NOTTINGHAM TRENT UNIVERSITY

60th Annual Meeting of the American Society of Hematology
San Diego, 1-4 December 2018

Adaptive Immune Gene Signatures Correlate with Response to Flotetuzumab, a CD123 × CD3 Bispecific DART® Molecule, in Patients with Relapsed/Refractory Acute Myeloid Leukemia

Sergio Rutella, MD, PhD, FRCPath[1], Sarah E. Church, PhD[2*], Jayakumar Vadakekolathu, PhD[1*], Elena Viboch, MSc[2*], Amy H. Sullivan, BSc[2*], Tressa Hood, MSc[2*], Sarah E. Warren, PhD[2*], Alessandra Cesano, MD, PhD[2], Ross La Motte-Mohs, PhD[3*], John Muth, MS[3*], Helene Lelièvre, PhD, PharmD[4*], Bob Löwenberg, MD, PhD[5], John F. DiPersio, MD, PhD[6] and Jan K. Davidson-Moncada, MD, PhD[3]

[1]John van Geest Cancer Research Centre, Nottingham Trent University, United Kingdom
[2]NanoString Technologies Inc., Seattle, WA
[3]MacroGenics Inc., Rockville, MD
[4]Servier, Paris, France
[5]Department of Hematology, Erasmus University Medical Center, Rotterdam, Netherlands
[6]Division of Oncology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO

Background

- Cytotoxic chemotherapy remains the standard-of-care for most patients with acute myeloid leukemia (AML), in spite of the recent approval of novel agents
  - The investigation of new molecularly-targeted and immuno-modulating agents remains a high priority
- 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 for predictive biomarkers to help identify patients who are more likely to respond to cancer immunotherapy
  - IFN-γ-related mRNA profiles ("Tumor Inflammation Signature" or TIS) predict response to pembrolizumab in 9 solid tumor types (Ayers M, *et al*. Journal of Clinical Investigation 2017; 127: 2930-40)
  - Tumor Mutational Burden (TMB) identifies responders to pembrolizumab in KEYNOTE clinical trials across 22 solid tumor types (Cristescu R, *et al*. Science 2018; 362:6411)
- Flotetuzumab, a CD123 × CD3 bispecific DART® molecule, is being tested in a phase 1 clinical trial of relapsed/refractory AML (NCT#02152956)
- See also presentation #764. Monday, December 3, 2018: 3:00PM
  - Dr. John DiPersio, Session #616. Acute Myeloid Leukemia: Novel Therapy
    Seaport Ballroom F (Manchester Grand Hyatt San Diego)

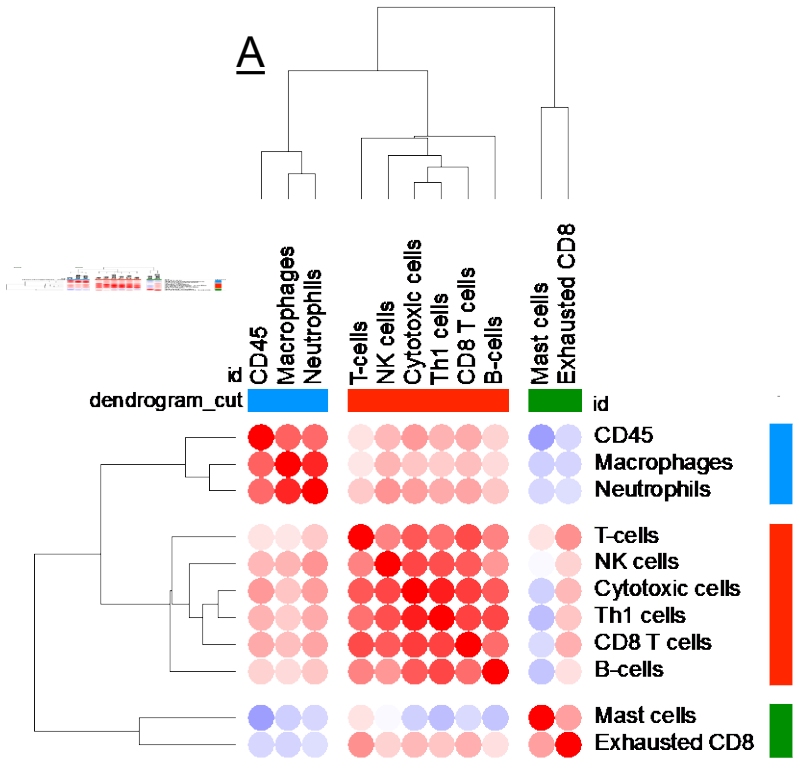
NTU

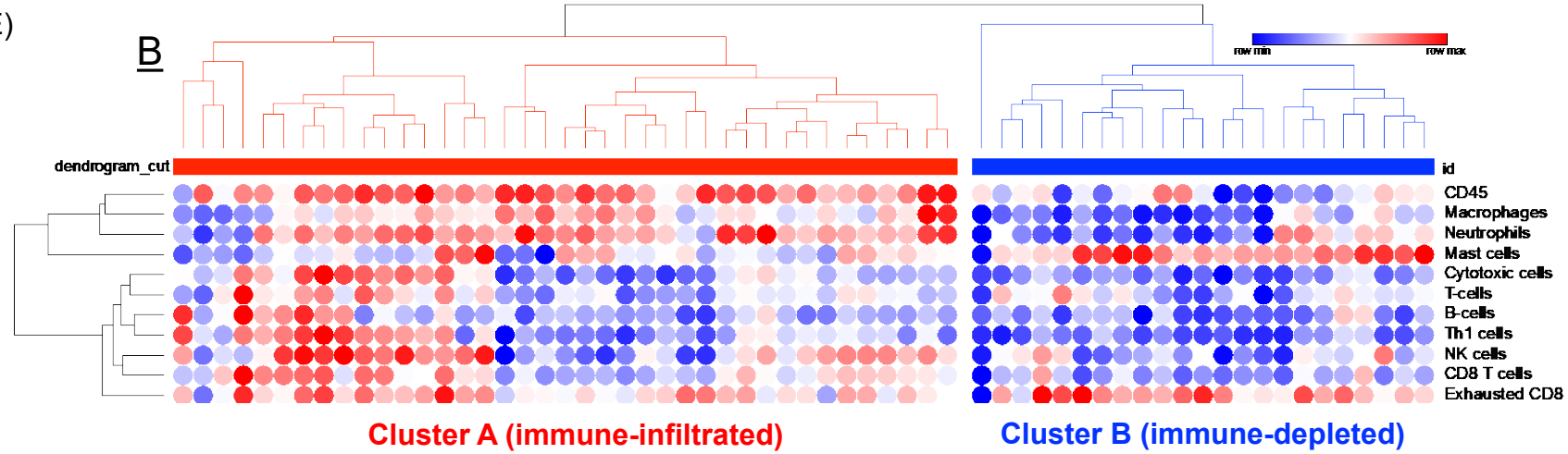
Diversity of immune landscapes in AML

Immune-inflamed TME is associated with resistance to cytotoxic chemotherapy

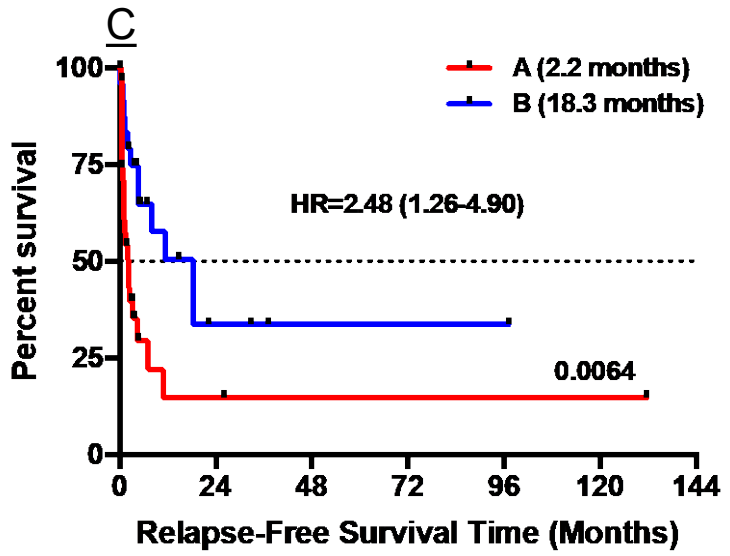
Immune profiles in the tumor microenvironment (TME)

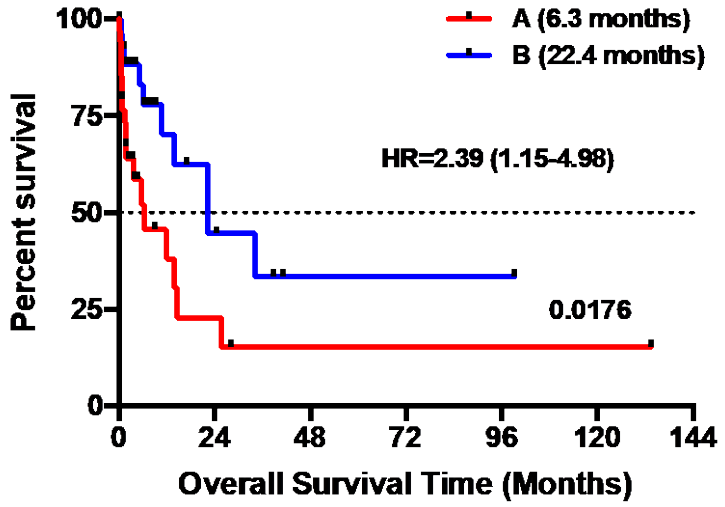
1. Innate (PMN, macrophages)
2. Adaptive (T, B, NK, CTL)
3. Mast cells, exhausted CD8⁺ T cells





Cluster A (immune-infiltrated) **Cluster B (immune-depleted)**





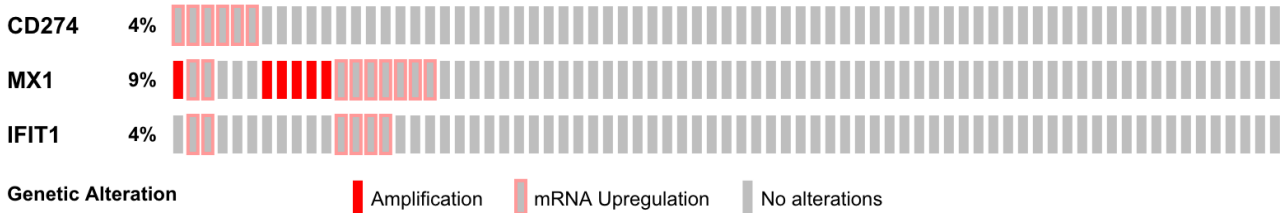
Discovery cohort (n=62)

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

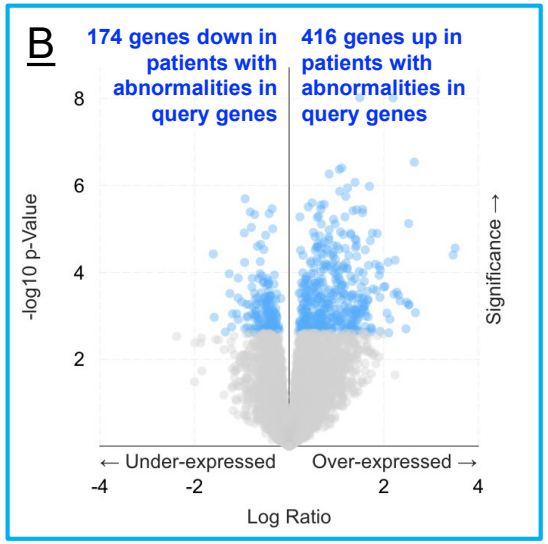
Vadakekolathu J, Patel T, Reeder S, et al. Blood 2017; 130: 3942A.

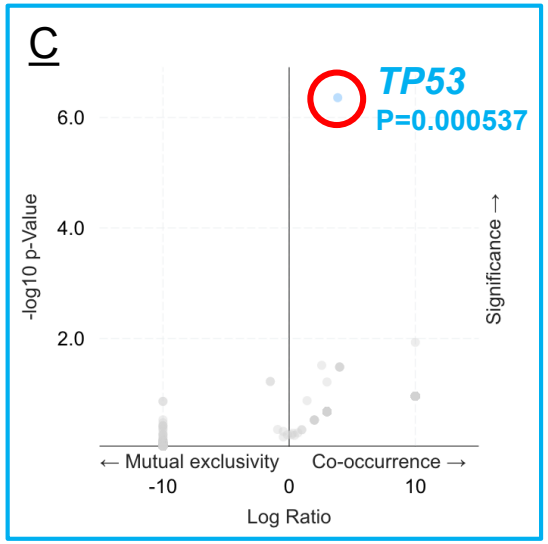
Expression of IFN-stimulated genes in BM associates with poor prognosis in AML

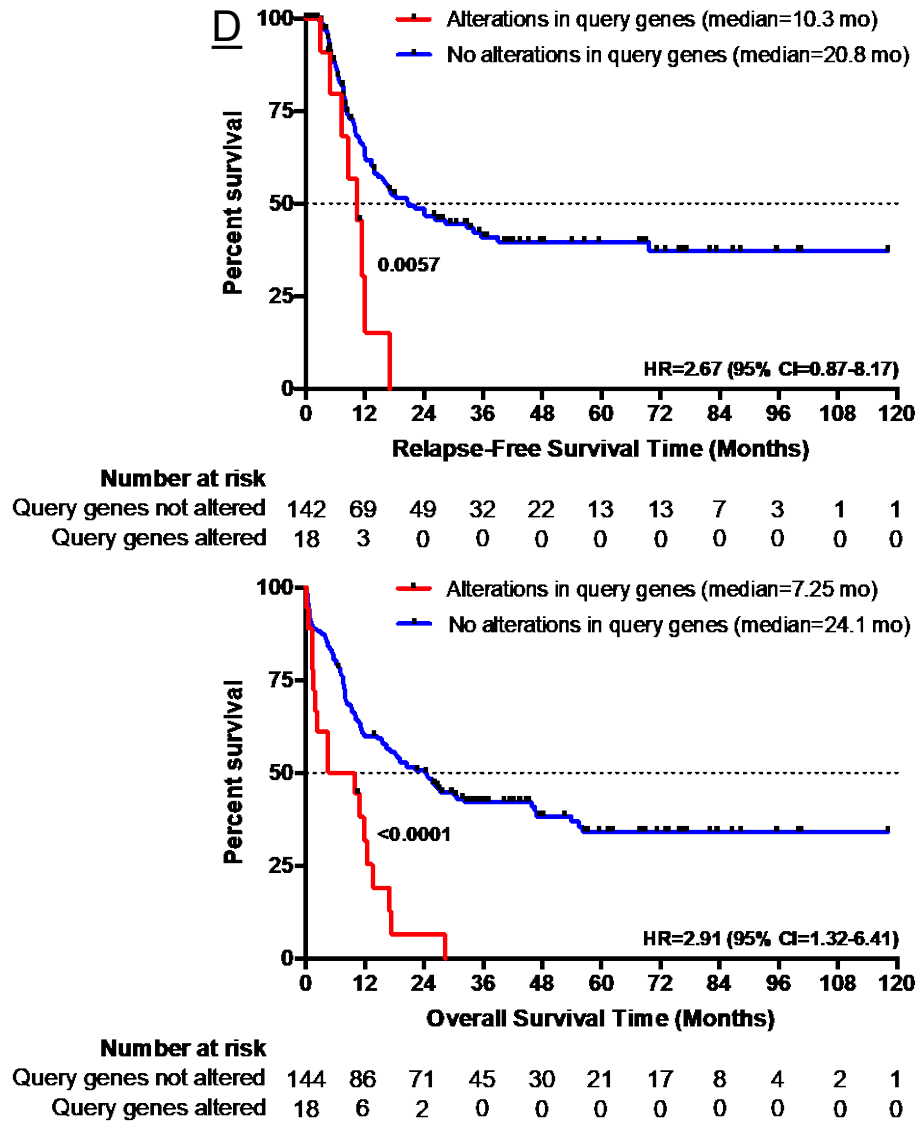
A Altered in 18 (11%) of 162 sequenced patients in TCGA-AML



ELN cytogenetic risk	Upfront treatment	HSCT	Treatment response
Intermediate (n=5) Adverse (n=13)	7+3 (n=9) HMA (n=6) Lenalidomide (n=1) None (n=2)	Yes (n=2 MUD; n=2 MRD) No (n=14)	CR (n=3) No CR (n=14) Persistent disease (n=1)







PLEASE DO NOT POST

Hood T, Viboch E, Church SE, Warren SE, Rutella S. Manuscript in preparation (October 2018).

Research questions

IFN-γ-related signatures reflecting an “inflamed” TME are associated with adverse prognosis in patients with AML receiving conventional chemotherapy

Are immune-infiltrated/inflamed TMEs, and IFN-γ gene signatures, associated with sensitivity to flotetuzumab?

Patients and methods

- Immune gene expression was analyzed in 65 bone marrow (BM) samples from patients with relapsed/refractory AML treated with flotetuzumab in **NCT#02152956** (Vey, *et al.* ESMO 2017; Uy, *et al.* ASH 2017; Uy, *et al.* ASH 2018)
  - 38 samples collected at baseline (35 with clinical outcome data)
    - 4 patients, 300 ng/kg/day
    - 28 patients, 500 ng/kg/day (RP2D)
    - 6 patients, 700 ng/kg/day
  - 27 samples collected “on treatment” (post-cycle 1)
- The NanoString PanCancer IO360™ assay interrogates 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

Patients' characteristics

Characteristic			All patients (n=38)
Age (median and range)			64 years (29-82)
Gender	Male		16 (42.1%)
	Female		22 (57.9%)
Disease status at time of enrolment	**Relapse**		**8 (21.1%)**
	Primary refractory (73.7%)§	Hypomethylating agents (HMA)	**12 (31.6%)**
		Chemotherapy	**16 (42.1%)**
	Not classifiable (Failed ≤ 2 cycles of HMA)		2 (5.2%)
2017 ELN risk stratification	Favorable		7 (18.4%)
	Intermediate		12 (31.6%)
	Adverse		**13 (34.2%)**
	Unknown		6 (15.8%)
Number of prior lines of therapy (median and range)			3 (1-11)

§Primary refractory: Chemotherapy-refractory (≥2 induction attempts or 1st CR with initial CR duration <6 months)
HMA-refractory (failure of ≥4 cycles of HMAs)

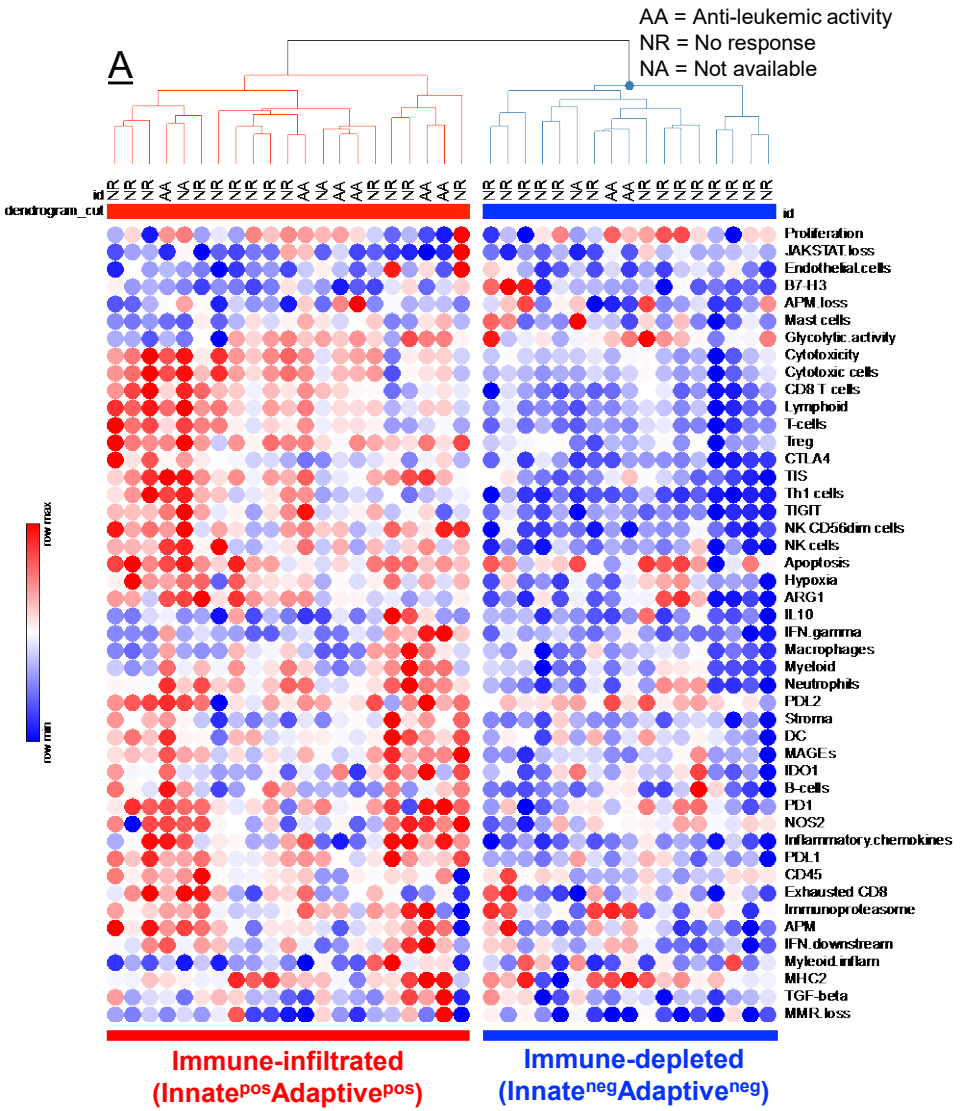
Response assessment criteria: Anti-leukemic activity (CR/CRi, PR, "other benefit"*)
Non-responders (treatment failure, stable disease, progressive disease)

*Other benefit defined as >30% decrease in BM blasts

NTU

Immune gene signatures at baseline (I)

A

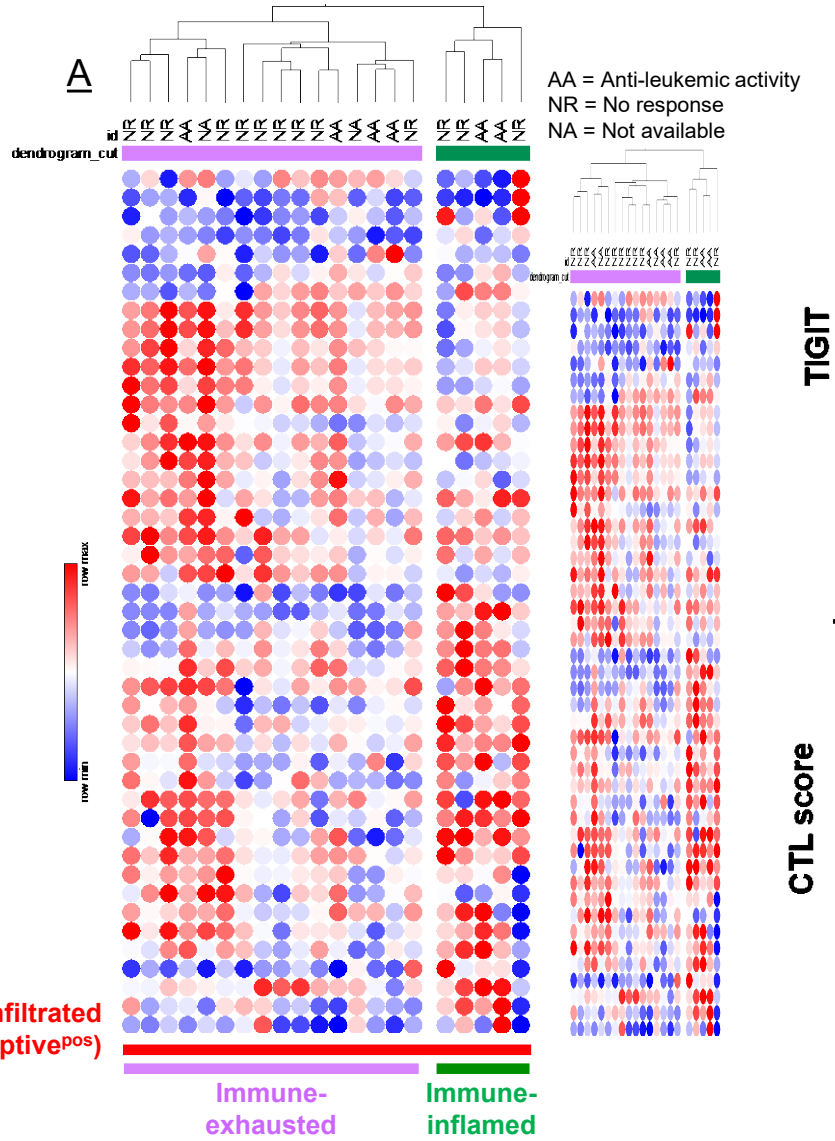


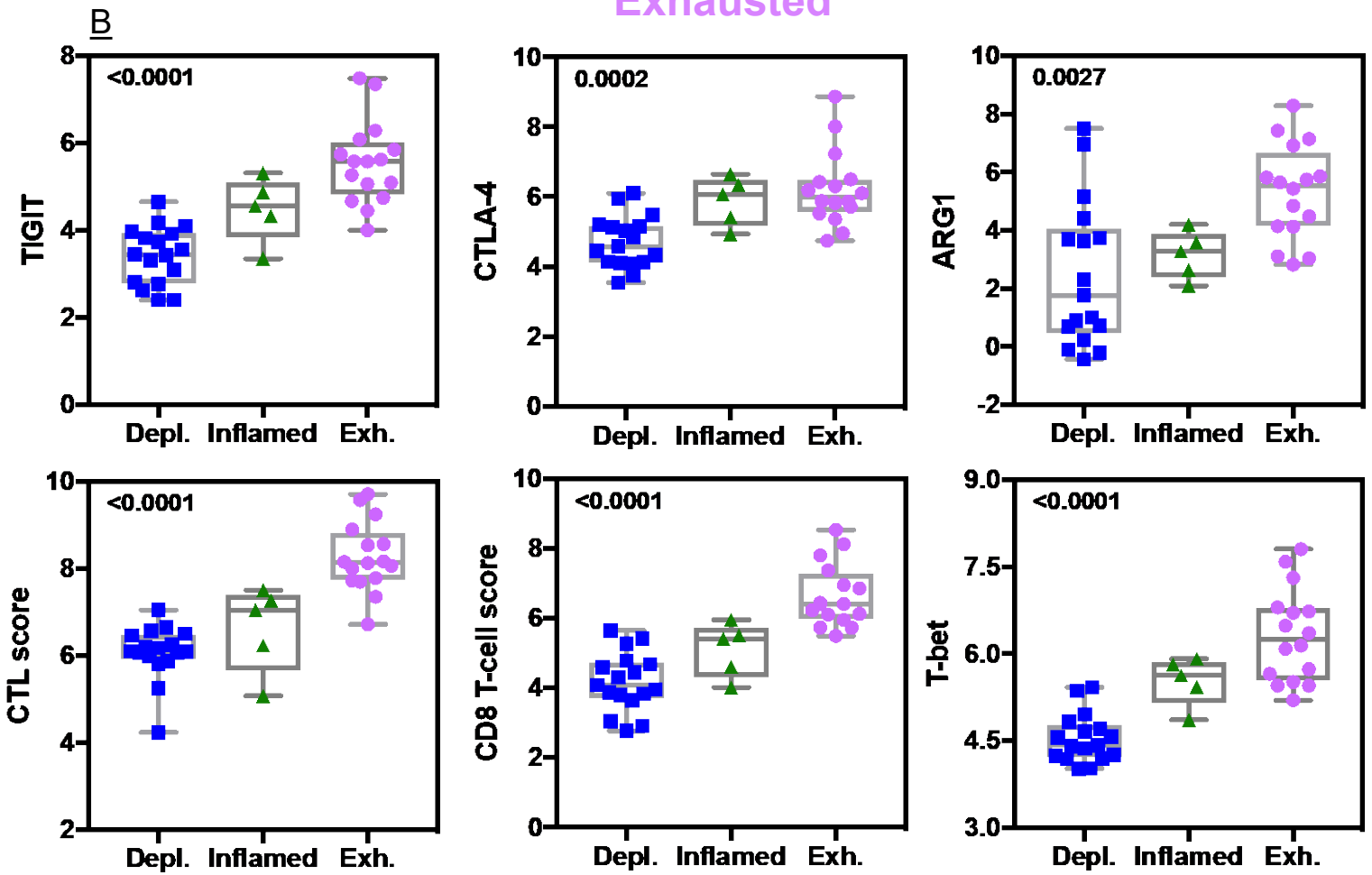
B

	Immune-infiltrated (Innate^{pos}Adaptive^{pos}) N=21	Immune-depleted (Innate^{neg}Adaptive^{neg}) N=17
Anti-leukemic activity	**31.6% (6/19)** 3 CR, 2 OB, 1 PR	**12.5% (2/16)** 1 CRi, 1 OB
No response	13/19	14/16
N.A.*	2/21	1/17
ELN cytogenetic risk at time of initial diagnosis (all patients)	Favorable (n=5) Intermediate (n=9) Adverse (n=5) N.A. (n=2)	Favorable (n=2) Intermediate (n=3) Adverse (n=8) N.A. (n=4)
ELN cytogenetic risk at time of initial diagnosis (patients with evidence of anti-leukemic activity)	Favorable (n=1) Intermediate (n=3) Adverse (n=1) N.A. (n=1)	Favorable (n=0) Intermediate (n=0) Adverse (n=2) N.A. (n=0)

*Response data available in 35/38 patients

Immune gene signatures at baseline (II)

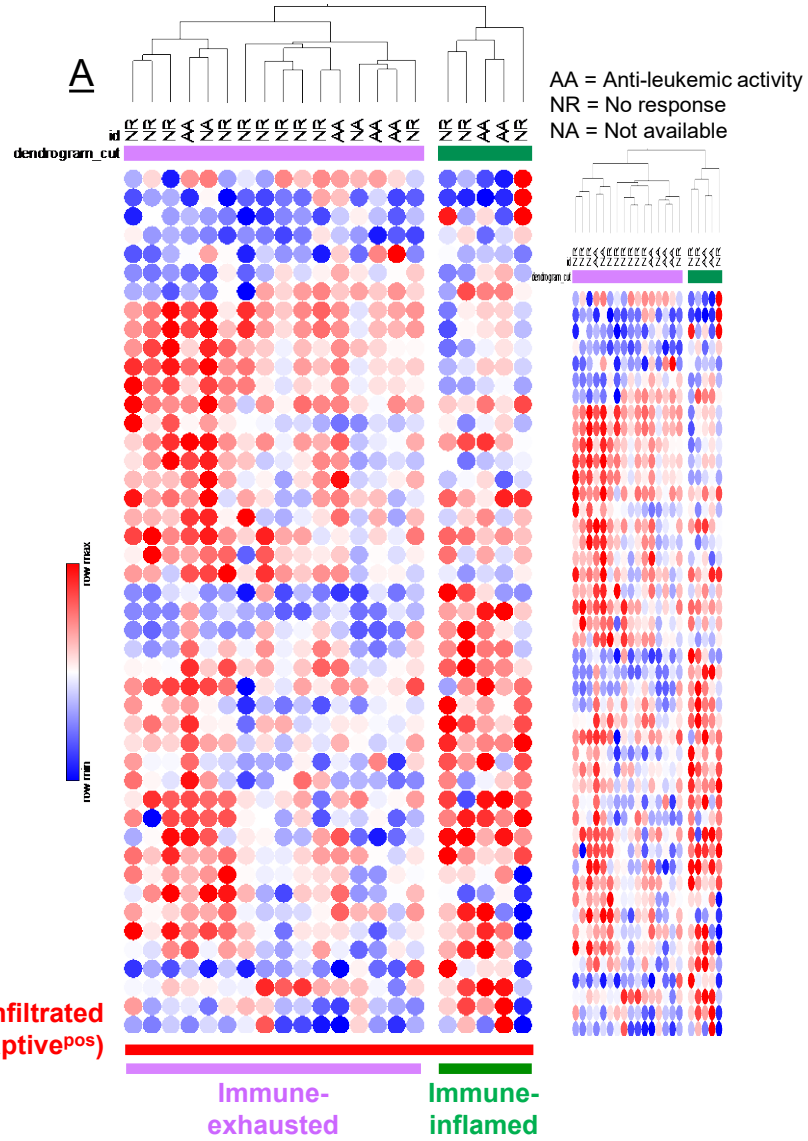


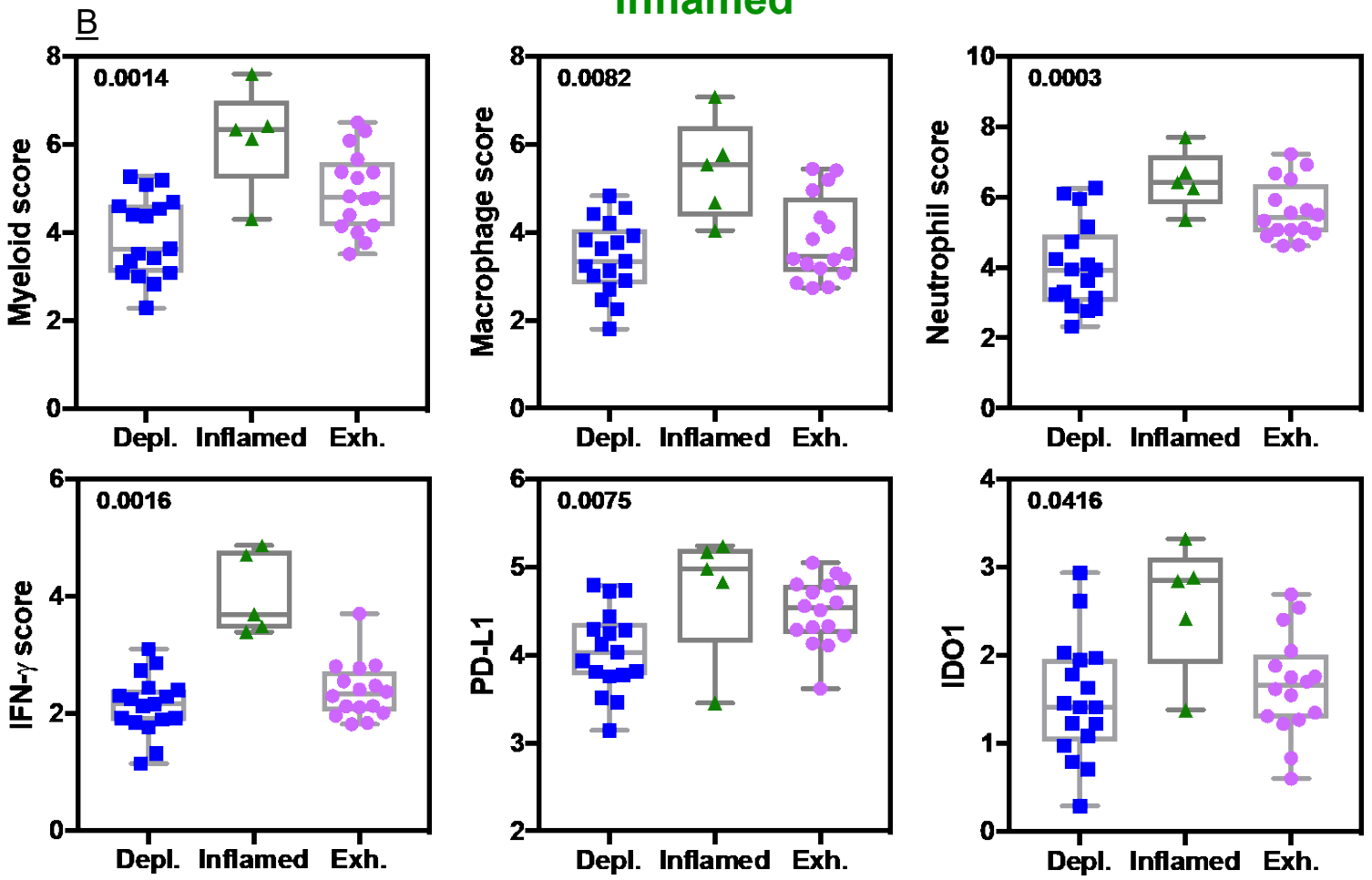


Dysfunctional T cells?

PLEASE DO NOT POST

Immune gene signatures at baseline (III)



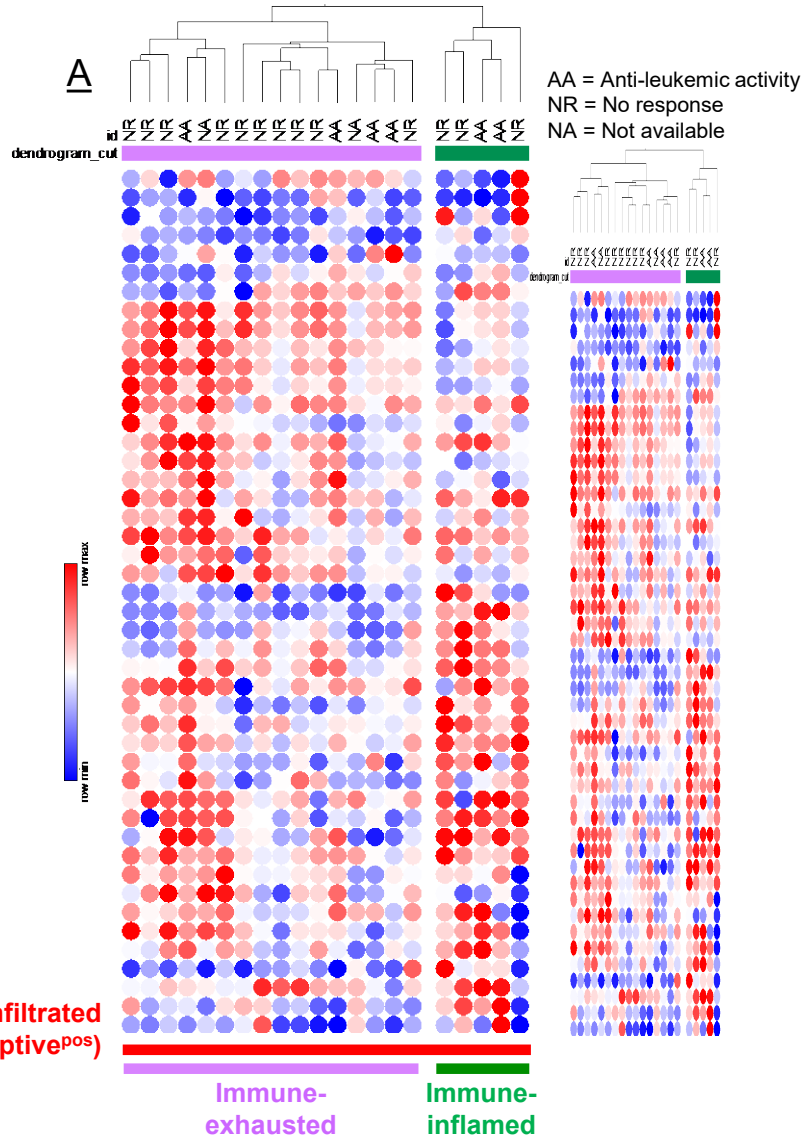


"IFN-γ-dominant" TME?

PLEASE DO NOT POST

Immune signatures and flotetuzumab response

A



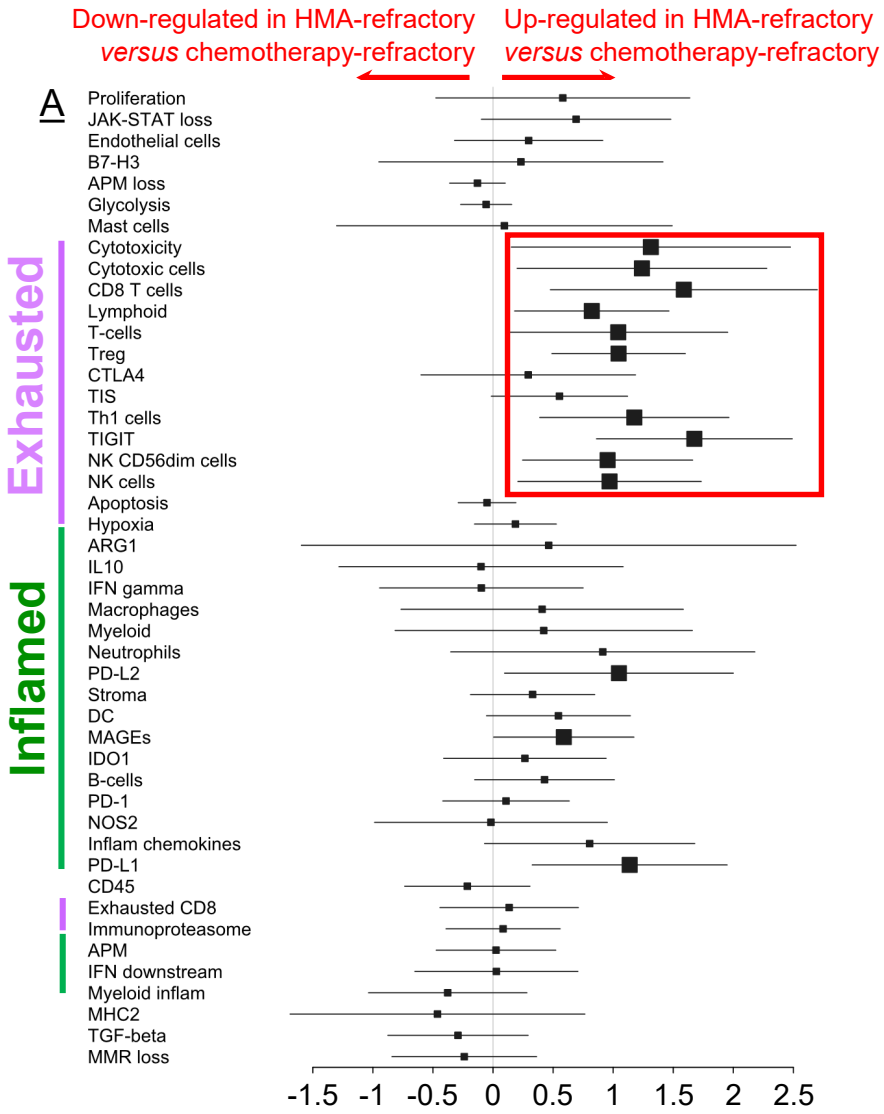
B

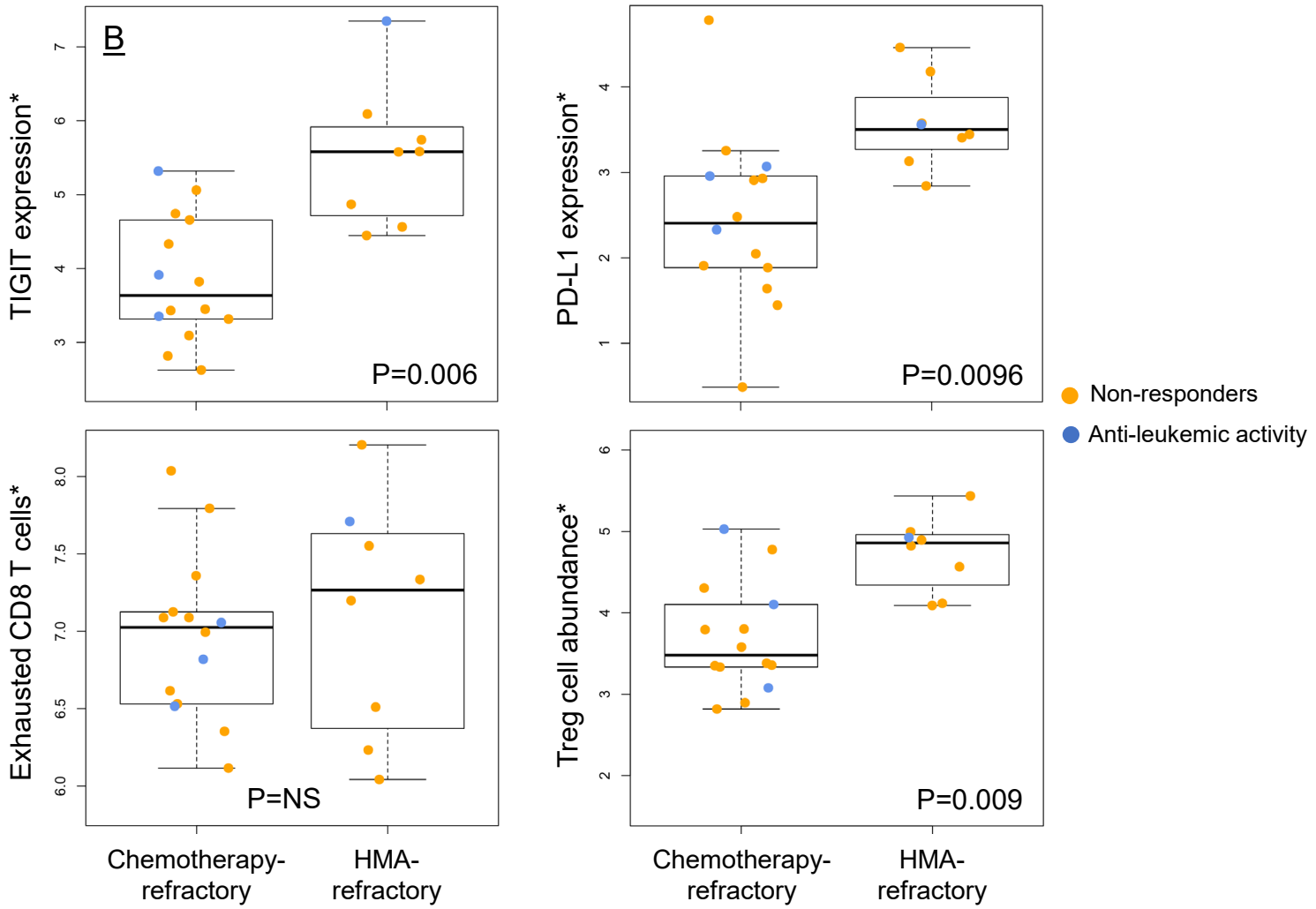
	Immune-inflamed (n=5)	Immune-exhausted (n=16)
Anti-leukemic activity	**40% (2/5)** 1 CR, 1 OB	**29% (4/14)** 2 CR, 1 OB, 1 PR
No response	3/5	10/14
N.A.*	0/5	2/16
Previous HMA treatment	40% (2/5)	62.5% (10/16)
ELN cytogenetic risk at time of initial diagnosis (all patients)	Favorable (n=1) Intermediate (n=0) Adverse (n=4) N.A. (n=0)	Favorable (n=4) Intermediate (n=9) Adverse (n=1) N.A. (n=2)
ELN cytogenetic risk at time of initial diagnosis (patients with evidence of anti-leukemic activity)	Favorable (n=1) Intermediate (n=0) Adverse (n=1) N.A. (n=0)	Favorable (n=0) Intermediate (n=3) Adverse (n=0) N.A. (n=1)

*Response data available in 35/38 patients

PLEASE DO NOT POST

Increased immune exhaustion signatures in HMA-refractory *vs.* chemotherapy-refractory patients

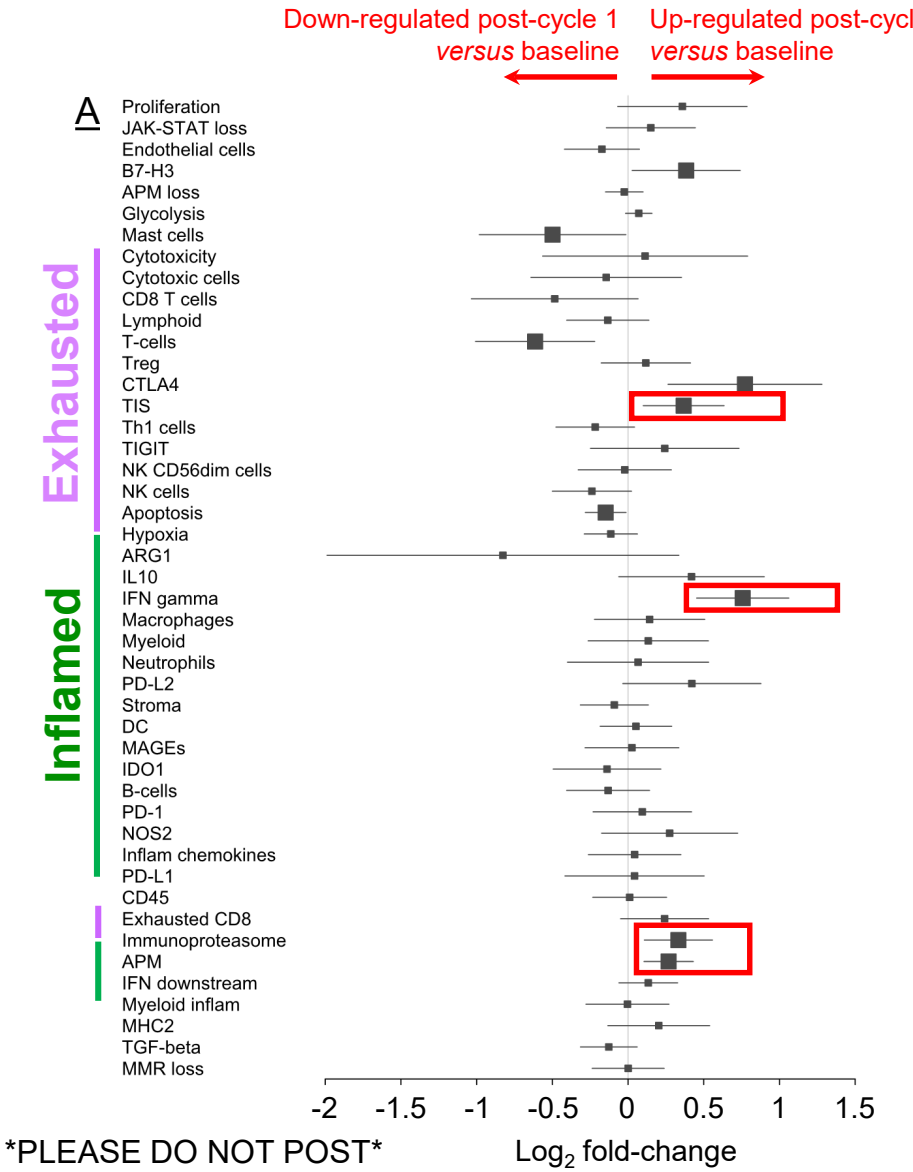


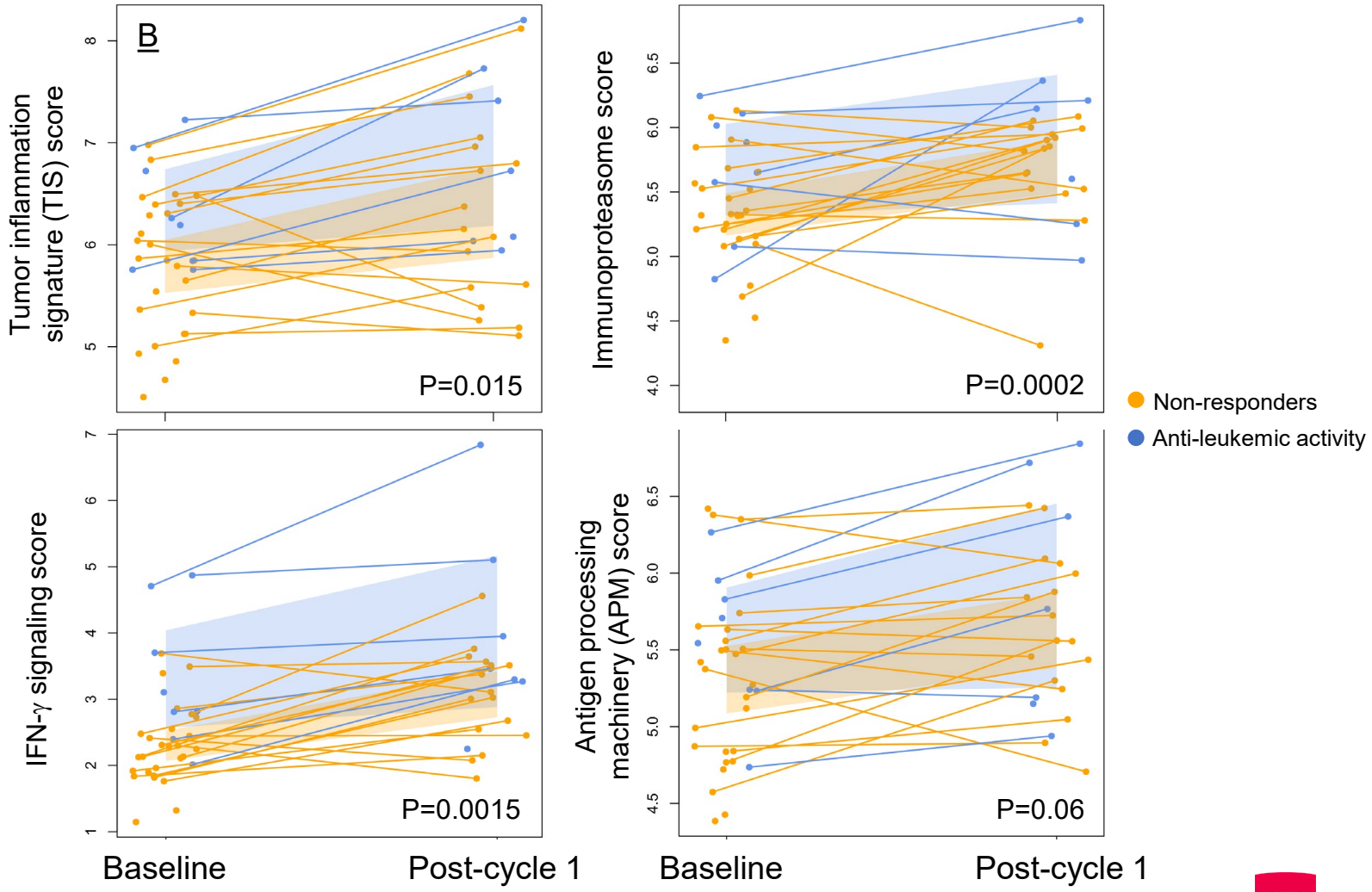


PLEASE DO NOT POST

*Evaluated in a subset of 22 patients (8 HMA-refractory, 14 chemotherapy-refractory)

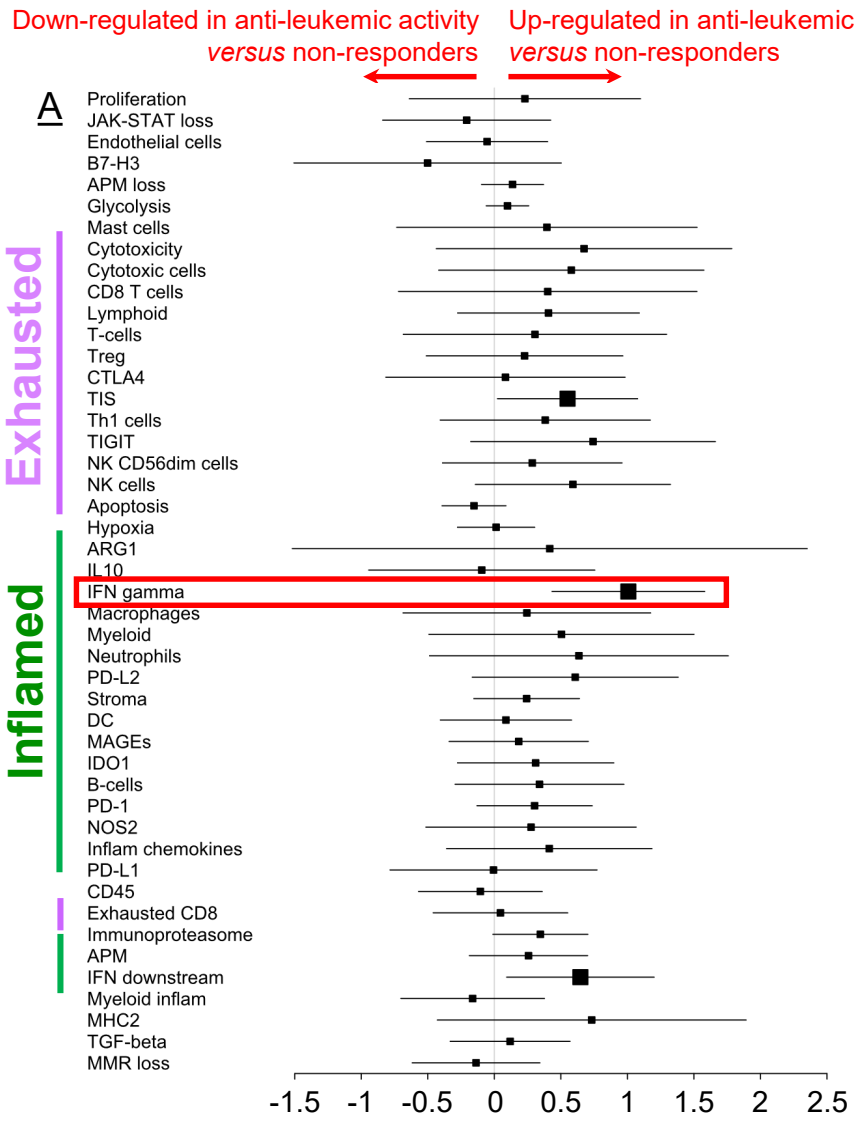
Flotetuzumab treatment enhances tumor inflammation, antigen presentation and IFN-γ signaling signatures



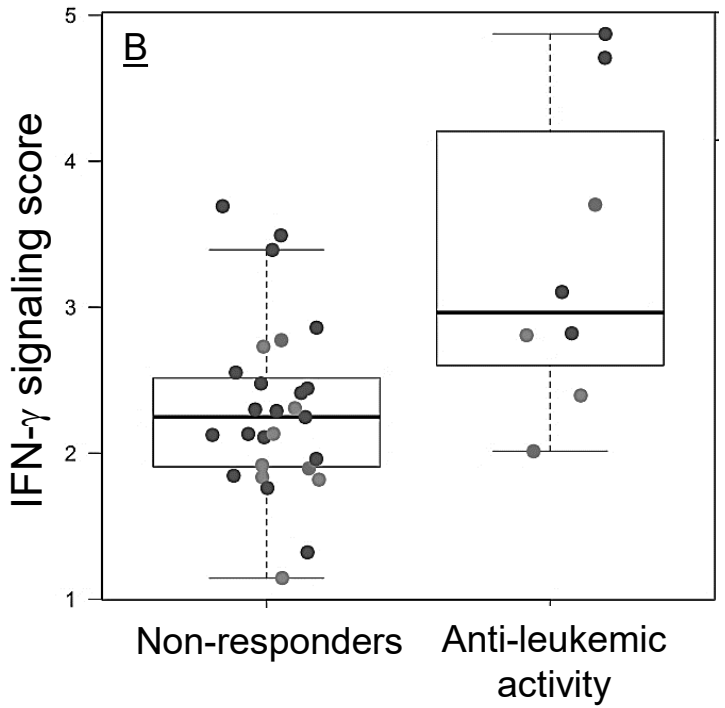


PLEASE DO NOT POST

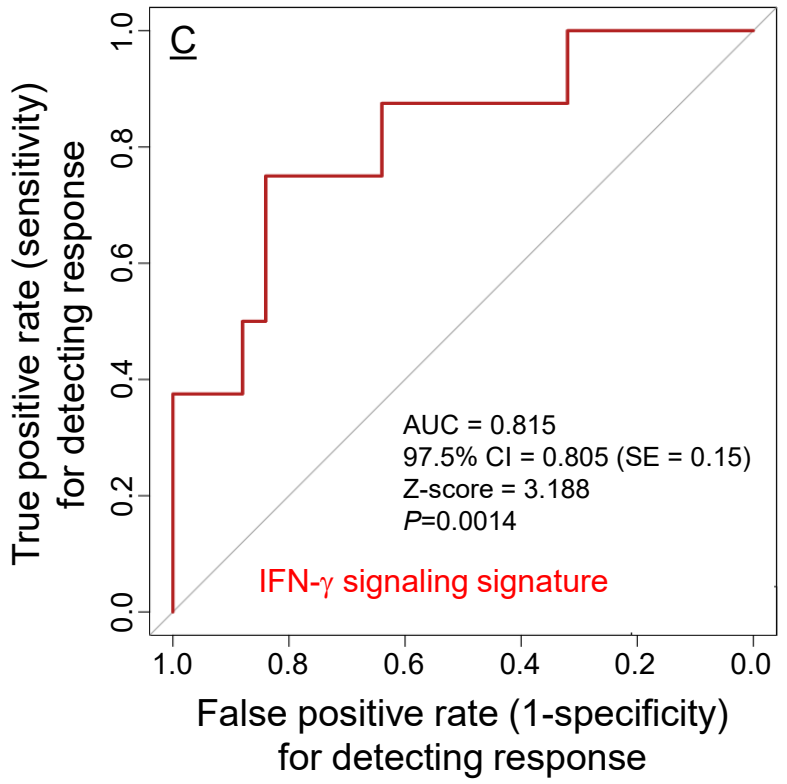
IFN-γ signaling scores are associated with response to flotetuzumab



IFN-γ signaling signature
STAT1, CXCL9, CXCL10, CXCL11

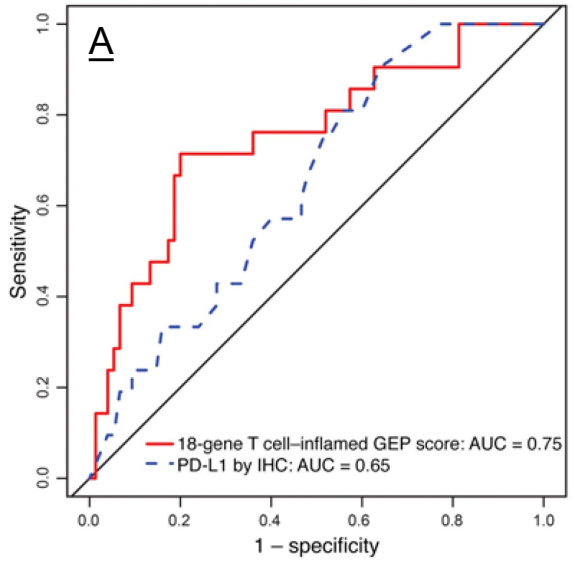


Receiver operating characteristic (ROC) curve analysis



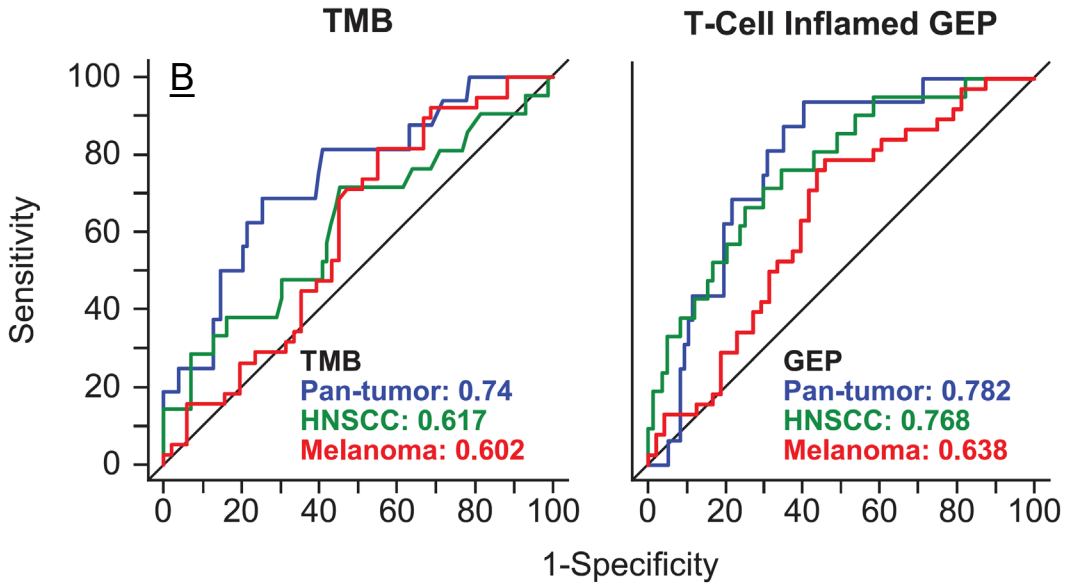
Predictors of ICB response in solid tumors

Ayers M, et al.
Journal of Clinical Investigation 2017; 127: 2930-40.



18-gene score
(Tumor Inflammation Signature)
for a cohort of 96 patients with HNSCC
from KEYNOTE-012

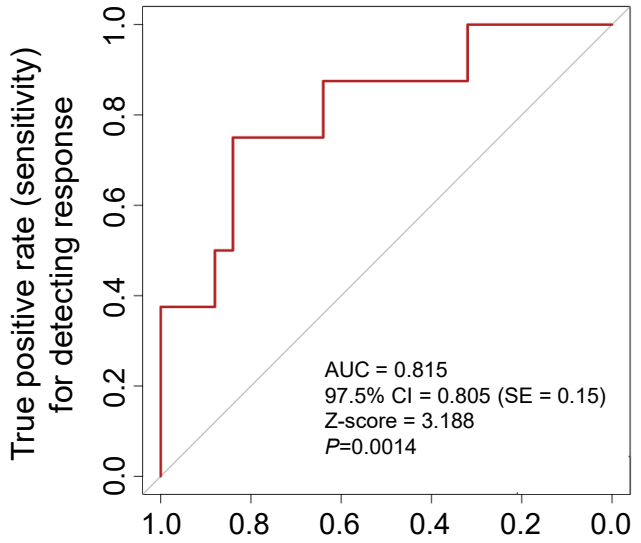
Cristescu R, et al.
Science 2018; 362 (6411): eaar3593



Pan-tumor cohort=119 patients
HNSCC cohort=107 patients
Melanoma cohort=89 patients

Pan-tumor cohort=113 patients
HNSCC cohort=105 patients
Melanoma cohort=86 patients

IFN-γ signaling signature for flotetuzumab



PLEASE DO NOT POST

Conclusions

- Evidence for a **range of immune profiles** in the AML TME was previously presented and confirmed here
- As opposed to prior experience with chemotherapy, most patients showing evidence of anti-leukemic activity with flotetuzumab [**6/8 (75%)**] in this initial data set had a gene signature consistent with higher immune infiltration in the bone marrow
- More specifically, **IFN-γ-related gene profiles** at baseline may associate with clinical response to flotetuzumab
- Patients previously treated with HMAs showed an immune-exhausted TME
  - We hypothesize that flotetuzumab could invigorate an immune-exhausted TME (increased tumor inflammation, antigen processing/presentation and IFN-γ signaling scores)
- Patients with an immune-infiltrated TME had increased immune checkpoint expression, suggesting potential enhanced benefit from flotetuzumab in combination with immune checkpoint blockade

Acknowledgements

Patients and Families!

Previous AML work
Tasleema Patel
Sarah K. Tasian
Division of Oncology and
Center for Childhood Cancer Research,
Children's Hospital of Philadelphia, PA

Heidi Altmann
Martin Bornhäuser
Jörn Meinel
Marc Schmitz
SAL Studienallianz Leukämie
Carl Gustav Carus Hospital, TU Dresden,
Dresden, Germany

Nottingham Trent University
Alan Graham Pockley
Stephen Reeder
Jayakumar Vadakekolathu

NanoString Technologies, Inc.
Alessandra Cesano
Sarah E. Church
Tressa Hood
Amy Sullivan
Elena Viboch
Sarah E. Warren

MacroGenics, Inc.
Jan K. Davidson-Moncada
Ross La Motte-Mohs
John Muth

Clinical Sites
Max Topp, Medizinische Klinik und Poliklinik II Universitätsklinikum Würzburg, Würzburg, Germany
Norbert Vey, Institut Paoli-Calmettes, Marseille, France
Fabio Ciceri, IRCCS Ospedale San Raffaele, Milan, Italy
Stefania Paolini, Policlinico S. Orsola-Malpighi, Bologna, Italy
Gerwin Huls, Universitair Medisch Centrum Groningen, Groningen, The Netherlands
Bob Löwenberg, Erasmus University Medical Center, Rotterdam, The Netherlands
Geoffrey Uy, Barnes-Jewish Hospital, Washington University School of Medicine, St. Louis, MO, U.S.A.
David Rizzieri, Duke University Medical Center, Durham, NC, U.S.A.
Martha Arellano, Winship Cancer Institute of Emory University School of Medicine, Atlanta, GA, U.S.A.
Matthew Foster, UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC, U.S.A.
John Godwin, Providence Cancer Center, Earle A. Chiles Research Institute, Portland, OR, U.S.A.
Farhad Ravandi, The University of Texas M D Anderson Cancer Center, Houston, TX, U.S.A.
Kendra Sweet, Moffitt Cancer Center, Tampa, FL, U.S.A.
Ibrahim Aldoss, City of Hope National Medical Center, Duarte, CA, U.S.A

الصندوق القطري لرعاية البحث العلمي
Qatar National Research Fund
Member of Qatar Foundation
National Priorities Research Programme, 2016-2018

RCF
Roger Counter Foundation
Dorset, 2017-2019

HIGHER EDUCATION FUNDING COUNCIL FOR ENGLAND hefce
Mainstream QR funding, 2017-2018

NTU