NEURAL REGENERATION RESEARCH
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Repurposing ibrutinib: therapeutic effects and implications for translational approaches in Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, and the number of patients with AD is estimated to double by 2060 (Alzheimer's Association, 2022). The AD mortality rate has increased by 145% over the last decade, the largest increase among the ten leading causes of death in the US (Alzheimer's Association, 2022). In 2022, the targets of novel disease-modifying agents for AD in phase 3 clinical trials expanded from amyloid β (Aβ)/tau to include synaptic plasticity, the gut-brain axis, oxidative stress, the vasculature, metabolism, and proteostasis (Cummings et al., 2022). Despite the identification of innovative AD therapeutic targets and high R&D investment in AD research, the clinical failure rate for AD therapeutics is 99.6% (Cummings et al., 2022). A major reason for this high failure rate may be the use of single-target approaches for AD drug development.

Developing polypharmacological therapeutics is a challenging strategy for treating and/or curing AD. Repurposing existing drugs as the novel, polypharmacological AD treatments might be a feasible therapeutic strategy. Researchers are increasingly turning to drug repurposing, in which novel therapeutic effects of existing Food and Drug Administration-approved drugs are identified, to provide new therapies in a cost-effective, safe, and time-saving manner.

Ibrutinib is an irreversible Bruton's tyrosine kinase (BTK) inhibitor that covalently binds to the adenosine triphosphate (ATP)-binding pocket in the kinase active site, thereby suppressing the proliferation and survival of malignant B lymphocytes (Berglof et al., 2015). The plasma concentration of ibrutinib peaks 1 to 2 hours (T_{max}) after oral absorption, and the half-life of ibrutinib is 4 to 6 hours (Bose et al., 2016). Ibrutinib inhibits the activity of its on-target, BTK, by 50% at a dose of 0.5 nM (IC_{50}) (Bose et al., 2016). The on-target therapeutic effects of ibrutinib have led to its approval by the Food and Drug Administration for the treatment of chronic lymphocytic leukemia, mantle cell lymphoma, and Waldenström macroglobulinemia (Berglof et al., 2015). However, BTK shares high homology in the ATP-binding region with other families of kinases, giving rise to off-target effects of ibrutinib (Berglof et al., 2015). These off-targets do not contribute to the therapeutic effects of ibrutinib on B-cell malignancy but are involved in the development of adverse effects such as hemorrhage, hypertension, and arrhythmia (Zimmerman et al., 2021).

We and others have demonstrated that ibrutinib penetrates the blood-brain barrier. Therefore, we investigated the effect of ibrutinib on the central nervous system and identified novel on- and off-target therapeutic effects on neuroinflammation-related diseases and neurodegenerative diseases, including AD, *in vitro* and *in vivo*. For example, ibrutinib attenuates lipopolysaccharide (LPS)-stimulated neuroinflammatory responses in wild-type mice (Nam et al., 2018). In addition, ibrutinib significantly improves cognitive function in 5xFAD mice, a model of AD, by promoting hippocampal dendritic spine formation (Lee et al., 2021). In the same study, we observed that ibrutinib significantly reduces Aβ plaque deposition, tau hyperphosphorylation, and neuroinflammatory responses in 5xFAD mice and P301S Tau Tg mice (Lee et al., 2021). Importantly, the effect of ibrutinib on tauopathy *in vitro* is not on-target (BTK) dependent (Lee et al., 2021). Overall, our findings suggest that ibrutinib is a therapeutic drug for AD and neuroinflammation-associated disease.

Given its effects on neuroinflammation, repurposing ibrutinib has therapeutic implications for other neurodegenerative diseases and their psychiatric symptoms. For instance, ibrutinib significantly attenuates the activation of the NLRP3/ASC/IL-1β inflammasome and the volume of brain injury induced by middle cerebral artery occlusion in a mouse model of ischemia (Ito et al., 2015). BTK is involved in NLRP3 activation, and inhibition of BTK suppresses NLRP3 inflammasome activation *in vitro* (Ito et al., 2015). In addition, ibrutinib reduces contusion injury-evoked gliosis and motor dysfunction in a rat model of thoracic spinal cord injury by inhibiting BTK expression and phosphorylation in the spinal cord (Yu et al., 2021). Surprisingly, ibrutinib reduces LPS-evoked anhedonia- and depression-like behaviors in a mouse model of depression (Li et al., 2021). Moreover, ibrutinib ameliorates LPS-induced dysregulation of dendritic spinogenesis/synaptic function, gliosis, NLRP3 inflammasome activation and subsequent proinflammatory responses in the hippocampus in mice with LPS-induced depression (Li et al., 2021). We found that ibrutinib downregulates LPS-mediated proinflammatory responses *in vitro* and *in vivo* (Nam et al., 2018). Our results and previous findings indicate that ibrutinib regulates neuroinflammatory/peripheral inflammatory responses through BTK and/or off-target effects, but additional studies are required to fully elucidate the underlying mechanisms.

Given that abnormal regulation of the on-target (BTK) and off-targets (Tec, epidermal growth factor receptor (EGFR), Src and Jak family kinases) of ibrutinib has been implicated in the pathogenesis of neurodegenerative diseases, it is essential to reveal whether on- and off-target inhibition by ibrutinib has differential therapeutic effects on the pathophysiology of neurological diseases. Translational research establishing the specific inhibition mechanisms of ibrutinib's novel therapeutic effects will narrow the gap for applying *in vivo* results to clinical research and reducing the failure rate of novel agents in clinical trials and the Food and Drug Administration approval process.

Several studies have demonstrated that on- and off-targets of ibrutinib are potential risk factors for the development of neurodegenerative diseases. BTK, the on-target of ibrutinib, is expressed in the cortical, hippocampal, striatal, and thalamic regions of the brain in AD mice and co-localizes with microglia (Keaney et al., 2019). BTK protein levels are markedly increased in the brains of 5xFAD mice and show a tendency to increase in the brains of P301S Tau Tg mice compared with their wild-type littermates (Keaney et al., 2019). AD patients also exhibit increased BTK expression in the temporal cortex compared with healthy controls (Keaney et al., 2019). BTK activates phospholipase C gamma 2 (PLCγ2) in the B-cell receptor signaling pathway via phosphorylation (Keaney et al., 2019). Given that *PLCγ2* polymorphisms are associated with genetic susceptibility to AD pathology development and that BTK is co-expressed with Aβ plaques in the brains of AD mice, inhibition of BTK may be a pivotal therapeutic strategy for AD treatment.

BMX (IC_{50}, 0.8 nM), an ibrutinib off-target and a member of the Tec kinase family, is distributed in the frontal cortex, hippocampus, and corpus callosum in a rat model of traumatic brain injury (Bose et al., 2016; Hsieh et al., 2017). BMX is involved in the STAT signaling cascade, a major pathway for neuroinflammation, and triggers the APBA2 signaling pathway, which regulates amyloid precursor protein (APP) proteolytic processing (Yeh et al., 2021). Increases in BMX, glial fibrillary acidic protein (a marker of astrocytic activation), and APP protein levels are correlated with the severity of traumatic injury in a traumatic brain injury rat model, suggesting that BMX may be related to AD pathoprogression via the regulation of glial activation and APP function (Hsieh et al., 2017).

More importantly, *in vivo* studies and clinical trials have clearly established the ibrutinib off-target EGFR (IC_{50}, 5.6 nM) as a risk factor for AD. Specifically, *EGFR* polymorphisms are observed in AD patients; in a mouse model of AD, EGFR phosphorylation is upregulated (Wang et al., 2012; Bose et al., 2016; Chen et al., 2018), and EGFR inhibition significantly improves Aβ-induced cognitive impairment (Wang et al., 2012). EGFR expression decreases after birth but remains detectable in memory-controlling regions of the brain, including the cerebral cortex and hippocampus, in adult rats (Romano and Bucci, 2020). Taken together, these findings suggest that repurposing ibrutinib is a practical strategy for the development of novel polypharmacological AD therapeutics.

Given that ibrutinib inhibits BTK and other off-target kinases via interaction with the kinase ATP-binding site, ibrutinib might modulate ATP-binding cassette (ABC) transporters. Interestingly, among ABC transporters, subfamily B member 1 (ABCB1) and subfamily C member 1 (ABCC1) regulate amyloid β accumulation/removal in the brain (Namasivayam et al., 2022). Moreover, the same study demonstrated that ABC transporter inhibitors/activators affect APP/Aβ metabolism

(Namasivayam et al., 2022). Therefore, ABC transporters could be regarded as pivotal interacting molecules through which ibrutinib regulates AD pathology.

Strikingly, a recent study demonstrated that c-terminal Src kinase, an off-target of ibrutinib, is closely related to severe adverse effects of ibrutinib and atrial fibrillation (Zimmerman et al., 2021). In addition, ibrutinib treatment induces hypertension and cardiac toxicity in chronic lymphocytic leukemia patients (Zimmerman et al., 2021). The recommended dose of ibrutinib for chronic lymphocytic leukemia patients (420 mg/per day) is much higher than the minimum dose for occupying BTK receptors (Zimmerman et al., 2021); thus, a lower dose of ibrutinib might be more effective for treating B-cell malignancy. Based on the literature, the optimal ibrutinib dose should be a critical consideration in repurposing ibrutinib as a novel therapeutic agent for AD to minimize adverse effects and clinical failure.

In conclusion, the antileukemia drug ibrutinib modulates various AD pathologies (e.g., amyloidogenesis, tauopathy, cognitive impairment/dendritic spinogenesis and neuroinflammation) in mouse models of AD (**Figure 1**). Therefore, repurposing ibrutinib as a novel polypharmacological AD therapeutic is a practical strategy, and unveiling the underlying mechanisms is critical for establishing an on- or off-target-dependent mode of action and dose optimization for the eventual treatment of AD.

This work was supported by the KBRI basic research program through KBRI funded by the Ministry of Science, ICT & Future Planning (grant Nos. 22-BR-02-03 and 22-BR-05-02, to HSH), and the National Research Foundation of Korea (grant No. 2022R1F1A1074320, to HL).

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Date of submission: November 18, 2022
Date of decision: December 20, 2022
Date of acceptance: January 10, 2023
Date of web publication: March 3, 2023

https://doi.org/10.4103/1673-5374.369108
How to cite this article: *Lee H, Hoe HS (2023) Repurposing ibrutinib: therapeutic effects and implications for translational approaches in Alzheimer's disease. Neural Regen Res 18(10):2194-2195.*

Open peer reviewer: *Swati S More, University of Minnesota Center for Drug Design, USA.*
Additional file: *Open peer review report 1.*

References

Alzheimer's Association (2022) 2022 Alzheimer's disease facts and figures. Alzheimers Dement 18:700-789.

Berglof A, Hamasy A, Meinke S, Palma M, Krstic A, Mansson R, Kimby E, Osterborg A, Smith CI (2015) Targets for ibrutinib beyond B cell malignancies. Scand J Immunol 82:208-217.

Bose P, Gandhi VV, Keating MJ (2016) Pharmacokinetic and pharmacodynamic evaluation of ibrutinib for the treatment of chronic lymphocytic leukemia: rationale for lower doses. Expert Opin Drug Metab Toxicol 12:1381-1392.

Chen X, Wang C, Zhou S, Li X, Wu L (2018) The impact of EGFR gene polymorphisms on the risk of Alzheimer's disease in a Chinese Han population: a case-controlled study. Med Sci Monit 24:5035-5040.

Cummings J, Lee G, Nahed P, Kambar M, Zhong K, Fonseca J, Taghva K (2022) Alzheimer's disease drug development pipeline: 2022. Alzheimers Dement (N Y) 8:e12295.

Hsieh TH, Kang JW, Lai JH, Huang YZ, Rotenberg A, Chen KY, Wang JY, Chan SY, Chen SC, Chiang YH, Peng CW (2017) Relationship of mechanical impact magnitude to neurologic dysfunction severity in a rat traumatic brain injury model. PLoS One 12:e0178186.

Ito M, Shichita T, Okada M, Komine R, Noguchi Y, Yoshimura A, Morita R (2015) Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. Nat Commun 6:7360.

Keaney J, Gasser J, Gillet G, Scholz D, Kadiu I (2019) Inhibition of Bruton's tyrosine kinase modulates microglial phagocytosis: therapeutic implications for Alzheimer's disease. J Neuroimmune Pharmacol 14:448-461.

Lee HJ, Jeon SG, Kim J, Kang RJ, Kim SM, Han KM, Park H, Kim KT, Sung YM, Nam HY, Koh YH, Song M, Suk K, Hoe HS (2021) Ibrutinib modulates Abeta/tau pathology, neuroinflammation, and cognitive function in mouse models of Alzheimer's disease. Aging Cell 20:e13332.

Li W, Ali T, He K, Liu Z, Shah FA, Ren Q, Liu Y, Jiang A, Li S (2021) Ibrutinib alleviates LPS-induced neuroinflammation and synaptic defects in a mouse model of depression. Brain Behav Immun 92:10-24.

Nam HY, Nam JH, Yoon G, Lee JY, Nam Y, Kang HJ, Cho HJ, Kim J, Hoe HS (2018) Ibrutinib suppresses LPS-induced neuroinflammatory responses in BV2 microglial cells and wild-type mice. J Neuroinflammation 15:271.

Namasivayam V, Stefan K, Gorecki L, Korabecny J, Soukup O, Jansson PJ, Pahnke J, Stefan SM (2022) Physicochemistry shapes bioactivity landscape of pan-ABC transporter modulators: anchor point for innovative Alzheimer's disease therapeutics. Int J Biol Macromol 217:775-791.

Romano R, Bucci C (2020) Role of EGFR in the nervous system. Cells 9:1887.

Wang L, Chiang HC, Wu W, Liang B, Xie Z, Yao X, Ma W, Du S, Zhong Y (2012) Epidermal growth factor receptor is a preferred target for treating amyloid-beta-induced memory loss. Proc Natl Acad Sci U S A 109:16743-16748.

Yeh SJ, Chung MH, Chen BS (2021) Investigating pathogenetic mechanisms of Alzheimer's disease by systems biology approaches for drug discovery. Int J Mol Sci 22:11280.

Yu CG, Bondada V, Iqbal H, Moore KL, Gensel JC, Bondada S, Geddes JW (2021) Inhibition of Bruton tyrosine kinase reduces neuroimmune cascade and promotes recovery after spinal cord injury. Int J Mol Sci 23:355.

Zimmerman SM, Peer CJ, Figg WD (2021) Ibrutinib's off-target mechanism: cause for dose optimization. Cancer Biol Ther 22:529-531.

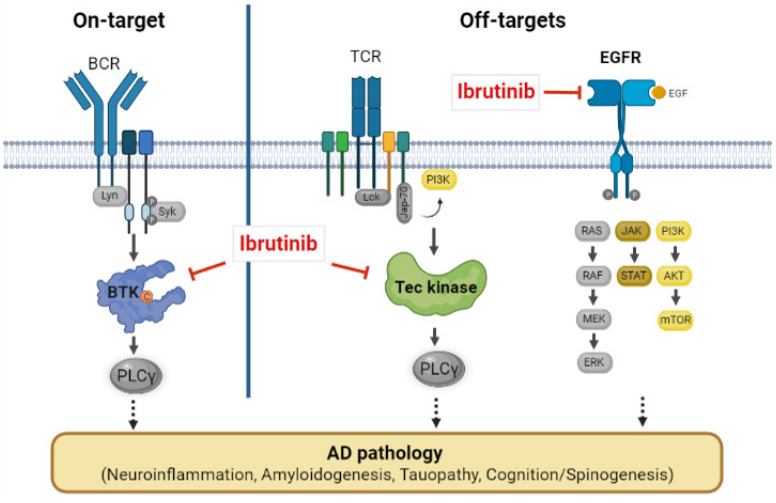

Figure 1 | **On- and off-target therapeutic effects of ibrutinib in AD.**
Ibrutinib modulates various aspects of AD pathophysiology and cognitive function by suppressing the activity of its on-target (BTK) and/or off-targets (Tec kinases and EGFR) *in vitro*, *in vivo*, and in human clinical studies. AD: Alzheimer's disease; BCR: B-cell receptor; BTK: Bruton's tyrosine kinase; EGFR; epidermal growth factor receptor; JAK: Janus kinase; mTOR: mammalian target of rapamycin; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; PLCγ: phospholipase C gamma; STAT: signal transducer and activator of transcription; TCR: T-cell receptor. Created with BioRender.com.

P-Reviewer: More SS; C-Editors: Zhao M, Sun Y, Qiu Y; T-Editor: Jia Y