NX-5948, a Selective Degrader of BTK With Activity in Preclinical Models of Hematologic and Brain Malignancies

Poster 2251

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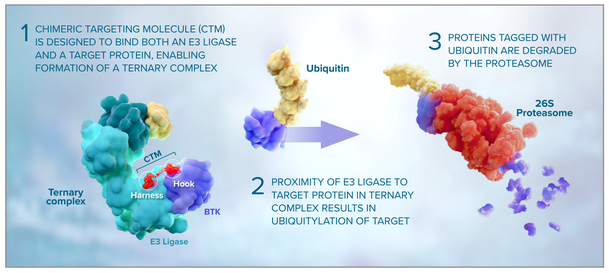
BACKGROUND

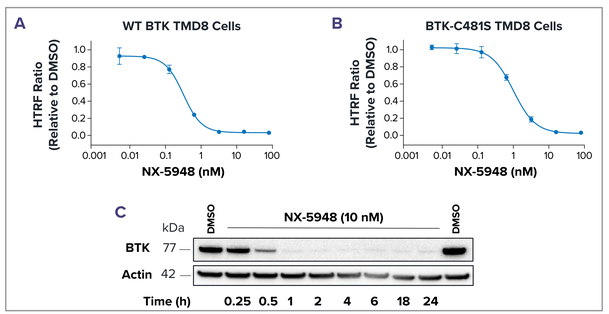
- Bruton's tyrosine kinase (BTK) plays a key role in cell survival in B cell malignancies, and covalent inhibitors of BTK, such as ibrutinib and acalabrutinib, have proven efficacious in chronic lymphocytic leukemia, mantle cell lymphoma, marginal zone lymphoma, and Waldenstrom macroglobulinemia[1]
- BTK inhibitors have also demonstrated clinical activity in small trials of patients with relapsed/refractory primary central nervous system lymphoma[2]
- The long-term efficacy of BTK inhibitors is limited by the emergence of resistance mutations, most commonly at C481 of BTK. These mutations preclude formation of a covalent bond with BTK and lead to diminished efficacy and disease progression[3]
- Several noncovalent BTK inhibitors, which do not require covalent binding to C481, are currently being investigated in clinical trials as potential therapies for patients with relapsed and refractory disease; however, other mutations have been shown to decrease the in vitro activity of these noncovalent BTK inhibitors, suggesting that mutations may ultimately limit the effectiveness of these compounds as well[4]
- Small molecule-induced protein degradation offers a unique approach to target BTK for the treatment of B cell malignancies
- Chimeric targeting molecules catalyze ubiquitylation and proteasomal degradation of target proteins and are composed of a target binding element ("hook"), a linker, and a ubiquitin ligase binding element ("harness"). NX-5948 is a chimeric targeting molecule that contains a BTK hook linked to a cereblon harness (**Figure 1**)
- Although some cereblon-binding drugs, such as lenalidomide and pomalidomide, promote the degradation of neo-substrate proteins, including the transcription factors Aiolos and Ikaros, NX-5948 has been engineered to avoid Aiolos and Ikaros degradation and therefore does not possess immunomodulatory agent activity

Figure 1. Chimeric Targeting Molecules



RESULTS

Figure 2. NX-5948 is a Potent and Rapid Degrader of Wildtype and C481S-Mutated BTK

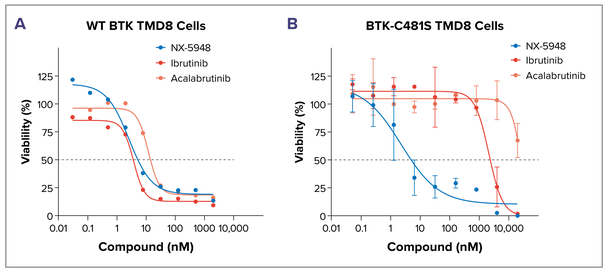


BTK-C481S, C481S-mutated BTK; DMSO, dimethyl sulfoxide; HTRF, homogeneous time resolved fluorescence; TMD8, Tokyo Medical and Dental University 8; WT, wildtype.

- NX-5948 catalyzes the degradation of 50% (DC_{50}) of (A) cellular WT BTK at 0.32 nM and (B) cellular C481S-mutated BTK at 1.0 nM concentrations in TMD8 cells. (C) Rapid degradation of BTK with 10 nM of NX-5948 occurs within 2 hours in Ramos cells

RESULTS (continued)

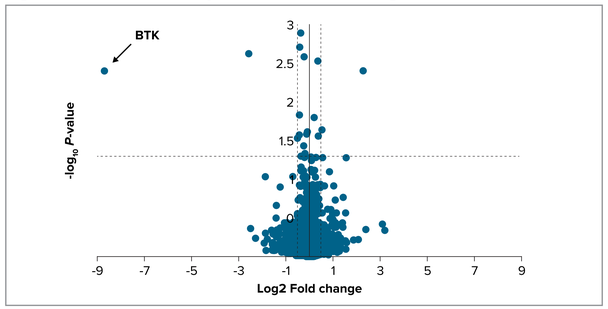
Figure 3. NX-5948 Decreases Viability of TMD8 Cells With Wildtype and C481S-Mutated BTK



% Viability measured as CellTiter-Glo normalized to DMSO. Variability in CellTiter-Glo readout can produce dose response curves that do not top out at exactly 100% viable in an individual experiment. Viability has been assessed in >6 independent experiments in WT TMD8 cells, and the tops of dose response curves for NX-5948, ibrutinib, and acalabrutinib all cluster around 100% viable.

- NX-5948 impairs viability of BTK-dependent TMD8 cells expressing (A) WT BTK and (B) C481S-mutated BTK

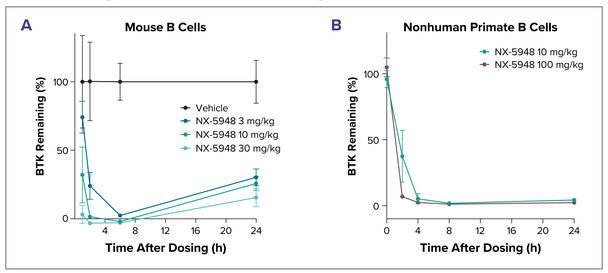
Figure 4. NX-5948 Catalyzes Selective BTK Degradation



TMD8 cells were treated with DMSO or 50 nM NX-5948.

- Proteomic analysis of NX-5948 demonstrates selective BTK degradation in TMD8 cells

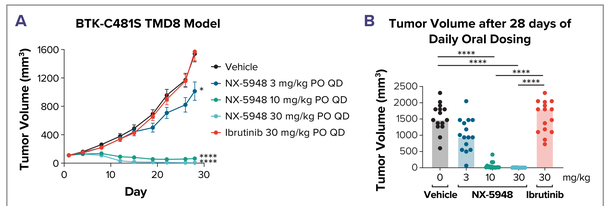
Figure 5. NX-5948 Promotes Rapid and Potent BTK Degradation in vivo in Circulating B Cells After Oral Dosing



Data plotted as mean ± standard deviation.

- After a single oral dose of NX-5948, BTK is degraded in (A) circulating mouse and (B) non-human primate B cells

Figure 6. Daily Oral Dosing of NX-5948 Leads to Regression of Tumors in a Mouse Model of Ibrutinib-Resistant DLBCL



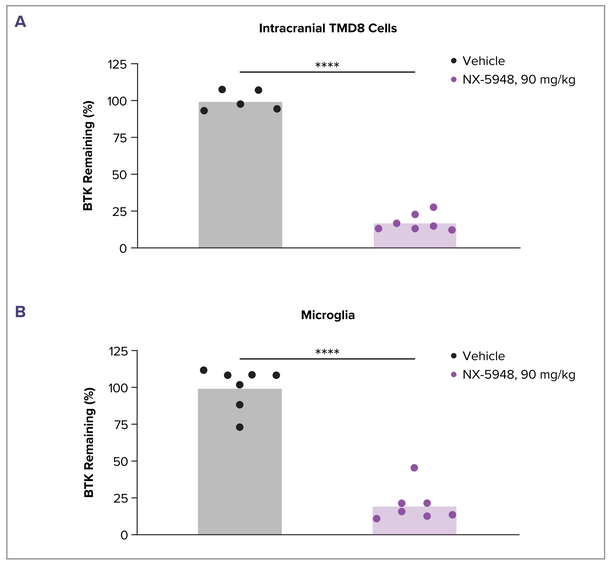
C

	Vehicle	NX-5948, mg/kg 3	10	30	Ibrutinib 30 mg/kg
%TGI (day 28)	—	36.3	99.0	99.7	-22.4
P value (vs vehicle)	—	.0173	<.0001	<.0001	.9990
P value vs ibrutinib	.9990	.0165	<.0001	<.0001	—

DLBCL, diffuse large B cell lymphoma; PO, orally; QD, once daily; TGI, tumor growth inhibition.
Statistical significance was assessed using (A) mixed-effects (REML) model with Tukey multiple comparisons post test day 1 to day 28 or (B) one-way Kruskal-Wallis ANOVA with Dunn multiple comparison post-test; *P<.05, ****P<.0001. TGI calculated from median tumor volumes.

- (A) In a murine xenograft model with TMD8 cells harboring the BTK-C481S mutation, daily oral administration of NX-5948 resulted in superior TGI compared with ibrutinib, (B) tumor volume after 28 days of daily oral dosing, (C) table comparing tumor growth inhibition

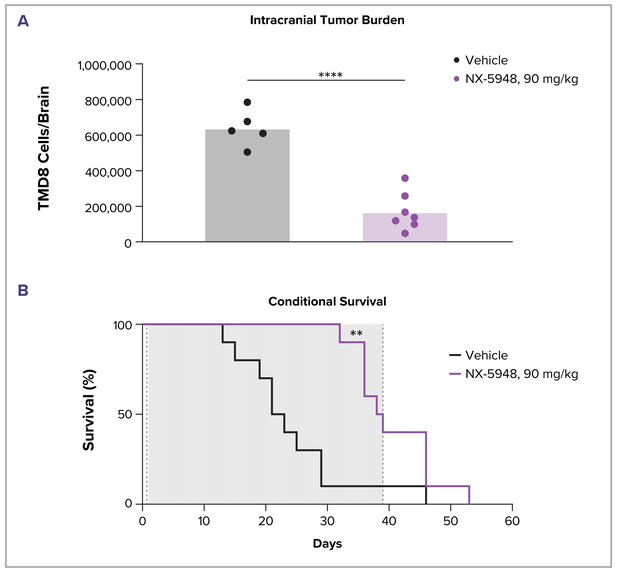
Figure 7. NX-5948 Promotes BTK Degradation in Brain-Resident TMD8 Tumor Cells and Microglia



(A,B) Statistical significance was assessed using Welch test ****P<.0001. Error bars represent mean values. Bars represent mean values. PO, QD treatment, day 1 to day 39 post implant.

- Daily oral administration of NX-5948 to mice promotes > 80% BTK degradation in (A) intracranially implanted TMD8 tumor cells and (B) microglia

Figure 8. NX-5948 Reduces Tumor Burden and Prolongs Survival in Mice With Intracranial TMD8 Tumors



(A) Statistical significance was assessed using Welch test and (B) Log-rank test; **P<.005, and ****P<.0001. Bars represent mean values. PO, QD treatment, day 1 to day 39 post implant (gray area in plot above).

- Daily oral administration of NX-5948 in mice significantly reduces intracranial tumor burden (A) and improves survival (B)

CONCLUSIONS

- NX-5948 is a selective degrader of BTK with potent antitumor activity in a mouse model of ibrutinib-resistant diffuse large B cell lymphoma
- NX-5948 crosses the blood-brain barrier and mediates BTK degradation in brain-resident tumor cells and microglia
- NX-5948 reduces tumor burden and extends survival in a mouse model of primary central nervous system lymphoma
- These findings support clinical development of NX-5948 for the treatment of B cell malignancies, including primary central nervous system lymphoma

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DISCLOSURES

DWR, MN, RR, MT, NB, CG, TI, DK, AK, ZK, JM, JMc, LP, CG: Employment and stock or other ownership in Nurix Therapeutics. **AT-M:** Employment with and stock or other ownership with Nurix Therapeutics and Gilead Sciences. **GH:** Employment and leadership with Nurix Therapeutics; stock or other ownership with Nurix Therapeutics and Lexicon Pharmaceuticals. **ATS:** Employment, leadership, and stock or other ownership with Nurix Therapeutics.

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