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Flotetuzumab: an Investigational CD123xCD3 Bispecific DART® Protein-Induced Clustering of CD3+ T Cells and CD123+ AML Cells in Bone Marrow Biopsies is Associated with Response to Treatment in Primary Refractory AML Patients

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Introduction

Infiltration of immune cells into tumors has been associated with therapeutic effects in preclinical models and patients with cancer. In acute myeloid leukemia (AML) we have previously reported that immune infiltrated TME is predictive of failure to cytotoxic chemotherapy, but associated with response to immunotherapy, specifically flotetuzumab[1,2]. Furthermore, flotetuzumab also affects the immune infiltration in TME[2]. NK cells play an important role in AML control[3]. Flotetuzumab is a humanized DART® molecule that bridges CD123 on AML with CD3 on T cells and mediates anticancer activity via T-cell activation and cytolytic activity against the bound cancer cell. While this is well described in vitro, little evidence of this interaction is available in vivo. For this report, we studied baseline and post-flotetuzumab Cycle 1 bone marrow (BM) tissue samples for 6 primary refractory AML patients.

Materials and Methods

- Patients were treated on the RP2D of flotetuzumab (multi-step lead-in dose followed by 500ng/kg/day, in 28-day cycles)
- Response assessment was performed at 25+3 days of each cycle
- Serial BM samples were evaluated using 2 different multiplex immunofluorescence (mIHC) staining panels on consecutive slides
  - Panel 1: T-cell immunopanel: PD-L1, FoxP3, CD8, CD3, CD103
  - Panel 2: Blast-NK panel: CD123, CD3, CD57, CD16
- Slides were stained using a Leica BondRx autostainer. Digital images were captured with Vectra Polaris scanner and analyzed with InForm Software
- A density-based clustering algorithm developed and run in QuPath was used to quantify CD3+ T cell clusters
- Percentage of total cells detected per each cell phenotype were reported

Results

- Evaluated patients were heavily pretreated (median prior lines of therapy 3, range 2–9), and had adverse cytogenetic risk[4]
- 3 patients had a complete response after 1 cycle of flotetuzumab (CR, CRh, CRi) and 3 were non-responders (2 SD, 1 PD)
- In baseline BM samples, CD3 and CD8 cell infiltrates were higher in CR patient vs non-responders (mean ± SEM)
  - CD3+ 18.3% ± 6.9 vs 9.3% ± 1.8
  - CD8+ 9.4% ± 3.5 vs 4.8% ± 1.2
- 2 of 3 patients with CR went on to receive allogeneic stem cell transplant (HSCT). These 2 patients developed CD3 clusters in their post-Cycle 1 biopsies, with 65 and 22 clusters, and an average of 34 and 17 T cells per cluster, respectively
- All clusters identified in 2 CR patients were found on or adjacent to CD123+ cells
- The BM biopsy of the CR patient with no detected clusters had no unequivocal evidence of residual/recurrent leukemic blasts. This patient had early dose interruption due to non-treatment related AE (infectious complication) and did not receive a full cycle of treatment; response was transient with relapse shortly thereafter
- Cytotoxic NK cells (CD57+CD16+) were increased in post-Cycle 1 biopsies of CR vs non-responders (0.93 ± 0.31 vs 0.27 ± 0.13 mean ± SEM) with the largest fold increase in CR (28 vs 9)
- Post-Cycle 1 PD-L1 expression was higher in non-responders vs CR (23% vs 16%), with non-responders exhibiting the largest fold change in total PD-L1 cells (10.9 vs 2.2)
- This increase in total PD-L1+ cells resulted in a post-Cycle 1 reduction in the ratio of CD3+ cells to PD-L1+ cells in non-responders

Summary of Individual Patient Results

Response	AML Disease Status	Patient ID	Blasts (Delta BM Blast %)	Visit	Visually CD3+ Clusters Identified	Computer Analysis #Clusters Detected*
CR	Primary Refractory	US008-0001	16 (-69) 0 0	Screen-D14 Cycle 1 Day 25 Cycle 2 Day 25	No clusters Clusters Clusters	0 65 (34) 37 (41)
Cri	Primary Refractory	US001-0035	23 (-80) 1	Screen-D14 Cycle 1 Day 25	No clusters Clusters	0 22 (17)
CRh	Primary Refractory	US011-0014	50 (-100) 0	Screen-D14 Cycle 1 Day 25	No clusters No clusters	0 0
SD	Primary Refractory	US011-0006	18 (-17) 15 25	Screen-D14 Cycle 1 Day 25 Cycle 2 Day 25	No clusters No clusters No clusters	0 0
SD	Primary Refractory	US004-0008	94 (-5) 89	Screen-D14 Cycle 1 Day 25	No clusters No clusters	0 0
PD	Primary Refractory	US001-0034	15 (340) 66	Screen-D14 Cycle 1 Day 25	No clusters No clusters	0 0

*Average #CD3 per cluster

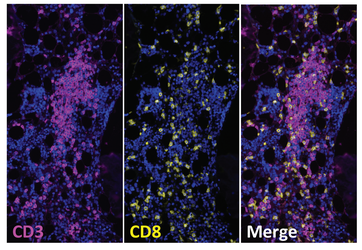


Figure 1. Multiplex IHC staining of bone marrow sample from AML US008-0001 patient at Cycle 1 Day 25. This is a representative cluster image showing the distribution of CD3+ T cells, CD3+CD8+ T cells and a merged image from the BM biopsy. CD3+ pattern distribution is dense and associated to CD123+ cells (not shown); however, the pattern of distribution of CD3+CD8+ T cells is more dispersed, not presenting as aggregations. Dapi in blue as a counterstained.

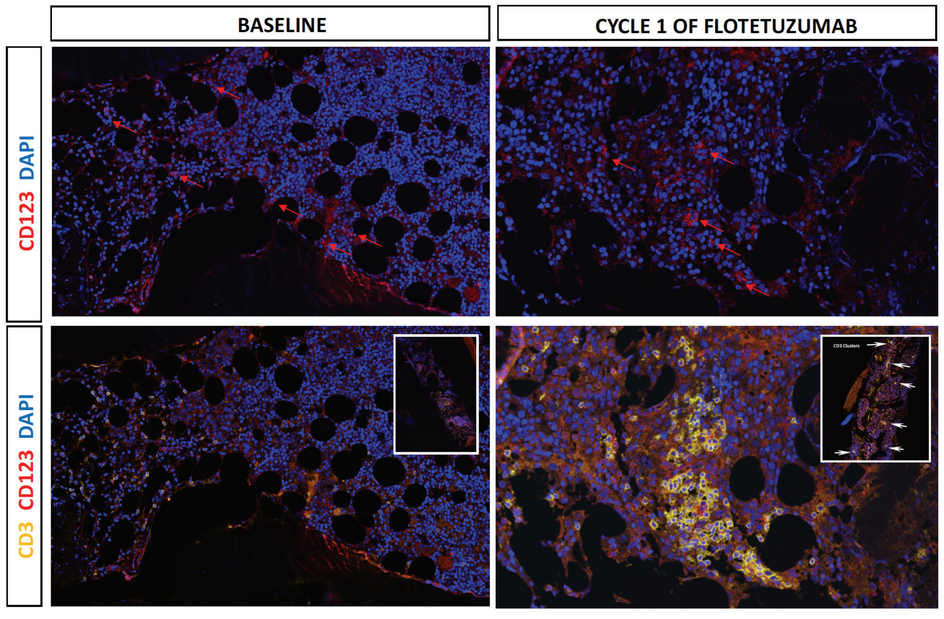


Figure 2. mIHC images of baseline and on flotetuzumab treatment on AML bone marrow biopsies for CR patient (US0008-0001). Baseline BM biopsies shown CD123+ cells and CD3+ T cells. On Cycle1 of flotetuzumab CD3+ T cells formed clusters around CD123+ cells. Dapi in blue as a counterstained.

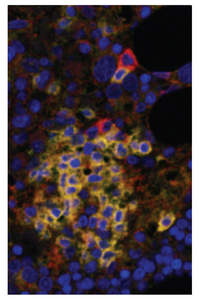
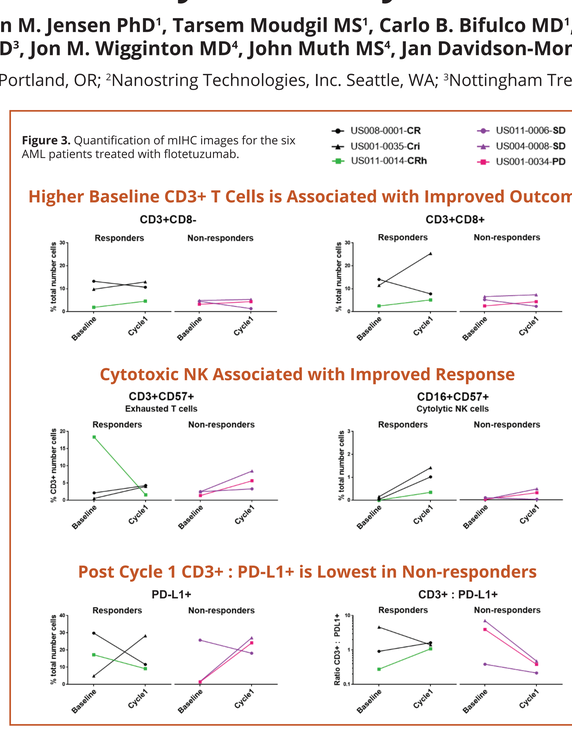
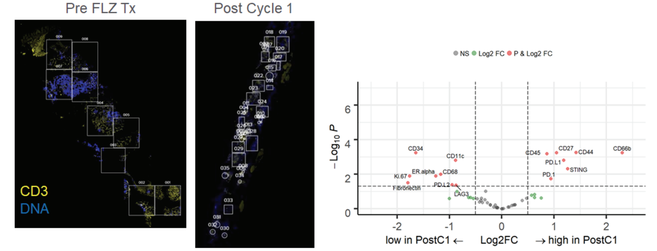


Figure 3. Quantification of mIHC images for the six AML patients treated with flotetuzumab.



GeoMx DSP Analysis Shows T-cell Markers are Increased Post Cycle 1 of Flotetuzumab



GeoMx digital spatial profiling of 50+ proteins was performed on a FFPE BM sample pre and post 1 cycle of FLZ treatment from a CR. ROIs were selected to profile the entire tissue sample. nCounter DSP protein marker counts were normalized using housekeeping and CD3 counts. Volcano plot shows differential expression of pre and postC1 ROIs. Analysis was performed using a linear mixed effect model. Vertical dotted lines are +/-0.5 log2 fold change and horizontal dotted line is a p-value of 0.05.

GeoMx DSP Analysis Shows Activation of T Cells in CD3+ Clusters Post Flotetuzumab

GeoMx digital spatial profiling of 50+ proteins was performed on FFPE BM samples post 1 or 2 cycles of FLZ treatment. At least 6 ROIs each were selected based on CD3 T cell clustered areas or CD3 non-clustered areas. nCounter DSP protein marker counts were normalized using housekeeping proteins. Volcano plot shows differential expression of T cell clustered and non-clustered ROIs. Analysis was performed using a linear model. Vertical dotted lines represent +/-0.5 log2 fold-change and horizontal dotted line is a p-value of 0.05.

Conclusions

- Consistent with flotetuzumab's proposed mechanism of action, these data highlight for the first time the dynamic induction of an increase in T-cell infiltration, and clustering around CD123 AML cells, in the bone marrow microenvironment of 2 AML patients that responded to treatment
- The baseline TME in responders was favorable compared to non-responders with greater numbers of CD3+ T cells
- Post-Cycle 1 cytolytic NK cells (CD57+CD16+) tend to be increased in responders
- Preliminary GeoMx DSP Analysis identifies: 1) An increase in T-cell activation markers post Cycle 1 in entire BM sample, 2) Increased expression of T-cell activation markers, including PD-1 and PD-L2, by cells in the CD3+ clusters vs non-clustered CD3+ ROIs
- Total PD-L1+ cells were similar in responders and non-responders but the post Cycle 1 CD3+ : PD-L1+ ratio was higher in responders. Combined with DSP data above, this suggests that PD-L1 and PD-L2 expression may be reducing therapeutic efficacy, and treatment with sequential anti-PD-1 might obviate this possible mechanism of resistance
- A trial combining flotetuzumab with sequential administration of a PD-1 inhibitor (MGA012; also known as INCMGA00012) is currently recruiting[5] (ASH 2019 poster 2662)

References

1. Uy ASH 2018. **2.** Rutella ASH 2018. **3.** Ruggieri Science 2012. **4.** ELN 2017. **5.** A Phase 1 Study of Flotetuzumab, a CD123 x CD3 DART® Protein, Combined with MGA012, an Anti-PD-1 Antibody, in Patients with Relapsed or Refractory Acute Myeloid Leukemia. Abtract #2662 ASH 2019. **6.** Grayson JM et al. *J Immunol.* 2000.

Presented at the 61st American Society of Hematology Annual Meeting, December 7–10, 2019, Orlando, FL