Rewriting Established Drug Design Rules with a CNS-Penetrant Catalytic Bruton's Tyrosine Kinase (BTK) Degrader

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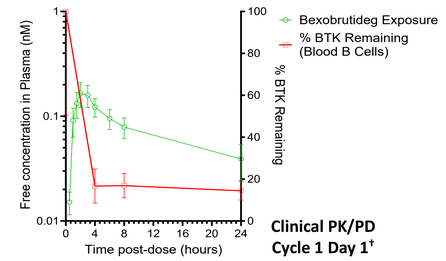
Abstract

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 physico-chemical 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.

Bexobrutideg has an event-driven mode of action and 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. One degrader molecule can promote degradation of thousands of copies of target protein, enabling 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 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 [1,2], supporting the therapeutic potential of bexobrutideg in B-cell malignancies with CNS involvement.

Figure 1. Bexobrutideg requires lower steady-state exposure than FDA approved BTK inhibitors to achieve pharmacodynamic responses



Clinical PK/PD Cycle 1 Day 1†

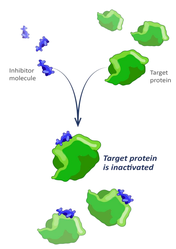
Steady-State Pharmacokinetics of BTK Agents

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

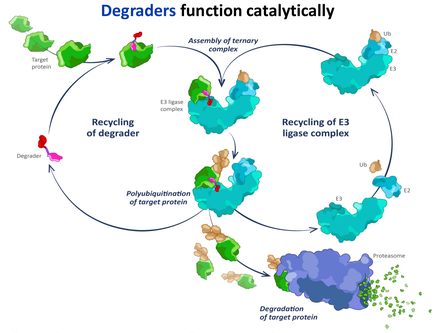
†NX-5948-301 Ph1a/b clinical study, Data cutoff: 01 Dec 2024
*clinically approved doses

Figure 2. Degraders are PK advantaged due to their catalytic mechanism of action

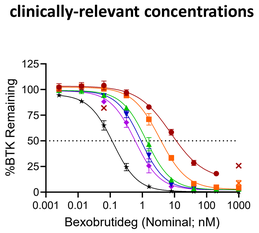
Inhibitors operate with 1:1 stoichiometry



Degraders function catalytically

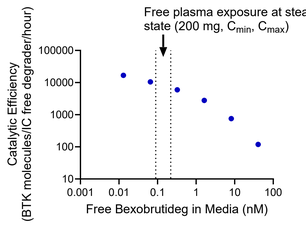


ONE molecule of BEXOBRUTIDEG catalytically degrades THOUSANDS of BTK proteins per hour at clinically-relevant concentrations



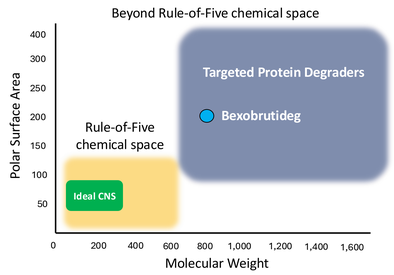
BTK DC_{50} (Nominal; nM)

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



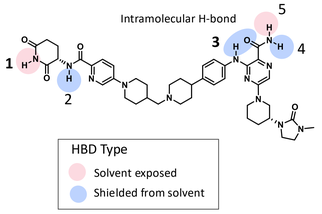
- BTK degradation kinetics were quantified by flow cytometry in TMD8 cells treated with bexobrutideg for 0.5-24 hours
- Catalytic efficiency was calculated using a modified version of an approach previously used for other BTK degraders [3]. The modified approach accounts for degradation half life, intracellular 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 3. Bexobrutideg's physico-chemical properties lie outside of the established norms for CNS drugs



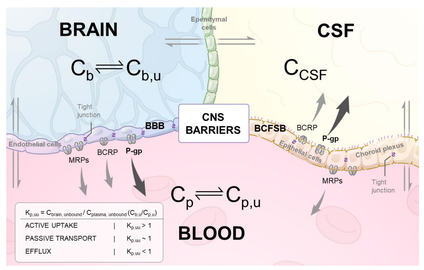
- Metrics for CNS penetration are more restrictive than the Rule-of-Five for oral bioavailability
- The chemical property space for an ideal CNS small molecule drug is very restrictive with more desirable MWs of less than 360 and a polar surface area (PSA) between 40 and 90 square angstroms
- Targeted protein degraders occupy a chemical property space with MW and PSA well beyond the typical small molecule and the typical CNS drug property space

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)**



- The CNS MPO score defines chemical properties that are optimal for CNS therapeutic agents
- 77% of marketed CNS drugs have an MPO score ≥ 4.0 [4]
- The calculated MPO score of 2.2 for bexobrutideg indicates it may have reduced CNS exposure relative to typical CNS drugs
- Bexobrutideg solvent-exposed hydrogen bond donor (eHBD) count was determined using temperature- and solvent-dependent ^{1}H NMR shift experiments [5]
- Accounting for eHBDs increases the score, but bexobrutideg still falls outside the range of typical CNS drugs

Figure 4. CNS penetration involves the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) with transporters that restrict access or active uptake of drugs into the CNS



- Drugs can cross the BBB by diffusion or active transport
- The BBB and BCSFB barriers express different transporters, and direct access can be more (or less) restrictive for one barrier over the other
- The free drug concentration in brain drives efficacy
- At steady-state, free drug concentration 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. Diagram was presented previously [6].

Figure 5. MDCK-MDR1 (P-gp) permeability assay indicates bexobrutideg has moderate passive permeability, but has potential to be a substrate for major efflux transporters

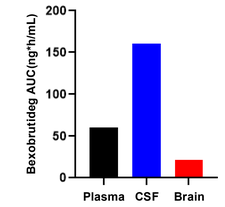
- Based on an analysis of drugs in CNS and non-CNS indications, compounds with passive permeability >1.5x10^{-6} cm/s and efflux ratio <2.5 were most likely to be CNS-penetrant [7]
- Bexobrutideg has an efflux ratio of 40 in MDCK-MDR1 cells, indicating the compound is a potential substrate for major active transporters like P-gp

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 6. Daily oral dosing of bexobrutideg in rats achieves free drug levels in the brain consistent with expectations for CNS-penetrant drugs

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



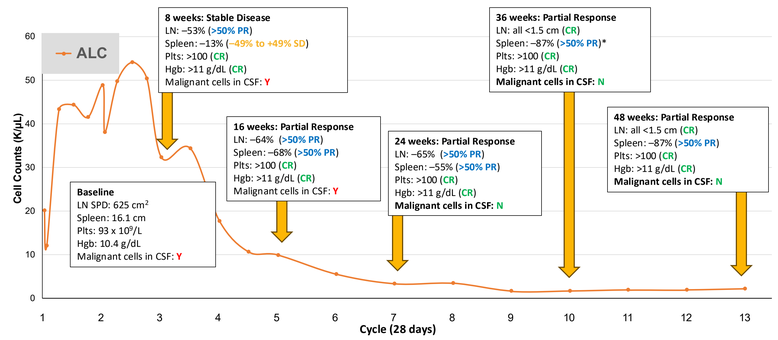
Bexobrutideg Exposure in Rat (Day 7, 100 mg/kg, QD dosing)

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%

- CSF levels are 2.7-fold higher than unbound plasma levels on day 7
- Compounds with brain $K_{p,uu}$ > 0.30 are considered brain penetrant [8]

Figure 7. Patient with CLL with CNS involvement treated with bexobrutideg showed deepening response over time approaching complete response



*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

Conclusions

- A potential new class of CNS drugs with Beyond-Rule-of-Five chemical properties is rapidly emerging
- Our data begin to redefine the rules that predict 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
- Eliminating P-gp-mediated drug efflux may not be necessary to achieve efficacious exposure of catalytic degraders in the CNS
- Bexobrutideg is a CNS-penetrant, orally bioavailable, BTK degrader in Phase 1 trials for the treatment of B-cell malignancies (NCT05131022) with the potential to treat patients with CNS involvement

References

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