EQ504: A Novel AhR Agonist That Promotes Immune Tolerance Through Modulating T Cell Function

POSTER 107

Jeanette Ampudia[1], Dalena Chu[1], Stephen Connelly[1], Cherie Ng[1]
[1]Equillium, Inc. San Diego, CA

Introduction

- Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that promotes immune tolerance at least in part by increasing the generation and function of regulatory T cells[1]. Activation of AhR is also linked to the induction of cytochrome P450 1A1 (CYP1A1), a key enzyme often used as a pharmacodynamic marker of AhR pathway activation.
- Efforts to target AhR have been hampered by the lack of drug candidates with good drug-like properties.
- The AhR pathway has been implicated in Ulcerative Colitis (UC) a type of inflammatory bowel disease (IBD), which is an autoimmune inflammatory diseases. In UC specifically, an imbalance between T_{regs} and T_h17 cells contributes to chronic intestinal inflammation, with reduced T_{reg} activity and elevated pathogenic T_h17 responses exacerbating disease severity. Expression levels of AhR are reduced in UC patients and endogenous AhR ligand availability is decreased in IBD overall, suggesting that insufficient AhR signaling may underlie impaired mucosal immune regulation. Decreased levels of endogenous AhR ligands have been reported in IBD patients compared with healthy controls. Therefore, AhR activity may be related to IBD symptoms[2].
- Here we test the impact of a new small molecule AhR agonist EQ504, a derivative of the endogenous ligand ITE (2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester)) with increased potency, on T_{regs} (anti-inflammatory) and T_h17 (pro-inflammatory) cells.

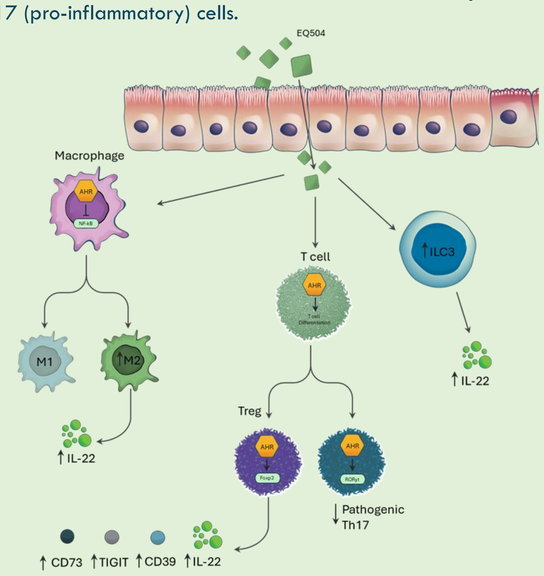

Figure 1. AhR Ligands Regulates Important Cellular Contributors to Colitis

Methods

Total PBMCs: naturally occurring T_{regs} (nT_{regs}) and endogenous T_h17 cells were assessed from total PBMCs. Total PBMCs were treated with EQ504, Vehicle control, other AhR agonists and CD3/CD28 stimulation for 72 or 96hrs. nT_{regs} were assessed by CD4+CD45RA-CD25hiCD127lo. Functional outcome of Tregs were assessed by markers such as CD39 and TIGIT. Endogenous T_h17 cells were identified by CD4+CD45RA-CCR6+, to further evaluate pathogenic T_h17 cells, cells were stained for CXCR6, CCR6 and RORγt.

Differentiated T_{regs} (iT_{regs}) and differentiated T_h17: cells were generated from naïve CD4 T cells using a STEMCELL kit. To induce iTregs, isolated naïve T cells were treated with a Treg differentiation cocktail (STEMCELL) and a low level CD3/CD28 stimulation for a total of 7 days. To induce T_h17 cells, isolated naïve T cells were treated with IL-1β, IL-6, TGF-β, IL-23 and low level CD3/CD28 stimulation for a total of 7 days. Cells were assessed by the same phenotypic markers as mentioned above (total PBMCs).

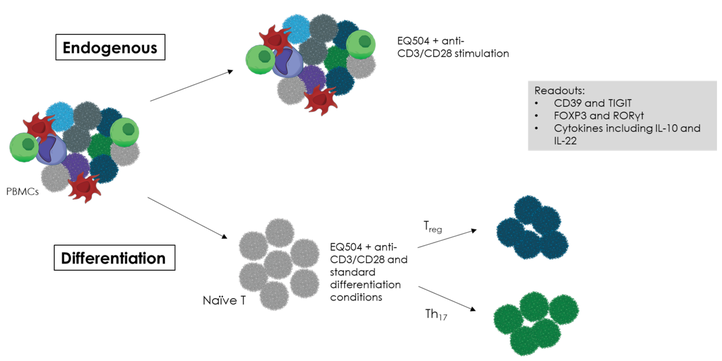

Figure 2. Schematic of the experimental approach to treat endogenous T_{regs}/T_h17 and/or differentiated T_{regs}/T_h17 cells.

Results

- **EQ504 Increases Foxp3 Expression While Simultaneously Decreasing T_{h17} Pathogenic Activity in total PBMCs**

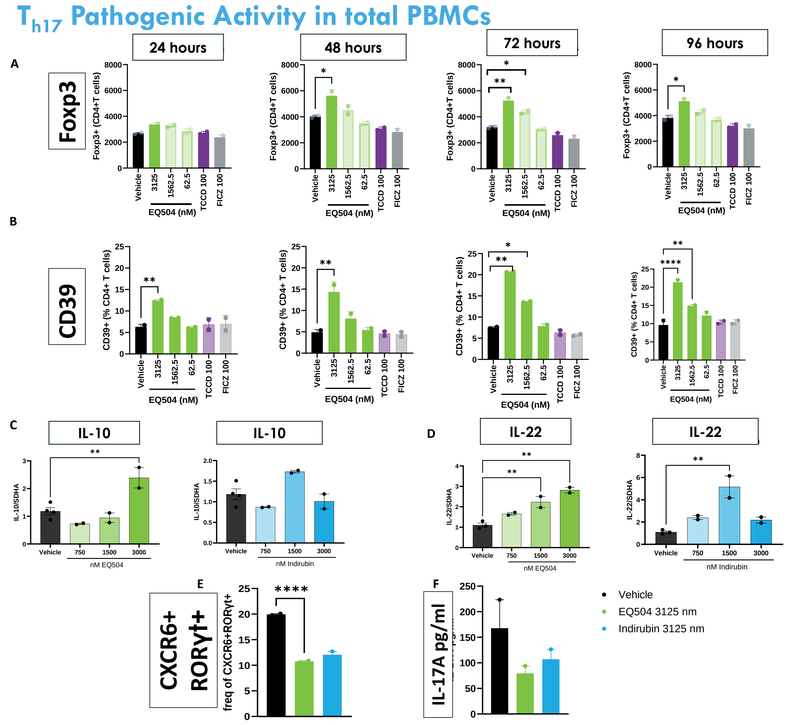

Figure 3. (A) Kinetics of Foxp3 expression and (B) CD39 on total CD4+ T cells in the presence of EQ504 or TCCD and FICZ. Total PBMCs were incubated with three different concentrations of EQ504 or Indirubin for 72hrs. PCR analysis was carried out for both IL-10 and IL-22. (C) IL-10 is detected at the highest concentration of EQ504. (D) However, there is a dose response of IL-22 detection by EQ504. (E) Total PBMCs were treated with EQ504 or Indirubin for 72 or 96hrs. A decrease in frequency of endogenous pathogenic T_h17 (CXCR6+RORγt+) as well as (F) IL-17A cytokine release can be observed.

- **EQ504 Decreases the Release of IL-17A While Inducing IL-22 of Differentiated T_h17**

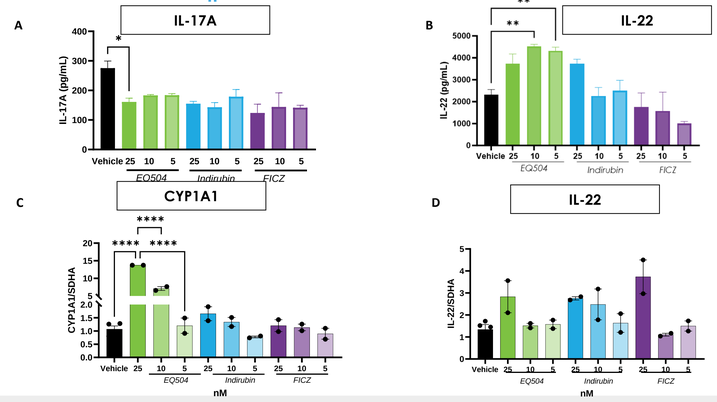

Figure 4. Differentiated naïve T cells treated with the IL-17 cocktail for 7 days in the presence of EQ504, Indirubin or FICZ. (A) EQ504 decreases IL-17A while inducing (B) IL-22 cytokine release and (D) protein expression. (C) EQ504 engages CYP1A1, a gene regulated by the AhR pathway.

- **EQ504 Increases Markers of T_{reg} Function During Differentiation**

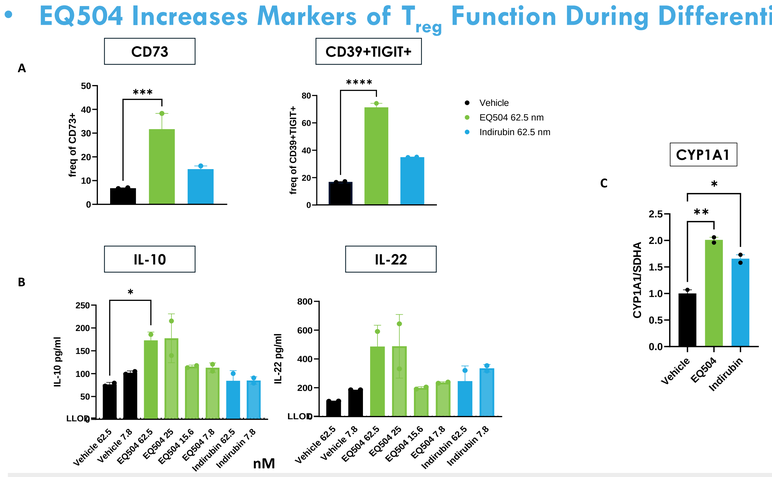

Figure 5. (A) CD73+ T_{regs} suppress proinflammatory activity by dendritic cells, macrophages and T cells through immunosuppressive adenosine production[4]. (B) TIGIT+ T_{regs} selectively inhibit pro-inflammatory T_h1 and T_h17 cells[5]. CD39hi T_{regs} have stronger stability and function under inflammatory conditions[6]. (B) EQ504 drives IL-10 and IL-22 cytokine release and engages (C) CYP1A1 genes regulated by the AhR pathway (both molecules at 100nM)

- **EQ504 Differentiated T_{regs} Have Greater Suppressive Function**

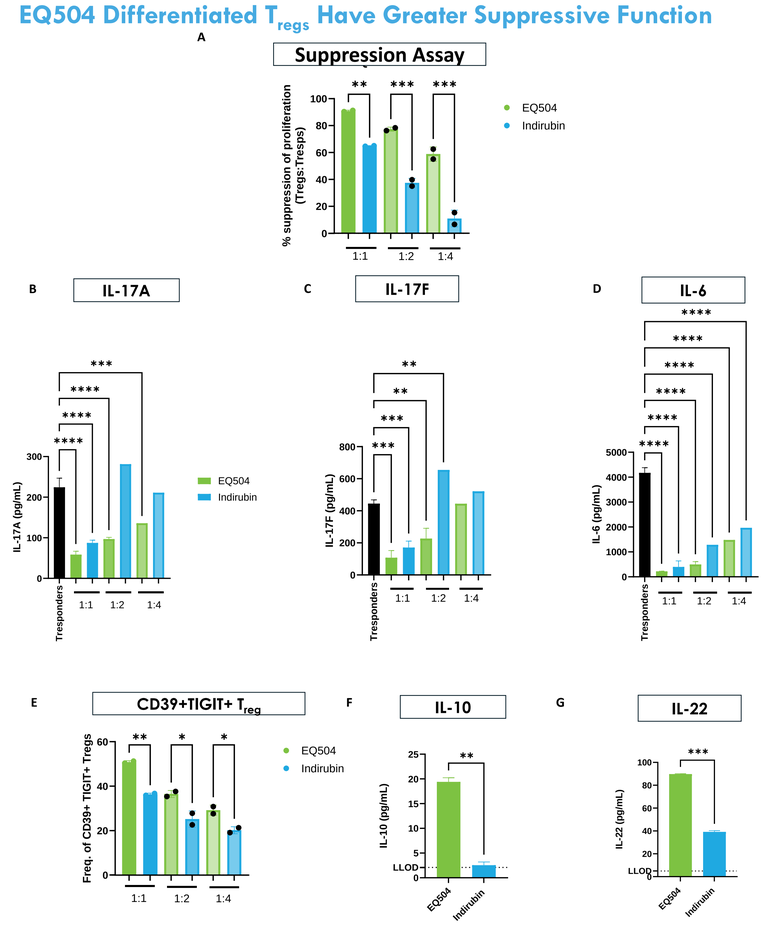

Figure 6. (A) Tresponders of the same donor were labeled with Cell Trace Violet. iT_{regs} differentiated for 7 days (100nM of EQ504 or Indirubin) were co-cultured with Tresponders at 1:1, 1:2, and 1:4 Treps:T_{reg} ratio. Suppression assay was stimulated with CD3/CD28 Immunocult 10ul/ml for 4 days. Suppression of T_{regs} differentiated with EQ504 have greater suppressive ability over Indirubin. Suppressive activity can also be observed by inhibition of (B) IL-17A, (C) IL-17F and (D) IL-6. (E) When measuring the iT_{regs} during suppression they continued to express higher levels of CD39+TIGIT+. We also measured iT_{regs} alone that underwent the same stimulation conditions with the suppression assay for 4 days. We observed an increase in (F) IL-10 and (G) IL-22. The expression of CD39, TIGIT plus the release of IL-10 and IL-22 could be contributing to the suppressive activity.

Conclusions

T_h17: EQ504 reduces the frequency of CXCR6$^+$ RORγt$^+$ pathogenic T_h17 cells in both endogenous and in vitro differentiated subsets. This is accompanied by decreased IL-17A levels and a modest increase in IL-22 production. EQ504 also upregulates CYP1A1, indicating activation of AhR responsive pathways.

T_{reg}: In total PBMCs, EQ504 enhances Foxp3 expression and promotes IL-22 secretion, suggesting improved mucosal immune regulation. iT_{regs} generated with EQ504 exhibit higher expression of suppressive markers CD39, TIGIT, and CD73 compared to those induced with Indirubin and maintain these markers during suppression. CYP1A1 induction is also observed, further supporting AhR pathway activation.

EQ504 drives T_{reg} stability and function while suppressing pathogenic T_h17 responses, highlighting AhR modulation as a promising strategy to rebalance T_{eff}/T_{reg} ratios in immunoinflammatory diseases.

Acknowledgments

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References

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Disclosures
This study was funded by Equillium, Inc., Jeanette Ampudia, Dalena Chu and Cherie Ng are currently employees and stockholders of Equillium.
Stephen Connelly is currently an employee, stockholder, and officer of Equillium.
To request permission or to ask any questions about the poster, please contact jeanetteampudia@equilliumbio.com