EQ101 and EQ102, Selective Multi-cytokine Antagonists, Inhibit Cytotoxic T cell and NK Activity

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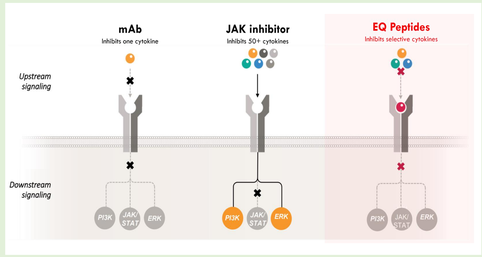
Introduction

- The common gamma chain (γc, CD132) receptor subunit is shared by 6 interleukin members, IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. These γc-cytokines play a crucial role in regulating major immune responses and contribute to various immune and inflammatory disorders in humans.
- Among these γc-cytokines, IL-2 has an immunoregulatory role and is critical for T-cell differentiation and activation. IL-15 functions in dictating T cell response and activating NK cells, which are involved in pathogenesis of various autoimmune and inflammatory diseases. Expression of IL-15 is commonly found on many different cell types (T cells, NK cells, Dendritic cells and Monocytes) and strongly regulated due to their pro-inflammatory nature [1].
- Treatment with monoclonal antibodies only inhibits a single cytokine and can be insufficient to adequately treat disease pathologies. In contrast, JAK inhibitors can lead to broad immuno-suppression. Therefore, selective multi-cytokine inhibitors that target at the receptor level, are advantageous in improving the therapeutic effect for diseases that are implicated by multiple cytokines.
- **EQ101 and EQ102** are selective multi-cytokine inhibitors that target IL-2/IL-9/IL-15 signaling and IL-15/IL-21 signaling, respectively (FIGURE 1).
- Here we sought to investigate the impact of these inhibitors on the activity of CTLs, effector/memory CD4+ T cells, and cytotoxic NK cells.

FIGURE 1. EQ101 & EQ102 SPECIFICALLY BLOCK THE COMMON RECEPTOR

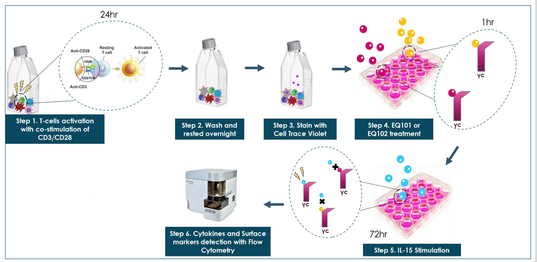


Methods

Experimental Design

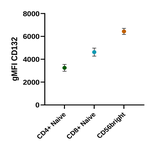
- **PBMCs Stimulation:** Frozen PBMCs (peripheral blood mononuclear cells) were thawed in complete media. Cells were stimulated with anti-CD3 and anti-CD28 antibodies through soluble method for 24hr at 37°C. After 24hr, cells were washed with complete media and rested at 37°C overnight.
- **Proliferation Labeling:** After resting, cells were collected and stained with a proliferation dye (Cell Trace Violet (CTV)).
- **Blockade of γc-receptor:** Cells were plated after CTV staining and treated with PEG40 peptide control and a titration of EQ101/EQ102 for 1hr at 37°C, allowing the inhibitors to block the common receptor.
- **Cytokine Stimulation:** After blocking, cells were stimulated with IL-15 for 72hr at 37°C.
- **Confirmation of inhibition:** At the end of incubation, supernatant was collected for detection of CD8, NK and CD4-relevant cytokines and cells were analyzed for surface activation markers by flow cytometry.

FIGURE 2. SCHEMATIC OF INHIBITION ASSAY



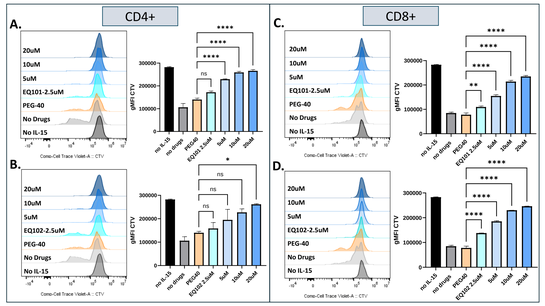
Results

FIGURE 3. γc/CD132 EXPRESSION ON CD4, CD8 NAÏVE T CELL, AND CD56BRIGHT NK CELLS



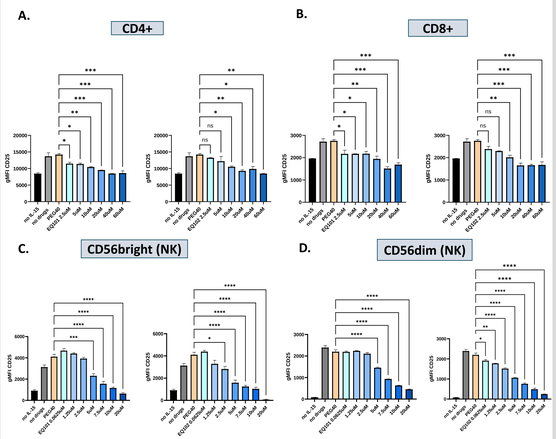
Upon stimulation with anti-CD3 and anti-CD28, the common receptor (γc, CD132) is highly expressed on CD4, CD8 T cell and CD56bright NK cells.

FIGURE 4. EQ101 AND EQ102 INHIBIT PROLIFERATION OF CD4+ AND CD8+ EFFECTOR MEMORY T CELLS



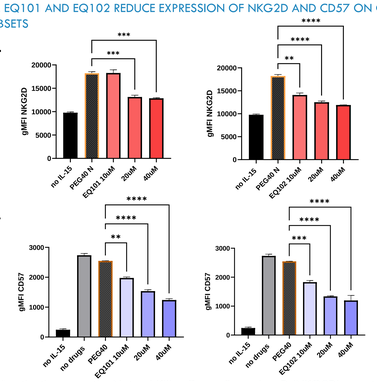
Flow cytometry analysis of Cell Trace Violet (CTV) proliferation staining are represented with histogram and geometric mean florescence intensity (gMFI). In comparison to no IL-15 conditions, stimulation with IL-15 leads to an increase in proliferation among CD4+ effector memory (EM) and cytotoxic CD8+ T cells. EQ101(A) and EQ102 (B) treatments decreased proliferation of CD4+ EM T cell (A,B). Also, significant inhibition of proliferation was observed on CD8+ EM T cell subset (C,D) by EQ101 (C) and EQ102 (D).

FIGURE 5. EQ101 AND EQ102 DOWNREGULATE CD25 EXPRESSION ON CD4+ AND CD8+ T CELLS



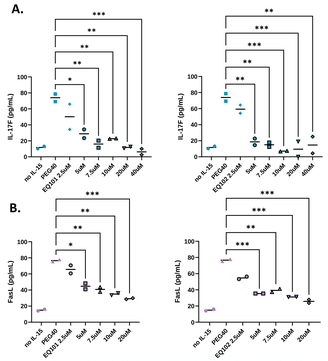
Cytotoxic CD8+, CD4+ EM T cells and CD56bright and CD56dim NK cells were further stimulated by IL-15 in comparison to no IL-15 condition, where cells were pre-activated with anti-CD3 and anti-CD28. Geometric MFI results of activation marker, CD25, revealed inhibition potential of EQ101 and EQ102 (A) on activation of CD4+ EM and cytotoxic CD8+ T cells (B) and both CD56bright (C) and CD56dim (D) NK sub populations.

FIGURE 6. EQ101 AND EQ102 REDUCE EXPRESSION OF NKG2D AND CD57 ON CD8+ MEMORY T CELL SUBSETS



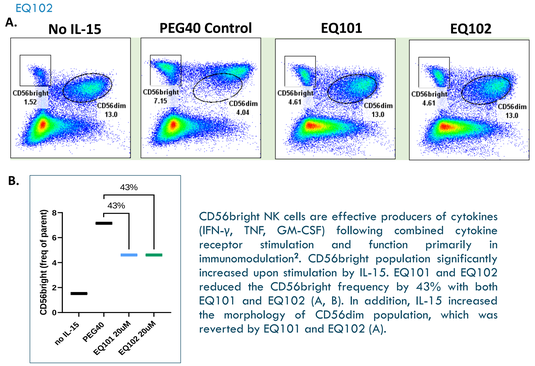
Expression of activating receptor, Natural Killer Group 2 member D (NKG2D) on cytotoxic CD8+ T cells were downregulated upon treatment by both multi-cytokine inhibitors (A). CD8+ CD57+ T cells express high level of IFN-γ, Granzyme B and Perforin, which enhance their cytotoxic potencies [3]. The expression of CD57 on CD8+ Memory T cell subsets were significantly reduced by EQ101 and EQ102 (B).

FIGURE 7. INHIBITION OF PRO-INFLAMMATORY IL-17F CYTOKINES AND FAS LIGAND PRODUCTION



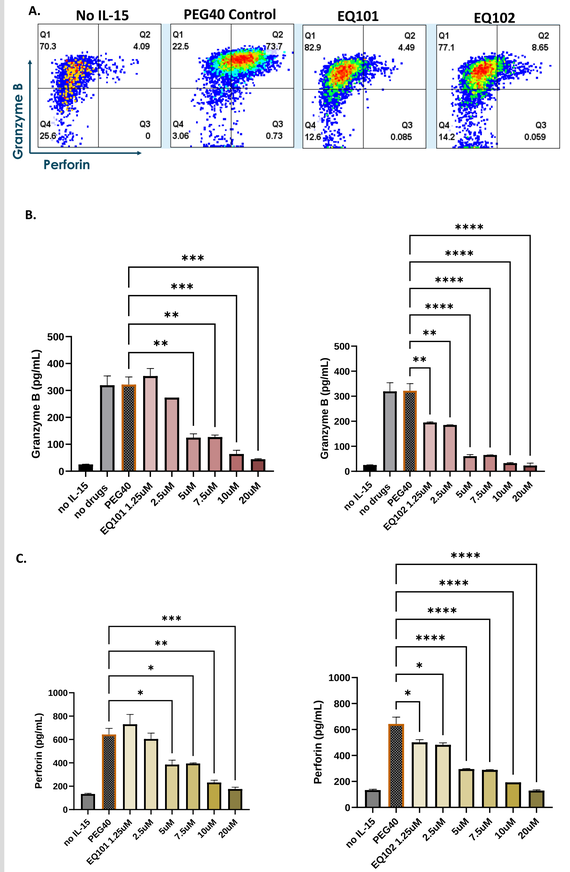
Cell supernatant from IL-15 stimulated cells was examined for human CD8 and NK cytokines. Results revealed an increase in pro-inflammatory IL-17F cytokine (A) and Fas Ligand (B) production upon activation with IL-15. This increase was significantly downregulated with the introduction of EQ101 and EQ102 (A, B).

FIGURE 8. MORPHOLOGY SHIFT OF CD56DIM NK POPULATION IS REGULATED BY EQ101 AND EQ102



CD56bright NK cells are effective producers of cytokines (IFN-γ, TNF, GM-CSF) following combined cytokine receptor stimulation and function primarily in immunomodulation[2]. CD56bright population significantly increased upon stimulation by IL-15. EQ101 and EQ102 reduced the CD56bright frequency by 43% with both EQ101 and EQ102 (A, B). In addition, IL-15 increased the morphology of CD56dim population, which was reverted by EQ101 and EQ102 (A).

FIGURE 9 . INHIBITION OF NATURAL KILLER CYTOTOXIC CYTOKINES GRANZYME B AND PERFORIN PRODUCTION



Granzyme B and Perforin are found in CD8+ T cells and NK cells, aiding their ability to mediate apoptosis of target cells. Intracellular staining of total PBMCs show high expression of Granzyme B and Perforin (A) upon stimulation, which were downregulated by EQ101 and EQ102 (A). Multiplex assay analysis of isolated NK supernatant results further confirmed EQ101 and EQ102 ability to inhibit the production of Granzyme B (B) and Perforin (C).

Conclusions

- Treatment of PBMCs with inhibitor EQ101 or EQ102 resulted in a decrease in proliferation of CD4+ effector/memory and cytotoxic CD8+ subsets. In addition, a decrease in CD56bright NK population was also observed.
- Activation of CD4+, CD8+, CD56bright and CD56dim populations were significantly reduced as observed with CD25, NKG2D and CD57 expressions.
- Analysis of the supernatant revealed EQ101 and EQ102 inhibited the production of CD8/NK relevant cytokines, IL-17F, FasL, Granzyme B, Perforin.
- These results demonstrate that the use of selective inhibitors for multiple key cytokines can be a more effective strategy in treating T and NK cell-driven autoimmune diseases in comparison to JAK inhibitors that can cause a non off target immuno-suppression.

References

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Disclosures

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Phoi Tiet, AJ Giovannone, Dalena Chu, Jeanette Ampudia, Stephen Connelly and Cherie Ng are currently employees and stockholders of Equillium.

Stephen Connelly is currently employee, stockholder, and officer of Equillium.

Additional Information

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