Bone Marrow T Cell Changes by Multiplex IHC After Treatment with Flotetuzumab, a CD123 x CD3 Bispecific DART® Protein, in a Primary Refractory t-AML Patient

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John E. Godwin[1], Carmen Ballesteros-Merino[1], Carlo B. Bifulco[1], Helene Lelièvre[2], Jon M. Wigginton[3], Jan Davidson-Moncada[3], Bernard A. Fox[1]

[1]Providence Portland Medical Center, Portland, OR; [2]Servier, Paris, France; [3]MacroGenics, Inc., Rockville, MD

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Background

- Therapy-related acute myelogenous leukemia (t-AML) is associated with adverse genetic lesions, complex karyotype, and TP53 mutation; it is challenging to treat and confers a poor prognosis

Case Report

74-year-old female with secondary-AML that developed 9 years after receiving 6 cycles of cytotoxic (FOLFOX) chemotherapy as adjuvant treatment of colorectal carcinoma

- Initial treatment consisted of 5 cycles of azacitidine (AZA), which failed to induce a response
- The patient was subsequently treated on a Phase 1 trial of flotetuzumab (MGD006/S80880) (FLZ), a novel T-cell redirecting (CD123 x CD3) DART protein [NCT02152956]
  - Patient received a total of 3 cycles (28 days/cycle) of FLZ
  - After one FLZ cycle, the patient achieved a complete response (CR), with normal cytogenetics, and residual IDH1 and TET2 mutations
  - Following 2 additional cycles of FLZ consolidation, the CR was maintained with loss of the IDH1 mutation but persistence of the TET2 mutation
  - CR was maintained with no additional therapy for approximately 7 months

Materials and Methods

Multiplex Immunohistochemistry (IHC)

Tissue sections were cut at 4 µm from formalin-fixed paraffin-embedded blocks. All sections were deparaffinized and subjected to heat-induced epitope retrieval in citrate buffer pH 9.0 (Biogenex). Multiplex IHC was performed for each tissue slide using the following antibodies: anti-FoxP3 (clone 236A/E7, dilution 1:100, Abcam), anti-PD-L1 (clone E1L3N, dilution 1:250, Cell signaling), anti-CD8 (clone SP16, dilution 1:50, Spring Bioscience), anti-CD3 (clone SP7, dilution 1:50, Spring Bioscience). Anti-flotetuzumab antibody: Biotinylated anti-EK coil antibody. Antigen-antibody binding was visualized with TSA-Opal520 (PerkinElmer), TSA-Opal690 (PerkinElmer), TSA-Opal570 (PerkinElmer), and TSA-Opal670 (PerkinElmer), respectively. Microwave treatment in citrate buffer pH 6.0 was performed between antibody addition to prevent cross-reactivity. Tissue slides were incubated with DAPI as counterstain and cover-slipped with VectaShield mounting media (Vector Labs). Controls tissue samples were stained for each marker. Hematoxylin and eosin (H&E) staining was performed on each sample and reviewed by a pathologist (CBB) to ensure the representative areas of the tissue sample.

Microscopy and Image Analysis

mIHC Images

Phenotype cell quantification in high-resolution images

- Digital images were captured with an Akoya Vectra Polaris (previously PerkinElmer). Bone marrow tissue was scanned at 20X for analysis. Multiplexed images were analyzed with InForm Software (PerkinElmer). The total number of cells were enumerated for specified phenotypes

H&E Images

- Tissue samples stained by conventional H&E were scanned using a Leica SCN400F scanner at 20X with magnification available to 200X–400X for immune infiltrate evaluation

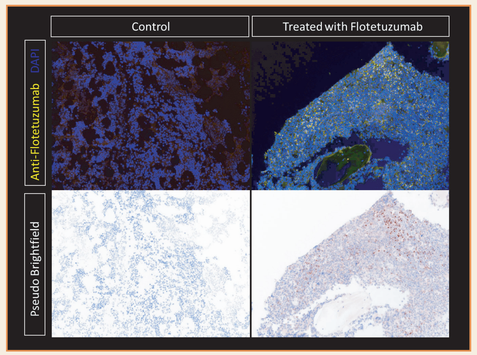
Statistical analysis

- Correlations were evaluated by the Pearson test. All P values were calculated using a two-tailed test. P values < 0.05 were considered statistically significant. Analyses were performed using GraphPad Prism

Results

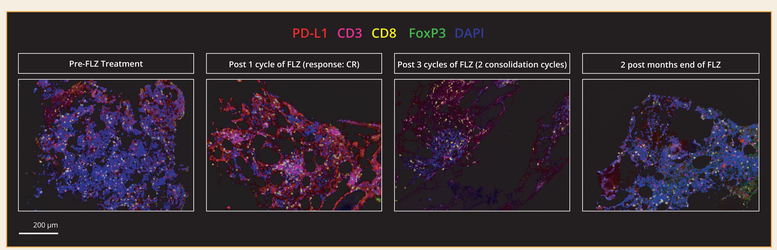
- Presence of flotetuzumab could be measured in BM sample of AML patient **(Figure 1)**
- AML exhibited clonal evolution while on treatment **(Table 1)**
- Serial BM samples were evaluated for T cells ($CD3^+$, CD4[$CD3^+$ $CD8^-$] , $CD8^+$, $FOXP3^+$ and $PD\text{-}L1^+$) using multiplex IHC **(Figure 2)**
  - BM T-cell expression was unchanged during AZA treatment, while treatment with FLZ led to significant increases in CD3, CD4, CD8, and FOXP3-positive T cells and PD-L1 expression **(Figure 3)**
  - $CD3^-$ and CD8-positive cells persisted in the BM 1 month beyond completion of 2 additional consolidation cycles of FLZ, while other T cell subsets and PD-L1 expression returned to baseline **(Figure 3)**
  - Three months after the last FLZ treatment, all T cell subsets had returned to baseline **(Figure 3)**. Early increase in leukemic blasts (8%) was noted, with normal cytogenetics **(Table 1)**
  - Five months after FLZ treatment blasts were unchanged, but a new abnormal cytogenetic clone, with additional mutations, accompanied by a rise in PD-L1 expression cells, was observed
  - Seven months after FLZ treatment, frank leukemia with 50% blasts developed, and all T cell subsets and PD-L1 expression had returned to pre-treatment levels

Figure 1. Presence of Flotetuzumab Detected in Bone Marrow



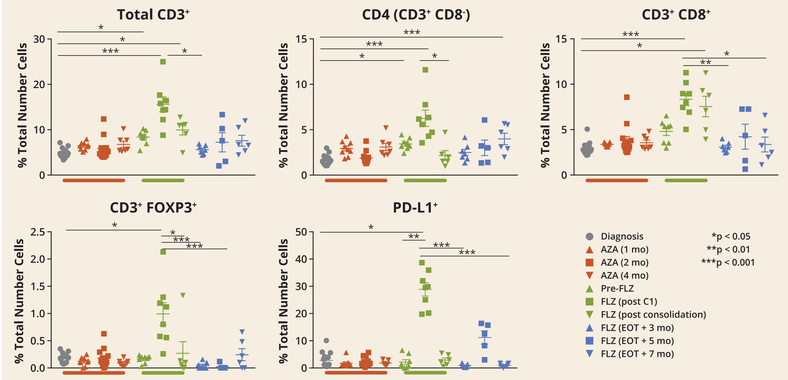
Single fluorescence IHC for flotetuzumab (anti-CD123 X CD3) detected using an anti-flotetuzumab mAb. The presence of anti-flotetuzumab was detected in the bone marrow of patient receiving drug. Upper left image is negative for anti-CD123 x CD3. Upper right image shows, in yellow, detection of drug using anti-flotetuzumab. The nuclei of cells are shown in blue. Images for this single staining are shown at the bottom. In the two lower quadrants a pseudo brightfield image is presented for the top two quadrants.

Figure 2. Multiplex IHC Staining of Bone Marrow Pre- and Post-Treatment



Multiplex IHC staining of bone marrow samples of AML patient. Four time points for the patient treated are shown above: Pre-FLZ treatment; following one cycle of FLZ (where the patient showed a complete response); after a total of 3 cycles of FLZ (1 cycle of induction therapy and 2 cycles of consolidation therapy); and 2 months post end of FLZ. PD-L1 expression is shown in red, $CD3^+$ and $CD8^+$ T cells in pink and yellow, respectively, T regs are shows in green and DAPI in blue as counterstained. Each image represents one of the regions of interest collected for analysis.

Figure 3. Quantification of Multiplex IHC Images



Graphs showing the quantification of the multiplex IHC images for the AML patient under study. Dates identify the time of BM biopsy and collection of tissue. To the extent possible, images of the entire BM specimen were collected and analyzed as regions of interest (ROI). Each dot represents the analysis of a single ROI. EOT: end of treatment. Green bar represents FLZ treatment, brown bar represents AZA treatment.

Table 1. Disease Evolution

Evaluation of patient's AML over time and association to treatments

Treatment	Diagnosis	AZA 1 month	AZA 2 months	AZA 4 months	Pre-FLZ	FLZ Post C1	FLZ post 2 cycles of consolidation	FLZ EOT + 3 mo	FLZ EOT + 5 mo	FLZ (EOT +7 mo)
CYTOGENETICS	46,XX[20]	46,XX[20]	No growth	Not done	92,XXXX,t(14;21)(q22;q22)x2[4]46,XX[16]	No growth	46,XX[20]	46,XX[20]	90,XXXX,-5,-7,t(14;21)(q22;q22)x2[3]/46,XX[17]	90–93,XXXX,add(5)(q11.2),-7,t(14;21)(q22;q22)x2,-16[cp2]46,XX[18]
BLASTS	35%	20%	30%	40%	**70%**	**1%**	**<1 %**	**8%**	**8%**	50%
FISH	tetraploid	Hyperdiploid Chr 3,4,5,5 7	Hyperdiploid Chr 3,4,5,6, 7,8, 9	Not done	Hyperdiploid Chr 3,4,5,6,7, 8	Normal	Normal	Normal	+5,+7,+8,+16,+20	Tetraploid; del 7, +5
Mutations (NGS)										
IDH1	+	+	+	+	+	+	–	–	–	–
TET2: p.E1826*	+	+	+	+	+	+	+	+	+	+
TET2: p.R1404*	+	+	+	+	+	–	+	+	+	+
SRSF2	+	+	+	+	+	+	+	+	+	+
RUNX1	–	–	–	–	–	–	+	+	+	+
ASXL1	–	–	–	–	–	–	+	+	+	+
PHF6	–	–	–	–	–	–	–	–	+	+

Conclusions

- Consistent with flotetuzumab's proposed mechanism of action, these data highlight for the first time the induction of an increase in T-cell infiltration, which persists beyond treatment with flotetuzumab, in the bone marrow microenvironment of an AML patient that also achieved a complete and durable anti-leukemic response
- Data show that flotetuzumab penetrates and binds into tumor microenvironment

Future Plans

- To further optimize and validate multiplex IHC of BM biopsies
- To evaluate expression of CD123 and checkpoint inhibitors in BM biopsies
- To determine the activation status of T cells infiltrating the BM

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