Bexobrutideg (NX-5948) is a CNS-Penetrant Catalytic Bruton's Tyrosine Kinase (BTK) Degrader That Breaks Established Design Rules for CNS Drugs

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Wylie Palmer, Mark Noviski, Paul Auger, Karthik Arumugam, Danielle Bautista, Nivetha Brathaban, Robert Cass, Ming Liang Chan, Ganesh Cherala, Philip Keyes, Aishwarya Kumar, Saloni Malla, Mitchell Lavarias, Filippo Marchioni, Jenny McKinnell, Jun Ma, Ryan Rountree, May Tan, Alexandra Trotier, Sarah Whelan, Davorka Messmer, Frederick Cohen, Gwenn M. Hansen
Nurix Therapeutics, Inc., San Francisco, CA, USA

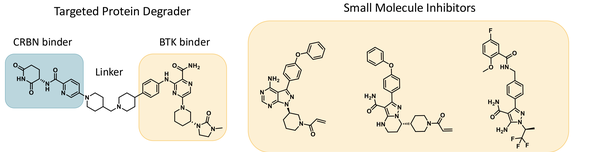
Abstract

Bexobrutideg (NX-5948) is a targeted protein degrader of Bruton's tyrosine kinase (BTK) in Phase 1 clinical development for the treatment of B-cell malignancies. Heterobifunctional degraders, such as bexobrutideg, occupy beyond Rule-of-Five chemical space where established guidelines for physicochemical properties associated with drug-likeness cannot easily be applied. In contrast to approved central nervous system (CNS) drugs, bexobrutideg exceeds several recognized chemical property metrics, such as molecular weight, polar surface area, and hydrogen-bond donor count. In addition, bexobrutideg does not conform with *in vitro* permeability and transporter efflux ratio guidelines that would predict for CNS penetration. Despite these unfavorable properties, bexobrutideg shows CNS exposure in preclinical models.

As a bifunctional degrader with an event-driven mode of action, bexobrutideg induces potent degradation of BTK in primary human B cells and malignant B cells. By performing a series of *in vitro* experiments measuring cellular partitioning and degradation kinetics, we calculated the catalytic efficiency of bexobrutideg and demonstrate that one degrader molecule can promote degradation of thousands of copies of target protein. This enables a very low concentration of free drug to sustain pharmacodynamic activity and efficacy *in vivo*. Bexobrutideg shows dose-dependent brain exposure in rodents with an unbound brain-to-plasma partition coefficient, or $K_{p,uu}$, value consistent with CNS penetration.

In the clinic, bexobrutideg is detectable in cerebrospinal fluid of patients with CNS-involved B-cell malignancies, with concentrations that exceed the minimum free plasma level that correlates with BTK degradation. Bexobrutideg has also demonstrated clinically meaningful responses in patients with primary CNS lymphoma or chronic lymphocytic leukemia with CNS involvement (O'Connor, P., 7th Annual TPD & Induced Proximity Summit, Boston, 2024; Linton, K., EHA Hybrid Congress, Madrid, Spain, 2024), supporting the therapeutic potential of bexobrutideg in B-cell malignancies with CNS involvement.

Figure 1. Bexobrutideg, a targeted degrader of BTK, and BTK inhibitors



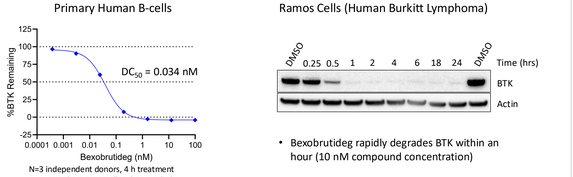
Bexobrutideg
Phase 1
Bifunctional degrader
MW = 807

Ibrutinib
FDA approved in 2013
Covalent
MW = 441

Zanubrutinib
FDA approved in 2019
Covalent
MW = 472

Pirtobrutinib
FDA approved in 2023
Non-Covalent
MW = 479

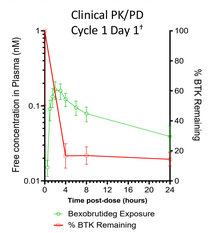
Figure 2. Bexobrutideg is a potent and rapid degrader of BTK



N=3 independent donors, 4 h treatment
SEM error bars are smaller than symbols

- Bexobrutideg rapidly degrades BTK within an hour (10 nM compound concentration)

Figure 3. Clinically active doses of bexobrutideg show lower unbound drug exposure than covalent and noncovalent BTK inhibitors in patients



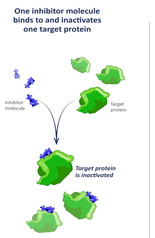
Bexobrutideg Requires Lower Steady-state Exposure than FDA Approved BTK Inhibitors for Pharmacodynamic Responses

	Bexobrutideg (200mg QD)†	Ibrutinib (560mg QD)*	Zanubrutinib (160mg BID)*	Pirtobrutinib (200mg QD)*
$C_{max,free}$ (nM)	0.22	8.0	40	540
$C_{min,free}$ (nM)	0.09	0.2	3.5	250

†NX-5948-301 Ph1a/b clinical study, Data cutoff: 01 Dec 2024
*clinically approved doses

Figure 4. Degraders are PK advantaged due to their catalytic mechanism of action

ONE inhibitor molecule inhibits ONE target protein



ONE degrader molecule promotes degradation of MULTIPLE target proteins

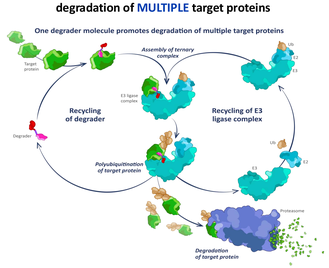
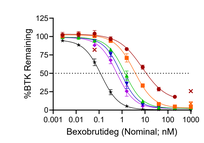
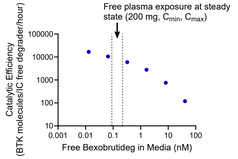


Figure 5. One molecule of bexobrutideg catalytically degrades thousands of BTK proteins per hour at clinically relevant concentrations



BTK DC_{50} (Nominal; nM)

0.5 hrs	11.6
1 hr	3.9
2 hrs	1.4
3 hrs	0.97
4 hrs	0.65
24 hrs	0.12



- BTK degradation kinetics were quantified by flow cytometry in TMD8 cells treated with bexobrutideg for 0.5-24 hours
- Catalytic efficiency was calculated using a modified version of an approach previously used for other BTK degraders (Lynch et al., *ACS Chemical Biology*, 2024*)*. The modified approach accounts for degradation half life, intracellular concentration of BTK protein, partitioning of bexobrutideg between cells and media, the free fraction of bexobrutideg in media and cellular homogenate, and loss of compound due to nonspecific adsorption.

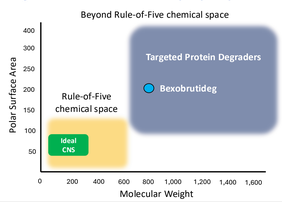
Figure 6. Metrics have been established for small molecule drugs to aid in achieving CNS exposure

- The blood-brain barrier (BBB) is a selective barrier that protects the brain from harmful compounds and precisely regulates its microenvironment
- The CNS multiparameter optimization score (MPO score) defines chemical properties that are optimal for CNS therapeutic agents
- Metrics for CNS penetration are more restrictive than the Rule-of-Five for oral bioavailability

Property	BBB Penetration CNS MPO*	Oral Bioavailability Small Molecule Rule-of-5
MW	≤ 360	≤ 500
HBD	as low as possible (ideally 0)	≤ 5
cLogP	≤ 3	≤ 5
TPSA	40 to 90	≤ 140

*CNS MPO criteria for highest score of 1 (Wager, et al., *ACS Chem Neuro*, 2016)

Figure 7. The properties of bexobrutideg suggest that CNS-penetrant targeted protein degraders will outline new property boundaries within a distinct chemical parameter space



- The chemical property space for the ideal CNS small molecule drug is very restrictive with more desirable MWs of less than 360 and a polar surface area between 40 and 90 square angstroms
- Targeted Protein Degraders occupy a chemical property space with MW and PSA well beyond the typical small molecule and the typical CNS drug property space
- The blue rectangle represents the bounds for observed CNS exposure from *in vivo* screening of bifunctional targeted protein degraders across several Nurix programs

Figure 8. Bexobrutideg's physico-chemical properties lie outside of the established norms for CNS drugs

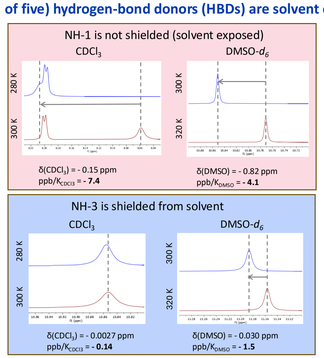
- Multiparameter optimization (MPO) scores can be useful guidelines for the design of small molecule CNS drugs
- 77% of marketed CNS drugs had an MPO score ≥ 4.0 (Wager, et al., ACS Chem Neuro, 2016)
- The calculated MPO score of 2.2 for bexobrutideg indicates it may have reduced CNS exposure relative to typical CNS drugs
- Accounting for solvent exposed HBDs (eHBDs) increases the score

Chemical Properties and Calculated MPO Score of Bexobrutideg

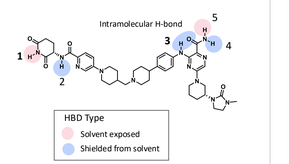
Property	More Desirable	Less Desirable	Bexobrutideg Property value	Score*
ClogP	≤ 3	> 5	3.6	0.7
ClogD	≤ 2	> 4	0.9	1.0
MW	≤ 360	> 500	807	0
TPSA	40 to 90	≤ 20, > 120	202	0
HBD	≤ 1	> 4	5 (2 eHBDs)	0 (0.5)
pKa	≤ 8	> 10	9.1 (measured)	0.45
			Bexobrutideg MPO score =	**2.2 (2.7)**

*Each property assigned a score from 0.0 to 1.0 and summed to give the MPO score

Figure 9. Temperature and solvent-dependent ^{1}H-NMR shift experiments indicate two (out of five) hydrogen-bond donors (HBDs) are solvent exposed (eHBD)



Bexobrutideg Hydrogen Bond Donors



- Large ^{1}H-NMR shifts for NH-1 and NH-5 (not shown) in both solvents indicate both HBDs are solvent exposed
- Small ^{1}H-NMR shifts for NH-3 in both solvents indicate it is not solvent exposed and engaged in an intramolecular H-bond with adjacent carbonyl.
- ^{1}H-NMR data (not shown) also indicates NH-2 is not exposed and NH-4 is less exposed to solvent.

Figure 10. MDCK-MDR1 (P-gp) permeability assay indicates bexobrutideg has moderate passive permeability, but has potential to be a substrate for major efflux transporters

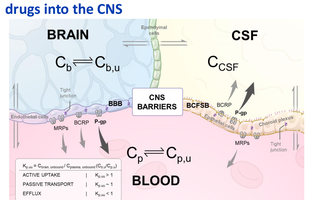
- Based on an analysis of drugs with CNS and non-CNS indications, compounds with passive permeability >1.5x10^{-6} cm/s and efflux ratio <2.5 were most likely to be CNS-penetrant*
- Bexobrutideg has an efflux ratio of 40 in MDCK-MDR1 cells indicating the compound is a potential substrate for major active transporters like P-gp

*Doan, et. al., *J. Pharmacol. Exp. Ther.* 303, 1029–1037.

Compound (3 µM)[1]	P_{app} A-B (10^{-6} cm/s)	P_{app} B-A (10^{-6} cm/s)	Efflux Ratio (B-A/A-B)	A-B Permeability Ranking[2]
Bexobrutideg	0.47	19	40	Low
Bexobrutideg + 100 µM Verapamil	3.8	5.5	1.4	Medium
Controls:				
Metoprolol	31	31	1	High
Imatinib	1.8	47	27	Medium

[1]Bexobrutideg aqueous solubility in PBS at pH 7.4 = 9.7 µM; conditions with bexobrutideg include 3% BSA
[2]Permeability ranking Papp(10^{-6} cm/s): Low < 1, high > 10

Figure 11. CNS penetration involves the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) with transporters that restrict access or actively uptake drugs into the CNS



- Drugs can cross the BBB by diffusion or active transport
- The BBB and BCSFB barriers have different transporters expressed and as such direct access can be more (or less) restrictive for one barrier over the other
- The free drug-concentration in brain drives efficacy and at steady-state is equal on both sides of a membrane unless active uptake or efflux is involved
- Unbound brain-to-plasma ratio:
  - $K_{p,uu}$ = [brain]$_u$/[plasma]$_u$

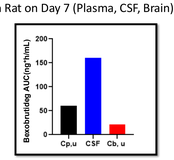
BBB blood-brain barrier, BCFSB blood-cerebrospinal fluid barrier, BCRP breast cancer resistance protein, CSF cerebrospinal fluid, C_b total brain concentration, $C_{b,u}$ unbound brain concentration, C_p total plasma concentration, $C_{p,u}$ unbound plasma concentration, MRPs multidrug resistance-associated proteins, P-gp P-glycoprotein. Light gray double-arrows represent passive diffusion across each barrier. Large arrows represent active transport. Black double-arrows represent equilibrium between protein bound and unbound drug.

Figure 12. Daily oral dosing of bexobrutideg in rat study demonstrates that bexobrutideg achieves free drug levels in the brain consistent with expectations for CNS-penetrant drugs

- CSF levels are 2.7-fold higher than unbound plasma levels on day 7
- Compounds with brain Kp,uu > 0.30 are considered brain penetrant*

*Kalvass, JC et al., *Drug Metab Dispos.* 2007, 25 (4) 660-666.

Bexobrutideg Unbound Exposures in Rat on Day 7 (Plasma, CSF, Brain)

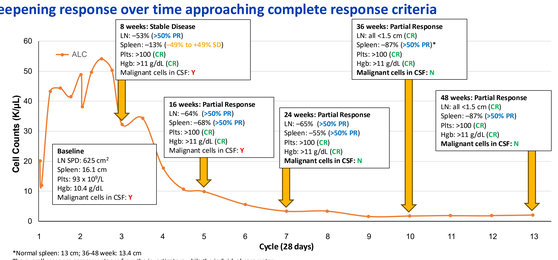


Bexobrutideg Exposure in Rat (Day 7, 100 mg/kg, QD dosing)

PK Parameters	Plasma	Brain	CSF
C_{min} (ng/mL)	153	98	2.21
$AUC_{0\text{-}24\,Total}$ (ng*h/mL)	11,153	3,228	160
$AUC_{0\text{-}24\,Unbound}$ (ng*h/mL)	60	21	160
$K_{p,uu}$ ($C_{b,u}$ or CSF/$C_{p,u}$)	-	**0.35**	**2.7**

$K_{p,uu}$ = unbound brain (or CSF)-to-plasma partition coefficient, $C_{b,u}$ = unbound brain concentration, $C_{p,u}$ = unbound plasma concentration
Oral bioavailability in this study, *F* = 17%

Figure 13. Patient with CLL and CNS involvement treated with bexobrutideg showed deepening response over time approaching complete response criteria



*Normal spleen: 13 cm; 36-48 week: 13.4 cm
The overall response assessments are from the investigators while the individual parameter response assessment criteria are calculated per IwCLL from the data entered
ALC, Absolute lymphocyte count; **CSF,** cerebrospinal fluid; **Hgb,** hemoglobin; **LN,** lymph nodes; **Plts,** platelets

NX-5948-301 Ph1a/b clinical study
Data cutoff: 10 June 2024

O'Connor, P. 7th Annual TPD & Induced Proximity Summit, Boston, 2024

Conclusion

- A potential new class of CNS drugs with Beyond-Rule-of-Five chemical properties is rapidly emerging
- Our data begin to define new "rules" to help predict potential for CNS exposure of degraders
- Physico-chemical properties of degraders may limit overall exposures; however, because of their catalytic MOA, sub-stoichiometric levels of a degrader can eliminate the target protein
- Bexobrutideg is a CNS-penetrant, orally bioavailable, BTK degrader in Phase 1 trials for the treatment of B-cell diseases with the potential to treat patients with CNS involvement

Presented at the American Association for Cancer Research, AACR; April 25-30, 2025