Abstract # 1675

NX-5948, a Selective Degrader of BTK, Significantly Reduces Inflammation in a Model of Autoimmune Disease

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Introduction

- Bruton's tyrosine kinase (BTK) transduces signals downstream of the B cell receptor (BCR), toll-like receptors, and Fc receptors in B cells and myeloid cells
- Overexpression of BTK in B cells can lead to hyperactive BCR signaling, plasma cell generation, and autoantibody secretion
- Aberrant activation of B cells and autoantibody mediated tissue damage are hallmarks of autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).
- NX-5948 is a chimeric targeting molecule (CTM) that engages the E3 ligase cereblon (CRBN) to promote the selective degradation of BTK

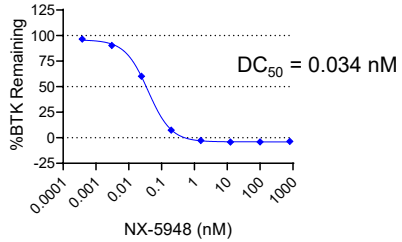
Results

- NX-5948 is a potent degrader of BTK in primary human B cells (DC_{50} = 0.34 nM) and inhibits BCR signaling
- NX-5948 is highly selective for BTK degradation by proteomic analysis with limited activity toward the CRBN neo substrate Aiolos (DC_{50} > 10 µM)
- In vivo, once daily oral administration of NX-5948 in mice and cynomolgus monkey demonstrated potent degradation of BTK in circulating B cells
- NX-5948 demonstrated significant anti-inflammatory activity and resulted in improvement of clinical symptoms in a mouse collagen-induced arthritis (CIA) model

Conclusions

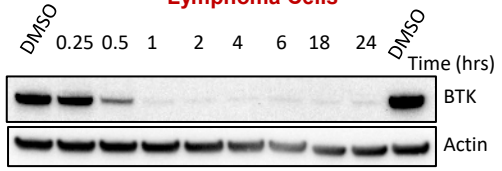
- NX-5948 mediates potent anti-inflammatory activity via BTK degradation with resultant inhibition of B cell activation
- Preclinical animal models support clinical development of NX-5948 to treat autoimmune diseases

NX-5948 is a Potent Degrader of BTK in Primary Human B Cells



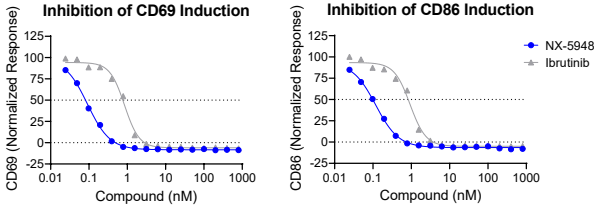
BTK Degradation Assays
Robust degradation of BTK was observed by flow cytometry in primary human B cells after 4 hour treatment with NX-5948.

NX-5948 Catalyzes Rapid BTK Degradation in Lymphoma Cells



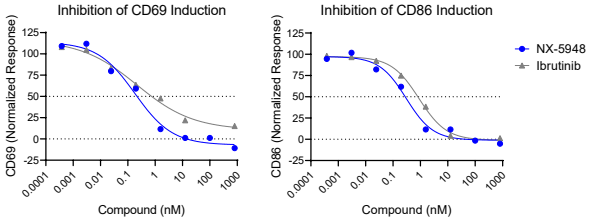
Degradation Time-Course Assay
Degradation of BTK is observed within 1 hour and is complete within 2 hours with 10 nM of NX-5948 in Ramos cells

NX-5948 is a Potent Inhibitor of Anti-IgM-Mediated B Cell Activation



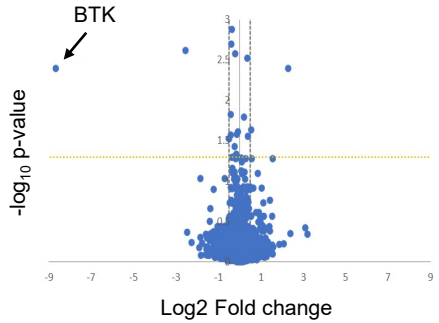
B Cell Activation Assay
NX-5948-mediated degradation of BTK prevents anti-IgM-induced upregulation of activation markers CD69 and CD86 on B cells. Human PBMCs were pre-incubated with NX-5948 or ibrutinib for 4 hours and then stimulated with 10 µg/ml anti-IgM for 18 hours. CD69 and CD86 levels on B cells were assessed by flow cytometry.

NX-5948 is a Potent Inhibitor of TLR7-Mediated B cell Activation



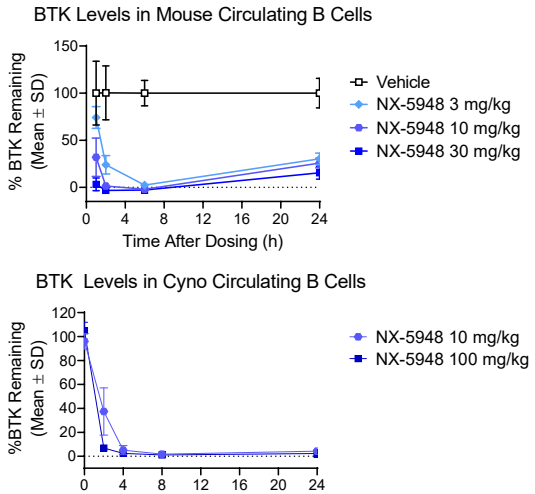
B Cell TLR7 Activation Assay
NX-5948-mediated degradation of BTK prevents Imiquimod-induced upregulation of activation makers CD69 and CD86 on B cells. Human PBMCs were pre-incubated with NX-5948 or ibrutinib for 4 hours and then stimulated with 5 µg/ml Imiquimod for 20 hours. CD69 and CD86 levels on B cells were assessed by flow cytometry.

NX-5948 is a Selective BTK Degrader



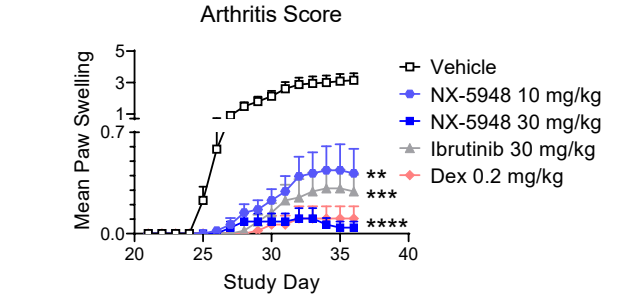
Proteomics Study
NX-5948 selectively degrades BTK in TMD8 cells. Cells were treated with DMSO or NX-5948 (50 nM) for 6 hours in triplicate. Effects on protein levels were analyzed using label-free proteomics.

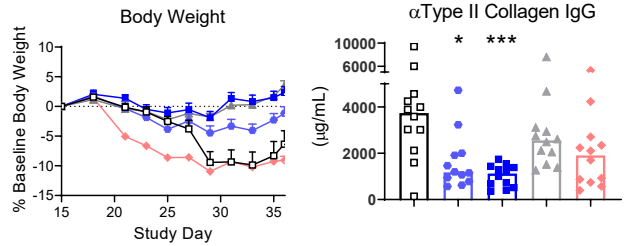
Oral Dosing of NX-5948 Promotes Rapid and Complete BTK Degradation in Mouse and Non-Human Primate B Cells

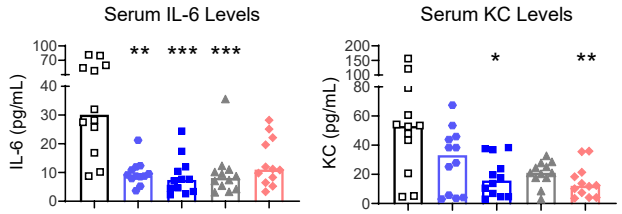


Mouse and Cynomolgus Monkey PD Studies
Dose- and time-dependent reduction in BTK levels was observed in circulating murine and non-human primate, cynomolgus monkey B cells following a single oral dose of NX-5948. BTK levels were fully suppressed in 1 to 6 hours. In mice, BTK levels increased 24 hours after dosing from BTK resynthesis. In cynomolgus monkeys, BTK levels remained suppressed at 24 hours. BTK levels were measured in circulating B cells by flow cytometry.

NX-5948 is Efficacious and Well-Tolerated in a Mouse Collagen-Induced Arthritis Model and Suppresses Antibody Titers and Cytokine Levels







Mouse Collagen-Induced Arthritis (CIA) Model
Daily oral treatment with NX-5948 at 30 mg/kg resulted in a lower mean arthritis score than ibrutinib and provided similar clinical benefit as dexamethasone with minimal body weight loss as compared to dexamethasone and vehicle. CIA was induced by immunization on Day 0 and boosted on Day 21 with type II collagen in complete Freund's adjuvant; treatment with vehicle or therapeutic agents was initiated on Day 18. At the end of the study on Day 36, serum was collected and serum levels of anti-type II collagen IgG, the inflammatory cytokine IL-6, or the neutrophil chemokine KC/CXCL1 were determined. Statistical significance was determined between vehicle control and treated groups with one-way Kruskal-Wallis ANOVA and Dunn's multiple comparisons test.

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