Robust hippocampal and cortical target engagement induced by ABX-002, a novel thyromimetic in development for major depressive disorder (MDD)

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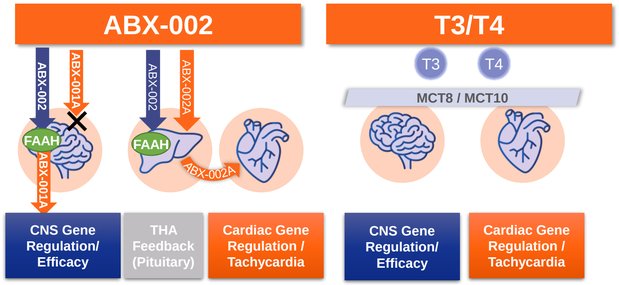
Introduction

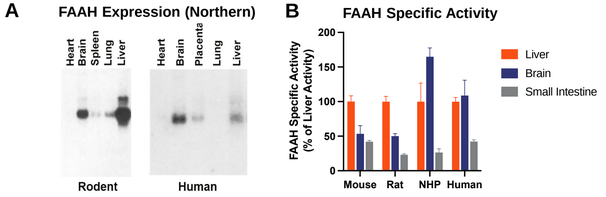
- Thyroid hormone (T_3) and thyroxine (T_4) are both used to treat depression typically as augmentation therapy. Dose is often limited by known tolerability limitations of thyroid hormone (e.g., cardiac effects).
- ABX-002 enhances brain delivery of a thyromimetic through the use of fatty acid amide hydrolase (FAAH) activated mechanisms. FAAH is highly expressed in the CNS and prodrugs enhance delivery [1,2]



Objectives of this work

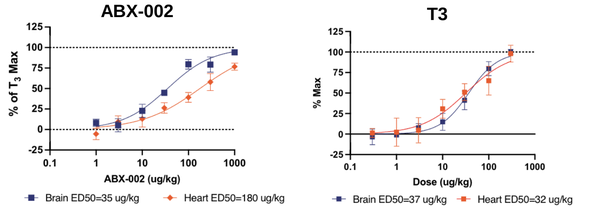
- Compare ABX-002 to T_3 in mouse for brain vs. heart target engagement (TE)
- Assess brain subregions to confirm activity in cortex & hippocampus → 2 regions involved in depression
- Evaluate brain & cardiac TE in NHP as a bridge to humans

FAAH Expression Across Species



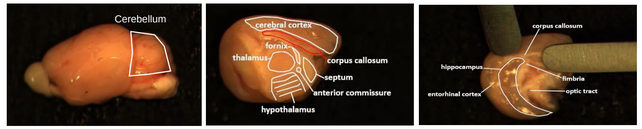
(A) Relative mRNA expression[3,4] & (B) Tissue FAAH activity (cleavage of AMC) in mouse, rat, NHP & human S9 fractions from different tissues

Tissue Target Engagement in Mouse



Gene expression in brain (blue) & heart (orange) after a single administration of ABX-002 (left) or T_3 (right) to C57Bl/6 mice. Hemibrains were harvested 4 or 8 hr after the dose and RNA analyzed by custom Nanostring panel. Graphs represent the average fold change on a $\log_2$ scale for 6 genes in the brain (*Dio3, Hr, Klf9, Sfrp5, Robo3, Flywch2*) and 4 genes in the heart (*Hr, Ucp3, Ppard, Abcd2*).

ABX-002 has a greater window b/t brain & heart TE than T_3 in the mouse



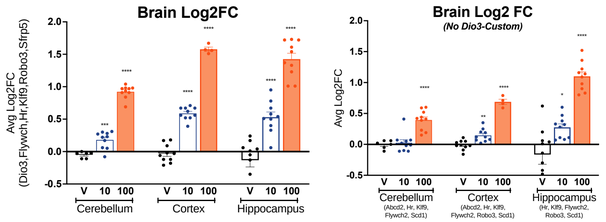
To investigate which subregions within the brain resulted in meaningful changes in gene expression, female C57BL/6 mice were dosed with vehicle, 10 or 100 μg/kg ABX-002 for 7 days. Brains were harvested 4 hrs after the last dose. Cerebellum was removed first with cortical and hippocampal enriched samples harvested as shown. RNA was analyzed using a custom Nanostring plexset panel. *Dio3* levels were BLOQ, so were analyzed separately by qPCR. Table lists the baseline counts in each region followed by the fold change for each gene compared with vehicle.

***Dio3* was the most sensitive gene induced by ABX-002 treatment**

	Cerebellum			Cortex			Hippocampus		
Gene	Baseline (counts)	Fold Change vs.V 10 μpk	Fold Change vs.V 100 μpk	Baseline (counts)	Fold Change vs.V 10 μpk	Fold Change vs.V 100 μpk	Baseline (counts)	Fold Change vs.V 10 μpk	Fold Change vs.V 100 μpk
Dio3	<50	6.9****	22****	<50	3.0****	39****	<50	4.3****	8.2****
Hr	2280 ± 170	1.1	1.7***	800 ± 17	1.3***	2.6 ****	432 ± 93	1.3	3.0****
Klf9	8000 ± 530	0.95	0.79	8680 ± 270	1.0	1.2***	772 ± 174	0.89	1.6*
Abcd2	865 ± 38	0.97	0.65**	741 ± 13	1.0	1.3****	BLOQ		
Sfrp5	105 ± 19	1.0	1.3	BLOQ			BLOQ		
Flywch2	697 ± 26	0.90	0.97	447 ± 21	1.1	1.5****	297 ± 20	1.5**	2.1****
Robo3	BLOQ			198 ± 11	1.2	2.5****	134 ± 19	1.5*	3.5****
Scd1	1290 ± 96	1.0	1.2	812 ± 27	1.1	1.2*	584 ± 32	1.2*	1.4****

* p<0.05,, **p<0.01, *** p<0.001, **** p<0.0001 by ANOVA w/Dunnet's post hoc test

Tissue Target Engagement in Mouse

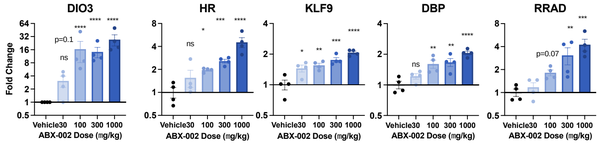


The fold change for each gene relative to vehicle-treated mice (on a $\log_2$ scale) was averaged to provide a single metric. In left panel, the same genes as used in the hemibrain studies were analyzed. In the right panel, *Dio3* was omitted since it can dominate the average & only measurable genes were included.

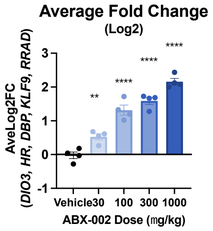
Hippocampus & cortex were more sensitive than cerebellum to thyromimetic stimulation by ABX-002 in the mouse

Tissue Target Engagement in NHP

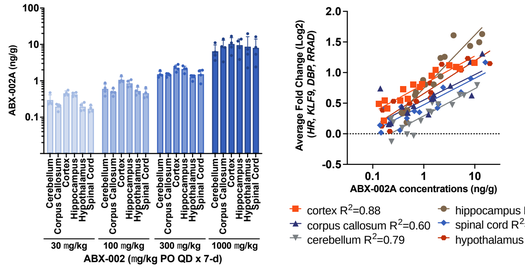
- To evaluate the effects of ABX-002 in a nonhuman primate, cynomolgus monkeys were dosed with QD PO ABX-002 (n=4/group) for seven days and samples harvested 8 hrs after last dose. Six CNS regions were harvested along with heart, liver and pituitary.
- In a pilot study, RNA sequencing was performed & key genes identified as T_3 responsive in NHP brain (not shown). *DIO3, HR, KLF9, DBP & RRAD* were probed with custom Nanostring panel in this study.
- In heart, RNA sequencing was inconclusive, so genes validated in rodents as relevant for cardiac phenotype were assessed (*MYH6, MYH7A, ATP2A2*)



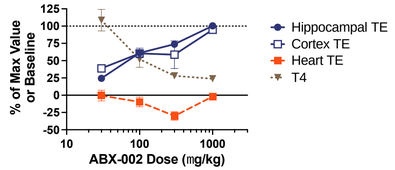
Dose-related target gene induction was observed in all regions for all genes probed with example data for hippocampus shown above. *DIO3* was most sensitive gene in all regions with up to 16x induction observed. Effects on multiple genes were combined into the average fold change (in $\log_2$ space). Since *DIO3* may dominate the metric, values were calculated both with and without that gene.



Tissue Target Engagement in NHP



Tissue concentrations of active metabolite (ABX-002A) were measured in the different regions of the CNS (left) and correlated with gene expression measured from each sample. **Strong TE was observed in all regions with cortex & hippocampus having greatest sensitivity & slightly better delivery than other regions.** Cerebellum had the least gene induction.



No effect on cardiac genes (*MYH6 MYH7A* or *ATP2A2* -- genes known to be involved in cardiac phenotype observed in hyperthyroidism). At 300 μg/kg those genes may be decreasing slightly, likely associated with pituitary-driven effects on the thyroid hormone axis.

Conclusions

- ABX-002 induces T_3-responsive gene expression in brains of both mice & NHP; cortex & hippocampus being most sensitive regions.
- ABX-002 enhances window between brain TE & cardiac TE in both mice & NHP with interspecies differences consistent with differences in FAAH expression.
- These data provide pharmacologic support for possible follow-on human studies in depressive disorders.

References

[1] Meinig et al., ACS Med Chem Lett 2019, 10, 111-116
[2] MacKenna et al., 73rd Annual Mtg of AAN, 2021, 1065-P
[3] Cravatt et al., Nature 1996 384, 83-87
[4] Giang & Cravatt, PNAS 1997, 94, 2238-2242
[5] EJN, https://www.youtube.com/watch?v=Upf15CB29V4

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