The Gamma Secretase Modulator, BMS-932481, Modulates Aβ Peptides in the Plasma and CSF of Healthy Volunteers.

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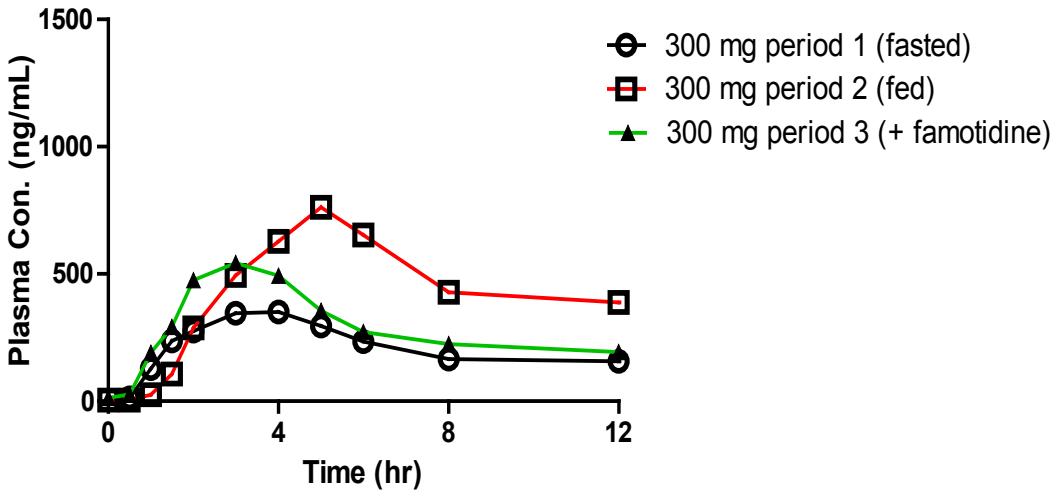
Supplementary Materials:

Supplemental Methods

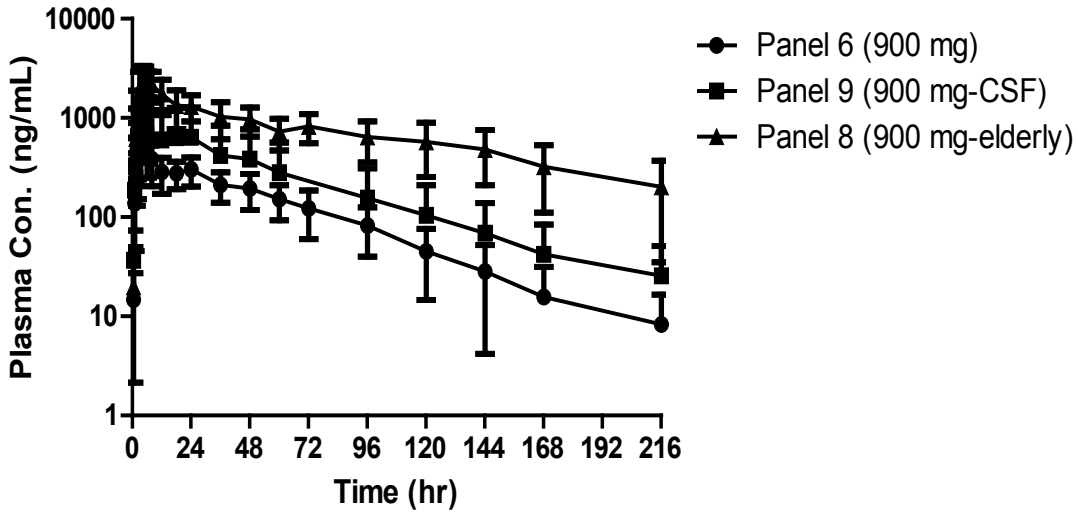
CSF Aβ peptides LC-MS/MS Supplemental methods.

Two multiplex quantitative LCMSMS assays were developed by PPD to measure Aβ peptides. One assay utilized 100µl for Aβ 14, Aβ15 and Aβ17 and used ^{15}N labeled Aβ15 used for Aβ14 and Aβ15 quantitation and ^{15}NAβ17 for Aβ17 quantitation. The other assay used 100ul for APL1-β28, Aβ34, Aβ37, Aβ38, Aβ39, Aβ40, Aβ42 and Aβ43. Corresponding ^{15}N labeled peptides were used for quantitation. Following addition of 15N labeled peptides to samples, samples were extracted with 100µL 5M guanidine HCl and mixed for 45 minutes at room temperature. Samples were then diluted with 100µl of 4% H_3PO_4 and subjected to solid phase extraction using a waters Oasis® MCX uElution 96 well plate. After sequential washes with 4% H_3PO_4 and 10% acetonitrile, peptides were eluted with ACN/water/conc NH_4OH (75:15:10, v/v/v) and extract diluted with 60µL H_20 (total vol 120µL) to reduce organic solvent content while maintaining analytes solubility. 50uL was injected onto LCMS/MS system (CTC LC-PAL

autosampler, agilent 1200 series LC binary pumps, AB Sciex API 5000 triple quandrupole LC-MS/MS with electrospray positive ionization (+ESI) multiple reaction monitoring (MRM). Analytes were separated under high pH NH_4OH-based reversed phase chromatographic conditions intended to minimize non-specific binding and aggregation. Waters ACQUITY UPLC® BEH300C18, 2.1x150mm,1.7µm) analytical columns were utilized for separation. Mobile phases for short Aβ peptide assay was H_20/ACN/NH_40H (98:2:0.1, v/v/v) followed by ACN/MeOH/IPA/H_2O (65:35:10:5, v/v/v/v) and for hydrophobic Aβ assay was 0.1% NH_40H in water and ACN/MeOH/TFE (70:25:5, v/v/v). Analyte MW, precursor ion, m/z charge, product ion, m/z charge, ion type for each of the peptides were as follows: **Aβ1-14** 1699, 567.1, +3m, 156.1, +1, y1; **Aβ1-15** 1827, 609.8, +3m, 284.0, +1, y2; **Aβ1-17** 2068, 690.3, +3, 646.4, +3, b2; **Aβ1-34** 3787, 947.8, +4, 1162.9, +3, b31; **APL1β28** 2586, 862.8, +3, 245.1, +1, b2; **Aβ1-38** 4132, 1033.9, +4, 1000.8, +4, b36; **Aβ1-40** 4330, 1083.6, +4, 1054.2, +4, b39; **Aβ1-42** 4514, 1129.4, +4, 1078.8, +4, b40; ***Aβ1-43*** *4615, 1154.8,* +4, *1124.9,* +4, *b42*

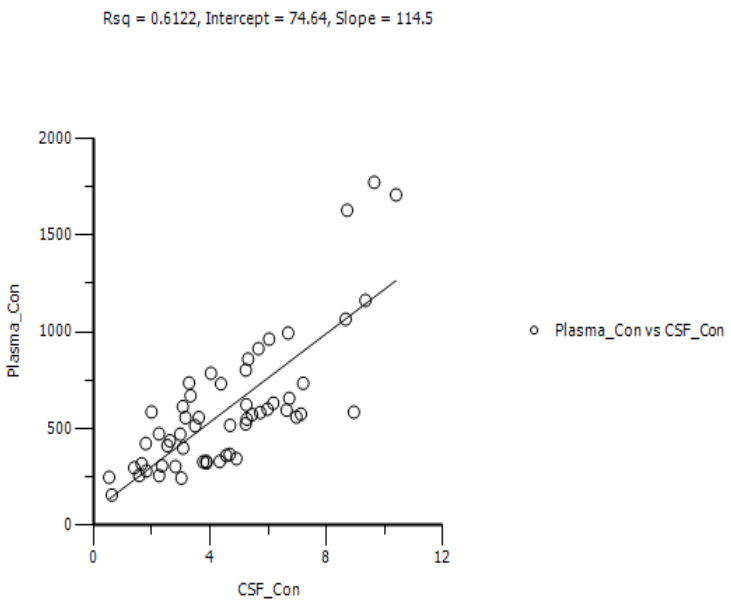


Supplemental Figure 1S. Effects of high fat coloric diet (fed) and 40 mg famotidine administration on plasma exposure.

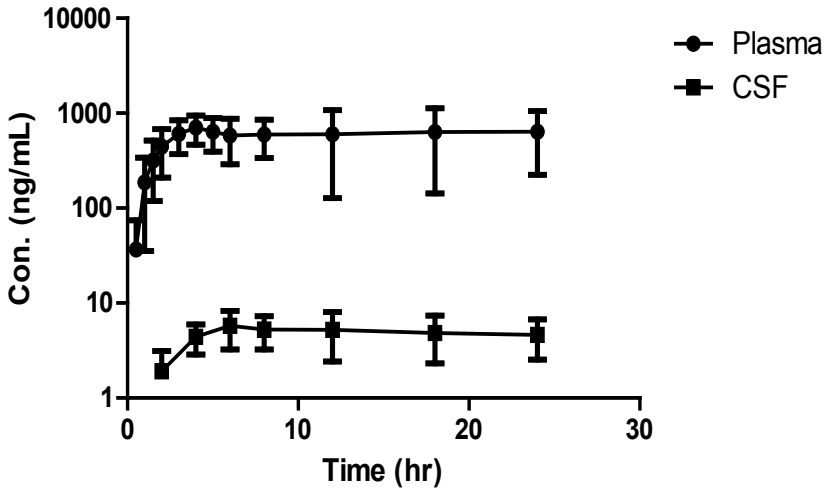


Supplemental Figure S2: Plasma exposures in healthy young (Panel 6 and 9) and elderly (panel 8) at 900 mg.

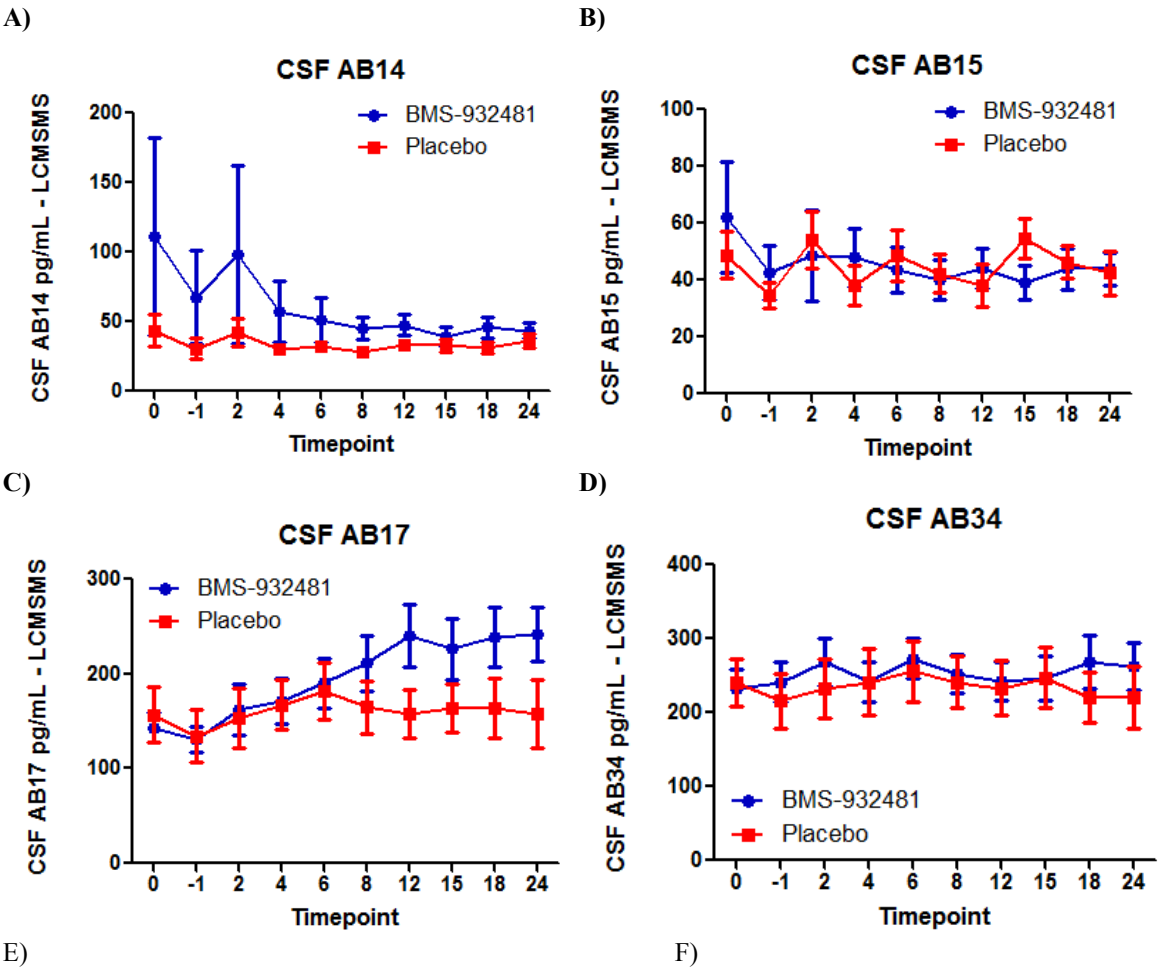
A)



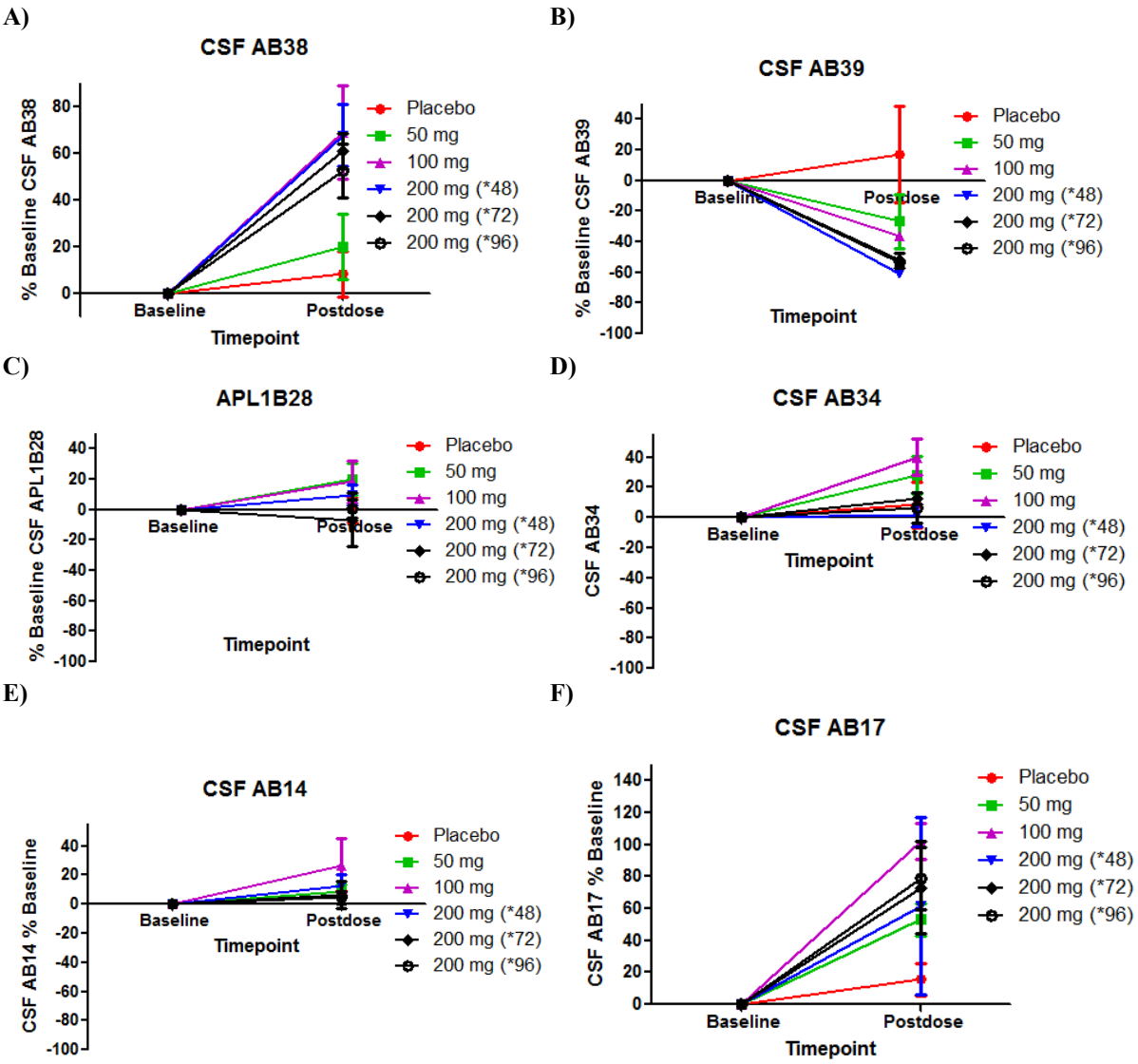
B)



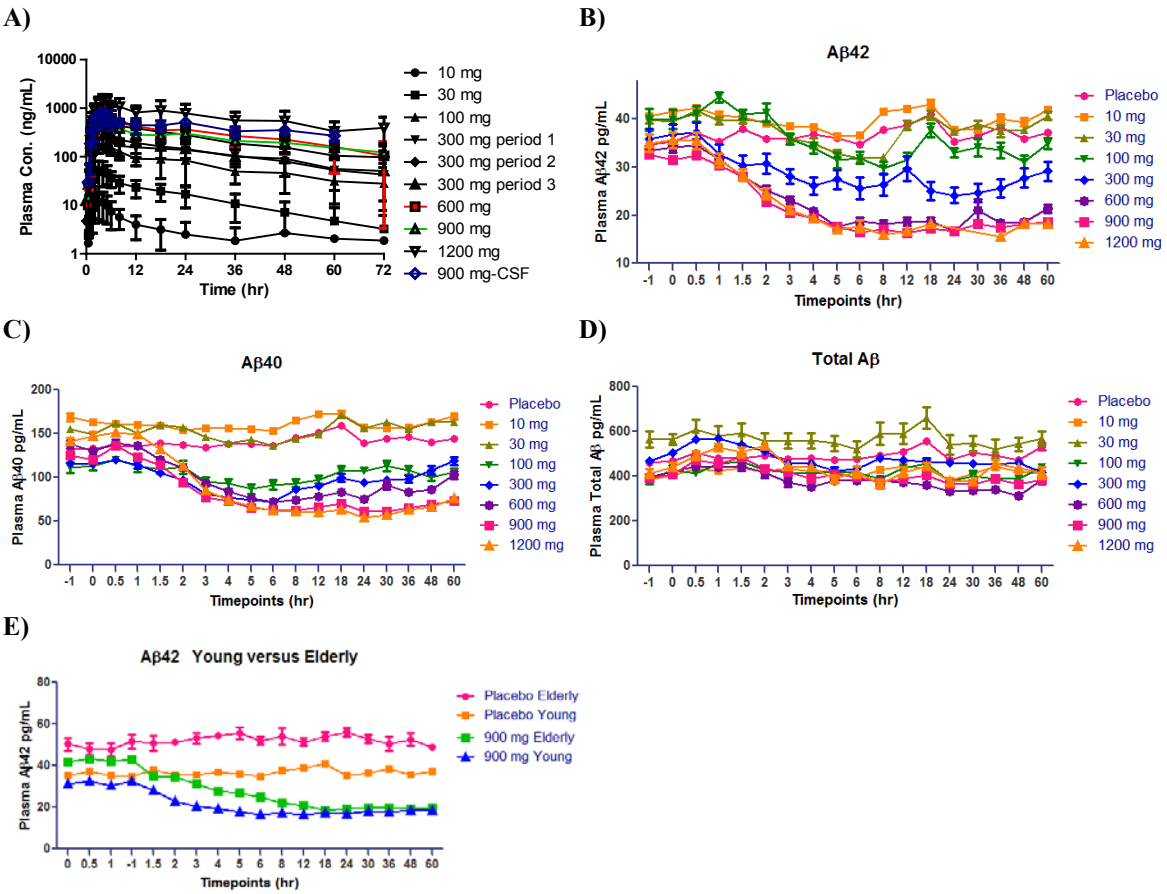
Supplemental Figure S3: Comparison of plasma and CSF exposures from Panel 9, 900mg healthy young cohort. (A) Plasma versus CSF concentrations ng/mL (B) Plasma and CSF concentrations over the first 24 hours following a single dose administration.



Supplemental Figure S4: Effect on CSF (A) Aβ14, (B) Aβ15, (C) AB17 and (D) AB34 E) AB39 following either 900mg BMS-932481 or placebo administration in the SAD study

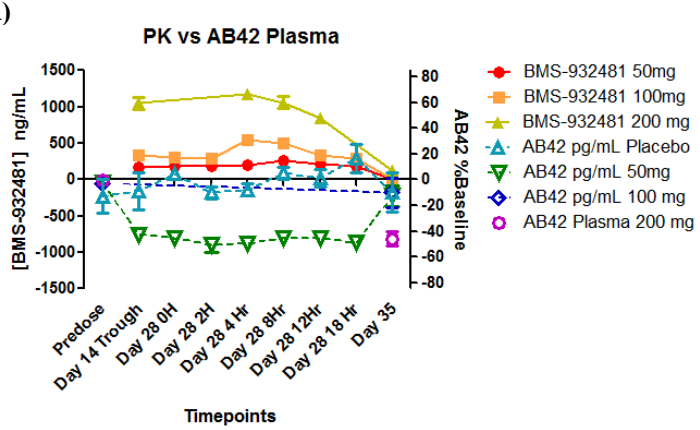


Supplemental Figure S5: Pharmacodynamic effects on CSF Aβ peptide fragments following multiple dose administration in healthy young volunteers. A) Aβ38, B) Aβ39, c) APL1β28, D) Aβ34, E) Aβ14, F) Aβ17

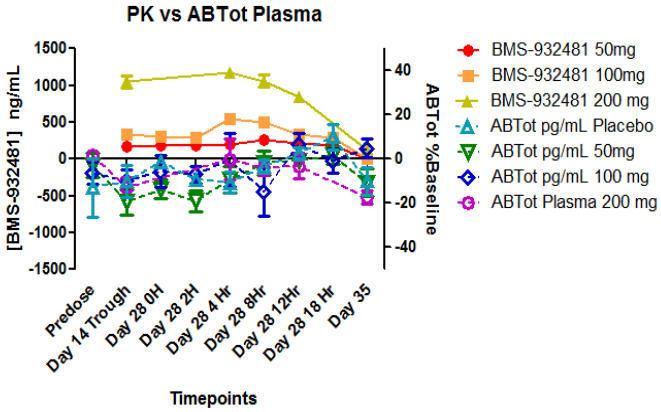


Supplemental Figure S6: Timecourse of BMS-932481 and pharmacodynamic effects on plasma Aβ following single dose administration of BMS-932481. Timecourse of (A) BMS-932481 drug levels (B) Aβ42 in healthy young and (C) Aβ40 in healthy young, (D) Total Aβ in healthy young and (E) Aβ42 in young vs. elderly. Values shown as mean + standard error of the mean.

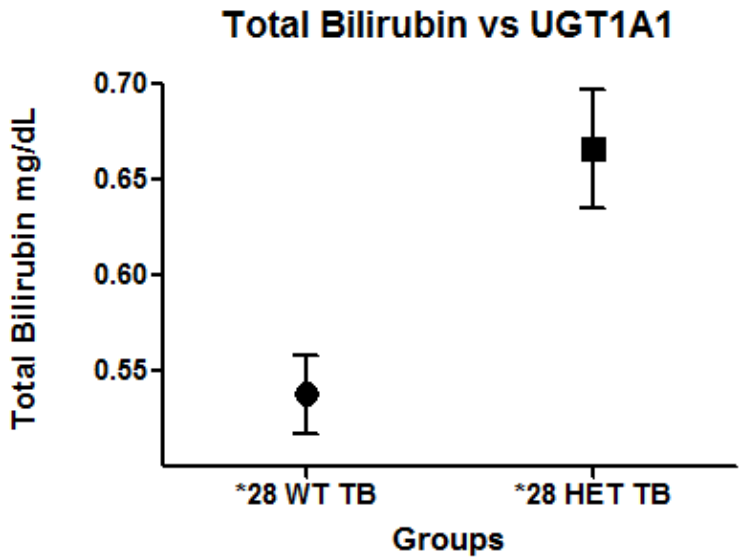
A)



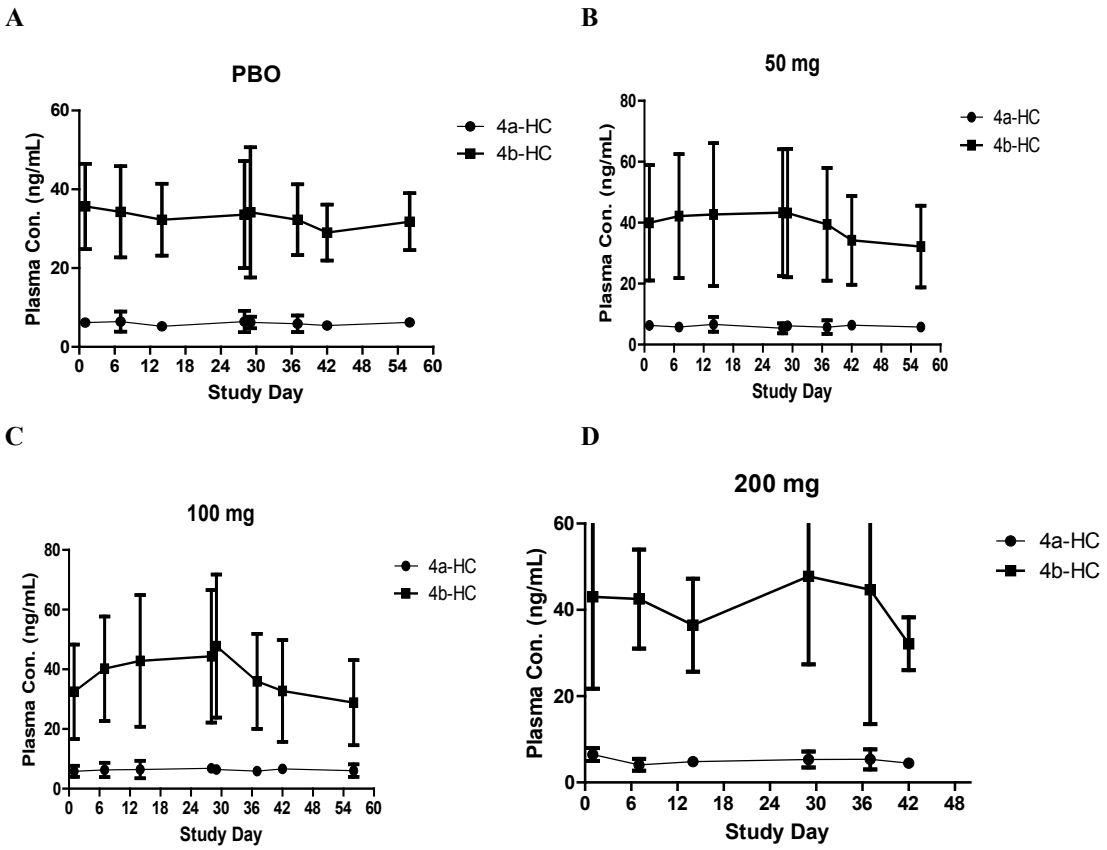
B)



Supplemental Figure S7: Timecourse of BMS-932481 drug levels and pharmacodynamic effects of BMS-932481 on plasma Aβ following multiple doses. (A) Aβ42 and (B) Total Aβ. Note, plasma Aβ42 level were below the limit of quantitation in the 100mg and 200mg dose cohorts for most of the post-dose observation timepoints.



Supplemental Figure 8S: Total Bilirubin levels in UGT1A1 *28 heterozygotes and wildtype carriers.



Supplemental Figure9S: 4 Beta and alpha hydroxycholesterol levels in BMS-932481 treated subjects following multiple dose administration. (A) Placebo, (B) 50 mg, (C) 100mg and (D) 200 mg.

Supplemental Table S1: Baseline CSF Aβ42 and Plasma Aβ42 vs ApoE genotoype.

ApoE Genotype	CSF Aβ42 Mean + STD (34)	Plasma Aβ42 Mean + (STD)
ε2ε2	0	53 (1)
ε2ε3	423 ± 154 (4)	43 + 10 (15)
ε2ε4	633 (1)	48 + 1 (2)
Total ε2	465 + 164 (5)	44 + 10 (18)*
ε3ε3	477 ±159 (21)	36 + 10 (57)
ε3ε4	503 ± 215 (8)	37 + 10 (23)
ε4ε4		23 (1)
Total ε4	503 + 215 (8)	36 + 10 (24)

*P<0.05 compared to total E3 and E4