Pharmacokinetic Details

We examined the effect of a single oral dose of 30 or 60 mg/kg JNJ-49146981 on the *in vivo* production of Aβ in brain in non-transgenic Swiss mice or APPPS1-21 mice (4 weeks of age) after oral treatment (n = 6 per time point) Mice were killed by decapitation and blood was collected in EDTA-treated collection tubes. Blood samples were placed immediately on ice and plasma was obtained following centrifugation at 4°C for 10 min at ~1900 g. Samples were stored at -18°C prior to pharmacokinetic analysis. Brains were excised and olfactory lobe and hindbrain were removed. Brain hemispheres were weighed, immediately frozen on dry ice and stored at -20°C or -80°C prior to pharmacokinetic (left hemisphere) or Aβ (right hemisphere) analysis, respectively. Homogenization of brain samples and subsequent analysis of Aβ and compound levels have been described in detail previously (*1*). Results demonstrated that a single oral dose of 60 mg/kg JNJ-49146981 produced a potent reduction of rodent Aβ1-40 levels in the brain compared to vehicle-treated mice at 4 and 7 hours after treatment in wild-type mice (Table 1). 24 hours after treatment, rodent Aβ1-40 levels returned to vehicle values. As it is known from previous studies that the presence of the APP-Swedish mutation reduces potency of BACE inhibitors (*2,3*), reduction in Aβ levels following a single oral dose of 30 mg/kg was compared in WT and APPPS1-21 mice 2 hours after treatment. Rodent Aβ1-40 levels were reduced to a similar extent (average 71% and 65% reduction in WT and APPPS1-21 mice, respectively). Human Aβ1-40 levels in APPPS1-21 mice were reduced to a lesser but still substantial extent (average 49% reduction). Compound levels in brain and plasma were comparable between WT and transgenic mice in this study (brain: average 1031 ± 246 and 840 ± 156 ng/g, respectively; plasma: 663 ± 91 and 706 ± 85 ng/ml, respectively).

REFERENCES

1. Van Broeck B, Chen JM, Tréton G, et al. Chronic treatment with a novel γ-secretase modulator, JNJ-40418677, inhibits amyloid plaque formation in a

mouse model of Alzheimer's disease. *Br J Pharmacol.* 2011;163:375–389.

2. Eketjäll S, Janson J, Jeppsson F, et al. AZ-4217: a high potency BACE inhibitor displaying acute central efficacy in different in vivo models and reduced amyloid deposition in Tg2576 mice. *J Neurosci.* 2013;33:10075–10084.

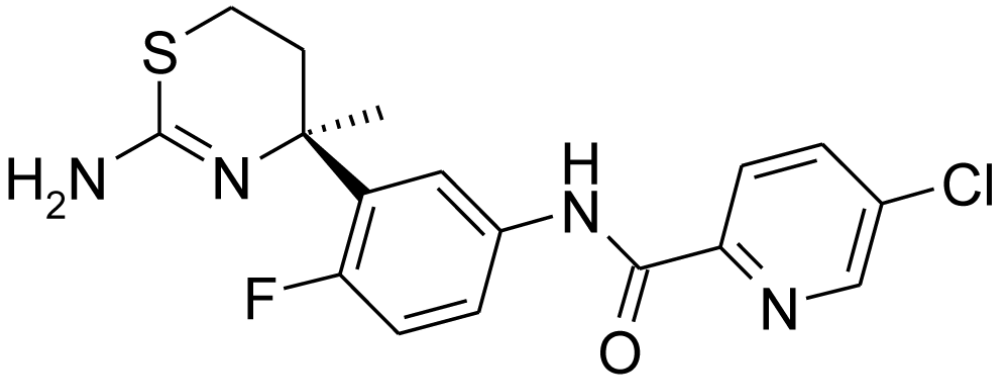
3. Yamakawa H, Yagishita S, Futai E, Ishiura S. beta-Secretase inhibitor potency is decreased by aberrant beta-cleavage location of the "Swedish mutant" amyloid precursor protein. *J Biol Chem.* 2010;285:1634–1642.

Supplemental Table 1. Pharmacokinetics of a single oral dose (60 mg/kg) of JNJ-49146981 in wild-type mice

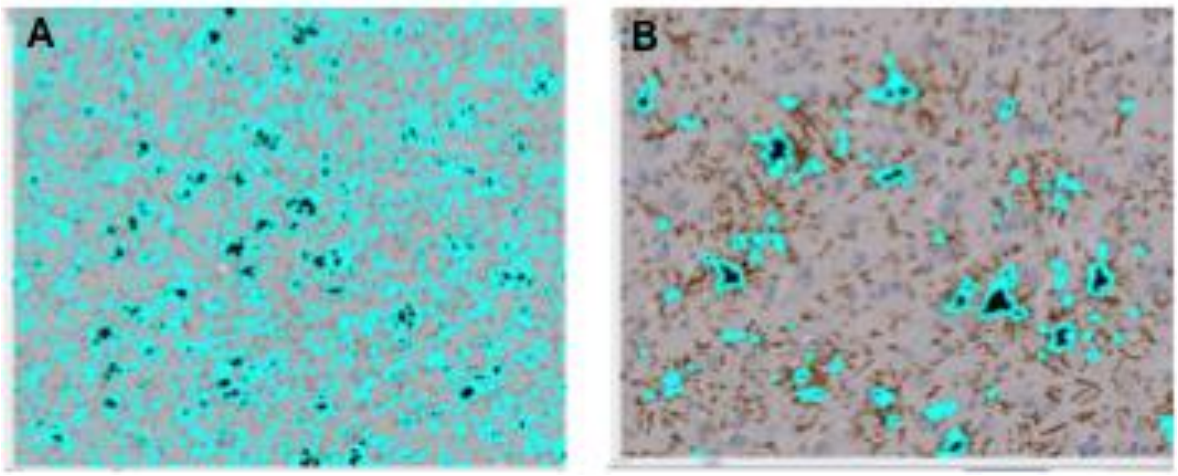
Time point	Average % reduction brain rodent Aβ1-40 concentration compared to vehicle-treated mice (wild-type)	Average JNJ-49146981 concentrations	
		Plasma (ng/ml)	Brain (ng/g)
4 hours	85	556	869
7 hours	83	520	807
24 hours	–26	13	19

Supplemental Table 2 The source and dilution of the primary antibodies used in the study

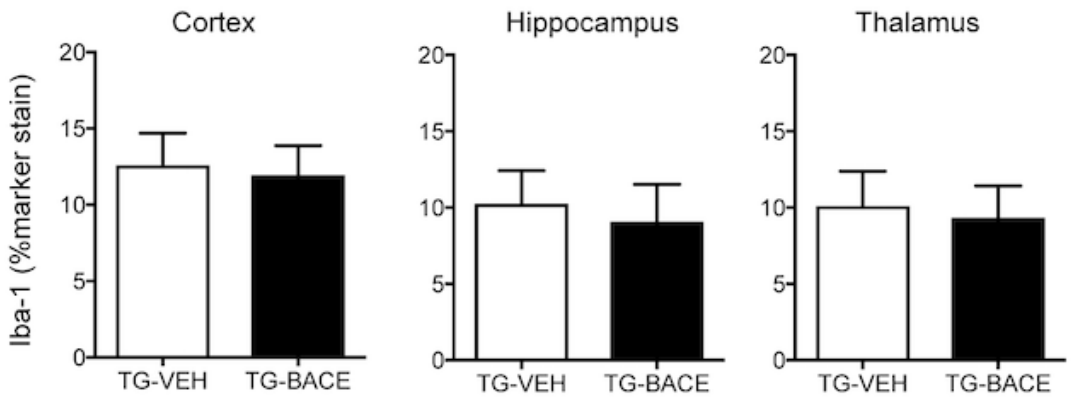
Antibody	Source	Dilution
4G8	Biolegend, San Diego, USA (SIG-39220)	1/2000
GFAP	Millipore, Temecula, USA (IF03L)	1/750
Iba1	Wako chemicals, Richmond, USA (019-19741)	1/750
NeuN	Millipore, Temecula, USA (MAB377)	1/2000



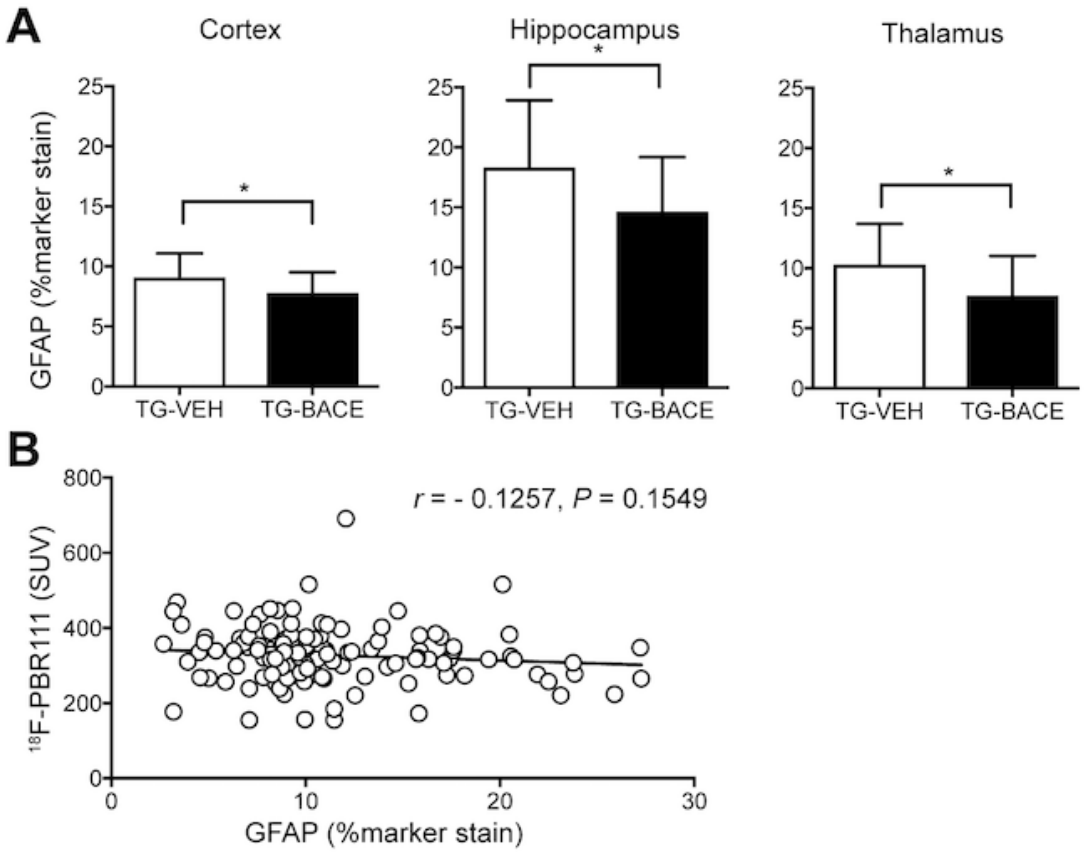
Supplemental Figure 1. Chemical structure of the BACE inhibitor JNJ-49146981



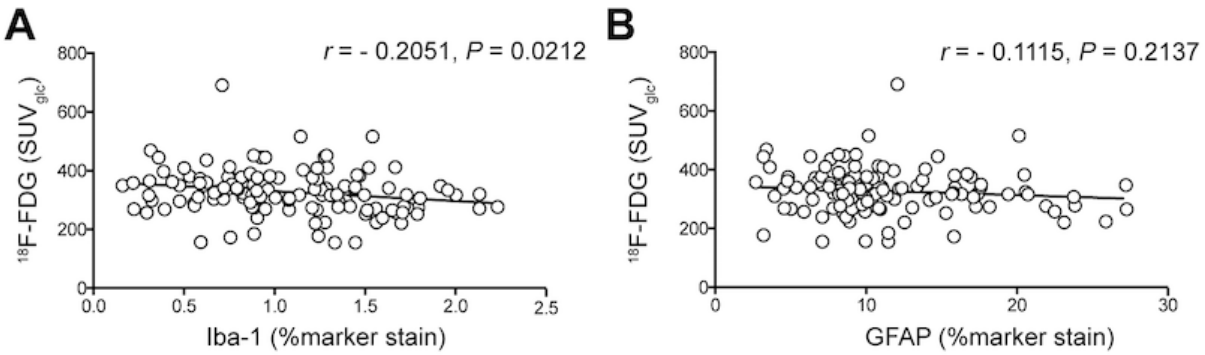
Supplemental Figure 2. Quantification of Iba-1 staining. (A) Quantification of both resting and active microglia when a low threshold is employed. (B) Quantification of activated microglia only using a higher threshold for detection.



Supplemental Figure 3. Quantification of Iba-1 staining with a low threshold. Data are presented as mean + standard deviation.



Supplemental Figure 4. Quantification of GFAP staining (A) and correlation to ^{18}F-PBR111 in TG mice (B). Data are presented as the mean + standard deviation in A.



Supplemental Figure 5. Correlation of ^{18}F-FDG uptake to Iba-1 (A) and GFAP (B) in TG mice.