Parkin-mediated reduction of nuclear and soluble TDP-43 reverses behavioral decline in symptomatic mice

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The transactivation DNA-binding protein (TDP)-43 binds to thousands of mRNAs, but the functional outcomes of this binding remain largely unknown. TDP-43 binds to Park2 mRNA, which expresses the E3 ubiquitin ligase parkin. We previously demonstrated that parkin ubiquitinates TDP-43 and facilitates its translocation from the nucleus to the cytoplasm. Here we used brain penetrant tyrosine kinase inhibitors (TKIs), including nilotinib and bosutinib and showed that they reduce the level of nuclear TDP-43, abrogate its effects on neuronal loss, and reverse cognitive and motor decline. Nilotinib decreased soluble and insoluble TDP-43, while bosutinib did not affect the insoluble level. Parkin knockout mice exhibited high levels of endogenous TDP-43, while nilotinib and bosutinib did not alter TDP-43, underscoring an indispensable role for parkin in TDP-43 sub-cellular localization. These data demonstrate a novel functional relationship between parkin and TDP-43 and provide evidence that TKIs are potential therapeutic candidates for TDP-43 pathologies.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease characterized by the destruction of motor neurons in the brain and spinal cord (1). In 2006, the primary component of ubiquitin-positive cytoplasmic inclusions in ALS was discovered to be the trans-activating response region DNA-binding protein known as TDP-43 (2). Full length TDP-43 is a 414 amino acid polypeptide that binds thousands of pre-mRNAs and affects the splicing patterns of many others (3). The protein possesses two RNA-recognition motifs (4,5), nuclear import and export signals (6), and a c-terminal region rich in glycine that is believed to engage in protein–protein interactions with components of the RNA splicing machinery (7). The precise function of TDP-43 remains unclear in ALS, and mutations in TDP-43 are found in fronto-temporal lobar dementia (FTLD) (2), consistent with the overlapping pathological and clinical features of these two disorders (8). Patients with sporadic and familial ALS show accumulations of TDP-43 in the cytoplasm of spinal cord motor neurons (2,9). TDP-43 also accumulates in cytoplasmic or nuclear inclusions in FTLD and ALS (10,11). Therefore, nuclear and cytoplasmic TDP-43 localization and level may be essential for its biological function. Because TDP-43-containing inclusions are found in sporadic and familial ALS, and FTLD, targeting TDP-43-mediated toxicity could benefit patients afflicted with both diseases.

Currently there is no cure, nor is there an effective treatment, to ease the suffering of ALS/FTLD patients. The only drug for the treatment of ALS is riluzole (rilutek), which is believed to extend survival by 3–6 months via possible glutamate mechanisms (12,13). The lack of “druggable” protein targets to prevent aggregated protein toxicity is a primary reason that no effective therapies have been developed in FTLD/ALS. We demonstrated that some brain-penetrant tyrosine kinase inhibitors (TKIs) may be a potential alternative strategy to ameliorate the pathology associated with protein aggregation in neurodegenerative diseases (14,15). TKIs, nilotinib and bosutinib, are clinically effective and well-tolerated FDA-approved treatments for chronic myeloid∗

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leukemia (16,17). Importantly, nilotinib and bosutinib lead to parkin ubiquitination and increase its endogenous level, thereby activity (14,18), and TDP-43 regulates parkin expression (3). Inversely, TDP-43 depletion down-regulates Park2 mRNA in human stem-cell derived motor neurons in sporadic ALS, and TDP-43 expression affects parkin levels in postmortem brains of ALS and FTLD (19). Furthermore, we previously demonstrated in lentiviral gene transfer animal models that parkin ubiquitimates TDP-43 and affects its localization and toxicity via reversal of TDP-43-induced amino acid dyshomeostasis, including glutamate (20,21).

To test our hypothesis we used transgenic mice that express nuclear or cytosolic wild type or mutant TDP-43 with endogenous parkin, as well as parkin null mice expressing endogenous TDP-43. Homozygous parkin+/− (22), hemizygous transgenic mice harboring wild type human TDP-43 (TDP-43 mice) (23) and hemizygous mice harboring the A315T mutation (A315T mice) (24) were treated with intraperitoneal (i.p.) injection of either nilotinib (10 mg/kg) or bosutinib (5 mg/kg) for 4 consecutive weeks. Cognitive and motor performances were assessed before the animals were sacrificed for end-point measurements of TDP-43 level, modification and localization.

RESULTS

TKIs promote translocation of nuclear TDP-43 to the cytoplasm

To determine the effects of nilotinib and bosutinib on TDP-43 translocation, GFP-tagged TDP-43 was expressed in human M17 neuroblastoma cells treated with 10 μM Nilotinib, 5 μM bosutinib or 1 μl DMSO for 24 h. GFP was observed predominantly within DAPI-stained nuclei in live M17 cells (Fig. 1A, n = 10), but nilotinib and bosutinib (Fig. 2B and C, inserts show higher magnification) led to more GFP signaling within the cytoplasm and cellular processes compared with DMSO treated cells (Fig. 1D, n = 10). These data are consistent with the role of parkin on TDP-43 localization in M17 cells (21). To verify these effects in vivo, 2–3 months old transgenic TDP-43, mutant A315T and parkin+/− mice, all generated on C57BL6 background, were i.p. treated with either nilotinib (10 mg/kg) or bosutinib (5 mg/kg) or 30 μl DMSO for 4 consecutive weeks. TDP-43 staining of 20 μm thick cortical sections shows endogenous TDP-43 localized predominantly to DAPI-stained nuclei (Fig. 1E, n = 8) in the cortex of DMSO (30 μl) treated C57BL6 control; and nilotinib (Fig. 1I) and bosutinib (Fig. 1M) did not change TDP-43 expression. TDP-43 was significantly higher in both DAPI-stained nuclei and cytoplasm of transgenic TDP-43 mice (Fig. 1F and Q, n = 8, P < 0.0001), but nilotinib significantly decreased the number of TDP-43-positive (by stereology) cells (Fig. 1J and Q, P < 0.001, n = 8) and led to less expression of nuclear TDP-43 (insert) compared with DMSO (insert). Bosutinib also significantly reduced the number of TDP-43-containing cells (Fig. 1N and Q, n = 8, P < 0.002) and led to less TDP-43 expression in the nucleus (insert), but the level of cytoplasmic TDP-43 was not different compared with DMSO (insert). TDP-43 was significantly higher in transgenic A315T mice (Fig. 1G and Q, n = 8, P < 0.0001), and again nilotinib significantly decreased the number of TDP-43-positive (by stereology) cells (Fig. 1K and Q, P < 0.003, n = 8) and reduced nuclear TDP-43 (insert) compared with DMSO. Bosutinib also reduced the number of TDP-43-bearing cells (Fig. 1O and Q, n = 8, P < 0.002) and attenuated nuclear TDP-43 level (insert) compared with DMSO. Interestingly, endogenous TDP-43 was significantly higher in parkin+/− mice (Fig. 1H and Q, n = 8, P < 0.001) compared with control DMSO (Fig. 1E), and neither nilotinib (Fig. 1L, n = 8) nor bosutinib (Fig. 1P, n = 8) altered the level or localization of TDP-43 in these mice, indicating an important role for parkin in TDP-43 expression and sub-cellular localization.

To further demonstrate the localization of TDP-43, sub-cellular fractionation was performed to isolate cytoplasmic (hypotonic solution) from nuclear TDP-43 as described in methods. Higher levels of mouse TDP-43 were detected by western blot (Fig. 1R, first blot, n = 4, P < 0.05) in the cytosolic fraction relative to actin from parkin+/− mice compared with control, but no human TDP-43 was detected as expected (Fig. 1R, second blot), further suggesting that parkin+/− mice over-express endogenous TDP-43. However, mouse (first blot) and human (second blot) TDP-43 were significantly higher in TDP-43 mice (n = 4, P < 0.00001), but nilotinib and bosutinib eliminated human TDP-43 and reduced mouse TDP-43 back to control level relative to actin. Significantly higher levels of TDP-43 relative to nuclear marker Poly [ADP-ribose] polymerase (PARP)-1 were observed in the nucleus of parkin+/− mice compared with control (Fig. 1S, n = 4, P < 0.05), but a significantly higher level of nuclear TDP-43 was present in TDP-43 mice (n = 4, P < 0.0001), while bosutinib and nilotinib reduced both mouse (first blot) and human (second blot) levels of nuclear TDP-43 relative to PARP-1. Further analysis showed that TDP-43 levels were significantly higher (30%) in the nucleus than the cytoplasm of TDP-43 mice (P < 0.05), however, the reduction of nuclear TDP-43 was 2-fold higher compared with cytoplasmic TDP-43 when these mice were treated with nilotinib or bosutinib. Taken together these data suggest that TKIs reduce the levels of both nuclear and cytosolic TDP-43. A different extraction method to isolate soluble (Supplementary Material, Fig. S1A) from 4 M urea insoluble (Supplementary Material, Fig. S1C) TDP-43 showed a decrease in both soluble (STEN buffer) and insoluble TDP-43 when animals were treated with nilotinib, but bosutinib only decreased the soluble fraction, suggesting that insoluble TDP-43 is a sequestration strategy.

TKIs reverse TDP-43-induced cell death in brain and spinal cord neurons

TDP-43 staining of 20 μm thick hippocampal sections showed endogenous TDP-43 in control mice treated with DMSO (Fig. 2A, n = 8) or nilotinib (Fig. 2E). The expression level of TDP-43 was higher in TDP-43 mice treated with DMSO and stereological counting of TDP-43-positive cells in the hippocampus was significantly higher than control (Fig. 2B, n = 8, P < 0.00001); while nilotinib (Fig. 2C, n = 8) and bosutinib (Fig. 2D, n = 8) significantly reduced the number of TDP-43 overexpressing cells (P < 0.002) compared with DMSO. The level of endogenous TDP-43 expression in parkin+/− mice (Fig. 2F, n = 8) was significantly higher than control with DMSO or nilotinib (P < 0.00001), but the number of TDP-43 cells in the hippocampus was not different than transgenic TDP-43 mice (Fig. 2B). Nilotinib (Fig. 2G) and bosutinib
Figure 1. Nilotinib and bosutinib alter the localization and level of TDP-43. GFP-tagged TDP-43 was expressed in DAPI-stained human M17 neuroblastoma cells (A) treated with (B) nilotinib, (C) bosutinib and (D) DMSO. Inserts are higher magnification ($n = 10$). TDP-43 staining of 20 μm thick sections shows TDP-43 in DAPI-stained cells in the cortex of (E) C57BL6, (F) TDP-43, (G) A315T and (H) parkin$^{-/-}$ mice treated with DMSO. Inserts are higher magnification ($n = 8$). TDP-43 in DAPI-stained cells in the cortex of (I) C57BL6, (J) TDP-43, (K) A315T and (L) parkin$^{-/-}$ mice treated with nilotinib. Inserts are higher magnification ($n = 8$). TDP-43 in DAPI-stained cells in the cortex of (M) C57BL6, (N) TDP-43, (O) A315T and (P) parkin$^{-/-}$ mice treated with bosutinib. Inserts are higher magnification ($n = 8$). (Q) Histograms represent stereological quantification of TDP-43 expressing cells. Asterisks indicate significantly different to control or as indicated, mean ± SEM, $n = 8$, ANOVA, Neumann–Keuls multiple comparison. WB analysis of total brain lysates on 4–12% SDS NuPAGE gel showing (R) cytoplasmic human (first blot) and mouse (second blot) TDP-43 relative to actin (third blot) and (S) nuclear human (first blot) and mouse (second blot) TDP-43 relative to PARP-1 (third blot), $n = 4$. 
Figure 2. Nilotinib and bosutinib reduce hippocampal TDP-43 level and brain caspase-3 activation. TDP-43 staining of 20 μm thick sections shows TDP-43 in DAPI-stained cells in the hippocampus of (A) C57BL6 mice treated with DMSO, and TDP-43 mice treated with (B) DMSO, (C) nilotinib and (D) bosutinib (n = 8). TDP-43 in the hippocampus of (E) C57BL6 mice treated with nilotinib, and parkin−/− mice treated with (F) DMSO, (G) nilotinib and (H) bosutinib (n = 8). Nissl staining in (I) C57BL6 mice treated with DMSO, and TDP-43 mice treated with (J) DMSO, (K) nilotinib and (L) bosutinib (n = 8). Histograms represent (M) stereological quantification of Nissl stained cells and (N) caspase-3 activity in whole brain extracts. Asterisks indicate significantly different to control or as indicated, mean ± SEM, n = 8, ANOVA, Neumann–Keuls multiple comparison.
(Fig. 2H) did not alter the number of TDP-43 positive cells in parkin−/− mice. Stereological counting of Nissl stained cells showed a significant decrease in Nissl cells in cortex and hippocampus (Fig. 2J and M, n = 8, P < 0.001) of TDP-43 mice compared with control (Fig. 2I) treated with DMSO, while nilotinib (Fig. 2K and M) and bosutinib (Fig. 2L and M) remained lower than control (Fig. 2I and M) and partially reversed the loss of Nissl stained cells compared with TDP-43 with DMSO. No difference was observed in Nissl-staining between parkin−/− mice and control (Fig. 2M). ELISA measurement of caspase-3 activity was detected in DMSO treated mice (Fig. 3F and Q) compared (Fig. 4A, n = 8, P < 0.02) with control treated with DMSO (Fig. 3A). However, nilotinib (Fig. 3C and Q) and bosutinib (Fig. 3D and Q) reduced the number of silver-positive cells back to control. Despite the high expression level of endogenous TDP-43 in parkin−/− mice, a non-significant trend (15–21%) of higher level of silver cells was detected in DMSO treated mice (Fig. 3F and Q) compared with control (Fig. 3E), and nilotinib (Fig. 3G) and Bosutinib (Fig. 3H) did not alter the expression pattern of silver cells in parkin−/− mice. A comparison of different brain regions showed that silver cells were significantly higher in the cortex and hippocampus (Fig. 3Q, n = 8, P < 0.02) in TDP-43 mice treated with DMSO compared with control, and nilotinib and bosutinib eliminated silver staining, indicating reduced cell death. Further analysis using toluidine blue staining of lumbar spinal cord fibers showed reduced overall staining intensity in the descending cortico-spinal tract (DCST) in TDP-43 mice (Fig. 3I, K and L) compared with control treated with DMSO (Fig. 3I). Counting toluidine blue stained axons showed a significant decrease in the number of in DCST fibers in the lumbar region in TDP-43 mice (Fig. 3J and R, n = 8, P < 0.05) compared with DMSO, and these effects were not observed in nilotinib (Fig. 3K and R) and bosutinib (3L and R) treated TDP-43 mice compared with control (Fig. 3M and K). No difference in DCST staining was observed in parkin−/− mice (Fig. 3N, O and P), but the diameter of individual axons was increased in parkin−/− mice compared with control.

**DISCUSSION**

These studies provide some evidence that alteration of nuclear TDP-43 localization and attenuation of its soluble level may be a protective mechanism that prevents TDP-43 toxicity. Our data show that TKIs, which increase parkin activity, can lead to translocation of nuclear TDP-43 to the cytoplasm, consistent with previous studies that demonstrated parkin over-expression to mediate TDP-43 ubiquitination and subsequent translocation (21). Additionally, TKIs decrease nuclear TDP-43 levels more efficiently than the cytoplasmic fraction, suggesting that increased levels of nuclear TDP-43 is a strong factor for its toxicity (6). Although both TKIs reduce TDP-43-induced neurodegenerative death, only nilotinib decreases insoluble TDP-43 (Supplementary Material, Fig. S1), suggesting that the soluble fraction may be toxic, while insoluble cytoplasmic TDP-43 is a sequestration strategy to prevent it from aberrant binding and mis-processing of mRNAs (7). This hypothesis is supported by data from postmortem human ALS/FTLD brains showing that insoluble cytoplasmic TDP-43 aggregates are detected in neurons that are not necessarily dead (7). We previously demonstrated that TKIs, including nilotinib and bosutinib, increase parkin level and activity (14,18), while parkin ubiquititates TDP-43 and facilitates its translocation from the nucleus to the cytoplasm, thus attenuating its toxicity (21). In these studies, we further demonstrate that TKIs depend on parkin to alter TDP-43 localization and sub-cellular level. Parkin deletion not only abrogates the effects of TKIs on TDP-43 localization, but also results in increased levels of endogenous TDP-43,
Figure 3. Nilotinib and bosutinib reduce brain cell and axonal spinal cord loss. Silver staining of 20 μm thick sections shows TDP-43 in DAPI-stained cells in the cortex of (A) C57BL6 mice treated with DMSO, and TDP-43 mice treated with (B) DMSO, (C) nilotinib and (D) bosutinib (n = 8). Silver staining in (E) C57BL6 mice treated with nilotinib, and parkin<sup>−/−</sup> mice treated with (F) DMSO, (G) nilotinib and (H) bosutinib (n = 8). Toluidine blue staining of 20 μm thick sections of lumbar spinal cord shows axonal myelination in (I) C57BL6 mice treated with DMSO, and TDP-43 mice treated with (J) DMSO, (K) nilotinib and (L) bosutinib (n = 8). Toluidine bluer in (M) C57BL6 mice treated with nilotinib, and parkin<sup>−/−</sup> mice treated with (N) DMSO, (O) nilotinib and (P) bosutinib (n = 8). Histograms represent (Q) stereological quantification of silver stained cells and (R) number of axons in the DCST lumbar spinal cord region. Asterisks indicate significantly different to control or as indicated, mean + SEM, n = 8, ANOVA, Neumann–Keuls multiple comparison.
Figure 4. Nilotinib and bosutinib reverse motor and cognitive decline in TDP-43, but not parkin−/− mice. Histograms represent (A) rotarod performance and (B) novel object recognition in TDP-43 and parkin−/− mice. (C) Water maze entry into the platforms and (D) the total distance traveled in TDP-43 mice. The number of animals is 30 before treatment and 10 for each treatment. Asterisks indicate significantly different to control or as indicated, mean + SEM, ANOVA, Neumann–Keuls multiple comparison.
underscoring an important role of parkin in the sub-cellular localization of TDP-43. A relationship between parkin and TDP-43 has been suggested in animal models and postmortem human brains (3, 21), and our results demonstrate a novel functional relationship between these two proteins. Despite the up-regulation of endogenous TDP-43 levels in parkin−/− mice, which did not exhibit the same level of neurodegeneration compared with TDP-43 mice, no TDP-43 fragments were detected (Supplementary Material, Fig. S1), suggesting that neurodegeneration may be attributed to TDP-43 cleavage (2, 25, 26). Furthermore, nilotinib is an Abelson (Abl) TKI and bosutinib is a SRC/Abl inhibitor. Although no data exist that show these tyrosine kinases are altered in ALS/FTLD brains, nilotinib and bosutinib can serve as a disease-modifying therapy to modulate TDP-43 level and localization in ALS/FTLD and other neurodegenerative diseases where Abl is activated (14, 15, 18).

The reduction of nuclear and soluble TDP-43 levels improved cognitive and motor performance in transgenic TDP-43 mice, but not parkin−/− mice, which were hyperactive, perhaps due to altered dopamine levels (27), and displayed cognitive defects on the NOR test. TKIs did not alter the behavioral outcomes in parkin−/− mice and did not change the high level or expression pattern of endogenous TDP-43. Supplementary Material, Figure S2 shows an increased level of mouse Aβ1-42 in TDP-43 and parkin−/− mice, consistent with our previous findings that TDP-43 over-expression increases β-secretase (BACE) level and alters the processing of amyloid precursor protein, resulting in higher levels of mouse Aβ1-42 peptides (28). Bosutinib and nilotinib again decreased the levels of mouse Aβ1-42 (which is believed not to be toxic) in a parkin-dependent manner (14), suggesting that cognitive improvement in TDP-43 mice may in part be due to the ability of neurons to reduce proteins that impair their physiological function. Additionally, we recently demonstrated that parkin reverses TDP-43 induced changes in glutamate metabolism (20), and more evidence exist that parkin is important for memory formation via modulation of the function and stability of glutamatergic synapses (29–32). Therefore, TKIs may reverse TDP-43-induced behavioral impairment via modulation of glutamate metabolism and reduction of toxic protein levels.

In conclusion, these data provide evidence that reduction of soluble TDP-43 levels may prevent aberrant mRNA processing, preventing cell death, and consequently, leading to improved cognitive and motor performance. Parkin is a critical factor that modulates TDP-43 sub-cellular localization and level, and TKIs that increase parkin function may constitute therapeutic strategies to modulate TDP-43 level and localization. These studies provide novel mechanistic insights into functional parkin-TDP-43 interaction and offer translational promises to treat TDP-43 linked pathologies, including ALS and FTLD.

**MATERIALS AND METHODS**

**Cell culture and transfection**

Human neuroblastoma M17 cells (seeding density 2 × 10^5 cells) were grown in 24 well dishes (Falcon) to 70% confluence in Dulbecco’s Modified Eagle Medium (DMEM; Invitrogen) plus 10% (v/v) heat-inactivated fetal bovine serum (Invitrogen), penicillin/streptomycin, and 2 mM L-glutamine at 37°C and 5% CO2, washed twice in phosphate-buffered saline. Transient transfection was performed with 3 μg TDP-43 cDNA, or 3 μg LacZ cDNA. Cells were transfected for 24 h. and then treated with 1 μl DMSO, 10 μM nilotinib or 5 μM Bosutinib for an additional 24 h and examined live under an AxioPlan fluorescent microscope.

**Nilotinib and bosutinib treatment**

Homozygous parkin−/− (22), hemizygous transgenic mice harboring wild type human TDP-43 (TDP-43 mice) (23), and hemizygous mice harboring the A315T mutation (A315T mice) (24) were i.p. treated with 30 μl DMSO, 10 mg/kg nilotinib or 5 mg/kg bosutinib for 4 consecutive weeks. All procedures were approved by the Georgetown University Animal Care and Use Committee (GUACUC).

**Western blot analysis**

The cortex was dissected out and homogenized in 1 × STEN buffer [50 mM Tris (pH 7.6), 150 mM NaCl, 2 mM EDTA, 0.2% NP-40, 0.2% BSA, 20 mM PMSF and protease cocktail inhibitor]. The pellet was then re-suspended in 4 mM urea and homogenized and centrifuged at 5000 g and the supernatant containing the insoluble protein fraction was collected. Total TDP-43 was probed either with (1:1000) mouse monoclonal (2E2-D3) antibody generated against N-terminal 261 amino acids of the full-length protein (Abnova) or (1:1000) Rabbit polyclonal (ALS10) antibody (ProteinTech, Cat#10782-2-AP). Rabbit polyclonal anti-parkin (Millipore) antibody was used (1:1000) for WB. Rabbit polyclonal anti-actin (Thermo Scientific) was used (1:1000). Western blots were quantified by densitometry using Quantity One 4.6.3 software (Bio Rad). Densitometry was obtained as arbitrary numbers measuring band intensity. Data were analyzed as mean ± SEM, using ANOVA, with Neumann–Keuls multiple comparison between treatment groups.

**Sub-cellular fractionation**

Total brains were homogenized in 2 ml hypotonic buffer [20 mM Tris–Hcl, 10 mM NaCl, 3 mM MgCl2 10% NP40, protease inhibitor (1:500)], incubated on ice for 15 min, and then centrifuged for 10 min at 3000 rpm at 4°C. The supernatant containing the cytosolic fraction was collected and the pellet was re-suspended in 0.5 ml complete cell extraction buffer (Invitrogen, Cat. no. FNN0011) for 30 min on ice with vortexing at 10 min intervals. The samples were then centrifuged for 30 min at 14,000 g at 4°C and the supernatant containing the nuclear fraction was collected.

**Immunohistology**

Immunohistochemistry was performed on 20 μm-thick sections of brain or lumbar spinal cord. TDP-43 was probed (1:200) with rabbit polyclonal (ALS10) antibody (ProteinTech, Cat#10782-2-AP). Toluidine blue and DAPI staining were performed according to manufacturer’s instructions (Sigma). Counting of toluidine blue staining of axons within 10 random fields of each slide was performed by a blind investigator. All staining experiments were scored by a blind investigator to the treatments. Caspase-3 activity was performed according to manufacturer’s protocol as described in (33, 34).
Stereological methods

Stereological methods were applied by a blinded investigator using unbiased stereology analysis (Stereologer, Systems Planning and Analysis, Chester, MD, USA) to determine the total positive cell counts in 20 cortical fields on at least 10 brain sections (~400 positive cells per animal) from each animal as previously explained (21).

Morris water maze

All animals were pre-trained (trials) to swim for 90 s in a water maze containing a platform submerged in water (invisible) for 4 consecutive days once a day. The pretraining trials ‘teach’ the swimming animals that to ‘escape’, they must find the hidden platform, and stay on it. The water maze ‘test’ was performed on Day 5 (35), when the platform was removed and 100 for the recognition session. Statistical calculations to estimate differences between sessions were performed by a pairwise t-test.

Novel object recognition

Mice were placed individually in a 22 × 32 × 30 cm testing chamber for a 5 min habituation interval and returned to their home cages. Thirty minutes later mice were placed in the testing chamber for 10 min with two identical objects (acquisition session), then returned to their home cages and 90 later placed back in the testing chamber in the presence with one of the original objects and one novel object of the same size but of a different color and shape (recognition session). Sessions were video recorded. Time spent exploring the objects were scored by blind observer. The recognition index was calculated as (time exploring one of the objects/time exploring both objects) × 100 for acquisition session, and (time exploring new object/time exploring both familiar and novel objects) × 100 for the recognition session. Statistical calculations to estimate differences between sessions were performed by a pairwise t-test.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

Conflict of Interest statement. The authors declare no conflict of interest is association with this manuscript. Dr C.E.-H.M. has a pending preliminary US patent application to use nilotinib and bosutinib for the treatment of neurodegenerative diseases.

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