IMU-935, a potent RORγt inverse agonist, effectively inhibits T helper 17 cells but maintains normal thymocyte development

Evelyn Peelen[1,*], Hongmin Wu[2,*], Andreas Muehler[1], Daniel Vitt[1], Zuoming Sun[2,†] and Hella Kohlhof[1,†]

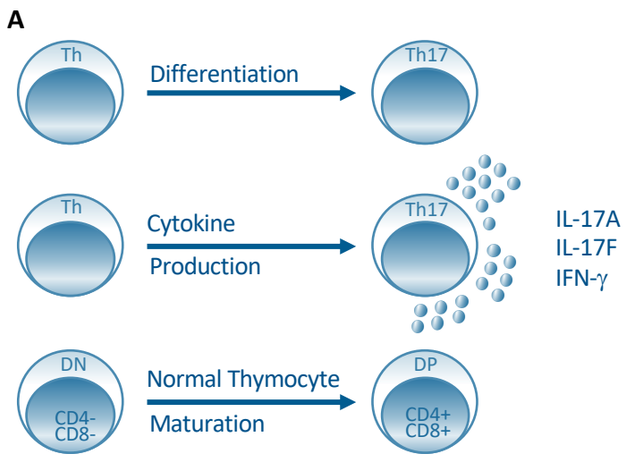
[1]Immunic AG, Lochhamer Schlag 21, 82166 Gräfelfing, Germany

[2]Department of Molecular Immunology, Beckman Research Institute of City of Hope, Duarte, CA 91010, USA

[*]Co-first authors, [†] Co-last authors

Background

The nuclear receptor RORγ exists in two isoforms. The shorter isoform RORγt is known to play a crucial role in thymocyte development and T helper 17 (Th17) differentiation (A). RORγt is critical for the maturation of single positive (SP) from double positive (DP) thymocytes (B)[1]. Th17 cells have been described to play a pivotal role in multiple autoimmune diseases. In addition, literature shows that absence or inhibition of RORγt inhibits Th17-mediated immunity and induces thymic aberrations.



Here, the effect of IMU-935 on Th17 cells and thymocytes was compared.

Methods[2]

***In Vitro* Assays**

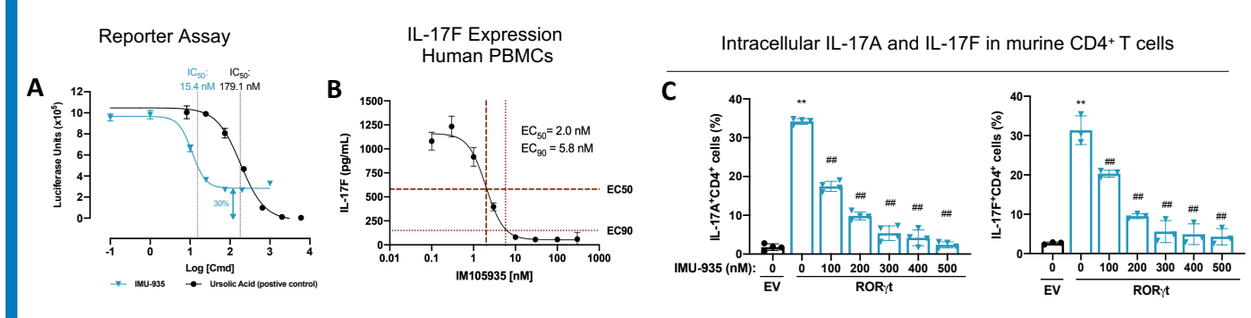
- **Human RORγt luciferase assay:** Performed according to manufacturers instructions[1]
- **Human PBMC activation:** Isolated PBMC were stimulated with PHA for 48h. Supernatant was collected and used for IL-17F detection by ELISA.
- **Murine Th17 cell differentiation:** Naïve CD4+ T cells isolated from spleens of RORγt$^{-/-}$ mice were transfected with a GFP expressing empty (EV-Flag) or RORγt (RORγt-Flag) containing retroviral vector and polarized towards Th17 cells. Cells were analyzed by flow cytometry for IL-17A and IL-17F expression or RNAseq (last 48h with/without compound).
- **Thymocytes (WT mice): Viability** was assessed over 36h and RNAseq was performed after 24h. For thymocyte **development**, sorted Thy1.2+CD4-CD8- thymocytes were cocultured with OP9-DL4 cells for 72h. Thymocytes from RORγt KO mice were used as control.

All assays were performed with/without compound.

***In Vivo* Models**

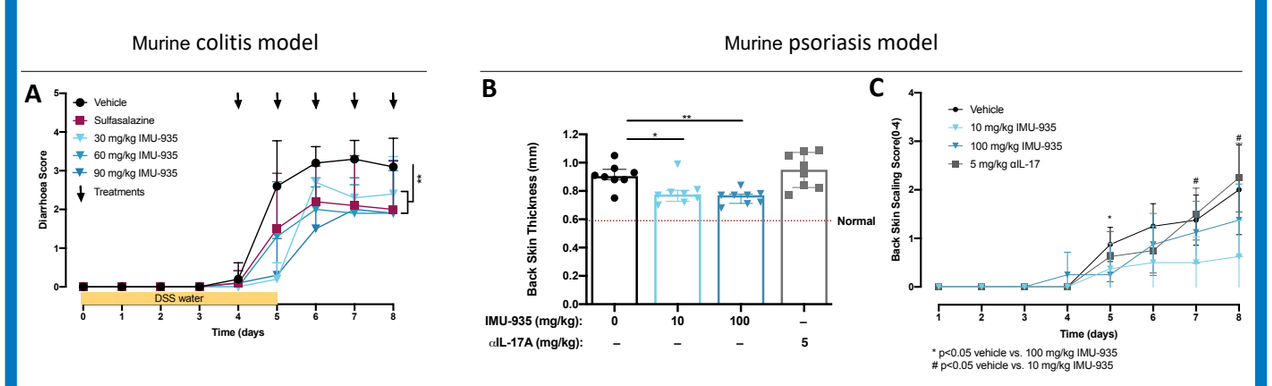
- **Murine DSS colitis:** Male C57BL/6J mice were treated with vehicle, 30, 60, 90 mg/kg IMU-935 or 60 mg/kg sulfasalazine per oral gavage at the day the first symptoms occurred for 5 days. Diarrhea score was assessed daily.
- **Murine imiquimod induced psoriasis:** Female BALB/c mice were treated with vehicle, 10 mg/kg or 100 mg/kg IMU-935 twice daily (oral) or 5 mg/kg anti-mouse IL-17A antibody q.o.d. (i.p.) for 8 days. Skin thickness and total back skin score (skin thickness, redness, scaling) were assessed.
- **Murine chronic thymic function:** Male C57BL/6j mice were administrated with IMU-935 (100 mg/kg) and Cpd1 (40 mg/kg) for 4 weeks (twice a day). Body weight, thymus size and thymic cellularity were assessed.

1. IMU-935 potently inhibits Th17 related cytokine expression and Th17 differentiation in human and murine cells



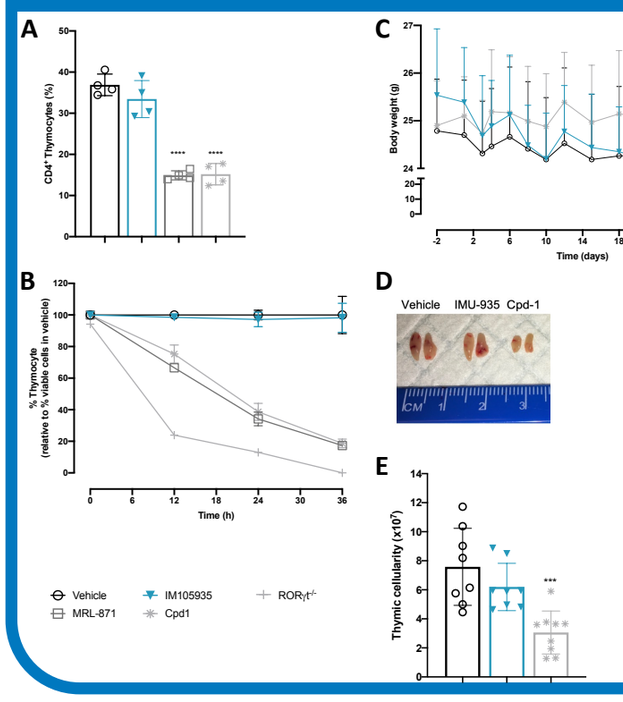
The representative graph of the luciferase reporter assay shows that IMU-935 inhibited RORγ activity potently at an IC_{50} of 15.4 nM (A). IMU-935 also potently inhibited IL-17A (not shown) and IL-17F (B) secretion by human PBMC activated by PHA for 48h. Since most studies are conducted in mice, IMU-935 was shown to inhibit the fraction of both IL-17A+ and IL-17F+ cells in RORγt overexpressing naïve murine CD4+ T cells polarized towards Th17 cells to a similar extent as cells that do not express RORγt (EV) (C).

2. IMU-935 shows activity in mouse models of autoimmune disease: DSS induced colitis and imiquimod induced psoriasis



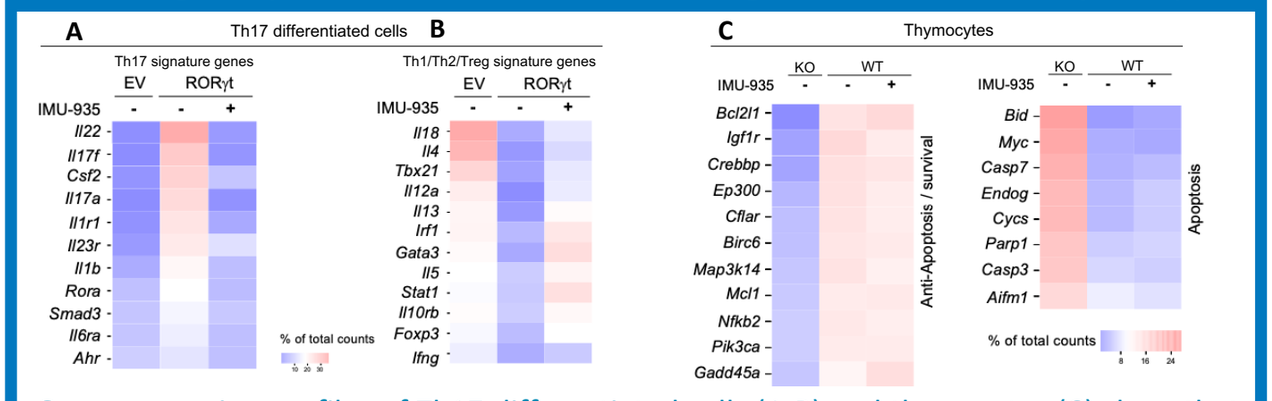
IMU-935 reduced the disease severity in a murine DSS induced colitis model in a therapeutic setting (A) and part of the symptoms related to an imiquimod induced murine psoriasis model, back skin thickness (B) and back skin scaling (C), in a prophylactic setting.

3. IMU-935 does not show aberrant effects on thymocytes *in vitro* or *in vivo*



In vitro, IMU-935 did not impair thymocyte maturation while MRL-871 and Cpd1, comparator compounds, as expected from literature[3,4], reduced thymocyte maturation (A). A similar effect is seen regarding 36h thymocyte viability (B). *In vivo* assessment of the thymus in mice shows that body weight (C) was not affected by the compounds. Also, IMU-935 did not affect the thymus, as shown in a representative image of thymic size (D) and by thymic cellularity (E), while Cpd1 reduced thymus size and thymic cellularity.

4. RNAseq analysis reveals that IMU-935 strongly reduces Th17 cell related gene profile, but does not affect genes critical for thymocytes



Gene expression profiles of Th17 differentiated cells (A,B) and thymocytes (C) show that IMU-935, comparable to cells without RORγt, did not upregulate Th17 related genes. While the presence of RORγt strongly downregulated the expression of Th1/Th2/Treg related genes upon Th17 cell differentiation, IMU-935 showed only a minor reduction of Th1 on related genes. However, in line with the *in vitro* and *in vivo* data, IMU-935 treated thymocytes did not downregulate survival genes or upregulate apoptosis related genes as was seen for thymocytes without RORγt.

Conclusions

- IMU-935 is pharmacologically active in the inhibition of human and murine Th17 cells.
- In contrast to the comparator RORγt inhibitors, IMU-935 does not affect thymocyte maturation and survival.
- Although IMU-935 metabolism is faster in mice compared to humans, *in vivo* activity in murine models for colitis and psoriasis could be observed.
- IMU-935 is currently being tested in moderate-to-severe psoriasis patients as part of a phase 1 clinical trial.

Contact: ZSun@coh.org

Contact: info@imux.com

References

1. Sun et al., 2000, Science
2. https://indigobiosciences.com/
3. Guo et al., 2016, Cell Reports (MRL-871)
4. Guntermann et al., 2017, JCI Insight (Cpd1)