Title: Evaluating the Safety, Tolerability, Pharmacokinetics and Receptor Occupancy of BMS-984923

ClinicalTrials.gov ID: NCT04805983

Date: 12/15/2021

Document: Protocol and SAP

HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
(2016-1)

Protocol Title: An Open-Label, Single-Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Receptor Occupancy of BMS-984923

Principal Investigator: Adam Mecca, MD, PhD

Version Date: November 29, 2021

(*If applicable*) Clinicaltrials.gov Registration #: NCT04805983

INSTRUCTIONS
This template is intended to help investigators prepare a protocol that includes all of the necessary information needed by the IRB to determine whether a study meets approval criteria. **Read the following instructions before proceeding:** 1. Use this protocol template for a PI initiated study that includes direct interactions with research subjects. Additional templates for other types of research protocols are available in the system Library. 2. If a section or question does not apply to your research study, type "Not Applicable" underneath. 3. Once completed, upload your protocol in the "Basic Information" screen in IRES IRB system.

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SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

   Primary Objective: To evaluate the safety, tolerability, and pharmacokinetics of BMS-984923 in healthy participants.

   Secondary Objective: A receptor occupancy substudy will determine drug receptor occupancy at each dose using [^{18}F]FPEB PET.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.

   Each participant will complete a screening period of up to 90 days, a treatment period of up to two days and a follow up period of seven days. The total duration of participation in this study is approximately 14 weeks.

   The study will enroll up to six cohorts of 6 participants each. Study drug will be administered in sequentially increasing dose groups. For all 6 participants at a dose cohort, a safety assessment review will be completed prior to advancing the next higher dose level.

   It is anticipated that enrollment will occur in less than 15 months and all protocol activities will be completed in less than 18 months, with up to 12 months for data analysis.

3. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

This project seeks to develop a novel disease-modifying compound for Alzheimer's disease (AD). Brain synapse loss in AD has been tightly correlated with cognitive symptoms and is triggered initially by amyloid beta (Aß) peptide accumulation. We have described a pathway in which soluble Aß oligomers (Aßo) bind to cellular prion protein (PRP^c), thereby engaging metabotrophic glutamate receptor subtype 5 (mGluR5) as a co-receptor, and activating PTK2B (Pyk2) and Fyn kinases to couple with Tau pathology and synapse loss. mGluR5 is a G protein-coupled receptor (GPCR), and multiple groups have shown that interrupting mGluR5 function rescues preclinical AD phenotypes, making it an attractive drug target. However, mGluR5 has a physiological role as a glutamate receptor, and full inhibition impairs function. Consequently, typical antagonists have a narrow therapeutic window.

We have identified a highly potent, orally bioavailable mGluR5 ligand that *does not* alter basal or glutamate activity, but *does block* Aßo/PrP^C activation of mGluR5. This compound is considered a silent allosteric modulator, or SAM, for mGluR5, meaning "silent" with regard to glutamate, while antagonistic with regard to Aßo/PrP^C. Preliminary studies demonstrate robust efficacy of this SAM compound for multiple preclinical mouse AD phenotypes. Drug treatment recovers synapse density, restores long term potentiation (LTP) and returns

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memory performance to wild-type (WT) levels. The overall goal is to develop disease-modifying oral therapy effective to slow, halt or partially reverse AD progression both in the Mild Cognitive Impairment (MCI) state and in mild dementia.

Significance:

Today, no disease-modifying therapy for AD is available. The hypothesis that the Aß peptide plays an early causative role is supported by pathology, human genetics and biomarker studies (Hardy 2002; Selkoe 2016). More specifically, Aßo triggers a toxic cascade that impairs synaptic function and leads to progressive synapse loss and cognitive dysfunction (Berman 2008; Cleary 2005; Hong 2016; Lacor 2007; Lambert 1998; Lauren 2009; Lesne 2006; Li 2009; Palop 2010; Shankar 2008; Walsh 2002). Recent clinical trials have focused on restricting production of Aβ by γ- or β-secretase inhibition, or on accelerating clearance of Aβ by active or passive immunization. As these strategies have thus far failed to produce substantial benefit, new treatment approaches are urgently needed. Thus, target-based therapies directed at suppressing AD-related synapse damage offer an alternate, unexplored strategy distinct from amyloid-lowering approaches, which may also be complementary to Aß-lowering strategies.

Aßo is thought to trigger the onset of AD, followed by a cascade of events over a multi-year course, involving synaptic loss, inflammation, tauopathy, and cell loss. The extent to which this process is subject to cessation or reversal upon elimination of synapse damage at different stages of progression is not fully defined. Effectiveness of synapse protection is predicted to be greatest in early disease. Therefore, the therapy envisioned here should be most valuable when administered to patients with amnestic MCI plus amyloid biomarkers, or those with mild AD dementia, rather than moderate-severe AD dementia.

Biological Rationale and Profile of the Therapeutic Agent:

The Amyloid hypothesis of AD is supported by the early and progressive accumulation of the Aβ peptide as well as the genetic effects of APP and PS1/2 mutations, suggesting that Aβ peptide species are the initial trigger (Hardy 2002; Selkoe 2016). Aβo have been implicated in initiating synaptic dysfunction, dendritic spine loss, inflammatory mediator recruitment and memory dysfunction, all of which are key characteristics of AD (Berman 2008; Cleary 2005; Hong 2016; Lacor 2007; M. P. Lambert 1998; Lauren 2009; Lesne 2006; Li 2009; Palop 2010; Shankar 2008; Walsh 2002). PrP^C is a high affinity receptor for Aβo, which we discovered by an unbiased genome wide screen. Pathological signals from Aßo/PrP^C are transmitted via its co-receptor metabotropic glutamate receptor 5 (mGluR5) to intracellular signaling (Figure 1) (Beraldo 2016; Gimbel 2010; Haas 2016a; Haas 2016b; Hu 2014; Kaufman 2015; Larson 2012; Lauren 2009; Um 2013; Um 2012). Aβo enhances the interaction of PrP^C with mGluR5, which is critically involved in AD disease pathogenesis (Haas 2014; Haas 2016a). Fyn regulation links mGluR5 to Tau pathology (Bhaskar 2010; Bhaskar 2005; Chin 2005; Chin 2004; Ittner 2010; Kaufman 2015; Larson 2012; Lee 1998; Lee 2004; Roberson 2011; Um 2013; Um 2012), and Pyk2 (PTK2B) regulation by mGluR5 and Fyn provides validation of this pathway by human

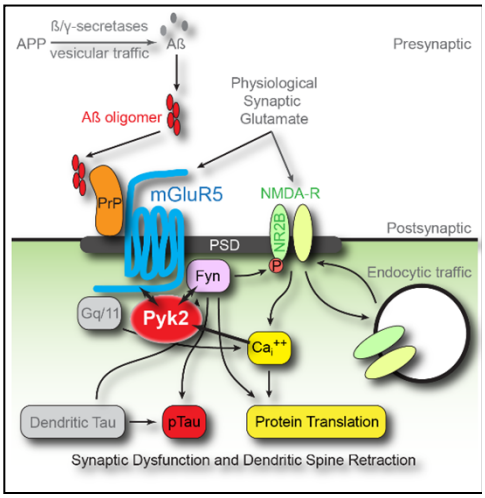

Figure 1 mGluR5 at the center of an Aβo-PrP^C-Fyn-Pyk2 cascade of AD synapse damage. Schematic illustrates the role of mGluR5 in linking cell surface Aßo–PrP^C complexes to intracellular Fyn/Pyk2, Tau and synaptic loss. Proteins are clustered in the PSD and alter NMDA-Rs, calcium and protein translation. Pyk2 (PTK2B) variation is a verified genetic risk for Late Onset AD and is associated with mGluR5 in an AD transduction network. Aberrant PrP^C–mGluR5–Fyn-Tau signaling leads to synaptic malfunction and loss.

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genetic risk assessment (Beecham 2014; Dourlen 2017; Haas 2016a; Haas 2016b; Kaufman 2015; Lambert 2013).

Separately from PrP^C coupling, multiple studies of AD models have implicated mGluR5 and altered glutamate signaling (Hamilton 2014; Hamilton 2016; Hu 2014; Overk 2014; Renner 2010; Wang 2004; Zhang 2015). Glu activation of mGluR5 leads to a range of signaling pathways, including phospholipase C, IP3, and intracellular calcium release, as well as Homer, Arc, eEF2 and FRMP regulation of protein translation at synapses, plus CamKII, Fyn and Pyk2 kinases (Bhakar 2012; Haas 2016a; Haas 2016b; Heidinger 2002; Kaufman 2015; Lesne 2006; Li 2009; Luscher 2010; Nicodemo 2010; Um 2013; Um 2012). The regulation of mGluR5 and synaptic plasticity by therapeutic molecules has been studied extensively, as mGluR5 is implicated in the pathogenesis of several neurodegenerative and psychiatric CNS disorders (Bruno 2001; Gasparini 2007; Gregory 2011; Gregory 2012; Molck 2014; Ribeiro 2010; Sheffler 2011).

Genetic loss and pharmacological inhibition studies by multiple groups have demonstrated that reduced mGluR5 activity alleviates synaptic and memory deficits in diverse AD-related models (Beraldo 2016; Haas 2016a; Hamilton 2014; Hamilton 2016; Hu 2014; Kumar 2015; Overk 2014; Raka 2015; Renner 2010; Um 2013; Wang 2004; Zhang 2017; Zhang 2015). Pharmacological inhibition studies have utilized the negative allosteric modulators (NAMs), MPEP, MTEP and CTEP, all of which reduce physiological glutamate signaling as well as pathological AD signaling. While this initially appears promising, it must be noted that full blockade of mGluR5 glutamate signaling genetically or pharmacologically impairs mouse learning and memory, and disrupts behavior in WT control animals (Abou Farha 2014; Campbell 2004; Lu 1997; Porter 2005; Rodriguez 2010; Um 2013; Xu 2009). Clinically, the mGluR5 NAM mavoglurant impairs cognitive performance at the 200 mg dosage, even though peak receptor occupancy reaches only 55% at 100 mg and 85% with 400 mg (Rutrick 2017). For this reason, separating blockade of glutamate signaling at mGluR5 from the beneficial effects of inhibiting Aβo/PrP^C/mGluR5 signaling is critical to enhance the therapeutic index of this target.

Here, we focus on mGluR5 ligands that distinguish between glutamate and Aβo/PrP^C signaling. Allosteric modulators of mGluR5 are subdivided into positive allosteric modulators (PAMs), negative allosteric modulators (NAMs), and silent allosteric modulators (SAMs). PAMs and NAMs shift the potency or efficacy of Glu-induced G-protein-mediated intracellular Ca^{2+} mobilization; some also alter basal activity via direct agonism or antagonism. In contrast, SAMs do not alter basal or Glu-induced Ca^{2+} signaling (Gregory 2011; Gregory 2010; Gregory 2012). Our Preliminary Studies (Haas 2017) showed that BMS-984923, compound 16 of (Huang 2016) potently inhibits the PrP^C-mGluR5 interaction and prevents pathological Aβo signaling without affecting physiological Glu signaling. Critically, inhibition of Glu signaling at mGluR5 is not essential for the benefits of blocking Aβo/PrP^C signaling at mGluR5 for *in vivo* AD models, thereby greatly expanding the therapeutic window for mGluR5 as a disease-modifying therapeutic target in AD.

BMS-**984923,** *High Potency SAM for mGluR5*

Our Preliminary Studies use a potent mGluR5 SAM to distinguish blockade of Glu/mGluR5 function from inhibition of AD-specific Aβo/PrP^C signaling through mGluR5 (Haas 2017). BMS-984923 was identified in an extensive drug development program initiated to identify mGluR5 PAMs for schizophrenia (Huang 2016). The compound is competitive antagonist of [3H]MPEPy binding to human mGluR5 with a K_i of 0.6 nM, but has no detectable agonist or

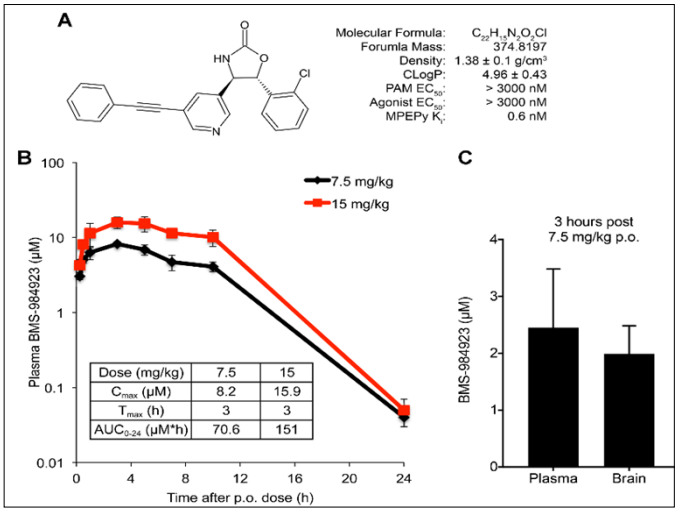

Figure 2 Pharmacokinetic properties of BMS-984923 in mice. **A.** Structure of BMS-984923, its chemical and pharmacological properties are summarized. Three separate batches have been generated, each >97% purity. **B.** The plasma levels of BMS-984923 in C57Bl6J male mice after a single oral dose are plotted as a function of time. Data are mean ± SEM, n=9. Calculated PK properties are provided. **C.** The brain and plasma levels of BMS-984923 in C57Bl6J male mice was measured 3 hours after a single 7.5 mg/kg dose. Data are mean ± SEM, n=3.

antagonist or PAM or NAM activity at mGluR5 signaling (Figure 2A) (Huang 2016).

We confirmed that the BMS-984923 does not affect glutamate/DHPG-induced Ca^{2+} signaling in mGluR5-overexpressing HEK-293T cells and extended these observations to DIV21 cortical neurons (Figure 3A) (Haas 2017). In contrast, a NAM, MTEP, strongly suppresses glutamate/DHPG-induced Ca^{2+} responses. Co-application of BMS-984923 and MTEP prevents MTEP-induced inhibition of glutamate/DHPG-induced Ca^{2+} responses (Figure 3A) (Haas 2017). This confirms that the BMS-984923 behaves as silent allosteric modulator of mGluR5, consistent with competition at the MTEP site.

BMS-984923 has good oral bioavailability and an apparently linear dose response (Figure 2B). At 7.5 or 15 mg/kg, plasma concentration exceeds 2 µM at 10 h. Brain concentrations are nearly as high 3 hours after a 7.5 mg/kg oral dose (Figure 2C). Compounds chemically related to the BMS-984923, but with PAM activity, induce seizures (Compound 5 of (Yang 2016)). To determine whether the BMS-984923 compound at the 7.5 mg/kg dose occupies brain mGluR5, we assessed the degree to which BMS-984923 prevented PAM-induced seizures (Haas 2017). During the 90 min after PAM administration, 50% of vehicle-pretreated mice exhibited seizures, whereas none of the SAM-pretreated mice did so (Haas 2017). Thus, the SAM effectively occupies brain mGluR5 sites to prevent PAM action. Based on the BMS-984923 pharmacokinetics, brain penetration and potency for mGluR5, we chose the BMS-984923 dose of 3.75 mg/kg by oral gavage twice a day as the dose to ensure essentially complete continuous receptor occupancy.

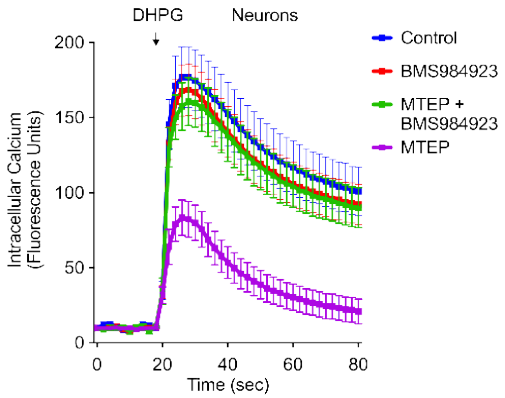

Figure 3 BMS-984923 does not interfere with glutamate signaling but competes with the NAM, MTEP. DIV21 mouse cortical neurons were exposed to SAM and/or MTEP for 20 min prior to recording intracellular Ca2+ concentration in response to 50 µM DHPG (mGluR5-specific analogue of Glu) at 18 s. Each line represents the induced mean ± SEM averaged from 18-20 wells.

It is known that higher doses of mGluR5 NAMs such as MTEP negatively affect EEG amplitude in mice (Um 2013). At 15 mg/kg/d B.I.D., MTEP is tolerated in mice, and has been applied successfully to recover AD-like phenotypes in transgenic mice (Um 2013). However, we observed that a 5-fold increased dose of MTEP (75 mg/kg) strongly impairs EEG amplitude and alters the power spectrum in mice (Haas 2017), demonstrating MTEP's limited therapeutic window. In contrast, neither a 1x dose (3.75 mg/kg) nor a 10x dose (37.5 mg/kg) of BMS-984923 impairs EEG amplitude or power spectrum in mice (Haas 2017). Thus, doses of SAM BMS-984923 well above the effective dose appear to be well tolerated with regard to CNS function in mice.

Protection from Aβo action by BMS-984923

While it is clear that BMS-984923 does not alter mGluR5 activation with or without glutamate, a key issue is whether it interrupts AD-related Aβo signaling via PrP^C through mGluR5. Aβo has been shown to enhance the association of PrP^C and mGluR5 detected by immunoprecipitation from transfected HEK293T cells (Haas 2014). With Aβo, mGluR5 interaction with PrP^C is increased two-fold (Figure 4A-C) (Haas 2017). BMS-984923 eliminates Aβo-driven association with an IC50 of 1-10 nM (Figure 4A-C), consistent with the concentrations required for BMS-984923 displacement of [3H]MPEPy from mGluR5 (Figure 2A). Thus, in this non-neuronal assay BMS-984923 has properties that allow selective blockade of Aβo action even though there is no effect on basal or glutamate signaling.

To determine whether inhibition of Aβo-enhanced binding of PrP^C to mGluR5 has functional consequences in neurons, we analyzed the effect of BMS-984923 with or without Aβo on mouse hippocampal slice synaptic plasticity in the CA3 to CA1 Shaffer collateral pathway (Fig. 3 of (Haas 2017)). Aβo are known to

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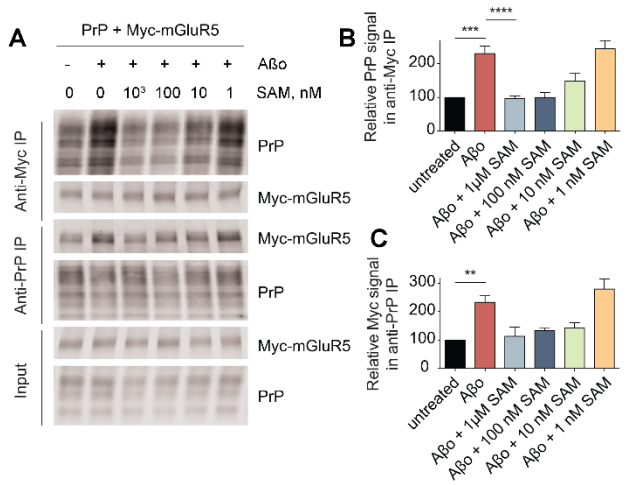

Figure 4 SAM (BMS-984923) reduces the interaction of PrPC with mGluR5 in membrane fractions. A. HEK-293 cells were co-transfected for PrPC and Myc-mGluR5. Membrane fractions were pre-incubated for 2 hours at 4°C with indicated concentrations of SAM, followed by 1 µM Aβo for 30 min (monomer equivalent concentration, estimated oligomer of 10 nM). NP-40 membrane extractions (input) and immunoprecipitates were immunoblotted with either anti-Myc or anti-PrPC. B-C. Data are mean ± SEM from n=3 independent experiments. B. Quantification of the PrPC signal in anti-Myc immunoprecipitates after treatment is normalized to the signal of untreated samples. Aβo trigger a significant enhancement of the co-immunoprecipitation of PrPC with Myc-mGluR5 in membrane fractions, which can be significantly reversed by 1 µM SAM (***, p<0.001; ****, p<0.0001 by one-way ANOVA with Tukey's post-hoc multiple comparisons test). C. Quantification of the Myc signal in anti-PrPC immunoprecipitates after treatment is normalized to the signal of untreated samples. Co-immunoprecipitation of Myc-mGluR5 with PrPC is significantly enhanced by Aβo only when SAM concentration is absent, or less than 10 nM (**, p<0.01 by one-way ANOVA with Tukey's post-hoc test).

wild type behavior (Figure 5A).

impair synaptic function by inhibiting long-term potentiation (LTP) acutely (Shankar 2008; Walsh 2002). The presence of 100 nM BMS-984923 does not alter basal fEPSP amplitude or theta-burst-induced LTP in the absence of Aβo (Haas 2017). Critically, preincubation of brain slices with BMS-984923 prevents Aβo-induced inhibition of LTP. Thus, BMS-984923 prevents Aβo-induced impairment of synaptic plasticity in brain slices.

SAM treatment of AD model mice rescues established memory deficits

To investigate whether BMS-984923 could ameliorate behavioral deficits of aged APP/PS1 transgenic mice (Jankowsky 2004; Jankowsky, Xu, Fromholt, Gonzales, & Borchelt, 2003), we assessed their behavior in multiple behavioral paradigms to 4 weeks of treatment with SAM or vehicle. Mice were randomized to treatment group, and all behavioral assessments were by an observer unaware of group. Since this mouse strain develops behavioral deficits starting at 6 months of age when Aβo levels rise (Gimbel 2010; Kostylev 2015; J. H. Park 2006), their deficits were well established when SAM treatment started at an average age of 14 months. Wild type mice express a clear preference for a novel over a familiar object, which is not affected by SAM treatment (Figure 5) (Haas 2017). Vehicle-treated transgenic mice lack a preference for either object, revealing a memory deficit in this paradigm. Importantly, SAM-treated transgenic mice show a significant preference for the novel object. Thus, BMS-984923 recovers memory by transgenic mice back to

We used the Morris water maze spatial navigation task as a second behavioral assessment. A total of 24 training trials were completed in three consecutive days, and mice were analyzed in a probe trial test on day four. During the last 8 trials, the vehicle-treated APP/PS1 group differs significantly from all other groups by one-way repeated measures ANOVA (RM-ANOVA) with Tukey's post-hoc multiple comparisons test, whereas all other comparisons are not significantly different (Figure 5B). A 2-way RM-ANOVA over the last 8 trials further shows a significant effect of genotype, treatment, and of their interaction (Figure 5B). The apparent improvement in learning by the transgenic mice after BMS-984923 treatment cannot be attributed to changes in swimming or overall activity, because the distance traveled to the platform during the last block of trials showed the same rescue of transgenic mouse performance by BMS-984923 (Figure 5C) and no differences in swim speeds (Figure 5D). During the memory probe trial 24 hours after completion of training, the vehicle-treated APP/PS1 group show a significant deficit compared to wild type (Figure 5E). Importantly, SAM-treated APP/PS1 mice have a significant improvement compared to vehicle-treated APP/PS1 mice. A 2-way ANOVA further reveals a significant effect of genotype, of treatment, and of their interaction (Figure 5E). Thus, BMS-984923 treatment rescues behavioral deficits of transgenic mice in the Morris Water Maze spatial navigation task.

Passive avoidance testing was used as a third behavioral assessment for mouse memory. Transgenic mice demonstrate clear deficits in passive avoidance testing, as can be observed by a reduced latency to enter the dark compartment in the retention test (Figure 5F). Importantly, BMS-984923 treatment significantly recovers memory deficits of

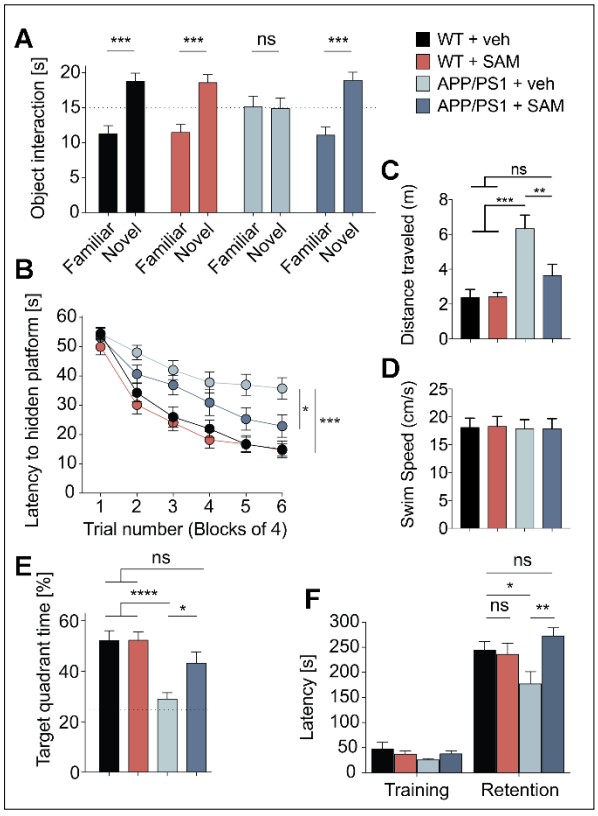

Figure 5 SAM (BMS-984923) reverses learning and memory deficits in APP/PS1 transgenic mice after 4 weeks of treatment. A. Time spent interacting with a novel object compared to the time spent interacting with a familiar object in plotted. Mean ± SEM, n=18-25 mice per group. Vehicle-treated APP/PS1 mice have no preference (p>0.05), while other groups show a preference for novel object (***, p<0.001). **B.** Spatial learning in Morris water maze is plotted as the latency to a hidden platform after 4 weeks of treatment. Mean ± SEM of n=21-28 mice per group. Performance was analyzed by 2-way analysis of variance with repeated measures (RM-ANOVA) over the last 8 trials and showed a significant effect of genotype (p=0.0001), of treatment (p=0.043), and of their interaction (p=0.045). The vehicle-treated APP/PS1 group differed from other groups over the last 8 trials (*, p<0.05, ***, p<0.001). **C-D.** Distance travelled to reach hidden platform and average swim speed during the last block of swim trials from B is plotted. Mean ± SEM of n=21-28 mice per group. Distance travelled for vehicle-treated APP/PS1 group differed from all other groups (**, p<0.01, ***, p<0.005), whereas all other comparisons were not significantly different (p>0.05). **E.** Plotted is percent time in the quadrant where platform had been located. Mean ± SEM of n=21-28 mice per group. Performance showed a significant effect of genotype (p=0.0001), of treatment (p=0.043), and of their interaction (p=0.046). Vehicle-treated APP/PS1 group differed significantly from other groups (*, p<0.05, ****, p<0.0001), whereas other comparisons were not different (p>0.05). Dashed line represents chance. **F.** Mice were trained to avoid a dark compartment in passive avoidance paradigm. Latency of vehicle-treated APP/PS1 mice to enter dark compartment 5 min after training was significantly decreased compared to WT (*, p<0.05). Deficit of APP/PS1 mice was rescued by SAM-treatment (**, p<0.01). Mean ± SEM, n=21-27 mice per group. For all, male:female ratio between 0.89-1.17.

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transgenic mice to wild type levels in passive avoidance testing (Figure 5F). Notably, BMS-984923 treatment does not affect wild type behavior in any behavioral assessment (Figure 5A-F).

Plaque and gliosis are not altered by BMS-984923

To investigate the underlying cause of behavioral recovery, we analyzed the brains of treated mice immunohistologically. No change in amyloid accumulation or gliosis was observed (Haas 2017). This is consistent with BMS-984923 blocking downstream action of mGluR5-mediated events at neuronal synapses, and not altering upstream Aβ accumulation or glial reaction to its accumulation.

BMS-984923 treatment reverses synapse loss in AD mice

We analyzed the density of synaptic markers by immunostaining, since synaptic depletion is a characteristic of AD tightly linked to memory deficits (Scheff 1990), and expected to be downstream of Aβo/PrP^C/mGluR5 signaling. By predetermined automated quantitation method applied without knowledge of group, we observed a significant loss of the presynaptic marker SV2a as well as the postsynaptic marker PSD95 in transgenic brain compared to wild type (Figure 6) (Haas 2017). Importantly, BMS-984923 treatment significantly recovers synaptic marker levels to wild type levels (Figure 6B, C). Thus, restoration of synapse density is a plausible explanation for memory recovery by SAM therapy, via interruption of Aβo signaling without changes in glutamate/mGluR5 coupling.

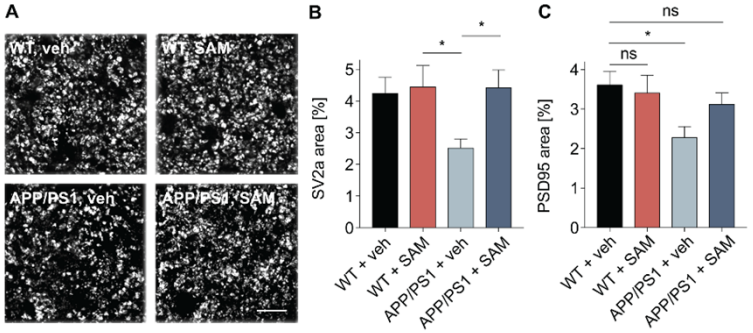

Figure 6 SAM (BMS-984923) recovers loss of synaptic markers in APP/PS1 mice after 5 weeks of treatment. A. Representative immunofluorescent images of the dentate gyrus of indicated groups stained with SV2a. Scale, 12 µm. B. Fractional area of immunoreactive puncta for SV2a in the dentate gyrus. One-way ANOVA with Tukey post hoc comparisons shows that vehicle-treated APP/PS1 group differs significantly from the SAM-treated WT group as well as the SAM-treated APP/PS1 group (*, p<0.05). Mean ± SEM, n=15-23 mice per group. C. Fractional area of immunoreactive puncta for PSD-95 in the dentate gyrus. Mean ± SEM, n=16-23 mice per group. One-way ANOVA with Tukey post hoc comparisons shows that the vehicle-treated APP/PS1 group differs from the vehicle-treated WT group (*, p<0.05), but the SAM-treated APP/PS1 group is rescued to WT levels (ns, p>0.05).

We found that Pyk2 as well as eukaryotic elongation factor 2 (eEF2) are aberrantly activated in transgenic AD model brain as measured by phosphorylation of specific sites (Haas 2016; Kaufman 2015; Um 2013). Specifically, the pPyk2/Pyk2 ratio in cortical lysates of APP/PS1 vehicle group is 1.57±0.14 relative to WT vehicle control ratio defined as 1.00±0.05 (p=0.0005 by ANOVA with Tukey post-hoc; mean±SEM, n=22-26). BMS-984923 therapy does not affect signaling in the wild type brain (Haas 2017), but significantly relieves signaling abnormalities in the APP/PS1 transgenic brain with a pPyk2/Pyk2 ratio of 1.13±0.07 (n.s. *vs* WT ; p=0.014 *vs* APP/PS1 veh). Thus, normalization of Aβo/PrP^C/mGluR5 signal transduction abnormalities in BMS-984923 treated transgenic mice is likely to underlie synaptic and behavioral recovery.

BMS-984923 rescues Tau pathology in triple transgenic mice

Pathological tau is a further characteristic of AD, but it is not modeled in the APP/PS1 transgenic model. Therefore, we studied triple transgenic (3xTg) mice expressing human mutant Tau, as well as mutant APP and PS1 (Oddo 2003), in which pathology driven by the Tau transgene is enhanced by the APP/PS1 transgene pathology.

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We treated 3xTg mice with 7.5 mg/kg/d of BMS-984923 over 4 weeks to investigate the effect of SAM on tau pathology. Phospho(S199/S202)-Tau is significantly enhanced in 3xTg brain compared to wild type brain in RIPA-soluble fractions (5.1±0.8 fold, mean±SEM, n=4), which is recovered close to wild type levels in 3xTg mice treated with BMS-984923 (2.5±0.2 fold, $p \leq 0.01$ *vs* 3xTG veh by ANOVA with Dunnett post-hoc) (Haas 2017). We also observed an increase of total Tau in formic acid extracted RIPA-insoluble brain fractions in 3xTg mice (1.7±0.1 fold), which is reduced to wild type levels by BMS-984923 therapy (1.2±0.1 fold, $p \leq 0.05$ *vs* 3xTG veh by ANOVA with Dunnett post-hoc). We further analyzed pathological Tau by immunostaining of 3xTg brain treated with vehicle or BMS-984923 compared to wild type brain. Without intervention, there is a trend to increased phospho(S199/S202)-Tau immunostaining in the hippocampal CA1 area as well as in the frontal cortex of 3xTg mice. The pathological accumulation is eliminated by BMS-984923 (Haas 2017). Thus, BMS-984923 induced conformational changes of the PrP^C-mGluR5 complex are beneficial for reducing Tau pathology.

BMS-984923 rescues synapse loss by PET in APP/PS1 and double KI mice

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To further maximize translational relevance, we conducted preclinical studies in which mice were aged to the point that synapse loss was detectable using SV2A PET, a new tool for monitoring synapse density with documented clinical utility in AD and other conditions. First generation scans utilized [11C]UCB-J (Chen 2018; Finnema 2016; Toyonaga 2019), but here we used the longer half-life, higher resolution [^{18}F]SDM-8 ligand (Li 2019) to further enhance clinical translation. At 12 months of age, synapse density is reduced in the hippocampus and cerebral cortex of APPswe/PS1ΔE9 (Jankowsky 2004) mice relative to WT (Figure 7A). Critically, rescans of the same mice after a one month treatment course with the mGluR5 SAM, showed a highly significant increase in hippocampal/brainstem [^{18}F]SDM-8 SUVR-1 to a level matching WT mice (Figure 7B, C).

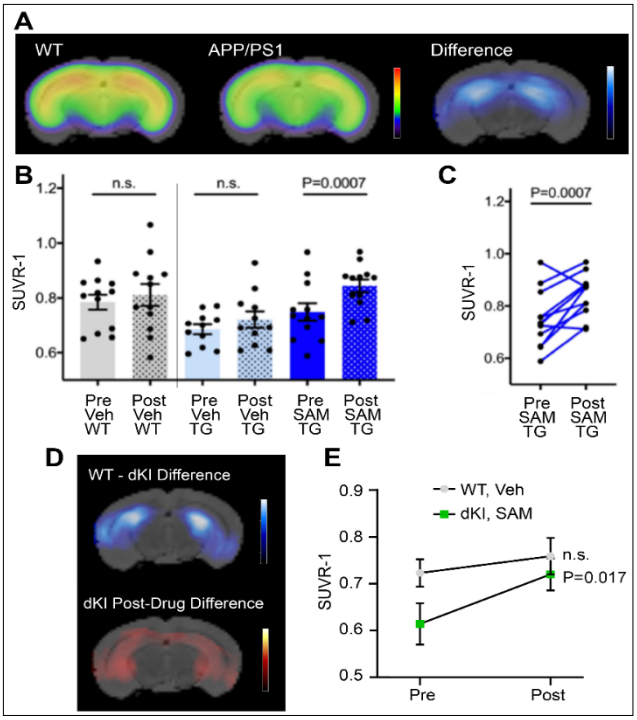

Figure 7 Rescue of Interruption of Sy
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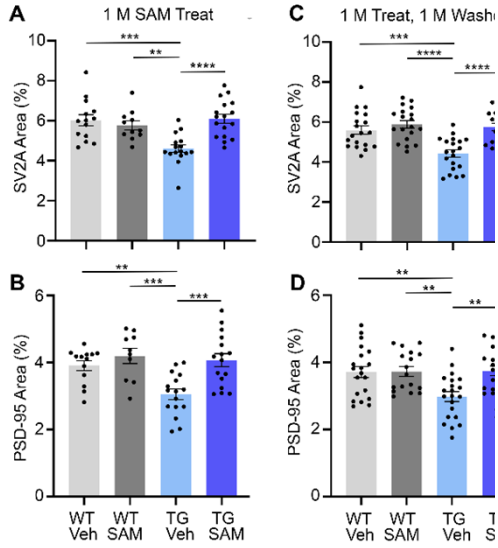

Figure 8 Disease-Modifying BMS984923 Benefit after Washout. (A, B) Transgenic APPswe/PS1ΔE9 mice at 12 months of age were treated by oral gavage with 3.75 mg/kg BMS984923 or vehicle twice a day. After one month treatment, brain was collected for immunohistology. Fractional area of immunoreactive puncta for SV2A (A) or PSD-95 (B) in the dentate gyrus is shown. Each mouse is shown as a dot, mean ± sem. One-way ANOVA with Tukey's post hoc comparisons as indicated. **, P<0.01, ***, P<0.001, ****, P<0.0001. (C, D) A separate group of transgenic APPswe/PS1ΔE9 was treated by oral gavage with 3.75 mg/kg BMS984923 or vehicle twice a day from 12-13 months of age and then treatment was stopped for one month prior to tissue collection and analysis for SV2A (C) and PSD-95 (D) by the same method as in A, B. Since the half-life of BMS984923 in mice is approximately 8 hours, the concentration of drug is nil after 3 days (9 half-lives) and washout persists for 3.5 weeks beyond this time. Statistics as in A, B.

Transgenic mouse overexpression models of AD (such as APPswe/PS1ΔE9 (Jankowsky 2004)) mimic certain aspects of AD, including Aß plaque, soluble Aßo, synapse loss, and age-dependent memory impairment. However, confidence in these phenotypes is restricted due to mutant human protein overexpression, competition with WT mouse genes and transgenic promotors. One way to overcome these issues is to utilize APP knock-in strains (Saito 2014; Saito 2019). We are using the NL-G-F knock-in allele, which inserts Aß-containing exons from human APP containing the Swedish, Arctic and Iberian mutations into the mouse locus. By replacing the relevant murine coding sequence, expression level and pattern are maintained. Such mice develop Aß plaque, decreases in synaptic markers and memory deficits (Saito 2014). In addition, Dr. Saido has

generated a strain in which the human wild type *MAPT* locus with its full sequence and splice sites replace the murine locus. In the *APP^{NL-G-F}/hMAPT* double knock-in strain (hTau-NLGF dKI, or simply dKI here) there is a moderate increase of MAPT phosphorylation at AD-relevant sites detected by AT8 and PHF-1 antibodies, as well as greater spread of misfolded human Tau aggregation (Saito 2019). We repeated the mGluR5 SAM treatment in this strain with PET imaging of SV2A sites. At 12 months of age, there is a decrement in synapse density in the dKI strain (Figure 7D). Importantly, the one month BMS984923 treatment restores the PET signal upon rescans of the same mice (Figure 7D, E). Thus, in multiple strains, blocking the PrP^{C}/mGluR5 pathway prevents synapse loss, allowing recovery.

We also tested whether the mGluR5 SAM (Figure 8) and anti-PrP^{C} treatment (Cox 2019) had a disease-modifying effect in the weeks after the treatment was stopped. Here, we used the APP/PS1 strain and monitored the area of SV2A and PSD-95 puncta in the hippocampus after one month treatment followed by one month washout of the compounds. In both cases, the decrement of synaptic area of transgenic mice was recovered by treatment, and the benefit persisted one month (Figure 8 and (Cox 2019)). We suspect that synapse loss would gradually recur at latter times; the initial synapse loss after Aß accumulation requires 4-6 months to develop. However, the data show that there is clearly a disease-modifying benefit of blocking the PrP^{C}/mGluR5 synaptic signaling pathway.

Receptor occupancy by FPEB-PET

The Yale PET Center has substantial experience in performing brain receptor occupancy studies. These are typically performed first in nonhuman primates (NHP), and subsequently in human participants. Examples of occupancy studies in nonhuman primates include occupancy at norepinephrine transporters (Gallezot 2011), serotonin receptors (Cosgrove 2011), dopamine receptors (Gallezot 2012), acetylcholine receptors (Hillmer 2016), glycine transporters (Castner 2014; Xia 2015), histamine receptors (Sawant-Basak 2017), kappa opioid receptors (Kim 2013), and synaptic vesicular protein (SV2A) (Nabulsi 2016; Nicolas 2016). In general, we have found there to be good correlation between occupancy in NHP and occupancy in humans, following adjustments for bioavailability and plasma free fraction of drug.

At Yale, we have developed [^{18}F]FPEB as a mGluR5 specific PET ligand (Esterlis 2017; Park 2015; Sullivan 2013). This included development of infusion methodology (Park 2015; Sullivan 2013), which allowed convenient application in clinical populations. Here, we assess receptor occupancy in brain by characterizing competitive displacement of the FPEB from the shared binding site after pre-administration of different BMS-984923 concentrations in multiple species. Our plan will allow receptor occupancy to guide dose equivalency across species in translation.

In mice, we measured the % decrease in brain FPEB uptake after dosing mice with the minimal SAM required to achieve rescue of transgenic AD mouse synapse density and memory. mGluR5 occupancy in mice was calculated using SUV

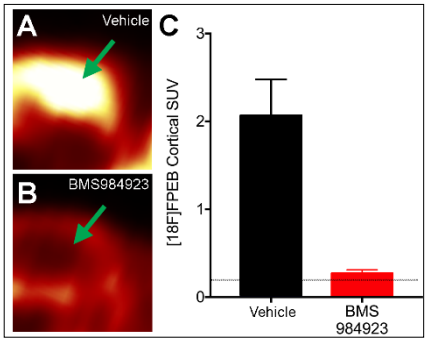

Figure 9 BMS-984923 blocks [^{18}F]FPEB brain uptake. A-B. Two mice received BMS-984923 (7.5 mg/kg by oral gavage, B) and two mice received vehicle (A) one hour prior to injection of [^{18}F]FPEB tracer. A static PET image was acquired on the Inveon microPET/CT for 15 min beginning 45 min post-injection of tracer. The image presents a sagittal view containing the brain (arrow) with rostral left, caudal right, dorsal up and ventral down. **C.** The CT was used to delineate a cortical region of interest. An average value for the region was generated and converted to Standard Uptake Values (SUV), with correction for weight and injected dose. Data are mean ± SEM. The 87% inhibition by BMS-984923 is likely an underestimate of receptor occupancy since no correction for non-displaceable binding was performed. Typical non-displaceable uptake level in the presence

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values. Our studies in adult WT mice demonstrated that BMS-984923 (7.5 mg/kg p.o. pretreatment) blocks 87% of total [^{18}F]FPEB brain uptake (Figure 9). PET imaging will be used to assess mGluR5 occupancy of BMS-984923 in human participants

The present clinical evaluation of BMS-984923 is being conducted in healthy participants. Safety and tolerability studies in this population will allow longer term safety studies and ultimately efficacy studies in participants with AD. Please refer to the Investigator's Brochure (IB) for a more detailed description of toxicology investigations.

Clinical Experience with BMS-984923

The present study represents the first evaluation of BMS-984923 in humans.

4. Research Plan: Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths. Describe the setting in which the research will take place.

   Overall Study Design

   This will be conducted at the Yale University Alzheimer's Disease Research Unit (ADRU), the Yale PET Center, and Yale Hospital Research Unit (HRU).

   Eligible participants will be between the ages of 50 to 80 years old, male or female, with normal cognition and without neuropsychiatric diagnoses.

   This study is an open-label, single ascending dose study in health participants. Up to six cohorts of 6 participants each are planned where the participants within each cohort are expected to receive the same dose of study drug. A total of approximately 36 participants will be enrolled and each will receive a single dose of BMS-984923.

   If the Maximum Tolerated Dose (MTD) is reached prior to the sixth cohort, the remaining participants may be enrolled to obtain further data at the tolerated dose levels. During this process, the Principal Investigator (PI) (Dr. Adam Mecca), the medical monitor, and the DSMB must agree to proceed with the study and escalate to the next dose cohort, independent of the influence of the IND holder (Dr. Stephen Strittmatter) and in compliance with the management of the Conflict of Interest for Dr. Strittmatter. The process is further described in Section 12 (Data and Safety Monitoring Plan). Briefly, a safety assessment will be completed and reviewed for all treated participants per cohort prior to opening enrollment into the next increased dose level.

   The study periods protocol are as follows:

   - Screening: (within 90 days prior to Day 1) Patients have 90 days from the time of signing informed consent to complete their screening assessments and, if needed, their washout period for prohibited concomitant medications. The screening laboratory tests must be completed within 30 days prior to Day 1.

   - Treatment period: Day 1: Admission, administration of study drug, and 2 night in-patient stay; Day 3: Discharge following the completion of all scheduled procedures.

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- **Follow-up:** Participants will have follow-up visits for up to 7 days post-dose as follows: Participants will return to clinic for visits on Study Days 4 and 7. Participants will receive a phone call on Study Day 5 to inquire about their general health.

Screening

The PI or designee is responsible for administering and obtaining freely given consent in writing from all participants using the study-specific Informed Consent Form (ICF) prior to undergoing any screening procedures; this may also include consent to participate in the optional receptor occupancy substudy. Screening procedures will take place within 90 days prior to Day 1. The participant's eligibility (per Inclusion and Exclusion Criteria listed in Section 8), demographics, medical history, and concomitant medications will be recorded. Screening/baseline assessments will be performed, and participants will undergo blood draws for laboratory tests. Urine samples will also be collected. If eligibility criteria are met, and the participant is enrolled in the receptor occupancy substudy, a brain MRI will be performed for quantitative evaluation of PET scans. The MRI will be reviewed and participants with anatomical abnormalities or findings indicating neurological disease will be excluded. A full list of procedures and assessments is included in Table 3: Schedule of Study Events.

If a participant is rescreened, the original screening MRI and expert review of the images may be used for the rescreening provided the MRI was completed within 6 months prior to study drug administration. Similarly, neurologic examinations and questionnaires do not need to be repeated provided these assessments were completed within 6 months prior to study drug administration.

In-Patient Treatment Period

Eligible participants will be admitted to the HRU on either the evening prior to or the day of dosing (Study Day 1) depending on participant needs. Following final pre-treatment assessments, participants will receive their single dose of study drug administered orally. Dosing will be according to Table 1.

Participants will remain as in-patients until discharge on Study Day 3 and will undergo safety assessments and close observation.

Blood samples will be obtained to assess safety and pharmacokinetics (PK). The timing for PK sample collection may be adjusted for subsequent cohorts based on data from the preceding cohorts.

Table 1: Treatment by Cohort

Cohort	Treatment	Number of Participants
1	10 mg BMS-984923	6
2	40 mg BMS-984923	6
3	70 mg BMS-984923	6
4	100 mg BMS-984923	6
5	150 mg BMS-984923	6

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6	200 mg BMS-984923	6

Follow-Up
Site will contact the participants via telephone on Study Days 5 to inquire as to their general health status.

Participants will return to the Clinic on Study Days 4 and 7 for PK sample collection, safety laboratory blood tests, vital signs, concomitant medications, AEs, and a general health status assessment according the Schedule of Study Events (Table 3).

Number of Participants
This study will enroll approximately 36 participants in cohorts of 6 participants each.

If the dropout rate is such that the power of the study could be compromised, or that the study objectives cannot reliably be achieved, the PI, in consultation with the medical monitor and agreement of Dr. Strittmatter (FDA IND holder) may elect to replace those participants who discontinue prematurely.

Treatment Assignment
Six sequentially-treated cohorts planned. BMS-984923 will be administered orally in a single dose.

The starting dose of 10 mg is estimated to be below the pharmacologically active dose. Dose escalation will proceed to 40 mg, 70 mg, 100 mg, 150 mg, and 200 mg contingent on safety and tolerability.

Dose Escalation Decisions
Determination of whether to open the next escalated dose cohort for enrollment will be made jointly by the PI, medical monitor, DSMB, and IND holder after review of all available clinical, safety, and PK data (See Section 12 for details of the Data and Safety Monitoring Plan).

Determination of whether to open the next escalated dose cohort for enrollment will be made jointly by the PI, medical monitor, DSMB, and IND holder (Dr. Strittmatter) after review of all available clinical, safety, and PK data. Dose escalation (for each sequential cohort) will not occur until all participants in the prior cohort have completed the study. The DSMB will perform an independent review. In addition, the PI and medical monitor will make a determination independent from the IND holder. Dr. Strittmatter will be consulted in scenarios where alterations to the planned dose escalation strategy are being considered. As the IND holder, Dr. Strittmatter must also agree with a decision to proceed with dose escalation before the next cohort is dosed.

Selection and Withdrawal of Participants
Participants must meet the inclusion and exclusion criteria as described in Section 8: Inclusion/Exclusion Criteria.

Participant Withdrawal and Discontinuation
Participants may withdraw from the study at any time at their own request, or they may be discontinued at any time at the discretion of the PI for safety, behavioral, or administrative reasons. The reason for a participant discontinuing from the study will be recorded in the source documents and case report form (CRF).

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A withdrawal or discontinuation occurs when an enrolled participant ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The PI must determine the primary reason for discontinuation. If a participant does not return for a scheduled visit, every effort will be made to contact the participant. A participant will be considered lost-to-follow-up after 3 failed attempts to contact participant are made, including two documented attempts by phone and one certified letter. In any circumstance, every effort should be made to document participant outcome, if possible. Withdrawal due to an adverse event will be distinguished from other withdrawal reasons and will include relevant details according to the definition of adverse event noted.

A discontinuation must be reported immediately if it is due to a serious adverse event. The treatment consists of a single dose of study drug. Therefore, every attempt will be made to perform all post dose assessments, since all are designed to monitor participant safety. If a participant is withdrawn or discontinued prior to receiving study drug, then no further assessments are required. The PI will record the reason for study discontinuation, provide or arrange for appropriate follow-up (if required), and document the course of the participant's condition.

If the participant withdraws from the study and withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The PI may retain and continue to use any data collected before such withdrawal of consent.

Treatment of Participants

This study involves a single-dose oral treatment for each participant with monitoring of safety as described in the protocol.

Description of Study Medication

"Study drug" in this protocol refers to the investigational product BMS-984923.

Formulation development was contracted by Yale University to the Aptuit (Verona) division of Evotec for the development of a formulation for non-clinical toxicology studies as well as the production of a final clinical dosage form. Study medication will be packaged and capsules containing 5 mg, 50 mg or 100 mg of nano-milled Active Pharmaceutical Ingredient (API) sprayed on granules will be prepared. Medication will be stored at Aptuit and transferred as needed to the Yale Investigational Drug Service. Study medication will be distributed to the participants from the Yale Investigational Drug Service. Doses for each cohort will be achieved using combinations of the capsules listed above.

Table 2: Study Drug

	Study Drug
Product Name:	BMS-984923
Route of Administration	Oral
Physical Description	Capsules containing nano-milled Active Pharmaceutical Ingredient (API) sprayed on granules.
Manufacturer	Aptuit (Verona) division of Evotec* Aptuit (Center for Drug Discovery & Development)

	Via Fleming, 4 37135 Verona (Italy)

*Study drug was produced by Aptuit through a contract with Yale University using NIH funds and will be supplied directly for this protocol. Allyx Therapeutics is licensing this technology from both Yale University and Bristol Meyers Squibb for future commercialization.

Concomitant Medications

All concomitant medications, whether prescription, over-the-counter, herbal treatments or other therapy, taken or used by the participant within 2 months of screening through end of study assessments will be recorded in the participant's medical record and in the Concomitant Medications CRF with start and stop dates.

Regular use of medications which, in the assessment of the PI or medical monitor, may confound efficacy and/or safety assessments is prohibited. Prohibited medications may include but are not limited to those listed in the inclusion/exclusion criteria, as well as those with CYP inhibitor/inducer activity listed in Appendix A.

Prospective participants who are taking prohibited medications at the time of screening (listed in Section 8: Inclusion/Exclusion Criteria or **Appendix A**) may stop them in consultation with their physician, after signing the ICF and receiving instructions on the discontinuation of these medications. In this case, the prohibited medication must be discontinued prior to study drug administration as outlined in the Inclusion/Exclusion Criteria. The prospective participant must be willing to discontinue treatment and the PI must deem this to be feasible and safe. If a prospective participant is taking prohibited medication(s) at the time of screening, they may be considered eligible at the time of study drug administration (Visit 2, Study Day 1) provided no reintroduction of the prohibited medication is being considered, the appropriate time window since last administration has elapsed, and the participant continues to meet other eligibility criteria. Upon signing the ICF and enrollment into the study, participants must remain off prohibited medications until all assessments are completed in the study (Visit 5, Study Day 7), unless the PI determines that a medication is required for participant safety and/or to treat an (S)AE. The PI will first discuss starting prohibited medications with the medical monitor unless that cannot be done due to an immediate safety need.

Treatment Compliance

Treatment compliance with study drug during the treatment period is expected to be high, as participants will be dosed directly at the research unit under well-controlled conditions. The date and time of study drug administration, dose group, and quantity of study drug administered will be recorded on the source documents and CRF. Non-compliance with other aspects of the study protocol (e.g., use of prohibited medications, missed study visits) will be documented on the participant's source document and on the CRF.

Randomization and Blinding

This study is open-label and does not include a reference therapy, therefore there is no randomization.

Study Procedures

Participants will participate in the study for a total duration of up to 14 weeks. Visits will be scheduled at:

- Screening Visit 1 (Study Days -90 to -1)
- In-patient Treatment Visit 2 (Study Days 1, 2, and 3)
- Post Treatment Follow-up
    - Visits 3 (Study Day 4)
    - Visit 4 (Phone call on Study Day 5)
    - Visit 5 (Study Day 7)

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Refer to the Schedule of Events (Table 3) for all the procedures and assessments. The following sections provide important details of the procedures.

Screening Period, Visit 1 (Study Days -90 through -1)

Participants will undergo informed consent and sign an ICF before any screening-related procedures are performed. The Screening Visit (Visit 1) should occur between Study Days -90 to -1 and screening procedures completed during this time.

The inclusion and exclusion criteria will be carefully assessed. The participant's demographics, medical history, and concomitant medications will be recorded. See the Schedule of Study Events (Table 3) for details on the assessments on each Study Day.

Potentially eligible participants will undergo a Screening Diagnostic Evaluation to ensure study eligibility. Assessments will consist of Clinical Dementia Rating (CDR) scale (Morris, 1993), Modified Hachinski Scale (MHS) (Rosen 1980), MMSE (Folstein 1975), Geriatric Depression Scale (GDS) (Sheikh 1986), Montreal Cognitive Assessment (MOCA), vital signs, Glasgow Coma Scale (GCS), ECG, physical examination, and blood collection for screening laboratory studies.

Screening laboratory studies will include a comprehensive metabolic panel [glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, bicarbonate, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate transaminase (AST), alanine transaminase (ALT)], phosphate, a lipid panel [total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides], a complete blood count and differential, lactate acid dehydrogenase (LDH), creatine kinase (CK), amylase, thyroid function tests (TSH, reflex FT4), Vitamin B12, and a urinalysis with reflex microscopic examination. For all women, menopausal status will be confirmed by serum follicle stimulating hormone (FSH) measurement or documentation of bilateral tubal ligation or hysterectomy.

Vital signs (blood pressure, pulse rate, respiratory rate, and oral body temperature) will be obtained after the participant has been in a sitting (or recumbent) position for 5 minutes. Blood pressure will be taken from the same arm each time when possible.

The 12-lead ECG will be taken in triplicate after the patient has been in a supine position for at least 5 minutes and before blood is drawn (whenever possible). All ECGs will be read by the PI or designee. The following parameters and intervals will be assessed: HR, RR, PR, QRS, and QTc. The occurrence of depolarization or repolarization disorders, arrhythmic disorders or other abnormalities will be noted. Any clinically significant finding must be reported as an adverse event.

If an individual is participating in the receptor occupancy substudy, a MRI must be completed, and results reviewed prior to the participant's admission to the HRU on Visit 2 (Pre-Dose). Results from the MRI will be read by a local expert.

If a participant fails to meet entry criteria prior to the end of the screening period it will be considered a screen failure. Participants who have screen failed are not required to return for additional visits (although a participant can be seen at any time for safety reasons). Participants who are screen failed due to lab values can be rescreened as determined by the PI in consultation with the study medical monitor. Participants who rescreen will

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be assigned a new screening number.

Serious AEs will be recorded and monitored starting at the time of participant signing the ICF. Non-serious AEs will only be collected after the administration of the study drug.

In-patient Treatment, Visit 2 (Study Days 1 to 3 [Pre-Dose and Post-Dose])

Eligible participants will be admitted to the HRU on the evening prior to or morning of the scheduled drug dose depending on scheduling needs. If, during this visit and prior to dosing, a participant is determined to no longer be eligible to continue in the study, the appropriate Screening CRFs will be completed and the participant deemed a Screen Failure and will not be required to return for additional visits (although a participant can be seen at any time for safety reasons.)

All inclusion and exclusion criteria, medical history, and concomitant medications will be reviewed to confirm eligibility prior to performing additional study procedures/assessments. New (S)AEs and concomitant medications reported by the participant since the Screening Visit (Visit 1) will be recorded on the CRF.

If participating in the receptor occupancy substudy, results obtained during the Screening Visit (Visit 1) including the results of the MRI scan must be reviewed by the PI prior to participant treatment with study drug to ensure participant remains eligible for the substudy.

Vital signs (blood pressure, pulse rate, respiratory rate, and oral body temperature) will be obtained after the patient has been in a sitting position for 5 minutes. 12- lead pre- and post-dose ECG will be taken in triplicate after the patient has been in a supine (resting) position for at least 5 minutes. An abbreviated physical exam will be performed.

If the participant remains eligible for study participation, visit information will be entered, and the required CRFs completed. The research pharmacist will be contacted regarding the participant's enrollment and for specifics about the preparation and availability of study drug necessary for oral administration of BMS-984923.

Study Drug BMS-984923 will be administered orally as a single dose. Details including dose and quantity administered, staff administering the study drug, and date and time of administration will be recorded on the CRF and in the participant's record. Post-dose, participants will be monitored for any adverse events.

After study drug administration, vital signs will be monitored every 1 hr for the first 8 hours and then every 3 hours until discharge on Day 3. An EKG will be performed at approximately 6 hours, 24 hours, and 48 hours after drug administration. The participant will have ready access to a call button and instructed in its use. There is a registered nurse present at the HRU and a physician who is on call at all times. The HRU is within Yale New Haven Hospital where a rapid response and "code" team are also available to respond if needed.

In order to monitor for changes in consciousness, the Glasgow Coma Scale (GCS) (Teasdale 1974) will be administered pre-dose and then every 2 hour for the first 8 hours and then every 3 hours until discharge on Day 3. Participants with a GCS below 15 will be evaluated for appropriate level of inpatient care. No participant will be discharged with a GCS below 15.

In order to monitor cognitive or psychiatric side effects, the Montreal Cognitive Assessment (MOCA) (Nasreddine 2005), Geriatric Depression Scale (GDS) (Sheikh 1986) and Neuropsychiatric Inventory Questionnaire (Cummings

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1994) will be administered prior to oral dose administration, again at approximately 6 hours post-dose (approximate peak concentration) and approximately 24 hours post-dose.

If a participant is found to be depressed, a clinician will perform further assessment and refer the participant to care as necessary. As part of ongoing adverse event monitoring, participants will be asked about changes in mood during in-person and phone visits. Additionally, they will be advised to call to inform the study team of changes in mood, specifically depression, between visits.

Blood will be collected for safety laboratory studies prior to drug dose administration and on Days 2 and 3. Safety laboratory studies will include a comprehensive metabolic panel [glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, bicarbonate, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate transaminase (AST), alanine transaminase (ALT)], phosphate, a lipid panel [total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides], a complete blood count and differential, lactate acid dehydrogenase (LDH), creatine kinase (CK), amylase, and a urinalysis with reflex microscopic examination.

Plasma will be collected for PK analysis as shown in Table 4.

A 24-hour urine collection will be performed starting at the time of study drug administration. A second 24-hour urine collection will be performed from 24-48 hours after study drug administration These samples will be used to measure urine drug and metabolite levels.

The participant will remain in the HRU on Study Days 1 and 2, and discharged on Study Day 3 after all required assessments have been completed. During the inpatient stay, abnormal assessments and laboratory values will be evaluated by the PI in collaboration with the medical monitor in real time. See the schedule of events (Table 3) for details on the assessments on each Study Day.

Post Treatment Follow-up Visits

See the schedule of events (Table 3) for details on the assessments on each Study Day.

Visit 3 (Day 4) – Vital signs (BP, pulse, respiratory rate, and oral body temperature) will be obtained. Plasma samples for PK assessments will be collected. Information on changes or newly administered concomitant medications as well as any new or ongoing (S)AEs will be collected at each visit.

Visit 4 (Day 5 phone call) - Participants do not have to visit the clinic for Post-treatment follow-up on Day 5 unless necessary to follow-up on adverse events. Participants will receive a telephone call from study site personnel inquiring as to their general health. Information on changes or newly administered concomitant medications as well as any new or ongoing (S)AEs will be assessed.

Visit 5 (Day 7) – Vital signs (BP, pulse, respiratory rate, and oral body temperature) will be obtained. The MOCA, GDS, NPI-Q, and safety laboratory assessments will be repeated on day 7. Plasma samples for PK assessments will be collected. Information on changes or newly administered concomitant medications as well as any new or ongoing (S)AEs will be collected at each visit.

Pregnancy Follow-up – Although we will exclude females of child-bearing potential, if a woman becomes pregnant during the study, we will follow it to outcome.

Table 3: Schedule of Study Events[1]

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Study Phase	Screening	Inpatient Treatment				Post-Treatment Follow-Up		
Study Day	-90 through -1	1		2	3	4	5	7 ± 1 days
		Pre-Dose	Post					
Visit Number	1	2				3	4[2]	5
Informed consent	X							
Assign Participant ID	X							
Inclusion & Exclusion Criteria	X	X						
CDR	X							
Modified Hachinski	X							
MMSE	X							
GDS	X	X	6 h	24 h				X
MOCA	X	X	6 h	24 h				X
NPI-Q		X	6 h	24 h				X
Instructions for potential washout period for concomitant medications	X							
Medical History	X	X						
Full Physical Examination	X							
Demographics	X							
Vital signs (BP, pulse, RR, oral temp)[3]	X	X	X	X	X	X		X
GCS[4]	X	X	X[4]	X[4]	X[4]	X		X
ECG[5]	X	X	X[5]	X[5]	X[5]			
Confirmation of non-child bearing potential for women	X							
MRI[6]	X							
Admission/Discharge to/from HRU		Admit			Discharge (48 h post-dose)			
Physical Examination (abbreviated)		X		X				
Dosing of Investigational Product[7]		X						
Study Drug Accountability			X					
Urine metabolites			24-hour urine collection	24-hour urine collection				
Plasma for PK[8]		X[8]	X[8]	X[8]	X[8]	X[8]		X[8]

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Clinical laboratory sample collection	X	X		X	X			X
Urinalysis	X	X		X				X
FSH (all females without documented bilateral tubal ligation or hysterectomy)	X							
[^{18}F]FPEB PET Scans[9]	X		X	X				
Concomitant Medications	X	X	X					
Adverse Events[10]		X	X					
Serious Adverse Events	X	X	X					

[1]Abbreviations: AE = adverse events, ECG = electrocardiogram, ID = identification, PK = pharmacokinetic, SAE = serious adverse event, GCS = Glasgow Coma Scale

[2]Visit 4 is a phone visit.

[3]Vital signs (blood pressure, pulse rate, respiratory rate, and oral body temperature) will be measured after the patient has been in a sitting or recumbent position for 5 minutes. Frequency noted below:

Screening visit - Once
Day 1 – Once pre-dose, once post-dose, then every 1 hour for the first 8 hours following dosing, then every 3 hours
Day 2 and 3- every 3 hours until discharge
Day 4, 7 - Pre-blood draw

[4]GCS will be administered pre-dose, post-dose, every 2 hours for the first 8 hours and then every 3 hours until discharge on Day 3.

[5]ECGs will be taken in triplicate and will be obtained after the patient has been in a supine (resting) position for at least 5 minutes. At Visit 2, triplicate ECGs will be performed pre-dose, approximately 6 hours post-dose, and approximately 24 and 48 hours post-dose.

[6]MRIs will be read by an expert prior to participant enrollment into the study and only performed for participants enrolled in the receptor occupancy sub-study.

[7]The study drug will be administered in a fasted state. Dosing will occur at least 8 hours after the last meal and no food will be provided for 1 hour post-dose.

[8]PK sampling will be done at the time points shown in Table 4. Participants in the receptor occupancy sub-study will also have up to 5 plasma PK samples collected during each PET scan for a total of up to 15 additional PK samples.

[9]Only for participants enrolled in the receptor occupancy sub-study. A pre-dose (baseline) scan will be performed within 60 days of study drug administration. Post-dose scans will be performed at approximately 4 and 24 hours after dose administration. The baseline [^{18}F]FPEB PET scan may be done after the inpatient and clinic follow-up visits if needed.

[10]AEs are only collected upon the start of study drug. Events occurring prior to this are to be reported as part of the participant medical history. However, SAEs are to be collected from the time of informed consent

Assessment of Efficacy and Pharmacokinetics

Efficacy

This study is focused on safety, tolerability, and pharmacokinetics in healthy participants. Therefore, no efficacy assessments will be performed as primary outcomes.

Exploratory endpoints will include drug receptor occupancy assessment with [^{18}F]FPEB PET by comparing within-participant changes in mGluR5 availability pre-treatment and at the time of presumed maximal brain drug concentration. This analysis will be performed in a subset of 2 participants per dose cohort for the 10-100 mg dose levels. PET scans may occur in a subset of 2 participants per cohort for dose level 150 mg and 200 mg.

Pharmacokinetics

Plasma samples will be collected for analysis, as shown in Table 4. The timing for plasma sample collection may be adjusted for subsequent cohorts based on PK data from the preceding cohorts.

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Table 4: Schedule for Pharmacokinetic Sampling*

	Day 1 (Dosing day)	Day 2	Day 3	Day 4	Day 7
Plasma Samples	Pre-Dose, and post-dose at 30 min (± 10 min), 1 h (± 10 min), 2 h (± 10 min), 4 h (± 10 min), 8h (± 1 h), 12 h (± 1 h)	24 h (± 1 h)	48 h (± 2 h)	72 h (± 6h)	Day 7 ± 1 day
Urine Samples	24 hour collection starting immediately post-dose	Second 24 hour collection from 24 to 48 hours post-dose			

*Participants in the receptor occupancy sub-study will have up to five additional PK samples collected during each PET scan. The timing of these samples will be with respect to [^{18}F]FPEB injection as follows: within 10 minutes prior to tracer injection, 1 h after tracer injection, and 2 hours after tracer injection.

Pharmacokinetic assessment will be carried out at Aptuit (Verona) Center for Drug Discovery and Development upon shipment to:

Luciana Romanelli/ Elena Bonesini/Michela Vecchini
Aptuit (Center for Drug Discovery & Development)
Via Fleming, 4 37135 Verona (Italy)
Email box: VERGMSampleManagement@evotec.com
Tel: + 39 045 8218342 or +39 045 8218854 or +39 045 821960

Remaining plasma and urine may be transferred to a separate (non GLP) experimental protocol wherein individual samples will be pooled and used to investigate the metabolism and excretion of study drug and related components.

Receptor Occupancy Substudy

The goal is to collect adequate PET scan data for at least 2 participants per cohort. Up to four participants in each cohort may participate in the Receptor Occupancy Sub-study. These participants will undergo an MRI during screening and three [^{18}F]FPEB PET Scans. PET scans will occur at screening (within 60 days of study drug administration), approximately 4 hours post-dose and approximately 24 hours post-dose. The timing of post-dose PET scans may be adjusted after review of human PK data collected in this study. Each participant may be asked to repeat up to one [^{18}F]FPEB PET scan if a scan fails quality control for a total of up to four [^{18}F]FPEB PET scans during the course of study participation. The baseline [^{18}F]FPEB PET scan may be done after the inpatient and clinic follow-up visits if needed.

The rationale for enrolling up to four participants from each Cohort in the sub-study is to help ensure that quality [^{18}F]FPEB PET scan data is collected at all three timepoints on at least two participants per cohort. This over-enrollment is necessary due to possibility of failed [^{18}F]FPEB PET tracer synthesis and the time sensitive nature of the post-dose [^{18}F]FPEB PET scans. The goal is to collect adequate PET scan data for 2 participants per cohort. We will not scan additional participants per cohort once adequate scan data has been captured.

MRI Procedures

Magnetic Resonance Imaging (MRI) will be performed for enrollment in the receptor occupancy substudy. To be screened by MRI, participants must meet all clinical eligibility criteria. MRI will be used for quantitative evaluation

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of PET scans, to screen for anatomical abnormalities, and to screen for neurological disease. MRI exclusion criteria will consist of focal or global atrophy, evidence of chronic or acute cerebrovascular disease, any space occupying lesion or other indication of underlying pathology. All MRIs will be read by a local expert to ensure consistent interpretation. MRIs will take place at the Anlyan Center, 300 Cedar Street, New Haven, CT.

MRI will be performed on a 3 Tesla system using pulse sequences selected to accommodate clinical reading, anatomical identification of VOIs, gray/white matter segmentation, and registration to a common space. Sequences will include:

- 3 Plane/Tri-Planar Scout
- Axial T2 Star/GRE
- Axial T2 FLAIR
- Axial fcMRI (Participant should have eyes OPEN)
- Sagittal 3D Accelerated MPRAGE/IRSPGR
- Axial DWI
- Axial DTI
- Axial PASL (eyes open)
- High Resolution Hippocampal T2

PET Scan Procedures

PET scanning sessions will take place at the Yale University PET Center, 801 Howard Ave, New Haven, CT. Participants with history of prior radiation exposure within the past year such that participation in this study would place them over FDA limits for annual radiation exposure will be excluded from this study.

Arterial Line Placement: A radial artery catheter will be inserted by an experienced provider before the PET scan to draw arterial blood samples for metabolite analysis and for determination of the fraction of plasma radioactivity unbound to protein. The goal of the arterial line is to be able to measure absolute physiological functions by mathematically relating the signal (from the PET scanner) to the tracer availability (from the blood). This approach is the gold standard for obtaining quantitative PET data and is required for receptor occupancy studies using orally administered drug. In scenarios where the arterial line cannot be placed or maintained, an additional intravenous catheter may be placed for blood sampling.

Radiotracer Injection: At each PET scanning session, participants will receive ≤ 5 mCi of [^{18}F]FPEB administered by bolus injection via a venous catheter. The participant's head will be immobilized and a transmission or CT scan will be obtained and used for attenuation correction. Vital signs will be taken immediately prior to injection of [^{18}F]FPEB and will be monitored during the scan. Any adverse events will be evaluated and recorded continuously through the PET imaging visit.

Scanning Session: The scanning period is 0 - 120 min post injection. Dynamic images of radioactivity concentration are reconstructed with corrections for attenuation, normalization, random events, scatter, and dead time.

Post-Scan: Upon completion of the PET Scanning days, the IV and arterial catheter will be removed and local pressure applied at the arterial catheter site for a minimum of 15 minutes to prevent bleeding under the skin. A pressure dressing will then be applied.

Inclusion of women of childbearing potential: Women who are pregnant or lactating, and women of

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childbearing potential will be excluded from the study. The PI will provide the PET center with documentation of non-childbearing potential status of female participants.

Radiation Exposure: Participants in this sub-study will receive a target radiation exposure from three injections of ≤ 5 mCi of [^{18}F]FPEB. This is equal to an effective dose of 0.930 rem. If a scan fails quality control, participants may be asked to repeat one [^{18}F]FPEB PET scan. If a scan is repeated, participants will receive radiation exposure from four injections of ≤ 5 mCi of [^{18}F]FPEB. This is equal to an effect dose of 1.24 rem.

Participants will also receive radiation exposure from either 3 low dose head CT scans or 3 transmission scans. If scans are completed on the High Resolution Research Tomograph (HRRT), participants will receive up to 0.0045 rem from transmission scans. If scans are completed on the mCT, participants will receive up to 135mrem (0.135 rem) from low dose head CT scans.

Participants who repeat a [^{18}F]FPEB PET scan will receive radiation exposure from an additional low dose head CT scan or transmission scan for a total of 4 low dose head CT scans or 4 transmission scans. If scans are completed on the HRRT, these participants will receive up to 0.006 rem from transmission scans. If scans are completed on the mCT, these participants will receive up to 180mrem (0.180 rem) from low dose head CT scans.

The targeted amount of radiation exposure participants will receive from this study is an effective dose of 1.065 rem. The maximum possible radiation exposure participants will receive from participating in this study, if a scan is repeated, is an effective dose of 1.42 rem.

5. Genetic Testing N/A ☒

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned

ii. the plan for the collection of material or the conditions under which material will be received

iii. the types of information about the donor/individual contributors that will be entered into a database

iv. the methods to uphold confidentiality

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?

C. Is widespread sharing of materials planned?

D. When and under what conditions will materials be stripped of all identifiers?

E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?

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i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?

F. Describe the provisions for protection of participant privacy

G. Describe the methods for the security of storage and sharing of materials

6. Subject Population: Provide a detailed description of the types of human subjects who will be recruited into this study.

The study will recruit healthy male and female adults. Up to six cohorts of six participants will be recruited for a total of 36 participants.

7. Subject classification: Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐Children	☒ Healthy	☐Fetal material, placenta, or dead fetus
☐Non-English Speaking	☐ Prisoners	☐Economically disadvantaged persons
☐Decisionally Impaired	☐ Employees	☐Pregnant women and/or fetuses
☐Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

8. Inclusion/Exclusion Criteria: What are the criteria used to determine subject inclusion or exclusion?

Participant Inclusion Criteria
Participants must meet all of the following inclusion criteria on the day of drug administration (Day 1) to be eligible for enrollment into the study:

- Men or women between the ages of 50 and 80 years, inclusive
- No history of cognitive impairment
- Capable of providing written informed consent and willing to comply with all study requirements and procedures
- Participant is not pregnant, lactating, or of childbearing potential
    1. Non-childbearing potential for women is defined as postmenopausal (last natural menses greater than 24 months; menopausal status will be documented with serum follicle stimulating hormone (FSH) or documentation of bilateral tubal ligation or hysterectomy
    2. Male participants who are sexually active with a woman of child-bearing potential must agree to use condoms during the trial and for 3 months after the last dose unless the woman is using an acceptable means of birth control. Acceptable forms of birth control include abstinence, birth

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control pills, or any double combination of: intrauterine device (IUD), male or female condom, diaphragm, sponge, and cervical cap.

3. Male participants must also agree not to donate sperm for 90 days after the last dose.

- GCS of 15 (Teasdale & Jennett, 1974)
- CDR of 0 (Morris 1993)
- Has a reliable study partner who has frequent contact with the participant (e.g., average of 10 hours per week or more), who can be available for study partner assessments, who can accompany the participant for 48 hours, without absence, after discharge from Visit 2.
- Score on the MMSE > 26 (Folstein 1975)

Participant Exclusion Criteria

Participants who meet any of the following criteria will be excluded from the study:

- Body mass index (BMI) > 38 kg/m2 or body weight < 50 kg.
- Significant cerebrovascular disease: Modified Hachinski score > 4.
- Any significant neurologic disease, such as AD, Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma followed by persistent neurologic defaults or known structural brain abnormalities.
- Major depression, bipolar disorder as described in DSM-IV within the past 1 year.
- Psychotic features, agitation or behavioral problems within 3 months, which could lead to difficulty complying with the protocol.
- History of schizophrenia (DSM IV criteria).
- History of alcohol or substance abuse or dependence within the past 2 years (DSM IV criteria).
- Clinically significant or unstable medical condition, including uncontrolled hypertension, uncontrolled diabetes, or significant cardiac, pulmonary, renal, hepatic, endocrine, or other systemic disease in the opinion of the PI, may either put the patient at risk because of participation in the study, or influence the results, or the patient's ability to participate in the study.
- Clinically significant abnormalities in B12 or TFTs that might interfere with the study.
- Use of psychoactive medications (typical neuroleptics, narcotic analgesics, antiparkinsonian medications, systemic corticosteroids, or medications with significant central anticholinergic activity) within 2 weeks or 5 half-lives (whichever is greater) prior to study drug administration and for the duration of the trial.
- Use of medications with significant CYP1A2, 2D6, or 3A4 inhibitor or inducer activity (See Appendix A) for a list of these medications) within 2 weeks or 5 half-lives (whichever is greater) prior to study drug administration and for the duration of the trial.
- Use of anticoagulants within 30 days or 5 half-lives (whichever is greater) prior to study drug administration and for the duration of the trial.
- Use of investigational amyloid lowering therapies within 2 months prior to study drug administration and for the duration of the trial.
- Use of another investigational agent within 30 days or 5 half-lives (whichever is greater) prior to screening and for the duration of the trial.
- Neutropenia defined as absolute neutrophils count of < 1,500/microliter.
- Thrombocytopenia defined as platelet count < 100,000/microliter.
- Clinically significant abnormalities in screening laboratories, including Aspartate aminotransferase (AST) >1.5 times ULN; Alanine aminotransferase (ALT) >1.5 times ULN; Total bilirubin >1.5 times ULN; Serum creatinine >2.0 times ULN.

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Receptor Occupancy Substudy Eligiblity Criteria

Inclusion Criteria

- Eligibility for and enrollment in Main Study
- Participant consent to the optional substudy

Exclusion Criteria

- Screening/baseline MRI scan with evidence of infection, infarction, or other focal lesions.
- Presence of multiple lacunes or lacunes in a critical memory structure in the brain as evidenced in an MRI.
- Any contraindications for MRI studies, including claustrophobia, the presence of metal (ferromagnetic) implants, metal fragments or foreign objects in the eyes, skin, or body or a cardiac pacemaker.
- Current or recent participation in research procedures involving radioactive agents such that the total radiation dose exposure to the participant in any given year would exceed the limits of annual and total dose commitment set forth in the US Code of Federal Regulations (CFR) Title 21 Section 361.1.

9. How will **eligibility** be determined, and by whom?
   See above question for specific eligibility criteria. Eligibility will be determined by the PI based on review of Inclusion/Exclusion criteria.

10. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

    Risks Associated with BMS-984923

    BMS-984923 has not previously been administered to humans. Toxicology studies have been completed in rat and non-human primate, with no adverse effects at the doses comparable to the planned doses. At doses higher than those planned for this study in humans [see Investigator Brochure (IB) section 4.3.2.1), serious adverse effects related to drug accumulation were observed in rats receiving repeated doses greater than 15 mg/kg/day. These adverse effects were gradual in onset over days and were manifest by lethargy, generalized stress responses, and death without organ specific or acute toxicity. This constellation of serious adverse effects was reversed after cessation of daily 100 mg/kg/day administration. There was no evidence for acute onset cardio-respiratory or CNS toxic effects. In order to mitigate the risk of similar adverse effects in humans, our planned maximal dose is well below the human equivalent of the rat and monkey no observed adverse effect level (NOAEL). Please refer to the Investigator Brochure (IB) for more detailed information on toxicology investigations.

    Risks Associated with Electrocardiograms (ECGs)

    There is no pain or discomfort during an ECG; however, removing the pads may cause some irritation to the skin.

    Risks Associated with Blood Drawing and IV Line Insertion

    Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. A total of approximately 70 mL (~ 5 tablespoons) of blood will be collected from each participant during this study. A total of approximately 140 mL (~10 tablespoons) of blood will be collected from each participant in Cohort 4 and subsequent dose cohorts.

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Psychological Stress
The tests used to assess the participant's mental performance may sometimes cause anxiety or fatigue.

Risks Associated with Unanticipated Events
The participant's health and safety will always be the primary concern of the PI and staff performing the study. In the event of an unanticipated event, all necessary medical action will be taken. Medication might be administered as needed, per the YNHH HRU/ Yale PET Center standard operating procedure for medical emergencies, in order to treat any unanticipated events/complications.

Receptor Occupancy Substudy Risks
Risks Associated with MRI
Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

Participants will be watched closely throughout the study. Some people may feel uncomfortable or anxious. If this happens, participants may ask to stop the study at any time and we will take the participant out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will have participants tell the research staff if they have these symptoms.

There are some risks with an MR study for certain people. Participants with a pacemaker or metal object inside their body will be excluded because the strong magnets in the MR scanner might harm the participant. Another risk is the possibility of metal objects being pulled into the magnet and hitting the participant. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once participants are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The PI, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a scan, a radiologist will be asked to review the relevant images. Based on his or her recommendation (if any), the PI or consulting physician will contact the participant, inform the participant of the finding, and recommend that the participant seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the participant and the participant's physician. The PI, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that the participant receives based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

The MRI scans will be read by an outside neuro-radiologist (Dr. Pradeep Varma) assess for exclusion criteria. In the event that Dr. Varma is unavailable for these readings, we will arrange for another qualified radiologist

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to evaluate the scans.

Risks Associated with Use of an Arterial Catheter

On the PET scanning day, a radial arterial catheter will be inserted. Certain individuals may feel light-headed during arterial catheter placement. Arterial catheter placement may be associated with mild-to-moderate pain, hematoma, inflammation, bleeding, or bruising at the puncture site. If any of these, or other symptoms occur and do not diminish within 24 to 72 hours after the arterial line removal, or in the event that they worsen, subjects will be advised to call the on-call doctor listed on the PET discharge instructions. In rare instances, blocking of the artery, tearing of the artery, arterial leakage, poor healing, nerve damage, or infection at the catheter insertion site may occur.

Risks Associated with Radiation

The RSC will review the use of radiation in this research study, and no participants will be scanned until approval is obtained. This research study involves exposure to radiation from [^{18}F]FPEB and associated transmission scans or low dose head CTs.

This study will involve 3 [^{18}F]FPEB PET scan sessions. The targeted amount of radiation an individual participant may receive from this study is from a maximum of three injections of ≤ 5 mCi of [^{18}F]FPEB plus three accompanying transmission scans or low dose head CTs.

Although each organ will receive a different dose, the amount of radiation exposure participants will generally receive during participation in this study is equal to an effective dose of 0.930 rem from [^{18}F]FPEB and 0.0045 rem from transmission scans if scans are performed on the HRRT or 0.135 rem from low dose head CTs if scans are performed on the mCT. The total radiation exposure from all three scanning sessions would be 0.935 rem if scans are performed on the HRRT and 1.065 rem if scans are performed on the mCT.

In the case of quality control failure, participants may be asked to repeat one [^{18}F]FPEB PET scan. In this case, the maximum amount of radiation exposure that a participant may receive from this study is 1.24 rem from four injections of ≤5 mci [^{18}F]FPEB and .006 rem from transmission scans if scans are performed on the HRRT or .180 rem from low dose head CTs if scans are performed on the mCT. The total radiation exposure from all four scans is 1.246 rem if scans are performed on the HRRT and 1.42 rem if scans are performed on the mCT.

The amount of radiation participants will receive in this study is below the dose guidelines established by the FDA and monitored by the Yale-New Haven Hospital Radiation Safety Committee for research participants. This guideline sets an effective dose limit of 5 rem per year. (FDA 21 CFR 361.1; 5 rads per year for whole body, active blood forming organs, lens of the eye and gonads; 15 rads per year for other organs).

Risks Associated with IV Line Insertion

To minimize risks associated with blood draws and infusions, experienced medical personnel will do all the blood drawing procedures. At the PET Center the risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic technique. To avoid injury due to fainting, the catheter will be inserted when the participants are in a recumbent position. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.

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11. Minimizing Risks: Describe the manner in which the above-mentioned risks will be minimized.

Risks Associated with Study Drug, BMS-984923

To minimize the potential risks of BMS-984923, participants with unstable medical conditions will be excluded. At doses higher than those planned for this study in humans, serious adverse effects related to drug accumulation were observed in rats. These adverse effects were gradual in onset over days, were reversed by cessation of daily administration, and were manifest by lethargy and generalized stress responses without organ specific or acute toxicity. There was no evidence for acute onset cardio-respiratory or CNS toxic effects. In order to mitigate the risk of similar adverse effects in humans, our planned maximal dose is well below the human equivalent of the rat and monkey NOAEL. Risks will be further minimized by the safety assessments detailed in the protocol, including neurological checks using the GCS, physical/neurological examinations, vital signs, ECGs, and clinical laboratories (See Table 3: Schedule of Study Events). Finally, safety of participants will be further enhanced according to the provisions of the Data Safety Monitoring Plan (DSMP).

Participants in the receptor occupancy sub-study will undergo increased monitoring while in the PET scanner. The monitoring will continue as described in the protocol and a PET technician and research coordinator will also be monitoring the participant for signs of physiologic or psychiatric distress during the scan. In additional, participants will have continuous blood pressure and heart rate monitoring by arterial line.

Risks Associated with Blood Drawing and IV Line Insertion

To minimize risks of associated with blood draws and IV line insertion, experienced medical personnel will do all the blood-drawing procedures.

Risks Associated with MRI

To minimize risks associated with MRI, the MRI's will be conducted by experienced personnel at The Anlyan Center at Yale. All participants will complete the MRI safety questionnaire prior to having the MRI.

Risks Associated with Psychological Stress

To minimize psychological stress, appropriate breaks will be provided to participants as needed

Minimization of Risks Associated with Receptor Occupancy Substudy

Risks Associated with MRI

To minimize risks associated with MRI, the MRI's will be conducted by experienced personnel at The Anlyan Center at Yale. All participants will complete the MRI safety questionnaire prior to having the MRI.

Risks Associated with Arterial Catheter

Risks of radial artery cannulation are minimized by having the procedure performed by an experienced health care provider. The health care provider will be either a physician or an advanced practice registered nurse (APRN) with experience in critical care and placement of arterial catheters, as is the practice at Yale-New Haven Hospital. For an APRN to place the arterial line at the Yale PET Center, they must meet the following criteria:

1.) Be currently credentialed at Yale-New Haven Hospital or similar institute and

2.) Perform 3 arterial line procedures supervised by a currently privileged PET Center physician.

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The 3 supervised arterial line placements will be documented and signed off by both the APRN and supervising physician. The completed document must be on file at the Yale PET Center prior to an APRN performing any arterial line catheterizations independently.

Pain is minimized by using local anesthesia. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion. After arterial catheter removal, bleeding is prevented by direct pressure applied to the site for a minimum of 15 minutes followed by a pressure dressing (coban) that should be kept clean and dry until evening. Subjects will have their hand and finger blood supply examined after arterial cannulation, throughout the study, and again following catheter removal. Also, subjects will be asked to abstain from aspirin and other NSAIDs for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal. Subjects will be provided a 24 hour emergency physician contact number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and treatment. A nurse will provide discharge instructions outlining general instructions in addition to post-arterial catheter precautions, problems to watch for, and procedures to follow should such problems occur.

Risks Associated with Radiation

The dose of radiation will be submitted for approval to the Yale institutional review board. All scans will be done in the presence of medical supervision and trained staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiotracers and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals. Participants will be asked about their previous radiation exposure and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study participants will be notified to the study doctor.

No PET studies will be performed on pregnant women or potentially pregnant women, as female participants of childbearing potential will be excluded from participation. If participants are breastfeeding, they will not be able to participate in this research study.

Risks Associated with Blood Drawing and IV Line Insertion

The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic technique. To avoid injury due to fainting, the catheter will be inserted when the participants are in a recumbent position.

12. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? Moderate. The assessment of the risks to participants associated with study participation is based on the fact that the study drug has extensive safety data in animals. However, this is the first in human study. Safety monitoring will be performed by the DSMB in conjunction with the PI

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and medical monitor. There is little uncertainty about the possible occurrence or nature of risks, and there is adequate surveillance and protections to discover adverse events promptly and minimize their effects.

b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? N/A

c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates for

   i. Minimal risk
   ii. Greater than minimal

Data and Safety Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) will be established, which will consist of individuals with expertise in: 1) Alzheimer's disease and clinical medicine, 2) clinical trials methodology and 3) biostatistics. Potential members will be appointed by the PI, after a review of experience and credentials.

The DSMB will provide an initial review of the final protocol and consent. They will provide ongoing review of safety data, participant accrual, and outcome data. The DSMB will review and approve changes to the protocol and consent forms, and provide routine oversight of both safety issues and data flow. They will receive serious adverse event (SAE) reports in real time and will review non-serious adverse events at the end of each dose cohort. Other safety data will be reported in summary tables to the DSMB within 3 days of each DSMB meeting. At their request, safety data will be reported in unblinded fashion.

The DSMB will meet following the last participant visit for each of the four dose cohorts. The PI will be responsible for scheduling the meetings (in-person or by teleconference) and to ensure that all materials needed for review are provided with sufficient time prior to the meeting. Each meeting will be divided into two parts. First, an open session in which the principal investigators may be present, at the request of the DSMB, to review the conduct of the trial and to answer questions from members of the DSMB. The focus in the open session may be on accrual, protocol compliance, and general toxicity issues. Following this session, a closed session involving only DSMB members will be held to allow the DSMB opportunity to discuss the general conduct of the trial and all outcome results, including toxicities and adverse events, develop recommendations, and take votes as necessary. A written report will be provided by the DSMB to the PI, IND holder (Dr. Strittmatter), and sponsors (NIA, Alzheimer's Association). At minimum this will include a recommendation to continue the protocol as planned and an approval of the dose escalation for the next dose cohort. Protocol changes or discontinuation may also be recommended.

Safety Assessment and Dose Escalation Decisions

For the first 3 participants enrolled in the study, drug dosing will be conducted at a minimum of 48 hours between the first and second, and between the second and third participants. For subsequent cohorts, there will be at least 24 hours between dosing the first and second participants, and between the second and third participants, to allow for a 24-hour safety assessment review by the PI and medical monitor for each prior participant.

Determination of whether to open the next escalated dose cohort for enrollment will be made jointly by the PI, medical monitor, DSMB, and IND holder (Dr. Strittmatter) after review of all available clinical,

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safety, and PK data. Dose escalation (for each sequential cohort) will not occur until all participants in the prior cohort have completed the study. The DSMB will perform an independent review. In addition, the PI and medical monitor will make a determination independent from the IND holder. Dr. Strittmatter will be consulted in scenarios where alterations to the planned dose escalation strategy are being considered. As the IND holder, Dr. Strittmatter must also agree with a decision to proceed with dose escalation before the next cohort is dosed.

Dose escalation decisions will be determined with the following approach. At the completion of each cohort, 0-72 h PK data will be used to assess the plasma exposure derived from the area under the curve (AUC) from 0-24 h. Our dose escalation decisions will be based on the human data relative to the rat 28 day GLP toxicology study to determine maximal NOAEL. In that study, the plasma exposure at NOAEL based on AUC (0-24h) was 192,000 ng*h/mL (see IB). Furthermore, the plasma exposure in corresponding Cynomolgus study was similar at 169,000 ng*h/mL.

The maximal doses for each cohort will be 10, 40, 70, 100, 150 and 200 mg. The human equivalent dose derived from the NOAEL dose in the 28D toxicology study in the most sensitive species (rat) is 140 mg. Thus, the starting dose is less than 10% of the 140 mg amount. Dose escalation in cohorts 2-6 will follow the plan above, but will be decreased based on the observed plasma exposure of the AUC (0-24 h) as detailed in the schedule below. The dose escalation for the next cohort will be based on the most recent cohort observed PK analysis as follows:

If plasma $AUC_{0\text{-}24h}$ < 24,000 ng*h/ml (12.5% NOAEL), then dose increase according to schedule
If plasma $AUC_{0\text{-}24h}$ 24,000-48,000 ng*h/(12.5-25% NOAEL), then dose increase maximum of 2 fold
If plasma $AUC_{0\text{-}24h}$ 48,001-96,000 ng*h/ml (25-50% NOAEL), then dose increase maximum of 1.5 fold
If plasma $AUC_{0\text{-}24h}$ 96,001-120,000 ng*h/ml (50-67.5% NOAEL), then dose increase maximum of 1.3 fold
If plasma $AUC_{0\text{-}24h}$ > 120,000 ng*h/ml (>67.5% NOAEL), then stop escalation

Any serious adverse events or other safety concern might also constitute a rationale to stop or reduce escalation.

Criteria for intervention discontinuation

The PI and medical monitor may elect to stop dosing or stop the study based on any treatment emergent concerns. Although not expected based on animal toxicology and PK studies, participants will be monitored for clinically significant neuropsychiatric symptoms with the GDS and NPI-Q, neurological symptoms by exam, cognitive toxicity with testing, and laboratory abnormalities. While the planned doses are below the human equivalent of the repeated doses which caused serious adverse effects with lethargy in rat, participants will be monitored by frequent repeated GCS observations. Dosing may be stopped due to the occurrence of any individual adverse events which, in the judgment of the PI and medical monitor, need further characterization with respect to progression and reversibility before further dosing is conducted. Such adverse events may include non-serious unusual events. If dosing is stopped, review of the data by the medical monitor and DSMB must occur with a positive recommendation before dosing can be resumed.

Attribution of Adverse Events

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- Definite: Adverse event(s) will clearly be related to investigational agent or other intervention and cannot be reasonably explained by an alternative explanation – i.e., concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive.
- Probable: Adverse event(s) will likely be related to investigational agent. The relationship in time is suggestive that the adverse event is likely related to the investigational agent. An alternative explanation is less likely – i.e., concomitant drug(s), concomitant disease(s).
- Possible: Adverse event(s) may be related to investigational agent. An alternative explanation – i.e., concomitant drug(s), concomitant disease(s), - is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
- Unlikely: Adverse event(s) will doubtfully be related to investigational agent(s). An adverse event for which an alternative explanation is more likely – i.e., concomitant drug(s), concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.
- Unrelated: Adverse event(s) will clearly not be related to the investigational agent

Plan for Grading Adverse Events

Adverse event (AE): An adverse event (AE) is any adverse change from the participant's baseline condition, regardless of relationship to study drug, including clinical or laboratory tests, or abnormalities which occur after informed consent is signed and up to 7 days after the study drug has been discontinued. Clinically significant adverse changes in clinical status, ECGs, and physical examinations are considered AEs. Any participant complaint associated with such an abnormal finding will also be reported as an AE. AEs are only collected upon the start of study drug. Events occurring prior to this are to be reported as part of the participant medical history. However, SAEs are to be collected from the time of informed consent.

Adverse events include but are not limited to: (1) worsening or change in nature, severity, or frequency of conditions or symptoms present at the start of the study; (2) participant deterioration due to primary illness; (3) intercurrent illness; and (4) drug interaction. An abnormal laboratory value will only be reported as an AE it if requires therapeutic medical intervention, if the investigator considers it to be an AE, or if it leads to the participant being withdrawn from the study.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs/symptoms. Symptoms and conditions present at the beginning of the study will be characterized, so that AEs can be defined as any new symptom, or any increase in frequency or severity of an existing symptom.

Following questioning and evaluation, all AEs, whether determined to be related or unrelated to the study drug by the Site Protocol Principal Investigator, must be documented in the participant's medical records, in accordance with the investigator's normal clinical practice. Each AE is evaluated for duration, severity, seriousness, and causal relationship to the study drug.

The intensity of each AE will be rated according to the following 3-point scale:

- Mild: Awareness of signs or symptoms, but no disruption of usual activity
- Moderate: Event sufficient to affect usual activity (disturbing)
- Severe: Inability to work or perform usual activities (unacceptable)

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Serious Adverse Event (SAE): Any untoward medical occurrence that at any dose: results in death, is life-threatening, require inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect (NIH Guide-6/11/99).

Note 1: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed in the definition above.

Note 2: Hospitalizations that fulfill one of the following conditions will not have to be reported as SAE:

- Hospitalizations for social reasons and thus unrelated to a deterioration of the participant's condition or adverse event (e.g., deterioration of the living conditions related to environmental factors rather than to a deterioration of the disease, lack of transportation to the investigational site, respite care for the caregiver)
- Hospitalizations for elective surgical interventions for which the date had already been determined prior to the study participation.

Unlisted (Unexpected) adverse event: An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., package insert/summary of product characteristics for an approved product (ICH and GCP)).

Life-Threatening: Any event in which the participant was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

Associated with the use of the drug: An adverse event is considered associated with the use of the drug if the attribution is possible, probable, or definite.

Abnormal Test Findings

The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- test result is associated with accompanying symptoms, and/or
- test result requires additional diagnostic testing or medical/surgical intervention, and/or
- test result leads to a discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or
- test result is considered to be an AE by the investigator in consultation with the medical monitor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

Adverse Events of Special Interest (AESI)

No Adverse Events of Special Interest (AESI) for this study have been identified in non-clinical toxicology studies with BMS-984923. Based on drug mechanism of action, the following are considered AESI for this study: central nervous system complications.

Plans for Reporting Adverse Events and Serious Adverse Events

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The PI and research staff will monitor the study procedures for this trial for overall safety and scientific relevance on an ongoing basis. Dr. Mecca (in conjunction with the medical monitor and DSMB as necessary) will evaluate every adverse event for safety and causality, and will determine whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol or consent form are required.

The PI will report the following types of adverse events to the Yale University HIC, DSMB, and IND holder within 48 hours of it becoming known to the investigator: a) all serious adverse events; b) non-serious adverse events occurring with a greater magnitude or frequency than expected; and c) other unanticipated problems involving risks to participants or others.

All adverse events post administration of a PET tracer will be reported to the PET center, the PET authorized user, and the YNHH RSC.

SAEs as defined in the HIC DSMP will be reported to the HIC, DSMB, and IND holder within 48 hours (using HIC Form 6A). In this report, the PI will give a brief description of the SAE including severity, duration, action taken, outcome, and plan. He will also give an opinion regarding the relationship of the SAE to the study drug and determine whether or not the protocol or consent form needs to be modified as a result of the SAE. If available, a report from the DSMB will accompany the HIC report submission. Reporting will not be delayed pending resolution or outcome of an event. If an outcome for an adverse event is not available at the time of the initial report, participant follow-up will proceed until such time as an outcome is known. A progress report of the study that includes all of the SAEs will be reported to the HIC in an annual request for renewal.

The DSMB will review all non-serious AEs at the completion of each dose cohort (approximately every 3 months). This will be done via teleconference.

The IND holder (Dr. Strittmatter) will report adverse events to the FDA following Guideline Section 312.32 IND Safety Reporting standards. In particular, the sponsor will notify FDA in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. Upon request from FDA, the IND holder will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request. The IND holder will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

13. Statistical Considerations: Describe the statistical analyses that support the study design.

Sample Size

The sample size was derived empirically from experience with previous single ascending dose clinical studies and PET occupancy studies in other disorders and is deemed appropriate to achieve the study objectives.

General Methods

Data will be tabulated by dose cohort, as well as by pooled (all participants combined), and no inferential statistics are planned.

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Handling of Missing Data

All data collected will be included in by-domain data listings, sorted by participant number and time point, or as appropriate. Every attempt will be made to collect data at each time point. For participants who discontinue treatment (for any reason), participants will continue to be followed and efficacy endpoints and safety data collected where possible.

Subgroups

The sample size precludes analysis by-subgroup. However, exploratory assessment of any trends among subgroups (eg, by dose) may be performed, after initial review if the data warrant.

Analysis Populations

Safety Population: Safety outcomes will be assessed for all participants who are given at least one dose of study drug.

Alpha Level Considerations

No inferential statistical testing is planned. However, if used for exploratory analysis, inferences will be assessed against a 5% alpha, and therefore p-values ≤ 0.05 will be considered statistically significant. All tests will be two-sided. No adjustment will be made to p-values for multiple testing.

Participant Disposition and Exposure

The numbers of participants completing or withdrawing, along with reasons for withdrawal, will be summarized by dose.

Tabulation of the number of doses of study drug, duration of treatment as well as total dose given will be provided.

Demographics and Baseline Characteristics Analyses

Demographic variables (age, sex, race, and ethnicity) as well as height (cm), weight (kg), Body Mass Index (BMI), temperature, heart rate, blood pressure and respiratory rate or pulse oximetry (from the vital signs) at baseline will be summarized using descriptive statistics. All demographic data will be provided in a data listing.

Efficacy Endpoints

No efficacy endpoints will be assessed since this is a sample of healthy participants.

Safety Endpoints

The safety analysis will be descriptive in nature. All safety data will be listed, and data will be tabulated by dose. Safety data include:

- AEs
    - Events occurring prior to the first dose of study drug will be defined as pre- treatment events.
    - TEAEs will be defined as any AE occurring during or after oral administration of the study drug. Therefore, this will include all events occurring through Day 7/ET.

Incidence of AEs will be summarized for each cohort by MedDRA system organ class (SOC) and preferred term (PT), sorted in descending frequency by SOC, and then by PT within SOC. These summaries will be given by dose in separate tables for each of the following TEAE event sets:

- All events

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- Treatment related events (defined by a relationship to study drug of possible, probable, or definite).
- Serious adverse events
- Events leading to premature discontinuation from study
- Events by maximum severity
- AEs of Special Interest, ie, events relating to cognitive decline or neuropsychiatric symptoms.

Other safety outcomes will include:

- Clinical laboratory tests presented as changes over time. Potentially clinically significant (PCS) ranges will be defined and used to determine the incidence of participants experiencing new- onset PCS laboratory values, where new-onset is defined as a PCS value for a participant following initiation of study treatment for which the participant did NOT have a PCS value for that analyte PRIOR to initiation of study treatment.
- Vital signs including: respiratory rate, heart rate, temperature, and blood pressure, as per the schedule of events. BMI will be assessed, with height collected only at screening.
- Physical examination
- 12-lead ECG. ECG parameters will be analyzed in a fashion similar to that of clinical laboratory parameters.

Concomitant treatments will be assessed according to the timing of their start/stop dates as they relate to the study drug treatment, as follows:

- Prior medications with start AND stop date before date of first dose of study drug.
- Concomitant medications with participant exposure that includes at least one dose of study drug.
- New-onset medications. Concomitant medications with a start date AFTER the first dose of study drug. New-onset medications are a subset of the full set of concomitant medications.

Changes from pre-treatment will be calculated in a similar fashion as for the efficacy endpoints, but no inferential statistics will be provided for safety endpoints. Shifts from baseline in ECG will be tabulated for heart rate and QTc. Other endpoints will be assessed according to the scale of the variable.

Pharmacokinetic Endpoints

Concentration data in plasma will be assessed descriptively over time. Correlation with safety and receptor occupancy outcomes may be performed, as the data warrant.

SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, check off N/A and delete the rest of the section.

A. RADIOTRACERS ☐N/A

1. Name of the radiotracer: [^{18}F]FPEB

2. Is the radiotracer FDA approved? ☐YES ☒NO

If NO, an FDA issued IND is required for the investigational use unless RDRC assumes oversight.

3. Check one: ☒IND# 150912

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or ☐RDRC oversight (RDRC approval will be required prior to use)

4. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this radiotracer is being administered to humans, include relevant data on animal models.

   [^{18}F]FPEB
   The radiotracer [^{18}F]FPEB has previously been used in human participants; 5 individuals at the Neurodegenerative Disorders (IND) in New Haven CT, and over 100 individuals at Yale University. No participants have reported any adverse event with this radiotracer. However, participants will be monitored carefully during and after the PET scan for any potential side effects.

   The radiation dose estimates are based on the biodistribution data for [^{18}F18]FPEB in 6 healthy adults (3 men and 3 women) and calculated using OLINDA/EXM software (Wong 2013). Based on these numbers, the critical organ is the gallbladder wall (0.191 mGy/MBq, i.e., 0.708 rad/mCi). Another radiation dose study (Kessler 2014) also reported gallbladder wall (0.193 mGy/MBq, 0.714 rad/mCi) as the critical organ based on the biodistribution data in 9 healthy adults (5 men and 4 women). However, Kessler et al. reported the urinary bladder wall as the critical organ (0.258 mGy/MBq, 0.955 rad/mCi) when no voiding takes place prior to a 3.5 h period. Thus, the maximum allowable injection doses for [F-18]FPEB to remain below the 21 CFR 361.1 dose limits for research participants are 5.2 mCi per single injection (calculations based upon the urinary bladder wall for a 3.5 h voiding), and 7 mCi per single injection (calculations based upon the gallbladder wall as the critical organs); 3 rads per single study for whole body, active blood forming organs, lens of the eyes and gonads; 5 rads for other organs per single study limit.

5. **Source:** Identify the source of the radiotracer to be used.
   [^{18}F]FPEB will be synthesized at the Yale University PET Center radiochemistry Laboratory under the supervision of Drs. Henry Huang & Nabeel Nabulsi.

6. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, method of sterilization and method of testing sterility and pyrogenicity.
   Due to the short half-life, PET drugs are prepared and formulated immediately before administration, and therefore there are no issues with storage or stability. PET drug products are stored at room temperature and are stable for at least 60 min after preparation.

   The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a 0.22 micron membrane filter during the last step of the formulation process. Prior to release for administration, a bubble point test is performed on the membrane filter used for terminal sterilization in order to validate and verify its integrity during the filtration process. Due to the short half-life, a sample of the PET drug product is tested for sterility after administration for further confirmation.

   The level of endotoxin in each batch of the final PET drug product is determined quantitatively prior to release for administration using the FDA approved Charles River Laboratory's Portable Testing System (Endosafe®-PTS).

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[^{18}F]FPEB will be prepared at the Yale PET Center in accordance with our Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in our FDA approved Drug Master File (DMF), under IND#059121.

B. DRUGS/BIOLOGICS ☐N/A

1. If an **exemption from IND filing requirements is** sought for a clinical investigation of a drug product that is lawfully marketed in the United States, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

N/A

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

   The present clinical evaluation of BMS-984923 is being conducted in healthy participants. Safety and tolerability studies in this population will allow longer term safety studies and ultimately efficacy studies in participants with AD.

   ### Nonclinical Summary
   Preclinical animal data are summarized in the background above for BMS-984923. Please refer to the IB for a more detailed description of toxicology investigations.

   ### Clinical Experience with BMS-984923
   The present study represents the first evaluation of BMS-984923 in humans.

3. **Source:** Identify the source of the drug or biologic to be used.

   a) Is the drug provided free of charge to subjects? ☒YES ☐NO
   If yes, by whom? Allyx Therapeutics, Inc.

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

   ### Study Drug Packaging and Labeling
   The clinical trial supply label will be in accordance with ICH GCP and local requirements for investigational product labelling. Investigational products are for investigational use only and the study drug supplied for this study is intended for use only within the context of this study. The study drug supplied for this study should be stored in a secure, temperature controlled, locked place with restricted access, maintained under adequate security until dispensed for participant use or returned to Investigational Drug Service.

   Please see page 16 “Description of Study Medication” for and Table 2 footnote for a description of how the drug is being provided for this study.

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Study Drug Storage
BMS-984923 is stored at 15-25° C.

Study Drug Management
The PI, pharmacist, or their designee will verify that study drug supplies are received intact and in the correct amounts by signing and dating the investigational product receipt log. The person receiving the supplies must verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable drug in a given shipment will be documented in the study files.

The site will maintain a Drug Inventory Log (includes, but not limited to, the following: lot number, number of units received, and number of capsules dispensed. The site will also maintain patient-specific drug dispensing logs.

Study Drug Accountability
Participants will be treated at the clinical site, in-patient clinical unit, or other approved location and therefore the Pharmacist or other investigational staff via documentation of receipt of the study drug and dosing/treatment given will perform/maintain drug accountability.

Study Drug Handling and Disposal
Records of receipt, dispensing records and inventory forms, as applicable, will be examined and reconciled during and at the end of the study. Both the study drug that is used during the study, as well as any remaining unused study drug, must be accounted for on a drug accountability record.

At the end of the study, all used and unused investigational drug capsules, accompanied by a packing slip, must be returned to the designated clinical supplies vendor for disposal or destroyed per site SOPs. In addition, a copy of all completed drug accountability records will be retained. The product is to be stored in a safe place (locked facility) at the appropriate temperature.

Check applicable Investigational Drug Service utilized:

☒ YNHH IDS ☐ CMHC Pharmacy ☐ West Haven VA
☒ PET Center ☐ None
☐ Other:

Note: *If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation*.

5. Use of Placebo: ☒Not applicable to this research project

If use of a placebo is planned, provide a justification which addresses the following:

a) Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.

b) State the maximum total length of time a participant may receive placebo while on the study.

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c) Address the greatest potential harm that may come to a participant as a result of receiving placebo.

d) Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Continuation of Drug Therapy After Study Closure** ☐**Not applicable to this project**
Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?

☐ **Yes** If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access. *Write here*

☒ **NO** If no, explain why this is acceptable. This is a Phase 1a study designed to determine the safety, tolerability, and efficacy of BMS-984923 in healthy participants. Therefore, the investigational treatments will not likely be available to patients beyond their participation in this clinical trial and would not be clinically indicated.

C. DEVICES ☒N/A

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment: Give the number of subjects:**
   a. Targeted for enrollment at Yale for this protocol: We plan to consent and enroll four cohorts of six participants each for a total of 24 participants. Up to three participants per cohort, or a total of 12 participants, will enroll in the Receptor Occupancy sub-study.

   b. If this is a multi-site study, give the total number of subjects targeted across all sites: N/A

2. **Indicate recruitment methods below.** Attach copies of any recruitment materials that will be used.

☐ Flyers	☐ Internet/web postings	☐ Radio
☐ Posters	☐ Mass email solicitation	☐ Telephone
☐ Letter	☐ Departmental/Center website	☐ Television
☒ Medical record review*	☐ Departmental/Center research boards	☐ Newspaper
☐ Departmental/Center newsletters	☐ Web-based clinical trial registries	☐ Clinicaltrails.gov
☒ YCCI Recruitment database	☐ Social Media (Twitter/Facebook):	

☒ Other: Physicians that are familiar with the ADRU may provide our contact information to his/her patients so that the patient can initiate contact with us.

* **Requests for medical records should be made through JDAT as described at**
http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. **Recruitment Procedures:**
a. Describe how potential subjects will be identified.
Participants may be identified from among those already known to the Alzheimer's Disease Research Unit (ADRU). Participants in the "Subject Recruitment Database" (HIC #25374) have either participated in a previous study or contacted the ADRU about participation and have asked to be contacted for future studies. In addition, physicians and other health professionals who are familiar with the ADRU may provide our contact information to their patients so that they may initiate contact with us.

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b. Describe how potential subjects are contacted.
Before contacting potential participants already known to the ADRU, we may perform reviews of charts that contain Protected Health Information to assess participant eligibility. We have permission from our participants to contact them for new studies, which is provided in the participant information forms (see "Subject Recruitment Database" HIC #25374).

For new participants who contact the ADRU (from the various sources advertising sources detailed above), after obtaining the required permission, we will conduct phone interviews that may include questions pertaining to Protected Health Information such as demographics, medical history, current medications, and family history (see "Subject Recruitment Database" HIC #25374).

c. Who is recruiting potential subjects?
The PI or members of his staff who work under his supervision.

4. Assessment of Current Health Provider Relationship for HIPAA Consideration:
Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?
☐Yes, all subjects
☐Yes, some of the subjects
☒No

If yes, describe the nature of this relationship.

5. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)
Choose one:
☐ For entire study
☒ For recruitment/screening purposes only
☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data:
N/A – requesting waiver of signed authorization only. We will obtain verbal authorization for use/disclosure of data.

ii. If requesting a waiver of signed authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data:

Information may be collected during a phone screen for recruitment purposes. At the end of a phone screen, individuals are read a paragraph for authorization for storing screening information (see HIC protocol 0307025374). If authorization is denied, all screening information is destroyed.

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The investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

6. Process of Consent/Assent: Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

   The specific steps in the informed consent process will be as follows:
   1. The informed consent form will be discussed with the participant.
   2. The participant will be given adequate opportunity to read the consent form.
   4. The Consent Personnel will answer any questions, correcting and discussing any misconceptions.
   5. The participant will sign the consent form if they wish to participate.

   The participant will not take part in any part of the clinical study, including the initial screening, until they have signed and dated the informed consent form to individuate that he or she understands and agrees to its contents.

7. Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent: Indicate how the personnel obtaining consent will assess the potential participant's ability and capacity to consent to the research being proposed.

   As this study is designed to determine the safety and tolerability of study drug and lacks potential benefit to participants, only participants capable of providing informed consent will be enrolled (no surrogate consent will be allowed). The PI or surrogate obtaining informed consent will make a judgment about whether the participant is capable of providing informed consent. This judgment will be based on an overall impression of the participant's ability to comprehend relevant information and make reasoned decisions.

   If the participant is judged capable of providing informed consent, they will sign the "Participant Informed Consent" page. Participants who are not able to provide informed consent at the start of the trial will be excluded from participation in the study.

8. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking participants. If enrollment of these participants is anticipated, translated copies of all consent materials must be submitted for approval prior to use.

   Only English-speaking participants will be enrolled.

As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? YES ☐ NO ☒

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Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

9. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐Not Requesting any consent waivers

☒Requesting a waiver of signed consent:

☒ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study (Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☒ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☒ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☐ NO☐
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☐

☐ Requesting a waiver of consent:

☐ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study

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For a full waiver of consent, please address all of the following:

- Does the research pose greater than minimal risk to subjects?
  ☐ Yes *If you answered yes, stop. A waiver cannot be granted.*
  ☐ No
- Will the waiver adversely affect subjects' rights and welfare? YES ☐ NO☐
- Why would the research be impracticable to conduct without the waiver? *Write here*
- Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date? *Write here*

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

2. Required private identifiable information about individuals, such as their medical history, current medications, clinical laboratory and EKG results, physical exam information, psychiatric problems, MRI and PET scan results, and family history will be collected by the research staff and be used for research purposes and charting after consent is obtained.

3. How will the research data be collected, recorded and stored?
   As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

   An CRF is required and should be completed for each included participant. The CRFs must be signed by the PI to attest that the data contained on the CRFs is true. Any corrections to entries made on the CRFs must be dated, initialed, and explained (if necessary) and should not obscure the original entry.

   In most cases, the source documents are the hospital's or the physician's participant chart. In these cases, data collected on the CRFs must match the data in those charts.

   In some cases, the CRF, or part of the CRF, may also serve as source documents. In these cases, a document should be available at the PI's site and clearly identify those data that will be recorded on the CRF, and for which the CRF will stand as the source document.

   To enable evaluations and/or audits from regulatory authorities, the PI will keep records, including the identity of all participants (sufficient information to link records, eg, CRFs and hospital records), all original signed ICFs, copies of all CRFs, SAE forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (eg, letters, meeting minutes, telephone calls reports). The records should be retained by the PI according to ICH, local regulations, or as specified in the Clinical Study Agreement, whichever is longer.

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4. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☐Portable Hard Drive ☒Secured Server ☐Laptop Computer ☐Desktop Computer ☐Other

5. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?

   Strict confidentiality will be maintained in all records of the study by identifying participants by code numbers. Information that is obtained in connection with this study and that can be identified with a participant will be kept confidential (participant's charts are kept in an area protected with a security system, in a locked room and file cabinet). Files will be kept in locked cabinets in a security system protected office. All desktop computers contain encryption software and are password protected.

All portable devices must contain encryption software, per University Policy 5100. If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url http://its.yale.edu/egrc or email it.compliance@yale.edu

6. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

   Identifiable information is stored in paper files in locked cabinets in a security system protected office. The link between participants' identities and their coded information will be kept for a maximum of 15 years, after which time the link will be destroyed and the data will become anonymous.

7. If appropriate, has a Certificate of Confidentiality been obtained? Not applicable.

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)
The expected benefit to society at large is a better understanding of the safety of BMS-984923. This study offers no direct individual benefit to participants. Participants in this study may derive subjective benefit from volunteering to take part in a study for the advancement of scientific knowledge.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?
   The alternative to participating in this study is to choose not to participate. Study participants are healthy individuals so there are not alternative treatments available outside of research.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

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Participants will be compensated for their time commitment and inconveniences necessary for completing the study. Participants will have no financial responsibilities for any portion of the study.

Compensation will be
- $75 for the initial screening visit ($25 for repeat screening, if needed)
- $100 for the MRI session
- $300 for each completed PET scan
- $50 for each Arterial Line
- $200 for administration of the study drug
- $250 for each overnight stay.

The expected compensation amounts are as follows:
For individuals not participating in the receptor occupancy sub-study: $775 for completion of the following: Screening, one dose of study drug, and 2 inpatient nights. This amount may increase if the participant requires a repeat screening visit or additional overnight stay during the in-clinic period.

For individuals participating in the receptor occupancy sub-study: $1925 for completion of the following: Screening, one MRI, three PET scans, three arterial lines, one dose of study drug, and 2 inpatient nights. This amount may increase if the participant requires a repeat screening visit, repeat MRI, repeat PET scan (and arterial line placement), or additional overnight stay during the in-clinic period.

An additional compensation of $300 will be provided if all procedures are completed. Individuals not participating in the receptor occupancy sub-study would receive $1075. Individuals participating in the receptor occupancy sub-study would receive $2225.

Because of the amount of compensation, only experienced research staff will be allowed to consent participants. This consent process will include an in-depth conversation regarding the risks of the study such that participants are given a complete understanding of the risks involved in the study.

Parking and transportation costs will not be reimbursed. These costs are considered to be covered in the study payment that participants will receive.

3. Costs for Participation (Economic Considerations): Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

   The entire medical evaluation, including physical examination, ECG and laboratory work, and all research procedures (PET and MRI) will be provided at no cost to the study participant.

4. In Case of Injury: This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

   a. Will medical treatment be available if research-related injury occurs? If a participant is injured as a result of participation in this study, medical treatment will be available to them. However, the participant's insurance carrier will be expected to pay for the cost of treatment.

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b. Where and from whom may treatment be obtained? Participants should receive medical treatment from wherever and however is appropriate (e.g., ED, primary physician, etc.)

c. Are there any limits to the treatment being provided? The participant's insurance carrier may have limits.

d. Who will pay for this treatment? The participant's insurance carrier will be expected to pay for the cost of treatment.

e. How will the medical treatment be accessed? As they normally would as appropriate (e.g., ED, primary physician, etc.)

5. **Conflict of Interest Disclosure:** Stephen Strittmatter, MD, PhD, Professor of Neurology, Yale School of Medicine, is the Investigational New Drug (IND) application holder for the study drug, as well as founder of Allyx Therapeutics, a company that seeks to license the study drug from Yale and develop it for clinical use in Alzheimer's disease. Dr. Strittmatter will not have any contact with study participants during the conduct of this trial.

IMPORTANT REMINDERS

Will this study have a billable service? **Yes ☒** **No☐**

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities?
Yes ☒ No ☐

If Yes, please answer questions a through c and note instructions below.

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a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? **Yes ☒ No ☐**

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? **Yes ☐ No ☒**

c. Will a novel approach using existing equipment be applied? **Yes ☐ No ☒**

If you answered "no" to question 4a, or "yes" to question 4b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. **By submitting this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH.**

Appendix A Excluded Medications based on CYP Inhibition/Induction

1A2 Inhibitors	2D6 Inhibitors	3A4 Inhibitors	3A4 Inducers
Ciprofloxacin Enoxacin	Bupriopion	Boceprevir	Apalutamide
Fluvoxamine	Fluoxetine	Clarithromycin	Carbamazepine
Interferon Alpha-2b	Paroxetine	Cobicistat	Enzalutamide
Vemurafenib	Quinidine	Conivaptan	Fosphenytoin
Amiodarone	Terbinafine	Grapefruit Juice	Lumacaftor
	Cinacalcet	Idelalisib	Mitotane
1A2 Inducers	Duloxetine	Indinavir	Phenytoin
Carbamazepine	Mirabegron	Itraconazole	Rifampin
Fosphenytoin	Rolapitant	Ketoconazole	St. John's Wort
Nicotine		Lopinavir/ritonavir	Bosentan
Phenytoin	2D6 Inducers	Nefazodone	Cenobamate
Rifampin	None	Posaconazole	Efavirenz
		Ritonavir	Etravirine
		Saquinavir	Lorlatinib
		Telaprevir	Modafinil
		Telithromycin	Nafcillin
		Voriconazole	Phenobarbital
		Aprepitant	Primidone

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		Atazanavir Ciprofloxacin Crizotinib Cyclosporin Diltiazem Dronedarone Erythromycin Fluconazole Fluvoxamine Fosnetupitant Imatinib Letermovir Netupitant Nilotinib Verapamil	Rifabutin Dexamethasone Nevirapine Oxcarbazepine Prednisone Rifapentine

REFERENCES

Abou Farha, K., Bruggeman, R., & Balje-Volkers, C. (2014). Metabotropic glutamate receptor 5 negative modulation in phase I clinical trial: potential impact of circadian rhythm on the neuropsychiatric adverse reactions-do hallucinations matter? *ISRN Psychiatry, 2014*, 652750. doi:10.1155/2014/652750

Beecham, G. W., Hamilton, K., Naj, A. C., Martin, E. R., Huentelman, M., Myers, A. J., . . . Montine, T. J. (2014). Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. *PLoS Genet, 10*(9), e1004606. doi:10.1371/journal.pgen.1004606

Beraldo, F. H., Ostapchenko, V. G., Caetano, F. A., Guimaraes, A. L., Ferretti, G. D., Daude, N., . . . Prado, M. A. (2016). Regulation of Amyloid beta Oligomer Binding to Neurons and Neurotoxicity by the Prion Protein-mGluR5 Complex. *J Biol Chem, 291*(42), 21945-21955. doi:10.1074/jbc.M116.738286

Berman, D. E., Dall'Armi, C., Voronov, S. V., McIntire, L. B., Zhang, H., Moore, A. Z., . . . Di Paolo, G. (2008). Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. *Nat Neurosci, 11*(5), 547-554. doi:10.1038/nn.2100

Bhakar, A. L., Dolen, G., & Bear, M. F. (2012). The pathophysiology of fragile X (and what it teaches us about synapses). *Annu Rev Neurosci, 35*, 417-443. doi:10.1146/annurev-neuro-060909-153138

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

Bhaskar, K., Hobbs, G. A., Yen, S. H., & Lee, G. (2010). Tyrosine phosphorylation of tau accompanies disease progression in transgenic mouse models of tauopathy. *Neuropathol Appl Neurobiol, 36*(6), 462-477. doi:10.1111/j.1365-2990.2010.01103.x

Bhaskar, K., Yen, S. H., & Lee, G. (2005). Disease-related modifications in tau affect the interaction between Fyn and Tau. *J Biol Chem, 280*(42), 35119-35125. doi:10.1074/jbc.M505895200

Bruno, V., Battaglia, G., Copani, A., D'Onofrio, M., Di Iorio, P., De Blasi, A., . . . Nicoletti, F. (2001). Metabotropic glutamate receptor subtypes as targets for neuroprotective drugs. *J Cereb Blood Flow Metab, 21*(9), 1013-1033. doi:10.1097/00004647-200109000-00001

Campbell, U. C., Lalwani, K., Hernandez, L., Kinney, G. G., Conn, P. J., & Bristow, L. J. (2004). The mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates PCP-induced cognitive deficits in rats. *Psychopharmacology (Berl), 175*(3), 310-318. doi:10.1007/s00213-004-1827-5

Castner, S. A., Murthy, N. V., Ridler, K., Herdon, H., Roberts, B. M., Weinzimmer, D. P., . . . Laruelle, M. (2014). Relationship between glycine transporter 1 inhibition as measured with positron emission tomography and changes in cognitive performances in nonhuman primates. *Neuropsychopharmacology, 39*(12), 2742-2749. doi:10.1038/npp.2014.4

Chen, M. K., Mecca, A. P., Naganawa, M., Finnema, S. J., Toyonaga, T., Lin, S. F., . . . van Dyck, C. H. (2018). Assessing Synaptic Density in Alzheimer Disease With Synaptic Vesicle Glycoprotein 2A Positron Emission Tomographic Imaging. *JAMA Neurol, 75*(10), 1215-1224. doi:10.1001/jamaneurol.2018.1836

Chin, J., Palop, J. J., Puolivali, J., Massaro, C., Bien-Ly, N., Gerstein, H., . . . Mucke, L. (2005). Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease. *J Neurosci, 25*(42), 9694-9703. doi:10.1523/JNEUROSCI.2980-05.2005

Chin, J., Palop, J. J., Yu, G. Q., Kojima, N., Masliah, E., & Mucke, L. (2004). Fyn kinase modulates synaptotoxicity, but not aberrant sprouting, in human amyloid precursor protein transgenic mice. *J Neurosci, 24*(19), 4692-4697. doi:10.1523/JNEUROSCI.0277-04.2004

Cleary, J. P., Walsh, D. M., Hofmeister, J. J., Shankar, G. M., Kuskowski, M. A., Selkoe, D. J., & Ashe, K. H. (2005). Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci, 8*(1), 79-84. doi:10.1038/nn1372

Cosgrove, K. P., Kloczynski, T., Nabulsi, N., Weinzimmer, D., Lin, S. F., Staley, J. K., . . . Carson, R. E. (2011). Assessing the sensitivity of [(1)(1)C]p943, a novel 5-HT1B radioligand, to endogenous serotonin release. *Synapse, 65*(10), 1113-1117. doi:10.1002/syn.20942

Cox, T. O., Gunther, E. C., Brody, A. H., Chiasseu, M. T., Stoner, A., Smith, L. M., . . . Strittmatter, S. M. (2019). Anti-PrP(C) antibody rescues cognition and synapses in transgenic alzheimer mice. *Ann Clin Transl Neurol, 6*(3), 554-574. doi:10.1002/acn3.730

Cummings, J. L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D. A., & Gornbein, J. (1994). The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology, 44*(12), 2308-2314.

Dourlen, P., Fernandez-Gomez, F. J., Dupont, C., Grenier-Boley, B., Bellenguez, C., Obriot, H., . . . Dermaut, B. (2017). Functional screening of Alzheimer risk loci identifies PTK2B as an in vivo modulator and early marker of Tau pathology. *Mol Psychiatry, 22*(6), 874-883. doi:10.1038/mp.2016.59

Esterlis, I., DellaGioia, N., Pietrzak, R. H., Matuskey, D., Nabulsi, N., Abdallah, C. G., . . . DeLorenzo, C. (2017). Ketamine-induced reduction in mGluR5 availability is associated with an antidepressant

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

response: an [11C]ABP688 and PET imaging study in depression. *Mol Psychiatry*. doi:10.1038/mp.2017.58

Finnema, S. J., Nabulsi, N. B., Eid, T., Detyniecki, K., Lin, S. F., Chen, M. K., . . . Carson, R. E. (2016). Imaging synaptic density in the living human brain. *Sci Transl Med, 8*(348), 348ra396. doi:10.1126/scitranslmed.aaf6667

Folstein, M. F., Folstein, S. E., & al, e. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatric Res, 12*, 189-198.

Gallezot, J. D., Beaver, J. D., Gunn, R. N., Nabulsi, N., Weinzimmer, D., Singhal, T., . . . Rabiner, E. A. (2012). Affinity and selectivity of [(1)(1)C]-(+)-PHNO for the D3 and D2 receptors in the rhesus monkey brain in vivo. *Synapse, 66*(6), 489-500. doi:10.1002/syn.21535

Gallezot, J. D., Weinzimmer, D., Nabulsi, N., Lin, S. F., Fowles, K., Sandiego, C., . . . Ding, Y. S. (2011). Evaluation of [(11)C]MRB for assessment of occupancy of norepinephrine transporters: Studies with atomoxetine in non-human primates. *Neuroimage, 56*(1), 268-279. doi:10.1016/j.neuroimage.2010.09.040

Gasparini, F., & Spooren, W. (2007). Allosteric modulators for mGlu receptors. *Curr Neuropharmacol, 5*(3), 187-194. doi:10.2174/157015907781695900

Gimbel, D. A., Nygaard, H. B., Coffey, E. E., Gunther, E. C., Lauren, J., Gimbel, Z. A., & Strittmatter, S. M. (2010). Memory impairment in transgenic Alzheimer mice requires cellular prion protein. *J Neurosci, 30*(18), 6367-6374. doi:10.1523/JNEUROSCI.0395-10.2010

Gregory, K. J., Dong, E. N., Meiler, J., & Conn, P. J. (2011). Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential. *Neuropharmacology, 60*(1), 66-81. doi:10.1016/j.neuropharm.2010.07.007

Gregory, K. J., Malosh, C., Turlington, M., Morrison, R., Vinson, P., Daniels, J. S., . . . Stauffer, S. R. (2010). Identification of a high affinity MPEP-site silent allosteric modulator (SAM) for the metabotropic glutamate subtype 5 receptor (mGlu5). In *Probe Reports from the NIH Molecular Libraries Program*. Bethesda (MD).

Gregory, K. J., Noetzel, M. J., Rook, J. M., Vinson, P. N., Stauffer, S. R., Rodriguez, A. L., . . . Conn, P. J. (2012). Investigating metabotropic glutamate receptor 5 allosteric modulator cooperativity, affinity, and agonism: enriching structure-function studies and structure-activity relationships. *Mol Pharmacol, 82*(5), 860-875. doi:10.1124/mol.112.080531

Haas, L. T., Kostylev, M. A., & Strittmatter, S. M. (2014). Therapeutic molecules and endogenous ligands regulate the interaction between brain cellular prion protein (PrPC) and metabotropic glutamate receptor 5 (mGluR5). *J Biol Chem, 289*(41), 28460-28477. doi:10.1074/jbc.M114.584342

Haas, L. T., Salazar, S. V., Kostylev, M. A., Um, J. W., Kaufman, A. C., & Strittmatter, S. M. (2016a). Metabotropic glutamate receptor 5 couples cellular prion protein to intracellular signalling in Alzheimer's disease. *Brain, 139*(Pt 2), 526-546. doi:10.1093/brain/awv356

Haas, L. T., Salazar, S. V., Smith, L. V., Zhao, H. R., Cox, T. O., Herber, C. S., . . . Strittmatter, S. M. (2017). Silent Allosteric Modulation of mGluR5 Maintains Glutamate Signaling while Rescuing Alzheimer's Mouse Phenotypes. *Cell Reports, 20*, 76-88. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5547898/pdf/nihms885511.pdf

Haas, L. T., & Strittmatter, S. M. (2016b). Oligomers of Amyloid beta Prevent Physiological Activation of the Cellular Prion Protein-Metabotropic Glutamate Receptor 5 Complex by Glutamate in Alzheimer Disease. *J Biol Chem, 291*(33), 17112-17121. doi:10.1074/jbc.M116.720664

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

Hamilton, A., Esseltine, J. L., DeVries, R. A., Cregan, S. P., & Ferguson, S. S. (2014). Metabotropic glutamate receptor 5 knockout reduces cognitive impairment and pathogenesis in a mouse model of Alzheimer's disease. *Mol Brain, 7*, 40. doi:10.1186/1756-6606-7-40

Hamilton, A., Vasefi, M., Vander Tuin, C., McQuaid, R. J., Anisman, H., & Ferguson, S. S. (2016). Chronic Pharmacological mGluR5 Inhibition Prevents Cognitive Impairment and Reduces Pathogenesis in an Alzheimer Disease Mouse Model. *Cell Rep, 15*(9), 1859-1865. doi:10.1016/j.celrep.2016.04.077

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*(5580), 353-356. doi:10.1126/science.1072994

Heidinger, V., Manzerra, P., Wang, X. Q., Strasser, U., Yu, S. P., Choi, D. W., & Behrens, M. M. (2002). Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current: mediation through the Pyk2/Src-family kinase pathway in cortical neurons. *J Neurosci, 22*(13), 5452-5461. doi:20026565

Hillmer, A. T., Zheng, M. Q., Li, S., Scheunemann, M., Lin, S. F., Holden, D., . . . Huang, Y. (2016). PET imaging evaluation of [(18)F]DBT-10, a novel radioligand specific to alpha7 nicotinic acetylcholine receptors, in nonhuman primates. *Eur J Nucl Med Mol Imaging, 43*(3), 537-547. doi:10.1007/s00259-015-3209-0

Hong, S., Beja-Glasser, V. F., Nfonoyim, B. M., Frouin, A., Li, S., Ramakrishnan, S., . . . Stevens, B. (2016). Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science, 352*(6286), 712-716. doi:10.1126/science.aad8373

Hu, N. W., Nicoll, A. J., Zhang, D., Mably, A. J., O'Malley, T., Purro, S. A., . . . Rowan, M. J. (2014). mGlu5 receptors and cellular prion protein mediate amyloid-beta-facilitated synaptic long-term depression in vivo. *Nat Commun, 5*, 3374. doi:10.1038/ncomms4374

Huang, H., Degnan, A. P., Balakrishnan, A., Easton, A., Gulianello, M., Huang, Y., . . . Macor, J. E. (2016). Oxazolidinone-based allosteric modulators of mGluR5: Defining molecular switches to create a pharmacological tool box. *Bioorg Med Chem Lett, 26*(17), 4165-4169. doi:10.1016/j.bmcl.2016.07.065

Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., . . . Gotz, J. (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell, 142*(3), 387-397. doi:10.1016/j.cell.2010.06.036

Jankowsky, J. L., Fadale, D. J., Anderson, J., Xu, G. M., Gonzales, V., Jenkins, N. A., . . . Borchelt, D. R. (2004). Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet, 13*(2), 159-170. doi:10.1093/hmg/ddh019

Jankowsky, J. L., Xu, G., Fromholt, D., Gonzales, V., & Borchelt, D. R. (2003). Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. *J Neuropathol Exp Neurol, 62*(12), 1220-1227. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14692698

Kaufman, A. C., Salazar, S. V., Haas, L. T., Yang, J., Kostylev, M. A., Jeng, A. T., . . . Strittmatter, S. M. (2015). Fyn inhibition rescues established memory and synapse loss in Alzheimer mice. *Ann Neurol, 77*(6), 953-971. doi:10.1002/ana.24394

Kessler, R. M., Seibyl, J., Cowan, R. L., Zald, D., Young, J. S., Ansari, M. S., & Stabin, M. G. (2014). Radiation Dosimetry of (18)F-FPEB in Humans. *J Nucl Med, 55*(7), 1119-1121. doi:10.2967/jnumed.113.133843

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

Kim, S. J., Zheng, M. Q., Nabulsi, N., Labaree, D., Ropchan, J., Najafzadeh, S., . . . Morris, E. D. (2013). Determination of the in vivo selectivity of a new kappa-opioid receptor antagonist PET tracer 11C-LY2795050 in the rhesus monkey. *J Nucl Med, 54*(9), 1668-1674. doi:10.2967/jnumed.112.118877

Kostylev, M. A., Kaufman, A. C., Nygaard, H. B., Patel, P., Haas, L. T., Gunther, E. C., . . . Strittmatter, S. M. (2015). Prion-Protein-interacting Amyloid-beta Oligomers of High Molecular Weight Are Tightly Correlated with Memory Impairment in Multiple Alzheimer Mouse Models. *J Biol Chem, 290*(28), 17415-17438. doi:10.1074/jbc.M115.643577

Kumar, A., Dhull, D. K., & Mishra, P. S. (2015). Therapeutic potential of mGluR5 targeting in Alzheimer's disease. *Front Neurosci, 9*, 215. doi:10.3389/fnins.2015.00215

Lacor, P. N., Buniel, M. C., Furlow, P. W., Clemente, A. S., Velasco, P. T., Wood, M., . . . Klein, W. L. (2007). Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci, 27*(4), 796-807. doi:10.1523/JNEUROSCI.3501-06.2007

Lambert, J. C., Ibrahim-Verbaas, C. A., Harold, D., Naj, A. C., Sims, R., Bellenguez, C., . . . Amouyel, P. (2013). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet, 45*(12), 1452-1458. doi:10.1038/ng.2802

Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., . . . Klein, W. L. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A, 95*(11), 6448-6453. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9600986

Larson, M., Sherman, M. A., Amar, F., Nuvolone, M., Schneider, J. A., Bennett, D. A., . . . Lesne, S. E. (2012). The complex PrP(c)-Fyn couples human oligomeric Abeta with pathological tau changes in Alzheimer's disease. *J Neurosci, 32*(47), 16857-16871a. doi:10.1523/JNEUROSCI.1858-12.2012

Lauren, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., & Strittmatter, S. M. (2009). Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature, 457*(7233), 1128-1132. doi:10.1038/nature07761

Lee, G., Newman, S. T., Gard, D. L., Band, H., & Panchamoorthy, G. (1998). Tau interacts with src-family non-receptor tyrosine kinases. *J Cell Sci, 111 (Pt 21)*, 3167-3177. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9763511

Lee, G., Thangavel, R., Sharma, V. M., Litersky, J. M., Bhaskar, K., Fang, S. M., . . . Ksiezak-Reding, H. (2004). Phosphorylation of tau by fyn: implications for Alzheimer's disease. *J Neurosci, 24*(9), 2304-2312. doi:10.1523/JNEUROSCI.4162-03.2004

Lesne, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., . . . Ashe, K. H. (2006). A specific amyloid-beta protein assembly in the brain impairs memory. *Nature, 440*(7082), 352-357. doi:10.1038/nature04533

Li, S., Hong, S., Shepardson, N. E., Walsh, D. M., Shankar, G. M., & Selkoe, D. (2009). Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron, 62*(6), 788-801. doi:10.1016/j.neuron.2009.05.012

Li, S. Y., Cai, Z. X., Wu, X. A., Holden, D., Pracitto, R., Kapinos, M., . . . Huang, Y. Y. (2019). Synthesis and in Vivo Evaluation of a Novel PET Radiotracer for Imaging of Synaptic Vesicle Glycoprotein 2A (SV2A) in Nonhuman Primates. *Acs Chemical Neuroscience, 10*(3), 1544-1554. doi:10.1021/acschemneuro.8b00526

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

Lu, Y. M., Jia, Z., Janus, C., Henderson, J. T., Gerlai, R., Wojtowicz, J. M., & Roder, J. C. (1997). Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci, 17*(13), 5196-5205. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9185557

Luscher, C., & Huber, K. M. (2010). Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. *Neuron, 65*(4), 445-459. doi:10.1016/j.neuron.2010.01.016

Molck, C., Harpsoe, K., Gloriam, D. E., Mathiesen, J. M., Nielsen, S. M., & Brauner-Osborne, H. (2014). mGluR5: exploration of orthosteric and allosteric ligand binding pockets and their applications to drug discovery. *Neurochem Res, 39*(10), 1862-1875. doi:10.1007/s11064-014-1248-8

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology, 43*(11), 2412-2414.

Nabulsi, N. B., Mercier, J., Holden, D., Carre, S., Najafzadeh, S., Vandergeten, M. C., . . . Huang, Y. (2016). Synthesis and Preclinical Evaluation of 11C-UCB-J as a PET Tracer for Imaging the Synaptic Vesicle Glycoprotein 2A in the Brain. *J Nucl Med, 57*(5), 777-784. doi:10.2967/jnumed.115.168179

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., . . . Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc, 53*(4), 695-699. doi:10.1111/j.1532-5415.2005.53221.x

Nicodemo, A. A., Pampillo, M., Ferreira, L. T., Dale, L. B., Cregan, T., Ribeiro, F. M., & Ferguson, S. S. (2010). Pyk2 uncouples metabotropic glutamate receptor G protein signaling but facilitates ERK1/2 activation. *Mol Brain, 3*, 4. doi:10.1186/1756-6606-3-4

Nicolas, J. M., Hannestad, J., Holden, D., Kervyn, S., Nabulsi, N., Tytgat, D., . . . Klitgaard, H. (2016). Brivaracetam, a selective high-affinity synaptic vesicle protein 2A (SV2A) ligand with preclinical evidence of high brain permeability and fast onset of action. *Epilepsia, 57*(2), 201-209. doi:10.1111/epi.13267

Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., . . . LaFerla, F. M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron, 39*(3), 409-421. doi:S0896627303004343 [pii]

Overk, C. R., Cartier, A., Shaked, G., Rockenstein, E., Ubhi, K., Spencer, B., . . . Masliah, E. (2014). Hippocampal neuronal cells that accumulate alpha-synuclein fragments are more vulnerable to Abeta oligomer toxicity via mGluR5--implications for dementia with Lewy bodies. *Mol Neurodegener, 9*, 18. doi:10.1186/1750-1326-9-18

Palop, J. J., & Mucke, L. (2010). Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nat Neurosci, 13*(7), 812-818. doi:10.1038/nn.2583

Park, E., Sullivan, J. M., Planeta, B., Gallezot, J. D., Lim, K., Lin, S. F., . . . Carson, R. E. (2015). Test-retest reproducibility of the metabotropic glutamate receptor 5 ligand [(1)(8)F]FPEB with bolus plus constant infusion in humans. *Eur J Nucl Med Mol Imaging, 42*(10), 1530-1541. doi:10.1007/s00259-015-3094-6

Park, J. H., Widi, G. A., Gimbel, D. A., Harel, N. Y., Lee, D. H., & Strittmatter, S. M. (2006). Subcutaneous Nogo receptor removes brain amyloid-beta and improves spatial memory in Alzheimer's transgenic mice. *J Neurosci, 26*(51), 13279-13286. doi:10.1523/JNEUROSCI.4504-06.2006

Porter, R. H., Jaeschke, G., Spooren, W., Ballard, T. M., Buttelmann, B., Kolczewski, S., . . . Malherbe, P. (2005). Fenobam: a clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J Pharmacol Exp Ther, 315*(2), 711-721. doi:10.1124/jpet.105.089839

Raka, F., Di Sebastiano, A. R., Kulhawy, S. C., Ribeiro, F. M., Godin, C. M., Caetano, F. A., . . . Ferguson, S. S. (2015). Ca(2+)/calmodulin-dependent protein kinase II interacts with group I metabotropic glutamate and facilitates receptor endocytosis and ERK1/2 signaling: role of beta-amyloid. *Mol Brain, 8*, 21. doi:10.1186/s13041-015-0111-4

Renner, M., Lacor, P. N., Velasco, P. T., Xu, J., Contractor, A., Klein, W. L., & Triller, A. (2010). Deleterious effects of amyloid beta oligomers acting as an extracellular scaffold for mGluR5. *Neuron, 66*(5), 739-754. doi:10.1016/j.neuron.2010.04.029

Ribeiro, F. M., Paquet, M., Cregan, S. P., & Ferguson, S. S. (2010). Group I metabotropic glutamate receptor signalling and its implication in neurological disease. *CNS Neurol Disord Drug Targets, 9*(5), 574-595. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20632969

Roberson, E. D., Halabisky, B., Yoo, J. W., Yao, J., Chin, J., Yan, F., . . . Mucke, L. (2011). Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. *J Neurosci, 31*(2), 700-711. doi:10.1523/JNEUROSCI.4152-10.2011

Rodriguez, A. L., Grier, M. D., Jones, C. K., Herman, E. J., Kane, A. S., Smith, R. L., . . . Conn, P. J. (2010). Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. *Mol Pharmacol, 78*(6), 1105-1123. doi:10.1124/mol.110.067207

Rosen, W. G., Terry, R. D., Fuld, P. A., Katzman, R., & Peck, A. (1980). Pathological verification of ischemic score in differentiation of dementias. *Ann. Neurol., 7*, 486-488.

Rutrick, D., Stein, D. J., Subramanian, G., Smith, B., Fava, M., Hasler, G., . . . Gomez-Mancilla, B. (2017). Mavoglurant Augmentation in OCD Patients Resistant to Selective Serotonin Reuptake Inhibitors: A Proof-of-Concept, Randomized, Placebo-Controlled, Phase 2 Study. *Adv Ther, 34*(2), 524-541. doi:10.1007/s12325-016-0468-5

Saito, T., Matsuba, Y., Mihira, N., Takano, J., Nilsson, P., Itohara, S., . . . Saido, T. C. (2014). Single App knock-in mouse models of Alzheimer's disease. *Nature Neuroscience, 17*(5), 661-+. doi:10.1038/nn.3697

Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., . . . Saido, T. C. (2019). Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation. *Journal of Biological Chemistry, 294*(34), 12754-12765. doi:10.1074/jbc.RA119.009487

Sawant-Basak, A., Chen, L., Shaffer, C. L., Palumbo, D., Schmidt, A., Tseng, E., . . . McCarthy, T. (2017). Quantitative projection of human brain penetration of the H3 antagonist PF-03654746 by integrating rat-derived brain partitioning and PET receptor occupancy. *Xenobiotica, 47*(2), 119-126. doi:10.3109/00498254.2016.1166531

Scheff, S. W., DeKosky, S. T., & Price, D. A. (1990). Quantitative assessment of cortical synaptic density in Alzheimer's disease. *Neurobiol Aging, 11*(1), 29-37. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2325814

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med, 8*(6), 595-608. doi:10.15252/emmm.201606210

Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., . . . Selkoe, D. J. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med, 14*(8), 837-842. doi:10.1038/nm1782

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

Sheffler, D. J., Gregory, K. J., Rook, J. M., & Conn, P. J. (2011). Allosteric modulation of metabotropic glutamate receptors. *Adv Pharmacol, 62*, 37-77. doi:10.1016/B978-0-12-385952-5.00010-5

Sheikh, J., & Yesavage, J. (1986). Geriatric Depression Scale (GDS): Recent evidence and development of a shorter version. In T. L. Brind (Ed.), *Clinical gerontology: A guide to assessment and intervention* (pp. 165-173). New York: Haworth Press.

Sullivan, J. M., Lim, K., Labaree, D., Lin, S. F., McCarthy, T. J., Seibyl, J. P., . . . Morris, E. D. (2013). Kinetic analysis of the metabotropic glutamate subtype 5 tracer [(18)F]FPEB in bolus and bolus-plus-constant-infusion studies in humans. *J Cereb Blood Flow Metab, 33*(4), 532-541. doi:10.1038/jcbfm.2012.195

Teasdale, G., & Jennett, B. (1974). Assessment of coma and impaired consciousness. A practical scale. *Lancet, 2*(7872), 81-84. doi:10.1016/s0140-6736(74)91639-0

Toyonaga, T., Smith, L. M., Finnema, S. J., Gallezot, J. D., Naganawa, M., Bini, J., . . . Carson, R. E. (2019). In Vivo Synaptic Density Imaging with (11)C-UCB-J Detects Treatment Effects of Saracatinib in a Mouse Model of Alzheimer Disease. *J Nucl Med, 60*(12), 1780-1786. doi:10.2967/jnumed.118.223867

Um, J. W., Kaufman, A. C., Kostylev, M., Heiss, J. K., Stagi, M., Takahashi, H., . . . Strittmatter, S. M. (2013). Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer abeta oligomer bound to cellular prion protein. *Neuron, 79*(5), 887-902. doi:10.1016/j.neuron.2013.06.036

Um, J. W., Nygaard, H. B., Heiss, J. K., Kostylev, M. A., Stagi, M., Vortmeyer, A., . . . Strittmatter, S. M. (2012). Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat Neurosci, 15*(9), 1227-1235. doi:10.1038/nn.3178

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., . . . Selkoe, D. J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature, 416*(6880), 535-539. doi:10.1038/416535a

Wang, Q., Walsh, D. M., Rowan, M. J., Selkoe, D. J., & Anwyl, R. (2004). Block of long-term potentiation by naturally secreted and synthetic amyloid beta-peptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5. *J Neurosci, 24*(13), 3370-3378. doi:10.1523/JNEUROSCI.1633-03.2004

Wong, D. F., Waterhouse, R., Kuwabara, H., Kim, J., Brasic, J. R., Chamroonrat, W., . . . Mozley, P. D. (2013). 18F-FPEB, a PET radiopharmaceutical for quantifying metabotropic glutamate 5 receptors: a first-in-human study of radiochemical safety, biokinetics, and radiation dosimetry. *J Nucl Med, 54*(3), 388-396. doi:10.2967/jnumed.112.107995

Xia, Y., Zheng, M. Q., Holden, D., Lin, S. F., Kapinos, M., Ropchan, J., . . . Carson, R. E. (2015). Measurement of Bmax and Kd with the glycine transporter 1 radiotracer (1)(8)F-MK6577 using a novel multi-infusion paradigm. *J Cereb Blood Flow Metab, 35*(12), 2001-2009. doi:10.1038/jcbfm.2015.163

Xu, J., Zhu, Y., Contractor, A., & Heinemann, S. F. (2009). mGluR5 has a critical role in inhibitory learning. *J Neurosci, 29*(12), 3676-3684. doi:10.1523/JNEUROSCI.5716-08.2009

Yang, F., Snyder, L. B., Balakrishnan, A., Brown, J. M., Sivarao, D. V., Easton, A., . . . Degnan, A. P. (2016). Discovery and Preclinical Evaluation of BMS-955829, a Potent Positive Allosteric Modulator of mGluR5. *ACS Med Chem Lett, 7*(3), 289-293. doi:10.1021/acsmedchemlett.5b00450

Zhang, D., Qi, Y., Klyubin, I., Ondrejcak, T., Sarell, C. J., Cuello, A. C., . . . Rowan, M. J. (2017). Targeting glutamatergic and cellular prion protein mechanisms of amyloid beta-mediated

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021

persistent synaptic plasticity disruption: Longitudinal studies. *Neuropharmacology, 121*, 231-246. doi:10.1016/j.neuropharm.2017.03.036

Zhang, H., Wu, L., Pchitskaya, E., Zakharova, O., Saito, T., Saido, T., & Bezprozvanny, I. (2015). Neuronal Store-Operated Calcium Entry and Mushroom Spine Loss in Amyloid Precursor Protein Knock-In Mouse Model of Alzheimer's Disease. *J Neurosci, 35*(39), 13275-13286. doi:10.1523/JNEUROSCI.1034-15.2015

APPROVED BY THE YALE UNIVERSITY IRB 12/15/2021