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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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-CD3 (clone SP7, dilution 1:50, Spring Bioscience), anti-CD8 (clone 32CD8, dilution 1:50, BioLegend), anti-CD19 (clone 6D5, dilution 1:50, DAKO), anti-CD20 (clone L26, dilution 1:50, DAKO), anti-CD4 (clone GK1.5, dilution 1:50, eBiosciences), anti-CD8 (clone 53-6.7.7, dilution 1:50, DAKO), anti-CD45 (clone 2F11, dilution 1:100, DAKO), anti-CD14 (clone 15G11, dilution 1:100, Abcam), anti-CD11b (clone KB11, dilution 1:100, Abcam), anti-CD123 (clone 8H10, dilution 1:100, BioLegend), anti-CD38 (clone 38-13, dilution 1:50, BioLegend), anti-CD56 (clone 1B1, dilution 1:100, BioLegend), anti-PDL1 (clone 29F1A, dilution 1:50, Biogenex), anti-PDL2 (clone 2C9, dilution 1:50, Biogenex), anti-ICOS (clone 1F10, dilution 1:50, R&D Systems), and anti-CD3 (clone SP7, dilution 1:50, Spring Bioscience).

Analyses were performed using GraphPad Prism®

Results

- Presence of flotetuzumab could be measured in BM sample of AML patient
- BM exhibited clonal evolution while on treatment (Table 1)
- Serial BM samples were evaluated for T cells (CD3+, CD4+CD3+ CD8+, CD4-CD3+ and CD8-CD3+) 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, was observed
  - Six months after FLZ consolidation, frank leukemia with 50% blasts developed, and all T cell subsets and PD-L1 expression had returned to pre-treatment levels

Conclusions

- Consistent with flotetuzumab's proposed mechanism of action, these data highlight the ability of the drug to target leukemic blasts and to reduce the 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

Table 1. Disease Evolution

<table>
<thead>
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<th>Treatment</th>
<th>Diagnosis</th>
<th>AZA (4 mo)</th>
<th>AZA (1 mo)</th>
<th>AZA (3 mo)</th>
<th>FLZ (EOT + 3 mo)</th>
<th>FLZ (EOT + 7 mo)</th>
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Graphs showing the quantification of the multiplex IHC images for the AML patient under study. Data 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 ROA. EOT: end of treatment. Green bar represents FLZ treatment, brown bar represents AZA treatment.