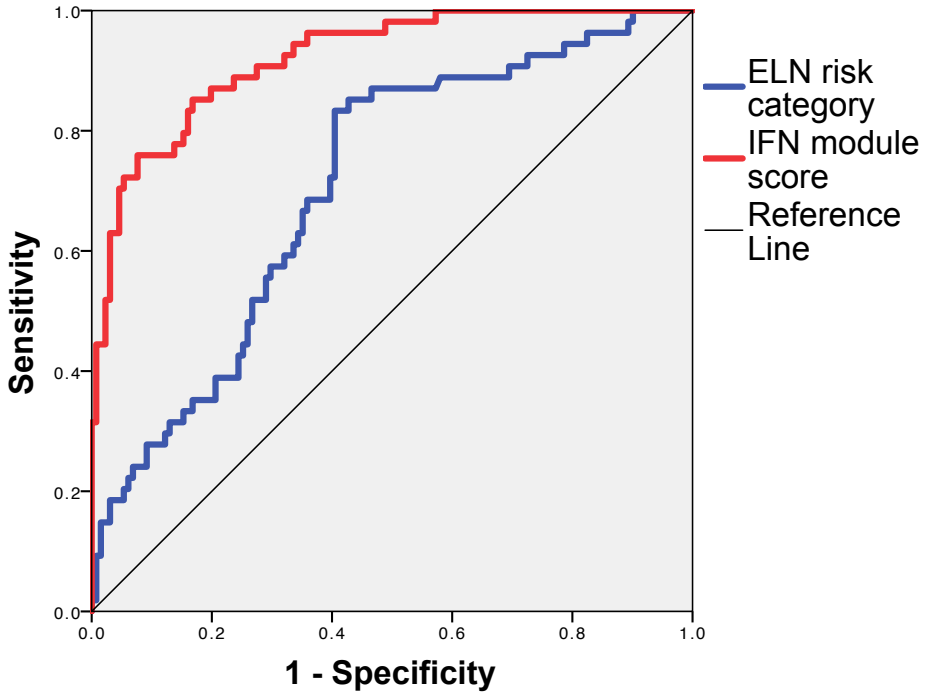
Therapy resistance ('3+7' backbone) was defined as failure to achieve CR in patients who survive at least 28 days (primary refractory AML) or as early relapse (less than 3 months after achieving CR)

A) PMCC discovery series (n=290)



Variable	AUROC	SE	95% CI
Immune scores	0.815	0.031	0.755-0.876
ELN risk	0.702	0.038	0.628-0.776

B) Beat-AML Master Trial validation series (n=196)



Variable	AUROC	SE	95% CI
IFN scores	0.921	0.04	0.88-0.961
ELN risk	0.709	0.021	0.629-0.788

Vadakekolathu J, et al. bioRxiv 2019; DOI: 10.1101/702001. Under revision.

Translational research question

IFN-γ-related gene signatures reflecting an immune-infiltrated TME are associated with adverse prognosis in patients with AML receiving conventional chemotherapy

Are immune-infiltrated TMEs, and IFN-γ gene signatures in particular, associated with sensitivity to targeted immunotherapy with flotetuzumab, a CD123 × CD3 DART bispecific molecule?

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Flotetuzumab immunotherapy

- Immune gene expression was analyzed in a subgroup of patients (n=30/50) with relapsed/refractory AML treated with flotetuzumab (**NCT#02152956**) at RP2D (500 ng/kg/day; Uy, *et al.* ASH 2017; Uy, *et al.* ASH 2018; Rutella, *et al*. ASH 2018)
  - 30 BM samples analyzed at baseline
  - 19 BM samples analyzed “on treatment” (post-cycle 1)
- The NanoString PanCancer IO360™ assay was used to interrogate the expression of 770 genes, including the abundance of 14 immune cell types and 32 immuno-oncology signatures
  - Signature scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets

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Patients and Methods

Characteristic			Patients (n=30)*
Age (median and range)			57 years (27-74)
Gender	Male		16 (53%)
	Female		14 (47%)
Disease status at study entry	Relapse (CR with initial duration >6 months)		7 (23%)
	Refractory	Primary induction failure (PIF; ≥2 induction attempts)	**17 (57%)**
		Early relapse (CR with initial duration <6 months)	6 (20%)
2017 ELN risk stratification	Favorable		6 (20%)
	Intermediate		7 (23%)
	Adverse		**17 (57%)**
Secondary AML			12 (40%)
Number of prior lines of therapy (median and range)			3 (1-9)

*Subgroup of 30/50 patients treated at the RP2D for whom BM samples were available

Response assessment criteria employed in analysis:

Anti-leukemic activity (ALA): CR/CRi, PR, “other benefit” (>30% decrease in BM blasts)

Non-responders (NR): treatment failure, stable disease, progressive disease

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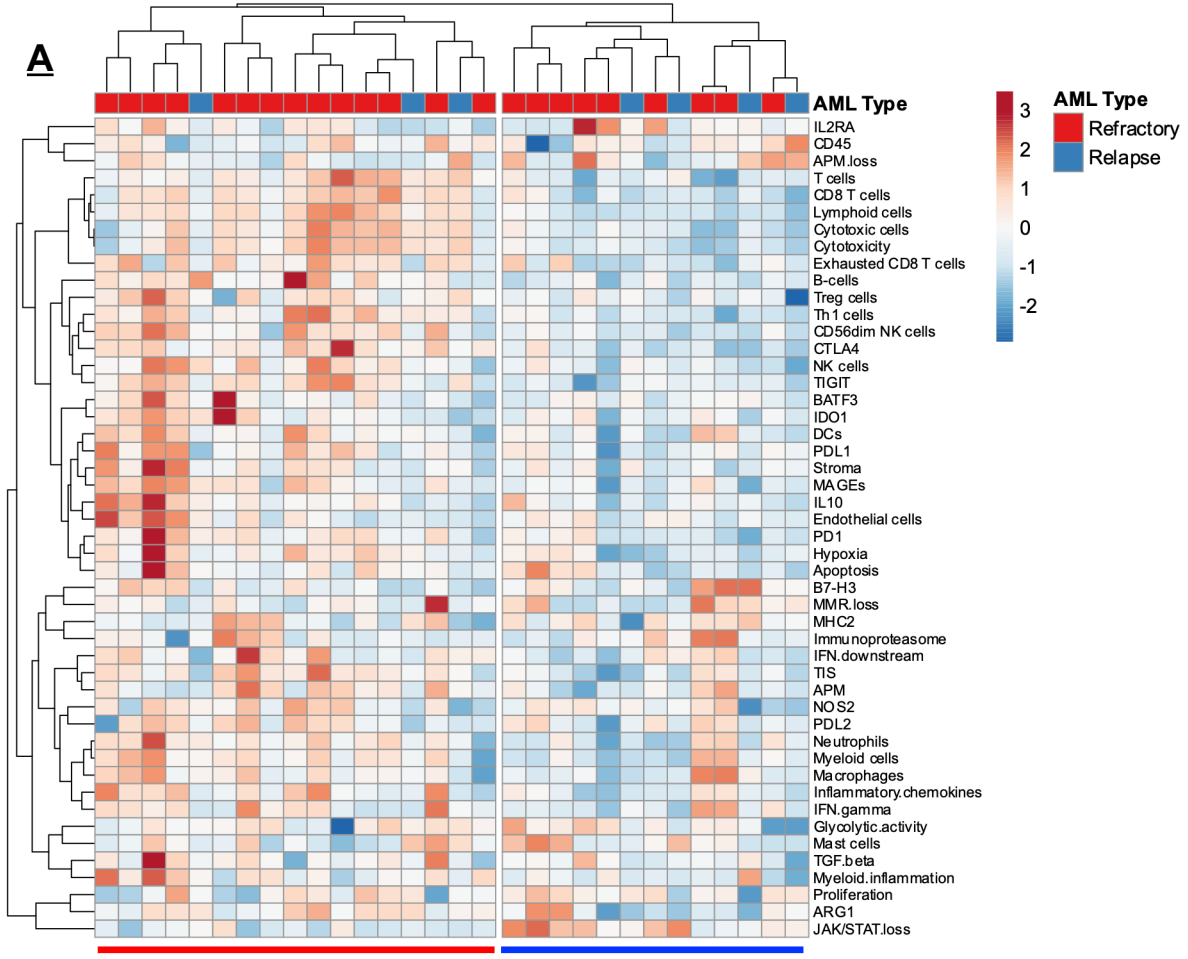
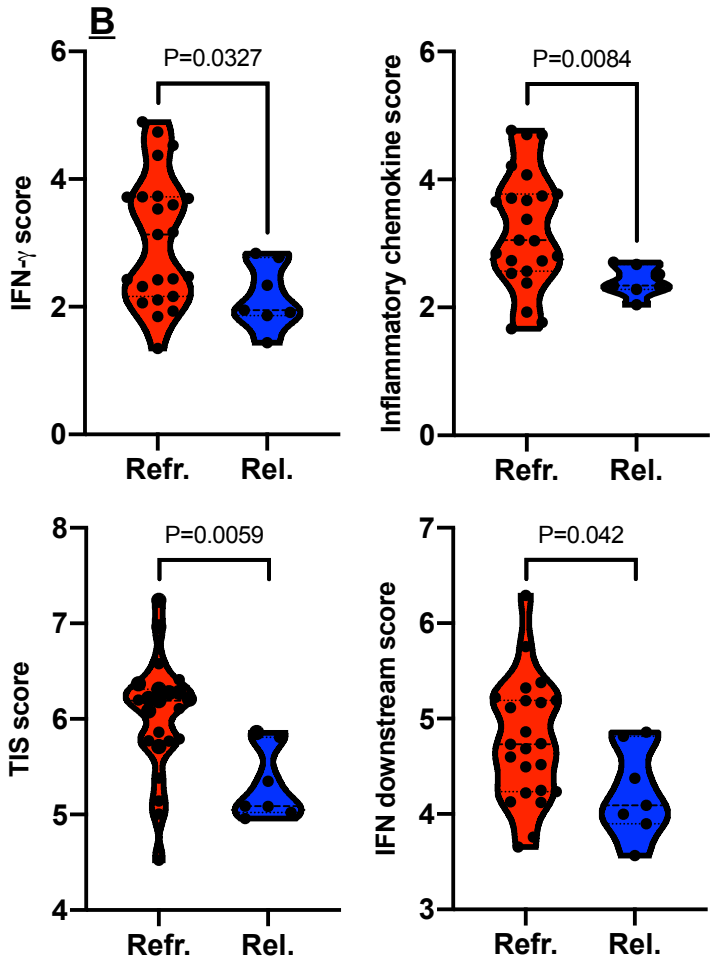
‘Hot’ TME in chemorefractory AML

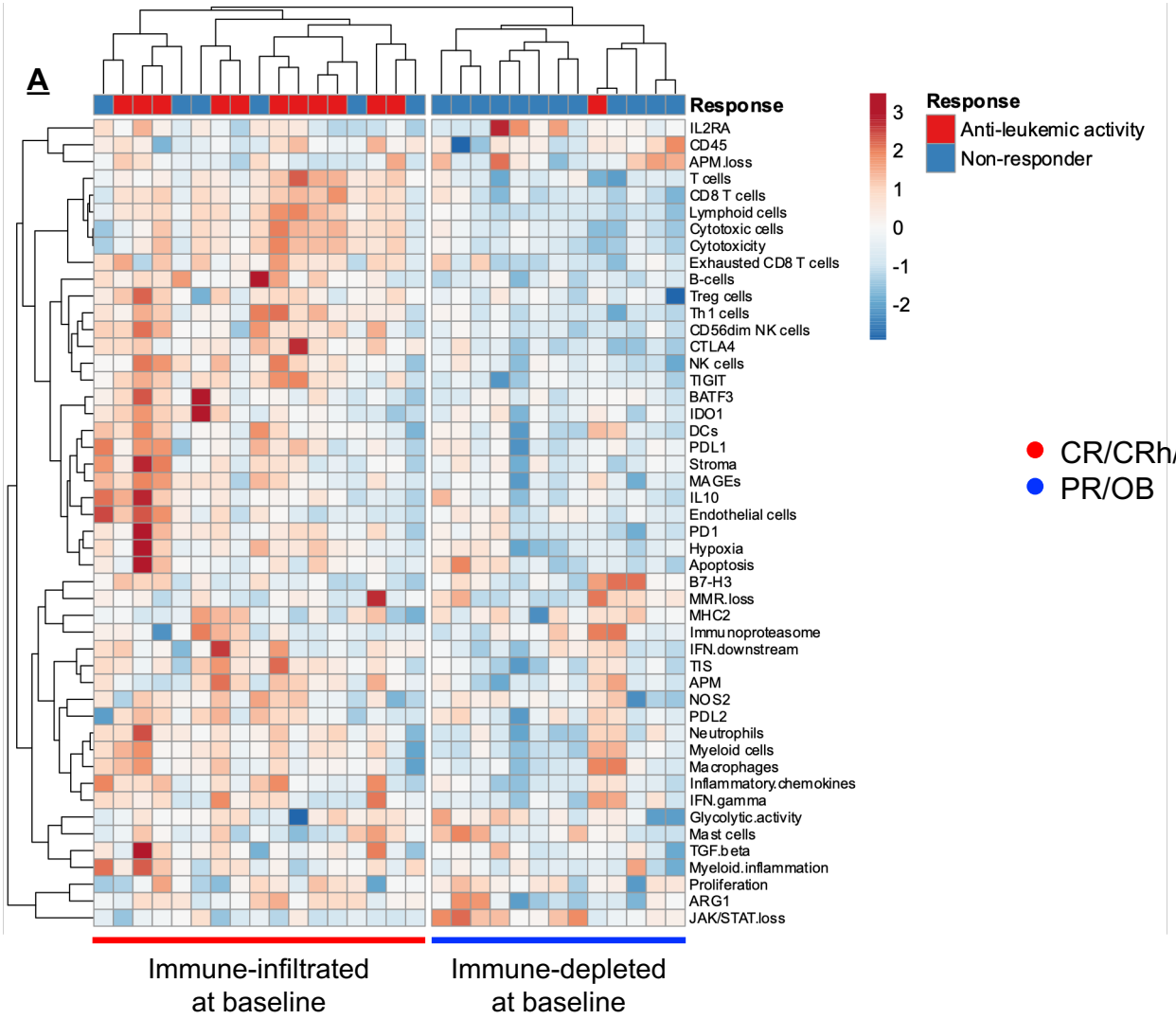


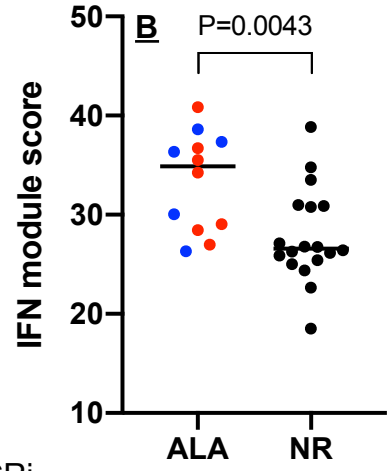


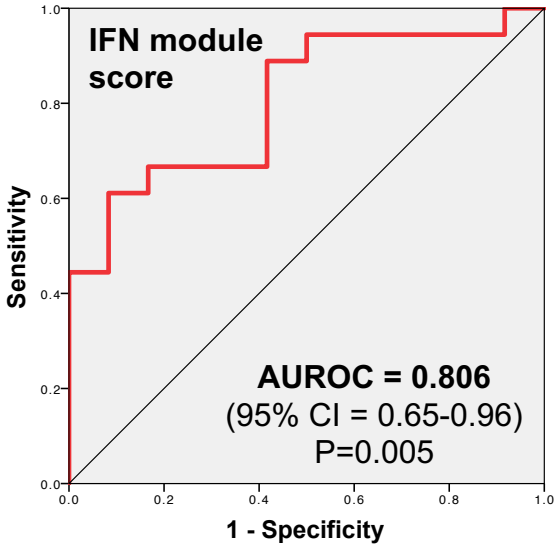
Mann Whitney *U* test for unpaired determinations
Refractory = Primary induction failure (PIF) + early relapse (ER)

Vadakekolathu J, et al. bioRxiv 2019; DOI: 10.1101/702001. Under review.

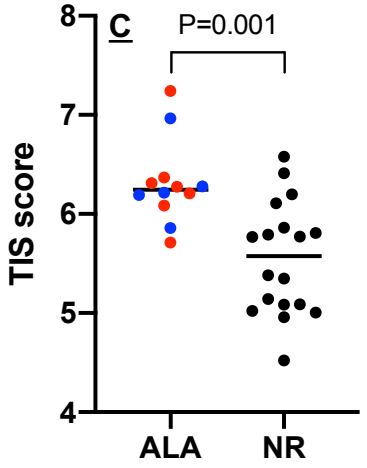
IFN-related profiles and response to flotetuzumab



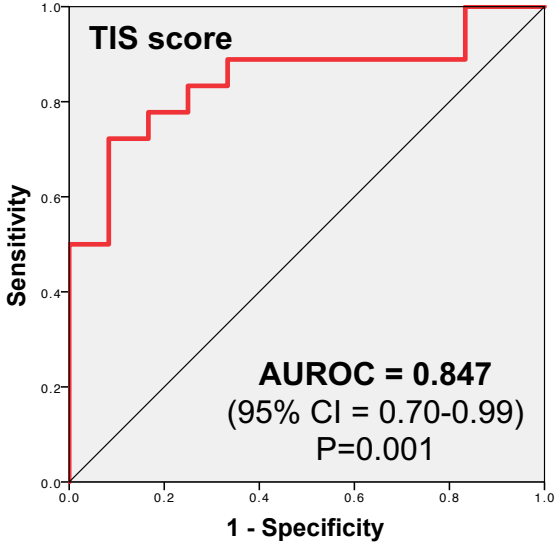




CR/CRh/CRi
PR/OB

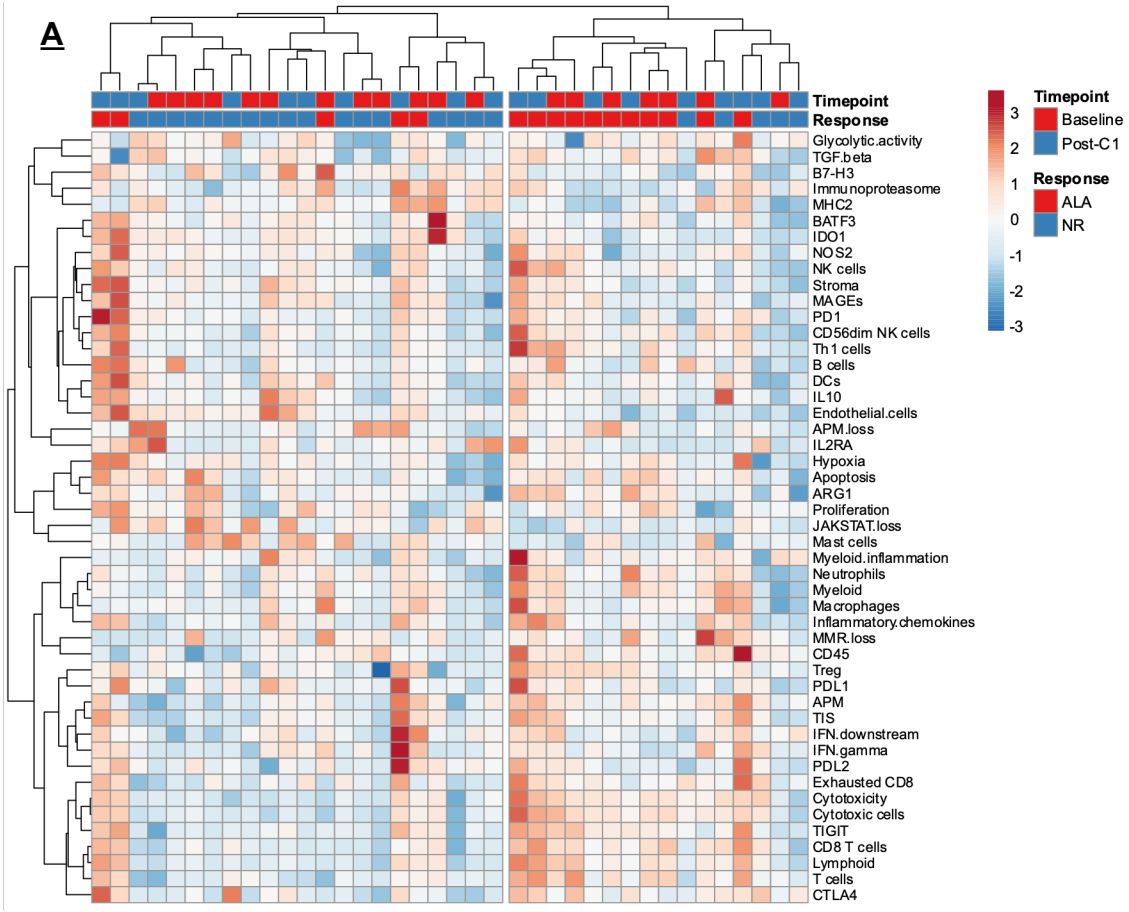


Mann Whitney *U* test for unpaired determinations

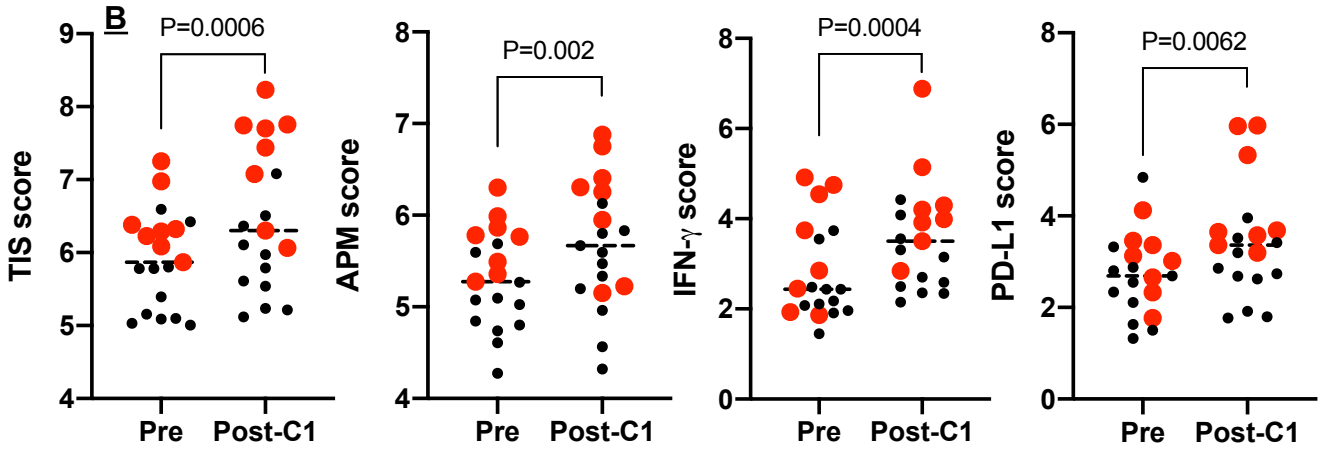


Vadakekolathu J, et al. bioRxiv 2019; DOI: 10.1101/702001. Under review.

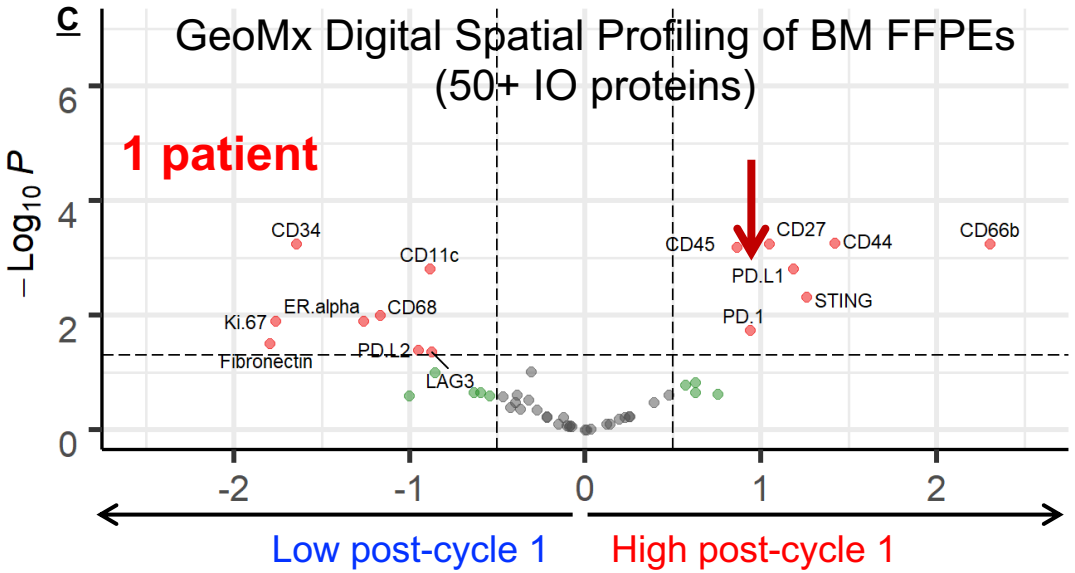
Flotetuzumab modulates the TME



Matched baseline-post-C1 BMs available for 19 patients treated with flotetuzumab



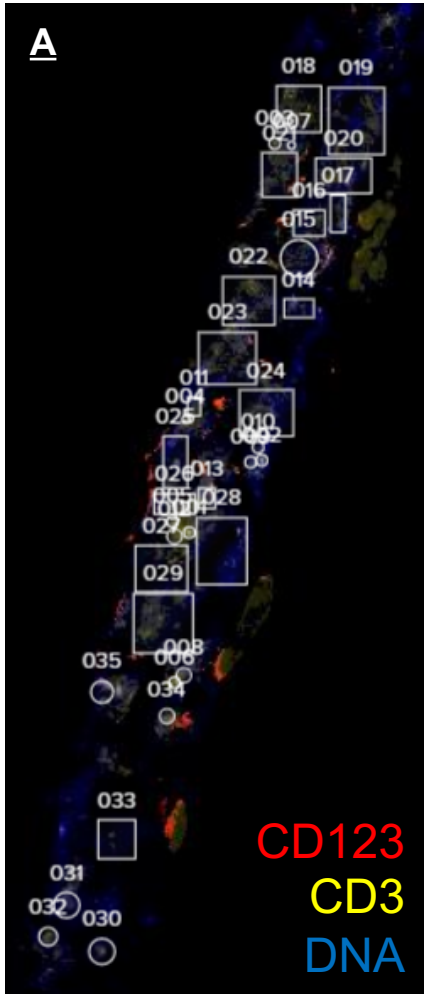
● Anti-leukemic activity ● No response Wilcoxon matched-pairs signed rank test



Vadakekolathu J, et al. bioRxiv 2019; DOI: 10.1101/702001. Under review and Unpublished.

Flotetuzumab modulates the TME

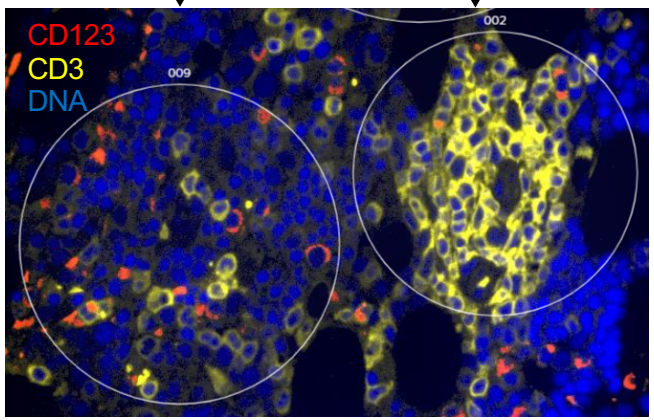
GeoMx Digital Spatial Profiling
of 2 BM FFPEs (50+ IO proteins)

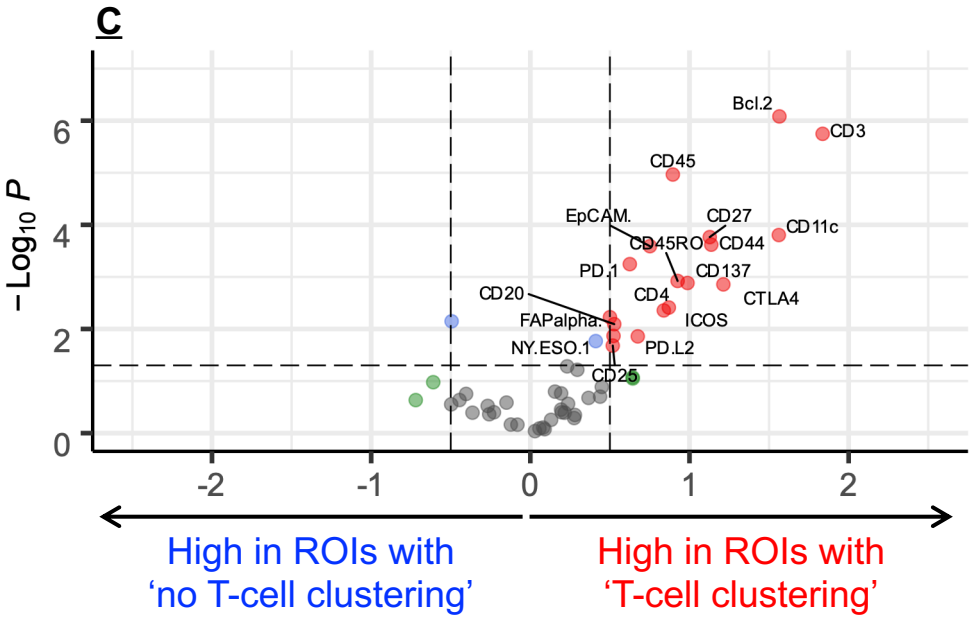


2 patients achieving CR

Region of interest (ROI) with
no 'T-cell clustering'

ROI with
'T-cell clustering'





- N.S.
- Log2 FC
- Adjusted *p* value
- Log2 FC + Adjusted *p* value

Rutella S, et al. 2019 (unpublished) and Godwin JE, et al. Poster #1410. ASH 2019.

Conclusions

- Transcriptional programs that reflect high immune infiltration and IFN-γ signaling enrich in a subset of patients with AML and predict chemotherapy resistance
- IFN-γ-related mRNA profiles at baseline correlate with anti-leukemic activity of flotetuzumab at the RP2D
- A subgroup of patients with an immune-infiltrated TME show high expression of immune checkpoints, including PD-L1, suggesting potential enhanced benefit from flotetuzumab in combination with ICB
  - A phase I study of flotetuzumab combined with MGA012, an anti-PD1 antibody, is ongoing in patients with R/R AML (Wei AH, *et al*. Poster #2662; ASH 2019)

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