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Disease-Modifying Benefit of Fyn Blockade Persists after Washout in Mouse Alzheimer's Model

Levi M. Smith^{a,b,d}, Rong Zhu^{a,d}, and Stephen M. Strittmatter^{a,c,*}

^aCellular Neuroscience, Neurodegeneration and Repair Program, Yale University School of Medicine, New Haven, CT, 06510 USA

^bDepartment of Cell Biology, Yale University School of Medicine, New Haven, CT, 06510 USA

^cDepartments of Neurology and of Neuroscience, Yale University School of Medicine, New Haven, CT, 06510 USA

Abstract

Alzheimer's disease remains without a disease-modifying therapy that improves symptoms after therapy withdrawal. Because no investigational agents have demonstrated disease-modifying effects clinically, we tested whether the Fyn inhibitor, saracatinib, provides persistent improvement in a transgenic model. Aged APP^{swe}/PS1^{E9} mice were treated with saracatinib or memantine for 4 weeks and spatial memory improved to control levels. After drug washout, there was sustained rescue of both memory function and synapse density by saracatinib, but a loss of benefit from memantine. These data demonstrate a disease-modifying persistent benefit for saracatinib in a preclinical Alzheimer's model, and distinguish its action from that of memantine.

Keywords

Alzheimer's disease; APP^{swe}/PS1^{E9} mice; saracatinib; AZD0530; memantine; Namenda; Fyn kinase

1. Introduction

Alzheimer's disease (AD) is a progressive and fatal, neurodegenerative disease characterized by a patient's presentation with confusion and memory loss that rapidly progresses to frank dementia. Definitive diagnosis of AD requires the presence of extracellular plaques of Amyloid Beta (A β) and intracellular neurofibrillary tangles of Tau on histological examination of brain tissue. Increasing age is the greatest risk factor for disease with incidence doubling every 6.3 years beyond the age of 60 (Martin, 2015). AD is responsible for up to 75% of the 50 million cases of dementia worldwide and is the 6th leading cause of

*To whom correspondence should be addressed: stephen.strittmatter@yale.edu.

^dThese authors contributed equally to this work

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death in the United States (Acosta and Wortmann, 2009; Martin, 2015; Weuve et al., 2014). By 2050, Alzheimer's disease is projected to account for 43% of adult deaths in the United States (Weuve et al., 2014). The impact of AD on both quality and duration of life will continue to grow in the absence of disease-modifying therapeutics as life expectancy rises worldwide (Martin, 2015).

A disease-modifying treatment for AD is one that delays the underlying pathophysiology and produces an improvement in clinical signs and symptoms (Committee for Medicinal Products for Human Use, 2008). The withdrawal study design has been proposed as a protocol capable of demonstrating these effects in clinical trials (Cummings, 2009; Leber, 1996). In the withdrawal study design, all patients initially receive drug treatment and are then randomized to either drug or placebo treatment. Benefits conferred by symptomatic treatments are not expected to persist after treatment withdrawal, resulting in clinical scores similar to patients who received placebo treatment throughout. Alternatively, the effects of a disease-modifying treatment are expected to be sustained, and while patients would begin declining again after treatment discontinuation, they would not decline to the level of placebo-only treated patients (Cummings, 2009). Over a 10-year observation period, Cummings et al. documented a 99.6% failure rate for Alzheimer's disease therapeutics that entered clinical development in the United States (Cummings et al., 2014). Of the 413 trials undertaken, 53% were for purported disease-modifying agents. Across all trials, 70% of the agents currently in development claim to be disease-modifying (Cummings et al., 2017). There are currently no drugs capable of preventing or modifying the progression of AD.

One explanation for the failure of pre-clinical success to translate to clinical efficacy is the quality of evidence required of pre-clinical efficacy studies. Traditionally, transgenic mice that express human genes with disease-causing mutations in amyloid beta precursor protein (*APP*) and/or presenilin 1 (*PSEN1*) are treated with an investigational agent in either a prevention or treatment modality and various measures of efficacy are assessed while the animals are on treatment. While improvements in the animals' performance in various learning and memory tasks are often accompanied by rescue of biomarkers of synapse density and neurotoxicity, the study design does not permit the distinction between disease-modifying and symptomatic agents. Notably, multiple classes of internationally-approved drugs for the symptomatic treatment of AD were successful in these traditional on-treatment paradigms (Dong et al., 2008; Filali et al., 2011; Martinez-Coria et al., 2010; Minkeviciene et al., 2004; Scholtzova et al., 2008; Unger et al., 2006).

Here, we assess the disease-modifying benefit of the Fyn inhibitor saracatinib (AZD0530) and compare it to the symptomatic agent memantine in a drug withdrawal study of a mouse model of AD. Fyn is a non-receptor tyrosine kinase related to Src. Fyn has been studied as a Tau kinase, and is a key connection of Amyloid beta oligomers ($A\beta_o$) to tauopathy (Chin et al., 2005; Chin et al., 2004; Ittner et al., 2010; Kaufman et al., 2015; Larson et al., 2012; Lee et al., 1998; Roberson et al., 2011; Rushworth et al., 2013; Shirazi and Wood, 1993; Um et al., 2012). Fyn activity is increased in AD brain and by exposure of neurons to $A\beta_o$, via PrP^C (Larson et al., 2012; Lauren et al., 2009; Um et al., 2012). Genetic deletion of *FYN* prevents $A\beta_o$ -induced cell death in hippocampus, and Fyn inhibition restores synapse density and memory function in transgenic mice (Kaufman et al., 2015; Lambert et al.,

1998). Although another kinase inhibitor, masitinib, is less potent and less selective than saracatinib as a Fyn inhibitor, the potential utility of both compounds in AD may depend on Fyn inhibition (Folch et al., 2015; Nygaard, 2017; Piette et al., 2011). We report that saracatinib has a persistent, disease-modifying benefit for memory function and synapse density in a transgenic mouse AD model.

2. Materials and Methods

2.1 Transgenic and Control Mouse strains

Mice were cared for by the Yale Animal Resource Center and all experiments were approved by Yale's Institutional Animal Care and Use Committee. Wild type and APP^{swe}/PS1^{E9} mice (Jankowsky et al., 2004) were purchased from Jackson Laboratory and maintained on a C57/Bl6J background as described previously (Gimbel et al., 2010; Um et al., 2013; Um et al., 2012). All experiments were conducted in a blinded fashion with respect to genotype and treatment, and groups were matched for age and sex.

2.2 Treatment

Mice were randomly assigned to treatment groups and the experimenter was unaware of both genotype and treatment group. Memantine was administered by twice daily intraperitoneal (i.p.) injection of 10 mg memantine per kg body weight in saline. Saracatinib was administered by twice daily oral gavage of 2.5 mg saracatinib per kg body weight in a vehicle of 0.5% w/v hydroxypropyl methylcellulose and 0.1% w/v polysorbate 80. To control for the different routes of administration, memantine treated animals were orally gavaged twice daily with vehicle and saracatinib treated animals were injected i.p. with saline twice daily. Vehicle treated animals were gavaged twice daily with vehicle and injected i.p. twice daily with saline. Animals were treated for 4 weeks prior to the on-treatment Morris water maze and throughout on-treatment assessment. A subset of mice was sacrificed after on-treatment studies, and the rest were continued for washout studies.

2.3 Behavioral testing

Each mouse was handled by the experimenter for five minutes each day for five days preceding behavioral assessment to reduce anxiety. Morris water maze testing was conducted as described (Haas et al., 2017; Kaufman et al., 2015; Morris, 1984; Um et al., 2013). Each day animals swam in two trials consisting of four swims each. Each swim lasted until the mouse located the hidden platform, or until 60 seconds had elapsed. Animals were given a 60 second rest period between swims in a dry cage with a heat lamp available. Trials were initiated twice daily, on three consecutive days. For each trial, a single animal's latency in the 4 swims was averaged and a group mean was calculated from these individual animal values. Twenty-four hours after the last learning swim, mice were tested in a probe trial. In the probe trial the hidden platform was removed and mice freely swam in the pool for 60 seconds. Each animal swam once for the probe trial. For comparison between on-treatment and washout performance in the Morris water maze probe trial, comparisons were only made between animals that swam in both trials as described in Fig. 1 legend.

Novel object recognition was performed as described (Haas et al., 2017; Kaufman et al., 2015; Um et al., 2013). The mice were exposed to two identical objects for 10 minutes. Forty-eight hours later, mice were again placed in a rat cage containing one familiar object and a novel object. The mice were allowed 10 minutes to accumulate 30 seconds of interaction time during each session. Animals that did not accumulate 30 seconds of interaction time during the allotted 10 minutes of familiarization or testing were excluded from analysis. The novel and familiar object were randomized, and the appearance of the novel in the left or right location of the cage was also randomized.

2.4 Immunohistology

Mice were euthanized, and perfused with ice-cold PBS for 2 minutes via cardiac perfusion (Haas et al., 2017; Kaufman et al., 2015; Um et al., 2013). The brain was then dissected and the hemispheres were separated. One hemisphere was placed in 4% PFA for 24 hours at 4°C, and then transferred to PBS containing 0.05% sodium azide. Brains were cut into 40 µm thick parasagittal slices using a vibratome (Leica VT1000S). Slices were permeabilized with 0.1% Triton X-100 in PBS and blocked with 4% horse serum in PBS. Primary antibodies and dilutions used were as follows: Aβ (CST #2454S, 1:250), GFAP (Sigma C9205, 1:500), CD68 (Bio-Rad MCA1957, 1:1000), PSD95 (Invitrogen 51-6900, 1:250) and were detected with appropriate Alexa Fluor conjugated (ThermoFisher Scientific) secondary antibodies. Tiled, confocal images were collected and then processed with ImageJ (Schneider et al., 2012). For Aβ, GFAP, and CD68, three slices per animal were imaged and a single tiled image comprising most of the cortex was collected and quantified. For each animal, values generated from the three images were averaged to yield a single value for each animal. For PSD95, three slices per animal were imaged and for each slice, three images in the polymorphic layer of the dentate gyrus were collected (Amaral et al., 2007). Three images from the same slice were averaged to yield a slice average value and three slice average values from three slices of the same animal were averaged to produce an animal average.

2.5 Statistics

All results are presented as means ± standard error of the mean (SEM). IBM SPSS Statistics version 21 and Prism 6 software were used for statistical analysis. Data was analyzed using one-way ANOVA (analysis of variance), followed by post-hoc Tukey's multiple comparisons test or Dunnett's multiple comparisons test. Only two-sided tests were used and all data analyzed met the assumption for the specific statistical test that was performed.

3. Results

3.1 Improvements in learning and memory persist after treatment washout for animals treated with saracatinib but not memantine

First, we verified on-treatment benefits using the Morris water maze as a measure of hippocampus-dependent learning and memory. In this task, animals must recall spatial cues to locate a hidden platform submerged below the surface of a pool. Learning and memory is evidenced as decreasing latency to find the hidden platform on subsequent swims. One-year-old APPswe/PS1 E9 transgenic mice were treated with saracatinib, memantine, or vehicle for five weeks and Morris water maze performance assessed (Fig. 1A). In the last two

learning trial blocks, vehicle-treated transgenic animals (Tg, Veh) were significantly impaired compared to wild-type vehicle-treated (WT, Veh) animals in latency to find the hidden platform (Fig. 2A,B, ANOVA $p < 0.001$). Transgenic mice treated with saracatinib (Tg, Sar) found the platform as quickly as WT (Fig. 2A,B). Transgenic mice treated with memantine (Tg, Mem) were significantly impaired compared to WT vehicle controls (Fig. 2A,B, ANOVA $p < 0.05$). Latency to escape was mirrored by the distance traveled (Supp. Fig. 1A) and was not due to differences in swim speeds (Supp. Fig. 2A). One day after completion of the training phase, the animals' ability to recall the platform location was assessed in a probe trial. Transgenic vehicle-treated mice spent less time in the target quadrant than did WT controls (Fig. 2C, ANOVA $p < 0.05$) indicating memory impairment. Differences in probe trial performance are due to memory recall as all groups swam at the same speed and traveled the same distance during the one minute trial (Supp. Fig. 1B and 2B). Treatment with either saracatinib or memantine increased the target time and both treatment groups performed as well as controls (Fig. 2C). Thus, both saracatinib and memantine can improve memory while mice are on-treatment.

To distinguish symptomatic from disease-modifying benefit, we re-tested a set of mice eight days after therapy cessation (Fig. 1A,B). Eight days is 12 times the steady-state saracatinib elimination half-life from mouse brain, thereby clearing all drug (Kaufman et al., 2015). In the water maze re-test, the hidden platform was moved to the quadrant opposite its previous location. Transgenic mice previously treated with saracatinib performed indistinguishably from WT controls after drug washout (Fig. 2D,E). In contrast, transgenic animals previously treated with memantine were significantly impaired in the washout re-test (Fig. 2D,E, ANOVA $p < 0.0001$). As for the on-treatment swims, differences in latency were due to the distance traveled before locating the hidden platform (Supp. Fig. 1C) and not due to different swim speeds (Supp. Fig. 2C). During the washout probe trial to the reverse platform location, the transgenic saracatinib group showed a non-significant trend to perform better than the transgenic control or memantine groups, and were not significantly different from the WT controls (Fig. 2F). Distance traveled and swim speeds were again the same between groups (Supp. Fig. 1D and 2D). To directly investigate changes in performance between on-treatment and washout assessments with statistical power, we compared performance in the washout probe trial to the same animals' performance in the on-treatment probe trial (Fig. 1B and 2G). Wild-type and transgenic mice treated with vehicle as well as transgenic animals treated with saracatinib spent similar time in the target quadrant during both probes. Transgenic animals treated with memantine spent significantly less time in the target quadrant during the washout probe trial compared to their on-drug trial (ANOVA $p < 0.01$). These data indicate that effects of saracatinib treatment persist after washout, whereas those of memantine do not.

The use of aversive stimulus and the lack of novel context in the reverse swim of the Morris water during washout prompted us to investigate effects of treatment washout in an additional learning and memory paradigm. We continued the washout period to 22 days (Fig. 1) and examined performance in novel object recognition. WT control mice prefer novelty, demonstrated by more time spent with the novel object (Fig. 3, ANOVA $p < 0.0001$). Transgenic mice are unable to distinguish between novel and familiar objects and their time with each object is similar. Transgenic mice previously treated with saracatinib spend

significantly more time with the novel object, whereas transgenic mice treated with memantine spend a similar time with both objects (Fig. 3). These data reveal that saracatinib treatment benefit continues at 22 days (>30 half-lives) post-treatment. The absence of a positive memantine effect is consistent with the decrement observed in water maze.

3.2 Rescue of synapse density by treatment with saracatinib persists up to one month post treatment

The role of the hippocampus in episodic memory in both mouse and man is well established and MRI studies have demonstrated robust loss of hippocampal volume in patients with mild cognitive impairment which worsens as severity progress to AD (Shi et al., 2009). Importantly, the APPswe/PS1dE9 transgenic mouse model of AD also demonstrates robust synapse loss in the hippocampus which is most pronounced in the dentate gyrus (Kaufman et al., 2015; Um et al., 2013). To determine whether the persistent improvements in memory following saracatinib treatment are due to synaptic changes in a brain region responsible for memory and learning, we measured synapse density in the dentate gyrus of mice 29 days after treatment discontinuation (Fig. 1). We observed a robust loss of PSD95 immunoreactive area in transgenic, vehicle-treated mice, which was fully restored to WT level by saracatinib, even after washout (Fig. 4A,B). Transgenic animals treated with memantine demonstrated a significant reduction in PSD95 area; indistinguishable from vehicle-treated transgenics, and significantly less than WT.

It is hypothesized that saracatinib confers benefit by action in neurons, rather than A β accumulation or inflammation (Kaufman et al., 2015; Um et al., 2012). To assess this, we quantified A β , glial fibrillary acid protein (GFAP), and CD68 immunoreactivity in cerebral cortex. Transgenic animals previously treated with saracatinib had similar A β burden as vehicle-treated transgenics whereas memantine-treated mice had somewhat greater cortical A β (Fig. 5A,B). We found no differences in astrogliosis between transgenics previously administered vehicle, saracatinib or memantine (Fig. 6A,B). Similarly, no differences were observed in microglial CD68 immunoreactivity in mice of the three groups (Fig. 6C,D). Previously, we observed a modest reduction of microglial Iba1 immunoreactivity with saracatinib (Kaufman et al., 2015), however microglial changes do not persist on washout. While reduced microgliosis by saracatinib may be beneficial, washout data suggest it is not essential for cognitive benefits and synapse recovery.

4. Discussion

In the current study, we demonstrate that saracatinib exhibits disease-modifying effects in a mouse AD model evidenced by preservation of treatment benefit following drug washout. These effects include persistent cognitive improvements in two learning and memory tasks, as well as persistent restoration of synapse density. By implementing the withdrawal study design in a preclinical model of AD, we distinguished the disease-modifying benefits of saracatinib from the symptomatic effects of memantine.

We observed no effect of treatment on astrogliosis or microgliosis, consistent with our understanding that both agents confer their benefits primarily on neurons. We have previously observed a modest reduction of Iba1 immunoreactivity during AZD0530

treatment, however this effect does not persist on washout (Kaufman et al., 2015). While this reduction in pathology may be beneficial, our washout data demonstrate that the decrement in microgliosis is not necessary for cognitive benefits and synapse recovery.

We have previously demonstrated that AZD0530 does not modulate any of the pools of A β and we confirmed that observation here with plaque staining in mouse cortex (Kaufman et al., 2015). We were surprised to find that amyloid plaque loads were increased in the brains of transgenic animals previously treated with memantine. Previous literature examining the efficacy of memantine in Alzheimer's model mice is highly varied. Many reports do not include an analysis of A β plaque burden (Filali et al., 2011; Minkeviciene et al., 2004; Van Dam et al., 2005; Van Dam and De Deyn, 2006). Others report no effect of treatment on formic-acid extracted A β (Martinez-Coria et al., 2010; Unger et al., 2006) and still others report decreases in plaque burden (Dong et al., 2008; Martinez-Coria et al., 2010; Scholtzova et al., 2008). The inconsistency in A β plaque response to memantine treatment could be due to variability in mouse strains used, age at treatment initiation, the duration of treatment, or treatment dose or route. As our study is the first to examine the effect of treatment washout in aged animals with established disease, plaque response may also be an effect of treatment washout. It is also worth noting that our analysis scored the entire cortex instead of a single image frame. This is important because plaque burden is heterogeneous and any one image may not accurately reflect amyloidosis.

While the phenotypes exhibited by the transgenic mice utilized in our studies did not return within one month following discontinuation of treatment with saracatinib, it is expected that disease progression would eventually recur. Treatment with saracatinib normalized synapse density and memory function while leaving plaque load and gliosis unaltered. This is similar to younger transgenic mice of approximately six months of age which have both plaque pathology and gliosis in the absence of synapse loss and memory impairments. We expect that if washout were continued, disease in these animals, much like younger, untreated animals would progress and synapse loss and memory impairments would recur.

The persistence of treatment effects in Alzheimer's model mice has only once been previously evaluated. Van Dam and De Deyn investigated the effects of the symptomatic agents galantamine and memantine in APP23 transgenic mice (Van Dam and De Deyn, 2006). Mice were implanted with osmotic minipumps infusing either galantamine or memantine at six weeks of age. Treatment continued for two months and was followed by three weeks of washout and assessment of performance in the Morris water maze. A high dose and low dose of each drug was compared to saline-treated transgenic animals. No treatment condition improved performance in the acquisition phase. In the probe trial, treatment with low-dose galantamine or high-dose memantine resulted in increased time in the target quadrant compared to vehicle-treated APP23 mice. High-dose memantine (Van Dam and De Deyn, 2006). It is important to note that while the APP23 mouse model exhibits impairments in the Morris water maze at three months of age, this is prior to the development of plaques, gliosis, and synapse loss (Boncristiano et al., 2005; Calhoun et al., 1998; Kelly et al., 2003; Reichwald et al., 2009; Sturchler-Pierrat et al., 1997; Van Dam et al., 2003). Furthermore, this model does not exhibit changes in long-term potentiation (Roder et al., 2003). This stands in stark contrast to the pathology observed in human

patients in which clinical symptoms are not apparent until amyloidosis has plateaued, neuroinflammation is ongoing, and neurodegeneration is well established (Morris and Price, 2001; Perrin et al., 2009; Shi et al., 2009; Tarkowski et al., 2003; Xiang et al., 2006).

As neurotoxicity progresses from dysfunction to synapse loss and on to neuron loss, the potential therapeutic intervention decreases. Importantly, transgenic AD model mice expression familial mutations in APP and/or PS1 exhibit a reduction in synapses but do not exhibit neurodegeneration. This allows the opportunity for therapeutic interventions to fully correct various histological and behavioral phenotypes.

We are currently investigating the clinical utility of saracatinib in AD with a Phase 2a study (NCT02167256). The primary outcome measures are the effect of saracatinib treatment on 12-month reductions in 18F-FDG PET, and the safety and tolerability. The novel and unprecedented evidence for disease modification in a pre-clinical model presented here provide further encouragement for investigation of saracatinib efficacy in AD.

The therapeutic effects of saracatinib treatment on multiple memory tasks and synapse density persist for at least one month after therapy cessation. This persistence of benefit is a unique attribute of saracatinib treatment and was not observed for memantine treatment, despite on-treatment efficacy. These results demonstrate the disease-modifying potential of saracatinib treatment and the utility of the withdrawal study design in differentiating disease-modifying and symptomatic agents in a pre-clinical model of Alzheimer's disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Disease-modifying effects of saracatinib or memantine were assessed *in vivo*.
- Saracatinib but not memantine exhibited persistent rescue of synapses and memory.
- Saracatinib benefit was observed at least one month after therapy discontinuation.
- Withdrawal study design can differentiate disease-modifying and symptomatic agents.

Fig. 1. Timeline of treatment paradigm and behavioral assessment

A. The schematic depicts the duration of treatment and at what times during washout the various behavioral assessments and tissue collection were performed. **B.** The flow diagram describes the initial population of animals randomized to receive vehicle, saracatinib, or memantine, and identifies instances where animals met exclusion criteria for the Morris water maze or were removed for potential on-treatment analysis.

Fig. 2. Persistent improvement in the Morris water maze after saracatinib treatment of aged APP/PS1 mice

A,B. Saracatinib and memantine improve learning and memory during treatment. **A.** Morris water maze. Latency for animals to find to hidden platform while on treatment is plotted as a function of training sessions. **B.** Latency of trials five and six while on treatment. One-way analysis of variance (ANOVA), each group compared to the mean of the WT, Vehicle group with Dunnett's multiple comparison test. **C.** Time spent in the target quadrant during the probe trial while on treatment. One-way ANOVA, each group compared to the mean of the WT, Vehicle group with Dunnett's multiple comparison test. **D,E** Cognitive effects of

saracatinib persist after drug washout. **D.** Morris water maze. Latency for animals to find the hidden platform after drug washout is plotted as a function of training sessions. **E.** Latency of trials five and six after drug washout One-way ANOVA, each group compared to the mean of the WT, Vehicle group with Dunnett's multiple comparison test. **F.** Time spent in the target quadrant during the probe trial after washout. One-way ANOVA, each group compared to the mean of the WT, Vehicle group with Dunnett's multiple comparison test. **G.** Comparison of time spent in the target quadrant during the probe trial during treatment and after drug washout. Repeated Measures two-way ANOVA with Sidak's multiple comparison test. For all experiments, data shown are mean $\pm$ s.e.m. WT = wild type, n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

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Fig. 3. Persistent benefit of saracatinib treatment on novel object recognition

Comparison of time spent with either the novel or familiar object. Repeated Measures two-way ANOVA with Sidak's multiple comparison test. For all experiments, data shown are mean $\pm$ s.e.m. WT = wild type, n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Fig. 4. Persistent recovery of synapse density after saracatinib treatment of APP^{swe}/PS1 E9 mice

A. Confocal images of PSD95 immunoreactivity in the dentate gyrus of mice sacrificed 29 days after receiving the indicated treatment. Scale bar = 10 μ m. **B.** Quantitation of PSD95 immunoreactivity in A. One-way ANOVA with Dunnett's multiple comparison test comparing each group to WT, Vehicle group.

Fig. 5. Amyloidosis is not altered by saracatinib treatment

A. Images of amyloid beta staining in the cortex of mice in the indicated groups.

Representative images are max intensity projections of 40 $\times$, tiled images obtained on a confocal microscope. **B.** Quantification of amyloidosis. One-way ANOVA with Dunnett's multiple comparison test comparing each group to APP/PS1, Vehicle. All data shown are mean $\pm$ s.e.m., n.s. = not significant, * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001.

Fig. 6. Astrocytosis and microgliosis are similar among transgenic animals

A. Images of mouse cortex stained for glial fibrillary acid protein (GFAP). Images are maximum intensity projections of 40× tiled z-stacks taken on a confocal microscope. Scale bars = 100 μm. **B.** Quantification of GFAP staining. One-way ANOVA with Dunnett's multiple comparison test comparing each group to APP/PS1, Vehicle. **C.** Images of CD68 staining in mouse cortex. Representative images are maximum intensity projections of 40× tiled z-stacks taken on a confocal microscope. Scale bars = 100 μm. **D.** Quantification of CD68 staining. One-way ANOVA with Dunnett's multiple comparison test comparing each

group to APP/PS1, Vehicle. All data shown are mean $\pm$ s.e.m., n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

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