TYK2 inhibition enhances Treg differentiation and function while preventing Th1 and Th17 differentiation

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THE JAK FAMILY IS A THERAPEUTIC TARGET IN AUTOIMMUNITY

The Janus kinase family (JAK1-3, TYK2) mediates cytokine receptor signalling.

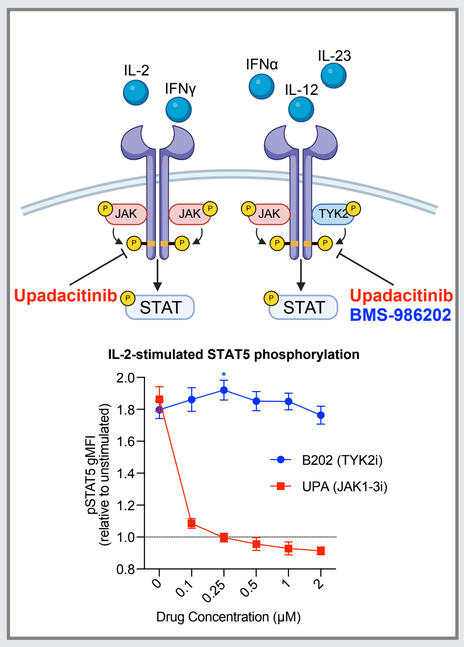
JAK1/3 inhibition (JAKi) has shown moderate efficacy in clinical trials in patients with type 1 diabetes[1] and other autoimmune/inflammatory diseases, like inflammatory bowel disease.

However, JAK1-3 inhibitors (e.g. Upadacitinib) may be less conducive to long-term tolerance because they block IL-2-stimulated JAK1/3 signalling, which is crucial for regulatory T cell (Treg) phenotype and function.

TYK2 inhibitors (TYK2i; e.g. BMS-986202) also disrupt many cytokine signalling pathways (e.g. type-I IFN, IL-12, IL-23), protect against islet autoimmunity in NOD mice[2], and reduce T cell-mediated lysis of SC-islets[3].

TYK2 is not involved in IL-2 signalling, so inhibition may better preserve Treg function.

Hypothesis: TYK2 inhibition better preserves Treg phenotype/function compared to JAK1-3 inhibition.



AIM

Compare the effect of JAK and TYK2 inhibition on Treg induction, phenotype, stability, and function

1. TYK2i and JAKi prevent Th1 and Th17 differentiation but only TYK2i spares Treg induction

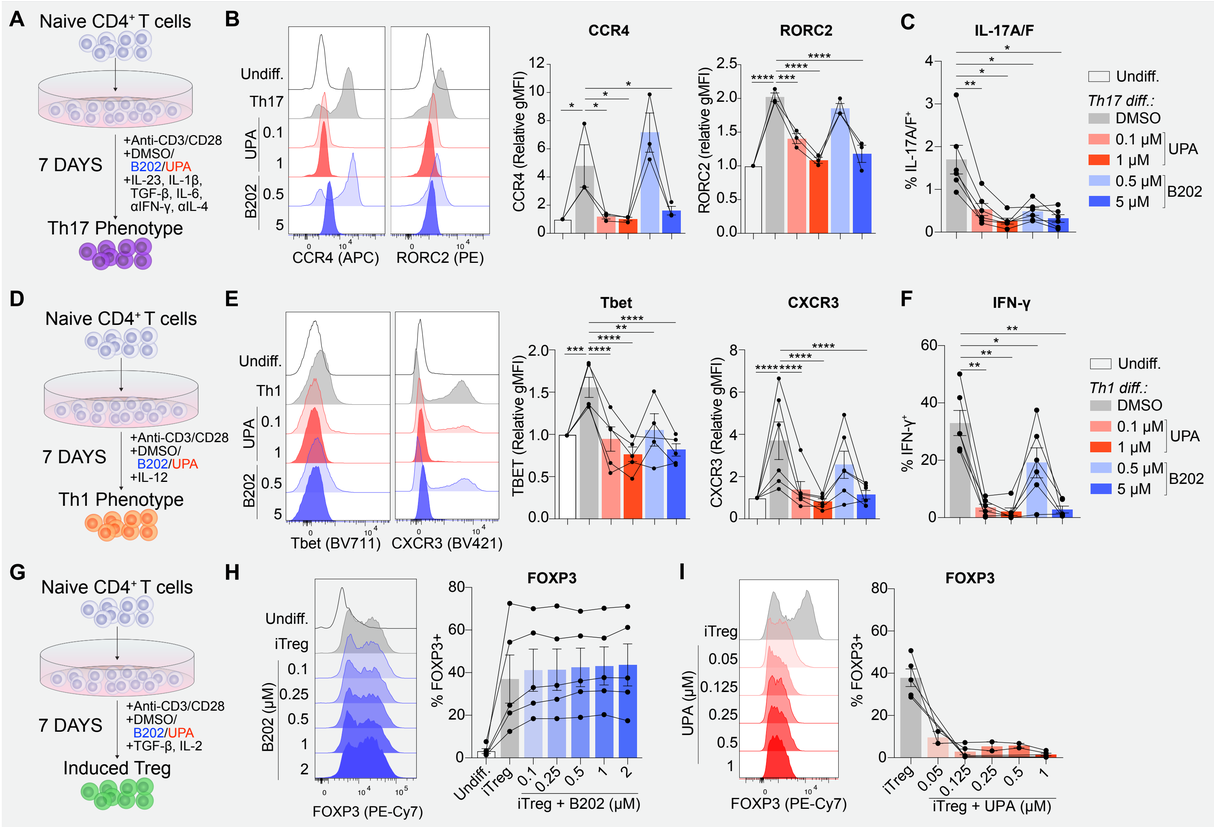


Figure 1 TYK2 inhibition reduces Th1 and Th17 differentiation of naive CD4+ T cells but preserves Treg induction. (A-C) Naïve CD4+ T cells were stimulated with anti-CD3/CD28 for 7 days in the presence of a Th17-inducing cytokine cocktail +/- BMS-986202 (B202) or upadacitinib (UPA) to induce Th17 differentiation. **(A)** Schematic of Th17 differentiation. **(B)** CCR4 and RORC2 expression relative to undifferentiated CD4+ T cells at day 7 (n=3). **(C)** Intracellular IL-17A/F expression after a 4 h stimulation with PMA/ionomycin (n=6). **(D-F)** Naïve CD4+ T cells were stimulated with anti-CD3/CD28 for 7 days in the presence of IL-12 +/- B202/UPA to induce Th1 differentiation. **(D)** Schematic of Th1 differentiation. **(E)** Tbet and CXCR3 expression relative to undifferentiated CD4+ T cells at day 7 (n=6). (F) Intracellular IFNg expression after a 4 h stimulation with PMA/ionomycin (n=6). **(G-I)** Naïve CD4+ T cells were stimulated with anti-CD3/CD28 for 7 days in the presence of TGF-β +/- B202/UPA to induce Treg differentiation. **(G)** Schematic of Treg differentiation. **(H-I)** FOXP3 expression at day 7 of treatment with B202 (H; n=5) or UPA (I; n=2-5). Statistically significant differences compared to DMSO-treated cells were determined by a repeated-measures one-way ANOVA with Dunnett's multiple comparisons test (B-C, E-F, H). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

2. TYK2i, but not JAKi, preserves Treg phenotype and enhances suppressive function

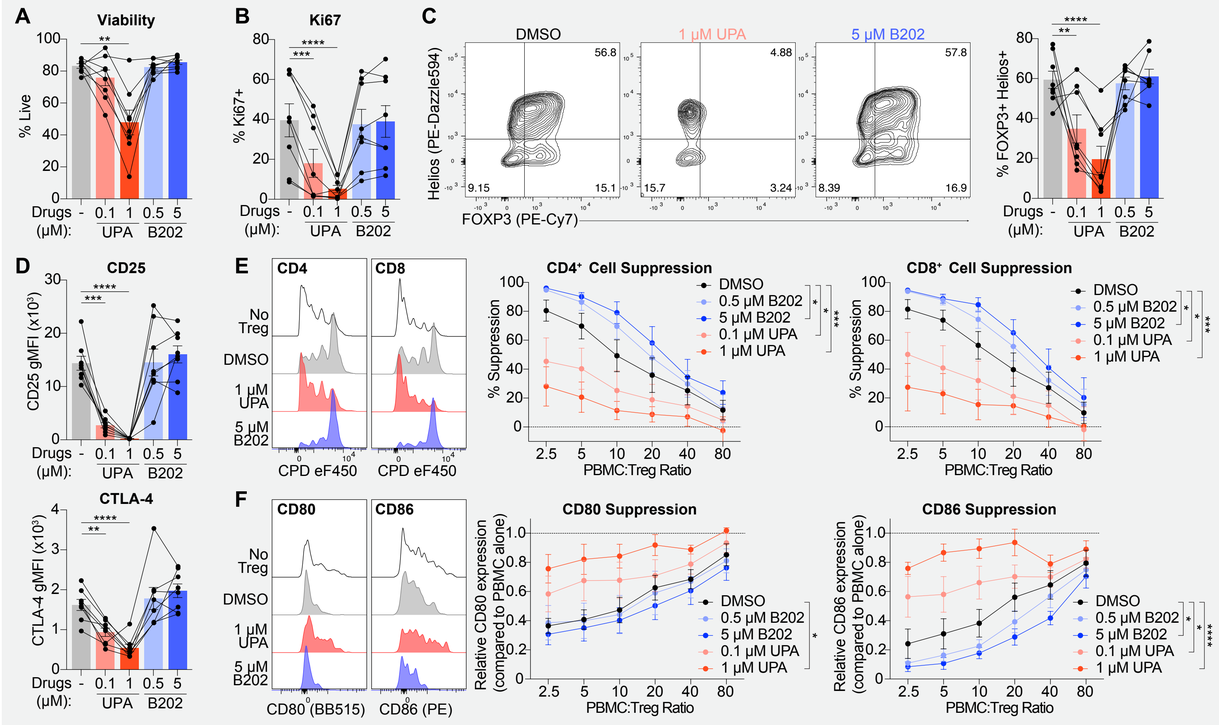


Figure 2 TYK2 inhibition maintains Treg phenotype and enhances suppressive function. Tregs (CD4+CD25hiCD127lo) were isolated from peripheral blood of healthy human donors, stimulated with anti-CD3/CD28, and cultured for 7 days in the presence of BMS-986202 (B202) or upadacitinib (UPA). **(A)** Treg viability, **(B)** percentage of Ki67+ cells, **(C)** percentage of FOXP3+/Helios+ cells, and **(D)** CD25 and CTLA-4 expression were determined after 7 days (n=4-8). **(E-F)** Responder PBMCs were stimulated with anti-CD3/CD28 Dynabeads in the presence of varying ratios of Tregs and cultured for 96 hours. **(F)** Percent suppression of CD4+ and CD8+ responder T cell proliferation was determined relative to PBMCs alone (dotted line) (n=7). **(G)** CD80 and CD86 expression on B cells relative to PBMC alone condition (dotted line) (n=6). Statistically significant differences compared to DMSO-treated cells were determined by one-way ANOVA with Dunnett's multiple comparisons test (A-D) or an uncorrected Fisher's LSD test (E-F). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

3. TYK2i enhances Treg stability in inflammatory Th1 and Th17 conditions

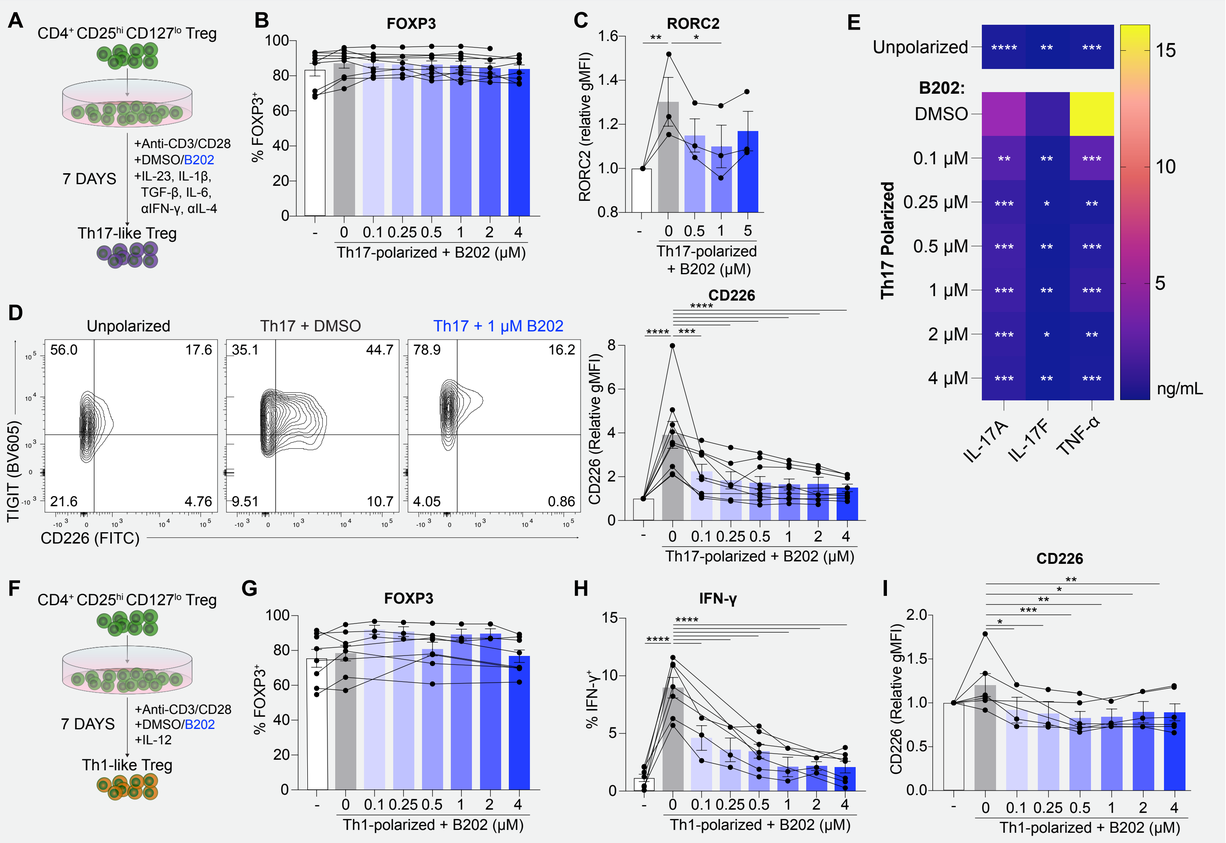


Figure 3. TYK2 inhibition enhances Treg stability in Th17 and Th1-polarising conditions. Tregs (CD4+CD25hiCD127lo) were isolated from peripheral blood of healthy human donors, stimulated with anti-CD3/CD28, and cultured for 7 days in a Th17 (A-E) or Th1 (F-I)-polarizing cocktail in the presence of BMS-986202 (B202). **(A)** Schematic of Th17 polarization protocol. **(B-D)** The proportion of FOXP3+ cells (B), expression of RORC2 (C), and expression of CD226 (D) was assessed after 7 days (n=3). **(E)** IL-17A, IL-17F, and TNFa concentration in supernatant after a 24 h re-stimulation with anti-CD3/CD28 (n=6). **(F)** Schematic of Th1 polarization protocol. **(G-I)** The proportion of FOXP3+ cells (G), IFN-γ+ cells (H), and CD226 expression (I) was assessed after 7 days (n=3-7).Statistically significant differences compared to DMSO-treated cells were determined by a repeated-measures one-way ANOVA with Dunnett's multiple comparisons test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

4. TYK2i redirects intestinal T cells towards a regulatory phenotype

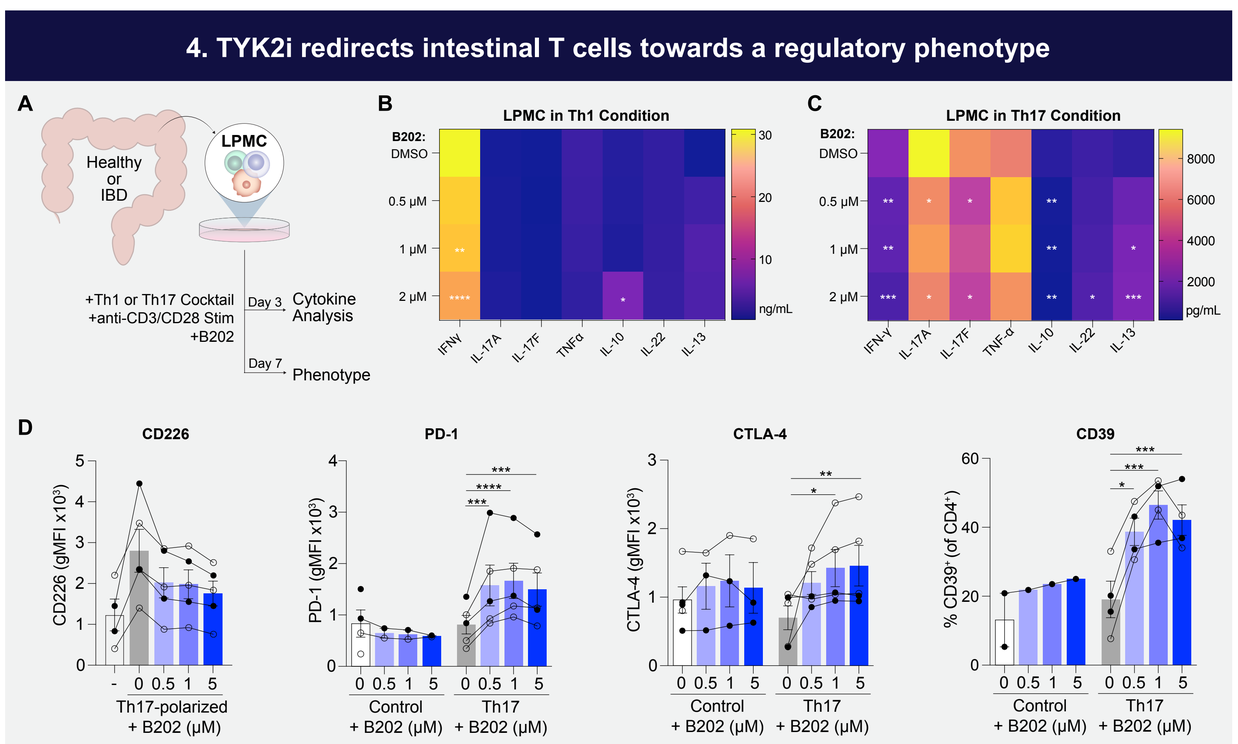


Figure 4 TYK2 inhibition redirects Th17-polarized LPMCs towards a regulatory phenotype. Lamina propria mononuclear cells (LPMCs) were isolated from colon biopsies of healthy or IBD patients, stimulated with anti-CD3/CD28 tetramer in the presence of a Th17 (C-E) or Th1 (B) cytokine cocktail and BMS-986202 (B202), and cultured for 7 days. **(A)** Schematic of LPMC culture. **(B-C)** Heatmap of cytokine secretion from LPMCs after 3 days of culture in Th1 (B) or Th17 (C) cytokine conditions. **(D)** Surface expression of CD226, PD-1, CTLA-4, and CD39 on LPMC CD4+ T cells cultured in Th17 cytokine conditions for 7 days. **(E)** Proportion of FOXP3+ cells of LPMC CD4+ T cells. Open circles (o) represent healthy patients. Closed circles (•) represent IBD patients. Statistically significant differences compared to DMSO-treated cells were determined by repeated-measures one-way ANOVA with Dunnett's multiple comparisons test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

SUMMARY

JAK inhibition

↓ Inflammatory Th polarization
↓ Treg induction
↓ Treg phenotype/function

TYK2 inhibition

↓ Inflammatory Th polarization
↑ Treg induction in inflammatory conditions
↑ Treg phenotype/function

Inhibition of JAK1/3 signalling is highly detrimental to Treg induction and natural Treg survival, proliferation, phenotype, and function.

TYK2 inhibition using BMS-986202 does not affect Treg induction, increases suppressive function, and prevents Th1 and Th17 polarization of Tregs.

TYK2 inhibition reduces inflammatory polarization of tissue-derived immune cells and promotes a regulatory T cell phenotype.

By enhancing the Treg compartment, TYK2 inhibition is more likely compared to JAK inhibition to promote long-term tolerance in autoimmunity.

Read the full paper on bioRxiv!

References:

[1] Waibel, M., Wentworth, J. M., So, M., Couper, J. J., Cameron, F. J., MacIsaac, R. J., ... & Kay, T. W. (2023). Baricitinib and β-cell function in patients with new-onset type 1 diabetes. New England Journal of Medicine, 389(23), 2140-2150.
[2] Mine, K., Nagafuchi, S., Akazawa, S., Abiru, N., Mori, H., Kurisaki, H., ... & Anzai, K. (2024). TYK2 signaling promotes the development of autoreactive CD8+ cytotoxic T lymphocytes and type 1 diabetes. Nature communications, 15(1), 1337.
[3] Chandra, V., Ibrahim, H., Halliez, C., Prasad, R. B., Vecchio, F., Dwivedi, O. P., ... & Otonkoski, T. (2022). The type 1 diabetes gene TYK2 regulates β-cell development and its responses to interferon-α. Nature Communications, 13(1), 6363.
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