

Robust Translation of γ -Secretase Modulator Pharmacology across Preclinical Species and Human Subjects[§]

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ABSTRACT

The amyloid- β peptide ($A\beta$)—in particular, the 42-amino acid form, $A\beta_{1-42}$ —is thought to play a key role in the pathogenesis of Alzheimer's disease (AD). Thus, several therapeutic modalities aiming to inhibit $A\beta$ synthesis or increase the clearance of $A\beta$ have entered clinical trials, including γ -secretase inhibitors, anti- $A\beta$ antibodies, and amyloid- β precursor protein cleaving enzyme inhibitors. A unique class of small molecules, γ -secretase modulators (GSMs), selectively reduce $A\beta_{1-42}$ production, and may also decrease $A\beta_{1-40}$ while simultaneously increasing one or more shorter $A\beta$ peptides, such as $A\beta_{1-38}$ and $A\beta_{1-37}$. GSMs are particularly attractive because they do not alter the total amount of $A\beta$ peptides produced by γ -secretase activity; they spare the processing of other γ -secretase substrates, such as Notch; and they do not cause accumulation of the potentially toxic processing intermediate, β -C-terminal fragment. This report describes the translation of pharmacological activity across species for two

novel GSMs, (S)-7-(4-fluorophenyl)-N2-(3-methoxy-4-(3-methyl-1H-1,2,4-triazol-1-yl)phenyl)-N4-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine-2,4-diamine (BMS-932481) and (S,Z)-17-(4-chloro-2-fluorophenyl)-34-(3-methyl-1H-1,2,4-triazol-1-yl)-16,17-dihydro-15H-4-oxa-2,9-diaza-1(2,4)-cyclopenta[d]pyrimidina-3(1,3)-benzenacyclonaphan-6-ene (BMS-986133). These GSMs are highly potent in vitro, exhibit dose- and time-dependent activity in vivo, and have consistent levels of pharmacological effect across rats, dogs, monkeys, and human subjects. In rats, the two GSMs exhibit similar pharmacokinetics/pharmacodynamics between the brain and cerebrospinal fluid. In all species, GSM treatment decreased $A\beta_{1-42}$ and $A\beta_{1-40}$ levels while increasing $A\beta_{1-38}$ and $A\beta_{1-37}$ by a corresponding amount. Thus, the GSM mechanism and central activity translate across preclinical species and humans, thereby validating this therapeutic modality for potential utility in AD.

Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia, placing a huge burden on patients, their families, and caregivers (Wimo and Prince, 2010). Treatments for

symptoms are available, but have limited benefit, and do not prevent or slow the progression of AD (Prince et al., 2011). The causes of AD are not fully understood, but evidence from human genetics, brain pathology, and experimental models has converged on the amyloid hypothesis (Hardy and Selkoe, 2002). This hypothesis implicates the accumulation of amyloid- β peptide ($A\beta$) in the brain, particularly the neurotoxic 42-amino acid form, $A\beta_{1-42}$, as a key factor in the disease

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ABBREVIATIONS: $A\beta$, amyloid- β peptide; ABEC, area between the $A\beta$ vehicle-dosed baseline and the $A\beta$ effect curve; AD, Alzheimer's disease; APP, amyloid- β precursor protein; AUC, area under the concentration-time curve; BMS-299897, (R)-4-(2-((4-chloro-N-(2,5-difluorophenyl)phenyl)sulfonamido)ethyl)-5-fluorophenyl)butanoic acid; BMS-698861, (R)-2-((4-chloro-N-(2-fluoro-4-(1,2,4-oxadiazol-3-yl)benzyl)phenyl)sulfonamido)-3-cyclopropylpropanamide; BMS-932481, (S)-7-(4-fluorophenyl)-N2-(3-methoxy-4-(3-methyl-1H-1,2,4-triazol-1-yl)phenyl)-N4-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine-2,4-diamine; BMS-986133, (S,Z)-17-(4-chloro-2-fluorophenyl)-34-(3-methyl-1H-1,2,4-triazol-1-yl)-16,17-dihydro-15H-4-oxa-2,9-diaza-1(2,4)-cyclopenta[d]pyrimidina-3(1,3)-benzenacyclonaphan-6-ene; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; FAD, familial AD; GSI, γ -secretase inhibitor; GSM, γ -secretase modulator; MS-MS, tandem mass spectrometry; NSAID, nonsteroidal anti-inflammatory drug; PK/PD, pharmacokinetics/pharmacodynamics.

(Findeis, 2007). A major therapeutic objective has therefore been to inhibit the synthesis of, neutralize, or clear $A\beta$ from the brain. Direct pharmacological targets include the proteolytic enzymes, amyloid- β precursor protein cleaving enzyme and γ -secretase, responsible for $A\beta$ production by cleavage of the amyloid- β precursor protein (APP; Karran et al., 2011), and $A\beta$ itself using anti- $A\beta$ antibodies (Karran, 2012). However, success in the clinic has been elusive, potentially because the drugs so far have had an insufficient impact on $A\beta$ -lowering at clinically tolerable doses (Toyn and Ahljianian, 2014).

γ -Secretase modulators (GSMs) are small molecules that are of particular interest because they selectively decrease $A\beta$ 1-42 production. GSMs bind to presenilin, the catalytic subunit of γ -secretase (Crump et al., 2011; Ebke et al., 2011; Ohki et al., 2011; Jumpertz et al., 2012; Pozdnyakov et al., 2013), and alter the amino acid positions at which substrate proteolysis takes place, resulting in the generation of a greater proportion of shorter $A\beta$ peptides, such as $A\beta$ 1-38 and $A\beta$ 1-37, relative to the longer peptides $A\beta$ 1-42 and $A\beta$ 1-40. An attractive feature of GSMs is that their effect on $A\beta$ peptides is essentially opposite that of presenilin mutations that cause familial AD (FAD). In presenilin FAD mutants, the proportion of $A\beta$ 1-42 relative to the levels of other $A\beta$ peptides is increased, and is associated with earlier age of onset (Duering et al., 2005; Kumar-Singh et al., 2006; Okochi et al., 2013). Thus, GSMs shift $A\beta$ peptide production in a direction opposite that of FAD, implying that GSMs have the potential to delay onset of dementia.

GSM activity was originally described for several nonsteroidal anti-inflammatory drugs (NSAIDs; Weggen et al., 2001). Subsequently, many other small molecules and natural products have been reported to exhibit selective lowering of $A\beta$ 1-42 (for review, see Tate et al., 2012). In general, the NSAIDs and their derivatives decrease $A\beta$ 1-42 and increase $A\beta$ 1-38, while having no effect on $A\beta$ 1-40 production. Other GSMs, with chemical structures unrelated to the NSAIDs (Caldwell et al., 2010; Kounnas et al., 2010; Wan et al., 2011a,b; Borggaard et al., 2012; Tate et al., 2012; Yu et al., 2014), decrease $A\beta$ 1-40 in addition to $A\beta$ 1-42, while simultaneously increasing one or more of the shorter peptides $A\beta$ 1-37, $A\beta$ 1-38, or $A\beta$ 1-39. The common element of all GSMs is that they decrease $A\beta$ 1-42 without inhibition of overall $A\beta$ 1-x production. GSMs have two advantages that derive from the lack of γ -secretase inhibition: first, they avoid inhibition of the many other protein substrates of γ -secretase (Haapasalo and Kovacs, 2011), and second, they avoid accumulation of the potentially toxic APP C-terminal fragments in the brain (Mitani et al., 2012, 2014). Low-potency NSAID GSMs, such as flurbiprofen, were reported to lower brain $A\beta$ 1-42 in some, but not all, studies in rodents (Eriksen et al., 2003; Lanz et al., 2005; Kukar et al., 2007). However, in clinical trials, flurbiprofen (tarenflurbil) had no effect on $A\beta$ 1-42 levels in cerebrospinal fluid (CSF) even at high doses (Galasko et al., 2007). High-potency GSMs have also entered early-stage clinical trials, but effects on $A\beta$ in CSF have not been reported (Nakano-Ito et al., 2014; Yu et al., 2014). Thus, lowering of central nervous system $A\beta$ 1-42 by GSMs has not been demonstrated previously in clinical trials.

(S)-7-(4-fluorophenyl)-N2-(3-methoxy-4-(3-methyl-1H-1,2,4-triazol-1-yl)phenyl)-N4-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine-2,4-diamine (BMS-932481) and (S,Z)-17-(4-chloro-2-fluorophenyl)-

34-(3-methyl-1H-1,2,4-triazol-1-yl)-16,17-dihydro-15H-4-oxa-2,9-diaza-1(2,4)-cyclopenta[d]pyrimidina-3(1,3)-benzenacyclonaphan-6-ene (BMS-986133) are novel bicyclic pyrimidines (Supplemental Fig. 1) related to analogs that were initially identified in a high-throughput screen (Toyn et al., 2014). Here, we show that GSM mechanism and $A\beta$ -lowering activity exhibit excellent translation across species. In rats, brain and CSF pharmacodynamics were found to be very similar, and the effects on CSF $A\beta$ peptides were found to be remarkably consistent between rats, dogs, monkeys, and healthy human subjects. BMS-932481 was chosen for clinical development, and provided a robust demonstration of GSM mechanism and central activity in human subjects.

Materials and Methods

Compounds. The novel GSMs BMS-932481 and BMS-986133 were prepared at Bristol-Myers Squibb, Wallingford, CT, using methods reported in Bristol-Myers Squibb patents (Boy et al. 2014a, b). The γ -secretase inhibitors (GSIs) used for comparisons in some experiments, (R)-4-(2-(1-((4-chloro-N-(2,5-difluorophenyl)phenyl)sulfonamido)ethyl)-5-fluorophenyl)butanoic acid (BMS-299897; Barten et al., 2005) and (R)-2-((4-chloro-N-(2-fluoro-4-(1,2,4-oxadiazol-3-yl)benzyl)phenyl)sulfonamido)-3-cyclopropylpropanamide (BMS-698861; Toyn et al., 2014), have been described previously.

$A\beta$ and Notch Assays. In overview, $A\beta$ peptides were quantified using a range of different immunoassays, using antibodies that are specific for the free C-terminal amino acids of $A\beta$ 1-42, $A\beta$ 1-40, $A\beta$ 1-38, or $A\beta$ 1-37 (Toyn et al., 2014). Immunoassays for $A\beta$ 1-x used antibodies that are not selective for the free C-terminal amino acid, and therefore measured the sum of the $A\beta$ peptides including $A\beta$ 1-42, $A\beta$ 1-40, $A\beta$ 1-38, and $A\beta$ 1-37. $A\beta$ x-42 represents an immunoassay capable of detecting N-terminally truncated $A\beta$ peptides while being selective for the C-terminal amino acid at position 42. The homogeneous time-resolved fluorescence immunoassays for $A\beta$ 1-42 and $A\beta$ 1-40 in H4-APPsw cell cultures and the Notch1 and Notch3 cell culture assays have been described previously (Gillman et al., 2010). The automated multiplex assay for simultaneous quantification of $A\beta$ x-42 and $A\beta$ 1-x in H4-APPsw cell cultures used a high-throughput homogeneous time-resolved fluorescence method in 1736-well format that will be described in detail elsewhere. In brief, $A\beta$ x-42 was detected by a combination of the monoclonals 4G8 ($A\beta$ 17-24 epitope; Covance, Princeton, NJ) and 565 ($A\beta$ 42 C-terminal cleaved epitope; Bristol-Myers Squibb), and therefore represents full-length and N-terminally truncated $A\beta$ peptides that have a C-terminal amino acid corresponding to position 42. $A\beta$ 1-x was simultaneously detected in the same assay wells by a combination of the monoclonals 4G8 and 26D6 (human $A\beta$ 1-12 epitope; Bristol-Myers Squibb), and therefore represents full-length and C-terminally truncated $A\beta$ peptides. The mesoscale 3-plex and 4-plex immunoassays and the $A\beta$ 1-x enzyme-linked immunosorbent assay (ELISA) were carried out as previously described (Toyn et al., 2014). The $A\beta$ 1-x ELISA used a combination of monoclonal 4G8, with 252Q6-horseradish peroxidase conjugate for rat or 26D6-horseradish peroxidase for monkey and human. For rat brain and CSF, $A\beta$ 1-42, $A\beta$ 1-40, $A\beta$ 1-38, and $A\beta$ 1-37 were quantified using conventional singleplex ELISA assays using appropriate combinations of monoclonals and enzyme-labeled conjugates. For dog and monkey CSF, $A\beta$ 1-42, $A\beta$ 1-40, $A\beta$ 1-38, and $A\beta$ 1-37 were quantified using the mesoscale 4-plex assay. For human subjects, $A\beta$ 1-42, $A\beta$ 1-40, and $A\beta$ 1-38 were quantified using the mesoscale 3-plex assay, and $A\beta$ 1-37 was quantified by ELISA using a combination of 26D6 and an $A\beta$ 1-37-selective rabbit polyclonal antibody (antibody provided by Pankaj Mehta, New York University School of Medicine, New York, NY). Concentrations of $A\beta$ peptides were determined by fitting the immunoassay readouts against calibration curves derived from a range of dilutions of the corresponding synthetic peptides on each

assay plate using a quadratic curve fit. Results were expressed in picomolar units corrected for sample dilution. The immunoprecipitation-matrix-assisted laser desorption/ionization mass spectroscopy assays for A β 1-42, A β 1-40, A β 1-38, and A β 1-37 were carried out as previously described (Toyn et al., 2014).

BMS-932481 Analytical Methods. BMS-932481 concentrations in animal plasma and brain samples were analyzed using an ultra-performance liquid chromatography–tandem mass spectrometry (MS-MS) method. The ultra-performance liquid chromatography–MS-MS system consisted of a Waters Acquity Ultra Performance LC Sample Organizer, Solvent Manager, and Sample Manager (Waters Corporation, Milford, MA); a Waters BEH C18, 1.7 μ m, 130Å, 2.1 mm \times 50 mm column operated at 60°C; and a SCIEX API 4000 QTRAP mass spectrometer (SCIEX, Concord, Ontario, Canada). The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid, delivered at 700 μ l/min using a gradient program. The initial elution condition was 5% B, which was maintained for 0.2 minute and increased to 95% B in 0.3 minute and maintained for 0.5 minute. It was then returned to 5% B in 0.1 minute and maintained for 0.2 minute. The MS-MS analysis was performed using turbo spray under positive ion mode with the source temperature at 600°C. The capillary voltage was 5500 eV, and the collision energy was 47 eV. Mass-to-charge ratios of 447 (precursor ion) and 431 (product ion) were used for multiple reaction mode monitoring of BMS-932481. The quantitation range for BMS-932481 was 10–5000 nM. Plasma samples were deproteinized and extracted with four portions of acetonitrile.

Animals and Dosing. All experimental procedures with animals followed National Institutes of Health guidelines and were authorized by and in compliance with the policies of the Bristol-Myers Squibb Animal Use and Care Committee. Animals were housed with a 12-hour light/dark cycle and allowed free access to food and water. For pharmacokinetics/pharmacodynamics (PK/PD) in rats, 10- to 12-week-old female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were dosed with BMS-932481 (1, 5, and 10 mg/kg), BMS-986133 (2.5, 5, and 15 mg/kg), or vehicle alone by intravenous injection at 1.5 ml/kg in vehicle consisting of polyethylene glycol with an average molecular weight of 400, ethanol, and Solutol HS 15 at a ratio of 93:5:2 (w/w/w). Rats were fasted between 16 hours prior to and 4 hours after dosing. Dosing was carried out in a randomized order within each time group (randomizing vehicle group, and all BMS-932481 and BMS-986133 dose groups together). Groups of rats ($n = 4$) were euthanized by asphyxiation in CO₂ at 10 minutes, 30 minutes, and 1, 3, 7, 12, and 24 hours after dosing. Blood was collected by cardiac puncture and placed into EDTA microtainer tubes for the preparation of plasma. CSF was collected from cisterna magna by syringe, centrifuged at 10,000 $\times g$ for 10 minutes, and supernatant was frozen in liquid nitrogen. Brain was separated into left and right halves, without the cerebellum, before freezing in liquid nitrogen. Distribution of BMS-932481 into rat brain was evaluated following oral administration of BMS-932481 and formulated at 10 mg/kg in polyethylene glycol with an average molecular weight of 400, Solutol, and D- α -tocopherol polyethylene glycol succinate at a ratio of 90:5:5 (w/w/w). Groups of rats ($n = 3$) were harvested at 1, 4, 8, and 24 hours postdose, and blood samples were collected from the jugular vein into EDTA-containing tubes and centrifuged at 4°C (1500–2000 $\times g$) to obtain plasma. Brain tissues were blotted dry, weighed, and homogenized with 4 volumes of sodium phosphate-buffered saline. The cerebellum was used for analysis of BMS-932481 levels. All samples were stored at –20°C before analysis by liquid chromatography–MS/MS.

For the dog CSF study, male beagle dogs (~1 year old; 8–11 kg) were implanted with an indwelling lumbar catheter to facilitate CSF collection from conscious, lightly restrained animals. Animals were singly housed, water was provided ad libitum, and food was provided once a day in the morning. The dogs were fasted 16 hours prior to dosing, and food was reintroduced 4 hours postdose. A total of four dogs were included in this experiment, which used a crossover design,

with a 1-week washout period between doses. In each given week, the assignment of dog to dose group was determined randomly. The dogs were dosed with BMS-932481 at 2, 5, and 30 mg/kg, or vehicle alone. Each dog had received all doses and vehicle at the completion of the experiment. Blood was collected from each dog at the following times relative to dosing: –24, –16, 0 (predose), 0.5, 1, 2, 4, 5, 6, 8, 10, 12, 24, 48, and 72 hours, and CSF was collected at –24, –16, 0 (predose), 2, 4, 6, 8, 10, 12, 24, 48, and 72 hours. BMS-932481-02 (HCl salt) was dosed by oral gavage (2 ml/kg) as a suspension prepared in vehicle containing 2% polyvinylpyrrolidone K 30, 0.01% Tween 80, 0.01 M HCl, and 0.09 M NaCl. All dosing was completed within 3 hours of compound formulation. Blood (2 or 4 ml) was collected from either the saphenous or cephalic vein into EDTA vacutainer tubes. CSF (0.2 ml) was collected from the lumbar catheter port into a low-protein binding polypropylene tube after the catheter dead volume was discarded (~0.2 ml). After each collection, the lumbar catheters were flushed with 0.3 ml of 0.9% sterile saline. Blood and CSF samples were kept on ice until centrifugation. Blood was centrifuged at 2880 $\times g$ for 10 minutes. The plasma was collected for determination of compound levels. CSF samples were centrifuged at 1330 $\times g$ for 10 minutes at 4°C.

For the monkey CSF study, male cynomolgus monkeys (~1 year old; 5–6 kg) were implanted with an indwelling lumbar catheter. Monkeys were singly housed, water was provided ad libitum, and food was provided once a day in the morning. Prior to dosing, animals were fasted overnight, within 16 \pm 2 hours prior to dosing. Food was reintroduced 4 hours postdose. A total of four monkeys were included in this experiment, which used a crossover design, with a 1-week washout period between doses. In each given week, the assignment of monkeys to doses or vehicle alone was determined randomly. Monkeys were dosed with BMS-986133 at 5 or 15 mg/kg, or vehicle alone. At the completion of the experiment, all four monkeys had received vehicle and both doses. Blood was collected from each monkey at the following times relative to dosing: 1, 2, 3, 4, 5, and 30 minutes, and 1, 2, 4, 7, 10, 24, and 48 hours, and CSF was collected at –24, 0 (predose), 1, 2, 4, 7, 10, 24, and 48 hours. Plasma and CSF samples were prepared and stored as described for the dog study.

Single-Dose Study in Human Subjects. Studies in human subjects were carried out in accordance with the Declaration of Helsinki and approved by the institutional review boards. Oral doses of BMS-932481 were given to human subjects in a single ascending dose study, in which subjects received doses of BMS-932481 ranging from 10 to 1200 mg. The single ascending dose study was designed and executed as a placebo-controlled, double-blinded study in healthy young subjects, and is described in detail in the accompanying manuscript by Soares et al. (2016). In one of the dose panels, longitudinal CSF samples were collected. This panel included 15 healthy young male and female subjects of non-child-bearing potential, who were given a single 900-mg oral dose of BMS-932481, or placebo, in the fasted state, and longitudinal CSF samples were collected via indwelling lumbar catheter at the following time points relative to dosing: –1, 0 (predose), 2, 4, 6, 8, 12, 15, 18, and 24 hours.

Results

In Vitro Effects of the GSM, BMS-932481, on A β Peptides and Notch. H4-APP^{sw} cell cultures were treated overnight with BMS-932481 at a range of concentrations to determine the dose response. In the A β multiplex assays, which quantify A β x-42 and A β 1-x simultaneously, IC₅₀ determinations for A β x-42 averaged 5.5 nM, whereas A β 1-x was reduced by no more than 30%, even at concentrations up to 50 μ M (Fig. 1A). For comparison, the GSI, BMS-299897, reduced A β x-42 and A β 1-x with approximately equal potency in the multiplex assays (Supplemental Fig. 2). Using ELISAs for A β 1-42 and A β 1-40, BMS-932481 potentially reduced A β 1-42

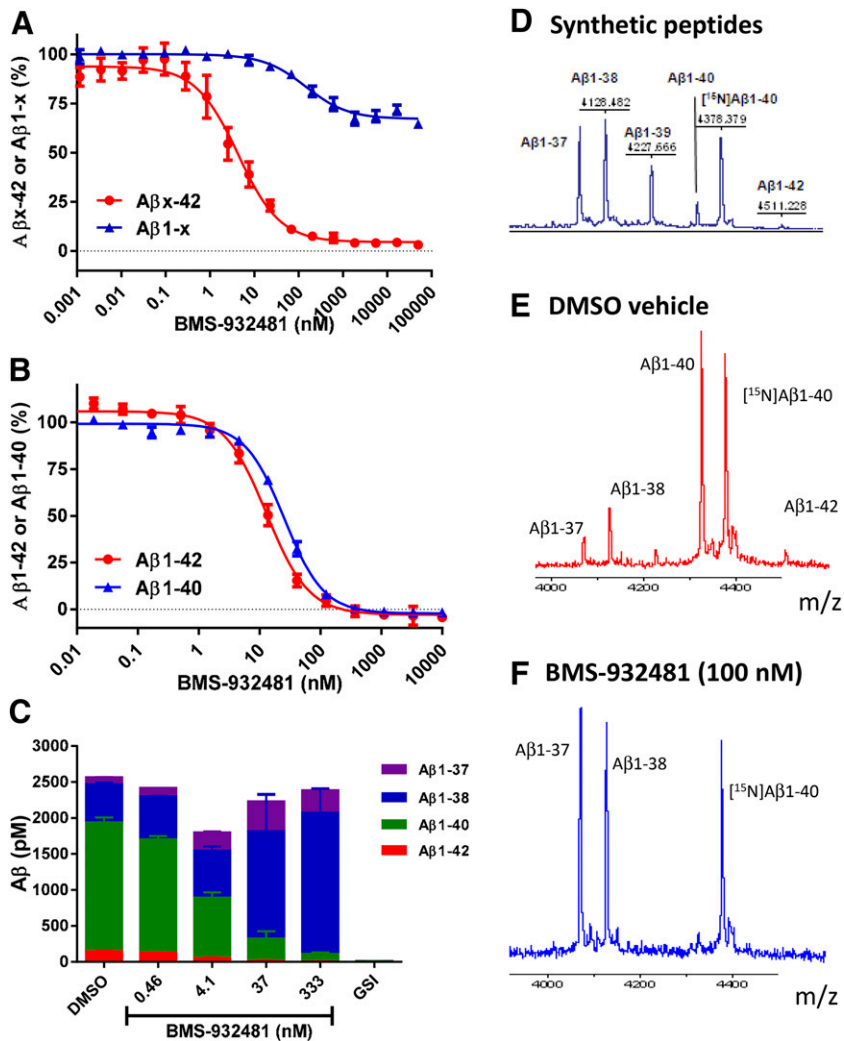


Fig. 1. In vitro activity of BMS-932481. (A) H4-APP^{sw} cell cultures were incubated overnight with BMS-932481 at a range of concentrations from 1 pM to 50 μ M. A β x-42 and A β 1-x concentrations were determined simultaneously using the automated multiplex homogeneous time-resolved fluorescence assays. Error bars show standard error for seven independent assays. IC₅₀ values (or inhibition percentage) with standard deviations are as follows: A β x-42 IC₅₀ = 5.5 \pm 3.6 nM; A β 1-x maximum inhibition = 30 \pm 10% at 50 μ M. (B) H4-APP^{sw} cell cultures were incubated overnight with BMS-932481 at a range of concentrations from 10 pM to 10 μ M. A β 1-42 and A β 1-40 were determined using automated homogeneous time-resolved fluorescence assays. Error bars show standard error for three independent assays in which both A β 1-42 and A β 1-40 were determined in parallel from the same cell cultures. IC₅₀ values with standard deviations are as follows: A β 1-42 IC₅₀ = 6.6 \pm 2.3 nM; A β 1-40 IC₅₀ = 25 \pm 7.9 nM. (C) H4-APP^{sw} cell cultures were incubated overnight with BMS-932481 at a range of concentrations from 0.46 to 333 nM, 0.1% dimethylsulfoxide (DMSO) vehicle, or GSI BMS-299897 at a concentration of 1 μ M. Concentrations were determined for A β 1-42, A β 1-40, A β 1-38, and A β 1-37 using the 4-plex Meso Scale Diagnostics assays. Error bars indicate standard error for two replicate wells. (D) An equimolar mix of synthetic peptides, including [¹⁵N]A β 1-40, was evaluated by matrix-assisted laser desorption/ionization mass spectroscopy. (E and F) H4-APP^{sw} cell cultures were incubated overnight with 0.1% dimethylsulfoxide (DMSO) (E) or BMS-932481 (F) at a concentration of 100 nM, then A β peptides were immunoprecipitated and evaluated by matrix-assisted laser desorption/ionization mass spectroscopy.

and A β 1-40 levels, yielding IC₅₀ values of 6.6 and 25.3 nM, respectively (Fig. 1B). Further evaluation of A β peptides using A β 1-42, A β 1-40, A β 1-38, and A β 1-37 immunoassays showed that, although BMS-932481 decreased A β 1-42 and A β 1-40, it increased A β 1-37 and A β 1-38 by an approximately corresponding amount. Despite the dramatic changes in the levels of individual peptides, there was little if any effect on the overall level of A β (Fig. 1C). For comparison, the GSI, BMS-299897, inhibited production of all four A β peptides (Fig. 1C).

The same changes in A β peptide levels can be visualized qualitatively by immunoprecipitation-matrix-assisted laser desorption/ionization mass spectroscopy (Fig. 1, D–F). Peptides with the expected masses for A β 1-42, A β 1-40, A β 1-38, and A β 1-37 were identified in vehicle-treated H4-APP^{sw} cell cultures (Fig. 1E). After overnight treatment with BMS-932481 at a concentration of 100 nM, no A β 1-42 or A β 1-40 was detected, whereas increased levels of the shorter A β peptides, A β 1-38 and A β 1-37, were observed (Fig. 1F).

Cell-based transcriptional reporter assays were also carried out to assess the effect of BMS-932481 on the γ -secretase substrates Notch1 and Notch3, and IC₅₀ values were determined to be approximately 1 μ M (not shown).

γ -Secretase Modulation of Brain A β and CSF A β Peptides in Rats. Rats were given single intravenous doses

of BMS-932481 (1, 5, or 10 mg/kg) or the related compound BMS-986133 (2.5, 5, or 15 mg/kg), or were dosed with vehicle only. The potency of BMS-986133 in the H4-APP^{sw} A β 1-42 immunoassay (IC₅₀ = 3.5 nM) is similar to that of BMS-932481 (IC₅₀ = 6.6 nM). The intravenous route of administration was used to minimize interanimal variability. Dose-dependent and time-dependent decreases in A β 1-42 and A β 1-40 and increases in A β 1-37 and A β 1-38 were observed for both compounds in both brain and CSF (Fig. 2; Supplemental Figs. 3–5). For BMS-932481, brain A β 1-42 and A β 1-40 levels were decreased by very similar amounts, to a maximum reduction of about 75% relative to vehicle-treated rats (Fig. 3, A and B). A β 1-37 and A β 1-38 exhibited increased levels, with ca. 2-fold maximal increase for A β 1-38 (Fig. 2C) and ca. 6-fold maximal increase for A β 1-37 (Fig. 2D). A β 1-x showed no significant changes at any dose or time point (Fig. 2E). Likewise, the sum of the A β 1-42, A β 1-40, A β 1-38, and A β 1-37 concentrations showed no significant changes (Fig. 2F). Thus, the relative amounts of the four A β peptides were changed after dosing with BMS-932481, but the overall amounts of A β peptides remained unchanged (Fig. 2, E–G). The concentrations of BMS-932481 in blood plasma from the same rats were dose-proportional (Fig. 2H). Similar dose- and time-dependent changes in A β 1-42, A β 1-40, A β 1-38, A β 1-37, and A β 1-x

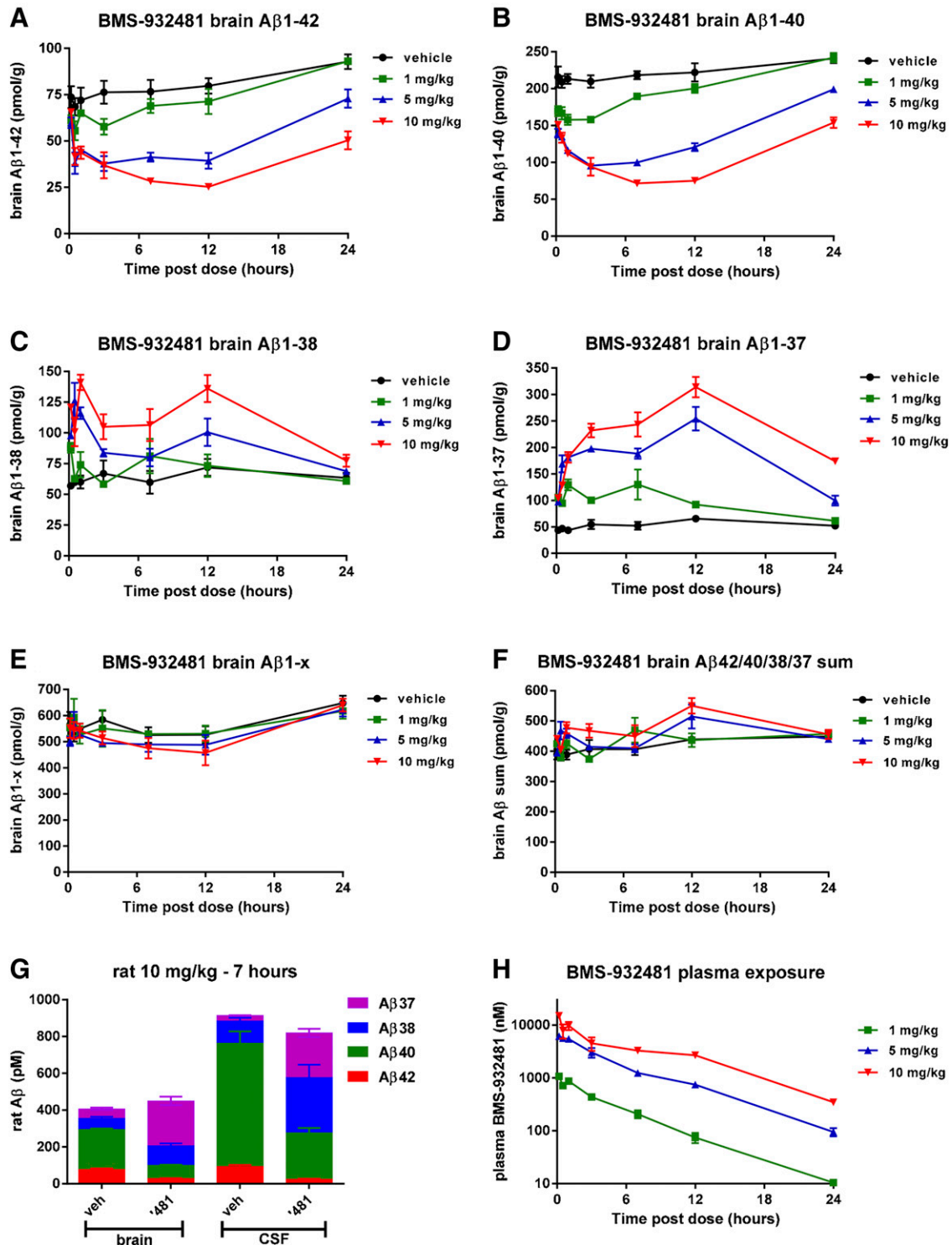


Fig. 2. Altered levels of brain A β peptides in rats given single doses of BMS-932481. Rats were given intravenous doses of BMS-932481 at 1, 5, and 10 mg/kg, or vehicle alone. After dosing, groups ($n = 4$) of rats were euthanized at intervals between 10 minutes and 24 hours. Plasma, brain, and CSF samples were taken. Brain A β 1-42 (A), brain A β 1-40 (B), brain A β 1-38 (C), brain A β 1-37 (D), brain A β 1-x (E), and the sum of brain A β 1-42, A β 1-40, A β 1-38, and A β 1-37 (F). (G) Stack chart showing amounts of A β 1-42, A β 1-40, A β 1-38, and A β 1-37 in brain and CSF of vehicle- and BMS-932481-dosed rats 7 hours after dosing. (H) Concentrations of BMS-932481 in blood plasma. Error bars indicate standard error. The concentrations of CSF A β 1-42, A β 1-40, A β 1-38, A β 1-37, and A β 1-x in the same rats are shown in Supplemental Fig. 3. The significance of treatment effects was analyzed by analysis of variance (Supplemental Tables 1 and 2).

peptides were observed in the CSF of BMS-932481-dosed rats (Supplemental Fig. 3). Likewise, similar profiles of dose dependence and time dependence in rats were observed for A β after treatment with the related GSM, BMS-986133, in brain (Supplemental Fig. 4) and CSF (Supplemental Fig. 5).

The extent of distribution of BMS-932481 into the brain in rats was evaluated in an additional time-course study (not shown). BMS-932481 exhibited moderate brain penetration. The brain-to-plasma ratio was ≥ 0.23 in samples collected at 1, 4, 8, and 24 hours. The ratio of brain area

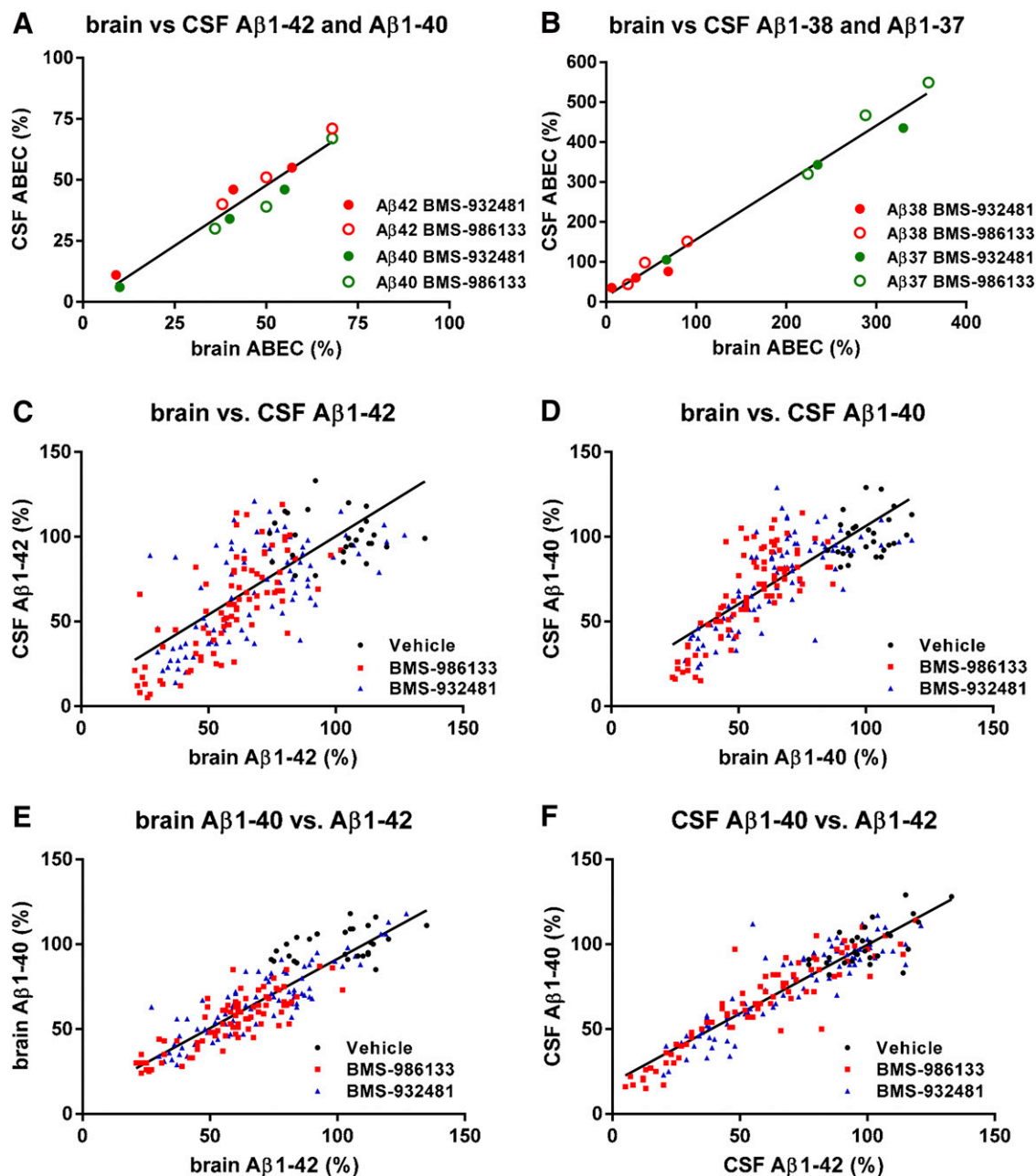


Fig. 3. The effects of GSMs on A β levels in brain and CSF are correlated. ABEC was calculated for brain and CSF A β 1-42, A β 1-40, A β 1-38, and A β 1-37 at each dose of BMS-932481 and BMS-986133 in the rat study illustrated in Fig. 2 and Supplemental Figs. 3–5. (A) ABECs for CSF A β 1-42 and CSF A β 1-40 were plotted against the corresponding ABECs for brain A β 1-42 and brain A β 1-40. Linear regression showed a best fit of $y = 0.99x - 1.7$, $r^2 = 0.93$, $P < 0.0001$. (B) ABECs for CSF A β 1-38 and CSF A β 1-37 were plotted against the corresponding ABECs for brain A β 1-38 and brain A β 1-37. Linear regression showed a best fit of $y = 1.4x + 14$, $r^2 = 0.98$, $P < 0.0001$. (C) Scatter plot for brain A β 1-42 and CSF A β 1-42 from individual rats (total of 196 rats). Linear regression showed a best fit of $y = 0.92x + 7.9$, $r^2 = 0.34$, $P < 0.0001$. (D) Scatter plot for brain A β 1-40 and CSF A β 1-40 from individual rats. Linear regression showed a best fit of $y = 0.922x + 14$, $r^2 = 0.61$, $P < 0.0001$. (E) Scatter plot for brain A β 1-42 and brain A β 1-40 from individual rats. Linear regression showed a best fit of $y = 0.82x + 9.8$, $r^2 = 0.78$, $P < 0.0001$. (F) Scatter plot for CSF A β 1-42 and CSF A β 1-40 from individual rats. Linear regression showed a best fit of $y = 0.81x + 19$, $r^2 = 0.85$, $P < 0.0001$.

under the concentration-time curve (AUC) to plasma AUC was ~ 0.6 . In addition, immunoprecipitation-western blots indicated no effect on amyloid- β precursor protein C-terminal fragment accumulation in the brains of rats treated with BMS-932481 (Supplemental Fig. 8).

γ -Secretase Modulation of A β Peptides Is Closely Correlated between Brain and CSF. To compare the pharmacology of γ -secretase modulation between brain and CSF, the area between the A β vehicle-dosed baseline and the

A β effect curve (ABEC) was calculated for each dose of BMS-932481 and BMS-986133 in brain and CSF from the experiment described earlier (Fig. 2; Supplemental Figs. 3–5). For A β 1-42 and A β 1-40, a scatter plot of CSF ABEC against brain ABEC exhibited a close linear correlation with a slope of one, indicating that the effect of GSMs on these peptides was near identical in brain and CSF (Fig. 3A). For A β 1-38 and A β 1-37, the ABEC scatter plot also showed a close linear correlation of the effects in brain and CSF; however, the magnitude of the

increase in these peptides was ca. 1.4-fold higher in CSF than in brain (Fig. 3B). This greater percentage increase in CSF relative to brain appears to be a reflection of the lower relative amount of A β 1-38 and A β 1-37 in CSF at baseline. A β 1-38 and A β 1-37 comprise approximately 17% of the A β in CSF, compared with 27% of A β in the brain (Fig. 2G).

A β peptide levels in brain and CSF can also be compared using scatter plots of the data from individual rats. A β 1-42 (Fig. 3C) and A β 1-40 (Fig. 3D) levels were significantly correlated between brain and CSF. However, considerable scatter exists on these plots, with no apparent correlation in the vehicle groups, i.e., animals not dosed with GSMs. Thus, aggregation of data, as shown using ABEC, was necessary to compare A β pharmacodynamics between brain and CSF. In contrast, the correlations between A β 1-42 and A β 1-40, at the level of individual rats, in brain (Fig. 3E) and CSF (Fig. 3F) are stronger, suggesting that A β 1-42 and A β 1-40 may be equally useful as CSF biomarkers for the evaluation of GSM pharmacodynamics. The similarity in the response of A β 1-42 and A β 1-40 is unexpected, given the different potencies of *in vitro*

inhibition by GSMs for these two peptides. Although this observation remains unexplained, it is highly reproducible.

γ -Secretase Modulation of CSF A β Peptides in Dog. Four dogs were given oral doses of BMS-932481 at 2, 5, or 30 mg/kg, or vehicle, in a crossover study design, so that all dogs received all doses and vehicle by the end of the study. CSF samples were taken predose and at intervals postdose via indwelling catheter. A maximum reduction of A β 1-42 (Fig. 4A) and A β 1-40 (Fig. 4B) of ca. 65% occurred 24 hours after the 30-mg/kg dose, whereas A β 1-38 (Fig. 4C) and A β 1-37 (Fig. 4D) were maximally increased by 1.8-fold and 5-fold, respectively. Despite the robust effects on A β 1-42, A β 1-40, A β 1-38, and A β 1-37, there was little, if any, effect on the sum of the four A β peptides (Fig. 4E). There was a transient rise in A β 1-42 and A β 1-40 levels of vehicle-treated dogs that returned toward baseline within 24 hours. In contrast, there was little, if any, transient rise apparent in A β 1-38 or A β 1-37 vehicle-treated dogs. Lowering of A β 1-42 and A β 1-40 was correlated (Fig. 4F). Plasma exposure in this experiment was variable between individual dogs, and three of the four dogs in the top dose

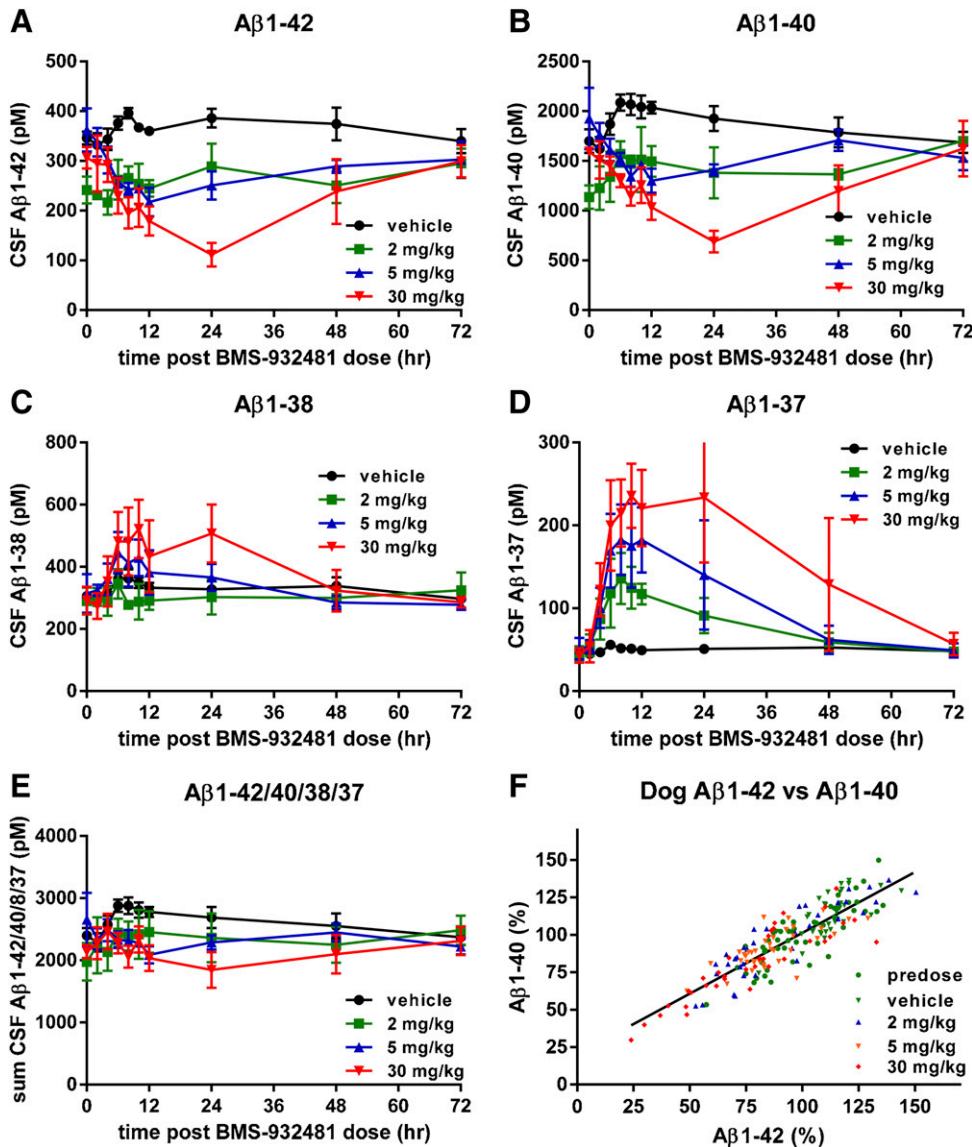


Fig. 4. Altered levels of CSF A β peptides in dogs given single doses of BMS-932481. Dogs surgically fitted with a cannula in the lumbar spinal cord were given oral doses of BMS-932481 at 2, 5, and 30 mg/kg, or vehicle alone in a cross-over study design (total of four dogs). Plasma and CSF samples were taken predose and at intervals after dosing up to 72 hours. CSF A β 1-42 (A), CSF A β 1-40 (B), CSF A β 1-38 (C), CSF A β 1-37 (D), and the sum of CSF A β 1-42, A β 1-40, A β 1-38, and A β 1-37 (E). (F) Scatter plot for CSF A β 1-42 and CSF A β 1-40 from all individual samples (total of 189 samples). Linear regression showed a best fit of $y = 0.81x + 20$, $r^2 = 0.77$, $P < 0.0001$. Error bars indicate standard error. Concentrations of BMS-932481 in blood plasma from the dogs are illustrated in Supplemental Fig. 6. The significance of treatment effects was analyzed by analysis of variance (Supplemental Table 3).

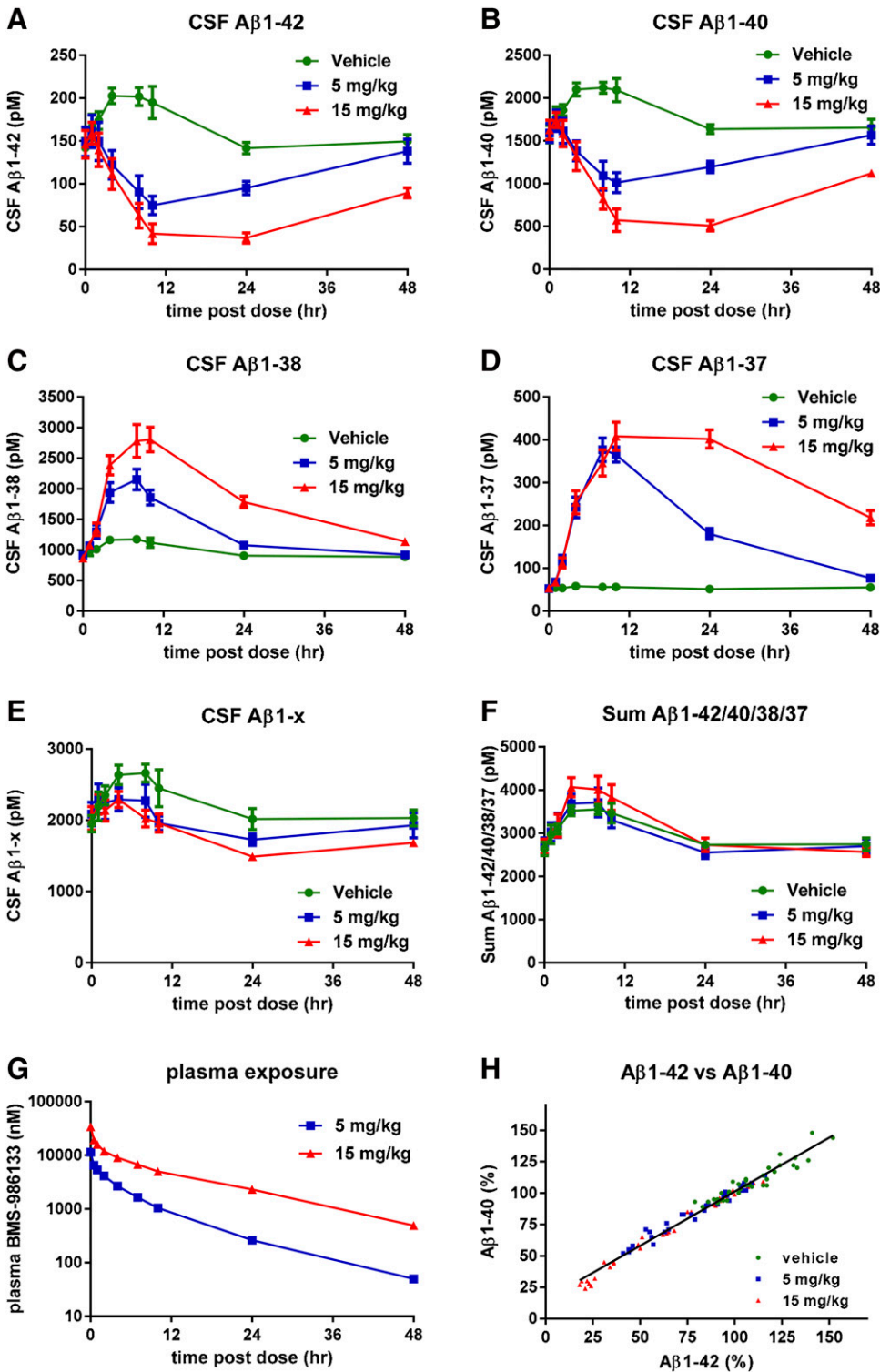


Fig. 5. Altered levels of CSF Aβ peptides in monkeys given single doses of BMS-986133. Monkeys surgically fitted with a cannula in the lumbar spinal cord were given intravenous doses of BMS-932481 at 5 and 15 mg/kg, or vehicle alone, in a crossover study design (total of four monkeys). Plasma and CSF samples were taken predose and at intervals after dosing up to 72 hours. CSF Aβ1-42 (A), CSF Aβ1-40 (B), CSF Aβ1-38 (C), CSF Aβ1-37 (D), CSF Aβ1-x (E), and the sum of Aβ1-42, Aβ1-40, Aβ1-38, and Aβ1-37 (F). (G) Concentrations of BMS-932481 in blood plasma. (H) Scatter plot for CSF Aβ1-42 and CSF Aβ1-40 from all individual samples (total of 108 samples). Linear regression showed a best fit of $y = 0.86x + 15$, $r^2 = 0.97$, $P < 0.0001$. Error bars indicate standard error. The significance of treatment effects was analyzed by analysis of variance (Supplemental Table 4).

group exhibited a secondary increase in exposure at 24 hours post dose, suspected due to coprophagia (Supplemental Fig. 6).

γ-Secretase Modulation of CSF Aβ Peptides in Monkey. BMS-986133 is a GSM with a related chemical structure and similar potency to BMS-932481. Four monkeys were given intravenous doses of BMS-986133 at 5 or 15 mg/kg, or vehicle, in a crossover study design, so that all monkeys received all doses and vehicle. CSF samples were taken

predose and at intervals postdose via indwelling catheter. A maximum reduction of Aβ1-42 (Fig. 5A) and Aβ1-40 (Fig. 5B) of ca. 75% occurred 12–24 hours after the 30-mg/kg dose, whereas Aβ1-38 (Fig. 5C) and Aβ1-37 (Fig. 5D) were maximally increased by ca. 3-fold and 8-fold, respectively. Despite the robust effects on Aβ1-42, Aβ1-40, Aβ1-38, and Aβ1-37, there was little, if any, effect on levels of CSF Aβ1-x (Fig. 5E) or the sum of the four Aβ peptides (Fig. 5F). There was a

transient rise in A β 1-42 and A β 1-40 levels of vehicle-treated monkeys that lasted for about 24 hours, a less pronounced rise in A β 1-38, but no apparent rise for A β 1-37 in vehicle-treated monkeys. Concentrations of BMS-986133 in blood plasma were dose-proportional (Fig. 5G). Levels of A β 1-42 and A β 1-40 were highly correlated in individual animals (Fig. 5H).

γ -Secretase Modulation of CSF A β Peptides in Human Subjects. As part of a single ascending dose study,

healthy human subjects were given a single 900-mg oral dose of BMS-932481, or placebo, and CSF was collected via indwelling lumbar catheter at a series of time points up to 24 hours after dosing. Additional details of the clinical program are described in the accompanying manuscript by Soares et al. (2016). A β 1-42 in the BMS-932481 group gradually decreased to 50% of predose levels after 24 hours, whereas in the placebo group, there was a transient increase of ca. 60% (Fig. 6A).

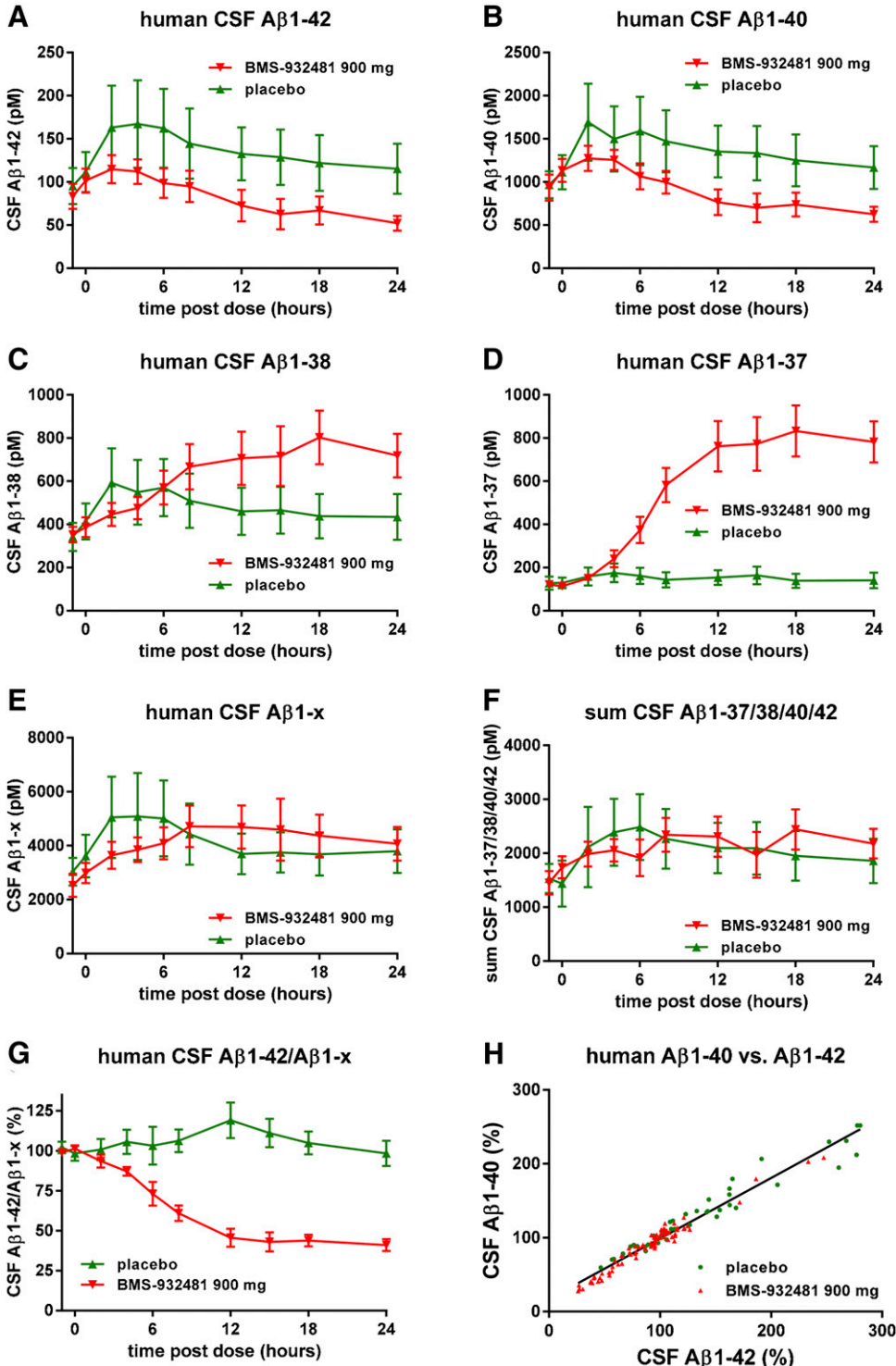


Fig. 6. Altered levels of CSF A β peptides in human subjects given a single oral dose of BMS-932481. Healthy human subjects were given a single 900-mg oral dose of BMS-932481 ($n = 10$) or placebo ($n = 5$), and CSF samples were taken through an implanted lumbar catheter at intervals up to 24 hours. CSF A β 1-42 (A), CSF A β 1-40 (B), CSF A β 1-38 (C), CSF A β 1-37 (D), CSF A β 1-x (E), and the sum of A β 1-42, A β 1-40, A β 1-38, and A β 1-37 (F). (G) A β 1-42 and A β 1-x were calculated as a percentage relative to predose levels, then each A β 1-42 value was divided by the corresponding A β 1-x value. (H) Scatter plot for CSF A β 1-42 and CSF A β 1-40 from all individual samples (total of 108 samples). Linear regression showed a best fit of $y = 0.81x + 17$, $r^2 = 0.96$, $P < 0.0001$. Error bars indicate standard error. The significance of treatment effects was analyzed by analysis of variance (Supplemental Table 5).

Likewise, A β 1-40 showed a decrease in the BMS-932481 group and a transient increase in the placebo group (Fig. 6B). A β 1-38 showed a gradual increase of about 2-fold in the BMS-932481 group and a transient increase in the placebo group (Fig. 6C). In contrast, A β 1-37 showed a ca. 12-fold increase in the BMS-932481 group, and no significant change in the placebo group (Fig. 6D). A β 1-x transiently increased by approximately 40% in both the BMS-932481 and placebo groups (Fig. 6E), as did the sum of the four peptides A β 1-42, A β 1-40, A β 1-38, and A β 1-37 (Fig. 6F). The increased CSF A β levels in the placebo groups are thought to be an artifact associated with frequent sampling of CSF via indwelling catheter (Bateman et al., 2007; May et al., 2011; Li et al., 2012a). The effect of this transient artifact can be effectively removed from the analysis by expressing A β 1-42 levels relative to A β 1-x levels, thereby emphasizing the biochemical effect on A β 1-42 specifically due to BMS-932481 treatment (Fig. 6G). The time-dependent changes in CSF A β levels in individual human subjects for each of the four A β peptides are further illustrated in Supplemental Fig. 7. When normalized relative to predose levels of each peptide, as well as to A β 1-x, the A β 1-42/A β 1-x and A β 1-40/A β 1-x ratios showed no overlap between BMS-932481 and placebo from 12 hours onward (Supplemental Fig. 7, A and B). The A β 1-37/A β 1-x ratio showed the greatest degree of separation between BMS-932481 and placebo, with complete separation of the groups after 6 hours (Supplemental Fig. 7C). For A β 1-38, the separation between BMS-932481 and placebo was less pronounced (Supplemental Fig. 7D). The concentration of BMS-932481 in human blood plasma after the 900-mg dose is illustrated in Supplemental Fig. 7E.

Pharmacological Comparison of γ -Secretase Modulation across Preclinical Species and Human Subjects.

To evaluate whether the PK/PD relationships were similar across preclinical species and human subjects, plasma exposure of BMS-932481 and A β 1-42 lowering for rat, dog, monkey, and human were compared. For each dose in each experiment, the maximal lowering (trough) of A β 1-42 was plotted against plasma AUC. This revealed a consistent relationship between plasma exposure and A β 1-42 lowering (Fig. 7A). A variant of this approach was to plot the A β 1-42 trough against plasma C_{max}, which also showed a consistent trend among all four species (Fig. 7B). Additional plots utilizing A β 1-42 ABEC instead of A β 1-42 trough showed the same trends, but with increased scatter (not shown). The same conclusions about consistent PK/PD across all four species can be drawn with respect to A β 1-40, because the effect of GSMs on A β 1-40 lowering was highly correlated with A β 1-42 lowering in all four species. Thus, the relationship of target engagement to peripheral exposure is consistent across species.

Lack of Notch-Related Effects and Observations on Human Safety. As part of an initial safety assessment, rats were given daily oral doses of BMS-932481 at 10, 30, or 100 mg/kg for 14 days. On day 14, AUC exposures averaged 892 μ M \cdot h in male rats and 508 μ M \cdot h in female rats at the top dose. There were no histologic changes suggestive of Notch inhibition, including lymphoid depletion in splenic marginal zones, intestinal goblet cell metaplasia, and ovarian atrophy, at any dose level. Thus, even at exposures far above those required for robust changes in A β peptides (area under the concentration-time curve in the time interval between dosing

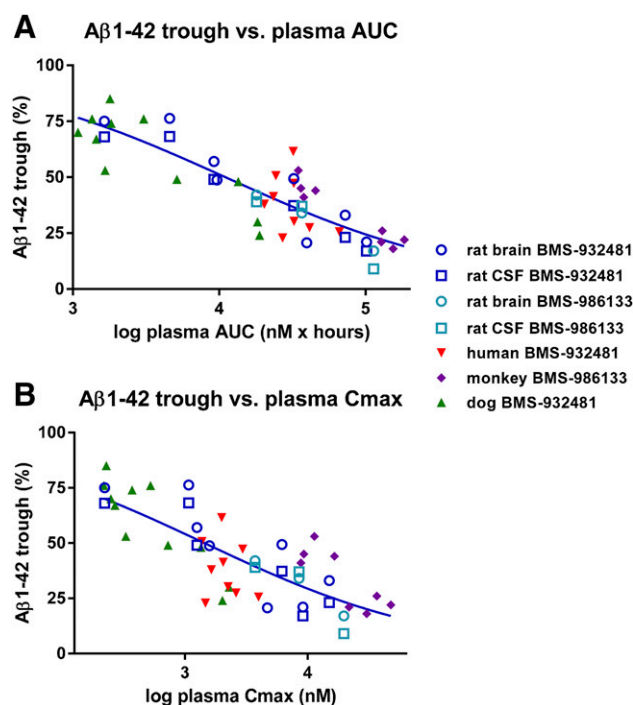


Fig. 7. Alignment of BMS-932481 PK/PD across species. (A) Scatter plot of CSF A β 1-42 trough versus plasma AUC: CSF A β 1-42 trough (minimum level of A β 1-42 after dosing) and plasma AUC were determined for each individual human subject, and for each dose in individual dogs and monkeys. For rats, A β 1-42 trough for brain, A β 1-42 trough for CSF, and plasma AUC were calculated using group means. Rat values were derived from the experiment illustrated in Fig. 2, Supplemental Figs. 3–5, and two additional time-course studies with BMS-932481 (not shown). Nonlinear fit of the entire data set indicates 50% inhibition at AUC = 11 μ M \cdot h. (B) Scatter plot of CSF A β 1-42 trough versus plasma C_{max}. Nonlinear fit indicates 50% inhibition at C_{max} = 1.5 μ M.

and 24 hours exhibited exposures of 4.5, 32, and 71 μ M \cdot h; see Fig. 2H), there was no evidence of Notch-related side effects. On the other hand, during a multiple-dose study in healthy human subjects, two subjects at the 200-mg dose exhibited increases in serum liver function enzymes that resolved upon cessation of the drug (Soares et al., 2016). When compared with the doses and exposure of the drug required to reduce A β 1-42 by an amount sufficient to constitute a valid test of the amyloid hypothesis (e.g., >25–50% reduction), the therapeutic index of BMS-932481 was considered to be insufficient to proceed safely with further clinical development of the molecule in patients.

Discussion

In this report, the potent novel GSMs, BMS-932481 and BMS-986133, were evaluated for their effects on the four main A β peptides: A β 1-42, A β 1-40, A β 1-38, and A β 1-37. Pharmacological potency was found to be consistent across cell cultures, rat, dog, monkey, and human subjects. In all four species, CSF A β 1-42 and A β 1-40 were decreased, whereas CSF A β 1-38 and A β 1-37 showed corresponding increases that conserved the overall level of CSF A β peptides. Furthermore, the PK/PD relationship was consistent across all species, thus confirming the value of preclinical species for prediction of pharmacology in humans. Additional details of the clinical program for BMS-932481 are reported in an accompanying manuscript (Soares et al., 2016).

Potency and Activity. The logic of analyzing the two GSMs, BMS-932481 and BMS-986133, together is based on their structural and pharmacological similarity. First, the two compounds have similar potencies for A β 1-42 lowering in vitro, with IC₅₀ values in the single-digit nanomolar range. Second, the PK/PD relationships of the two compounds proved to be essentially identical when compared head-to-head in the rat. Third, both compounds showed the same robust decreases in A β 1-42 and A β 1-40, accompanied by simultaneous increases in A β 1-38 and A β 1-37, resulting in conservation of the overall levels of A β peptides. Fourth, the PK/PD for BMS-986133 in rats and monkeys was well aligned with the PK/PD of BMS-932481 in rats, dogs, and humans.

PK/PD in Brain versus CSF. In rats treated with BMS-932481 and BMS-986133, decreases in A β 1-42 and A β 1-40 and increases in A β 1-38 and A β 1-37 were observed within minutes of dosing, followed by a return toward baseline after 7–12 hours. The effects of dose and time were similar in brain and CSF, although maximum effects on the peptides occurred slightly earlier in CSF in the 7-hour sample (Supplemental Fig. S3 and S5) than in brain in the 12-hour sample (Fig. 2; Supplemental Fig. 4). Likewise, several studies, using either GSIs or GSMs, have shown greater and more rapid effects in CSF than in brain (Abramowski et al., 2008; Martone et al., 2009; Hawkins et al., 2011; Albright et al., 2013). The differences between brain and CSF A β time-course profiles have been ascribed to a greater rate of clearance (K_{out}) for CSF A β relative to brain A β , as well as to a limitation in the maximal extent of brain A β lowering (I_{max}) relative to CSF A β (Lu et al., 2011; Tai et al., 2012). Notably, the differences in pharmacological parameters were independent of the mechanism of A β lowering, being similar for GSMs, GSIs, and β -APP cleaving enzyme inhibitors (Lu et al., 2012). However, in our rat studies, the differences between brain and CSF pharmacodynamics appeared less pronounced, consistent with several previous studies (Barten et al., 2005; Best et al., 2005, 2006; Lanz et al., 2010).

Significant correlations were found between A β 1-42 and A β 1-40 peptides in CSF and brain of individual rats treated with BMS-932481 or BMS-986133 (Fig. 3, C and D), consistent with previous reports using GSIs (Shapiro et al., 2012; Tai et al., 2012). However, the baseline levels of brain and CSF A β 1-42 in vehicle-treated rats showed little evidence of correlation. Therefore, to evaluate the effect of GSMs independently of the individual rat A β variation, the effect on the overall time course was taken into account. Thus, the ABEC was calculated for each dose. The percentage decrease in ABEC was found to be nearly identical between brain and CSF for A β 1-42 and A β 1-40. Likewise, the increases in A β 1-38 and A β 1-37 ABEC also showed a linear relationship, but the increase in ABEC was about 1.4-fold greater in CSF than in brain. Thus, the pharmacodynamic effects were closely equivalent between brain and CSF, confirming the potential of CSF A β peptides as pharmacodynamic biomarkers for brain A β peptides in the clinic.

A β Placebo Rise. A complicating factor was the A β placebo rise, which is a transient increase in CSF A β levels known to occur during frequent sampling via lumbar catheter in human subjects (Bateman et al., 2007; May et al., 2011; Li et al., 2012). The A β placebo rise is independent of drug treatment, and was observed in CSF cannulated dogs and monkeys, as well as in human subjects (Figs. 4–6). It was not

observed in rats because only terminal CSF samples were taken. The rise was most pronounced for A β 1-42, A β 1-40, and A β 1-38, but absent for A β 1-37, perhaps suggesting an analytical recovery artifact associated with the more hydrophobic peptides. Although there is still no clear explanation for the placebo rise, it is clearly not related to drug treatment.

Translation across Species. The GSMs exhibited similar PK/PD in rats, dogs, monkeys, and humans. In particular, the A β 1-42 trough (lowest level of A β 1-42 observed after dosing) at each dose was closely related to the plasma AUC exposure across all preclinical species and human subjects (Fig. 7). The same conclusion can be drawn for A β 1-40, because the GSMs caused closely correlated decreases in A β 1-42 and A β 1-40 peptides in all species. The decrease in A β 1-42 and A β 1-40 was balanced by corresponding increases in A β 1-38 and A β 1-37 production, such that the total combined level of the four peptides remained constant in all species. Likewise, the A β 1-x ELISA assay, which detects all four peptides, showed no significant changes upon GSM treatment in all species.

One consequence of the noninhibitory mechanism and conservation of A β levels is that the proportion of A β 1-42 goes down in the presence of GSMs. For example, A β 1-42 lowering by 50% would decrease the ratio of A β 1-42 relative to the total A β peptide levels by 50%. Increased levels of the shorter A β peptides inhibit A β 1-42 aggregation (Watanabe et al., 2006; Murray et al., 2009) and thereby attenuate its neurotoxic properties (Kuperstein et al., 2010). This is opposite to the effect of presenilin FAD mutants, which increase the relative proportion of A β 1-42, and are associated with earlier AD age of onset (Duering et al., 2005; Kumar-Singh et al., 2006). Thus, GSMs are attractive not only because they lower the proportion of the disease-associated A β 1-42 peptide, but also because they affect A β peptides in a direction opposed to that of presenilin FAD mutations.

Off Targets. In cellular and animal studies, BMS-932481 did not appear to effect the proteolytic processing of Notch at concentrations or exposures that resulted in maximal modulation of A β peptides. Despite achieving plasma exposures in human subjects in the range that inhibited Notch processing in the in vitro assay, no adverse effects generally believed to be attributable to inhibition of Notch processing were observed clinically (e.g., untoward gastrointestinal effects or skin abnormalities). This is likely due to the high degree of non-specific plasma protein binding exhibited by the compound (>90%), rendering the free plasma concentration significantly less than both the reported total plasma concentration and the potency in the in vitro Notch assay (performed in the absence of serum). The lack of effect of BMS-932481 on Notch processing is consistent with the mechanism of GSMs and is anticipated to be a significant advantage over enzymatic inhibitors of γ -secretase. On the other hand, BMS-932481 produced increases in plasma concentrations of liver function enzymes in multiple-day dosing studies, which is generally interpreted as a sign of hepatic inflammation or damage. These changes were transient in that the plasma concentration of these markers resolved following discontinuation of the administration of BMS-932481. Because this effect on safety precluded robust A β lowering by oral dosing, further clinical development of the molecule was terminated. The mechanism underlying the observed increases in hepatic enzymes is unclear, although it is unlikely to be mechanism-based. Due

to the high degree of nonspecific protein binding and lipophilicity of BMS-932481, the plasma exposures that were required to achieve significant modulation of γ -secretase in brain were relatively high for a therapeutic intended to be administered chronically, i.e., in the micromolar range. In addition, in preliminary experiments (data not shown), we found that molecules structurally related to BMS-932481 exhibited accumulation in the liver following single and multiple doses in rats. Thus, the observed changes in plasma concentrations in hepatic enzymes are likely due to the high level of xenobiotic load and accumulation in the liver at exposures required for a desirable range of modulation of γ -secretase.

In conclusion, these studies demonstrate proof of the GSM mechanism in normal human subjects and illustrate the utility of building comprehensive PK/PD data sets in pre-clinical species to make predictions of pharmacological activity in human subjects. A remaining challenge is to predict non-mechanism-related or off-target safety effects to identify safe compounds for clinical studies. Nevertheless, these studies suggest that the GSM mechanism has therapeutic potential, and that GSMs with improved clinical safety profiles should be tested in AD.

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Authorship Contributions

Participated in research design: Toyn, Boy, Raybon, Meredith, Denton, Thompson, Lentz, Padmanabha, Drexler, Macor, Albright, Gasiar, Olson, Soares, AbuTarif, Ahlijanian.

Conducted experiments: Robertson, Guss, Hoque, Sweeney, Clarke, Snow, Morrison, Berisha, Cook, Furlong, Wei.

Contributed new reagents or analytic tools: Boy, Zhuo, Zuev.

Performed data analysis: Toyn, Raybon, Denton, Grace, Wang, Hong.

Wrote or contributed to the writing of the manuscript: Toyn, Ahlijanian.

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