

ABS-1230, a Selective Oral Small Molecule Inhibitor of KCNT1 Broadly Inhibits Pathogenic Mutants and Reduces Seizures in a Mouse Model of KCNT1-related Epilepsy

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Abstract

Rationale

Pathogenic gain-of-function mutations in *KCNT1* (Slack) cause early onset developmental and epileptic encephalopathy characterized by a high seizure burden and resistance to anti-seizure medications. Development of small molecule inhibitors of the KCNT1 K⁺ channel for the treatment of KCNT1-related epilepsy has been limited by the large number of distinct pathogenic mutations, many of which are resistant to known inhibitors. A campaign was undertaken to identify a selective small molecule oral inhibitor of KCNT1 with high brain penetrance and activity against all pathogenic mutations and culminated in the discovery of ABS-1230. ABS-1230 was characterized for its pharmacologic properties and potency against KCNT1 mutations in vitro, oral bioavailability and brain penetrance in preclinical species, and efficacy to reduce seizures in a KCNT1 related epilepsy mouse model.

Methods

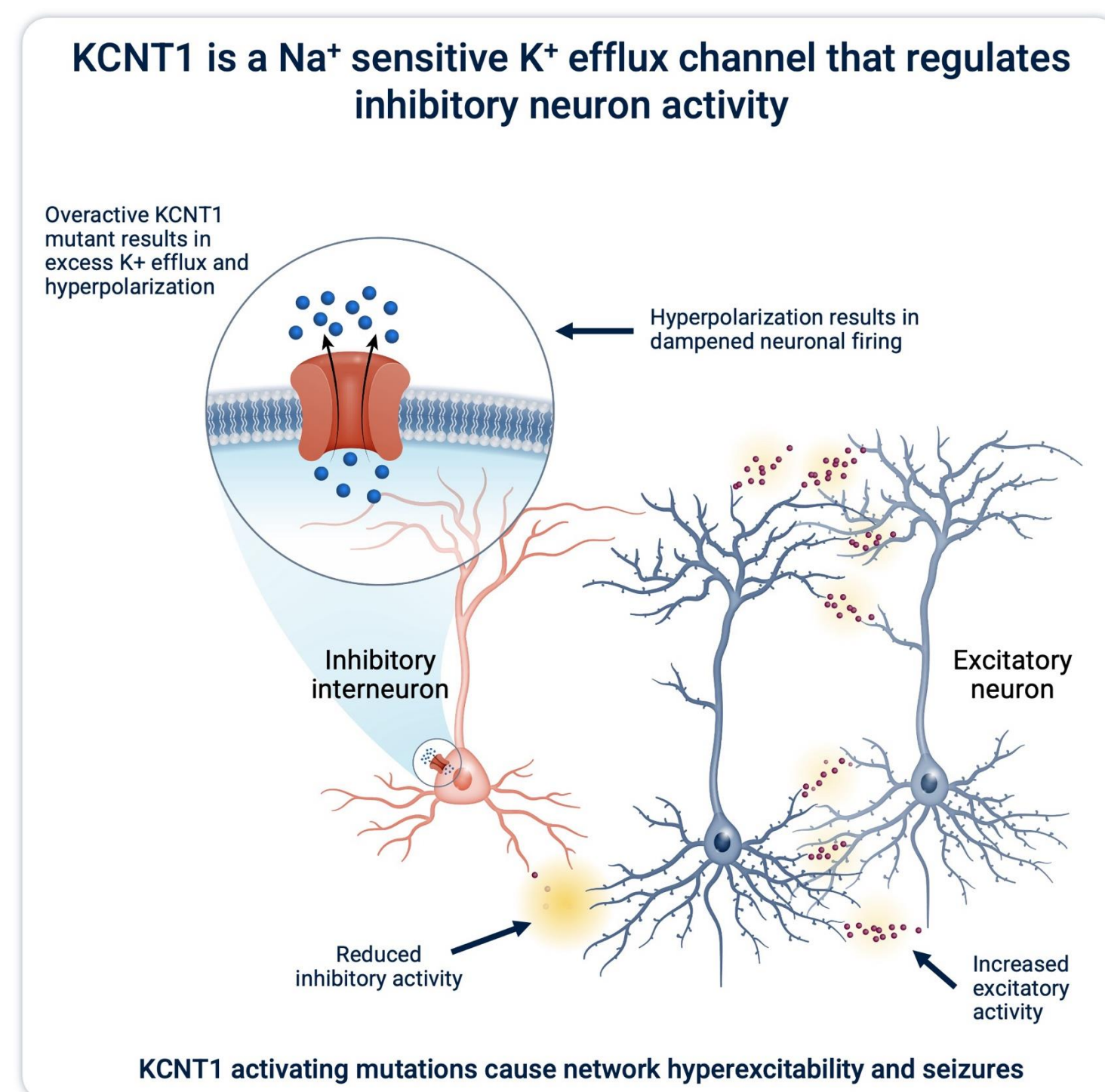
Human and mouse wild type (WT) *KCNT1* and mutants were expressed in HEK293 cells and *KCNT1* activity was assessed by manual patch clamp electrophysiology or by loxapine-induced thallium (Tl⁺) influx following treatment with ABS-1230. ABS-1230 selectivity was profiled in commercial kinase, ion channel, and safety panels. The oral bioavailability and brain penetrance of ABS-1230 was assessed in mouse, rat, dog, and monkey by comparing systemic, brain or CSF exposures. *Kcnt1*^{R409Q/R409Q} mice were dosed by intraperitoneal injection with a vehicle control followed by ABS-1230, and longitudinal monitoring of seizures was assessed by electroencephalography.

Results

ABS-1230 inhibited human and mouse *KCNT1*-dependent K⁺ current with IC₅₀ values of 0.25 and 0.12 nM, respectively, and showed no meaningful activity against > 500 off-target proteins examined (IC₅₀ values > 1000 nM). ABS-1230 also potently inhibited K⁺ current generated by 3 prevalent *KCNT1* mutants that are known to differentially impact channel properties (R428Q, R474H and Y796H mutants with ABS-1230 IC₅₀ values of 1.26, 1.96, and 0.15 nM, respectively). Mutant profiling was expanded to 63 of 67 known pathogenic or likely pathogenic mutants that could be assayed for *KCNT1*-dependent influx of Tl⁺ induced by loxapine. *KCNT1* mutants demonstrated increased response to loxapine, consistent with a gain-of-function mechanism, and ABS-1230 potently inhibited all 63 mutants with IC₅₀ values of 0.8 – 8.8 nM, which was similar to the IC₅₀ for WT *KCNT1* in this assay (1.6 nM) (Figure 1). ABS-1230 displayed high oral bioavailability (70 - >100 %) and brain penetration (K_{p,uu,brain} 0.3-0.7) across species. Administration of ABS-1230 (0.4, 1.25, 2.5 mg/kg BID) to *Kcnt1*^{R409Q/R409Q} mice dose-dependently reduced seizures relative to vehicle control treatment (Figure 2).

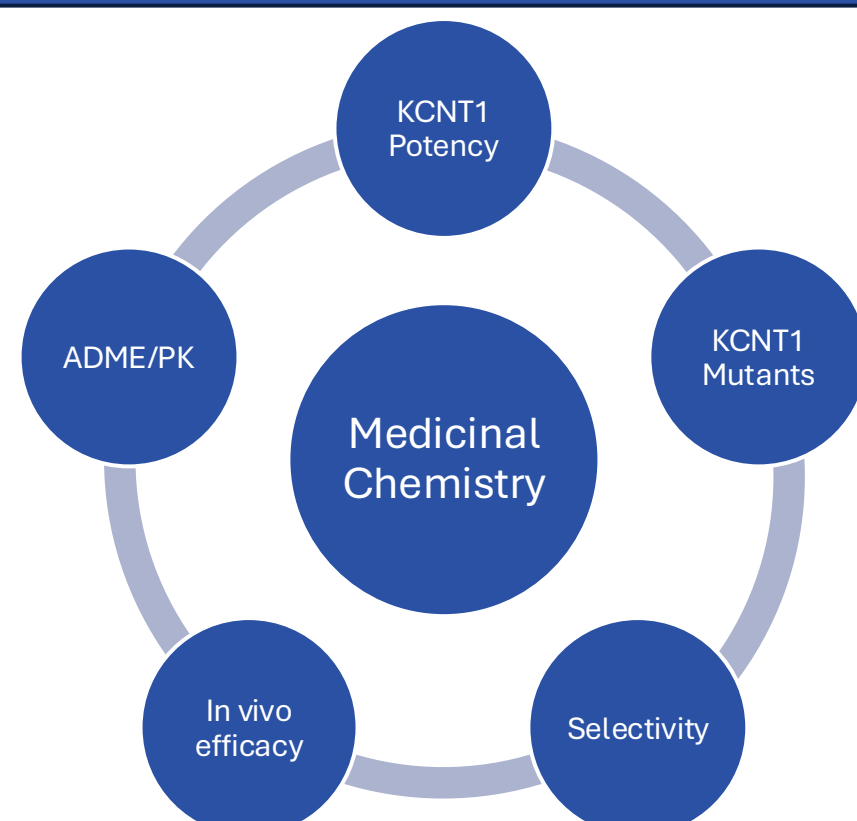
Conclusions

ABS-1230 is a selective inhibitor of *KCNT1* with high oral bioavailability and brain penetrance that potently inhibits known *KCNT1* mutations in vitro and strongly reduces seizures in *Kcnt1*^{R409Q/R409Q} mice in vivo. These data support ABS-1230 as an oral targeted therapy with potential to treat all patients with *KCNT1*-related epilepsy.



Methods

- ✓ Potency against wild type *KCNT1*
- ✓ Broad activity against mutants
- ✓ Selectivity for *KCNT1* over other channels/protein
- ✓ Seizure reduction in *KCNT1* mutant mouse model
- ✓ ADME/PK properties to enable oral bioavailability, CNS penetration



KCNT1 drug discovery campaign led to discovery of ABS-1230

Results (1)

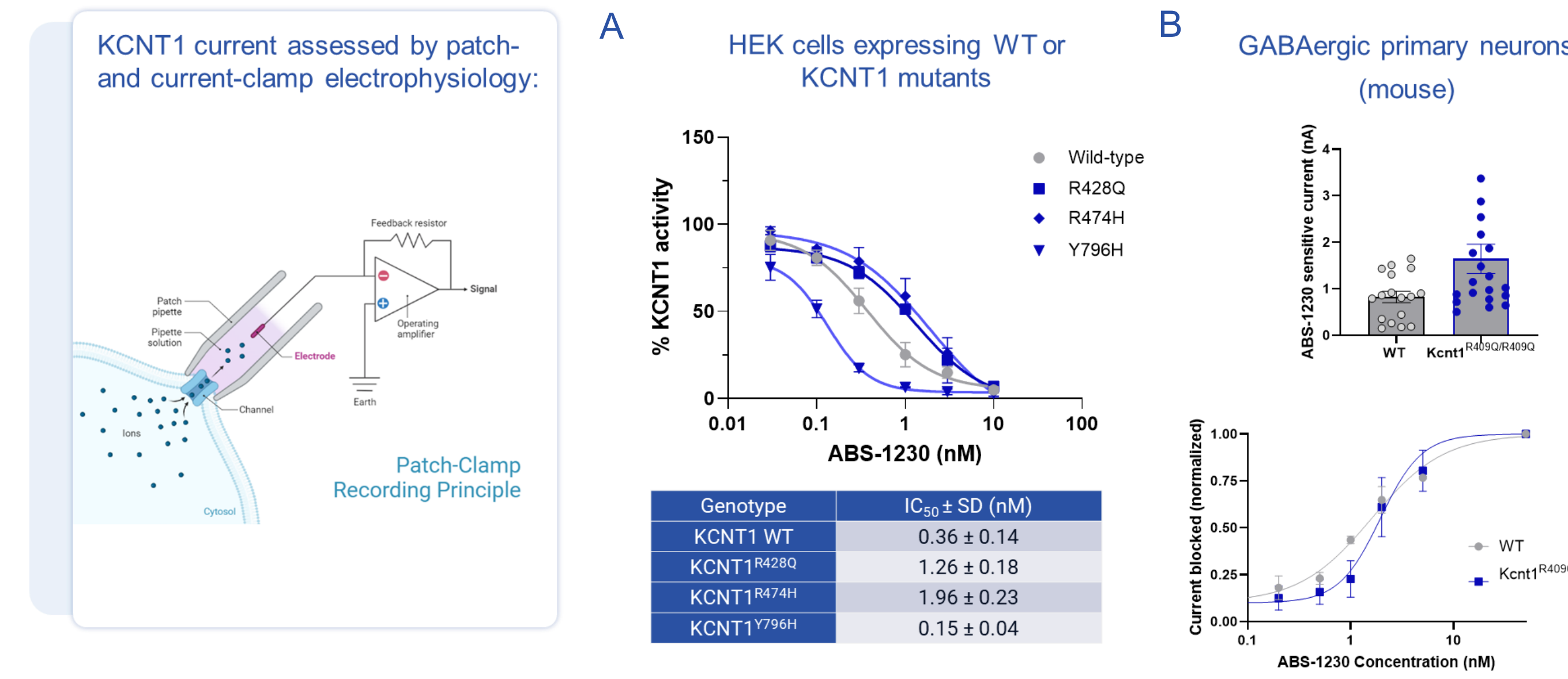


Figure 1. ABS-1230 demonstrated to be a potent inhibitor of *KCNT1*

A) Wild type (WT) or mutant *KCNT1* proteins were expressed in HEK293 cells and current was measured by patch clamp electrophysiology in the absence or presence of ABS-1230 to determine an IC₅₀ value. B) The ABS-1230-sensitive current was measured in GABAergic neurons isolated from WT and *Kcnt1*^{R409Q/R409Q} mice. *Kcnt1*^{R409Q} is the mouse equivalent of the human R428Q mutation.

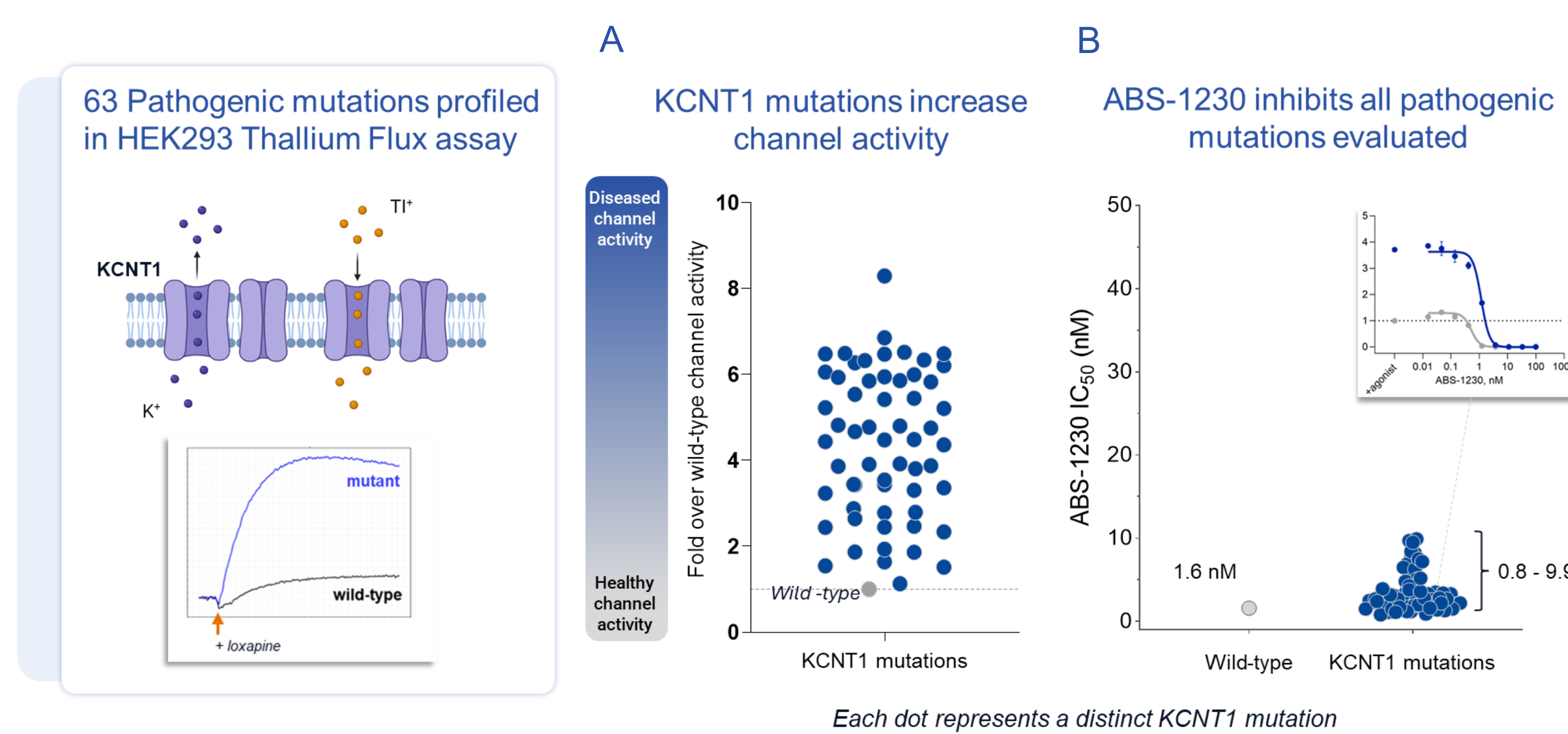


Figure 2. ABS-1230 shown to broadly inhibit *KCNT1* pathogenic mutations

Wild type or 63 distinct pathogenic or likely pathogenic *KCNT1* mutants were expressed in HEK293 cells and *KCNT1* activity was evaluated in a loxapine-induced Thallium (Tl⁺) flux assay (2). A) The relative activity of mutants compared to wild type *KCNT1* is shown (58 of 63 *KCNT1* mutations showed elevated activity compared to wild type *KCNT1*). B) ABS-1230 dose-response experiments were conducted on each *KCNT1* mutant to determine an IC₅₀ value.

ABS-1230 tested for off-target activity

- ✓ 22 Potassium (K⁺) channels
- ✓ 9 Sodium (Na⁺) channels
- ✓ 2 Calcium (Ca²⁺) channels
- ✓ 11 additional ion channels
- ✓ >40 GPCRs and transporters*
- ✓ >400 kinases*

*not shown

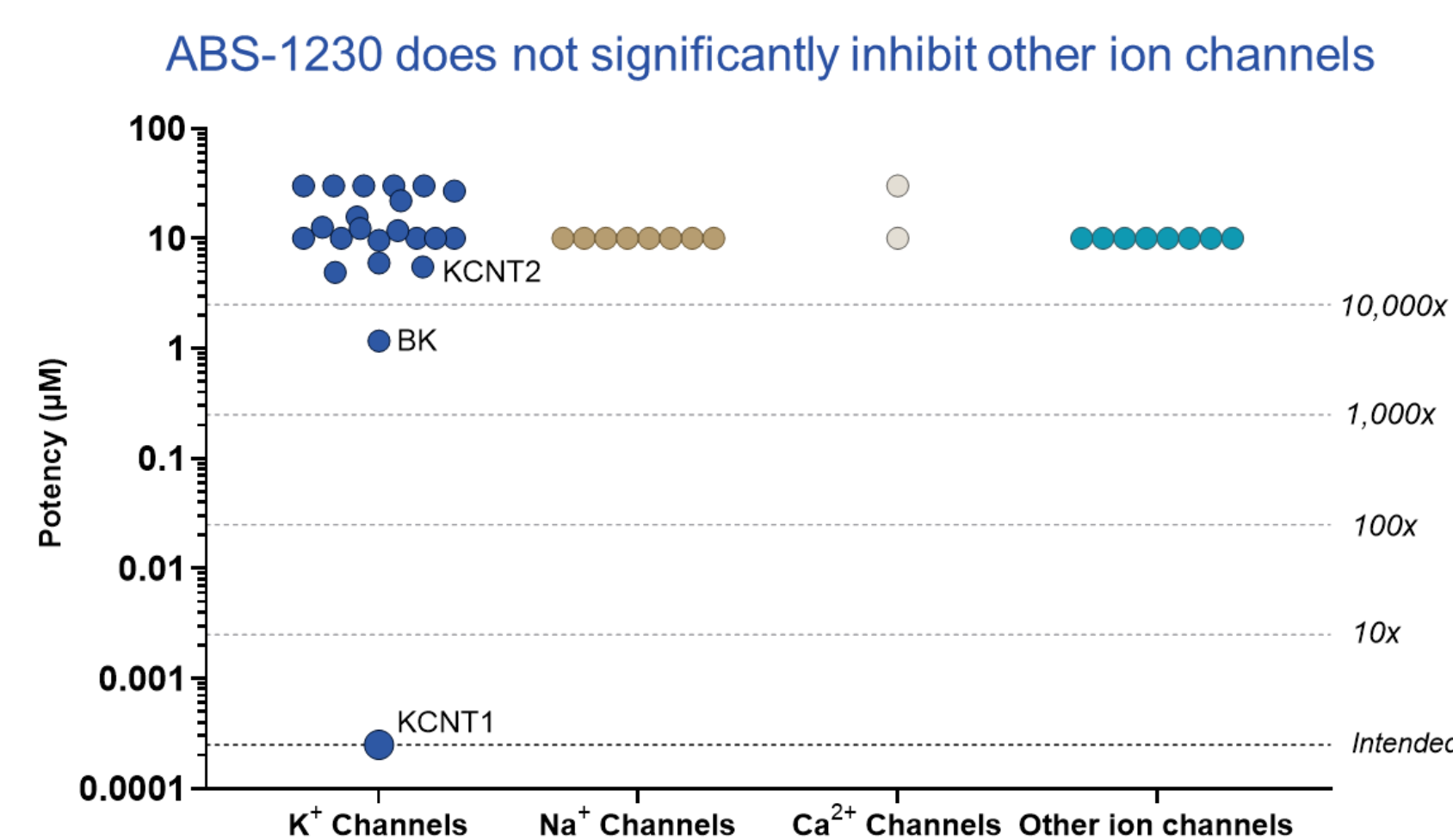


Figure 3. ABS-1230 demonstrated high selectivity for *KCNT1*

The off-target binding and activity of ABS-1230 was evaluated in a series of commercial panels encompassing ion channels, GPCRs, transporters, kinases and other enzymes. Right, data is shown for Ion Channels. Ion channel screening was conducted by patch clamp electrophysiology or FLIPR assays performed at Charles River and Metron Biosciences. Additional screening panels included the Cerep CNS SafetyScreen panel (49 CNS safety related targets, Eurofins), SafetyScan47 Panel (Eurofins), KinSight Kinome Profile (Assay Quant). At a concentration of 1µM, ABS-1230 did not display significant (>30%) activity against any protein assayed other than *KCNT1*.

Results (2)

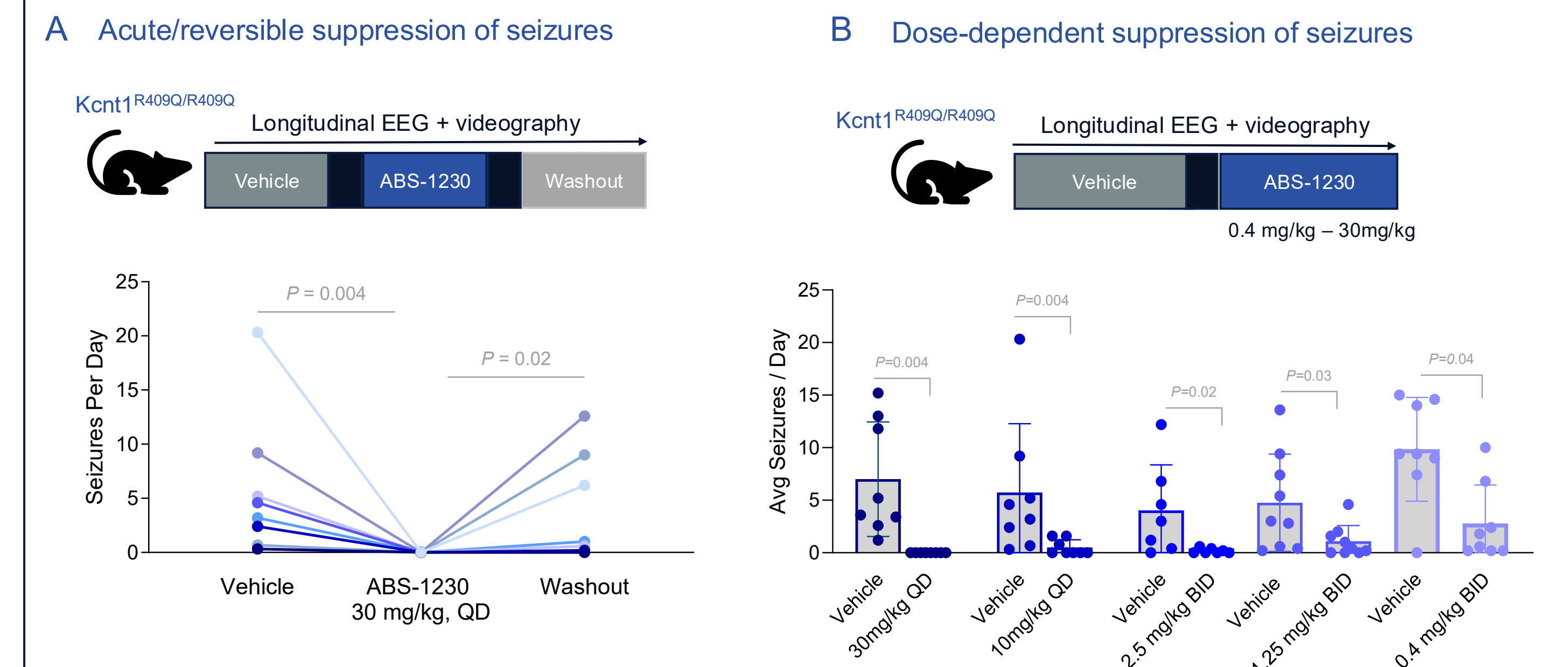


Figure 4. ABS-1230 demonstrated seizure control in *Kcnt1*^{R409Q/R409Q} mice

Spontaneous seizures were assessed at ~ 8 weeks of age by longitudinal EEG and videography. *Kcnt1*^{R409Q} is equivalent to human *KCNT1*^{R428Q}, a recurrent mutation associated with DEE and epilepsy of infancy with migrating focal seizures A) *Kcnt1*^{R409Q/R409Q} mice were treated with vehicle for 3-5 days followed by treatment with ABS-1230 at 30 mg/kg once daily by intraperitoneal injection for 5 days, followed by a 14-day drug washout period and another 5-day recording period. The average number of seizures per day is shown over each period, each line representing a different mouse (n=8 mice). B) *Kcnt1*^{R409Q/R409Q} mice were treated with vehicle and then different doses of ABS-1230, once daily of twice daily.

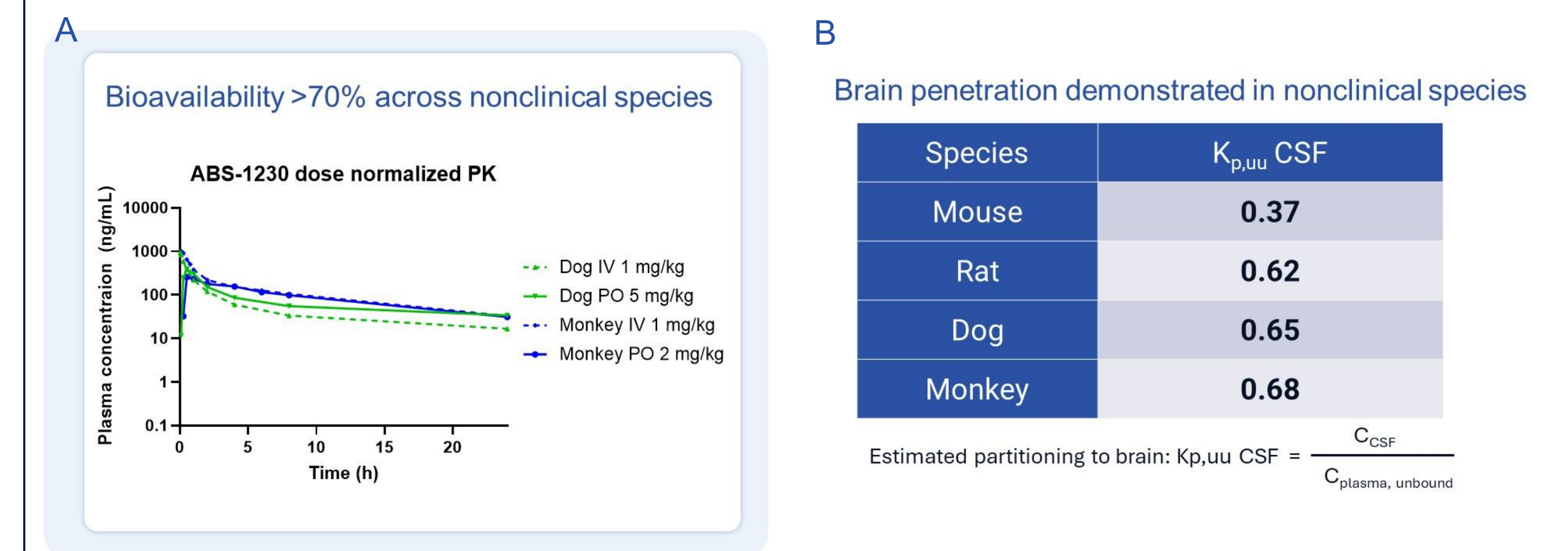


Figure 5. ABS-1230 demonstrated high oral bioavailability and CNS penetration in preclinical species

A) ABS-1230 was administered orally (PO) or intravenously (IV) at the indicated doses and plasma concentrations were analyzed at different time points post-dosing. Plasma concentrations were normalized to the dose given to enable direct comparison of PO and IV exposures and determination of oral bioavailability. B) CNS penetration was determined across different species by comparing drug concentrations in CSF to protein unbound levels in plasma.

Summary

- *KCNT1*-related epilepsy is a devastating DEE that lacks effective treatment options
- ABS-1230 demonstrated to be a potent and selective inhibitor of *KCNT1* and pathogenic mutations that reduce seizures in *Kcnt1*^{R409Q} mice
- ABS-1230 had high oral bioavailability and CNS penetration in preclinical species
- Phase 1a healthy participant trial in progress with favorable PK and exposure observed, a Phase 1b/2a first-in-patient trial planned for 2026

Acknowledgements

- *KCNT1* Epilepsy Foundation and *KCNT1* Families **KCNT1 EPILEPSY**
HOPE IS ON THE HORIZON

References

1. Shore et al., 2020 Cell Reports 33, 108303.
2. Biton et al., 2012 J. Pharmacol Exp Ther. 230(3): 706-15.