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

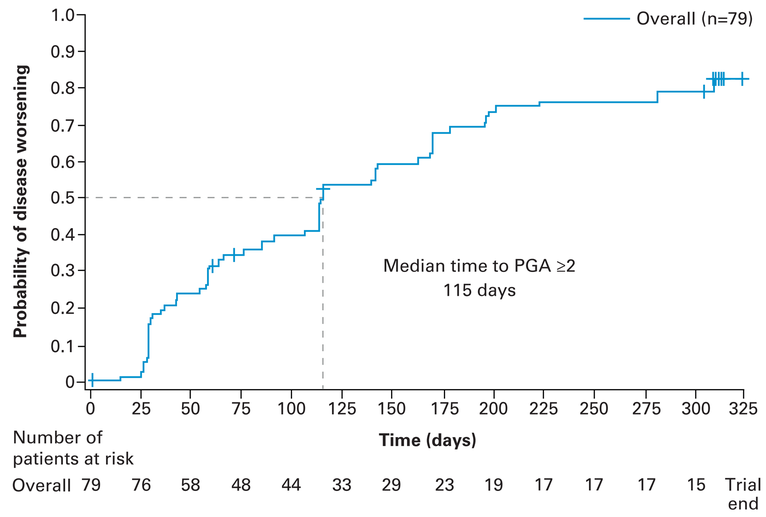
Nathan Mooney,[1] Jessica E. Teague,[1] Ahmed E. Gehad,[1] Kimberly McHale,[2] David S. Rubenstein,[2] Rachael A. Clark[1]

[1]Brigham and Women's Hospital, Boston, MA, USA; [2]Dermavant Sciences, Morrisville, NC, USA

BACKGROUND

- Tapinarof (VTAMA®; Dermavant Sciences, Inc.) is a first-in-class, non-steroidal, topical, aryl hydrocarbon receptor (AhR) agonist approved by the Food and Drug Administration for the treatment of plaque psoriasis in adults,[1] and under investigation for the treatment of psoriasis in children down to 2 years of age and for atopic dermatitis in adults and children down to 2 years of age
- Tapinarof cream 1% once daily treatment has been observed to induce a remittive effect in the phase 3 psoriasis long-term extension trial, PSOARING 3[2]
  - Patients who achieved Physician Global Assessment (PGA) of 0 (clear) after topical tapinarof treatment remained clear for a median of ~4 months after therapy was discontinued, defined as a PGA of 0 or 1 (almost clear) while off therapy after achieving complete disease clearance (**Figure 1**)[2]
- Resident memory T cells (T_{RM}) drive lesional recurrence in psoriasis and are affected by AhR signaling[3–5]
- AhR binds to toxins, endogenous and exogenous ligands, and has published effects on T cell differentiation[6,7]

Figure 1. Duration of Remittive Effect Among Patients Entering PSOARING 3 with a PGA Score of 0 (Clear): Maintenance of a PGA Score of 0 or 1 (Almost Clear) While Off Therapy



PGA, Physician Global Assessment.

OBJECTIVE

- To study the effect of tapinarof on T_{RM} using in vitro assays

MATERIALS AND METHODS

T Cell Activation and Cytokine Production Assessment

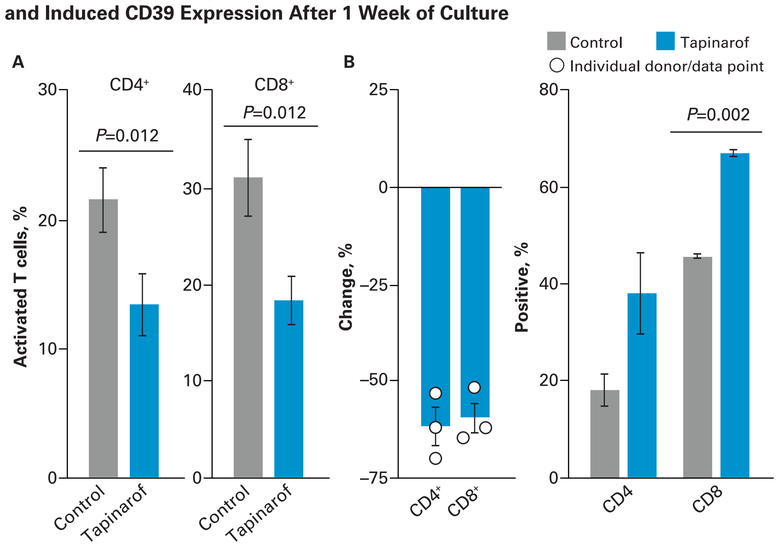
- Blood-derived T cells were cultured with anti-CD2/CD3/CD28 activation beads for 1 week
  - Activation was assessed at 24 hours (CD69 expression)
  - Expression of other markers (CD69, CD103, etc.) was determined after 1 week of culture
- Cytokine production was quantified using intracellular cytokine staining and flow cytometry analysis
- An antigen-presenting cell assay was used to quantify the T_{RM} survival niche in skin cells
- Dermatomed samples of human skin were cultured in Th17-skewing conditions[8] 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=0.012) 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**)

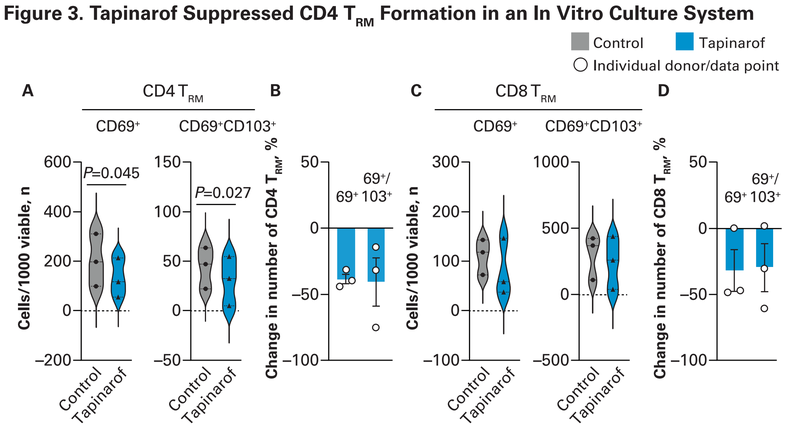
Figure 2. Tapinarof Significantly Suppressed Early T Cell Activation After 24 Hours and Induced CD39 Expression After 1 Week of Culture



In Vitro Generation of T_{RM}

- CD4 T_{RM} formation was significantly reduced ($CD69^+$, P=0.045 and $CD69^+CD103^+$, P=0.027) and there was a trend for reduced CD8 T_{RM} formation with tapinarof compared with control (**Figure 3**)

Figure 3. Tapinarof Suppressed CD4 T_{RM} Formation in an In Vitro Culture System

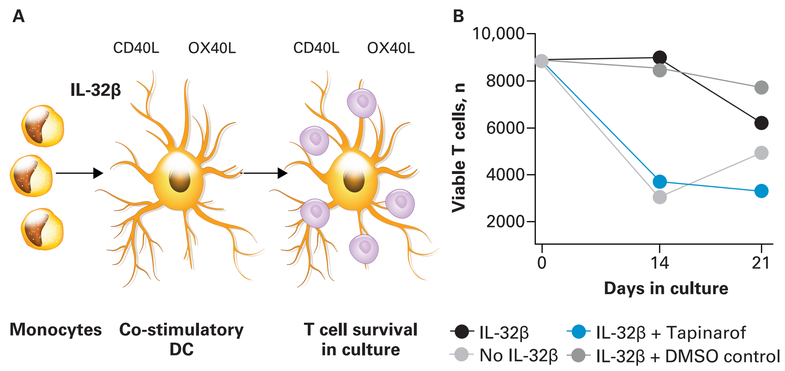


T_{RM}, resident memory T cell.

In Vitro Survival

- The antigen-presenting cells used IL-32β produced by T cells to induce differentiation of monocytes into OX40L/CD40L-expressing dendritic cells (DC), which may support T cell survival in vitro (**Figure 4A**)
- Tapinarof did not inhibit the formation of DC (not shown) but blocked T cell survival, suggesting it may interfere with DC:T cell signaling (**Figure 4B**)

Figure 4. Tapinarof Suppressed T Cell Survival in an In Vitro Antigen-Presenting Cell: T Cell Support Assay

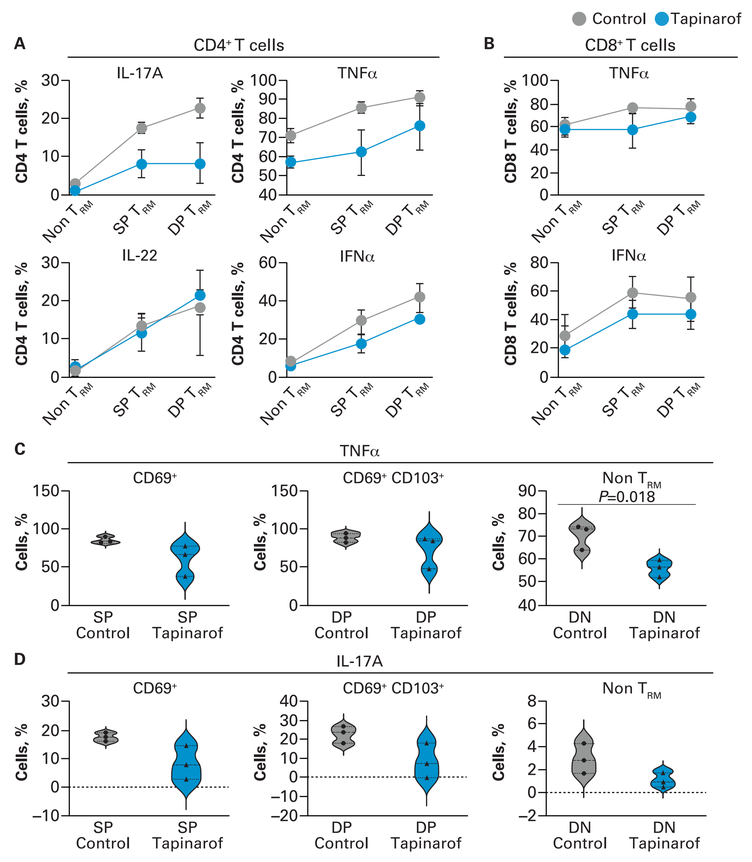


DC, dendritic cells; DMSO, dimethyl sulfoxide; IL, interleukin.

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}: $CD69^+CD103^+$

Figure 5. Tapinarof Suppressed Cytokine Production in CD4 and CD8 T_{RM} Cells Generated In Vitro

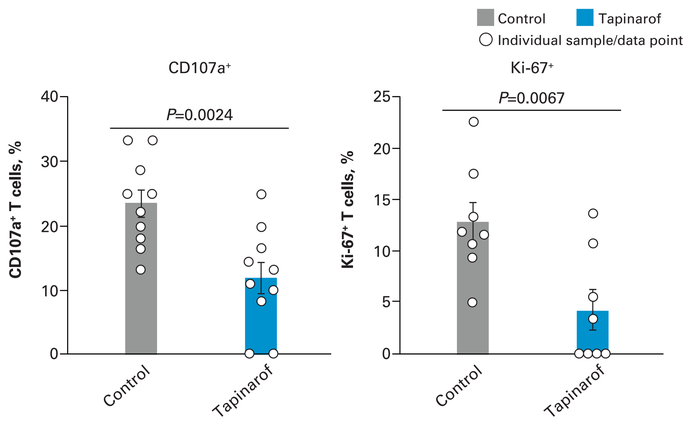


DN, double negative; DP, double positive; IFN, interferon; IL, interleukin; SP, single positive; T_{RM}, resident memory T cell; TNF, tumor necrosis factor.

T_{RM} Activation and Proliferation

- Tapinarof treatment significantly reduced T_{RM} activation (CD107a; P=0.0024) and proliferation (Ki-67; P=0.0067) (**Figure 6**)

Figure 6. Tapinarof Suppressed T_{RM} Activation and Proliferation in Human Skin



CONCLUSIONS

- Our initial results suggest tapinarof:
  - Reduced early activation in $CD4^+$ and $CD8^+$ blood-derived T cells
  - Upregulated 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 allogeneic 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[2]

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ACKNOWLEDGMENTS

Dermavant Science, Inc. provided material for these studies under a material transfer agreement. Adult skin samples were generously provided by Drs. Simon G. Talbot, Shailesh Agarwal, and Dennis P. Orgill of the Division of Plastic Surgery at Brigham and Women's Hospital, Boston, MA. Drs. Qian Zhan and Ira Kim performed immunostaining and analysis for the activation and proliferation experiment. Dr. Yoshinori Watanabe performed the antigen-presenting cell:T cell survival assays. K.M. and D.S.R. are employees of Dermavant Science, Inc. with stock options. R.A.C. has served as a scientific advisory board member for Science Immunology, AnaptysBio, Micreos, and SEDEC Therapeutics. Editorial support under the guidance of the authors was provided by ApotheCom, UK, and was funded by Dermavant Sciences, Inc. in accordance with Good Publication Practice (GPP) guidelines (*Ann Intern Med*. 2022;175:1298–1304).

Contact Dr Kimberly McHale at kimberly.mchale@dermavant.com with questions or comments.