Flotetuzumab and Other Cellular Immunotherapies Upregulate MHC Class II Expression on Acute Myeloid Leukemia Cell *In Vitro* and *In Vivo*

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ASH 2020 Annual Meeting, Poster Session

December 7, 2020
Relative Financial Disclosures

• None
Introduction

- Up to 50% of AML patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) experience relapse.\(^1\)
- Thirty to fifty percent of AML samples from patients relapsing after allo-HCT have downregulated MHC class II (MHC-II) expression.\(^2-3\)
- Reinduction of MHC-II expression may lead to re-engagement of immune effectors and restoration of the graft-versus-malignancy (GvM) effect.
- Interferon gamma (IFNg) can restore MHC-II\(^2-3\) but would likely cause significant and life-threatening toxicities if administered systemically.
- T cell immunotherapies are known to cause T cell activation and localized IFNg release.
- **T cell immunotherapies targeting AML cells will lead to T cell activation, localized IFNg release, and upregulation of MHC-II on AML cells.**

Methods

• For *in vitro* studies, THP1 cells (THP1s), which have intermediate MHC-II expression, or primary human AML samples with low MHC-II from a patient relapsing after allo-HCT (AML-low cells) were used.

• The following T-cell immunotherapies were tested:
  • Flotetuzumab (FLZ), an investigational CD123 x CD3 bispecific DART® molecule (MacroGenics, Rockville, MD)
  • CD33 x CD3 bispecific molecule (Creative Biolabs, Shirley, NY)
  • CD123-directed chimeric antigen receptor (CAR) T cells

• MHC-II expression was measured by flow cytometry.

• IFNg concentrations were measured via Luminex immunofluorescence assay.

• THP1 IFNg receptor-1 (IFNgR1) knockout cell lines were generated using CRISPR-Cas9.

• To rescue THP1’s from FLZ-induced death and allow for longitudinal evaluation, a transwell plate system was used.

• For *in vivo* experiments, NOD-scid IL2Rgamma-null mice expressing human IL-3, GM-CSF, and SCF (NSG-S) were used.
Figure 1. T cell immunotherapies upregulate MHC-II expression on THP1s and primary AML-low cells in vitro
Figure 2. FLZ-induced MHC-II upregulation lasts 48-72 hours and activates MHC-mismatched CD4+ T cells.

0.4um Pores allow transfer of small proteins (i.e. cytokines) but not cells:
- THP1: 16.5um diameter
- T lymphocyte: 7.3um
- IFNg: 3-4nm

IFNgR: Interferon gamma receptor
○: Interferon gamma
Figure 2. FLZ-induced MHC-II upregulation lasts 48-72 hours and activates MHC-mismatched CD4+ T cells.

2A. Transwell Experimental Design

2B. FLZ-induced MHC-II upregulation peaks at 48-72 hours.

2C. THP1s with FLZ-induced MHC-II upregulation activate MHC-mismatched T cells in a 48-hour mixed lymphocyte reaction.

IFNgR: Interferon gamma receptor
●: Interferon gamma
Figure 3. FLZ-induced MHC-II upregulation is mediated by IFNg.

3A. THP1 + T cell + FLZ co-cultures contain high levels of IFNg at 48 hours.

3B. IFNg and IFNgR1 blocking antibodies inhibit FLZ + T cell induced MHC-II upregulation on AML-low cells at 24 hours.

Figure 3C. IFNgR-KO THP1 do not upregulate MHC-II after a 24 hour co-culture with IFNg or FLZ + T cells.
Figure 4. JAK inhibitors prevent FLZ-induced MHC-II upregulation.

4A. Forty-Eight hour co-culture with THP

4B. Forty-Eight hour co-culture with AML-low
Figure 5. FLZ with T cells upregulates MHC-II expression on AML-low cells in an in vivo xenograft model.

20 NSG-S Mice  →  250 rad; Inject 1E6 AML Cells (contain hT cells) → Engraftment (approximately ~5.5 weeks) → Control 2mg/kg DART 10e7 hT Cells hT Cells + DART → Bleed Mice at 24, 48 hrs; Harvest BM/Spleen at 48hrs → HLAII Expression on AML T Cell activation markers

Figure 5B. AML-low cells engrafted well in the bone marrow after 5.5 weeks.

Figure 5C. FLZ + T cells upregulates MHC-II expression on AML-low cells engrafted in the bone marrow.
Discussion

- FLZ and other T cell immunotherapies targeting AML antigens can upregulate MHC-II expression in vitro. FLZ can upregulate MHC-II expression in vivo.
- This effect peaks at 48-72 hours in an in vitro transwell system and leads to activation of MHC-mismatched CD4+ T cells.
- This effect is mediated by IFNg and is blocked by IFNg antibody blockage, KO of IFNgR1, and JAK inhibition.
- Single cell RNA sequencing of AML-low cells harvested from in vivo experiments is ongoing.
- Future studies include evaluation of the kinetics of MHC-II upregulation on AML-low cells in the in vitro and in vivo settings; evaluation of other primary AML samples; and determining whether FLZ-mediated MHC-II upregulation on AML cells can lead to FLZ-independent, MHC-II mediated allogeneic T cell activation in an in vivo model.
- These preclinical results show that FLZ may potentially stimulate donor cell recognition and increase the GvM effect. However, IFNg can also stimulate increased checkpoint inhibitor expression. Further research is needed to better understand the end result of these opposing effects.
- Based on these preclinical results, a clinical trial evaluating FLZ for AML patients relapsing after allo-HCT is planned.