Preliminary Translational Results from an Ongoing Phase 1 Study of Flotetuzumab, a CD123 x CD3 DART® Protein, in AML/MDS: Rationale for Combining Flotetuzumab and Anti-PD-1/PD-L1 Immunotherapies

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Background

- Immunotherapy has transformed cancer treatment
- Although checkpoint inhibitors (CPI) have demonstrated striking clinical activity and are approved for the treatment of patients with various solid tumors and Hodgkin's disease, their role in the treatment of acute myeloid leukemia (AML) remains unclear
- Activated T-cells can upregulate PD-1 expression, and become functionally exhausted when PD-1 is engaged by ligands including PD-L1 and PD-L2
- As part of our ongoing Phase I study of flotetuzumab, a novel CD123 x CD3 DART protein, in patients with AML and myelodysplastic syndrome (MDS), we have completed translational studies that support a rationale for combining flotetuzumab with PD-1/PD-L1 inhibition

Phase 1 Flotetuzumab Study Design (CP-MGD006-01)

Dose Escalation
Single patient dose escalation (3, 10, 30, 100 ng/kg/day)

→ **Dose Escalation**
3 + 3 Multi-patient dose escalation 4-week cycles

→ **Expansion Cohort**
Relapsed/Refractory AML

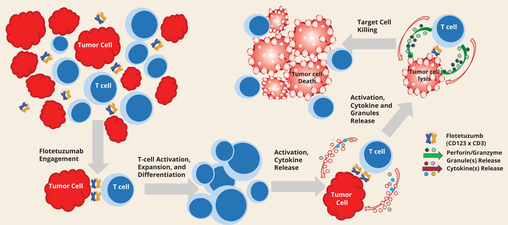
→ **Expansion Cohort**
Hypomethylation Failure MDS

- **Patient Population:** Patients with relapsed/refractory AML and hypomethylation failure Int-2/High Risk MDS
- **Dosing Regimen:** Continuous intravenous infusion with lead-in dosing
- Anti-leukemic activity in patients with relapsed/refractory AML has been reported previously (ESMO 2017; ASH 2017 Oral Presentation #637)

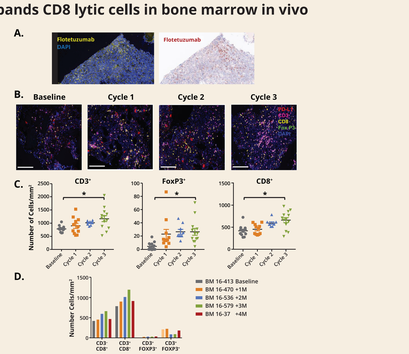
Methods

Flotetuzumab Mode of Action

Flotetuzumab redirects T-cells to lyse AML cells in conjunction with induction of T-cell activation, cytokine release and proliferation



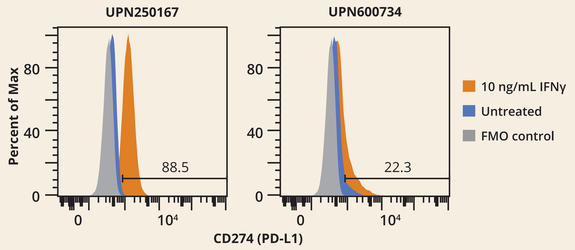
Flotetuzumab expands CD8 lytic cells in bone marrow in vivo



Multiplex IHC staining of bone marrow from AML patient treated with flotetuzumab. FFPE bone marrow sections were immunolabeled and quantifies for MGD006 **(A)** as well as PD-L1, CD3, CD8, FoxP3 and DAPI **(B)**. Analysis showed a statistically significant increase in the cell density of $CD3^+$, $FoxP3^+$ and $CD8^+$ (1.58-fold increase, p=0.0013) cells on treatment (*p<0.05) compared to baseline **(C)**. $CD8^+CD3^+$ T cells and $CD8^-CD3^+$ cells also demonstrated an increase in cell density post flotetuzumab treatment **(D)**.

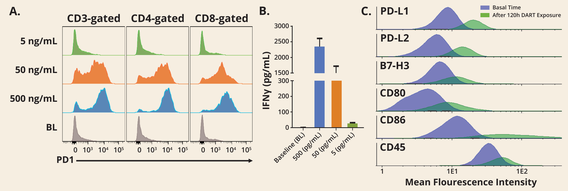
Results

IFNγ Upregulates PD-L1 in AML Blasts



Cryopreserved cells from two AML patients were cultured on HS27 stromal cells for 48 hours. Samples were left untreated or treated with IFNγ (10 ng/mL) for an additional 48 hours. PD-L1 expression on the AML blasts was evaluated by flow cytometry.

Flotetuzumab Upregulates PD-1, PD-L1 Expression and IFNγ Secretion Ex Vivo

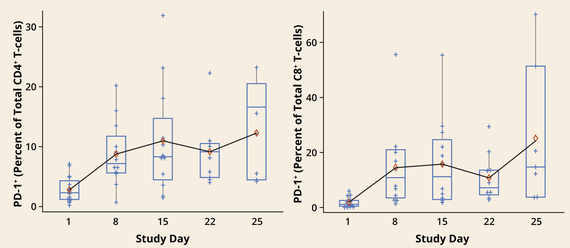


Flow cytometric analysis of PD-1 expression on T cell subsets **(A)** and IFNγ secretion in supernatants **(B)** from an AML-patient donor PBMC (24% CD123-positive cells and 14.2% CD3-positive T cells). PBMC were cultured for 48 hours in the absence (grey histograms, baseline (BL)) or presence of 5 (green histograms), 50 (orange histograms), and 500 (blue histograms) pg/mL of flotetuzumab.

Flow cytometric analysis of checkpoint (PD-L1, PD-L2, and B7-H3) and costimulatory (CD80, CD86, and CD45) molecules at base line (blue histograms) or 120 hours post-incubation of 50 ng/mL of flotetuzumab (green histograms) in AML blasts **(C)**.

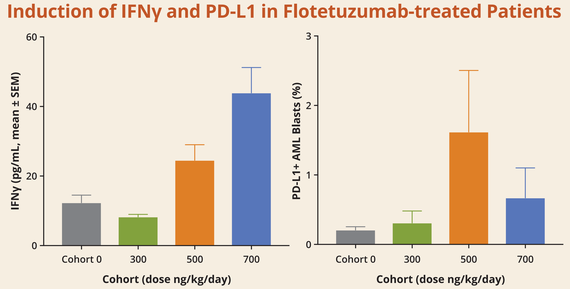
Flotetuzumab Upregulates PD-1 In Vivo

PD-1 up-regulation observed on both CD4 and CD8 T cells



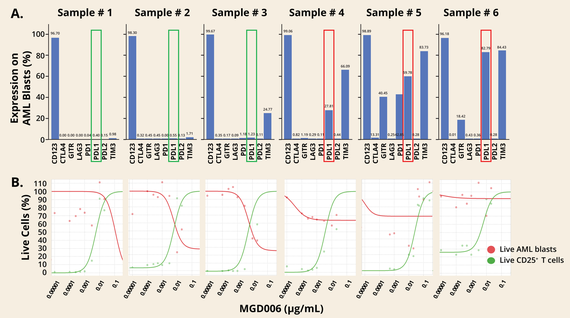
PD-1 expression on CD4 and CD8 T cells from patients treated on CP-MGD006-01 study. Graphs show median PD-1 expression of all patients treated on study.

Induction of IFNγ and PD-L1 in Flotetuzumab-treated Patients



Patient samples from CP-MGD006-01 were evaluated for circulating IFNγ levels (left panel) and PD-L1 expression on circulating AML blasts across four dose levels in Cohort 0 (each ≤100 ng/kg/day), 300 ng/kg/day, 500 ng/kg/day and 700 ng/kg/day.

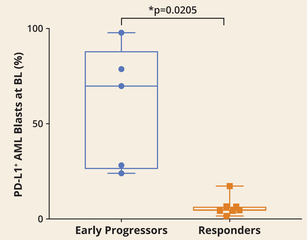
PD-L1 Expression is Associated With Decreased Flotetuzumab Anti-leukemic Activity In Vitro



Primary AML samples were co-incubated with flotetuzumab at different concentrations for 144 hours in vitro.

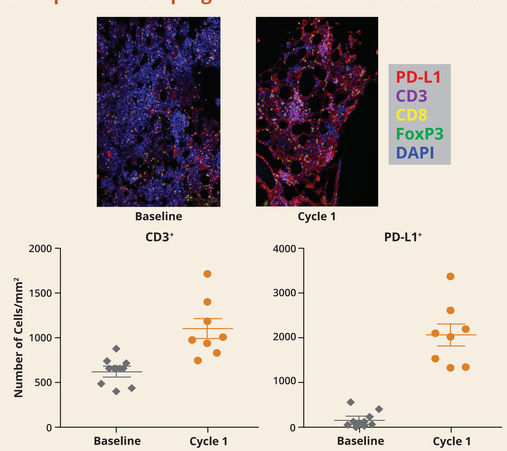
- **Figure A:** Immunophenotyping of AML samples expressed as percent of total cells. Boxes highlight expression on PD-L1 in flotetuzumab sensitive samples (green) vs resistant samples (red).
- **Figure B:** Flotetuzumab concurrently induced T-cell activation (as measured by increased cell counts of $CD25^+$ T cells (green line)) and depleted AML blasts cells (red line) in concentration-dependent manner.

PD-L1 Expression is Associated With Decreased Flotetuzumab Activity In Vivo



Patients that progressed early (<15 days) on flotetuzumab treatment had higher baseline levels of PD-L1 on AML cells than patients that had evidence of anti-leukemic activity (SD, OB, PR, CR). AML samples collected during screening were analyzed for PD-L1 expression by flow cytometry. Data is expressed as mean ± distribution.

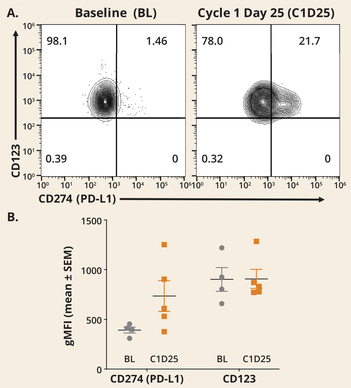
PD-L1 Expression Is Upregulated in Bone Marrow Blasts



Multiplex immunohistochemical staining of bone marrow from AML patient treated with flotetuzumab. FFPE bone marrow sections were immunolabeled and quantifies for flotetuzumab as well as PD-L1, CD3, CD8, FoxP3 and DAPI. Analysis showed a statistically-significant increase in the cell density of CD8 (p=0.0025) and PD-L1 (p<0.001) after one cycle of treatment.

PD-L1 Upregulation in Residual Bone Marrow Blasts

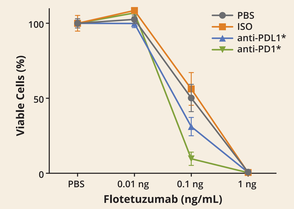
Patients on flotetuzumab with residual disease showed stable CD123 expression and increased expression of PD-L1 positive AML blasts vs. basal levels



Representative flow cytometry analysis of CD123 and PD-L1 in AML blast bone marrow samples pre (BL D-14) and post flotetuzumab (C1D25) **(A)**. Geometric mean fluorescent intensity (gMFI) of PD-L1 and CD123 expression in responding patients at baseline (BL, grey symbols) or at cycle 1 day 25 (C1D25, orange squares) **(B)**.

PD-1/PD-L1 Blockade Enhances Flotetuzumab Activity

Synergistic in vitro cytotoxicity was observed after treatment of an AML cell line with flotetuzumab in presence of CPI



KG1A cells incubated with human T cells at E:T ratio 0.25:1 in presence of flotetuzumab ±10 μg/mL anti-checkpoint antibody or isotype control. *Statistically significant (p<0.05)

Conclusions

- Flotetuzumab has demonstrated clinical activity in patients with relapsed/refractory AML
- Treatment with flotetuzumab enhances T-cell infiltration and activation in the bone marrow
- T-cell activation is associated with enhanced PD-1 expression and IFN-gamma secretion, and upregulation of PD-L1 on AML blasts
- Enhanced PD-L1 expression by AML blasts was associated with reduced flotetuzumab activity in vitro and in vivo
- Residual bone marrow AML blasts show higher expression of PD-L1 positive compared to baseline
- Synergistic in vitro T-cell mediated cytotoxicity was observed after treatment of an AML cell line with flotetuzumab in combination with PD-1/PD-L1 inhibitors
- These data suggest that combination with anti-PD-1/PD-L1 directed therapy could enhance the anti-leukemic effects of flotetuzumab in patients with relapsed/refractory AML

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