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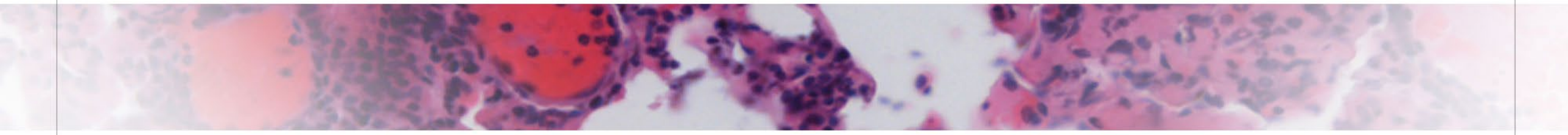

Microenvironmental Immune Senescence and Exhaustion in Acute Myeloid Leukemia Associate with Response to Flotetuzumab, an Investigational CD123×CD3 Bispecific DART® Molecule

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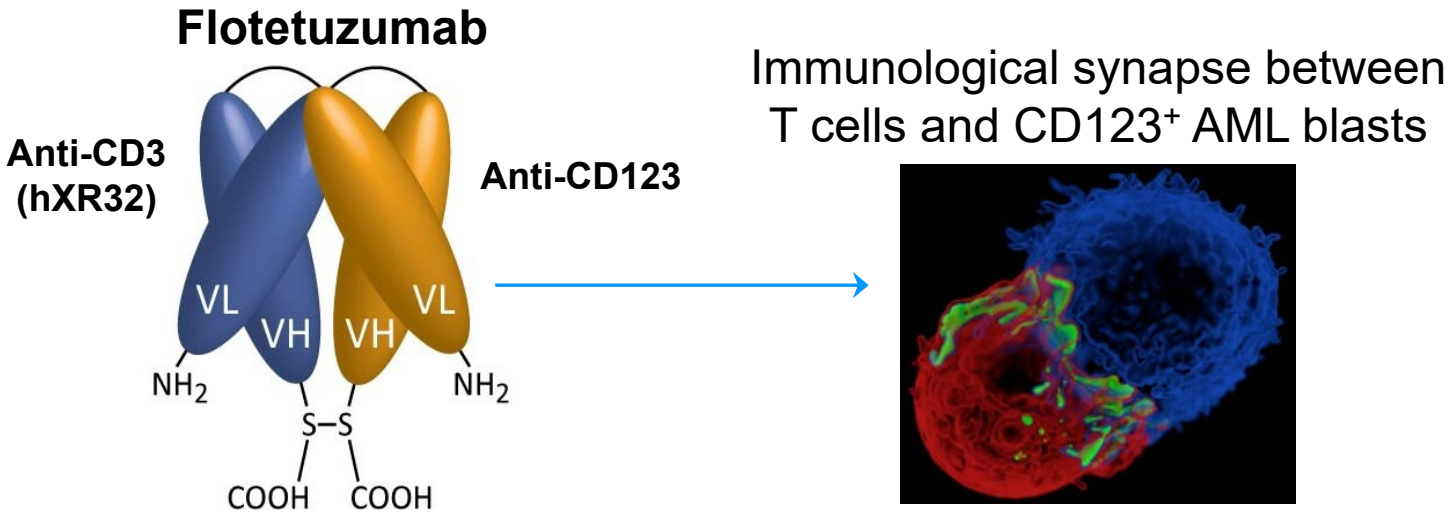
Background

- Chemotherapy refractoriness and disease relapse continue to be significant obstacles to therapeutic success in AML
- We have recently shown that bone marrow (BM) RNA profiles stratify patients with AML into immune-infiltrated and immune-depleted subtypes, and that type I/II interferon (IFN)-related gene signatures associate with complete response to flotetuzumab (Vadakekolathu J, *et al*. *Sci. Transl. Med.* 2020; 12: eaaz0463)
- Within the AML tumor microenvironment (TME), $CD8^+$ T cells exhibit features of immune exhaustion and senescence (IES; Knaus HA, et al. *JCI Insight* 2018; 3: e120974)
- IES are dysfunctional states driven by metabolic alterations in the TME, and are emerging targets for cancer immunotherapy

- To determine whether gene sets reflective of IES in the BM TME predict response of relapsed-refractory (R/R) AML to flotetuzumab in the CP-MGD006-01 clinical trial (NCT#02152956)



Patients and Methods

	All patients (n=71)
Age (years, median and range)	61 (27-82)
Males/Females, n/n	46/25
AML risk stratification (2017 ELN; n, %)	
Favorable	6 (8.4%)
Intermediate	18 (25.4%)
Adverse	47 (66.2%)
Secondary AML (n, %)	29 (40.8%)
Disease status at study entry (n, %)	
Primary induction failure*	30 (42.2)
Early relapse (CR1 duration < 6 months; ER6)	10 (14.1)
Late relapse (CR1 duration ≥ 6 months; LR)	31 (43.7)
Number of prior lines of therapy (median and range)	2 (1-4)

- 139 BM samples from 71 patients with R/R AML treated with FLZ at the RP2D of 500 ng/kg/day (Uy J, *et al*. Blood 2020); BMs collected at time of study entry (n=71; n=66 with response data) and longitudinally post-cycle (PC) 1 (n=40), PC2 (n=18), PC3 and PC4 (n=4) and end of treatment (n=6);
- Microenvironmental RNAs were profiled using the PanCancer IO 360™ gene expression panel on the nCounter® platform;
- Disease status was assessed by modified International Working Group (IWG) criteria (Uy J, *et al*. *Blood* 2020).

*Lack of response to at least two cycles of induction chemotherapy

Derivation of an IES Gene Expression Score

TCGA and Beat AML Master Trial Cohorts

↓

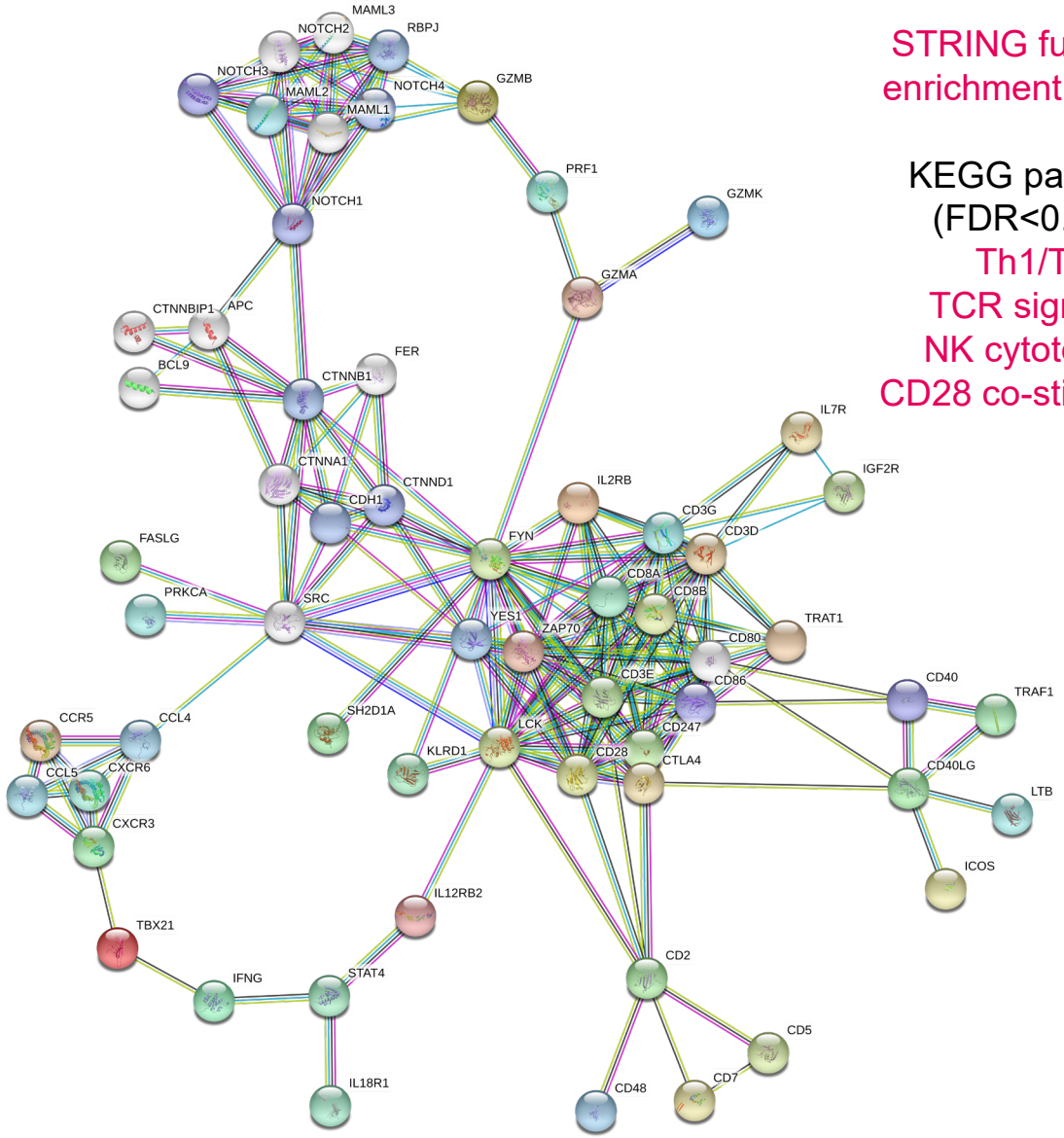
"Immunologic signature" gene sets
(C7; n=4,872) (MSigDB)

Gene Set Enrichment Analysis
(GSEA)

↓

68-gene IES signature score

An Immune Senescence and Exhaustion-Related RNA Profile Predicts Clinical Outcomes in Acute Myeloid Leukemia (Vadakekolathu J, *et al*. ASH Oral Presentation #33; December 5th, 2020)

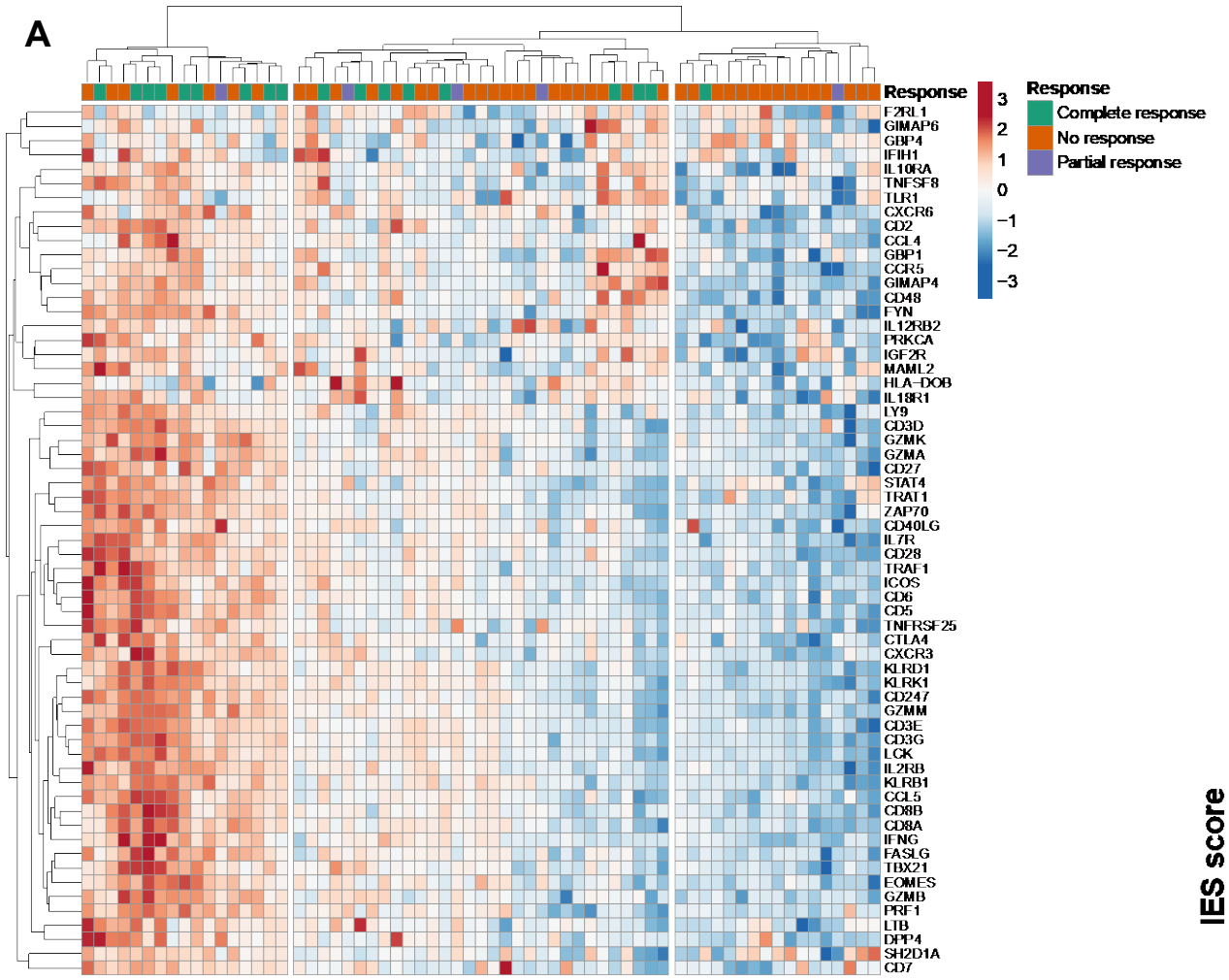


STRING functional enrichment analysis

KEGG pathways (FDR<0.001):
Th1/Th2
TCR signaling
NK cytotoxicity
CD28 co-stimulation

IES Stratify BM Samples and Associate with Response to Flotetuzumab

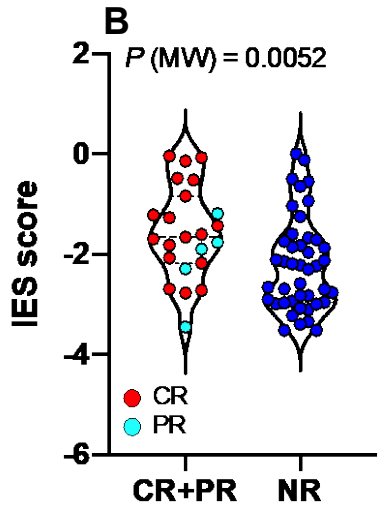
Baseline BM samples (n=66 patients evaluable for response)

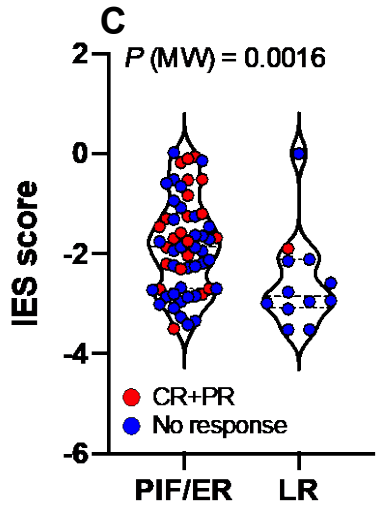


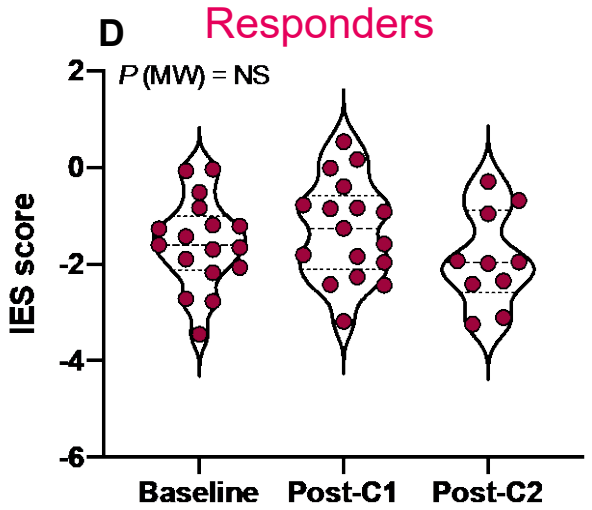
IES^{high}
CR+PR=11/19
(58%)

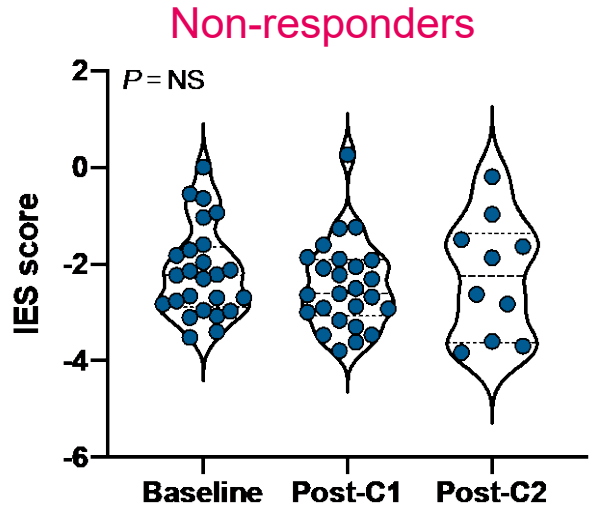
IES^{int}
CR+PR=10/32
(31.2%)

IES^{low}
CR+PR=2/15
(13.3%)

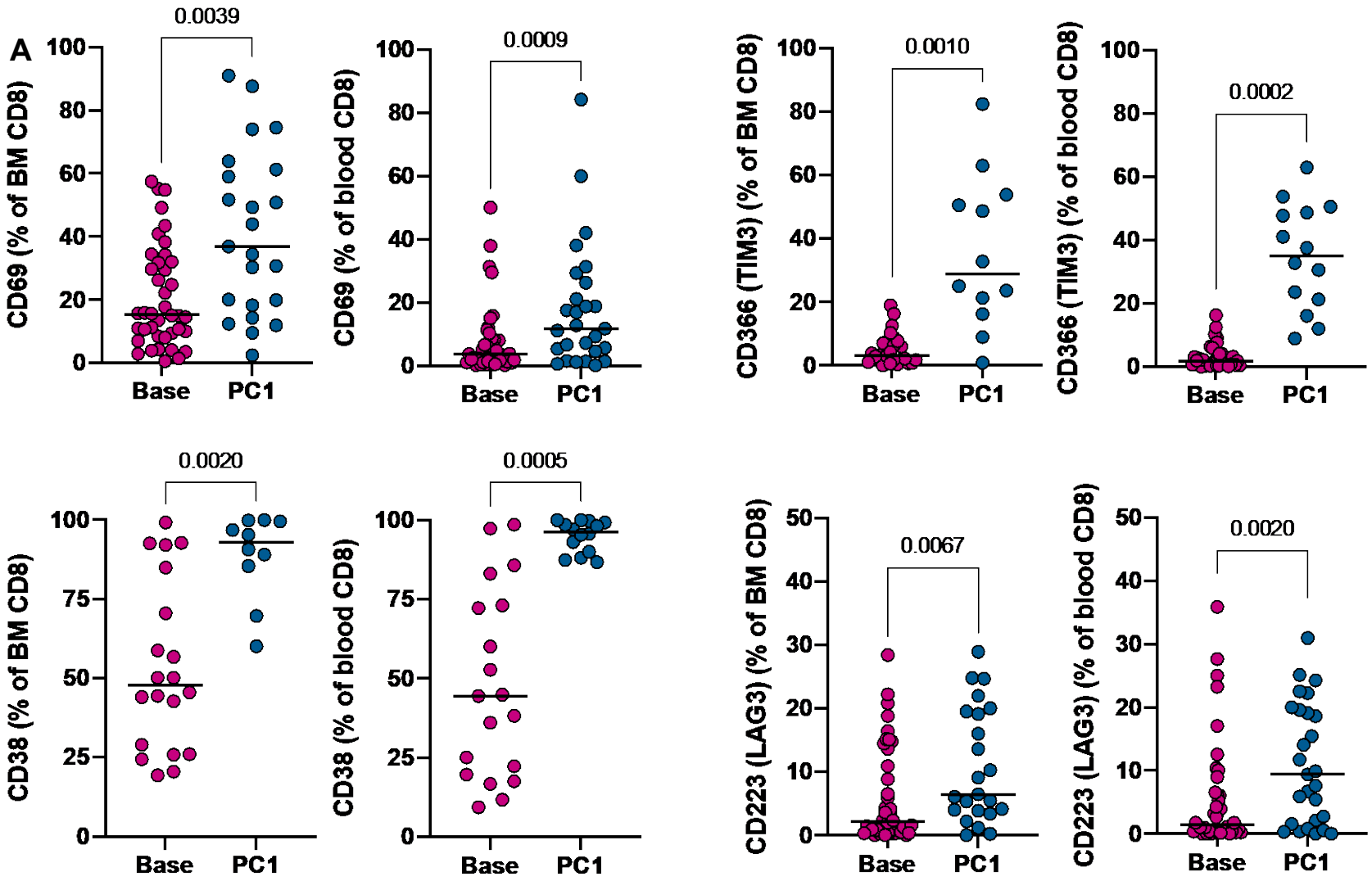






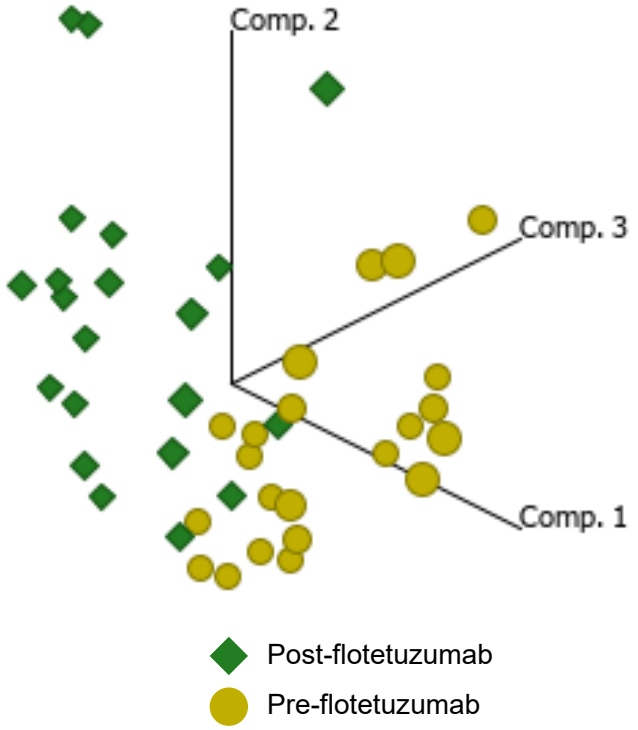


Flotetuzumab Modulates the Immunological TME

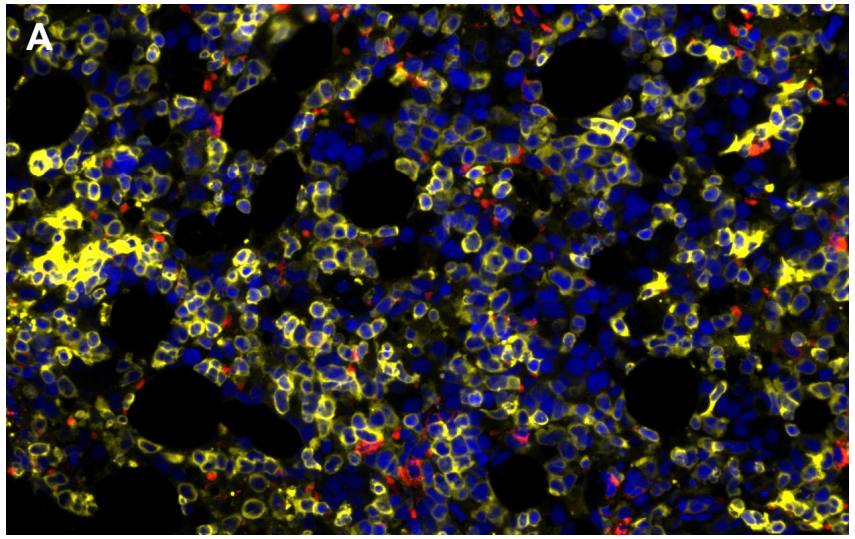


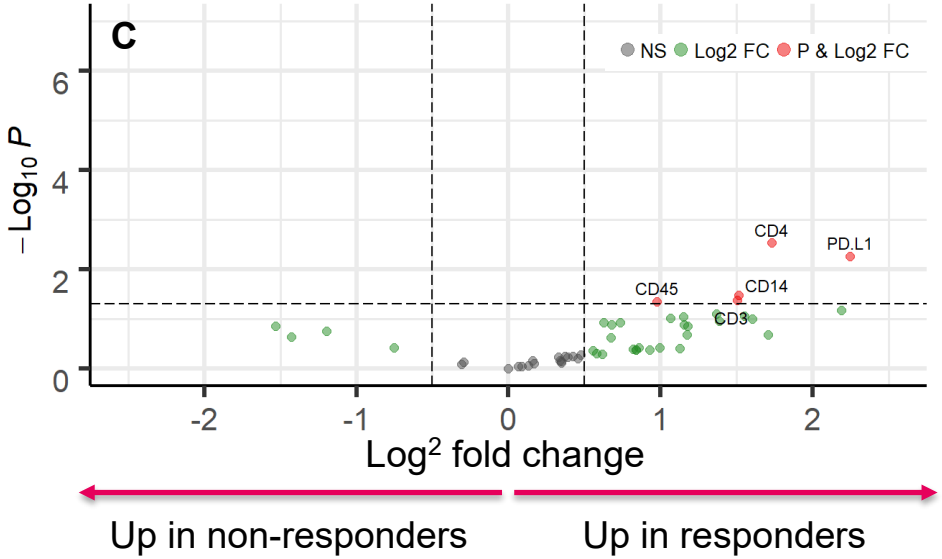
B

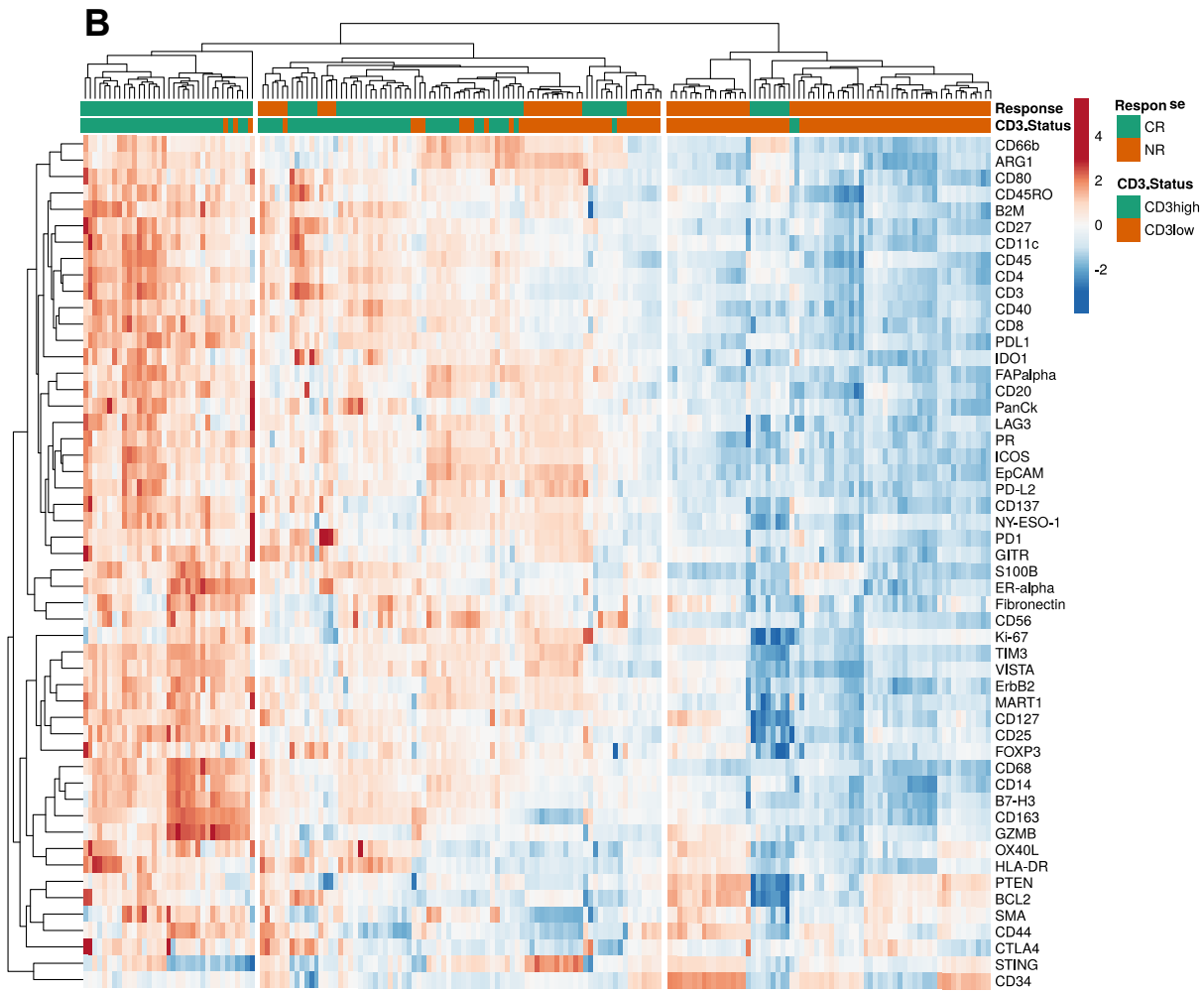
BM aspirates of 22 subjects, prior to and post flotetuzumab treatment (21-marker flow cytometry panel)



GeoMx Digital Spatial Profiling of the TME Identifies Protein Changes after Flotetuzumab







8 FFPE BM biopsies
183 Regions of interest/Areas of illumination

Conclusions

- Immune exhaustion and senescence gene sets are over-expressed in BM samples from patients with evidence of chemotherapy resistance (PIF/ER6) compared with LR
- Flotetuzumab modulates the immunological TME by enhancing T cell activation and the expression of immune checkpoints
- Transcriptomic features of immune and exhaustion and senescence are associated with response (CR+PR) to flotetuzumab
- T cell functional rejuvenation by flotetuzumab could benefit patients with R/R AML by counteracting pre-existing immune dysfunction

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