Tapinarof Inhibits the Formation, Cytokine Production, and Persistence of Resident Memory T Cells In Vitro

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MATERIALS AND METHODS
T Cell Activation and Cytokine Production Assessment
Blood-derived T cells were cultured with anti-CD2/CD3/CD28 activation beads for 1 week.
- Activation was assessed at 24 hours (CD69 expression).
- Expression of other markers (CD69, CD103, etc.) was determined after 1 week of culture.
Cytokine production was quantified using intracellular cytokine staining and flow cytometry analysis.
An antigen-presenting cell assay was utilized to quantify the T_Rm survival niche in skin cells.
Dermatolymph node samples of human skin were cultured in TH17-skewing conditions for 3 days, and analyzed for T cell activation (CD107a) and entry into the cell cycle (Ki-67) by immunostaining.

RESULTS
Suppression of Early T Cell Activation and Induction of CD39 Expression
A significantly lower proportion of tapinarof-treated T cells (both CD4+ and CD8+ cells; P=0.002) were activated compared with control, following treatment for 24 hours (Figure 2A).
Numerically higher proportions of CD4 cells and significantly higher proportions of CD8 cells (P=0.002) expressed CD39 following tapinarof treatment compared with control after 1 week (Figure 2B).

Cytokine Production by In Vitro Generated T_Rm
IL-17A, TNFα, and IFNγ production was reduced among in vitro generated T_Rm (Figure 5).
Non T_Rm CD69+CD103+; SP T_Rm CD69+CD103+; DP T_Rm CD69+CD103+.

CONCLUSIONS
Our initial results suggest tapinarof:
- Reduced early activation in CD4+ and CD8+ blood-derived T cells.
- Uregulated CD39 on CD4+ and CD8+ blood-derived T cells.
- Reduced the in vitro generation of CD4+ T_Rm.
- Reduced IL-17A, IFNγ, and TNFα production by CD4+ T_Rm.
- Reduced T cell survival in an in vitro antigen-presenting cell T cell support assay.
- Reduced activation and entry into the cell cycle of T_Rm in healthy skin cultured under TH17-skewing conditions.
- Additional donors will be tested to confirm statistical significance.
- Future studies will also test the effect of tapinarof in vivo using NSG mice grafted with human skin and infused with autologous peripheral blood mononuclear cells (PBMCs).
The demonstrated effects on T_Rm may explain the ability of tapinarof to induce a remittive effect in psoriasis clinical trials.

REFERENCES
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