NX-5948, a Clinical-Stage BTK Degrader, Achieves Deep Suppression of BCR, TLR, and FcR Signaling in Immune Cells and Demonstrates Efficacy in Preclinical Models of Arthritis and Other Inflammatory Diseases

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Abstract

Bruton's tyrosine kinase (BTK) mediates signaling downstream of the B cell receptor (BCR), toll-like receptors (TLRs), and Fc receptors (FcRs). This makes BTK an attractive therapeutic target in antibody-mediated autoimmune and inflammatory diseases, as targeting BTK can reduce both the generation of new antibodies and the inflammation induced by existing antibodies.

Although BTK inhibitors are currently under development for the treatment of autoimmune and inflammatory diseases, recent studies have shown that BTK functions through a combination of both enzymatic activity and kinase-independent scaffolding activity [1-2], suggesting that inhibition alone may not achieve complete pathway suppression.

Targeted protein degradation (TPD) utilizes small molecules to recruit an E3 ubiquitin ligase to a target protein and induce its ubiquitylation and degradation. In contrast to inhibitors, TPD removes both the enzymatic and scaffolding functions of a target protein. NX-5948 is an orally active degrader of BTK currently in Phase 1 clinical development for the treatment of B cell malignancies (NCT05131022). We compared the ability of NX-5948 and BTK inhibitors to suppress BCR, TLR, and FcR signaling in immune cells in vitro and assessed efficacy in multiple in vivo models of autoimmune and inflammatory disease.

Human PBMCs or individual immune cell types were stimulated with BCR, TLR, and FcR agonists, and activation was assessed by flow cytometry or ELISA. For in vivo studies, NX-5948 or BTK inhibitors were orally administered to mice daily at dose levels ranging from 3 to 30 mg/kg. Multiple preclinical models were evaluated, including collagen-induced arthritis (CIA), experimental autoimmune encephalomyelitis (EAE), passive cutaneous anaphylaxis (PCA), and antibody-induced glomerulonephritis (AGN).

NX-5948 promotes potent and rapid BTK degradation in primary human B cells and monocytes, with DC_{50} = 0.056 and 0.034 nM at 4 hours, respectively. It effectively suppresses BCR-, TLR-, and FcR-mediated activation in both B and myeloid cells with sub-nanomolar potency, showing equal or greater suppression compared to BTK inhibitors. In naive mice, oral administration of NX-5948 led to significant degradation of BTK in circulating and brain-resident immune cells. In comparison to BTK inhibitors, NX-5948 displayed similar or superior efficacy in the models tested. In the established CIA model at 30 mg/kg, 10/12 mice treated with NX-5948 displayed complete resolution of paw swelling, compared to 2/12 mice treated with rilzabrutinib and 7/12 mice treated with ibrutinib.

NX-5948 is a clinical stage BTK degrader that potently suppresses BCR, TLR, and FcR signaling in vitro and demonstrates efficacy across multiple disease models. Regardless of model type, NX-5948 displayed comparable or better efficacy than BTK inhibitors, supporting the hypothesis that BTK degradation confers a significant therapeutic benefit over BTK inhibition by removing both kinase and scaffolding functions. These preclinical results support initiation of clinical development of NX-5948 in autoimmune and inflammatory disease settings.

Figure 1: BTK is a nexus for autoimmune, inflammatory, and allergic processes

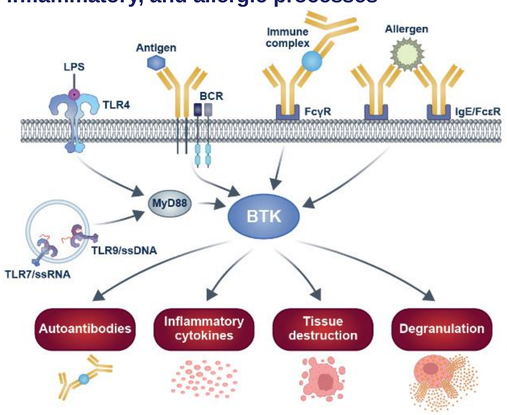

Figure 1. BTK transduces signals downstream of the B cell receptor, toll-like receptors, and Fc receptors. BTK-dependent signaling drives various autoimmune and inflammatory processes, including autoantibody production, cytokine secretion, tissue destruction, and mast cell and basophil degranulation.

Results

Figure 2: NX-5948 potently degrades BTK in primary B cells and suppresses proximal BCR signaling

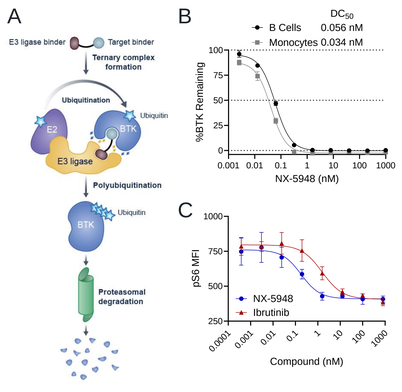

Figure 2. (A) NX-5948 mechanism of action. NX-5948 brings BTK into the proximity of the E3 ligase Cereblon, inducing ubiquitylation and proteasomal degradation of BTK. (B) BTK degradation in primary human B cells and monocytes following 4 hours of NX-5948 treatment. (C) S6 phosphorylation in primary human B cells pre-treated with NX-5948 or ibrutinib for 4 hours and then stimulated with 10 μg/mL anti-IgM for 5 minutes. Results are averaged from n = 3 independent donors, and mean ± SEM is displayed.

Figure 3: NX-5948 potently suppresses B cell activation following both soluble and particulate antigen stimulation

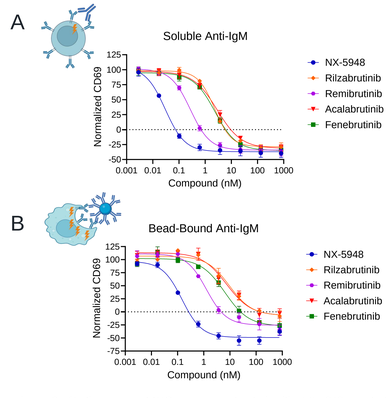

Figure 3. (A-B) Human PBMCs were pre-treated with NX-5948 or BTK inhibitors for 4 hours and then stimulated for 19 hours with either (A) 10 μg/mL soluble anti-IgM or (B) streptavidin-coated particles treated biotinylated anti-IgM and washed. CD69 levels were quantified on CD20+ HLA-DR+ B cells and normalized (0 = untreated, 100 = pre-treated with DMSO and stimulated). Results were generated in n = 3 independent donors and mean ± SEM is displayed. Diagrams created with BioRender.com

Figure 4: NX-5948 suppresses TLR4, TLR7, and FcγR signaling

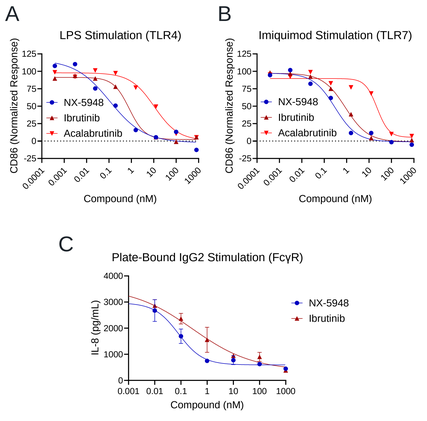

Figure 4. (A-B) Peripheral blood mononuclear cells (PBMCs) were pre-treated with compound for 4 hours and stimulated with LPS (10 μg/mL) or imiquimod (5 μg/mL) for 20 hours in the continued presence of compound. CD86 expression on B cells was quantified by flow cytometry. (C) PBMCs were pre-treated with NX-5948 or ibrutinib for 4 hours and then transferred onto IgG2-coated plates and incubated overnight. IL-8 levels in cell culture supernatant were quantified by MSD.

Figure 5: Oral administration of NX-5948 promotes *in vivo* BTK degradation in circulating B cells and myeloid cells in the brain

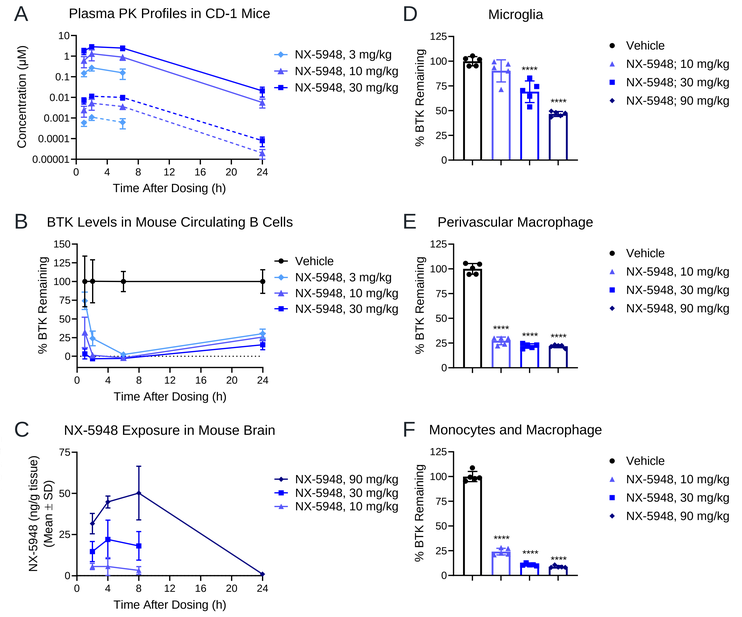

Figure 5. (A) Plasma concentration of NX-5948 in CD-1 mice following a single oral dose of NX-5948. Solid lines show total concentration; dashed lines show free concentration. (B) Time- and dose-dependent BTK degradation in circulating blood B cells from mice in (A), normalized to vehicle control. (C) Brain exposure of NX-5948 in CB.17 SCID mice after a single oral administration of NX-5948. (D-F) Degradation of BTK in brain-resident microglia, perivascular macrophage, and monocytes and macrophage in the brains of C57BL/6J mice 8 hours after the third daily oral administration of NX-5948. Mean ± SD displayed. **** p < 0.0001 compared to vehicle control (One-Way ANOVA, Dunnett's post-test)

Figure 6: NX-5948 is strongly efficacious in a mouse model of established collagen-induced arthritis and reduces plasma cell numbers

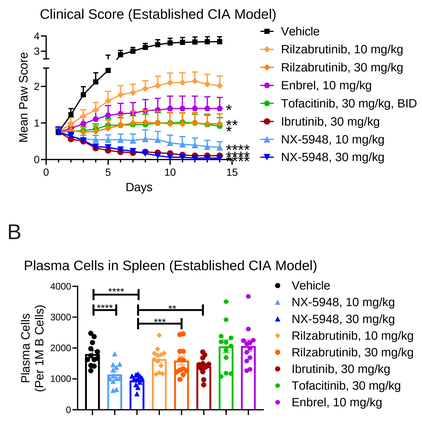

Figure 6. (A) Clinical scores in mice with established collagen-induced arthritis and treated QD or BID with the designated agents beginning on day 1. (B) Plasma cell counts in the spleens of mice on day 14. *p<0.05, **p < 0.01, ***, p < 0.001, ****p < 0.0001 compared to vehicle control (A) or between designated groups (B).

Figure 7: NX-5948 displays strong efficacy in mouse models of glomerulonephritis, passive cutaneous anaphylaxis, ALPS, and experimental autoimmune encephalitis

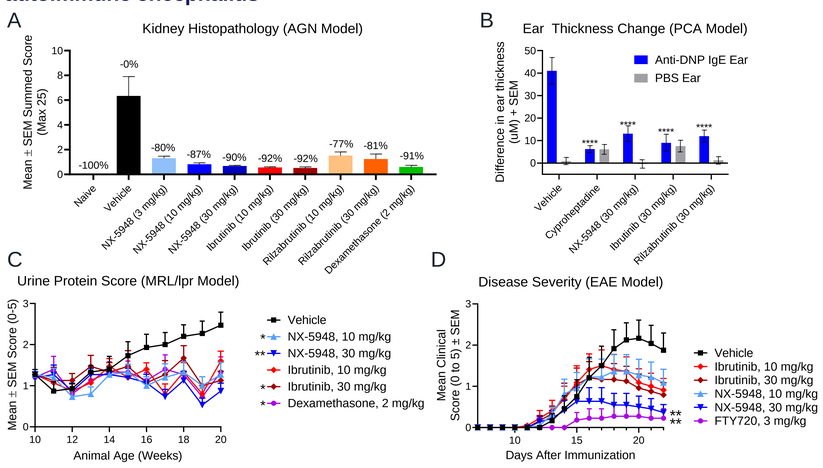

Figure 7. (A) Summed kidney histopathology score on Day 10 in a mouse model of antibody-mediated glomerulonephritis (AGN). Mice were immunized against sheep IgG in CFA on Day -5 then treated daily with the listed agents starting on Day -1 and challenged with sheep anti-glomerular basement membrane antibody on Day 0. (B) Ear thickness change in a mouse model of passive cutaneous anaphylaxis (PCA). Mice were mice pre-treated with anti-2,4-dinitrophenol (DNP) IgE and challenged by injection of ears with either DNP-protein conjugate or PBS. (C) Urine protein score in the MRL/lpr autoimmune lymphoproliferative syndrome (ALPS) model with daily treatment of the listed agents from weeks 10 to 20. (D) Clinical score in preventative mouse experimental autoimmune encephalitis (EAE) model of multiple sclerosis where mice were treated prior to immunization with human MOG_{1-125}. (B) Stats determined by one-way ANOVA with Dunnett's multiple comparisons test: ** p < 0.01; **** p < 0.0001. (C) *p<0.05, **p < 0.01 compared to vehicle control (Week 20 scores; Kruskal-Wallis test with Dunn's post-hoc analysis). (D) *p < 0.05, **p < 0.01 compared to vehicle control (Wilcoxon's non-parametric test on average end clinical score).

Conclusions

- NX-5948 promotes potent and selective BTK degradation in primary B cells and monocytes (DC_{50} = 0.056 and 0.034 nM, respectively) and suppresses proximal BCR signaling more potently than ibrutinib, which inhibits several other kinases in addition to BTK [3].
- BTK agents efficiently suppress B cell activation upon soluble antigen stimulation, but NX-5948 provides deeper suppression upon particulate antigen stimulation.
- NX-5948 provides potent suppression of TLR4- and TLR7-mediated activation of B cells and FcγR-mediated activation of PBMCs.
- NX-5948 displays strong efficacy in a therapeutic rheumatoid arthritis model (CIA) and, by contrast to BTK inhibitors, can reduce splenic plasma cells.
- NX-5948 also demonstrates comparable or superior efficacy in models of glomerulonephritis (AGN), SLE (MRL/lpr), MS (EAE), and anaphylaxis (PCA) compared to BTK inhibitors.
- These results provide mechanistic rationale to investigate NX-5948 as a treatment for diverse autoimmune and inflammatory diseases.

Disclosures

All authors are past or current employees of Nurix Therapeutics and hold company stock or stock options.

References

[1] Montoya, et al., Science, 2024
[2] Li, et al., Mol. Cancer Ther, 2024
[3] Kaptein et al., Blood, 132 (Supplement 1), 2018

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