POSTER 242

EQ504: A Novel AhR Modulator That Promotes Immune Tolerance Through IL-10 & IL-22 Cytokine Release

Jeanette Ampudia[1], Rosa Quinn Closser[1], Phoi Tiet[1], Mary Casis[1], Valeria Marrocco[1], AJ Giovannone[1], Stephen Connelly[1]

[1]Equillium, Inc. San Diego, CA

Introduction

- The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that promotes immune tolerance by enhancing development and regulatory function of T cells (T_{reg}) and macrophages (M2).
- AhR signaling exerts it's immunoregulatory function partly through induction of IL-10 (an immuno-modulatory peacekeeper) and IL-22 (an epithelial protector & healer) that synergize to maintain mucosal homeostasis.
- More specifically, IL-10 mediates immune regulation, resolves inflammation and supports tolerance and resolution of inflammation across immune and epithelial compartments, while in parallel, IL-22 acts on intestinal epithelial cells to reinforce barrier integrity and promote epithelial regeneration[1,2].
- Activation of AhR in immune cells, including T_{reg} cells and antigen presenting cells (APC) such as macrophages, enhances anti-inflammatory pathways and limits pathogenic immune responses.
- Despite its therapeutic potential inflammatory bowel disease (IBD), clinical development of AhR modulators has been limited by the lack of potent, selective and drug-like molecules[3,4].

➢ **We evaluated EQ504, a novel small-molecule AhR modulator derived from the endogenous ligand ITE. Human immune cells were treated with compound to assess AhR activation, T_{reg} cell differentiation, and macrophage polarization. Cytokine production of IL-10 and IL-22 and phenotypic markers were analyzed to define immunoregulatory outcomes.**

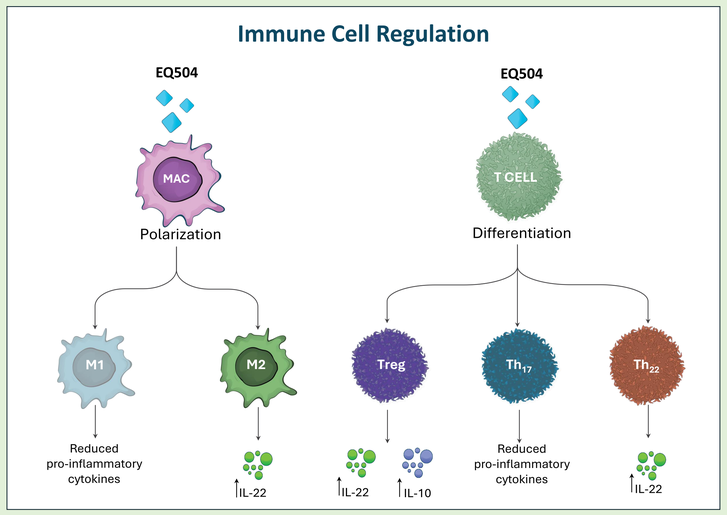

Figure 1. AhR Ligands Regulates Important Cellular Contributors to Colitis

Methods

Total PBMCs: naturally occurring T_{regs} (nT_{regs}) and endogenous T_h17/T_h22 cells were assessed from total PBMCs. Total PBMCs were treated with EQ504, Vehicle control, other AhR agonists and anti-CD3/CD28 stimulation for 72 or 96hrs. nT_{regs} were assessed by CD4+CD45RA-CD25^{hi}CD127^{lo}. Functional outcome of T_{regs} were assessed by markers such as CD39, TIGIT, CD73 and Foxp3 (data previously shown AAI 2025). Endogenous T_h17 cells were identified by CD4+CD45RA-IL-17A+, endogenous T_h22 by CD4+CD45RA-IL-22+.

Differentiated T_{regs} (iT_{regs}) and differentiated M2 Macrophages: cells were generated from naïve CD4 T cells using a STEMCELL kit. To induce iT_{reg}s, isolated naïve T cells were treated with a T_{reg} differentiation cocktail (STEMCELL) and a low level CD3/CD28 stimulation for a total of 7 days. To induce M2 macrophage differentiation, monocytes were isolated using a STEMCELL kit. MCSF was introduced along with EQ504, Vehicle control, other agonists for 5 days. At day 5 there was a media refresh and the addition of IL-6, IL-4 and IL-13 to further drive polarization. Cells were then evaluated at day8. M2s were assessed by CD124 and CD206, functional outcome by CD80, CD83 and cytokine.

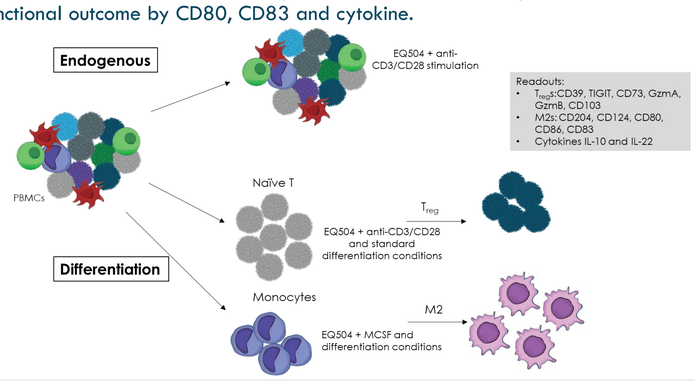

Figure 2. Schematic of the experimental approach to treat PBMCs, endogenous T_{reg}s and/or differentiated T_{reg}s and M2 Macrophages.

Results

EQ504 Suppresses APC Driven Immune Recruitment and Activation of M2 Macrophages

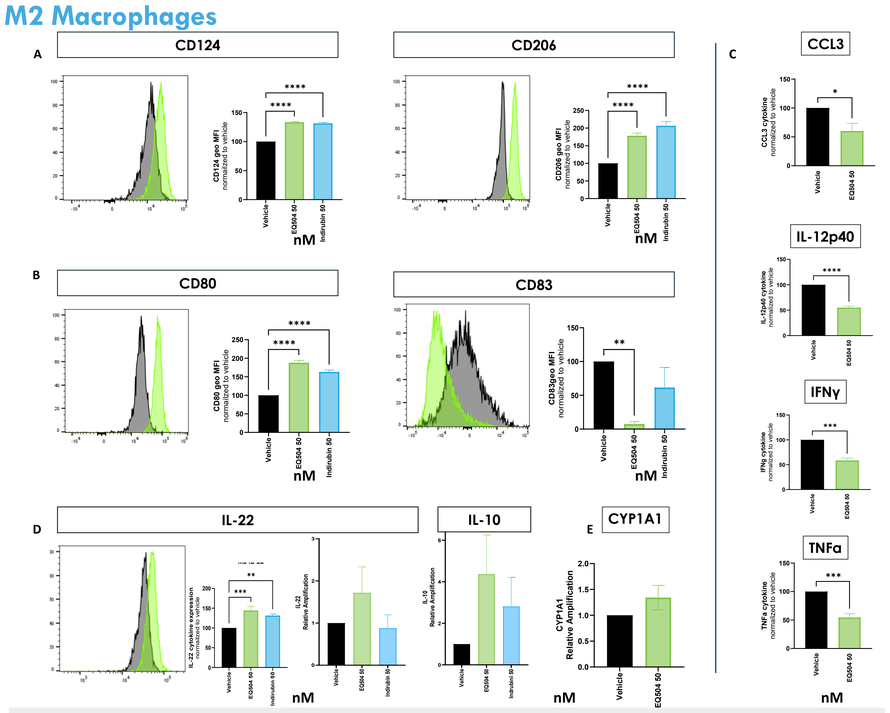

Figure 3. Isolated monocytes polarized towards an M2 phenotype with EQ504 or Indirubin for a total of 8 days. (A) EQ504 skewed M2 polarization marked by increased CD124 and CD206 expression. Increased CD124 leads to anti-inflammatory responses and tissue repair[5]. (B) Functional outcomes were measured by CD80 and CD83. An increase in CD80 may modulate the suppressive activity of T_{reg} cells while a decrease in CD83 may induce tolerance[6]. (C) EQ504 decreased several pro-inflammatory cytokines. (D) EQ504 increases IL-22 cytokine release confirmed by FACS and gene expression. Macrophage-derived IL-10 promotes intestinal mucosal wound healing[7]. (E) AhR engagement confirmed through CYP1A1 gene expression.

EQ504 Inhibits IL-17A and Enhances IL-22 Production in CD4$^+$ T Cells, with Greater Responses in UC Patient-derived Cells

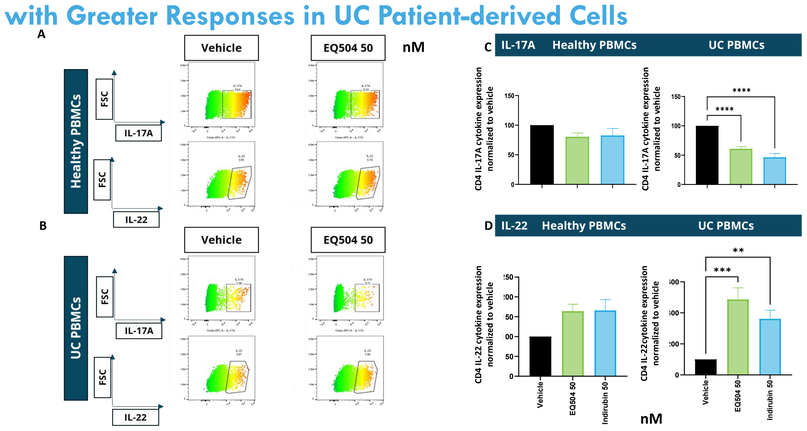

Figure 4. (A,B) Total PBMCs from healthy and UC donors were stimulated for a total of 3 days with anti-CD3/CD28 stimulation and EQ504 or Indirubin. (C,D) EQ504 decreases IL-17A while simultaneously increasing IL-22 in total CD4+ T cells. Greater IL-22 responses in UC donors.

EQ504 Preferentially Increases IL-22 in UC-patient Derived nT_{reg} Cells Compared to Healthy Donors

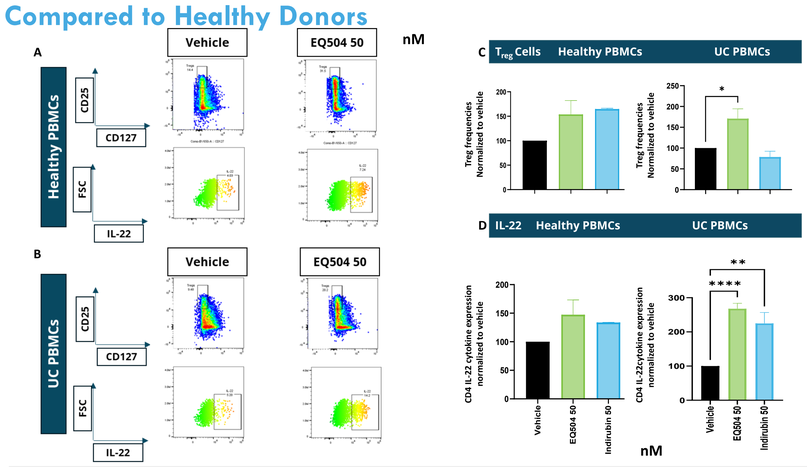

Figure 5. (A,B) Total PBMCs from healthy and UC donors were stimulated for a total of 3 days with anti-CD3/CD28 stimulation and EQ504 or Indirubin. (C,D) EQ504 increases T_{reg} cell frequencies as determined by CD25^{hi}CD127^{lo} while also increasing IL-22 within this population. Greater T_{reg} frequencies and IL-22 responses in UC donors can be observed.

EQ504 Programs iT_{reg} Cells Toward a Gut-Homing, Immunoregulatory Phenotype

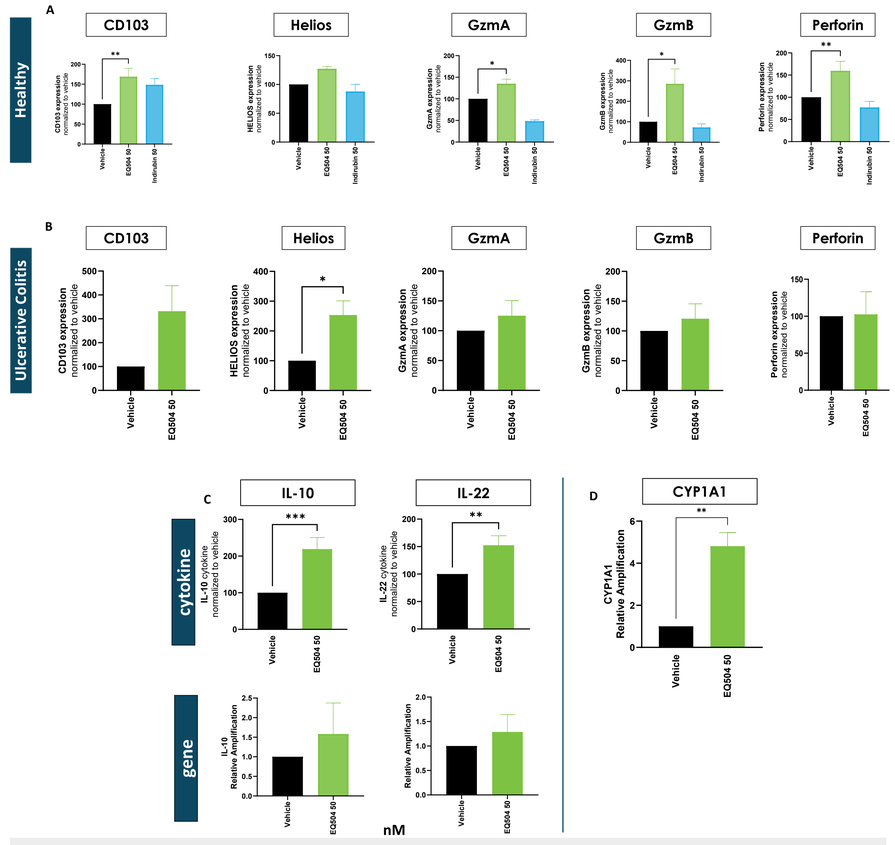

Figure 6. Naïve T cells treated with a standard T_{reg} cell differentiation cocktail and a low anti-CD3/CD28 stimulation for a total of 7 days with EQ504 or Indirubin. (A) Cells were assessed for markers associated with colonic tissue resident T_{reg} cells such as CD103, Helios, Granzyme A and B as well as Perforin[8,9] (B) UC differentiated T_{reg} cells had a strong response to EQ504 with an increase in both CD103 and Helios. (C) IL-10 and IL-22 are both increased in iT_{reg} cells data confirmed by cytokine release as well as gene expression. (D) AhR engagement confirmed through CYP1A1 gene expression. This data supports that T_{reg} cells differentiated with EQ504 express greater CD103 with suppressive activity which is required for T_{reg} cell localization[8,9].

Conclusions

M2 Macrophages: EQ504 activation of AhR skews macrophages toward an M2, anti-inflammatory phenotype marked by increased CD124 and CD206, reduced pro-inflammatory cytokines, and elevated IL-22 production. These effects, together with modulation of CD80, CD83 and enhanced macrophage-derived IL-10, support immune tolerance and mucosal repair.

T_{reg} Cells: Our data highlights AhR signaling as a key regulator of T_{reg} cell stability and function and highlight therapeutic AhR modulation as a strategy to restore T_{eff}/T_{reg} balance in immunoinflammatory disease. T_{reg} cells differentiated with EQ504 display increased CD103 expression and suppressive capacity, supporting an AhR-dependent program that couples regulatory function with tissue localization.

These findings demonstrate EQ504 as a potent AhR modulator with a unique immunoregulatory profile, promoting immune tolerance and tissue repair in cells highly relevant to maintaining intestinal homeostasis and the treatment of IBD.

Acknowledgments

We would like to acknowledge the Equillium research team specifically **Phoi Tiet** for her schematic of the immune cell regulation pathway impacted by EQ504. We would also like to acknowledge a previous Equillium team member for her contributions to the M2 macrophage work, Dalena Chu.

References

1. Keir M, Yi Y, Lu T, Ghilardi N. The role of IL-22 in intestinal health and disease. J Exp Med. 2020 Feb 13;217(3):e20192195.
2. Minutti, Carlos et al. Local amplifiers of IL-4Rα-mediated macrophage activation promote repair in lung and liver. Science 2020
3. Rothhammer, V., Quintana, F.J. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. Nat Rev Immunol 19, 184–197 (2019).
4. Neavin DR, Liu D, Ray B, Weinshilboum RM. The Role of the Aryl Hydrocarbon Receptor (AHR) in Immune and Inflammatory Diseases. Int J Mol Sci. 2018 Dec 3;19(12):3851.
5. Keir M, Yi Y, Lu T, Ghilardi N. The role of IL-22 in intestinal health and disease. J Exp Med. 2020 Feb 13;217(3):e20192195.
6. Minutti, Carlos et al. Local amplifiers of IL-4Rα-mediated macrophage activation promote repair in lung and liver. Science 2020
7. O' Brien et al. Pro-inflammatory polarization primes Macrophages to transition into a distinct M2-like phenotype in response to IL-4 Journal of Leukocyte Biology, Volume 111, Issue 5, May 2022, Pages 989–1000
8. Zheng Y et al. CD86 and CD80 Differentially Modulate the Suppressive Function of Human Regulatory T Cells. The Journal of Immunology, Volume 172, Issue 5, March 2004, Pages 2778–2784.
9. Miguel Quiros et al. Macrophage-derived IL-10 mediates mucosal repair by epithelial WISP-1 signaling. JCI 2017
10. Yusuke Yoshimats et al.Volume 39, Issue 6110773May 10, 2022.Aryl hydrocarbon receptor signals in epithelial cells govern the recruitment and location of Helios+ Tregs in the gut.
11. Jian Ye, et al.Volume 21, Issue 8p2277-2290November 21, 2017. The Aryl Hydrocarbon Receptor Preferentially Marks and Promotes Gut Regulatory T Cells

Disclosures

This study was funded by Equillium, Inc., all authors listed are currently employees and stockholders of Equillium.
Stephen Connelly is currently an employee, stockholder, and president of Equillium.
To request permission or to ask any questions about the poster, please contact IR@equilliumbio.com