Apabetalone modulates Th1 responses in diabetes and CVD through intrinsic and extrinsic mechanisms: in vitro and in human study

Norman C. Wong [1], Li Fu [1], Laura Tsujikawa [1], Brooke D. Rakai [1], Sylwia Wasiak [1], Dean Gilham [1], Stephanie C. Stotz [1], Michael Sweeney [2], Jan O. Johansson [2], and Ewelina Kulikowski [1]

[1]Resverlogix Corp. Calgary, Canada, [2] Resverlogix, San Francisco, USA

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

Abstract

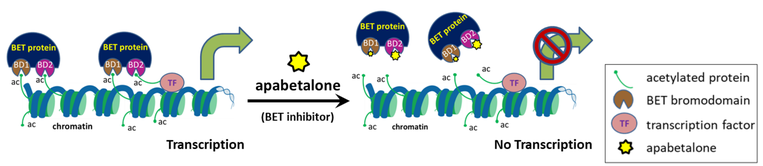
Background: T cells play a dominant role in promoting chronic inflammation and insulin resistance during type 2 diabetic mellitus (T2DM) progression and the development of associated complications such as cardiovascular disease (T2DM/CVD). Upon stimulation, circulating T cells enter into tissues/organs where they change their phenotype to either the Th1 tissue damage state or the Th2 tissue regeneration state. This phenotypic switching process, known as Th1/Th2 polarization, is regulated through intrinsic pathways (e.g. intracellular transcription factors) and extrinsic mechanisms (e.g. extracellular cytokines). In the process of T2DM and onset of CVD, naïve T cells polarize to Th1 phenotype and produce pro-inflammatory Th1 cytokines leading to tissue damage. Apabetalone (RVX-208) is an oral compound that regulates gene transcription by blocking the activity of the epigenetic readers, bromodomain and extra-terminal (BET) proteins. In phase 2 trials, apabetalone treatment reduced the incidence of major adverse cardiovascular events (MACE) by 44% in CVD patients and by 57% in diabetic CVD patients. Here we examined whether apabetalone contributes to lowering CVD risk by affecting genes involved in Th1 responses.

Materials and methods: PBMCs derived from T2DM/CVD patients or normal donors were stimulated with CD3 and CD28 antibodies and co-treated with apabetalone. Th1 marker changes driven by apabetalone were determined by mRNA assays. To further evaluate apabetalone induced changes, we assessed levels of circulating inflammatory mediators in plasma from patients in phase 2 trials via a SOMAscan® proteomic analysis (a measure of ~1300 analytes).

Results: PBMCs express lower basal levels of pro-inflammatory cytokines (e.g. IL2 and IL17) in diabetic patients receiving glycemic treatments and/or cholesterol lowering therapies relative to normal controls. However, upon CD3 and CD28 antibody stimulation, their resting T cells become hyper-activated and increase the expression of IL2 and TNFα by approximately 7- and 4- fold compared to controls. Apabetalone attenuates this over production by up to 65%. Anti-CD3 and anti-CD28 stimulation induces similar levels of IFN-γ in T2DM vs control. However, apabetalone still suppresses the induced IFN-γ by 40%. T-bet is the key transcription factor that drives Th1 polarization and thus exacerbates chronic inflammation. With in vitro anti-CD3 and anti-CD28 stimulation, T-bet is induced up to ~5 fold in PBMCs from T2DM patients, and apabetalone diminishes this upregulation by 50%. Consistent with the in vitro results, in diabetic CVD patients treated with 200mg apabetalone for 12 weeks, IL2 and IFN-γ abundance in plasma were reduced by 11% and 21% versus baseline, respectively. In contrast, plasma IL2 was increased by 22% and IFN-γ was reduced by 2% in patients treated with placebo.

Conclusion: For the first time, we demonstrate the immunomodulatory action of apabetalone on T cells from patients with T2DM and CVD through intrinsic (preventing the upregulation of the transcription factor T-bet) and extrinsic (inhibiting the production of Th1 cytokines) mechanisms. Downregulation of Th1 responses by apabetalone may contribute to the reduction in CVD events observed in phase 2 studies. The ability of apabetalone to prevent MACE in post-acute coronary syndrome patients with T2DM and low HDL-C is being assessed in the phase 3 trial (BETonMACE).

Mechanism of Action



BET protein: Bromodomain & extraterminal domain protein

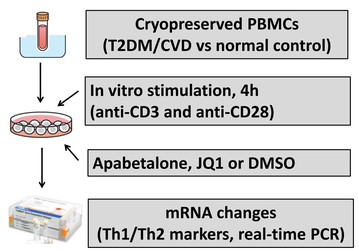
Yellow star indicates selectivity of apabetalone for BD2

Aims

- **Assess baseline characteristics of Th1/Th2 markers in T2DM/CVD patients (pts) relative to normal controls to determine inflammatory state in T2DM/CVD**
- **Examine apabetalone effects on T cell responses in the setting of T2DM/CVD (*in vitro* assays and *in vivo* human study) to understand the biology of apabetalone in reducing MACE events**

Methods

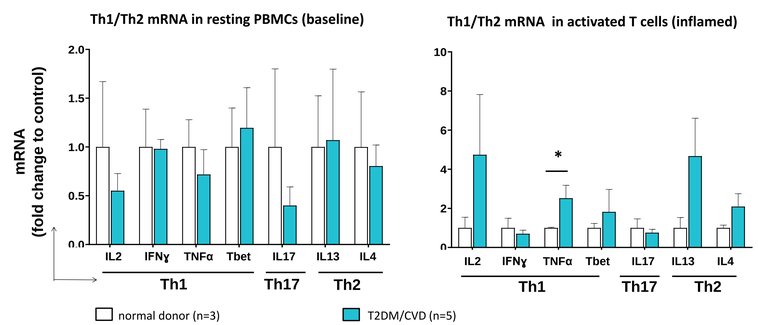
- ***In vitro* pilot study**: Th1 and Th2 changes driven by apabetalone



- Protein levels of Th1 markers were assayed in ASSERT a phase 2 trial in which stable CVD pts were treated with apabetalone (100mg b.i.d) or placebo. Plasma protein changes within 12 weeks were examined by SOMAscan® proteomic analysis

Results

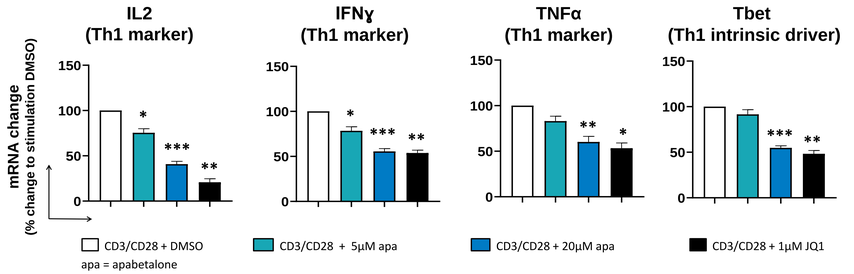
Fig 1. Th1 genes are overexpressed in activated T cells from T2DM/CVD patients



PBMCs derived from T2DM/CVD pts (n=5) and normal donors (n=3) were stimulated with anti-CD3 and anti-CD28 antibodies or media control for 4hrs. The cells were harvested for real time-PCR analysis of Th1 and Th2 genes in resting PBMCs (media control, baseline) or activated T cells (anti-CD3 and anti-CD28 stimulation, inflamed).
Bar graph statistics: Mann-Whitney test * $p<0.05$

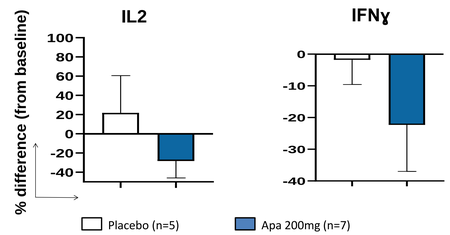
Results (cont'd)

Fig 2. Apabetalone inhibits transcription of induced Th1 genes in activated T cells from T2DM/CVD patients



PBMCs from T2DM/CVD pts (n=5) were stimulated with anti-CD3 and anti-CD28 antibodies (CD3/CD28) plus apabetalone (5 or 20 μM) co-treatment, pan BET inhibitor, JQ1 (1μM), or vehicle (DMSO) for 4 hrs prior to cell harvest for real time-PCR analysis. Statistics: mixed effects analysis followed by Dunnett's multiple comparison test relative to stimulation control (DMSO with CD3+CD28) *** $p<0.001$, ** $p<0.01$, * $p<0.05$

Fig 3. Apabetalone reduces Th1 protein abundance in plasma of T2DM/CVD patients



In the ASSERT phase 2 trial, stable CVD pts were treated with placebo or 100 mg b.i.d apabetalone for 12 weeks. Plasma was collected at day 0 and week 12 and analyzed for protein abundance with SOMAscan® proteomic assay. Percent differences of IL2 and IFNγ relative to baseline in the T2DM/CVD sub-group treated with placebo or apabetalone are shown.

Summary and Conclusion

1. **Th1 markers are overexpressed in stimulated PBMCs from T2DM/CVD pts vs controls**
2. **Apabetalone suppresses transcription of Th1 markers involved in intrinsic (T-bet) and extrinsic (IL2, IFNγ, TNFα) T cell activation pathways (*in vitro* assays)**
3. **Apabetalone reduces protein abundance of Th1 markers in plasma of T2DM/CVD pts**
4. **Attenuation of Th1 mediated inflammation by apabetalone may contribute to the reduced MACE observed in phase 2 trials, which is currently being tested in the phase 3 trial, BETonMACE**