Published in final edited form as:

J Alzheimers Dis. 2017 ; 60(2): 461–481. doi:10.3233/JAD-170429.

Pazopanib reduces phosphorylated tau levels and alters astrocytes in a mouse model of tauopathy

Monica Javidnia^{a,b}, Michaeline L. Hebron^a, Yue Xin^a, Nikolas G. Kinney^a, and Charbel E-H. Moussa^a

^aDepartment of Neurology, Laboratory for Dementia and Parkinsonism, Translational Neurotherapeutics Program. 4000 Reservoir Rd. NW. Building D, Room 267

^bDepartment of Pharmacology & Physiology. Georgetown University Medical Center, 3900 Reservoir Rd. NW. Medical-Dental Building, SE402. Washington, DC 20007-2145

Abstract

Hyperphosphorylation and aggregation of tau protein is a critical factor in many neurodegenerative diseases. These diseases are increasing in prevalence, and there are currently no cures. Previous work from our group and others has shown that tyrosine kinase inhibitors (TKIs) can stimulate autophagy, decrease pathological proteins, and improve symptoms in models of neurodegeneration. Here we examined the role of pazopanib in mouse models that express either human mutant P301L tau (TauP301L) or triple mutant amyloid precursor protein (3x-A β PP). The TauP301L mouse expresses P301L tau under the control of a prion promoter in both neurons and astrocytes, reminiscent of some human tauopathies. Pazopanib crosses the blood-brain barrier with no detectable peripheral off-side effects, and decreases p-tau in TauP301L mice. Pazopanib reaches a brain concentration sufficient for inhibition of several tyrosine kinases, including vascular endothelial growth factor receptors (VEGFRs). Further, pazopanib does not affect microglia but reduces astrocyte levels toward nontransgenic controls in TauP301L mice. Pazopanib does not alter amyloid beta levels or astrocytes in 3x-A β PP mice but modulates a number of inflammatory markers (IP-10, MIP-1 α , MIP-1 β , and RANTES). These data suggest that pazopanib may be involved in p-tau clearance and modulation of astrocytic activity in models of tauopathies.

Keywords

Alzheimer's disease; tauopathies; amyloid beta; tau; pazopanib

Corresponding author: Charbel E-H. Moussa, Department of Neurology, Laboratory for Dementia and Parkinsonism, Translational Neurotherapeutics Program, 4000 Reservoir Rd. NW., Building D, Room 203-C., Washington, DC 20007-2145, cem46@georgetown.edu, Telephone: (202) 687-7328, FAX: (202) 687-7378.

Conflict of Interest: Dr. Charbel Moussa is listed as an inventor on a Georgetown University intellectual property patent to use tyrosine kinase inhibitors for the treatment of neurodegenerative diseases.

Introduction

The microtubule-associated protein ‘tau’ is a phosphoprotein primarily expressed within neurons in the central nervous system (CNS), where it stabilizes microtubules and aids in cargo transport along the axon. While basal phosphorylation of tau is normal, tau modifications, predominantly hyperphosphorylation of tau, are critical in the pathogenesis of numerous neurodegenerative disorders known as the tauopathies. These diseases include frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP), Alzheimer's disease (AD), corticobasal degeneration (CBD), among others [1-6]. Further, tau modifications are found in the majority of neurodegenerative diseases [7-10]. In addition to pathological tau (tangles), AD patients exhibit plaques composed of amyloid beta ($A\beta$), a cleavage product of the amyloid precursor protein ($A\beta$ PP). There are no disease-modifying drugs for these diseases, and symptom-management medications provide only mild relief.

Our group has previously shown that FDA-approved tyrosine kinase inhibitors (TKIs) nilotinib (Tasigna®) and bosutinib (Bosulif®) are potential therapeutics for neurodegenerative diseases [11-17]. These findings led to phase II clinical trials currently underway. Both drugs are multi-target agents, but they primarily inhibit the non-receptor tyrosine kinase Abelson (Abl), a protein believed to contribute to neurodegenerative disease pathology [18-20]. To determine whether inhibition of other tyrosine kinases could have similar effects, we screened several FDA-approved TKIs and identified pazopanib (Votrient®) as a drug of interest. Pazopanib was developed as a potent inhibitor of angiogenesis and is used for advanced renal cell carcinoma at an oral dose of 800mg daily. It is primarily an inhibitor of the tyrosine kinases vascular endothelial growth factor receptors 1-3 (VEGFR1-3) (IC_{50} 10-47nM), platelet-derived growth factor receptors alpha and beta (PDGFR α , PDGFR β) (IC_{50} 71-84nM), and mast/stem cell growth factor receptor (KIT) (IC_{50} 74nM) [21]. The safety effects in the periphery are well characterized for this FDA-approved drug, though little is known about how it would work within the brain. This study aims to determine the central safety and efficacy of pazopanib within the context of neurodegenerative tauopathies.

The current study examined the central and peripheral effects of 3.5 week treatment with a daily intraperitoneal injection of 5mg/kg pazopanib in mouse models that express either human mutant P301L tau (TauP301L) or triple mutant $A\beta$ PP (3x- $A\beta$ PP). The TauP301L mouse expresses P301L tau under the control of a prion promoter in both neurons and astrocytes. We found that pazopanib crosses the blood-brain barrier (BBB) with no detectable peripheral off-side effects, and reduces p-tau in TauP301L mice.

Materials and Methods

Transgenic animals

Approximately 15 months old (± 1 month) male and female hemizygous TauP301L (015815, Jackson Labs) and nontransgenic control littermates as well as approximately 17 months old (± 1 month) homozygous 3x- $A\beta$ PP (34843-JAX, Jackson Labs) and age-matched control C57BL/6 (000664, Jackson Labs) mice were used in these experiments. This age group was

selected to allow for adequate expression and accumulation of the respective pathological protein. Animals were age-matched in their respective treatment cohorts. For the TauP301L mice, we modified the rTg4510 mouse model [22] which expresses the FTDP-17-associated P301L mutation in human four-repeat microtubule-associated protein tau (4R0NTau P301L) under a mouse prion protein promoter. rTg4510 mice are hemizygous and have the Tet-responsive element as well as a mouse prion protein promoter sequence directing expression of the human P301L tau. In the original model, hemizygous rTg4510 mice are bred to CaMKIIa-tTA mice resulting in the rTg4510 bi-transgenic model with conditional P301L tau expression in the forebrain. In the current experiments, only hemizygous mice expressing the P301L tau were crossed, leading to the general of homozygous, hemizygous, and nontransgenic littermates. 3x-A β PP [23] mice express the 770 isoform of human amyloid precursor protein with Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutations under the mouse thymus cell antigen 1 theta promoter (Thy1). Animals were housed on a 12-hour light/dark cycle with food and water provided *ad libitum*. Mice were genotyped using quantitative polymerase chain reaction (TransnetYX). Genotyping results were verified post-mortem with enzyme-linked immunosorbent assay analysis of human phosphorylated tau levels or amyloid beta. All experiments were in compliance with the Georgetown University Animal Care and Use Committee.

Drug treatment and sacrifice

Animals were treated daily with an intraperitoneal (IP) injection of 5mg/kg pazopanib (Cat S3012, Selleckchem) dissolved in dimethyl sulfoxide (DMSO) in a volume of up to 30 μ L at 5mg/mL and compared to vehicle (DMSO) for 3.5 weeks (pilot study for 1 week). In addition to checks by the veterinary staff, we performed a gross observation of the health of the mice daily, weighed them once per week during drug treatment, and sacrificed them between 2-4 hours of the final drug administration. Animals were deeply anesthetized with a cocktail of 20% ketamine (100mg/mL) and 10% xylazine (20mg/mL) in sterile saline. For Western blotting, ELISA, and MILLIPLEX® experiments, blood was collected via cardiac puncture then the remaining blood was flushed with 1X phosphate-buffered saline (PBS) for 5 min. Brains were quickly dissected then the cerebrum was removed and homogenized in 1X STEN buffer (50 mM Tris (pH 7.6), 150mM NaCl, 2mM EDTA, 0.2% NP-40, 0.2% BSA, 20mM phenylmethylsulfonyl fluoride) with protease and phosphatase inhibitors (Cat 78440, ThermoFisher), and centrifuged at 10,000 $\times$ g for 20 minutes at 4°C. The supernatant containing the STEN-soluble protein fraction was collected. Protein concentrations were measured with the Pierce BCA Protein Assay Kit (Cat 23227, ThermoFisher) and normalized. Blood was allowed to clot for 15-30 min, centrifuged at 2,000 $\times$ g for 10 minutes at 4°C, and the supernatant (serum) was collected. For experiments involving staining of brain tissue, animals were perfused with 1X PBS for 5 minutes then with 4% paraformaldehyde (PFA) for 5 min. Brains were quickly dissected out and immediately stored in 4% PFA for 24h at 4°C then transferred to 30% sucrose at 4°C. Tissues were cut using a cryostat at -20°C into 20 μ m thick sections and stored at -20°C. For the pilot study, we treated 3 TauP301L mice per group. In the first TauP301L cohort, we treated 6 NonTg-DMSO, 5 NonTg-Pazo, 7 TauP301L-DMSO, and 8 TauP301L Pazo. Upon finding no major differences between NonTg-DMSO and NonTg-Pazo, the subsequent TauP301L cohort had

8 NonTg-DMSO, 9 TauP301L-DMSO, and 10 TauP301L-Pazo. In the 3x-A β PP cohort, we had 3 C57-DMSO, 4 C57-Pazo, 7 3x-A β PP-DMSO, and 8 3x-A β PP-Pazo.

Pharmacokinetic analysis

C57BL/6 mice received a single IP dose of 5mg/kg pazopanib or 10mg/kg pazopanib, and brain and serum were collected at 2, 4, 6, or 8 hours (n=3 per dose and time point). Animals injected with vehicle (DMSO) were used for background subtraction. Drug levels were determined at the Georgetown University Proteomics and Metabolomics Shared Resources (PMSR). Stock solution for pazopanib (approximately 1 mg/mL each) was prepared in methanol/dichloromethane (50:50). The serial dilutions for each of the standards were produced for the study separately in methanol/HPLC grade water (50:50). Preparation of the calibration curve standards and quality samples (QC) was performed by mixing the stock solutions in blank samples. The final calibration concentration for pazopanib ranged 0.1ng/mL to 100ng/mL. The QC concentrations were 30ng/mL, 3ng/mL and 0.3ng/mL, respectively. Serum and brain samples were stored at -80°C then thawed to room temperature prior to preparation. The thawed serum samples (20 μ L) were transfused to a tube containing 100 μ L of water. The 500 μ L extraction solvent, acetonitrile/methanol (50:50) was added to the sample. The mixture was vortexed and incubated on ice for 20 minutes to accelerate protein precipitation. After incubation, the samples were vortexed again and centrifuged at 13,000 rpm for 20 minutes at 4°C. The supernatant was then collected and transferred to a new tube, was dried using speed vac, and reconstituted in 200 μ L of methanol/water (50:50). The mixture was spun again at 13,000 rpm for 20 minutes at 4°C. The supernatant was then collected into a mass spec sample tube cap and run in the mass spectrometer. For brain, a small section of the thawed brain sample from each animal was transferred to a flat bottom tube. 200 μ L of methanol/water (90:10) was added, and the tissue was homogenized. Acetonitrile was then added to the mixture facilitating protein precipitation. The mixture was then incubated on ice for 10 minutes. After incubation, the samples were vortexed and centrifuged at 13,000 rpm for 20 minutes at 4°C. The supernatant was then collected and transferred to a new tube, dried using speed vac, and reconstituted in 200 μ L of methanol/water (50:50). The mixture was centrifuged at 13,000 rpm for 20 minutes at 4°C. The supernatant was collected into a mass spec sample tube cap and run in the mass spectrometer. The samples were resolved on an Acquity UPLC BEH C18 1.7 μ m, 2.1 $\times$ 50mm column online with a triple quadrupole mass spectrometer (Xevo-TQ-S, Waters Corporation, USA) operating in the multiple reaction monitoring (MRM) mode. The sample cone voltage and collision energies were optimized for both analytes to obtain maximum ion intensity for parent and daughter ions using “IntelliStart” feature of MassLynx software (Waters Corporation, USA). The instrument parameters were optimized to gain maximum specificity and sensitivity of ionization for the parent [m/z = 438.25] and daughter ions [m/z = 357.33]. Signal intensities from all MRM Q1/Q3 ion pairs for analytes were ranked to ensure selection of the most intense precursor and fragment ion pair for MRM-based quantitation. This approach resulted in selection of cone voltages and collision energies that maximized the generation of each fragment ion species. Analysis was performed with a six to eight-point calibration curve, the sample queue was randomized and solvent blanks were injected to assess sample carryover. MRM data were processed using TargetLynx 4.1. The relative quantification values of analytes were determined by

calculating the ratio of peak areas of transitions of samples normalized to the peak area of the internal standard.

Cell culture and shRNA

M17 human neuroblastoma cells were cultured in Dulbecco's Modified Eagle Medium (Cat 10-017-CV, Corning) with 10% vol/vol fetal bovine serum (Cat 35-010-CV, Corning), penicillin/streptomycin (Cat 30-001-CI, Corning), at 37°C and 5% CO₂. Human shRNA constructs targeting vascular endothelial growth factor receptor 2 (VEGFR2) were purchased from GE Healthcare Life Sciences. The mature, antisense sequences were as follows: V2LSH 133709 -ATTTGAAGAGTTGTATTAG, V2LSH 133711 - TTGTCACTGAGACAGCTTG, V2LHS 133712- ATAAATATCCCGGGCCAAG, V2LHS 133714 - TACTTCTGGTTCTTCTAAC, V3LHS 339400- TCTTCTACATCACTGAGGG, V3LHS 339401 - AGACTGGTAACCTTCATCA. Clones were abbreviated to the last 3 numbers (ex: V2LSH 133709 as 709). Cells were transfected with human P301L tau cDNA and/or VEGFR2 shRNA using Fugene HD (Cat E2312, Promega), as per manufacturer's protocol, and collected after 48 hours.

Western blotting

Samples were normalized and prepared with NuPAGE LDS Sample Buffer [4X] (Cat NP0008, Life Technologies). Samples were run on either a 10% or 4-12% NuPAGE Novex Bis-Tris gel (Cat NP0315 NP0335, ThermoFisher), transferred to a 0.45µm nitrocellulose membrane (sigma, Z741975), then probed for phosphorylated tau (threonine-231) (1:1000) (Cat MN1040, ThermoFisher), Actin (1:2000) (Cat 4180, Cell Signaling), Beclin-1 (Cat 3495, Cell Signaling), Atg7 (1:1000) (Cat 8558, Cell Signaling), Atg12 (1:1000) (Cat 4180, Cell Signaling), LC3A/LC3B (1:1000) (PA1-16931), mTOR (1:1000) (Cat 2983, Cell Signaling), phosphorylated mTOR (serine-2448) (1:1000) (Cat 2971, Cell Signaling), TAU-5 (ab80579), or VEGFR2 (1:1000) (Cat 2479, Cell Signaling) followed by anti-mouse or anti-rabbit IgG antibody (Cat 7076P2, 7074S, Cell Signaling). Blots were visualized using Super Signal™ West Dura Extended Duration Substrate (Cat 37071, ThermoFisher) on the Amersham™ Imager 600 (GE Healthcare Life Sciences). Western blots were quantified using ImageJ software (National Institutes of Health). Briefly, individual lanes on blots were selected, and profile plots were generated representing the relative density of the lane contents. The region containing the band was selected, and the area of the signal was measured. Band areas were normalized to the densitometry of the actin (and when appropriate, total tau) bands probed on the same membrane.

Enzyme-linked immunosorbent assay (ELISA)

Protein samples were normalized, and p-tau levels were measured with human tau (threonine-181) ELISA (Cat KHB0061, Invitrogen) and human tau (serine-396) ELISA (Cat KHB7031, Invitrogen), as per manufacturer's protocols.

MILLIPLEX® assays

To probe numerous analytes, we utilize the Luminex xMAP technology [11, 15, 17, 24]. Kidney injury analytes were measured in serum with the Mouse Kidney Injury Magnetic

Bead Panel 1 (Cat MKI1MAG-94K, EMD Millipore). Cytokines and chemokines were measured in serum and brain with the Mouse Cytokine/Chemokine Magnetic Bead Panel (Cat MCYTOMAG-70K, EMD Millipore). Human amyloid beta was measured in brain using the Human Amyloid Beta and Tau Panel (Cat HNABTMAG-68K, EMD Millipore). AKT/mTOR signaling cascade phosphoproteins were measured using the Akt/mTOR Phosphoprotein Magnetic Bread 11-Plex Kit (Cat 48-611MAG, EMD Millipore). Assays were performed according to the manufacturer's protocols.

Safety assays

Animals were monitored daily and weighed once per week during drug treatment. Alanine transaminase (ALT) levels were measured using the BioVision™ ALT Activity Colorimetric/Fluorometric Assay Kit (Cat K-752-100, BioVision) as per manufacturer's instructions for the colorimetric protocol. Serum samples were diluted 1:3 using the provided assay buffer. Creatinine was measured using the QuantiChrom™ Creatinine Assay Kit (Cat DICT-500, BioAssay Systems) as per manufacturer's instructions for the blood protocol. Total protein was measured using the Pierce™ BCA Protein Assay Kit (Cat 23225, ThermoFisher), as per manufacturer's instructions.

Immunohistochemistry

Immunohistochemistry was performed to probe for p-tau (AT180) (1:500) (Cat OPA-03156, ThermoFisher), glial fibrillary acidic protein (GFAP) (1:300) (Cat ASTRO6, ThermoFisher), ionized calcium-binding adapter molecule 1 (IBA1) (1:300) (Cat 019-19741, WAKO), and collagen IV (1:200) (Cat ab19808, Abcam). Stereological quantifications were obtained by a blinded investigator using unbiased stereological methods (Stereology Resource Center, Chester, MD). A total of 6 brain regions of 1 mm² per animal were randomly selected within a coronal section of the brain that included the hippocampus and cortex. The number of GFAP or IBA-1 or tau positive cells were counted and normalized to the total number of detected cells within the same region. The number of positively stained cells were averaged and expressed as total number of positive cells relative to total number of cells per mm². Colocalization quantification was performed using ImageJ, Manders' Coefficient with a standardized threshold.

Nissl staining

Nissl staining was performed using FD Cresyl Violet Solution™ Regular Strength (Cat PS102-01, FD NeuroTechnologies) as per manufacturer's instructions.

Silver staining

Silver staining was performed using FD NeuroSilver™ (Cat PK301, FD NeuroTechnologies) as per manufacturer's instructions.

Motor and cognitive testing

Rotarod: Animals were placed on a rotarod apparatus which sped up to 50 rpm over the course of 5 minutes for 5 trials with a minimum for 2 minutes between trials. Open field test: Mice were placed in the open field arena for 60 minutes. Animals were tracked and data

were collected with ANY Maze software. Morris water maze: Animals were placed in random locations in the pool and pre-trained for four consecutive days, 5 times per day, to swim and locate a submerged platform. On the fifth day, the platform was removed, and mice were placed in the quadrant diagonal to the quadrant formerly containing the platform to test spatial memory. Information was collected with ANY Maze software.

Data analysis

All statistical analyses were performed using GraphPad Prism, version 5.0 (GraphPad Software). In the shRNA experiment, significance was determined using one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test, comparing all groups to the P301L tau-transfected group. In all other experiments, significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001), and n=number of animals. Unless otherwise indicated, data are presented as mean $\pm$ standard error of the mean.

Results

Pazopanib penetrates the blood-brain barrier

Following a single intraperitoneal (IP) injection of pazopanib of 0, 5, or 10mg/kg in dimethyl sulfoxide (DMSO) in C57BL/6 mice, we found that the drug penetrates the BBB and peaks at approximately 4 hours (T_{max}) (Fig. 1A). The peak concentration (C_{max}) in the brain at the 4-hour point was 450ng/mL for the 5mg/kg dose and 583.33 ng/mL for the 10 mg/kg dose, corresponding to approximately 1 μ M and 1.3 μ M (Fig. 1C), respectively. Therefore, the amount of pazopanib entering the brain is not linear compared to drug concentration, but it is sufficient to engage the known receptor tyrosine kinase targets [21], including VEGFRs, PDGFR α/β , and KIT. The blood (serum) characteristics (Fig. 1B) were consistent with prior observations in humans [25], as it can display large inter-individual variability. Further the drug has been shown to be influenced by food, and animals were not fasted prior to dosing in our experiments. We performed a 7-day pilot study in TauP301L mice using 5mg/kg and 10mg/kg of pazopanib to determine whether the lower dose would decrease levels of p-tau. Pazopanib treatment shows a dose-dependent decrease in p-tau (serine-396) levels, as measured by ELISA (Fig. 1D). While the 5mg/kg dose did not significantly reduce p-tau levels, we chose to proceed with this dose due to its brain penetrance and to ameliorate some of the potential side effects known to be associated with the drug.

Pazopanib displays no peripheral off-target side effects after treatment

Primary concerns regarding pazopanib are hepatotoxicity, diarrhea, hypertension, fatigue, appetite, and weight loss. Although effects of pazopanib on the periphery have been well established, we were still interested in reproducing this work in addition to determining the effects of the drug on the brain. We treated male and female hemizygous TauP301L (015815, Jackson Labs) and homozygous 3x-A β PP (34843-JAX, Jackson Labs). Littermate controls were used for the TauP301L mice, and C57BL/6 mice (000664, Jackson Labs) were used as controls for 3x-A β PP mice. While C57BL/6 mice are not littermate controls to the 3x-A β PP mice, they are Jackson's suggested control. Animals were treated with a daily IP

injection of either 5mg/kg pazopanib or vehicle (DMSO) in a volume of up to 30μL. Transgenic 3x-AβPP mice express the 770 isoform of human amyloid precursor protein with Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutations under the mouse thymus cell antigen 1 theta promoter (Thy1) [23]. TauP301L mice were modified from the rTg4510 mouse model [22] which expresses the FTDP-17-associated P301L mutation in human four-repeat microtubule-associated protein tau (4R0NTau P301L) under a mouse prion protein promoter. rTg4510 mice are hemizygous and have the Tet-responsive element as well as a mouse prion protein promoter sequence directing expression of the human P301L tau. Hemizygous rTg4510 mice are bred to CaMKIIa-tTA mice resulting in the rTg4510 bi-transgenic model with conditional doxycycline-off P301L tau expression in the forebrain. To avoid the effects of doxycycline and potential interference with pazopanib treatment, only hemizygous mice expressing the P301L tau under the control of mouse prion protein promoter were crossed to generate homozygous, hemizygous, and nontransgenic littermates (see methods). P301L is a well-characterized and aggressive tau mutation [26-28] as well as the most common tau mutation, associated with FTDP-17 [29, 30]. In the TauP301L cohorts, pazopanib treatment did not lead to changes in body weight, compared to DMSO-treated groups (Fig. 2A). Further, pazopanib did not lead to changes in wet, whole brain weight (Fig. 2B).

The main warning for pazopanib is hepatotoxicity, and it is recommended to monitor hepatic function. Alanine transaminase (ALT) is a clinical indicator of liver damage, with blood levels typically increasing following damage. There were no significant differences in groups, as measured by ALT activity assay (Fig. 2C). In addition to hepatotoxicity, we were interested in the effects of pazopanib on kidney-injury markers due to the importance of VEGF signaling within the kidney [31-33]. Pazopanib treatment did not lead to changes in beta-2-microglobulin (Fig. 2D), tissue inhibitor of metalloproteinases-1 (TIMP-1) (Fig. 2E), or renin (Fig. 2F). Renin is of particular importance, due to its role in the renin-angiotensin system and blood pressure and the potential for pazopanib treatment to lead to hypertension. There were also no changes in creatinine levels associated with drug treatment (Fig. 2G). No changes were found in total protein levels between groups (Fig. 2H). In 3x-AβPP animals, no significant changes were found in weight, ALT, kidney markers, or total protein (data not shown). Further, throughout the treatment period, we did not observe diarrhea in TauP301L or 3x-AβPP cohorts.

We performed rotarod, Morris water maze (MWM), and open field test of the mice prior to sacrificing them to determine whether there was an effect of treatment on motor and cognitive abilities. At the age ranges tested, we did not detect a significant difference between transgenic and non-transgenic mice (data not shown). This may be due to sample size or the sensitivity of the behavioral tests. It is important to note that a side effect of pazopanib is fatigue, and the mice treated with pazopanib did not show impairments in these tests. They were able to remain on the rotarod, swim, and had a similar travel distance in the MWM and open field as the DMSO-treated animals. In future studies, we will perform a more extensive behavioral analysis using different age groups and homozygous TauP301L mice.

Pazopanib significantly decreases phosphorylated tau levels but does not affect amyloid beta

To determine the efficacy of pazopanib to reduce p-tau, we measured brain levels of human p-tau (threonine-181) following a 3.5 week treatment. Confirming genotyping results, hemizygous TauP301L-DMSO mice show significant increases in the levels of human p-tau181 (Fig. 3A) compared to nontransgenic mice. Homozygous mice die prematurely so it was very difficult to use them in this study. Pazopanib significantly decreased p-tau 181 in TauP301L mice. Conversely, pazopanib did not appear to affect the concentration of A β ₄₀ (Fig. 3B) or A β ₄₂ (Fig. 3C) in 3x-A β PP mice, as measured by MILLIPLEX® ELISA.

Because pazopanib primarily inhibits VEGFRs, we transfected P301L tau or A β cDNA with or without VEGFR2 shRNA into M17 human neuroblastoma cells to ascertain whether inhibition of VEGFR2 could reduce p-tau and/or A β _{40/42}. We used six different shRNAs to knockdown VEGFR2, and Western blot analysis shows that 4 clones resulted in VEGFR2 knockdown, with clone 400 showing up to 44% receptor knockdown (Fig. 3D, E). VEGFR2 knockdown significantly reduced tau levels, compared to P301L tau-transfected cells (Fig. 3F), as measured by MILLIPLEX® ELISA. Co-transfection with P301L tau cDNA and VEGFR2 shRNA led to tau reduction ranging from ~10-44%, depending on the clone. We did not have detectable p-tau levels in cells, as we did in transgenic animals. This could be due to the duration of gene expression or perhaps the low levels of hyperphosphorylation of tau below the detection level of the assays used. Further, A β levels were unchanged with VEGFR2 shRNA (data not shown).

We performed immunohistochemistry for AT180 (p-tau, threonine-231) in the hippocampus of nontransgenic littermates (Fig. 4A) and TauP301L mice (Fig. 4B) treated with DMSO and nontransgenic (data not shown) and TauP301L mice treated with pazopanib (Fig. 4C). Quantification via stereological methods (Fig. 4M) of AT180 staining levels revealed significant increases in the CA1/2 (Fig. 4E and M), CA3 (Fig. 4H and M), and dentate gyrus (Fig. 4K and M) compared to nontransgenic control littermates (Fig. 4D, G, J). Treatment with pazopanib in TauP301L mice (Fig. 4F, I, L) restores AT180 levels toward nontransgenic control levels (Fig. 4D, G, J, M). We then measured AT180 via Western blot and confirmed the stereology results; showing that TauP301L mice have significantly higher levels of p-tau compared to nontransgenic controls, and pazopanib treatment significantly decreases it (Fig. 4N, O). These data expand upon the initial findings showing pazopanib leads to a reduction in p-tau (serine-396) (Fig. 1D) as well as threonine-181 and threonine-231.

The reduction in phosphorylated tau levels is not due to a loss of neurons

To determine whether pazopanib was reducing p-tau by killing neurons, we performed cresyl violet (Nissl) as well as cupric silver stains [34] of brain tissue in treated and non-treated mice. Quantification via stereological methods (Fig. 5P) shows that TauP301L-DMSO mice have significantly lower levels of Nissl-positive cells (Fig. 5B, P) compared to nontransgenic mice (Fig. 5A, P) with no significant difference between TauP301L-Pazo mice and their respective controls (Fig. 5C, P). With cupric silver staining, we found significantly increased levels of cell death in regions of TauP301L-DMSO mice brains (Figure 5E) such as the CA1/2 (Fig. 5H), CA3 (Fig. 5K), and dentate gyrus (Fig. 5N),

compared to corresponding regions in nontransgenic mice (Fig. 5D, G, J, M). Pazopanib treatment in TauP301L mice appeared to prevent cell death in those regions (Fig. 5 F, I, L, O) as well as others. Quantification of silver staining via stereological methods (Fig. 5Q) shows that TauP301L-DMSO mice have significantly higher levels of silver-positive cells, indicative of degenerating neurons (Fig. 5E, Q) compared to nontransgenic (Fig. 5D, Q) mice, and TauP301L-Pazo mice are not significantly different from their respective nontransgenic controls. In both measures, pazopanib treatment not only did not cause cell death but appears to be preventing cell death.

Clearance of phosphorylated tau is independent of beclin-1-mediated autophagy

Previous work from our group with TKIs shows they decrease levels of neurodegenerative proteins by stimulating beclin-mediated autophagy and promoting the maturation of autophagic vacuoles to autophagosomes [12-14]. To determine whether pazopanib has a similar mechanism of action, we performed Western blots probing for beclin-1 (Fig. 6A, B), but we did not detect any changes in beclin-1 levels between groups. Additionally, we measured levels of the pre-lysosomal proteins autophagy-related protein 7 (Atg7) and 12 (Atg12) as well as light chain protein 3 (LC3). We were unable to assess autophagic flux using LC3B-II, as it was undetectable by western blot in our study. Atg7 is an E1-like protein that conjugates Atg12 to other proteins. This chain of events leads to a complex which accumulates on pre-autophagosomal structures prior to forming the autophagosome. Further, Atg7 aids in LC3 conjugation, which is also involved in the formation of the autophagosome. We found no changes in levels of Atg 7 (Fig. 6C, D), Atg12 (Fig. 6E, F), or LC3 (Fig. 6G, H) between groups. These data suggest that pazopanib is likely not stimulating autophagy.

Pazopanib does not alter levels of Akt/mTOR signaling cascade proteins

Tau interacts with several proteins in the Akt and mTOR signaling cascades, including Akt [35, 36], ribosomal protein S6 kinase beta-1 (p70S6K) [37, 38], mTOR [39, 40], phosphatase and tensin homolog (PTEN) [41, 42], glycogen synthase kinases 3 alpha and beta (GSK3 α , GSK3 β) [43-46], and many others, leading to actions such as tau phosphorylation and degradation. For example, GSK3 β is known to phosphorylate tau at numerous sites [45]. We measured levels of the aforementioned kinases as well as tuberous sclerosis complex 2 (TSC2) and ribosomal protein S6 (RPS6) to determine whether treatment with pazopanib alters their phosphorylation levels. We found these analytes to be unchanged between treatment groups, indicating human P301L tau does not alter these levels in the transgenic mice, and pazopanib's kinase inhibition is not affecting the phosphorylation of these proteins (Supplemental Fig. 1).

Pazopanib restores inflammatory markers in 3x-A β PP mice but does not significantly alter markers in TauP301L mice

Pazopanib is an FDA-approved drug and has been extensively researched for cancer treatment. We were particularly interested in the potential for differential effects in the periphery (blood) and in the brain. Pazopanib did not significantly affect brain levels of granulocyte-colony stimulating factor (G-CSF), granulocyte monocyte-colony stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP-10, CXCL10), keratinocyte

chemoattractant (KC, interleukin-8), monocyte chemotactic protein 1 (MCP-1, CCL2), macrophage inflammatory protein 1- α (MIP-1 α , CCL3), or RANTES (CCL5) in TauP301L and nontransgenic mice (Table 1). We were concerned of the potential for pazopanib to alter components of vasculogenesis in the brain. However, VEGF levels (and putative receptors, data not shown) were not significantly altered in the TauP301L cohort. There were no significant differences in blood inflammatory markers tested in both TauP301L (Table 2) and 3x-A β PP (Table 4) cohorts of animals, suggesting pazopanib safety at the 5mg/kg dose. In the 3x-A β PP cohort, there were striking differences between 3x-A β PP and C57 groups when we measured brain levels of chemokines and cytokines (Table 3). 3x-A β PP-DMSO animals had significantly higher levels of IP-10, MIP-1 α , MIP-1 β (CCL4), and RANTES. Treatment with pazopanib reverses these levels in 3x-A β PP mice and did not alter levels in C57 mice. IP-10, MIP-1 α , MIP-1 β , and RANTES have been found by other groups to be upregulated in Alzheimer's disease models and patients and are generally believed to be pathogenic [47-55]. It is important to note pazopanib did not have a significant effect on nontransgenic or C57 mice, compared to nonTg-DMSO or C57-DMSO mice in any analyte we measured, further demonstrating its safety at this dose.

Pazopanib reduces astrocyte levels in TauP301L mice

Within the hippocampus of nontransgenic mice (Fig. 7A), we found basal levels of GFAP in various regions such as CA1/2 (Fig. 7D) and CA3 (Fig. 7G). GFAP staining was quantified via stereological methods (Fig. 7M). In TauP301L-DMSO mice (Fig. 7B), GFAP levels were increased in the CA1/2 (Fig. 7E, M) and CA3 (Fig. 7H, M) regions, as well as others such as the dentate gyrus (data not shown). However, pazopanib treatment of TauP301L mice (Fig. 7C, M) shows that the GFAP-staining was reversed toward nontransgenic levels (Fig. 7F, I, M). We also performed stereological quantification of IBA-1 staining in nontransgenic (Fig. 7J, N), TauP301L-DMSO (Fig. 7K, N), and TauP301L-Pazo (Fig. 7L, N). We did not observe many microglia within the hippocampus but found staining in layers V and VI of the cortex. We did not find any changes between nontransgenic, TauP301L-DMSO, and TauP301L-Pazo groups, indicating no change in microglia numbers (Fig. 7N). In 3x-A β PP mice, while GFAP and IBA-1 levels were significantly increased compared to C57 mice, there were no changes between DMSO and pazopanib treated animals (data not shown).

Because of the changes we observed in astrocytes, we sought to determine whether p-tau is detectable in astrocytes via co-staining for GFAP and AT180. We found little association between p-tau and astrocytes in nontransgenic mice (Fig. 8A, B, C). However, in the TauP301L-DMSO mice, a significant increase GFAP colocalization with p-tau was observed (Fig. 8D, E, F, Supp. Fig. 2), and pazopanib treatment significantly reduced p-tau colocalization with GFAP (Fig. 8G, H, I, Supp. Fig. 2).

Discussion

The TauP301L mouse is a novel model of tauopathy, where p-tau is significantly increased in both neurons and astrocytes. This is in contrast to findings in another P301L mouse model, in which reactive astrocytes did not show tau inclusions [56]. Furthermore, astrocyte activation was independent of changes in microglial activity and other markers of

inflammation. These data provide some insights into p-tau accumulation in astrocytes. Under physiological conditions, tau is predominantly expressed in the axons of neurons and not found in astrocytes [57]. In TauP301L mice, the human mutant tau is expressed under the mouse prion protein promoter, and it has been shown that astrocytes do translate prion protein mRNA [58]. Other groups have shown prion proteins can accumulate in astrocytes and spread to neurons [59, 60] and that alpha-synuclein can be endocytosed by astrocytes [61]. It is unclear whether expression of tau under the prion protein promoter results in p-tau accumulation in astrocytes or whether p-tau is secreted from neurons and deposited into astrocytes. Tau-positive glia have been found in human tauopathies such as PSP, CBD, and Pick's disease [62, 63]. This suggests either a lack of p-tau clearance in glial cells or propagation of p-tau via secretion from neurons to astrocytes. It is unknown whether, in this model, astrocytes are contributing to the propagation of p-tau or are attempting to clear p-tau. However, in PSP, it has been shown that astrocyte pathology is degenerative rather than reactive [64]. Treatment of TauP301L mice with pazopanib significantly reduces p-tau and GFAP levels. This is of particular importance, as astrocytes serve numerous roles within the brain such as regulation of cerebral blood flow, housekeeping and detoxification, and contribute to synaptic function. There are numerous studies about astrocytes containing p-tau with relation to the tauopathies, but little research has been performed to determine the crosstalk between p-tau and astrocyte biology. One group has shown expressing tau under the GFAP promoter leads to disruption of the BBB and neurodegeneration [65] as well as a decrease in astrocytic glutamate transporters in the brain stem and spinal cord of mice [66], suggesting tau burden in astrocytes can lead to microenvironments with heightened glutamate toxicity. Tau in glia can also lead to both neuronal and glial toxicity and cell death [67]. A limitation of this study is that the P301L mutation is associated with FTDP-17, and the mice express a 4R0N isoform of tau. These findings may not be generalizable to all tauopathies, given the variety of known mutations and isoforms. Future research will examine additional forms of tau to further determine the efficacy of pazopanib treatment at p-tau clearance.

We previously showed increased levels of GFAP staining in 3x-A β PP mice, compared to control animals [15] which is consistent with findings in the current studies. In contrast to the effects on p-tau and GFAP in TauP301L mice, we did not detect any changes in A β levels or GFAP in 3x-A β PP mice with pazopanib treatment. Another group has shown increased levels of GFAP in 5xFAD mice and impaired A β uptake by aged astrocytes [68]. Moreover, tau is an intracellular protein while A β is extracellular. Thus, the localization of the protein may contribute to whether pazopanib could lead to its clearance. Pazopanib inhibits PDGFR β , and studies with PDGFR $\beta^{+/-}$ mice crossed with A β PP mice (with the Swedish mutation) show that pericyte deficiency leads to tau pathology, neuronal loss, and heightened A β_{40} and A β_{42} pathology [69]. Interestingly in our studies, potential inhibition of PDGFR β by pazopanib did not lead to increases in A β_{40} and A β_{42} , though we did not specifically look at how the drug may have influenced pericytes. Pericytes and astrocytes are closely associated with the BBB and the differential effects of pazopanib on p-tau and A β may partly be due to the compromised BBB and vascular abnormalities in 3x-A β PP mice [70]. We measured the level of collagen IV as a potential marker of brain vasculature, and we did not detect any differences in collagen IV staining (data not shown) upon pazopanib

treatment, suggesting no effect on vasculogenesis. Additionally, it has been shown that astrocytes can promote A β -induced neuronal death as well as promote A β -induced tau phosphorylation [71]. Future studies will test pazopanib in a mouse which expresses both A β PP/A β as well as P301L tau to determine whether tau pathology triggers A β or vice versa. This will also allow us to determine the effects of tau on astrocytes/pericytes as well as whether pazopanib clearance of p-tau is hindered by A β pathology. Moreover, p-tau reduction does not appear to be mediated via autophagy or microglial changes, suggesting an alternative mechanism of pazopanib effects on p-tau clearance, possibly via astrocytic mechanisms. This hypothesis is the focus of future investigation. Numerous studies have shown A β -containing astrocytes in AD brains [72-74], and both astrocytes [75] and microglia are capable of degrading A β , likely via scavenger receptors [76, 77]. Moreover, an imbalance between complement protein C1q and scavenger receptor B1 has been implicated in impaired uptake of A β by astrocytes [68]. These studies focused on A β and had little or no mention of tau. Growing research shows the influence of T cells on glia within the CNS (reviewed in [78]). In our upcoming studies, we aim to determine potential mechanisms of tau uptake and degradation by glia in relation to A β as well as the potential contribution of T cells.

The reduction in levels of IP-10, MIP-1 α , MIP-1 β , and RANTES in 3x-A β PP mice suggests pazopanib regulates neuroinflammation. These proteins have been shown to be upregulated in AD [47-55], however it is unclear whether the change in levels of these inflammatory markers is neuroprotective or neurotoxic. Within the brain, the cytokines MIP-1 α , MIP-1 β , IP-10, and RANTES have been found in numerous cell types including neurons, microglia, astrocytes, and microvessel endothelial cells as well as infiltrating peripheral immune cells [47, 48, 52, 79-81]. Synergistic expression between these proteins has also been found. For example, IP-10 is found in both resting and reactive astrocytes, but the levels are greatly increased in reactive astrocytes and co-localize with MIP-1 β [47]. This finding lends us insight into the roles of these proteins within the context of AD and neuroinflammation, as their reduction did not appear to lead to negative side effects nor did it change the levels of A β . Though pazopanib has its highest affinity to VEGFR, it is possible mechanisms of action could include alteration of the neuroimmune environment through KIT inhibition. Masitinib, a KIT inhibitor (and to a lesser extent, PDGFR inhibitor) has shown a potential therapeutic value as an adjunct treatment in a phase II clinical trial in mild-to-moderate AD [82], potentially through the modulation of mast cells at the blood-brain barrier. Taken together, pazopanib is a potential drug candidate for repurposing in neurodegenerative tauopathies. We will continue studying the effects of the drug and expanding animal numbers, namely in the control-treatment animal group as well as testing it in other animal models of neurodegenerative disease. We acknowledge the variability in mean values between groups, and these appear almost exclusively in the serum safety and inflammation assays. Pazopanib is FDA-approved and has had substantial research on its effects in the periphery. We try to gain as much data as we can from each mouse, given the limited amount of tissue they have, and we plan to keep increasing the 'n' of the inflammation and safety work as we continue to study the drug.

The pharmacokinetic profile of pazopanib has been previously studied using *in vitro* assays [21], in mice receiving single doses [21, 83], and in human plasma following single or

chronic doses [84-88]. Knowing the pharmacokinetics of pazopanib in the brain with chronic treatment would help us to better understand how pazopanib may be working. Interestingly, tau has been shown to be phosphorylated by lymphocyte-specific protein tyrosine kinase (Lck) [89], which is also inhibited by pazopanib (IC₅₀ 411 nM) [21]. With the brain penetrance of pazopanib at 5 mg/kg IP, the dose entering the brain (1 µM) is sufficient to engage this target (Lck) and potentially decrease p-tau directly through inhibition of Lck. Furthermore, another group has shown pazopanib putatively shares similar acetylcholine esterase interactions with a known AD drug donepezil (Aricept®) [90] and restores cognitive deficits in rats insulted with quinolinic acid and treated with 15 mg/kg pazopanib (less at 3.75 and 0.94 mg/kg doses). Moreover, pazopanib targets receptor-interacting serine/threonine-protein kinase 1 (RIPK1) [91], a protein when inhibited leads to increased levels of the growth factor progranulin [92]. Increasing progranulin levels has long been believed to be a treatment method for AD and frontotemporal dementia [93-95]. Given this information, in addition to our work, it is likely pazopanib is working through multiple mechanisms of action to have its beneficial effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Alzheimer's Association, Cure Foundation, and Georgetown University support to CEM, stipend support to MJ through NCATS TL1 [TL1-TR001431], and the Georgetown-MedStar CERSI Scholars program. The Georgetown University Center of Excellence in Regulatory Science and Innovation (CERSI) is a collaborative effort between the university, its partners, and the U.S. Food and Drug Administration to promote regulatory science through innovative research and education. This research does not necessarily reflect the views of the FDA. We would like to thank Hannah Brown, Ding Dan, Xiaokong Gao, Whitney Hosein, and Ruochong Wang for their help with experiments. CEM designed the experiments. MJ, MLH, YX, and NK conducted the experiments. MJ, MLH and CEM analyzed the results. MJ and CEM wrote the manuscript.

References

1. See TM, LaMarre AK, Lee SE, Miller BL. Genetic causes of frontotemporal degeneration. *J Geriatr Psychiatry Neurol.* 2010; 23:260–268. [PubMed: 20938042]
2. Short RA, Graff-Radford NR, Adamson J, Baker M, Hutton M. Differences in tau and apolipoprotein E polymorphism frequencies in sporadic frontotemporal lobar degeneration syndromes. *Arch Neurol.* 2002; 59:611–615. [PubMed: 11939896]
3. Baker M, Litvan I, Houlden H, Adamson J, Dickson D, Perez-Tur J, Hardy J, Lynch T, Bigio E, Hutton M. Association of an extended haplotype in the tau gene with progressive supranuclear palsy. *Hum Mol Genet.* 1999; 8:711–715. [PubMed: 10072441]
4. Ihara Y, Nukina N, Miura R, Ogawara M. Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. *J Biochem.* 1986; 99:1807–1810. [PubMed: 2427509]
5. Feany MB, Dickson DW. Widespread cytoskeletal pathology characterizes corticobasal degeneration. *Am J Pathol.* 1995; 146:1388–1396. [PubMed: 7778678]
6. Joachim CL, Morris JH, Kosik KS, Selkoe DJ. Tau antisera recognize neurofibrillary tangles in a range of neurodegenerative disorders. *Ann Neurol.* 1987; 22:514–520. [PubMed: 2963585]
7. Sengupta U, Guerrero-Munoz MJ, Castillo-Carranza DL, Lasagna-Reeves CA, Gerson JE, Paulucci-Holthauzen AA, Krishnamurthy S, Farhed M, Jackson GR, Kaye R. Pathological Interface Between Oligomeric Alpha-Synuclein and Tau in Synucleinopathies. *Biological Psychiatry.* 2015; 78:672–683. [PubMed: 25676491]

8. Fernandez-Nogales M, Cabrera JR, Santos-Galindo M, Hoozemans JJM, Ferrer I, Rozemuller AJM, Hernandez F, Avila J, Lucas JJ. Huntington's disease is a four-repeat tauopathy with tau nuclear rods. *Nature Medicine*. 2014; 20:881–885.
9. Anderson JM, Hampton DW, Patani R, Pryce G, Crowther RA, Reynolds R, Franklin RJM, Giovannoni G, Compston DAS, Baker D, Spillantini MG, Chandran S. Abnormally phosphorylated tau is associated with neuronal and axonal loss in experimental autoimmune encephalomyelitis and multiple sclerosis. *Brain*. 2008; 131:1736–1748. [PubMed: 18567922]
10. Grossman M, Elman L, McCluskey L, McMillan CT, Boller A, Powers J, Rascovsky K, Hu W, Shaw L, Irwin DJ, Lee VM, Trojanowski JQ. Phosphorylated tau as a candidate biomarker for amyotrophic lateral sclerosis. *JAMA Neurol*. 2014; 71:442–448. [PubMed: 24492862]
11. Hebron ML, Lonskaya I, Olopade P, Selby ST, Pagan F, Moussa CE. Tyrosine Kinase Inhibition Regulates Early Systemic Immune Changes and Modulates the Neuroimmune Response in alpha-Synucleinopathy. *J Clin Cell Immunol*. 2014; 5:259. [PubMed: 25635231]
12. Lonskaya I, Hebron ML, Desforges NM, Franjie A, Moussa CE. Tyrosine kinase inhibition increases functional parkin-Beclin-1 interaction and enhances amyloid clearance and cognitive performance. *EMBO Mol Med*. 2013; 5:1247–1262. [PubMed: 23737459]
13. Hebron ML, Lonskaya I, Moussa CE. Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of alpha-synuclein in Parkinson's disease models. *Hum Mol Genet*. 2013; 22:3315–3328. [PubMed: 23666528]
14. Lonskaya I, Hebron ML, Desforges NM, Schachter JB, Moussa CE. Nilotinib-induced autophagic changes increase endogenous parkin level and ubiquitination, leading to amyloid clearance. *J Mol Med (Berl)*. 2014; 92:373–386. [PubMed: 24337465]
15. Lonskaya I, Hebron ML, Selby ST, Turner RS, Moussa CE. Nilotinib and bosutinib modulate pre-plaque alterations of blood immune markers and neuro-inflammation in Alzheimer's disease models. *Neuroscience*. 2015; 304:316–327. [PubMed: 26235435]
16. Pagan F, Hebron M, Valadez EH, Torres-Yaghi Y, Huang X, Mills RR, Wilmarth BM, Howard H, Dunn C, Carlson A, Lawler A, Rogers SL, Falconer RA, Ahn J, Li Z, Moussa C. Nilotinib Effects in Parkinson's disease and Dementia with Lewy bodies. *J Parkinsons Dis*. 2016; 6:503–517. [PubMed: 27434297]
17. Heyburn L, Hebron ML, Smith J, Winston C, Bechara J, Li Z, Lonskaya I, Burns MP, Harris BT, Moussa CE. Tyrosine kinase inhibition reverses TDP-43 effects on synaptic protein expression, astrocytic function and amino acid dis-homeostasis. *J Neurochem*. 2016; 139:610–623. [PubMed: 27507246]
18. Cancino GI, Perez de Arce K, Castro PU, Toledo EM, von Bernhardt R, Alvarez AR. c-Abl tyrosine kinase modulates tau pathology and Cdk5 phosphorylation in AD transgenic mice. *Neurobiol Aging*. 2011; 32:1249–1261. [PubMed: 19700222]
19. Ko HS, Lee Y, Shin JH, Karuppagounder SS, Gadad BS, Koleske AJ, Pletnikova O, Troncoso JC, Dawson VL, Dawson TM. Phosphorylation by the c-Abl protein tyrosine kinase inhibits parkin's ubiquitination and protective function. *Proc Natl Acad Sci U S A*. 2010; 107:16691–16696. [PubMed: 20823226]
20. Imam SZ, Zhou Q, Yamamoto A, Valente AJ, Ali SF, Bains M, Roberts JL, Kahle PJ, Clark RA, Li S. Novel regulation of parkin function through c-Abl-mediated tyrosine phosphorylation: implications for Parkinson's disease. *J Neurosci*. 2011; 31:157–163. [PubMed: 21209200]
21. Kumar R, Knick VB, Rudolph SK, Johnson JH, Crosby RM, Crouthamel MC, Hopper TM, Miller CG, Harrington LE, Onori JA, Mullin RJ, Gilmer TM, Truesdale AT, Epperly AH, Boloor A, Stafford JA, Luttrell DK, Cheung M. Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. *Mol Cancer Ther*. 2007; 6:2012–2021. [PubMed: 17620431]
22. Santacruz K, Lewis J, Spire T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH. Tau suppression in a neurodegenerative mouse model improves memory function. *Science*. 2005; 309:476–481. [PubMed: 16020737]
23. Davis J, Xu F, Deane R, Romanov G, Previti ML, Zeigler K, Zlokovic BV, Van Nostrand WE. Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic

- mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor. *J Biol Chem.* 2004; 279:20296–20306. [PubMed: 14985348]
24. Moussa C, Hebron M, Huang X, Ahn J, Rissman RA, Aisen PS, Turner RS. Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease. *J Neuroinflammation.* 2017; 14:1. [PubMed: 28086917]
 25. Di Gion P, Kanefendt F, Lindauer A, Scheffler M, Doroshenko O, Fuhr U, Wolf J, Jaehde U. Clinical Pharmacokinetics of Tyrosine Kinase Inhibitors Focus on Pyrimidines, Pyridines and Pyrroles. *Clinical Pharmacokinetics.* 2011; 50:551–603. [PubMed: 21827214]
 26. Guo JL, Lee VM. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem.* 2011; 286:15317–15331. [PubMed: 21372138]
 27. Gotz J, Chen F, Barmettler R, Nitsch RM. Tau filament formation in transgenic mice expressing P301L tau. *J Biol Chem.* 2001; 276:529–534. [PubMed: 11013246]
 28. Alonso Adel C, Mederlyova A, Novak M, Grundke-Iqbal I, Iqbal K. Promotion of hyperphosphorylation by frontotemporal dementia tau mutations. *J Biol Chem.* 2004; 279:34873–34881. [PubMed: 15190058]
 29. Dumanchin C, Camuzat A, Campion D, Verpillat P, Hannequin D, Dubois B, Saugier-Verber P, Martin C, Penet C, Charbonnier F, Agid Y, Frebourg T, Brice A. Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism. *Hum Mol Genet.* 1998; 7:1825–1829. [PubMed: 9736786]
 30. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature.* 1998; 393:702–705. [PubMed: 9641683]
 31. Sison K, Eremina V, Baelde H, Min W, Hirashima M, Fantus IG, Quaggin SE. Glomerular structure and function require paracrine, not autocrine, VEGF-VEGFR-2 signaling. *J Am Soc Nephrol.* 2010; 21:1691–1701. [PubMed: 20688931]
 32. Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, Kalluri R. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem.* 2003; 278:12605–12608. [PubMed: 12538598]
 33. Veron D, Reidy KJ, Bertuccio C, Teichman J, Villegas G, Jimenez J, Shen W, Kopp JB, Thomas DB, Tufro A. Overexpression of VEGF-A in podocytes of adult mice causes glomerular disease. *Kidney Int.* 2010; 77:989–999. [PubMed: 20375978]
 34. Lonskaya I, Hebron M, Chen W, Schachter J, Moussa C. Tau deletion impairs intracellular beta-amyloid-42 clearance and leads to more extracellular plaque deposition in gene transfer models. *Mol Neurodegener.* 2014; 9:46. [PubMed: 25384392]
 35. Ksiezak-Reding H, Pyo HK, Feinstein B, Pasinetti GM. Akt/PKB kinase phosphorylates separately Thr212 and Ser214 of tau protein in vitro. *Biochim Biophys Acta.* 2003; 1639:159–168. [PubMed: 14636947]
 36. Dickey CA, Koren J, Zhang YJ, Xu YF, Jinwal UK, Birnbaum MJ, Monks B, Sun M, Cheng JQ, Patterson C, Bailey RM, Dunmore J, Soresh S, Leon C, Morgan D, Petrucelli L. Akt and CHIP coregulate tau degradation through coordinated interactions. *Proc Natl Acad Sci U S A.* 2008; 105:3622–3627. [PubMed: 18292230]
 37. Pei JJ, An WL, Zhou XW, Nishimura T, Norberg J, Benedikz E, Gotz J, Winblad B. P70 S6 kinase mediates tau phosphorylation and synthesis. *FEBS Lett.* 2006; 580:107–114. [PubMed: 16364302]
 38. Taga M, Mouton-Liger F, Paquet C, Hugon J. Modulation of oxidative stress and tau phosphorylation by the mTOR activator phosphatidic acid in SH-SY5Y cells. *FEBS Lett.* 2011; 585:1801–1806. [PubMed: 21510936]

39. Caccamo A, Magri A, Medina DX, Wisely EV, Lopez-Aranda MF, Silva AJ, Oddo S. mTOR regulates tau phosphorylation and degradation: implications for Alzheimer's disease and other tauopathies. *Aging Cell*. 2013; 12:370–380. [PubMed: 23425014]
40. Tang Z, Iojia E, Bereczki E, Hultenby K, Li C, Guan Z, Winblad B, Pei JJ. mTor mediates tau localization and secretion: Implication for Alzheimer's disease. *Biochim Biophys Acta*. 2015; 1853:1646–1657. [PubMed: 25791428]
41. Zhang X, Li F, Bulloj A, Zhang YW, Tong G, Zhang Z, Liao FF, Xu H. Tumor-suppressor PTEN affects tau phosphorylation, aggregation, and binding to microtubules. *FASEB J*. 2006; 20:1272–1274. [PubMed: 16645045]
42. Chen Z, Chen B, Xu WF, Liu RF, Yang J, Yu CX. Effects of PTEN inhibition on regulation of tau phosphorylation in an okadaic acid-induced neurodegeneration model. *Int J Dev Neurosci*. 2012; 30:411–419. [PubMed: 22906543]
43. Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH. Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett*. 1992; 147:58–62. [PubMed: 1336152]
44. Lovestone S, Reynolds CH, Latimer D, Davis DR, Anderton BH, Gallo JM, Hanger D, Mulot S, Marquardt B, Stabel S, et al. Alzheimer's disease-like phosphorylation of the microtubule-associated protein tau by glycogen synthase kinase-3 in transfected mammalian cells. *Curr Biol*. 1994; 4:1077–1086. [PubMed: 7704571]
45. Cho JH, Johnson GV. Glycogen synthase kinase 3 β phosphorylates tau at both primed and unprimed sites. Differential impact on microtubule binding. *J Biol Chem*. 2003; 278:187–193. [PubMed: 12409305]
46. Asuni AA, Hooper C, Reynolds CH, Lovestone S, Anderton BH, Killick R. GSK3 α exhibits beta-catenin and tau directed kinase activities that are modulated by Wnt. *Eur J Neurosci*. 2006; 24:3387–3392. [PubMed: 17229088]
47. Xia MQ, Bacskaï BJ, Knowles RB, Qin SX, Hyman BT. Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: in vitro ERK1/2 activation and role in Alzheimer's disease. *J Neuroimmunol*. 2000; 108:227–235. [PubMed: 10900358]
48. Xia MQ, Qin SX, Wu LJ, Mackay CR, Hyman BT. Immunohistochemical study of the beta-chemokine receptors CCR3 and CCR5 and their ligands in normal and Alzheimer's disease brains. *Am J Pathol*. 1998; 153:31–37. [PubMed: 9665462]
49. Zhu M, Allard JS, Zhang Y, Perez E, Spangler EL, Becker KG, Rapp PR. Age-related brain expression and regulation of the chemokine CCL4/MIP-1 β in APP/PS1 double-transgenic mice. *J Neuropathol Exp Neurol*. 2014; 73:362–374. [PubMed: 24607962]
50. Marciniak E, Faivre E, Dutar P, Alves Pires C, Demeyer D, Caillierez R, Laloux C, Buee L, Blum D, Humez S. The Chemokine MIP-1 α /CCL3 impairs mouse hippocampal synaptic transmission, plasticity and memory. *Sci Rep*. 2015; 5:15862. [PubMed: 26511387]
51. Tripathy D, Thirumangalakudi L, Grammas P. Expression of macrophage inflammatory protein 1- α is elevated in Alzheimer's vessels and is regulated by oxidative stress. *J Alzheimers Dis*. 2007; 11:447–455. [PubMed: 17656823]
52. Tripathy D, Thirumangalakudi L, Grammas P. RANTES upregulation in the Alzheimer's disease brain: a possible neuroprotective role. *Neurobiol Aging*. 2010; 31:8–16. [PubMed: 18440671]
53. Haskins M, Jones TE, Lu Q, Bareiss SK. Early alterations in blood and brain RANTES and MCP-1 expression and the effect of exercise frequency in the 3xTg-AD mouse model of Alzheimer's disease. *Neurosci Lett*. 2016; 610:165–170. [PubMed: 26547034]
54. Cudaback E, Yang Y, Montine TJ, Keene CD. APOE genotype-dependent modulation of astrocyte chemokine CCL3 production. *Glia*. 2015; 63:51–65. [PubMed: 25092803]
55. El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, Freeman MW, Luster AD. CD36 mediates the innate host response to beta-amyloid. *J Exp Med*. 2003; 197:1657–1666. [PubMed: 12796468]
56. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet*. 2000; 26:127. [PubMed: 10973265]

57. Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol.* 1985; 101:1371–1378. [PubMed: 3930508]
58. Jackson WS, Krost C, Borkowski AW, Kaczmarczyk L. Translation of the prion protein mRNA is robust in astrocytes but does not amplify during reactive astrocytosis in the mouse brain. *PLoS One.* 2014; 9:e95958. [PubMed: 24752288]
59. Hollister JR, Lee KS, Dorward DW, Baron GS. Efficient uptake and dissemination of scrapie prion protein by astrocytes and fibroblasts from adult hamster brain. *PLoS One.* 2015; 10:e0115351. [PubMed: 25635871]
60. Victoria GS, Arkhipenko A, Zhu S, Syan S, Zurzolo C. Astrocyte-to-neuron intercellular prion transfer is mediated by cell-cell contact. *Sci Rep.* 2016; 6:20762. [PubMed: 26857744]
61. Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, Hwang D, Masliah E, Lee SJ. Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *J Biol Chem.* 2010; 285:9262–9272. [PubMed: 20071342]
62. Arai T, Ikeda K, Akiyama H, Shikamoto Y, Tsuchiya K, Yagishita S, Beach T, Rogers J, Schwab C, McGeer PL. Distinct isoforms of tau aggregated in neurons and glial cells in brains of patients with Pick's disease, corticobasal degeneration and progressive supranuclear palsy. *Acta Neuropathol.* 2001; 101:167–173. [PubMed: 11271372]
63. Ferrer I, Lopez-Gonzalez I, Carmona M, Arregui L, Dalfo E, Torrejon-Escribano B, Diehl R, Kovacs GG. Glial and neuronal tau pathology in tauopathies: characterization of disease-specific phenotypes and tau pathology progression. *J Neuropathol Exp Neurol.* 2014; 73:81–97. [PubMed: 24335532]
64. Togo T, Dickson DW. Tau accumulation in astrocytes in progressive supranuclear palsy is a degenerative rather than a reactive process. *Acta Neuropathol.* 2002; 104:398–402. [PubMed: 12200627]
65. Forman MS, Lal D, Zhang B, Dabir DV, Swanson E, Lee VM, Trojanowski JQ. Transgenic mouse model of tau pathology in astrocytes leading to nervous system degeneration. *J Neurosci.* 2005; 25:3539–3550. [PubMed: 15814784]
66. Dabir DV, Robinson MB, Swanson E, Zhang B, Trojanowski JQ, Lee VM, Forman MS. Impaired glutamate transport in a mouse model of tau pathology in astrocytes. *J Neurosci.* 2006; 26:644–654. [PubMed: 16407562]
67. Colodner KJ, Feany MB. Glial fibrillary tangles and JAK/STAT-mediated glial and neuronal cell death in a Drosophila model of glial tauopathy. *J Neurosci.* 2010; 30:16102–16113. [PubMed: 21123557]
68. Iram T, Trudler D, Kain D, Kanner S, Galron R, Vassar R, Barzilai A, Blinder P, Fishelson Z, Frenkel D. Astrocytes from old Alzheimer's disease mice are impaired in Abeta uptake and in neuroprotection. *Neurobiol Dis.* 2016; 96:84–94. [PubMed: 27544484]
69. Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A, Zlokovic BV. Pericyte loss influences Alzheimer-like neurodegeneration in mice. *Nat Commun.* 2013; 4:2932. [PubMed: 24336108]
70. Miao J, Xu F, Davis J, Otte-Holler I, Verbeek MM, Van Nostrand WE. Cerebral microvascular amyloid beta protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotropic mutant amyloid beta precursor protein. *Am J Pathol.* 2005; 167:505–515. [PubMed: 16049335]
71. Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W. Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture. *Cell Death Dis.* 2011; 2:e167. [PubMed: 21633390]
72. Thal DR, Schultz C, Dehghani F, Yamaguchi H, Braak H, Braak E. Amyloid beta-protein (Abeta)-containing astrocytes are located preferentially near N-terminal-truncated Abeta deposits in the human entorhinal cortex. *Acta Neuropathol.* 2000; 100:608–617. [PubMed: 11078212]
73. Funato H, Yoshimura M, Yamazaki T, Saido TC, Ito Y, Yokofujita J, Okeda R, Ihara Y. Astrocytes containing amyloid beta-protein (Abeta)-positive granules are associated with Abeta40-positive diffuse plaques in the aged human brain. *Am J Pathol.* 1998; 152:983–992. [PubMed: 9546359]
74. Lasagna-Reeves CA, Kaye R. Astrocytes contain amyloid-beta annular protofibrils in Alzheimer's disease brains. *FEBS Lett.* 2011; 585:3052–3057. [PubMed: 21872592]

75. Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J. Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med.* 2003; 9:453–457. [PubMed: 12612547]
76. Alarcon R, Fuenzalida C, Santibanez M, von Bernhardi R. Expression of scavenger receptors in glial cells. Comparing the adhesion of astrocytes and microglia from neonatal rats to surface-bound beta-amyloid. *J Biol Chem.* 2005; 280:30406–30415. [PubMed: 15987691]
77. Zhang H, Su YJ, Zhou WW, Wang SW, Xu PX, Yu XL, Liu RT. Activated scavenger receptor A promotes glial internalization of abeta. *PLoS One.* 2014; 9:e94197. [PubMed: 24718459]
78. Korn T, Kallies A. T cell responses in the central nervous system. *Nat Rev Immunol.* 2017; 17:179–194. [PubMed: 28138136]
79. McManus CM, Brosnan CF, Berman JW. Cytokine induction of MIP-1 alpha and MIP-1 beta in human fetal microglia. *J Immunol.* 1998; 160:1449–1455. [PubMed: 9570566]
80. Shukaliak JA, Dorovini-Zis K. Expression of the beta-chemokines RANTES and MIP-1 beta by human brain microvessel endothelial cells in primary culture. *J Neuropathol Exp Neurol.* 2000; 59:339–352. [PubMed: 10888363]
81. Guo CJ, Douglas SD, Lai JP, Pleasure DE, Li Y, Williams M, Bannerman P, Song L, Ho WZ. Interleukin-1beta stimulates macrophage inflammatory protein-1alpha and -1beta expression in human neuronal cells (NT2-N). *J Neurochem.* 2003; 84:997–1005. [PubMed: 12603824]
82. Piette F, Belmin J, Vincent H, Schmidt N, Pariel S, Verny M, Marquis C, Mely J, Hugonot-Diener L, Kinet JP, Dubreuil P, Moussy A, Hermine O. Masitinib as an adjunct therapy for mild-to-moderate Alzheimer's disease: a randomised, placebo-controlled phase 2 trial. *Alzheimers Res Ther.* 2011; 3:16. [PubMed: 21504563]
83. Minocha M, Khurana V, Mitra AK. Determination of pazopanib (GW-786034) in mouse plasma and brain tissue by liquid chromatography-tandem mass spectrometry (LC/MS-MS). *J Chromatogr B Analyt Technol Biomed Life Sci.* 2012; 901:85–92.
84. Glade Bender JL, Lee A, Reid JM, Baruchel S, Roberts T, Voss SD, Wu B, Ahern CH, Ingle AM, Harris P, Weigel BJ, Blaney SM. Phase I pharmacokinetic and pharmacodynamic study of pazopanib in children with soft tissue sarcoma and other refractory solid tumors: a children's oncology group phase I consortium report. *J Clin Oncol.* 2013; 31:3034–3043. [PubMed: 23857966]
85. Heath EI, Forman K, Malburg L, Gainer S, Suttle AB, Adams L, Ball H, LoRusso P. A phase I pharmacokinetic and safety evaluation of oral pazopanib dosing administered as crushed tablet or oral suspension in patients with advanced solid tumors. *Invest New Drugs.* 2012; 30:1566–1574. [PubMed: 21811833]
86. Yau T, Chen PJ, Chan P, Curtis CM, Murphy PS, Suttle AB, Gauvin J, Hodge JP, Dar MM, Poon RT. Phase I dose-finding study of pazopanib in hepatocellular carcinoma: evaluation of early efficacy, pharmacokinetics, and pharmacodynamics. *Clin Cancer Res.* 2011; 17:6914–6923. [PubMed: 21831954]
87. Hurwitz HI, Dowlati A, Saini S, Savage S, Suttle AB, Gibson DM, Hodge JP, Merkle EM, Pandite L. Phase I trial of pazopanib in patients with advanced cancer. *Clin Cancer Res.* 2009; 15:4220–4227. [PubMed: 19509175]
88. Imbs DC, Paludetto MN, Negrier S, Powell H, Lafont T, White-Koning M, Chatelut E, Thomas F. Determination of unbound fraction of pazopanib in vitro and in cancer patients reveals albumin as the main binding site. *Invest New Drugs.* 2016; 34:41–48. [PubMed: 26572909]
89. Scales TME, Derkinderen P, Leung KY, Byers HL, Ward MA, Price C, Bird IN, Perera T, Kellie S, Williamson R, Anderton BH, Reynolds CH. Tyrosine Phosphorylation of Tau by the Src Family Kinases Lck and Fyn. *Molecular Neurodegeneration.* 2011; 6
90. Yang YL, Li GH, Zhao DY, Yu HY, Zheng XL, Peng XD, Zhang X, Fu T, Hu XQ, Niu MS, Ji XF, Zou LB, Wang J. Computational discovery and experimental verification of tyrosine kinase inhibitor pazopanib for the reversal of memory and cognitive deficits in rat model neurodegeneration. *Chemical Science.* 2015; 6:2812–2821. [PubMed: 28706670]
91. Fauster A, Rebsamen M, Huber KV, Bigenzahn JW, Stukalov A, Lardeau CH, Scorzoni S, Bruckner M, Gridling M, Parapatics K, Colinge J, Bennett KL, Kubicek S, Krautwald S,

- Linkermann A, Superti-Furga G. A cellular screen identifies ponatinib and pazopanib as inhibitors of necroptosis. *Cell Death Dis.* 2015; 6:e1767. [PubMed: 25996294]
92. Mason AR, Elia LP, Finkbeiner S. The Receptor-Interacting Serine/Threonine Protein Kinase 1 (Ripk1) Regulates Progranulin Levels. *J Biol Chem.* 2017
93. Laird AS, Van Hoecke A, De Muynck L, Timmers M, Van den Bosch L, Van Damme P, Robberecht W. Progranulin is neurotrophic in vivo and protects against a mutant TDP-43 induced axonopathy. *PLoS One.* 2010; 5:e13368. [PubMed: 20967127]
94. Minami SS, Min SW, Krabbe G, Wang C, Zhou Y, Asgarov R, Li Y, Martens LH, Elia LP, Ward ME, Mucke L, Farese RV Jr, Gan L. Progranulin protects against amyloid beta deposition and toxicity in Alzheimer's disease mouse models. *Nat Med.* 2014; 20:1157–1164. [PubMed: 25261995]
95. Martens LH, Zhang J, Barmada SJ, Zhou P, Kamiya S, Sun B, Min SW, Gan L, Finkbeiner S, Huang EJ, Farese RV Jr. Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. *J Clin Invest.* 2012; 122:3955–3959. [PubMed: 23041626]

Abbreviations

Aβ	amyloid beta
Abl	Abelson
AD	Alzheimer's disease
ALT	alanine transaminase
AβPP	amyloid precursor protein
Atg	autophagy related protein
CBD	corticobasal degeneration
KIT	mast/stem cell growth factor receptor
FTDP-17	frontotemporal dementia with Parkinsonism linked to chromosome 17
G-CSF	granulocyte-colony stimulating factor
GFAP	glial fibrillary acidic protein
GM-CSF	granulocyte monocyte-colony stimulating factor
GSK3	glycogen synthase kinase 3
IBA1	ionized calcium-binding adapter molecule 1
IP	intraperitoneal
IP-10	interferon gamma-induced protein 10
KC	keratinocyte chemoattractant
LC3	light chain protein 3
Lck	lymphocyte-specific protein tyrosine kinase
MCP-1	monocyte chemoattractant protein 1

MIP-1	macrophage inflammatory protein 1
mTOR	mammalian target of rapamycin
p70S6k	ribosomal protein S6 kinase beta-1
PSP	progressive supranuclear palsy
PTEN	phosphatase and tensin homolog
RIPK1	receptor-interacting serine/threonine-protein kinase 1
RPS6	ribosomal protein S6
TIMP-1	tissue inhibitor of metalloproteinases-1
TDP-43	transactive response DNA binding protein 43 kDa
TKI	tyrosine kinase inhibition/inhibitor
TSC2	tuberous sclerosis complex 2

Figure 1. Pazopanib penetrates the blood-brain barrier at 5mg/kg and 10mg/kg intraperitoneal doses

A. Brain levels of pazopanib. B. Serum levels of pazopanib. C. Pharmacokinetics of pazopanib in the brain. D. Pilot study with 5mg/kg and 10mg/kg doses of Pazopanib show a decrease in p-tau levels. T_{max} = time it takes to reach peak concentration of pazopanib. C_{max} = peak concentration. AUC_{0-t} = area under the curve, represents total exposure to the drug. $n=3$ /data point. Data are shown as mean + SEM. Significance was determined by ANOVA with Tukey's method for multiple comparisons. Asterisks denote significant differences (* <0.05 , ** <0.01 , *** <0.001 , **** <0.0001).

Figure 2. Safety profile of pazopanib

Pazopanib has no significant effects on body weight, brain weight, liver, or kidney injury markers in non-transgenic and TauP301L mice. A. Change in body weight between baseline and the end of treatment. NonTg-DMSO n=9, NonTg-Pazo n=5, TauP301L-DMSO n=16, TauP301L-Pazo n=18. B. Wet, whole brain weight following treatment. NonTg-DMSO n=8, TauP301L-DMSO n=9, TauP301L-Pazo n=10. C. Serum alanine transaminase levels, n=3/group. D. Serum beta-2-microglobulin levels. NonTg-DMSO n=7, NonTg-Pazo n=3, TauP301L-DMSO n=9, TauP301L-Pazo n=9. E. Serum TIMP-1 levels. NonTg-DMSO n=7, NonTg-Pazo n=3, TauP301L-DMSO n=9, TauP301L-Pazo n=9. F. Serum renin levels. NonTg-DMSO n=7, NonTg-Pazo n=3, TauP301L-DMSO n=9, TauP301L-Pazo n=9. G. Serum creatinine levels. NonTg-DMSO n=4, TauP301L-DMSO n=5, TauP301L-Pazo n=5. H. Serum total protein levels. NonTg-DMSO n=4, TauP301L-DMSO n=5, TauP301L-Pazo n=5. Data are shown as mean $\pm$ SEM. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001).

Figure 3. Pazopanib significantly reduces brain levels of human phosphorylated tau in TauP301L mice through VEGFR2 inhibition

A. ELISA of human p-tau (threonine-181) levels. NonTg-DMSO n=6, NonTg-Pazo n=3, TauP301L-DMSO n=9, TauP301L-Pazo n=9. Data are shown as a percentage of the TauP301L-DMSO group. B. A β ₄₀ levels. C57-DMSO n=3, C57-Pazo n=4, 3x-A β PP-DMSO n=4, 3x-A β PP-Pazo n=5. C. A β ₄₂ levels. C57-DMSO n=3, C57-Pazo n=4, 3x-A β PP-DMSO n=4, 3x-A β PP-Pazo n=5. D. Western blot of VEGFR2 and actin levels in cell lysates. E. Quantification of Western blots, normalized to actin. n=6 for 709, 711, 712, 714, n=4 for 400, 401. F. Tau levels in untransfected, P301L tau cDNA-transfected, and P301L tau cDNA and VEGFR2 shRNA co-transfected M17 cells. n=4 for untransfected and P301L tau, n=5 for P301L tau + VEGFR2 shRNA groups. Data are shown as mean $\pm$ SEM. Significance was determined by unpaired, two-tailed Student's t-test in 3A-C and one-way ANOVA with Dunnett's multiple comparison test in 3F. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001).

Figure 4. Pazopanib restores AT180 levels in TauP301L toward nontransgenic levels

A. 4X image of AT180 in cortex and hippocampus of NonTg-DMSO animal. B. 4X image of AT180 in cortex and hippocampus of TauP301L-DMSO animal. C. 4X image of AT180 in cortex and hippocampus of TauP301L-Pazo animal. D. 20X image of AT180 in CA1/2 region of NonTg-DMSO animal. E. 20X image of AT180 in CA1/2 region of TauP301L-DMSO animal. F. 20X image of AT180 in CA1/2 region of TauP301L-Pazo animal. G. 20X image of AT180 in CA3 region of NonTg-DMSO animal. H. 20X image of AT180 in CA3 region of TauP301L-DMSO animal. I. 20X image of AT180 in CA3 region of TauP301L-Pazo animal. J. 20X image of AT180 in dentate gyrus region of NonTg-DMSO animal. K. 20X image of AT180 in dentate gyrus region of TauP301L-DMSO animal. L. 20X image of AT180 in dentate gyrus region of TauP301L-Pazo animal. M. Densitometry of AT180 staining (images not shown for NonTg-Pazo). NonTg-DMSO n=6, NonTg-Pazo n=3, TauP301L-DMSO n=6, TauP301L-Pazo n=6. Data are shown as mean ± SEM. Scale bar marks 1000µm in 4X and 200µm in 20X images. Histograms represent stereological quantification. N. Western blot of AT180 levels. O. Western blot quantification of p-tau relative to total and actin (p-tau/tau/actin), n=6/group. Data are shown as mean ± SEM. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001).

Figure 5. Pazopanib does not cause cell death in neurons

A. 4X image of nissl stain in cortex and hippocampus of NonTg-DMSO animal. B. 4X image of nissl stain in cortex and hippocampus of TauP301L-DMSO animal. C. 4X image of nissl stain in cortex and hippocampus of TauP301L-Pazo animal. D. 4X image of silver stain in cortex and hippocampus of NonTg-DMSO animal. E. 4X image of silver stain in cortex and hippocampus of TauP301L-DMSO animal. F. 4X image of silver stain in cortex and hippocampus of TauP301L-Pazo animal. G. 20X image of silver stain in CA1/2 region of NonTg-DMSO animal. H. 20X image of silver stain in CA1/2 region of TauP301L-DMSO animal. I. 20X image of silver stain in CA1/2 region of TauP301L-Pazo animal. J. 20X image of silver stain in CA3 region of NonTg-DMSO animal. K. 20X image of silver stain in CA3 region of TauP301L-DMSO animal. L. 20X image of silver stain in CA3 region of TauP301L-Pazo animal. M. 20X image of silver stain in dentate gyrus region of NonTg-DMSO animal. N. 20X image of silver stain in dentate gyrus region of TauP301L-DMSO animal. O. 20X image of silver stain in dentate gyrus region of TauP301L-Pazo animal. P. Nissl+ cells. NonTg-DMSO n=6, NonTg-Pazo n=3, TauP301L-DMSO n=6, TauP301L-Pazo n=6. Q. Silver+ cells. NonTg-DMSO n=6, NonTg-Pazo n=3, TauP301L-DMSO n=6, TauP301L-Pazo n=6. Scale bar marks 1000µm in 4X and 200µm in 20X images. Histograms represent stereological quantification. Data are shown as mean ± SEM. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001).

Figure 6. Pazopanib-mediated clearance of phosphorylated tau is beclin-1-independent
 A. Western blot of beclin-1 levels. B. Quantification of beclin-1 Western blots. C. Western blot of Atg7 levels. D. Quantification of Atg7 Western blots. E. Western blot of Atg12 levels. F. Quantification of Atg12 Western blots. G. Western blot of LC3 levels. H. Quantification of LC3 Western blots. n=3/group. Data are shown as mean $\pm$ SEM. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001).

Figure 7. Pazopanib reduces GFAP levels in TauP301L mice

A. 4X image of DAPI stain in cortex and hippocampus of NonTg-DMSO animal. B. 4X image of DAPI stain in cortex and hippocampus of TauP301L-DMSO animal. C. 4X image of DAPI stain in cortex and hippocampus of TauP301L-Pazo animal. D. 20X image of GFAP in CA1/2 region of NonTg-DMSO animal. E. 20X image of GFAP in CA1/2 region of TauP301L-DMSO animal. F. 20X image of GFAP in CA1/2 region of TauP301L-Pazo animal. G. 20X image of GFAP in CA3 region of NonTg-DMSO animal. H. 20X image of GFAP in CA3 region of TauP301L-DMSO animal. I. 20X image of GFAP in CA3 region of TauP301L-Pazo animal. J. 20X image of IBA1 in cortex of NonTg-DMSO animal. K. 20X image of IBA1 in cortex of TauP301L-DMSO animal. L. 20X image of IBA1 in cortex of TauP301L-Pazo animal. M. GFAP+ cells (n=4/group). N. IBA1+ cells NonTg-DMSO n=4, TauP301L-DMSO n=4, TauP301L-Pazo n=5. Histograms represent stereological quantification. Data are shown as mean ± SEM. Scale bar marks 1000µm in 4X and 200µm in 20x images. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances (* <0.05, ** <0.01, *** <0.001, **** <0.0001).

Figure 8. Phosphorylated tau is localized inside astrocytes in TauP301L mice

A-C. 40X images of CA3 region in NonTg-DMSO animals. D-F. 40X images of CA3 region in TauP301L-DMSO animals. G-I. 40X images of CA3 region in TauP301L-Pazo animals. Scale bar marks 25 μ m.

MILLIPLEX® analysis of chemokines and cytokines in nontransgenic and TauP301L mouse brain**Table 1**

Brain	NonTg-DMSO	NonTg-Pazo	TauP301L-DMSO	TauP301L-Pazo
G-CSF	4.33 ± 1.37	5.03 ± 2.10	4.52 ± 1.06	5.56 ± 1.71
GM-CSF	9.89 ± 4.28	6.09 ± 1.97	6.38 ± 1.94	11.59 ± 3.58
IP-10	74.17 ± 10.89	107.01 ± 9.22	73.37 ± 8.30	68.44 ± 11.01
KC	54.47 ± 11.39	84.43 ± 6.02	55.14 ± 9.23	55.22 ± 10.19
MCP-1	51.67 ± 9.05	31.83 ± 3.91	33.23 ± 4.46	36.45 ± 4.88
MIP-1a	114.97 ± 25.07	39.16 ± 10.80	102.16 ± 21.90	88.91 ± 20.09
RANTES	2.07 ± 0.45	N.D.	1.59 ± 0.31	1.52 ± 0.38
VEGF	3.90 ± 1.05	7.22 ± 1.04	4.97 ± 1.03	4.64 ± 1.03
p value summary	NonTg-DMSO vs. NonTg-Pazo	TauP301L-DMSO vs. TauP301L-Pazo	NonTg-DMSO vs. TauP301L-DMSO	NonTg-Pazo vs. TauP301L-Pazo
G-CSF	0.7681	0.6133	0.9147	0.8756
GM-CSF	0.5951	0.2377	0.4492	0.4171
IP-10	0.1069	0.7221	0.9531	0.0765
KC	0.1415	0.9954	0.9640	0.1301
MCP-1	0.2083	0.7734	0.1217	0.6176
MIP-1a	0.0958	0.6618	0.7058	0.2012
RANTES	-	0.8951	0.3921	-
VEGF	0.0898	0.8266	0.4952	0.1923

Data are shown as mean ± SEM with p values. N.D. = not detected. NonTg-DMSO n=7, NonTg-Pazo n=3, TauP301L-DMSO n=9, TauP301L-Pazo n=9. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances

* <0.05,

** <0.01,

*** <0.001,

**** <0.0001.

Table 2
MILLIPLEX® analysis of chemokines and cytokines in nontransgenic and TauP301L mouse serum

Serum	NonTg-DMSO	NonTg-Pazo	TauP301L-DMSO	TauP301L-Pazo
G-CSF	84.68 ± 29.95	44.15 ± 16.42	70.30 ± 12.39	82.53 ± 16.55
IP-10	112.54 ± 18.79	111.84 ± 15.75	109.62 ± 17.77	131.01 ± 13.16
KC	33.55 ± 8.95	44.18 ± 38.63	35.14 ± 5.66	70.84 ± 21.50
MCP-1	7.65 ± 1.36	17.00 ± 8.94	11.17 ± 4.35	20.68 ± 4.80
RANTES	1.86 ± 0.40	N.D.	2.64 ± 0.96	3.09 ± 1.03
p value summary	NonTg-DMSO vs. NonTg-Pazo	TauP301L-DMSO vs. TauP301L-Pazo	NonTg-DMSO vs. TauP301L-DMSO	NonTg-Pazo vs. TauP301L-Pazo
G-CSF	0.5159	0.5465	0.6437	0.3279
IP-10	0.9826	0.3477	0.9125	0.4592
KC	0.7050	0.1279	0.8781	0.5521
MCP-1	0.0855	0.1646	0.4797	0.7384
RANTES	-	0.8909	0.7053	-

Data are shown as mean ± SEM with p values. N.D. = not detected. NonTg-DMSO n=7, NonTg-Pazo n=3, TauP301L-DMSO n=9, TauP301L-Pazo n=9. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances

* <0.05,

** <0.01,

*** <0.001,

**** <0.0001.

Table 3
MILLIPLEX® analysis of chemokines and cytokines in C57 and 3x-A β PP mouse brain

Brain	C57-DMSO	C57-Pazo	3x-A β PP-DMSO	3x-A β PP-Pazo
G-CSF	5.74 $\pm$ 0.90	4.92 $\pm$ 0.37	4.62 $\pm$ 0.40	3.53 $\pm$ 0.34
GM-CSF	9.73 $\pm$ 3.32	9.79 $\pm$ 1.54	9.08 $\pm$ 0.74	7.81 $\pm$ 0.89
IP-10	62.86 $\pm$ 5.41	67.57 $\pm$ 7.08	218.36 $\pm$ 25.63	155.26 $\pm$ 9.85
KC	19.40 $\pm$ 0.96	19.79 $\pm$ 2.07	22.00 $\pm$ 1.98	16.81 $\pm$ 1.07
MCP-1	28.01 $\pm$ 3.95	26.70 $\pm$ 2.27	52.95 $\pm$ 10.73	35.94 $\pm$ 2.53
MIP-1 α	32.73 $\pm$ 2.44	29.92 $\pm$ 1.23	82.87 $\pm$ 5.36	54.40 $\pm$ 1.45
MIP-1 β	9.85 $\pm$ 2.36	7.41 $\pm$ 2.05	60.95 $\pm$ 15.43	24.28 $\pm$ 1.14
RANTES	3.23 $\pm$ 0.16	3.06 $\pm$ 0.49	6.58 $\pm$ 1.08	4.15 $\pm$ 0.15
p value summary	C57-DMSO vs. C57-Pazo	3x-A β PP-DMSO vs. 3x-A β PP-Pazo	C57-DMSO vs. 3x-A β PP-DMSO	C57-Pazo vs. 3x-A β PP-Pazo
G-CSF	0.3891	0.0878	0.3220	* 0.0273
GM-CSF	0.9855	0.3693	0.8583	0.2803
IP-10	0.6419	* 0.0403	** 0.0039	*** 0.0002
KC	0.8855	* 0.0445	0.3419	0.2155
MCP-1	0.7706	0.1738	0.1158	* 0.0346
MIP-1 α	0.3127	*** 0.0006	** 0.0010	**** <0.0001
MIP-1 β	0.5168	* 0.0309	0.0923	*** 0.0006
RANTES	0.7177	* 0.0390	* 0.0470	* 0.0280

Data are shown as mean $\pm$ SEM with p values. C57-DMSO n=3, C57-Pazo n=4, 3x-A β PP-DMSO n=4, 3x-A β PP-Pazo n=5. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances

*
<0.05,

**
<0.01,

<0.001,

<0.0001.

Table 4
MILLIPLEX® analysis of chemokines and cytokines in C57 and 3x-AβPP mouse serum

Serum	C57-DMSO	C57-Pazo	3x-AβPP-DMSO	3x-AβPP-Pazo
G-CSF	40.54 ± 9.82	68.21 ± 13.04	85.95 ± 20.93	43.91 ± 4.54
IP-10	155.73 ± 14.06	158.65 ± 20.47	184.71 ± 7.51	236.29 ± 54.20
KC	25.51 ± 12.41	110.76 ± 38.31	42.98 ± 3.07	69.78 ± 6.61
MCP-1	30.01 ± 13.05	31.55 ± 13.78	40.77 ± 8.93	11.70 ± 2.88
RANTES	7.48 ± 4.03	5.90 ± 0.71	6.18 ± 0.29	10.18 ± 3.03
p value summary	C57-DMSO vs. C57-Pazo	3x-AβPP-DMSO vs. 3x-AβPP-Pazo	C57-DMSO vs. 3x-AβPP-DMSO	C57-Pazo vs. 3x-AβPP-Pazo
G-CSF	0.1743	0.0973	0.1423	0.129
IP-10	0.9179	0.3822	0.1065	0.2287
KC	0.1262	*0.0103	0.1723	0.3323
MCP-1	0.9444	*0.0438	0.5283	0.2315
RANTES	0.6668	0.3594	0.7626	0.2583

Data are shown as mean ± SEM with p values. N.D. = not detected. C57-DMSO n=3, C57-Pazo n=4, 3x-AβPP-DMSO n=4, 3x-AβPP-Pazo n=5. Significance was determined by unpaired, two-tailed Student's t-test. Asterisks denote significances

*
<0.05,

**
<0.01,

<0.001,

<0.0001.