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Inhibition of Epigenetic Reader BET Proteins by Apabetalone Counters Inflammation in Activated Innate Immune Cells from Fabry Disease Patients Receiving ERT

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†**Disclosure:** Authors are employees of Resverlogix & hold stock or stock options.

INTRODUCTION

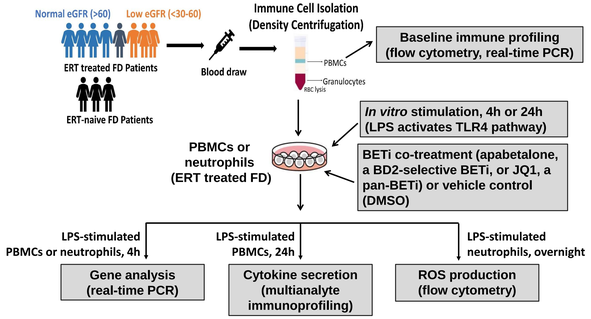
- Fabry disease (FD) is a rare genetic disorder resulting in a deficit in the degradation pathway of a glycolipid, globotriaocylceramide (Gb3)
- Gb3 deposits in cells and tissues evoke immune-mediated systemic inflammation through activation of the TLR4 pathway, driving the progression of FD complications
- Enzyme replacement therapy (ERT) is less effective in the late phases of FD, partially due to uncontrolled inflammation and fibrosis that contribute to life-threatening consequences in multiple organs (e.g., the heart and kidney)
- Apabetalone (RVX-208) is a clinical-stage drug candidate that regulates gene transcription by blocking the activity of the epigenetic readers, bromodomain and extra-terminal (BET) proteins. Apabetalone is well-tolerated in >1,900 patients tested in multiple clinical trials (phase 1, 2 and 3)

AIM

- Assess immune profiles in unstimulated peripheral blood mononuclear cells (PBMCs) from FD patients ± ERT (ERT-naïve and ERT treated FD patients)
- Examine the effects of BET inhibitor (BETi), apabetalone, on TLR-initiated pro-inflammatory responses in FD patients

METHOD

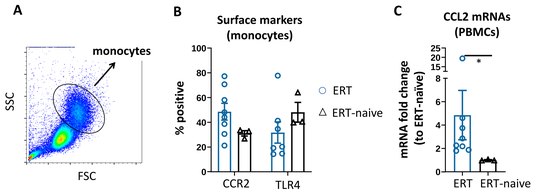
- Baseline immune profiling: flow cytometry and real-time PCR (unstimulated PBMCs from FD patients)
- *Ex vivo* apabetalone treatment: PBMCs or neutrophils from ERT treated FD patients were stimulated with LPS (a TLR4 ligand) with BETi co-treatment (4h or overnight)
- Cytokine secretion: multianalyte immunoprofiling, real-time PCR
- ROS production: flow cytometry



RESULTS

Fig 1. Dysregulated immune cell activation in PBMCs from ERT treated FD patients

Baseline immune profiles of PBMCs from FD patients. ERT treated FD patients (n=8) are undergoing continuous ERT treatment, whereas ERT-naïve patients have no history of ERT upon FD diagnosis (n=3). Surface markers were measured by flow cytometry, pro-inflammatory gene expression was determined by real-time PCR



Pro-inflammatory gene analysis of unstimulated PBMCs from ERT treated FD patients with normal eGFR (n=4) start versus end of the study

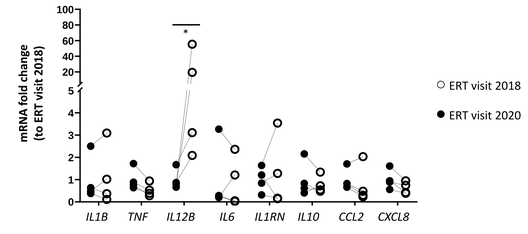
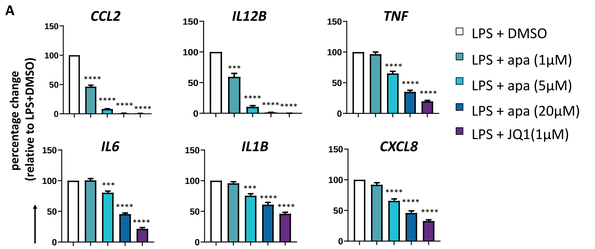


Fig 2. Ex vivo apabetalone-mediated BET inhibition reduces pro-inflammatory responses in LPS-stimulated PBMCs from ERT treated FD patients

Quantification of pro-inflammatory gene expression in cultured PBMCs from ERT treated FD patients (n=8) stimulated with LPS ± BETi co-treatment (4h)



Quantification of secreted cytokines by cultured PBMCs from ERT treated FD patients (n=6) stimulated with LPS ± BETi co-treatment overnight

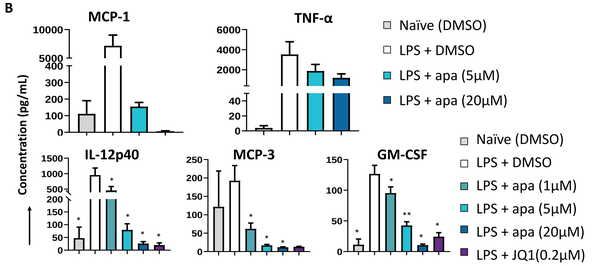


Fig 3. The expression of pro-inflammatory genes is regulated through a BET-dependent mechanism

Pro-inflammatory gene analysis of LPS-stimulated PBMCs from one ERT treated FD patient in the condition of BET protein degradation. PBMCs were pre-treated with MZ-1 (BET degrader) or apabetalone for 4h and then stimulated with LPS for additional 2h

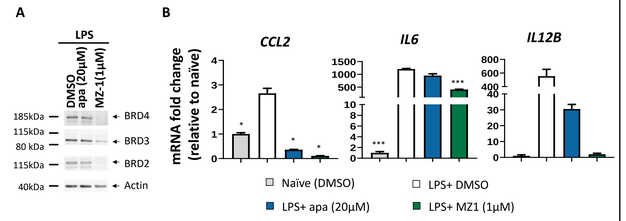
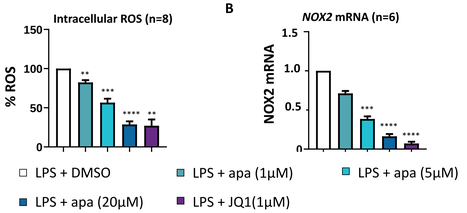


Fig 4. Apabetalone attenuates ROS production in LPS-stimulated neutrophils from ERT treated FD patients

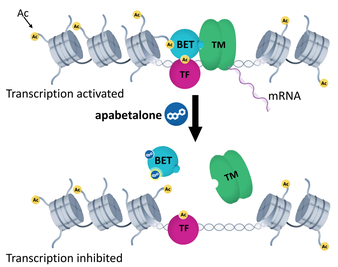
Assessment of ROS production from LPS-stimulated neutrophils from ERT treated FD patients (n=8) ± BETi co-treatment. Intracellular ROS production (overnight) was examined by flow cytometry, expression of *NOX2* (encoding a major factor generating ROS) were assessed by real-time PCR



Statistical analysis: Mann Whitney test in Figure 1 and one-way ANOVA followed by Dunnett's multiple comparison test relative to LPS+DMSO in Figures 2-4. *p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001. Note: apa denotes apabetalone in Figures 2-4

Apabetalone Mechanism of Action

Ac: acetylation TF: transcription factors TM: transcriptional machinery



BET proteins recruit other transcriptional elements → forms the transcriptional complex → activates gene transcription

Apabetalone blocks the interaction of BET proteins with acetylated histones and transcription factors → disrupts BET-dependent transcriptional complexes → inhibits gene transcription

CONCLUSIONS

- Baseline immune profiles demonstrate immune dysfunction in ERT treated FD patients
  - Upregulated *CCL2* expression in PBMCs from ERT treated FD patients
  - Elevated *IL12B* expression in PBMCs from FD patients over 2-years of continuous ERT
- *Ex vivo* apabetalone treatment mitigates TLR4-initiated pro-inflammatory responses in immune cells isolated from ERT treated FD patients
  - Reduces cytokine production in LPS-stimulated PBMCs via transcriptional regulation
  - Attenuates ROS production in LPS-stimulated neutrophils partially through regulation of *NOX2*
- Apabetalone treatment may reduce pathological inflammation and oxidative stress in FD patients and thus complement ERT to optimize patient outcomes, warranting further investigation of apabetalone as a therapeutic for FD

ACKNOWLEDGEMENTS

The authors would like to thank all the participants who made the study possible, as well as the nurses and staff at M.A.G.I.C. clinic for their valuable contributions.

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