Enhancing autophagy in Alzheimer’s disease through drug repositioning

Mehdi Eshraghi a,1, Mazaher Ahmadi b,c,1, Saeid Afshar d, Shahrokh Lorzadeh a, Aida Adlimoghaddam c,e, Nahid Rezvani Jalal b,1, Ryan West f, Sanaz Dastghaib g, Somayeh Igder h, Seyed Reza Naeimi Torshizi d, Amir Mahmoodzadeh 1, Pooneh Mokarram c,p, Tayyebeh Madrakian b,c, Benedict C. Albensi e,j,k, Marek J. Łos i, Saed Ghavami a,c,m,n,⁎,⁎⁎, Stevan Pecić f ecological author at: Department of Human Anatomy and Cell Science, University of Manitoba College of Medicine, Winnipeg, MB R3E 0V9, Canada.

Abstract

Alzheimer’s disease (AD) is one of the biggest human health threats due to increases in aging of the global population. Unfortunately, drugs for treating AD have been largely ineffective. Interestingly, downregulation of macroautophagy (autophagy) plays an essential role in AD pathogenesis. Therefore, targeting autophagy has drawn considerable attention as a therapeutic approach for the treatment of AD. However, developing new therapeutics is time-consuming and requires huge investments. One of the strategies currently under consideration for many diseases is “drug repositioning” or “drug repurposing”. In this comprehensive review, we have provided an overview of the impact of autophagy on AD pathophysiology, reviewed the therapeutics that upregulate autophagy, and highlighted the potential of drug repositioning for AD treatment.

Keywords:
Autophagy induction

Abbreviations:
Aβ, Amyloid β; AAV, Adeno-associated virus; ABL1, Abelson Murine Leukemia Viral Oncogene Homolog 1; AD, Alzheimer’s diseases; AgNPs, Silver nanoparticles; AgTNP, Silver triangular nanoplates; AICD, APP intracellular domain; AJs, Adherent junctions; Akt, Alpha Serine/Threonine-Protein Kinase; ALS, Amyotrophic lateral sclerosis; AMPK, AMP-dependent protein kinase; ApoE2, Apolipoprotein E2; ApoE3, Apolipoprotein E3; APP, Amyloid precursor protein; ARG, Abelson related gene; ATGs, Autophagy related genes; AuNPs, Gold nanoparticles; BACE, β-secretase enzyme; BBB, Blood-brain barrier; BDNF, Brain-derived neurotrophic factor; BECN1, Beclin 1; BE-MC, Baicalein loaded poly(ethylene glycol)-block-poly(D, L-lactide) micelles; BNP3, BCL2 Interacting Protein 3; BRB, Berberine; CAMs, Coronary arterial myocytes; CD8, Carbon dots; c-kit, Stem-cell factor receptor; CML, Chronic myeloid leukemia; CNS, Central nervous system; CNTs, Carbon nanotubes; CPP, Cell-penetrating peptide; CSF, Cerebrospinal fluid; CTCL, Cutaneous T cell lymphoma; DAPK, Death associated protein kinase; DDR, Discoidin domain receptor; DHA, Docosahexaenoic acid; DLB, Dementia with Lewy bodies; ENL, Erythema nodosum leprosum; ERT, Enzyme replacement therapy; FKBP12, FK506 binding protein 12; FLRT3, FMS-like tyrosine kinase 3; FRDA, Friedreich’s ataxia; FTD, Frontotemporal dementia; G-proteins, Gastrointestinal stromal tumors; GIs, Gap junctions; GLUT1, Glucose transporter-1; GNBS, Gold nanorods; GQDs, Graphene quantum dots; GSK, Glycogen synthase kinase; HADC, Histone deacetylase; HCC, Hepatocellular carcinoma; HD, Huntington’s disease; HDACs, Histone deacetylase inhibitors; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HTS, High-throughput screening; IP, Intraportal; IUC, Light chain 3; Lck, Lymphocyte-specific protein 1; LdS, Lysosomal storage diseases; MAPK, Mitogen-Activated Protein Kinase; MCI, Mild cognitive impairments; MM, Mammalian target of rapamycin; MDR, Multidrug resistance; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTR, Mammalian target of rapamycin; mTOR, Mammalian target of rapamycin; mTORC1, Mammalian target of rapamycin complex 1; mTORC2, Mammalian target of rapamycin complex 2; MWCNTs, Multi-walled carbon nanotubes; NDD, Neurodegenerative diseases; NPs, Nanoparticles; PAMAM, Poly(amine-diamine); PD, Parkinson’s disease; PDE5, Phosphodiesterase type 5 inhibitor; PEFK, Phosphofructokinase; PFG, Phagocytosis; PIGA, Polypeptide-γ-glutamic acid; PMLA, Polyamine-like (L-malic acid); PSEN1/P5, 1, Presenilin 1; prP, PRP-C, Prion protein C; RCC, Renal cell carcinoma; Res, Resveratrol; HDL, Reconstituted high-density lipoproteins; RVG, Rabies virus glycoprotein peptide; SGLT2, Sodium-glucose transport protein 2; SOD1, Superoxide dismutase 1; SNARE, Single-walled carbon nanotubes; TDP-43, TAR DNA-binding protein 43; TJs, Tight junctions; Tks, Tyrosine kinases; UPR, Unfolded protein response; v-AIPase, Vacuolar ATPase; VLS, Virtual (in silico) ligand screening; ZAK, Zipper-containing kinase; ZBG, Zinc-binding group; α-Synuclein.
1. Introduction

Alzheimer’s disease (AD) is the most prevalent form of neurodegenerative diseases (NDD) and the major cause of age-dependent dementia. It is estimated that 15% of people >60 years of age suffer from mild cognitive impairments (MCI); each year 10–15% of these individuals develop clinical dementia, mainly characterized as AD. Indeed, it is predicted that by 2050, 100 million people worldwide will suffer from dementia (Lopez & Kuller, 2019). Despite all the efforts made over the last two decades, only two new pharmacological compounds have been approved for the treatment of AD in the USA (i.e., memantine and aducanumab) (Cummings, 2021; Kishi et al., 2017). Importantly, at present there is no approved pharmacological intervention available for MCI (Ballard et al., 2020).

The etiology of AD is still poorly understood (Burns & Iliffe, 2009). Currently, proteinopathy is the hypothesized dominant cause for the pathogenesis of AD. Amyloid plaques and neurofibrillary tangles (NFTs), two now established hallmark features of AD, were characterized by Dr. Alois Alzheimer in 1906. In line with the proteinopathy hypothesis, the most prominent factor underlying AD is the deposition of amyloid β (Aβ) plaques in the brain. Thus, amyloid precursor protein (APP) is cleaved by β-secretase enzyme (BACE) and γ-secretase forming Aβ peptides, which accumulate and deposit as Aβ plaques (Müller, Deller, & Korte, 2017; Wilquet & Strooper, 2004). Aβ40 and Aβ42, the two most commonly observed amyloidogenic Aβ peptides (Qiu, Liu, Chen, Zhao, & Li, 2015). Of these, Aβ42 has been shown to oligomerize faster and represents the predominant Aβ peptide in senile plaques in AD brains, even though Aβ40 peptides are present in higher concentrations than Aβ42 in the brain (Gu & Guo, 2013) (Fig. 1).

Autophagy is a physiological cellular stress response that clears harmful and/or redundant materials from the cells through the function of lysosomes (Siri et al., 2021a). It has been suggested that microautophagy in the brain has important roles in the maintenance of synapses, however, further studies are necessary to de

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mammalian target of rapamycin (mTOR) enzymatic complex 1 (mTORC1) by its upstream regulators, the kinase activity on Unc-51 like autophagy activating kinase 1 (ULK1) complex is terminated resulting in the dephosphorylation and activation of this complex (Alizadeh, Lorzadeh, & Ghavami, 2021; Eshraghi et al., 2021; L. Liu, Yan, Liao, Wu, & Shi, 2020). The activated ULK1 complex subsequently phosphorylates and activates class III phosphoinositide 3-kinase (PI3K) complexes, which promotes the formation of the autophagic vesicle membrane (i.e., phagophore) (Lorzadeh, Kohan, Ghavami, & Azarpira, 2021; X. Yu, Long, & Shen, 2015). The next step of autophagy involves phagophore membrane elongation and the formation of autophagosome vesicles, and is navigated by the function of two ubiquitin-like conjugating protein complexes: ATG12 and ATG8 (Nakatogawa, 2013; S. Shojaei, Suresh, Klionsky, Fig. 1. Mechanism of Aβ and hyperphosphorylated of tau aggregation in the brain. A. Aβ protein is generated through series of proteolytic digestions the single-pass transmembrane, amyloid precursor protein (APP). First β-secretase (BACE) cleaves APP, releasing the soluble App fragment (sAPPβ) into the extracellular space and leaves the C terminal fragment (CTFβ) attached to the membrane. Subsequently, CTFβ is cleaved by an intramembrane-cleaving aspartyl protease complex, γ-secretase to generate APP intracellular domain (AICD) and Aβ isoforms (Aβ40 or Aβ42). The major product is a 40 amino acid long Aβ (Aβ40) while the 42 amino acid residues Aβ42 oligomerize faster and form Aβ plaques in Alzheimer’s brains. B. Mechanism of aberrant hyper-phosphorylation of tau protein that leads to microtubule depolymerization. Further, aggregation of oligomerized hyper-phosphorylated tau forms neurofibrillary tangles (NFTs) which accounts for neuronal death. Autophagy is suggested to involve in the clearance of Aβ and hyper-phosphorylated tau aggregates, restricting generation of Aβ plaques and NFTs, respectively. Autophagy inducer drugs can potentially enhance autophagic degradation of aggregated Aβ and tau proteins.
Autophagy plays important roles in neuronal development and homeostasis. Neurons, as post-mitotic cells, are not able to dilute protein aggregates or damaged organelles through cell division. Indeed, neurons have limited capacity for regeneration and the majority survives for the entire lifetime of the organism. Nevertheless, neurons are vulnerable to the accumulation of toxic protein species and rely mainly on the removal of protein aggregates and defective organelles through autophagy flux (Lim & Yue, 2015).

Anatomically, neurons are divided into three distinct compartments: the soma, axon, and dendritic tree. Axonal autophagy contributes to the turnover of damaged mitochondria (Cuervo & Wong, 2014; Maday, Wallace, & Holzbaur, 2012), synaptic vesicles (Okerlund et al., 2017), and aggregated proteins (Ravikumar et al., 2005). Autophagosomes are generated within distal areas of axons from where they are transported to the soma in a retrograde manner to fuse with endosomes and lysosomes in proximal axonal areas (X.-T. Cheng, Zhou, Lin, Cai, & Sheng, 2015b; X. T. Cheng, Zhou, et al., 2015b; George, Hayden, Stanton, & Brockheroff, 2016; Neisch, Neufeld, & Hays, 2017; Stavoe, Hill, Hall, & Colón-Ramos, 2016). Only a limited number of lysosomes can be detected within distal areas of axons, whereas they are more prevalent in the proximal axons and soma. Furthermore, axonal late endosomes and lysosomes are less acidified than those in soma (Gowrishankar et al., 2015). Interestingly, autophagosomes are continuously produced in the axons even under nutrition-rich conditions; this is in contrast to non-neuronal cells where food starvation acts as the main inducer of autophagy (Scivo, Bourdenx, Pampiglia, & Cuervo, 2018; Stavoe & Holzbaur, 2019).

Dendritic autophagy specifically contributes to the regulation of cell surface expression of neurotransmitter receptors, which implies autophagy in neurons is involved in the modulation of synaptic transmission and function (Z.-W. Chen, Chang, Leil, & Olsen, 2007; Rowland, Richmond, Olsen, Hall, & Bamber, 2006). Indeed, this phenomenon has been shown to regulate both GABA and AMPA receptor surface expression in PC12 cells, primary hippocampal neurons, and Xenopus oocytes (Y. Chen et al., 2020; Rowland et al., 2006). Autophagy also regulates dendritic branching. Thus, knockdown or overexpression of autophagy genes modulates dendritic arborization and terminal branching in Drosophila sensory neurons (S. G. Clark et al., 2018), and ATG7 deletion leads to dystrophy of dendritic trees of dopaminergic neurons and forebrain excitatory neurons in mouse brain (Friedman et al., 2012; G. Tang et al., 2014).

Autophagosomes generated in soma are less dynamic and mature in comparison to those derived from axons (Maday & Holzbaur, 2014; Maday & Holzbaur, 2016). From a functional perspective, autophagy in the soma is primarily involved in mitochondrial quality control (MQC). It has been demonstrated that mutations in genes involved in selective mitophagy, such as PINK1, Parkin and OPTN, lead to loss of integrity of somal mitochondria (Cornelissen et al., 2018; Devireddy, Liu, Lampe, & Hollenbeck, 2015; McWilliams et al., 2016; Sung, Tandarich, Nguyen, & Hollenbeck, 2016).

Generally, the rate of autophagosome biogenesis reduces with aging in neuronal cells (Sepe et al., 2014), despite of the fact that the expression levels of autophagy-related genes remain constant during this process (J. T. Chang, Kumsta, Hellman, Adams, & Hansen, 2017; Stavoe & Holzbaur, 2019). In addition, the fusion of lysosomes with autophagosomes is reduced with aging, exacerbating the destructive effects of accumulated proteins and damaged organelles (Lipinski et al., 2010). On the other hand, enhanced autophagy delays aging and extends the lifespan of neurons, probably through removal of damaged mitochondria by mitophagy (Lane, Carroll, Hewitt, & Korolchuk, 2013; Wager & Russell, 2013). Accumulation of damaged mitochondria can lead to production of excessive amounts of reactive oxygen species (ROS) as well as induction of lipid peroxidation and DNA damage (Cuervo & Wong, 2014; Gelino & Hansen, 2012; He, Lu, & Yue, 2013; Ori et al., 2015) (Fig. 2).

2.2. Autophagy is impaired in Alzheimer’s disease

As mentioned before AD is associated with aggregation of misfolded Aβ peptide in the brain (Fig. 1). Autophagy is one of the main processes for the removal of misfolded proteins. It is commonly accepted that autophagy dysregulation, mostly presented as accumulation of autophagosomes as well as downregulation of autophagy proteins, is involved in the pathogenesis of AD. Genome-wide association studies have shown that proteins involved in endocytic trafficking such as PICALM/CALM (phosphatidylinositol binding clathrin assembly protein) contribute to AD pathology through autophagy modulation and alteration of the clearance of tau protein, both in vitro and in vivo (Moreau et al., 2014). In line with these observations, downregulation of Beclin 1 (BECN1) enhances the aggregation of Aβ plaques and tau aggregates in AD (Salminen, Kaarniranta, & Kauppinen, 2013). A mutation in presenilin 1 (PS1/PSE1/PS-1), one of the loss-of-function mutations observed in AD, dysregulates lysosomal homeostasis and transcription of autophagy-related genes (Cacace, Sleezers, & Van Broeckhoven, 2016; Chong et al., 2018; Lee et al., 2015). Furthermore, accumulation of p62 and light chain 3 (LC3) proteins is evident in post-mortem brains of AD patients (Butzlaff et al., 2015). In addition, pathological species of Aβ and αs interfere with the autophagy process resulting in a vicious circle of harmful events (Hou, Watzlawik, Fiesel, & Springer, 2020).

2.3. Autophagy modulation as an effective strategy for the treatment of AD

Based on a growing body of evidence, autophagy modulation has emerged as an interesting therapeutic option in the fight against AD. As indicated, there appears to be a vicious circle between impaired autophagy and protein aggregation in AD (Kulkarni, Chen, & Maday, 2018; Maday, 2016; Suresh, Chakravorty, Giridharan, Carmella, & Manjithaya, 2020). Deletion of ATG7 and ATG5 in adult animals leads to neurodegeneration and death mainly through aggregation of ubiquitinated protein complexes (Karsli-Uzunbas et al., 2014; M. Kim et al., 2016a; Towers & Thorburn, 2016). Conversely, enhanced autophagy flux reverses protein accumulation and promotes cellular and organismal health (Suresh et al., 2020).

Indeed, autophagy has recently been proposed as a potential therapeutic target for treatment of several NDDs including AD (Heras-Sandoval, Pérez-Rojas, & Pedraza-Chaverri, 2020; S. Liu & Li, 2020; Rahman et al., 2020). Thus far, two distinct approaches for autophagy modulation in the CNS have been considered: small molecule therapeutics (e.g., berberine, sirolimus or trehalose) and genetic intervention (i.e., gene therapy with TFEB or BECN1) (Scrivo et al., 2018). Both approaches showed promising outcomes in several in vitro and in vivo models of AD (Castillo et al., 2013; Martini-Stoica, Xu, Ballabio, & Zheng, 2016; Menzies et al., 2017; Polito et al., 2014; Shoji-Kawata et al., 2013; Torra et al., 2018). For example, oral administration of the small molecule berberine (a compound extracted from plants like European barberry) promoted tau clearance by modulating the PI3K/BECN1 autophagic pathway in a 3xTg AD mice model (Y. Chen et al., 2020).

Although induction of autophagy has emerged as a promising approach in the treatment of AD, several unanswered questions remain (Scrivo et al., 2018). Primarily, which step of autophagy (induction, autophagosome formation or maturation) should be targeted to achieve the desired therapeutic outcome(s)? This presents a complicated issue as, for example, inducers of autophagy may not necessarily lead to removal of aggregated proteins, but instead increase the accumulation of immature autophagosomes and even exacerbate disease pathology (Ajobalaby et al., 2021). In addition, it has been
reported that upregulated mitophagy can lead to non-apoptotic cell death and neuronal loss in neurons (Carroll, Hollville, & Martin, 2014) and, in some cases, autophagy modulators have been associated with non-autophagic activities (Galluzzi & Green, 2019; Subramani & Malhotra, 2013). For example, rapamycin and its analogues induce autophagy through inhibition of mTORC1 (Lin, Han, Weng, Wang, & Chen, 2018; Sarkar, Ravikumar, Floto, & Rubinsztein, 2009a); however, mTORC1 is also involved in various
non-autophagic pathways and inhibiting its activity may interfere with other important cellular processes such as synaptic plasticity (Hashemi et al., 2020; Menzies, Fleming, & Rubinsztein, 2015).

### 3. Drug repurposing for autophagy modulation in Alzheimer's disease

#### 3.1. Drug repurposing as an effective strategy to identify new treatments for AD

In order to avoid long and expensive drug discovery processes, including safety assessment, dosing, and other pharmacokinetic and -dynamic characterizations, many academic institutions and pharmaceutical companies employ a strategy known as repurposing (or repositioning) of approved drugs for alternative indications. Drug repurposing can be broadly defined as the process of re-evaluating existing (or abandoned) drugs for new avenues (Appleby, Nacopoulos, Milano, Zhong, & Cummings, 2013; Boguski, Mandl, & Sukhatme, 2009; Sonaye, Sheikh, & Doifode, 2021; Toney, Fasick, Singh, Beyrer, & Sullivan Jr., 2009). Several detailed and comprehensive reviews have been published that describe this approach (with representative case studies), which contributed to the identification of various important 'novel' therapeutics (Durães, Pinto, & Sousa, 2018; Gns, Gr, Murahari, & Krishnamurthy, 2019; Pushpakom et al., 2019; Talevi & Bellera, 2020). Some of these repurposed drugs were found by serendipity (e.g., sildenafil (Goldstein et al., 1998)), but many were identified by specific target searching. Thalidomide was originally developed in Germany as a sedative during the late 1950s but it was never approved in the USA as a sedative due to severe teratogenic side effects (Ito, Ando, & Handa, 2011). However, following re-evaluation, thalidomide was repurposed and approved by the Food and Drug Administration (FDA) in 1998 for the treatment of erythema nodosum leprosum (ENL) (Upputuri, Pallapati, Tarwater, & Srikantam, 2020), and later in 2006 in combination with dexamethasone for treating multiple myeloma (Cavallo, Boccadoro, & Palumbo, 2007). Currently, it is being evaluated as an anti-inflammatory therapy for Crohn’s disease, myelofibrosis, and COVID-19-induced pneumonia and acute lung injury (Khalil, Kumar, & Nemer, 2020).

Several strategies have been introduced for drug repurposing, of which high-throughput (HTS) and virtual (in silico) ligand screening (VLS) appear most popular. Both methodologies can be used in parallel to increase the chance of positive outcomes in the repurposing process. Using these approaches, already approved drugs can be identified directly or lead compounds similar in structure to the original drug can be further modified and optimized for the new target (purpose) (Doan, Pollastri, Walters, & Georg, 2011).

HTS of known drugs has several advantages: it can be automated, employ different types of assays to confirm the observed effect(s) on the desired targets, and obtained data can be interpreted, clustered, and thoroughly analyzed using various available software programs (Attene-Ramos, Austin, & Xia, 2014; Zhong, Guo, & Che, 2015). However, HTS of approved drugs for repurposing can still be costly and time-consuming depending on the costs and availability of in vitro assay components as well as the time required to conduct the experiments. The National Institutes of Health–National Center for Advancing Translational Sciences (NIH NCATS) have generated a publicly available library of approved and new investigational drugs for HTS purposes (Mullard, 2012). In addition, many other profit and non-profit initiatives can provide researchers with clinically approved drugs for HTS as well (S. G. Clark et al., 2018; Wilkinson & Pritchard, 2015).

The identification of new antiviral therapies is a well-known example of the potential of HTS. In 2014, a library of 348 FDA-approved drugs was screened against SARS-CoV and MERS-CoV, which rendered four compounds (chloroquine, chlorpromazine, loperamide and lopinavir) with effective inhibitory actions (in the micromolar range) on MERS- and SARS-CoV (de Wilde et al., 2014). Similarly, several therapeutic candidates for the treatment of Ebola were discovered utilizing HTS on a library of FDA-approved drugs (Johansen et al., 2015; Kouznetsova et al., 2014; Vincent et al., 2005). Even though only some of the above-mentioned findings were validated in pre-clinical and/or clinical studies, they clearly demonstrate the potential of HTS to identify novel indications for pre-existing FDA-approved drugs. Furthermore, these HTS data provided a strong foundation for currently ongoing SARS-CoV2 research (Oscanoa, Romero-Ortuno, Carvajal, & Savarino, 2020).

In silico screening applies computational methods, such as network analysis (Advani & Kumar, 2021) and VLS, or structure/scaffold-based drug screenings to identify known therapeutics or specific drug part(s) that bind to the target of interest, respectively. Compared to HTS, these methods are innovative, highly automated, less expensive, but also more theoretical in nature.

VLS typically consists of performing docking experiments using a compound database (e.g., FDA-approved library) to identify binding pockets or the catalytic sites of a protein/enzyme of interest and rank them based on binding energy (usually expressed as a docking score), and suggest the best candidates for the next phase, usually in vitro testing (Kontoyianni, 2017). Thus, the general purpose of VLS and in silico experiments is to decrease the time and cost of the drug repurposing process by reducing the number of molecules moving to the HTS phase (Sohraby, Bagheri, & Aryanpour, 2019). Due to an unmet need for treatment for many conditions as well as the complexity of multifactorial diseases such as AD, the modern drug discovery process is adapting and moving from the traditional target-based strategy toward polypharmacology and designed multiple ligands (DML) approaches (Murphy & Rankovic, 2005; Proschak, Stark, & Merk, 2019; S. Wilt et al., 2020; Stephanie Wilt et al., 2021). These are innovative and unique and have been designed to identify a single drug acting on multiple targets in a disease pathway of interest. Interestingly, from a traditional drug screening perspective, these so-called “off-target” effects were considered as the source of drug toxicity and adverse effects. The main aim of a polypharmacological approach is the discovery of unknown “off-targets” for existing drugs that will yield desirable treatment strategies for indications other than those for which the drugs were initially developed (Chaudhari, Tan, & Zhang, 2017; Santos, Chand, & Chaves, 2016). The polypharmacology approach strongly depends on multidisciplinary scientific sources and requires collecting and processing of data from preclinical and clinical work, medicinal and synthetic chemistry, in vitro/in vivo pharmacological testing, in silico screening, and molecular modeling.

Currently, only a few clinical trials on potential new therapeutics for AD are being conducted in comparison to those for other chronic conditions such as cancers (Ballard & Murphy, 2018). The development of effective treatments is hindered even further by the high failure rate of clinical trials for AD (Hawkes, 2018).

Autophagy research has received a lot of interest over recent years (Dikic & Elazar, 2018; Graef, 2020; L. Yu, 2020). This is not surprising considering autophagy has been implicated in fundamental pathophysiological processes of various human diseases, including cancer, infectious diseases, and AD (Essick & Sam, 2010; P. Jiang & Mizushima, 2014; Levine & Kroemer, 2008). In the following section, we will provide a detailed evaluation of drugs currently repurposed as autophagy modulators for AD.

#### 3.2. Anticancer drugs for repositioning as autophagy modulators in AD

Anticancer drugs constitute a very important group of therapeutics in modern medicine, and there is a long and diverse list of compounds that have been approved for the treatment of human malignancies. Several anticancer drug classifications exist, of which we considered the one provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) very useful and comprehensive (Table 1) (LiverTox., 2012).
Most chemotherapy agents induce autophagy and, depending on the extent of induction, this may either contribute to the death of cancer cells or, in most of the cases, enhance their survival and promote chemoresistance (Behrouj, Seghatoeslam, Mokarram, & Ghavami, 2021; Moghadam et al., 2018; Samiei, Seyfoori, Toyota, Ghavami, & Akbari, 2020; S. Shojaei et al., 2020a; Stefanek et al., 2021). Indeed, the induction of autophagy in response to anticancer drugs (e.g., alkylating agents) might contribute to chemoresistance in the long-term (Bordin et al., 2013; Cordani & Somoza, 2019; Shahla Shojaei, Suresh, et al., 2020b). When evaluating the repurposing of anticancer agents for the induction of autophagy in the context of AD, it is important to consider that these agents target crucial subcellular compartments (e.g., the genome or mitochondria) and disable fundamental biological processes such as gene expression and energy production. For example, alkylating agents inflict a wide range of DNA damage within the cells (Hurley, 2002). This is of relevance, as DNA damage has been reported in several neurodegenerative disorders and activation of its downstream signaling, including the p53 pathway, contributes to the neurodegenerative

<table>
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<th>Table 1 Classification of anticancer drugs.¹</th>
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<td><strong>Pharmaceutical family</strong></td>
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<td><strong>Alkylating Agents</strong></td>
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<td><strong>Miscellaneous</strong></td>
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* Source of the Table: NIH LiverTox – produced by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK).
process (J. R. Chang et al., 2012; Madabhushi, Pan, & Tsai, 2014; Pessina et al., 2021; Szybiszka & Leśniak, 2017). Antibiotics, such as dactinomycin and doxorubicin, are another class of anticancer drugs that exhibit cytotoxic effects against cancer cells through their anti proliferative and pro-apoptotic properties (Y. Gao, Shang, et al., 2020b). Although these anticancer agents have been reported to induce autophagy in targeted cells, associated (severe) toxic (cardiotoxicity) and detrimental effects on the normal human microvilli deem them unsuitable for AD treatment (Y. Gao, Shang, et al., 2020b; Koleini & Kardani, 2017). For this review, our criteria for selecting and discussing which anticancer agents might be of interest for repurposing as autophagy enhancers in AD included: 1) direct effect(s) on autophagy or its direct regulators, and 2) minimal toxicity against non-targeted cellular compartments and biologic processes.

3.2.2.1. Rapamycin.

The “protein kinase inhibitors” class of anticancer drugs meets the abovementioned criteria to be considered for repurposing for autophagy modulation in AD. Regulation of the autophagy process involves a multitude of protein kinases and targeting them is becoming a popular approach for the treatment of different human diseases, including cancers (Xiang et al., 2020). Examples of kinases that regulate autophagy include, but are not limited to, mTOR, AMP-dependent protein kinase (AMPK), Abelson murine leukemia viral oncogene homolog 1 (ABL1), PI3K, alpha serine/threonine-protein kinase (Akt), several members of mitogen-activated protein kinase (MAPK) family, protein kinase C (PKC), and death associated protein kinase (DAPK) (Sridharan, Jain, & Basu, 2011). A more comprehensive list is depicted in the Table 2.

Most of these kinases are associated with tumorigenesis and tumor metastasis. As a result, several small molecule libraries have been developed to facilitate (specific) targeting and implement effective and safe therapeutic interventions in different types of human malignancies (Berndt, Karim, & Schönbroun, 2017). However, a big barrier for repurposing such compounds for the treatment of AD is their poor penetration through the blood–brain barrier (BBB) (Benn & Dawson, 2020). This issue will be discussed as part of the following sections on individual therapeutics.

3.2.2.2. Inhibitors of mammalian target of rapamycin (mTOR)

mTOR is a highly conserved serine/threonine kinase and an essential component of two multiprotein complex structures, mTORC1 and 2, both of which are involved in cell metabolism, growth, and maintenance. mTOR is one of the main intracellular inhibitors of autophagy and its inhibition results in an increase in autophagy flux and catabolism of proteins and organelles (Johnson, Sangesland, Kaerberlein, & Rabinovitch, 2015). On the other hand, increased activity of mTOR leads to the accumulation of damaged proteins and organelles and is linked to aging (Nixon & Yang, 2012). Accordingly, increased mTOR activity has been reported in several NDDs. For example, mTOR plays important pathogenic roles in AD by inhibiting the autophagic degradation of Aβ and phospho-tau (ptau) aggregates. Importantly, several studies have shown that mTOR inhibition leads to increased autophagic flux and alleviates the severity of several neurodegenerative disorders such as AD (Aricò, Danesino, Pende, & Moretta, 2001; Feng et al., 2005; M. Perluigi, Di Domenico, & Butterfield, 2015; Querfurth & Lee, 2021; Talboom, Velazquez, & Oddo, 2015).

3.2.2.2.1. Rapamycin. Rapamycin is a natural product that was initially identified as an antifungal metabolite produced by Streptomyces hygroscopicus from a soil sample (Arriola Apelo & Lamming, 2016). Rapamycin also shows immunosuppressive properties and has been approved for patients with kidney transplants. Later on, rapamycin was found to exhibit antitumor effects as evidenced by its ability to suppress the expansion of cancer cells in numerous solid cancers (e.g., liver, gastric, bladder, breast, prostate, non-small cell, lung, and ovarian cancers) (Hua et al., 2019).

Rapamycin binds to the intracellular FK506 binding protein 12 (FKBP12) and this FKBP12-rapamycin complex subsequently inhibits the activity of mTORC1 (Fig. 3) (Chiu, Katz, & Berlin, 1994) (Lamming, 2016). This likely represents the mechanism through which rapamycin induces autophagy in cells. Chronic treatment with rapamycin can also inhibit mTORC2.

Beneficial effects of rapamycin have been demonstrated in several AD models. As indicated, NFTs and amyloid plaques are two major pathological hallmarks of AD (Selkoe, 2001). Administration of rapamycin has been shown to reduce the amount of both and improve cognitive function in several animal models of AD. Indeed, long-term administration of rapamycin reduced the levels of Aβ42 peptide and rescued cognitive deficits in a mouse model of AD (Spilman et al., 2010). Also, prophylactic administration of rapamycin significantly reduced Aβ plaques and NFTs and prevented cognitive deficits in an AD transgenic mouse (Majumder, Richardson, Strong, & Oddo, 2011) (Fig. 5). In a murine model of pure tauopathy, Ozcelik et al. studied the effects of short- and long-term rapamycin treatment regimens and observed a significant reduction in the numbers of cortical tau aggregates and lower levels of hyper-phosphorylated tau as well as an increased abundance of the autophagy-associated proteins p62 and LC3 in the cortex (Ozcelik et al., 2013). Furthermore, systemic rapamycin treatment of mice using a viral vector-based model of AD slowed down the progression of AD by alleviating tau-mediated neurotoxicity (Siman, Cocca, & Dong, 2015).

One of the problems of using rapamycin for neurological disorders is its relatively poor penetration across the BBB. The levels of rapamycin within the brain are exponentially correlated with its levels in the blood; thus, high doses of rapamycin need to be administered systematically to achieve therapeutic levels of rapamycin within the brain (Banerjee, Gianino, Gao, Christians, & Gutmann, 2011). Accordingly, definitive mTORC1 inhibition could not be observed following intraperitoneal (IP) injections of rapamycin (Banerjee et al., 2011).

Overall, systemic administration of (high levels of) rapamycin appears to induce autophagy in the brain, inhibit pathologic mechanisms underlying neurodegeneration, and alleviate the phenotypes accompanying AD; however, further studies are undergoing to study its efficacy in clinical settings.

3.2.2.2.2. Temsirolimus. As mentioned above, rapamycin has less than ideal pharmacokinetic properties and, therefore, several rapamycin analogs were designed and evaluated for better (local) bioavailability. Temsirolimus (3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate) is a soluble ester of rapamycin and represents a second generation mTOR inhibitor (Amato, Stepankiw, & Ochuka, 2014; Schollar, 2009). Temsirolimus exhibits better anticancer properties and is approved by the FDA for the treatment of advanced renal cell carcinoma (RCC) (Hudes, 2009; Maisere, Chong, Shang, & Wang, 2013; Marzia Perluigi et al., 2015). The mechanism of mTOR inhibition by temsirolimus is similar to rapamycin: after formation of the drug-FKBP12 complex, binding to mTOR via the piperidine region of the macrolide promotes dimerization and inactivation of mTOR (Fig. 3).

Temsirolimus induces Aβ phagy flux both in in vitro and in vivo models of AD (T. Jiang et al., 2014). To further investigate the effects of temsirolimus on autophagy, the authors used an APP transgenic mouse model of AD and showed that treatment with temsirolimus for 60 days significantly promoted clearance of Aβ (indicated by reduced levels Aβ40 and Aβ42) through autophagy in the brain. In addition, an improvement in spatial cognitive functions was observed in the AD mice (T. Jiang et al., 2014). Based on these studies, chronic treatment with the polar rapamycin analog temsirolimus appears promising for the treatment of AD as it promotes autophagic Aβ clearance and reduces NFTs density leading to significant improvement in cognitive function.

3.2.2.2.3. Dactolisib (NVP-BEZ235). The imidazoquinoline derivative dactolisib is a dual PI3K/mTOR inhibitor that induces G(1) arrest in
Table 2
The protein kinase inhibitor class of anticancer drugs and their effects on autophagy.

<table>
<thead>
<tr>
<th>Name</th>
<th>Mechanism of action</th>
<th>Effect on autophagy</th>
<th>Disease model</th>
<th>Experimental model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abemaciclib</td>
<td>CDK4/6 inhibitor</td>
<td>↑</td>
<td>myeloma</td>
<td>In vitro; myeloma cell lines</td>
<td>[Iriyama et al., 2018]</td>
</tr>
<tr>
<td>Acalabrutinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>Not investigated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afinatinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Head and neck squamous cell carcinoma (HNSCC)</td>
<td>In vitro; HNSCC cells</td>
<td>(X. Liu et al., 2021)</td>
</tr>
<tr>
<td>Alectinib</td>
<td>ALK inhibitor</td>
<td>↑</td>
<td>Lung cancer</td>
<td>In vitro; H1322 and H2228 EML4-ALK+ lung cancer</td>
<td>(Schläfli et al., 2021)</td>
</tr>
<tr>
<td>Axitinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>Not investigated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binimetinib</td>
<td>MAPKs inhibitor</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Proteasome inhibitor</td>
<td>↑</td>
<td>Breast cancer</td>
<td>In vitro; estrogen receptor positive (ER+) breast cancer cell</td>
<td>(Periyasamy-Thandavan et al., 2010)</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Alzheimer's Disease</td>
<td>In vivo; mouse model of AD</td>
<td>(L. Lonskaya, Hebron, Desforges, Franjie, &amp; Moussa, 2013a)</td>
</tr>
<tr>
<td>Brigatinib</td>
<td>ALK inhibitor</td>
<td>↑</td>
<td>Colorectal cancer (CRC)</td>
<td>In vitro; CRC cell line</td>
<td>(Z. Zhang et al., 2019b)</td>
</tr>
<tr>
<td>Cabozantinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Metastatic colorectal cancer (mCRC)</td>
<td>In vivo; xenograft mouse model of mCRC</td>
<td>(Scott et al., 2018)</td>
</tr>
<tr>
<td>Carfilzomib</td>
<td>Proteasome inhibitor</td>
<td>↑</td>
<td>Head and neck squamous cell carcinoma (HNSCC)</td>
<td>In vitro; HNSCC cell line</td>
<td>(Zang et al., 2012)</td>
</tr>
<tr>
<td>Ceritinib</td>
<td>ALK inhibitor</td>
<td>↓</td>
<td>Pancreatic cancer (PC)</td>
<td>In vitro; PC cell line</td>
<td>(Jamsheed et al., 2020)</td>
</tr>
<tr>
<td>Cobimetinib</td>
<td>MAPKs inhibitor</td>
<td>↑</td>
<td>Pancreatic ductal adeno carcinoma (PDA) chaperone-mediated autophagy (CMA) evaluation</td>
<td>In vitro; xenograft mouse model of PDA</td>
<td>(Kinsey et al., 2019)</td>
</tr>
<tr>
<td>Copanlisib</td>
<td>PI3K inhibitor</td>
<td>↑</td>
<td></td>
<td>In vitro and in vivo; NIHST3 cells, AML12 cells, mMCD3 cells and mouse</td>
<td>(Endicott, Zieme, Beckmann, Boynton Jr., &amp; Miller, 2020)</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Lung cancer</td>
<td>In vitro; xenograft mouse model of lung cancer</td>
<td>(You et al., 2015)</td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>RAF inhibitor</td>
<td>↑</td>
<td>Melanoma</td>
<td>In vitro; human melanoma cell lines A375 and ME624</td>
<td>(C. Ji et al., 2016)</td>
</tr>
<tr>
<td>Dacominib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Ovarian cancer</td>
<td>In vitro; ovarian cancer cells</td>
<td>(Xu et al., 2016)</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Ovarian cancer</td>
<td>In vitro; ovarian cancer cells</td>
<td>(Spilman et al., 2010)</td>
</tr>
<tr>
<td>Duvelisib</td>
<td>PI3K inhibitor</td>
<td>↑</td>
<td>B-acute lymphoblastic leukemia (B-ALL)</td>
<td>In vitro; Philadelphia chromosome-positive B-ALL cell lines</td>
<td>(Ultimo et al., 2017)</td>
</tr>
<tr>
<td>Enasidenib</td>
<td>IDH2 inhibitor</td>
<td>↑</td>
<td>Acute Myeloid Leukemia (AML)</td>
<td>In vitro; AML cells</td>
<td>(Y. Kim et al., 2020)</td>
</tr>
<tr>
<td>Encorafenib</td>
<td>RAF inhibitor</td>
<td>↑</td>
<td>Melanoma</td>
<td>In vitro; BRAFV600E melanoma cells</td>
<td>(Z. Li et al., 2016)</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Non-small cell lung cancer (NSCLC)</td>
<td>In vitro; NSCLC cell lines (HCC827, HCC4006, H358 and H1975)</td>
<td>(Y. Y. Li, Lam, Mak, Zheng, &amp; Ho, 2013)</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Lung cancer</td>
<td>In vitro; patient derived lung cancer cells</td>
<td>(Zhou, Yu, Li, &amp; Ouany, 2016)</td>
</tr>
<tr>
<td>Giltertinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>Unknown</td>
<td>Glioblastoma</td>
<td>In vitro; patient derived glioblastoma cells</td>
<td>(Zdazlik-Bielecka et al., 2022)</td>
</tr>
<tr>
<td>Glasdegib</td>
<td>SHI inhibitor</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibrutinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Glioblastoma</td>
<td>In vitro; LN229 and U87 cells</td>
<td>(J. Wang et al., 2017b)</td>
</tr>
<tr>
<td>Idelalisib</td>
<td>PI3K inhibitor</td>
<td>↑</td>
<td>Acute lymphoblastic leukemia (ALL)</td>
<td>In vitro; pre-B acute lymphoblastic leukemia cell lines</td>
<td>(Sheikh-Zeineddini et al., 2019)</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Chronic myelogenous leukemia (CML)</td>
<td>In vitro; CML cells</td>
<td>(Ertmer et al., 2007)</td>
</tr>
<tr>
<td>Ivosidenib</td>
<td>IDH2 inhibitor</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ixazomib</td>
<td>Proteasome inhibitor.</td>
<td>↑</td>
<td>colorectal cancer (CRC)</td>
<td>In vivo; xenograft mouse model of CRC</td>
<td>(Yue &amp; Sun, 2019)</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>CML</td>
<td>In vitro; K562 cells</td>
<td>(H. L. Huang et al., 2011)</td>
</tr>
<tr>
<td>Larotrectinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>Not investigated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levnavatinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Gallbladder cancer (GBC)</td>
<td>In vitro; xenograft mouse model of CGB</td>
<td>(Ye et al., 2021)</td>
</tr>
<tr>
<td>Lorlatinib</td>
<td>ALK inhibitor</td>
<td>↑</td>
<td>Lung cancer</td>
<td>In vitro; H1322 and H2228 cells</td>
<td>(C. Lu et al., 2019)</td>
</tr>
<tr>
<td>Midostaurin</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neratinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>pancreatic cancer</td>
<td>In vitro; pancreatic cancer cells</td>
<td>(Dent et al., 2020)</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>↑</td>
<td>Hepatocellular carcinoma (HCC)</td>
<td>In vivo; xenograft mouse model of HCC</td>
<td>(H.-C. Yu et al., 2013)</td>
</tr>
<tr>
<td>Niraparib</td>
<td>PARP inhibitor</td>
<td>↑</td>
<td>Ovarian cancers</td>
<td>In vivo; xenograft mouse model of ovarian cancer</td>
<td>(Santiago-O’Farrill et al., 2020)</td>
</tr>
<tr>
<td>Olaparib</td>
<td>PARP inhibitor</td>
<td>↑</td>
<td>Breast cancer</td>
<td>In vitro; breast cancer cell lines</td>
<td>(Arun, Akar, Gutierrez-Barrera, Hortobagyi, &amp; Ospolat, 2015)</td>
</tr>
</tbody>
</table>

(continued on next page)
Thus, TK inhibitors could represent safe and effective tools in the fight against neurodegenerative conditions. Indeed, a few are already under investigation for the treatment of AD (see below). Importantly, a long list of TK inhibitors approved for other clinical purposes (i.e., human malignancies) is publicly available (see Table 2), which provides scientists with a unique opportunity to consider the repositioning of these drugs as autophagy modulators for the treatment of AD.

Masitinib is a potent inhibitor of several protein TKs, including the stem-cell factor receptor (c-kit) (Dubreuil et al., 2009). Masitinib has been approved as an anti-cancer drug since 2008 (Hahn et al., 2008). Preclinical studies reported some beneficial effects of masitinib in animal models of AD (T. Li et al., 2020a). Further investigations are required to determine how these effects are related to autophagy induction by this compound. Currently, it is under clinical investigation for the treatment of AD (ClinicalTrials.gov Identifier: NCT00976118) (alzforum.org, 2021e).

<table>
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<tr>
<th>Name</th>
<th>Mechanism of action</th>
<th>Effect on autophagy</th>
<th>Disease model</th>
<th>Experimental model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osimertinib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>Non-small cell lung cancer (NSCLC)</td>
<td>In vitro; NSCLC cells</td>
<td>(Z. H. Tang, Cao, Su, Chen, &amp; Lu, 2017)</td>
</tr>
<tr>
<td>Palbociclib</td>
<td>CDK4/6 inhibitor</td>
<td>↑</td>
<td>Gastric cancer</td>
<td>In vitro; gastric cancer cells</td>
<td>(Valenzuela, Vargas, Martinez, Bravo, &amp; Brown, 2017)</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>Bladder cancer (BC)</td>
<td>In vitro; BC cell lines</td>
<td>(Santoni et al., 2013)</td>
</tr>
<tr>
<td>Pexidartinib</td>
<td>Tyrosine kinase</td>
<td>Not investigated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ponatinib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>Chronic Myeloid Leukemia (CML)</td>
<td>In vitro; imatinib-resistant CML and leukemia stem cells (LSC)</td>
<td>(Kayabasi et al., 2017)</td>
</tr>
<tr>
<td>Regorafenib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>Hepatocellular carcinoma</td>
<td>In vitro; HepG2 and Hep3B cells</td>
<td>(Han &amp; Li, 2018)</td>
</tr>
<tr>
<td>Ribociclib</td>
<td>CDK4/6 inhibitor</td>
<td>Not investigated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rucaparib</td>
<td>PARP inhibitor</td>
<td>↑</td>
<td>Ovarian cancer</td>
<td>In vivo; xenograft mouse model of ovarian cancer</td>
<td>(Santiago-O'Farrell et al., 2020)</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>JAK inhibitor</td>
<td>↑</td>
<td>Multiple myeloma</td>
<td>In vitro; ARH-77 multiple myeloma cell line</td>
<td>(Kusoglu, Bagca, Ay, Saydam, &amp; Avci, 2020)</td>
</tr>
<tr>
<td>Selumetinib</td>
<td>MAPKs inhibitor</td>
<td>↑</td>
<td>Colorectal cancer (CRC)</td>
<td>In vitro; colorectal cancer (CRC) cells</td>
<td>(Grasso et al., 2016)</td>
</tr>
<tr>
<td>Sonidegib</td>
<td>SHH inhibitor</td>
<td>↑</td>
<td>BCR-ABL-positive chronic myeloid leukemia</td>
<td>In vitro; CML cells</td>
<td>(X. Zeng et al., 2015)</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>Hepatocellular carcinoma</td>
<td>In vitro; HCC cells</td>
<td>(T. Sun, Liu, &amp; Ming, 2017)</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>Glioblastoma (GBM)</td>
<td>In vitro; LN229 and U87 cells</td>
<td>(B. Wang, Lu, Xuan, &amp; Hu, 2017a)</td>
</tr>
<tr>
<td>Talazoparib</td>
<td>PARP inhibitor</td>
<td>↑</td>
<td>Ovarian cancer</td>
<td>In vivo; xenograft mouse model of ovarian cancer</td>
<td>(Santiago-O'Farrell et al., 2020)</td>
</tr>
<tr>
<td>Trametinib</td>
<td>MAPKs inhibitor</td>
<td>↑</td>
<td>Pancreatic ductal adenocarcinoma (PDA)</td>
<td>In vivo; xenograft mouse model of PDA</td>
<td>(Kinsey et al., 2019)</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>Tyrosine kinase</td>
<td>↑</td>
<td>non-small cell lung cancer (NSCLC)</td>
<td>In vitro; NSCLC cell line Calu-6</td>
<td>(Y. Zhou et al., 2015)</td>
</tr>
<tr>
<td>Vemurafenib</td>
<td>RAF inhibitor</td>
<td>↑</td>
<td>Thyroid cancer</td>
<td>In vitro; thyroid cancer cells</td>
<td>(Run et al., 2021)</td>
</tr>
<tr>
<td>Vismodegib</td>
<td>SHH inhibitor</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zanubrutinib</td>
<td>Tyrosine kinase</td>
<td>N/A</td>
<td>Idiopathic pulmonary fibrosis (IPF)</td>
<td>In vivo; mouse model of pulmonary fibrosis</td>
<td>(X. Li et al., 2021)</td>
</tr>
</tbody>
</table>

Thus, TK inhibitors could represent safe and effective tools in the fight against neurodegenerative conditions. Indeed, a few are already under investigation for the treatment of AD (see below). Importantly, a long list of TK inhibitors approved for other clinical purposes (i.e., human malignancies) is publicly available (see Table 2), which provides scientists with a unique opportunity to consider the repositioning of these drugs as autophagy modulators for the treatment of AD.

Masitinib is a potent inhibitor of several protein TKs, including the stem-cell factor receptor (c-kit) (Dubreuil et al., 2009). Masitinib has been approved as an anti-cancer drug since 2008 (Hahn et al., 2008). Preclinical studies reported some beneficial effects of masitinib in animal models of AD (T. Li et al., 2020a). Further investigations are required to determine how these effects are related to autophagy induction by this compound. Currently, it is under clinical investigation for the treatment of AD (ClinicalTrials.gov Identifier: NCT00976118) (alzforum.org, 2021e).
developed and approved as anticancer agents, including imatinib, nilotinib, dasatinib, bosutinib, ponatinib and radotinib, of which only nilotinib, dasatinib and bosutinib demonstrate a relatively effective brain penetration (Lindholm et al., 2016). c-Abl plays important roles in the development and function of the CNS (Perez de Arce et al., 2010). It has been reported that aberrant c-Abl activation in the brain results in neuroinflammation and degeneration (Schlatterer, Tremblay, Acker, & Davies, 2011b). Accordingly, c-Abl is upregulated in AD and inhibition of c-Abl might therefore represent a promising strategy for the treatment of this disease (Lindholm et al., 2016; Schlatterer, Acker, & Davies, 2011a). Currently, the three c-Abl inhibitors with acceptable BBB penetrating properties (nilotinib, dasatinib and bosutinib) are in Phase 2 clinical trials for the treatment of several neurodegenerative disorders including AD (Alzforum.org, 2021a, 2021b, 2021g). Repurposing of c-Abl inhibitors for the treatment of AD conditions is an interesting and promising strategy, which we will discuss in more detail below.

3.2.4.1. Imatinib. Imatinib (STI571 or Gleevec) is a c-Abl TK inhibitor originally developed to treat Philadelphia-chromosome-positive chronic myeloid leukemia (Cohen, Cross, & Jänne, 2021; Joensuu & Dimitrijevic, 2001). In addition to c-Abl, imatinib modulates the activity of several other proteins such as Abl related gene (ARG), platelet-derived growth factor receptor (PDGFR), FMS-like tyrosine kinase 3 (FLT3), lymphocyte-specific protein (W. Zhang, Mehta, Tong, Esser, & Voelcker), MAPK, APP intracellular domain (AICD), αS and c-kit
(Pardanani & Tefferi, 2004). Of particular relevance for this review, it has been reported that imatinib induces autophagy in a wide variety of mammalian cell types in a dose-dependent manner (Ertmer et al., 2007).

The therapeutic efficacy of imatinib was investigated in several neurological pathologies, including AD. Administration of imatinib suppressed the APP conversion to Aβ by inhibiting the interaction of the γ-secretase activating protein (GSAP) and γ-secretase in a mouse model of AD injected with LPS (Weintraub et al., 2013) (Fig. 4). Although these results are promising, the poor penetration through the BBB remains a significant barrier for the use of imatinib for the treatment of AD (Senior, 2003).

3.2.4.2. Nilotinib. Nilotinib (Tasigna®, AMN107) is a novel c-Abl TK inhibitor approved by the FDA for the treatment of patients with CML (Deremer, Ustun, & Natarajan, 2008). The inhibition of other kinases, including MAPK, sterile alpha motif and leucine zipper-containing kinase (ZAK), c-kit, discoidin domain receptor (DDR), and PDGFR, qualifies nilotinib as a potential treatment option for other types of cancer, such as melanoma, gastrointestinal stromal tumors (GIST), and breast cancer (Manley et al., 2010). Nilotinib shows great penetration across the BBB (Hebron, Lonskaya, & Moussa, 2013). In addition, nilotinib improved cognitive functions in animal models of AD (Pagan et al., 2016; Turner et al., 2020).

The activity of c-Abl is significantly increased in post-mortem AD brains (Imam et al., 2011; M. A. Tremblay, Acker, & Davies, 2010) and phosphorylated Abl can be detected in NFTs (Z. Jing, Caltagarone, & Bowser, 2009; S. D. Schlatterer, Acker, & Davies, 2011a). In addition, Abl inhibition has been shown to induce autophagy, clear tau deposits, and improve cognitive functions in several models of AD (Lonskaya, Hebron, Chen, Schachter, & Moussa, 2014a; I. Lonskaya, Hebron, et al., 2013a). Furthermore, c-Abl phosphorylates tau in AD (M. A. Tremblay et al., 2010) and quantitative proteomic analyses revealed a connection between c-Abl activity and phosphorylated tau (M. A. Tremblay et al.,...)

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**Fig. 4. Schematic representation of imatinib mode of action:** Aβ aggregation is reportedly associated with an increase in c-Abl, a ubiquitously expressed nonreceptor tyrosine kinase. c-Abl directly phosphorylates and activates p73 protein, which drives it toward the nucleus. p73 is structurally similar to P53, hence can activate the 53 responsive genes involved in apoptotic cell death. Activated c-Abl can also directly induce tau phosphorylation leading to the occurrence of neurofibrillary tangles, exacerbating Alzheimer’s disease pathology. Imatinib binds to the ATP-binding sites of c-Abl, reducing the p73 and tau phosphorylation preventing cell death. Imatinib can also inhibit the interaction between γ-secretase and the γ-secretase activating protein (GSAP), leading to a decrease in Aβ production.
Although cancer involves uncontrolled cell proliferation and AD is primarily caused by (neuronal) cell loss, the earliest deficits in the pathological progression of both disorders, preceding respective oncogene and Aβ/tau expression, are associated with mitochondrial dysfunction (Adlimoghaddam et al., 2019; Porporato, Filigheddu, Pedro, Kroemer, & Galluzzi, 2018). Recently, we reported significant reductions in brain glucose uptake rates and a decreased activity of the terminal mitochondrial enzyme (COX or Complex IV) in our 3Txg mouse model of AD as compared to controls (Adlimoghaddam et al., 2019). Additionally, we showed that nilotinib enhanced mitochondrial metabolism, improved mitochondrial biogenesis, and increased the activity of COX in brain astroglia in 3Txg-AD mice (Adlimoghaddam, Odero, Glazner, Turner, & Albensi, 2021). These results further support that targeting mitochondrial function may be an effective strategy for AD treatment. Cancer metabolic reprogramming promotes non-apoptotic function, which indicates that the apoptotic pathway is associated with metabolic pathways (Bhat, Kumar, Chaudhary, Yadav, & Chandra, 2015) and that targeting mitochondrial function might benefit cancer therapy as well (Amsalem, Arif, Shteinfer-Kuzmine, Chalifa-Caspi, & Shoshan-Barmatz, 2020). Thus, the common mechanistic link between cancer and AD creates a rationale for repurposing existing anticancer drugs such as nilotinib for AD.

Mitochondrial dysfunction is associated with increased expression of autophagic genes and the elimination of dysfunctional mitochondria via mitophagy. A growing body of experimental evidence in AD research suggests that impaired mitophagy leads to an accumulation of damaged mitochondria that contributes to Aβ/tau pathology and, ultimately, neuronal loss and memory impairment (Adlimoghaddam & Albens, 2021; Eshraghi et al., 2021). In cancer, alterations in mitophagy influence energy production and result in a loss of cellular adaptation and cell death (Chourasia, Boland, & Macleod, 2015). Notably, decreased brain expression is associated with defects in autophagic clearance of Aβ and tau in AD brains, indicating parkin is altered as a function of aging and neurodegeneration (I. Lonskaya, Hebron, et al., 2013a; I. Lonskaya et al., 2013b). In addition, activated parkin mediates mitophagy and clears autophagic vacuoles in AD mice (Geisler et al., 2010; Khandelwal, Herman, Hoe, Rebeck, & Moussa, 2011; I. Lonskaya, Shekoyan, et al., 2013b; Vives-Bauza et al., 2010). Interestingly, c-Abl contributes to the inhibition of activated parkin; therefore, inhibition of c-Abl may result in the upregulation of parkin-dependent mitophagy (Imam et al., 2011).

The solubility of parkin is significantly decreased in AD which leads to parkin instability and reduced parkin–BECN1 interactions. In AD brains, nilotinib boosted the autophagic machinery, which increased the level of parkin and functional parkin–BECN1 interaction activity. Such an enhanced functional interaction between parkin and BECN1 in response to autophagic changes improves Aβ clearance and cognitive function (I. Lonskaya, Hebron, et al., 2013a; Lonskaya, Hebron, Desorges, Schachter, & Moussa, 2014b).

Protein phosphatase 2 (PP2A)-AMPK/autophagy signaling has been explored as a novel therapeutic target of nilotinib. Thus, nilotinib-induced autophagic cell death is a result of AMPK activation and PP2A deactivation in hepatocellular carcinoma (HCC) cell lines (H. C. Yu et al., 2013). Other studies in HCC cells revealed that nilotinib treatment diminished microtubule-associated protein 1A/1B LC3 cleavage when AMPK was knocked out and enhanced LC3 cleavage in the presence of an AMPK activator (H. C. Yu et al., 2013), suggesting that AMPK is a critical trigger of autophagy in nilotinib-treated cells. Moreover, nilotinib induced autophagic cell death in an in vivo xenograft tumor model, which led to a significant reduction in PLC5 tumor size (H. C. Yu et al., 2013). Collectively, these results strongly indicate that nilotinib triggers autophagic cell death by activation of AMPK.

Currently, nilotinib is subject to several Phase 2 clinical trials for AD (Alzforum.org, 2021g). Indeed, there is strong evidence to suggest that nilotinib represents a promising therapeutic for AD.

### 3.2.4.3. Bosutinib

Bosutinib is a drug from the C-Abl Inhibitor family as an effective treatment against chronic myeloid leukemia (CML) was approved by the FDA in 2012. The mechanism of action of this drug in the case of CML cancer is that Bosutinib can be effective by inhibiting the kinases activity of Bcr-Abl chimERIC protein. Also, this drug can have an inhibitory effect on the kinases activity of the Src family and thus prevent cancer cell proliferation (Cortes et al., 2012).

This drug can be effective in curing NDDs, especially AD. Research results show that Bosutinib can cause increase autophagy and Aβ peptides clearance by enhance the interaction of beclin-1 and parkin. Actually pre-clinical animal models and others have shown benefits of Bosutinib on inflammation and neurotropic protein clearance in neurodegeneration. However, to determine the ability of this drug in the treatment of AD based on autophase stimulation, further studies are needed to differ-ent phases of clinical trial (Advani et al., 2020). As mentioned earlier, another advantage of this drug is its permeability to the CNS, which can effectively run Bosutinib for the treatment of AD.

### 3.2.5. Taxanes

Taxanes are a family of natural products that target and stabilize microtubules (MTs) and are approved for the treatment of human malignancies due to their antimitotic properties (Abal et al., 2003). MTs are one of the major components of the cytoskeleton and play crucial roles in the maintenance of neuronal shape and axonal transport (GM, 2000). MTs importantly contribute to autophagy flux in neurons by facilitating the formation of autophagosomes, the transport of autophagosomes into the axons, the maturation of autophagosome, and the formation of autolysosomes (Köchl, Hu, Chan, & Tooze, 2006; Mackeh et al., 2013). The dynamics of MTs are profoundly affected in AD and this contributes hugely to the impaired autophagy seen in AD (Kurt R. Brunden, Lee, Smith 3rd, Trojanowski, & Ballatore, 2017). In AD, hyperphosphorylation of axonal tau (a MT stabilizer protein) results in aggregation of tau molecules, destabilization of axonal MTs, and consequently defective autophagosome transport and maturation (Kurt R. Brunden et al., 2017; Cash et al., 2003). Indeed, accumulation of immature autophagic vacuoles is one of the main (but neglected) pathological features reported in AD (Wolfe et al., 2013).

Several studies have shown that several members of the taxane family (including cabazitaxel, docetaxel and paclitaxel) have pro-autophagic, anticancer properties and enhance autophagy flux in treated cancer cells (Cristofani et al., 2018; F. Hu et al., 2018a; R. Huo et al., 2016; Zamora et al., 2019). For example, cabazitaxel induced autophagy in a lung cancer cell line through activation of PI3K/Akt/mTOR pathway (Ruiachao Huo et al., 2016).

Paclitaxel is approved as an anticancer agent; it induces tumor cell death by enhancing tubulin polymerization and mitosis arrest (Mekhail & Markman, 2002). Results of in vitro studies indicated that paclitaxel protects neurons from Aβ toxicity (Michaelis et al., 1998), reduces tau hyperphosphorylation, and prevents activation of cdk5 induced by Aβ (G. Li et al., 2003). Paclitaxel has been reported to induce autophagy in cancer cells (Xie et al., 2021); however, it exhibits very poor BBB penetration (Fellner et al., 2002) which would limit its applicability as an autophagy enhancer for the treatment of AD.

TPI 287 is an abeotaxane, a synthetic derivative of the taxane diterpenoid drugs, which has mainly been investigated for the treatment of human malignancies and in particular brain tumors because of its effective penetration through the BBB (Fitzgerald et al., 2012; S. Tremblay, Soucy, Towers, Cunnion, & Breau, 2004). Currently, TPI 287 is under clinical investigation for treatment of AD (alzforum.org, 2021h). To the best of our knowledge, no reports are available on the
potential effects of TPI 287 on autophagy flux. However, based on its class of action, we predict that it is a potent inducer of autophagy and might be of therapeutic values for neurodegenerative disorders.

Epothilone D is a non-taxane MT-stabilizing agent that is derived from myxobacterium Sorangium cellulosum. As for taxanes, this compound has anticancer properties through inhibition of mitosis (J. A. Clark, Chuckowree, Dyer, Dickson, & Blizzard, 2020). It exhibits excellent BBB permeability and has been evaluated for the treatment of AD (Kurt R. Brunden et al., 2011; K. R. Brunden et al., 2010; B. Zhang et al., 2012). However, additional studies are required for to validate its clinical efficacy in AD. Mechanistically, epothilone B has been demonstrated to enhance autophagy, albeit only by one study (Jianhua Zhou et al., 2020); therefore, the role of autophagy in the effects of epothilone D remains to be fully elucidated.

3.2.6. Histone deacetylase inhibitors (HDACs)

HDACs are small molecules (either natural or synthetic) which inhibit the function of histone deacetylase (HADC). Currently, several HDACs are approved for treatment of human malignancies (Eckschlager et al., 2017). Also, they have been proposed to be beneficial for the treatment of AD. However, due to the presence of a polar zinc-binding group (ZBG), most HDACIs have poor BBB permeability (Hiranaka et al., 2018).

The acetylation of lysine residues of core histones results in less compact and more available DNA for transcription (i.e., euchromatin). HDAC are enzymes that remove these acetyl groups from core histones and have important roles in the epigenetic regulation of gene expression (Thiagalingam et al., 2003). Targeting HDACs has shown to have therapeutic effects in a variety of human diseases, including malignancies as well as psychiatric, neurological, inflammatory, and infectious diseases (Adcock, 2007; Eckschlager et al., 2017; Elliott et al., 2014; Kazantsev & Thompson, 2008; Surawee, O’Byrne, & Richard, 2018).

Hypoacetylation of core histones has been reported in AD and is believed to play a pathological role in this disease (Saha & Pahan, 2006). Interestingly, emerging evidence indicates that HDACs have beneficial effects in AD by reversing this condition to a hyperacetylated chromatin state (S. Shukla & Tekwani, 2020). In this regard, several studies reported that treatment with specific HDAC6 inhibitors (including tubastatin A and ACY-1215) rescues pathological changes and behavioral deficits in various animal models of AD (Kazantsev & Thompson, 2008; Surabhi Shukla & Tekwani, 2020). Other mechanisms have also been proposed to account for the beneficial effects of HDACs in AD. Acetylation of α-tubulin is one of the posttranslational modifications (PTM) associated with more stable MTs; on the other hand, deacetylation of α-tubulin results in MT instability (L. Li & Yang, 2015). It has been suggested that HDAC6 targets and deacetylates neuronal α-tubulin in several AD (Hubbert et al., 2002). As mentioned, autophagosomes are formed within distal areas of axonal projections and subsequently transported toward soma to fuse with lysosomes. Therefore, the integrity of axonal MTs is a crucial factor for autophagic flux in the neurons. Indeed, retrograde transport of axonal autophagosomes is impaired in AD (Tammineni, Ye, Feng, Aikai, & Cai, 2017). As such, by improving the stability of MTs, HDACs might contribute to improved autophagic flux in neurons. This is supported by the observation that some HDACs induce autophagy in treated cells (Gammoh et al., 2012; J. Zhang et al., 2015). For instance; It is proved that targeted suppression of HDAC6 leads to the induced retrograde flux of LC3-positive vesicles, more somatic neuronal autophagosome–lysosome fusion, higher LC3-II and lower p62 protein amounts in tubastatin A (TBA)-treated neurons which obviously indicated the intensified autophagosomal biosynthesis and productive autophagic removal to promote the autophagic clearing of Aβ and hyperphosphorylated tau (p-tau) oligomers (H. Choi et al., 2017).

Increasing autophagic flux stimulated by class III HDAC SirT1 activation displays a strong preventative role against prion neurotoxic pathways and further implies that this respective control of autophagic cell death can provide a new therapeutic target for the suppression of deposing of plaque-like abnormal prion proteins in infected neurons. Numerous studies on this approach have been suggested that the repression of mTOR signaling alone, or combined with PI3K-Akt-mTOR signaling following ER stress are involved in a transcriptional up-regulation of autophagic indicators like ATG, Beclin-1, and LC3 which initiate through HDACi-induced autophagy (Frohlich, 2017; Y.-L. Liu et al., 2010). In order to assist the autophagy-induced cell death, HDAC class III inhibitors can also modulate ROS accumulations respond to oxidative stress activity and acetylation of the tumor-suppressor TP53 (Makrovic, Kleinehein, & Frohlich, 2017; J. Wang et al., 2012).

Several HDACs are currently under clinical investigation for the treatment of AD; these include nicotinamide (NCT00589931), and vorinostat (NCT03056495).

Nicotinamide is a water-soluble form of vitamin B3 (niacin) and a substrate for the production of NADH that plays crucial roles in cellular metabolism and energy production (Belkomy, Bogan, & Brenner, 2007). It is a selective inhibitor of HDACs and improves mitochondrial quality through enhancing autophagy in human cells (Harrison, Powell, & Dexter, 2019; H. T. Kang & Kwang, 2009).

Nicotinamide inhibits neuropathology and cognitive impairment in a model-AD mouse over a mechanism including enhanced brain mitochondrial bioenergetics and the induced opposing autophagic process of sirtuin pathways. Accordingly, pharmacological normalization of NAD+/NADH rate has been demonstrated to preserve hypospic myocardial cells by positive regulating the autophagy and mitochondrial mass that requests repression of mTOR activity (Cabrillo et al., 2019; Maisse, 2015, 2020). Furthermore, intervention with nicotinamide proceeds the PI3K/Akt efflux in the human epitheloid carcinoma cell line A-431 and prevents the anti-tumoral behavior of TRAIL-mediated colon cancer cell via autophagy flux activation (S.-W. Kim et al., 2016b; H. Sun et al., 2014).

Vorinostat (Suresh, et al.), also known as suberanilohydrylic acid (Saha & Pahan), is an approved medication for cutaneous T cell lymphoma (CTCL) (“Vorinostat,” 2012). It is currently under investigation for repurposing as a therapeutic for several NDDs, including AD (Athira, Sadanandan, & Chakravarty, 2021). Vorinostat has been demonstrated to induce autophagy by inhibiting the deacetylation of key autophagic molecules such as LC3-II (Patra, Praharaj, Klionsky, & Bhutia, 2022). The opportunity to use histone deacetylase inhibitors SAHA specifically in the promotion of cellular autophagy system is yet controversial. For example, SAHA activity implicated in the Tp53 wild type-mediated apoptosis in cancer cells, while the lack or breakdown of cytoplasmic p53 results in stimulated autophagic cell death (Frohlich, Makrovic, Smole, & Zatloukal, 2016; Zhang et al., 2015). It is also recognized that SAHA catalyses the autophagic cell death across the trigger of ER stress (acetylation of GRP78) directed to separation and activation of PERK, supplying an immediate adaptive reaction to ER stress along with the downregulation of Akt/ mTOR pathway. Moreover, simultaneous overexpression of ATG genes following SAHA medication can be caused by deregulated NF-κB p65 signaling in prostate cancer cells (Y.-L. Liu et al., 2010; Shulak et al., 2014). On the contrary, on-study biopsies exhibited an overexpressed aspartic proteolytic activity of lysosomal cathepsin D coexisted with LC3B and p62 signal accumulation, in line with autophagy abolishment. These outcomes were associated with lowered cancer autophagy by Vor plus Hydroxycloroquine (HCQ) in metastatic colorectal cancer (mCRC) (S. P. Arora et al., 2019). The aforementioned contradictions can be attributed to differences in human tumor cells the applied orthotopic models, and targeted Vor dose-response.

Various nanotechnology strategies have been explored to improve Vor penetration through the BBB and decrease its systemic toxicity. These approaches included the use of several types of nanoparticles, such as poly(ethylene glycol)-b-poly(DL-lactic acid) micelles, Vor-pluronic F127 micelles, encapsulated iron complexes of Vor into PEGylated liposomes, human serum albumin bound Vor nanoparticles,
and magnetically guided layer-by-layer assembled nanocarriers, as the carrier for the delivery of VOR to the brain (Athira et al., 2021). We will discuss the application of different types of nanoparticles as drug carriers in section 4.

3.3. Metabolic agents to modulate autophagy in neurodegenerative conditions

Cell metabolism is a complex system consisting of a set of chemical reactions to provide energy and building blocks required for the proper execution of biological processes (Saghir, 2014). Aberrant metabolism is involved in a multitude of chronic health conditions (e.g., diabetes and hyperlipidemia) and many therapeutics have been developed in an attempt to rectify these abnormalities (Tune, Goodwill, Sassoon, & Mather, 2017).

In addition to its housekeeping function of removing redundant and/or harmful intracellular materials, autophagy participates in cell metabolism by recycling macromolecules into energy and nutrient molecules (Rubinszttein, Codogno, & Levine, 2012). Indeed, autophagy constitutes an integrated part of cellular metabolism and plays crucial roles under conditions of metabolic stress (e.g., when cellular energy is low) (Rabinowitz & White, 2010). For example, autophagy is upregulated during starvation and promotes survival by providing essential nutrients to cells (Takeshige, Baba, Tsuboi, Noda, & Ohsumi, 1992). Conversely, defective autophagy has been linked to several metabolic disorders. These classically include lysosomal storage diseases (LSDs) which are caused by primary deficiencies of lysosomal enzymes and lack of autophagosomal maturation. Synthetic forms of these enzymes are currently used as enzyme replacement therapy (ERT) (Platt, Boland, & van der Spoel, 2012) and repositioning of these drugs is under investigation for the treatment of AD (Klein & Mazzulli, 2018).

Given the close relationship between the cellular metabolic state and autophagy induction rate, it is conceivable that autophagy could be manipulated by treating cells with metabolic agents. Inhibitors of sodium-glucose transport protein 2 (SGLT2) are a group of antidiabetic drugs that reduce blood glucose levels by inhibiting renal glucose reabsorption (Anderson & Marts, 2012). SGLT2 inhibitors induce a fasting-like state accompanied by ketogenesis, which results in activation of SIRT1 and AMPK signaling and, consequently, induction of autophagy flux (Packer, 2020). Recently, SGLT2 inhibitors have been proposed as appropriate candidates to be repurposed for the treatment of AD (Esterline, Oscarson, & Burns, 2020).

Several classifications exist for metabolic drugs, of which one stood out specifically for the purpose of this manuscript (Table 3) (Drugs.com, 1986-2021c). Due to space limitations, only some examples of drugs (of the long list of agents approved for human metabolic conditions) are provided; we refer to (Drugs.com, 1986-2021c) for the complete list. Unfortunately, relatively few of these drugs have been evaluated as autophagy inducers or potential candidates for the treatment of AD. Here, we discuss the metabolic agents with known autophagy-inducing effects that are under investigation for the treatment of AD.

3.3.1. Antidiabetic agents

As indicated, the metabolic state of cells importantly affects the rate of autophagy flux. For example, autophagy flux increases dramatically during starvation (catabolic state). This is mainly due to the suppression of mTORC1 (a major inhibitor of autophagy) and upregulation of AMPK (a sensor of low intracellular energy levels and an autophagy inducer) (González, Hall, Lin, & Hardie, 2020). Type II diabetes is a metabolic disorder in which cells do not (adequately) respond to insulin (i.e., insulin resistance), while mTORC1 is over-activated and AMPK activity is diminished (Guillén & Benito, 2018; Ruderman, Carling, Prentki, & Cacicedo, 2013). Under these conditions, autophagy is impaired in most tissues. One of the effects of antidiabetic drugs is to address this issue by improving autophagy flux (Cahova, 2015). Indeed, many antidiabetic agents, such as thiazolidinediones (Cerquetti et al., 2011), SGLT-2 inhibitors (Packer, 2020), metformin (De Santi et al., 2019), and sulfonylureas (Jiali Zhou et al., 2019), exhibit pro-autophagy properties. Several antidiabetic drugs are currently under clinical investigation for the treatment of AD (Table 4). Although the beneficial effects of antidiabetics in AD have thus far been primarily attributed to reversing the “insulin resistance” phenotype reported in AD, their pro-autophagic properties undoubtedly contribute to improving/restoring cellular health by removing undesirable and harmful protein aggregates characteristic of these disorders (Ashrafzadeh, Yaribeygi, Atkin, & Sahebkar, 2019). In this regard, we will discuss the beneficial effects of metformin in AD.

Dimethylbiguanide was initially synthesized from the herb Gelega officinalis. Metformin (1,1-dimethylbiguanide hydrochloride) was introduced as a medication in 1957 by Jean Sterne, and became one of the preferred antidiabetic agents after the United Kingdom Prospective Diabetes Study (UKPDS) highlighted its reduced cardiovascular risk compared to other therapeutics in 1998 (Bailey, 2017). The mechanism of action of metformin is still poorly understood and likely involves a variety of molecular reactions (Rena, Hardie, & Pearson, 2017) (Baur & Birnbaum, 2014; Flory & Lipska, 2019). The mitochondria are the predominant cellular target of metformin. It inhibits complex 1 of the electron transport chain, which eventually leads to an increased AMP/ATP ratio and the activation of AMPK (Fig. 5) (Fauret, Vincent, Poffenberger, & Jones, 2015; Rena et al., 2017). AMPK is one of the main regulators of autophagy and induces autophagy through direct phosphorylation of ULK1 (Kim, Kundu, Viiolet, & Guan, 2011). Metformin has been reported to induce autophagic cell death in several type of cancers (Nazim et al., 2016; Yan Wang et al., 2018; Xiao et al., 2017). Diabetes increases the risk of developing dementia and AD; ~60% of people with diabetes suffer from cognitive impairments (Gudala, Bansal, Schifano, & Bhansali, 2013; W. Li & Huang, 2016). Moreover, diabetes and AD share several pathological (molecular) mechanisms such as insulin resistance, oxidative stress, inflammation, and autophagy dysfunction. Indeed, some investigators have used the term type 3 diabetes to refer to AD in recent years (Gudala, Bansal, Schifano, & Bhansali, 2013; W. Li & Huang, 2016). Metformin improved behavioral phenotypes while decreasing Aβ and tau pathologies in several preclinical models of AD (Craig, Parvez, & Isbøerner, 2019). In addition, metformin might have some beneficial effects in AD patients (Luchsinger et al., 2016). Although not fully understood, metformin is thought to provide neuroprotection by activating AMPK/autophagy pathways (Paudel, Angelopoulou, Piperi, Shaikh, & Othman, 2020). In vivo studies suggest that metformin helps to increase available acetylcholine by reducing degradation by acetylcholinesterase (Cole & Vassar, 2008; Markowitz-Piazecka et al., 2017). Several studies reported negative or neutral effects of metformin on the cognitive performance of older patients with diabetes (Imfeld, Bodmer, Jick, & Meier, 2012; Moore et al., 2013). However, further studies revealed that metformin-induced vitamin B12 deficiency could be the cause of its negative effects on cognition (J. M. Campbell et al., 2018b). A placebo-controlled crossover study performed with high-dose metformin in non-diabetic patients with mild cognitive impairment and early-stage dementia due to AD showed that metformin could be detected in the cerebrospinal fluid (CSF), indicating that metformin crosses the BBB, and that treatment improved executive functioning (Koenig et al., 2017). Overall, these results suggest that metformin treatment can have salutary effects on cognitive functions, even after disease onset. Metformin is currently in Phase 2/3 clinical trials for further evaluation of its clinical potential for the treatment of AD (Alzforum.org, 2021f). Metformin is currently in Phase 2/3 clinical trials for further evaluation of its clinical potential for the treatment of AD (Alzforum.org, 2021f) (Fig. 5).

3.3.2. Antihyperlipidemic agents

The link between dyslipidemia and impaired autophagy is well established. Hypercholesterolemia suppresses autophagy and promotes apoptosis through the mTOR pathway (Yao, Li, & Zeng, 2020). One
Table 3
Classification of metabolic agents.

<table>
<thead>
<tr>
<th>Drug category</th>
<th>Drug Subcategory</th>
<th>Examples</th>
<th>Mechanism of action</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antidiabetic Agents</td>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose, miglitol</td>
<td>This class of drugs blocks reabsorption of filtered glucose in the kidney</td>
<td>(Guerciolini, 1997)</td>
</tr>
<tr>
<td></td>
<td>Amylin analogs</td>
<td>Pramlintide</td>
<td>This class of drugs increases intracellular cyclic AMP (cAMP) leading to insulin release</td>
<td>(Panus et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Dipeptidyl peptidase 4 inhibitors</td>
<td>Alogliptin, linagliptin, saxagliptin, sitagliptin</td>
<td>This class of drugs lower blood glucose by targeting different key receptors (e.g. IGF1) and enzymes (e.g. SGLT-2) within glucose metabolism pathway</td>
<td>(Shorr et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Incretin mimetics</td>
<td>Alfaglutide, dulagludtide, exenatide, liraglutide, lixisenatide</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Panus et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Insulin hormone</td>
<td>Insulin degludec, insulin glargine, insulin detemir,</td>
<td>This class of drugs function by prevention of synthesis and secretion of uric acid</td>
<td>(Panus et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Meglitinides</td>
<td>Nateglinide, repaglinide</td>
<td>This class of drugs act by inhibiting HMGCoA reductase, a key enzyme which regulates intracellular cholesterol synthesis</td>
<td>(Egom &amp; Hafeez, 2016)</td>
</tr>
<tr>
<td></td>
<td>Sulfonylureas</td>
<td>Chlorpropamide, glimepiride, glipizide, glyburide, tolazamide, tolbutamide</td>
<td>This class of drugs act by preventing synthesis and secretion of uric acid</td>
<td>(Bernárdes, Coelho, Araújo, &amp; Saúde-Guimarães, 2019)</td>
</tr>
<tr>
<td></td>
<td>Non-sulfonylureas</td>
<td>Metformin</td>
<td>This class of drugs act by inhibiting mineralization or resorption of the bone</td>
<td>(Menci &amp; Sakai, 2015)</td>
</tr>
<tr>
<td></td>
<td>SGLT-2 inhibitors</td>
<td>Rosiglitazone, pioglitazone</td>
<td>This class of drugs act by inhibiting reabsorption of uric acid at the proximal convoluted tubule.</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Thiazolidinediones</td>
<td>Evolocumab, alirocumab</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td>Antihyperlipidemic Agents</td>
<td>Statins</td>
<td>Rosuvastatin, atorvastatin, pitavastatin, simvastatin, pravastatin, lowastatin, fluvastatin, simvastatin, cerivastatin</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Bile acid sequestrants</td>
<td>Colestevlem, cholestyramine, colestipol</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Cholesterol absorption inhibitors</td>
<td>Ezetimibe</td>
<td>This class of drugs act by inhibiting reabsorption of uric acid at the proximal convoluted tubule.</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Fibric acid derivatives</td>
<td>Fenofibric acid, fenofibrate, gemfibrozil</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>PCSK9 inhibitors</td>
<td>Evolocumab, alirocumab</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Miscellaneous antihyperlipidemic agents</td>
<td>Colchicine, allopurinol, probenecid, sulfinpyrazone</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td>Antigout Agents</td>
<td>Cystic fibrosis Drugs</td>
<td>CFTR potentiators</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Glucose Elevating Agents</td>
<td>–</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Lysosomal Enzymes</td>
<td>–</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Peripheral Acting Anti-obesity Agents</td>
<td>–</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Transthyretin Stabilizers</td>
<td>–</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Urea Cycle Disorder Treatment Agents</td>
<td>–</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td>Bone Resorption Inhibitors</td>
<td>Bisphosphonates</td>
<td>Zoledronic acid, ibandronate, alendronate, risedronate, tiludronate, pamidronate</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Miscellaneous bone resorption inhibitors</td>
<td>Denosumab, gallium nitrate, onasozumab</td>
<td>This class of drugs function by reducing or inhibiting of bile acid uptake (exogenous pathway) or suppressing of endogenous pathways of bile acid synthesis</td>
<td>(Shorr et al., 2007)</td>
</tr>
</tbody>
</table>

Table 4
Anti-diabetic agents repurposed for treatment of Alzheimer’s.

<table>
<thead>
<tr>
<th>Antidiabetic agent</th>
<th>Mechanism of action</th>
<th>Study stage</th>
<th>ClinicalTrials.gov Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapagliflozin</td>
<td>SGLT2 inhibitor; blocks reabsorption of filtered glucose in the kidney</td>
<td>Alzheimer’s (Phase 1/2)</td>
<td>NCT03801642</td>
</tr>
<tr>
<td>Empagliflozin</td>
<td>SGLT2 inhibitor; blocks reabsorption of filtered glucose in the kidney</td>
<td>Alzheimer’s (Phase 1)</td>
<td>NCT05081219</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>Increases intracellular cyclic AMP (cAMP) leading to insulin release</td>
<td>Alzheimer’s (Phase 2)</td>
<td>NCT01843075</td>
</tr>
<tr>
<td>Metformin</td>
<td>Inhibits gluconeogenesis</td>
<td>Mild Cognitive Impairment (Phase 2/3)</td>
<td>NCT04098666</td>
</tr>
<tr>
<td>Nasal insulin</td>
<td>Promotes cellular uptake of glucose</td>
<td>Mild Cognitive Impairment (Phase 2)</td>
<td>NCT05081219</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>PPAR-γ activator, improves glucose metabolism</td>
<td>Alzheimer’s (Phase 2)</td>
<td>NCT00982202</td>
</tr>
<tr>
<td>Semaglutide</td>
<td>Increases the production of insulin</td>
<td>Alzheimer’s (Phase 3)</td>
<td>NCT04777409</td>
</tr>
</tbody>
</table>
study showed that a high fat diet induces cardiomyocyte apoptosis through inhibition of flux autophagy flux (Hsu, Chen, Lee, & Chen, 2016). Although the modulatory effects of lipid compounds on autophagy vary among different types of lipids, some lipid species have been shown to exhibit pro-autophagic properties. For example, docosahexaenoic acid (DHA), a member of the omega-3 family polyunsaturated fatty acids, profoundly promotes autophagy (Bonofiglio, Lanzino, Giordano, Catalano, & Andò, 2016; K. Jing et al., 2011), and is currently in a Phase 4 clinical study for the treatment of AD (Alzforum.org, 2021c).
Autophagy itself has a pivotal role in lipid metabolism. Thus, inhibition of autophagy reduces the degradation of lipids and increases intracellular accumulation of lipid molecules (Miao, Zang, Cui, & Zhang, 2020). Some researchers propose a central role for autophagy in the dysregulations of lipid metabolism and have suggested that the modulation of autophagy might serve as a viable therapeutic strategy for the treatment of different types of hyperlipidemia and its associated consequences such as atherosclerosis (M. Yang, Zhang, & Ren, 2018). To date, several types of antihyperlipidemic agents have been approved for these clinical applications. Interestingly, emerging evidence suggests that the beneficial effects of these agents are partly due to their pro-autophagic properties (Ashrafzadegan, Ahmadi, Farkhondeh, & Samarghandian, 2020; S. H. Kim et al., 2017; Luo et al., 2020; Parihar et al., 2014; Y. Wang et al., 2017c; J. Zhang et al., 2013b).

Gemfibrozil, simvastatin and vascpea are three antihyperlipidemic agents currently under investigation to be repurposed for AD treatment (alzforum.org, 2021d). The induction of autophagy by gemfibrozil has been suggested as one of the main mechanisms by which this compound enhances Aβ clearance (Luo et al., 2020). Statins are widely used to treat hyperlipidemia; however, compelling evidence has emerged over the years strongly indicating effects independent of lipid lowering ability contributing to their therapeutic impact.

Simvastatin, a semi-synthetic derivative of lovastatin, was approved in 1988 for the treatment of hypercholesterolemia. The rate-limiting step in cholesterol biosynthesis is the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate and is regulated by the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR). Statins are potent HMGR inhibitors of HMGR and cause a decrease in levels of mevalonate and cholesterol. In addition, statins exhibit “pleiotropic” properties, including antioxidant, anti-inflammatory, anti-apoptotic, anti-neoplastic, and neuroprotective effects, that are independent of their lipid-lowering ability (Ahmadi, Madrakian, Ghoorchian, Kamalabadi, & Afkhami, 2020; Cho, Kim, Park, & Heo, 2019; Shabla Shojaei et al., 2018) (Fig. 5). Simvastatin has an impact on various signaling pathways (and associated crosstalk) that drive critical biological processes. This includes modulatory effects on cell cycle progression, unfolded protein response (UPR), autophagy, fibrosis, adhesion and migration (Emami et al., 2019; Sheikholeslami et al., 2019; Shojaei, Koleini, et al., 2020a). In keeping with the scope of this review, simvastatin has been reported to reduce the incidence of AD in human (Wolozin et al., 2007) and improves cognition and memory in an animal model of AD and AD patients (W. Huang, Li, Zhao, & Zhao, 2017). In the latter study, administration of simvastatin reduced the mRNA expression of inflammatory cytokines, suppressed apoptosis of neural stem cells, and improved the survival rate of neurons. In addition, simvastatin has been shown to decrease the levels of Aβ by inhibition of both α- and β-secretases in patients with AD (Sjögren et al., 2003). Dhakal et al. showed that simvastatin effectively reduced levels of cellular Aβ42 protein in a dose-dependent manner in yeast, providing further mechanistic insights into the way how simvastatin might exert its protective role (Dhakal, Subhan, Fraser, Gardiner, & Macreadie, 2019). Neuroprotective effects of simvastatin have also been demonstrated in a rat model of spinal cord injury, and were mediated through inhibition of the PI3K/Akt/mTOR signaling pathway and subsequent induction of autophagy (K. Gao et al., 2015; Ramos, Sierra, Ramirez, Velasco, & Burgos, 2012). A recent pre-clinical study revealed that long-term high doses of simvastatin could inhibit apoptosis through significantly increasing Bcl2 (Hu, Song, Fang, Li, & Medicine, 2018b). Li et al. demonstrated that transgenic mice treated with simvastatin (50 mg/kg) for an extended period of time showed improved spatial memory and learning independently of loading amyloid into the brain (Ling Li et al., 2006). In contrast to these observations, other work showed that 12-weeks simvastatin treatment (80 mg/day) did not result in any changes in lipid profile and AD biomarkers (Aβ40, Aβ42, phosphorylated tau, and total tau) in plasma and CSF in patient with AD (Serrano-Pozo et al., 2010). Furthermore, a randomized placebo-controlled trial showed that simvastatin had no beneficial effect on the progression of symptoms of mild-moderate AD despite significantly decreasing blood cholesterol level (Sano et al., 2011). Despite the promising observation from the large-scale epidemiology study that simvastatin was unique among statins in reducing the incidence of AD (Wolozin et al., 2007), these conflicting results question the therapeutic potential of simvastatin for the treatment of AD and warrant the need for further in-depth clinical and mechanistic studies. Currently, simvastatin is in a Phase 4 clinical study to evaluate its effects at earlier stages of AD (i.e., amnestic mild cognitive impairment/prodromal AD) and in cognitively healthy people at elevated risk of developing AD (alzforum.org).

4. Applying nanotechnology for the treatment of neurodegenerative disorders

As one of the most complex organs of the body, the brain is isolated from the blood circulation system by the BBB (Agrawal et al., 2020). The BBB is a biological barrier located between the blood circulation and the neurons within the CNS. The main role of the BBB is to provide a suitable ambiance to maintain proper functioning of the brain and protect it from external harmful materials (Ahlawat et al., 2020). Structurally, the BBB consists of a layer of tightly adhered endothelial cells, which are polarized into two (a luminal (blood-facing) and abluminal (brain-facing)) plasma membrane domains (L. Roberts et al., 2008). The endothelial cells are connected via tight junctions (TJs), adherent junctions (AJs), and gap junctions (GJs). TJs constitute the main compositional element of the BBB and exert transendothelial resistance (Ahlawat et al., 2020), whereas AJs restrict transcellular passage and paracellular transport (Re, Gregori, & Masserini, 2012). Pericytes, astrocytes, microglia, and adjacent neurons also contribute to the function and integrity of the BBB. Pericytes (perivascular smooth muscle cells) regulate blood flow in the brain capillaries (Ahlawat et al., 2020), astrocytes are specialized glial cells that participate in neuronal homeostasis and function, and microglia are the primary immune cells of the brain that become activated in response to either systemic inflammation or trauma (Redzic, 2011).

The ability of a drug to cross the BBB is affected by several parameters, including the properties of the drug itself and various environmental factors. Thus, the intrinsic characteristics of a drug such as conformation, size, molecular weight, lipophilicity, ionization, and the ability to form hydrogen bonds crucially influence transport across the BBB. Generally, the partition coefficient (log D) is used to assess the lipophilicity of a drug (Teixeira, Lopes, Amaral, & Costa, 2020). The relationship between hydrogen bonding and BBB penetration is inverse. Therefore, compounds like peptides (comprising amide and hydroxyl functional groups that can form hydrogen bonds) decrease both the log D and membrane permeability, which reduces the ability to cross the BBB. On the other hand, very small and lipophilic molecules can effectively cross the BBB due to the lipophilic nature of the endothelial cells (Henna et al., 2020). Peripheral factors also affect the transport of drugs over the BBB and include the binding affinity of plasma proteins, the rate of clearance, and uptake of neurotherapeutics by non-target tissues (Teixeira et al., 2020).

AD is progressive in nature, identified by damage/loss of neurons in the CNS, and can be triggered by a combination of different genetic and environmental factors (S. Arora, Sharma, & Singh, 2020b; Do, Li, Chreifi, Poulos, & Silverman, 2019; Nikpour, Sharafi, Hamidi, & Andalib, 2020). Neurons are post-mitotic cells and do not regenerate; therefore, disruption of nerve cells can lead to their death and irreversible loss of some parts of the brain. Depending on the affected area, the destruction of nerve cells can lead to cognitive and/or movement deficits (Goldsmith, Abramovitz, & Peer, 2014). The direct delivery of drugs to the brain through injection or surgery is highly invasive, inconvenient for patients, and carries the risk of severe side effects. For example, when considering intracerebral implants for the direct delivery of drugs to the brain, the possibility of dislodging of the needle, blockage
of the catheter and infection just exemplify some of the disadvantages (Henna et al., 2020). Indeed, drug delivery to the brain through the BBB remains the safest and most convenient strategy for medical treatment of AD. Fortunately, drug transport over the BBB can be improved via enhancing transcellular diffusion pathways, regulating efflux transport, chemically modifying the drug, or transiently disrupting TJs and using a carrier that is able to penetrate the BBB. Finding a carrier that is capable of easily passing the barrier and effectively deliver the therapeutic compound into the brain is a challenging task. Chemical modification of drugs is primarily aimed at increasing the influx through transcellular pathways and enhancing their solubility in lipids (Barchet & Amiji, 2009). The lipophilicity of a hydrophilic molecule can be improved by surrounding it with a layer of lipids (lipidation), which in turn facilitates penetration into the BBB. Polar groups can be replaced by nonpolar functional groups unable to form hydrogen bonds (Poovaiah et al., 2018). For example, heroin and codeine formation by morphine O-acetylation and O-methylation, respectively, are classical lipidization techniques. When two hydrogen bonds are removed during the formation of codeine, the permeability of the BBB increases by ten folds (Oldendorf, Hyman, Braun, & Oldendorf, 1972). Thus, improving drug lipophilicity might be a promising strategy for the modification and repurposing of previously approved therapeutics.

4.1. Different mechanisms of BBB crossing

The entry of ions and essential nutrients as well as the efflux of compounds through the BBB is regulated by various carrier mechanisms. In theory, targeted drug delivery to the brain is possible via this route. There are three major pathways for passing molecules through the BBB: the paracellular, transcellular and carrier-mediated pathway, the latter of which can be subcategorized into efflux transport and transporter-, receptor-, and adsorptive-mediated transcytosis (Fig. 6) (Ahlawat et al., 2020; Alexander et al., 2019; Teixeira et al., 2020).

Fig. 6. Three major pathways for passing molecules through the BBB: the paracellular, transcellular, and carrier-mediated pathway. The latter can be subcategorized into efflux transport and transporter-, receptor-, and adsorptive-mediated transcytosis. The paracellular BBB transport mechanism is the passive diffusion of molecules down a concentration gradient through/between the brain endothelial cells. The transcellular pathway involves the transport of small lipophilic molecules from the blood to the brain across the luminal and abluminal membranes of the endothelial layer down a concentration gradient. Carrier-mediated pathways are bi-directional systems consisting of carriers using receptors, transporters, and vesicles.
4.1.1. Paracellular pathway

The paracellular BBB transport mechanism is the passive diffusion of hydrophilic or small molecules down a concentration gradient through/ between the brain endothelial cells, but it is limited by the regulation of the transient relaxation of tight junctions between the endothelial cells (Barar, Rafi, Pourseif, & Omid, 2016). These tight junctions produce hydrophilic channels of ~0.8 nm diameter between two neighboring endothelial cells (Jena, McErlean, & McCarthy, 2020). Although significant, it has limited bearing on nanocarrier-based medicinal delivery to the brain.

4.1.2. Transcellular pathway

The transcellular pathway involves the transport of small lipophilic molecules from the blood to the brain across the luminal and abluminal membranes of the endothelial cell layer according to the concentration gradient, which is a non-saturable mechanism (Erickson & Banks, 2018). Small lipid-soluble molecules of low molecular weight (<400 Da), such as hormones, alcohol, and gases (CO2, O2) can passively diffuse through the plasma membrane of the endothelial cells (W. Zhang, Mehta, Tong, Esser, & Voelcker, 2021).

4.1.3. Transporter-mediated transcytosis

Transporter-mediated transcytosis (or carrier-mediated transcytosis, CMT) comprises a bi-directional system consisting of carriers located on both luminal and abluminal surfaces and is important for the transport of essential nutrients to the brain. CMT principally transports relatively small molecules such as glucose, amino acids, nucleotides, vitamins, hormones, and choline into the brain using carriers (Lajoie & Shusta, 2015; Pardridge, 2007). Therefore, these small molecules or their derivatives can be utilized as carriers to mediate the nanoparticles across BBB through CMT. Insomuch glucose is the brain’s main energy source, glucose transporter-1 (GLUT1) has a relatively high concentration when compared to other receptors or carriers. In addition, Choline transporter (ChT) is another potential CMT since it can easily transport choline compounds without interfering with the supply of CNS choline (J. Li et al., 2011; Winkler et al., 2015). Other carriers present in BBB are large neutral amino acid transporter (LAT1), cationic amino acid transporter (CAT1), and concentrative nucleoside transporter (CNT2) for transporting the large neutral amino acids, cationic amino acids and nuclear base, respectively. Also, lactate and pyruvate cross the BBB via the mono-carboxylic acid transporter (MCT1) (Dube, Chibh, Mishra, & Panda, 2017). Considering its specificity, this transport system could represent a suitable option for the targeted delivery of therapeutics to the brain.

4.1.4. Receptor-mediated transcytosis

Receptor-mediated transcytosis (RMT) involves both endocytosis and exocytosis: it transfers a macromolecule by the formation of intracellular vesicles that contain the ligand attached to its receptor. RMT is highly selective due to the requirement of receptor-ligand recognition. The vesicles cross the endothelial cytoplasm and release their contents into the brain milieu. This system is also suitable for the delivery of drugs of low BBB permeability. In this pathway, functionalized nanoparticles surface with different ligands can attach to the bind specific receptors on the brain endothelial cells and resulting promote endocytosis. The RMT pathway involves four key steps such as (i) a ligand binds to its cognate receptor at the apical membrane of the brain endothelial cell, (ii) endocytosis occurs through membrane invagination and the creation of an intracellular vesicle containing receptor-ligand complexes, (iii) The newly formed intracellular vesicle can follow various traffic routes including recycling to the apical membrane or routing to the basolateral membrane where membrane fusion permits the vesicle content to be released, (iv) the vesicle is shuttled to the basolateral plasma membrane and exocytosis occurs, releasing the contents of vesicles into the brain parenchyma (Pulgar, 2019). Several receptors and transporters have been used as targets expressed on the surface of brain capillary endothelium of BBB, such as transferrin receptor (TIR) (Marino et al., 2019), lactotransferrin receptor (LIR) (Fang et al., 2016), low density lipoprotein (LDL) receptor (Dheouck et al., 1997), single domain Llama antibodies (Muruganandam, Tanha, Narang, & Stanimirovic, 2002), Insulin-like growth factor 1 receptor (IGF1R) (Terstappen, Meyer, Bell, & Zhang, 2021), insulin receptor (IR) (Bell & Ehlers, 2014). TIR is the most widely studied and independently validated target protein for RMT-based brain delivery approaches. TIR is expressed at a high level at the BBB and mediates iron delivery to the brain via binding and intracellular trafficking of the iron-binding protein transferrin (Tf). Therefore, the internalization of the transferrin-iron complex is triggered by the binding of Tf to the receptor followed by endosome formation. The subsequent acidification releases iron, and Tf-TIR is recycled back to the luminal side of the BBB. Such properties make the TIR interesting in the potential use of brain-targeted drug delivery (Azarmi, Maleki, Nikkam, & Malekinejad, 2020). Since the presence of high bloodstream levels of Tf requires competition with the endogenous ligand for TIR binding, alternative methods involving anti-TIR antibodies have been developed (Pulgar, 2019). Lactoferrin can adsorb on procationic liposomes due to the electrostatic interaction (H. Chen et al., 2010). However, this structure may not be stable, and the ligands may break off during blood circulation. To solve this problem, most research employ chemical conjugation to modify the ligands (Ding et al., 2010). The insulin receptor (IR) is expressed at the BBB and is responsible for the import of bloodborne insulin into the brain via RMT. Similarly to the TIR targeting, anti-IR antibodies are employed for drug delivery into the brain (Lajoie & Shusta, 2015). Insulin-like growth factor 1 receptor (IGF1R) is expressed in the brain and the cerebral vessels. Engagement of IGF1R by anti-bodies that do not interfere with IGF1 binding may be a promising strategy to deliver biotherapeutics across the BBB (Terstappen et al., 2021). Low density lipoprotein (LDL) receptor, a single transmembrane glycoprotein able to recognize LDL particles and promote their endocytosis. Also, LDL receptor-related proteins are expressed at both luminal and basolateral membrane of the endothelium and mediate the transport of lipoproteins and other ligands through RMT (Y. Li et al., 2020b; Pulgar, 2019). Single domain antibodies (sDNAs) are naturally occurring heavy chain fragments that lack the light chain. sDNAs such as the variable domain of heavy-chain antibodies (VHs) and variable new antigen receptors (VNARs) have been used to reach the brain allowing them to be used as therapeutic, diagnosis, or transporter tools. The first VHs found to perform RMT across the BBB are FCS and FC44. These two VHs were obtained from a naive llama phage-displayed library followed by a phanning on human endothelial cells forming BBB (Pulgar, 2019). Table 5 indicates some of the RMT cargo delivery through the BBB. Finally, the study of the blood–brain barrier can lead to the discovery of new transporters on BBB endothelial cells that could be used in brain drug delivery. Table 6 shows some of the RMT-based drug delivery attempts to the central nervous system.

4.1.5. Adsorptive-mediated transcytosis

Adsorptive-mediated transcytosis (AMT) provides a mechanism for carrying molecules to the brain by non-specific electrostatic interactions. AMT uses vesicles to endocytose and shuttle larger proteins and molecules across the BBB. Positively charged molecules can attach to the negatively charged heparin sulfate proteoglycans present on the endothelial cells of BBB, such as transferrin (TfR) (Barar, Rafi, Pourseif, & Omid, 2016), lactoferrin (LIR) (Fang et al., 2016), low density lipoprotein (LDL) receptor (Dheouck et al., 1997), single domain Llama antibodies (Muruganandam, Tanha, Narang, & Stanimirovic, 2002), Insulin-like growth factor 1 receptor (IGF1R) (Terstappen, Meyer, Bell, & Zhang, 2021), insulin receptor (IR) (Bell & Ehlers, 2014). TIR is the most widely studied and independently validated target protein for RMT-based brain delivery approaches. TIR is expressed at a high level at the BBB and mediates iron delivery to the brain via binding and intracellular trafficking of the iron-binding protein transferrin (Tf). Therefore, the internalization of the transferrin-iron complex is triggered by the binding of Tf to the receptor followed by endosome formation. The subsequent acidification releases iron, and Tf-TIR is recycled back to the luminal side of the BBB. Such properties make the TIR interesting in the potential use of brain-targeted drug delivery (Azarmi, Maleki, Nikkam, & Malekinejad, 2020). Since the presence of high bloodstream levels of Tf requires competition with the endogenous ligand for TIR binding, alternative methods involving anti-TIR antibodies have been developed (Pulgar, 2019). Lactoferrin can adsorb on procationic liposomes due to the electrostatic interaction (H. Chen et al., 2010). However, this structure may not be stable, and the ligands may break off during blood circulation. To solve this problem, most research employ chemical conjugation to modify the ligands (Ding et al., 2010). The insulin receptor (IR) is expressed at the BBB and is responsible for the import of bloodborne insulin into the brain via RMT. Similarly to the TIR targeting, anti-IR antibodies are employed for drug delivery into the brain (Lajoie & Shusta, 2015). Insulin-like growth factor 1 receptor (IGF1R) is expressed in the brain and the cerebral vessels. Engagement of IGF1R by anti-bodies that do not interfere with IGF1 binding may be a promising strategy to deliver biotherapeutics across the BBB (Terstappen et al., 2021). Low density lipoprotein (LDL) receptor, a single transmembrane glycoprotein able to recognize LDL particles and promote their endocytosis. Also, LDL receptor-related proteins are expressed at both luminal and basolateral membrane of the endothelium and mediate the transport of lipoproteins and other ligands through RMT (Y. Li et al., 2020b; Pulgar, 2019). Single domain antibodies (sDNAs) are naturally occurring heavy chain fragments that lack the light chain. sDNAs such as the variable domain of heavy-chain antibodies (VHs) and variable new antigen receptors (VNARs) have been used to reach the brain allowing them to be used as therapeutic, diagnosis, or transporter tools. The first VHs found to perform RMT across the BBB are FCS and FC44. These two VHs were obtained from a naive llama phage-displayed library followed by a phanning on human endothelial cells forming BBB (Pulgar, 2019). Table 5 indicates some of the RMT cargo delivery through the BBB. Finally, the study of the blood–brain barrier can lead to the discovery of new transporters on BBB endothelial cells that could be used in brain drug delivery. Table 6 shows some of the RMT-based drug delivery attempts to the central nervous system.

4.1.6. Efflux transport

The activity of the efflux pumps, such as P-glycoprotein (Pgp), multi-drug resistance proteins (MRPs), multi-drug resistance proteins (Mrps), and breast cancer resistance protein (BCRP), located at the brain capillary endothelium prevents from the drugs entering into the...
Table 5
Some of the receptor systems RMT cargo delivery through the BBB.

<table>
<thead>
<tr>
<th>Target/Transport mechanism</th>
<th>Ligands/Antibodies/Peptides</th>
<th>Major outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferin receptor (TIR)</td>
<td>Human TIR fused to iduronate 2-sulfatase (IDS): JR-141.</td>
<td>Immunoreactivity of JR-141 found in brain in TFRC-KI/Ids-KO mice. Phase I/I clinical trial of JR-141 for mucopolysaccharidosis II (MPSII) currently underway. (Sonoda et al., 2018)</td>
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<tr>
<td>OX26 (murine mAb to rat TIR)</td>
<td>BDNF-polyethylene glycol (PEG)-biotin-OX26 conjugates increased brain uptake, minimized rapid clearance upon PEGylation, and decreased the infract volume at higher doses of the conjugate. The conjugates also reduced stroke volume in a rat model of permanent middle cerebral artery occlusion (MCAO). (Pardridge, Wu, &amp; Sakane, 1998; D. Wu &amp; Pardridge, 1999; V. Zhang &amp; Pardridge, 2001)</td>
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<tr>
<td>8D3 (Rat mAb to mouse TIR)</td>
<td>Biotinylated human basic fibroblast growth factor (bFGF) coupled to OX26 showed 66% and 34% of reduced stroke/infract volumes at doses of 25 μg/kg and 5 μg/kg, respectively, within 1 h of arterial occlusion. No effect was seen if administered at 2 h or 3 h after MCAO. (Song, Vinters, Wu, &amp; Pardridge, 2002)</td>
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<td>Ox26-immunoliposomes loaded with GDNF plasmids provided sustained release of GDNF in a rat model of Parkinson's disease. Expression of the transgene was higher in organs expressing tyrosine hydroxylase (TH). TH promoted the expression of the GDNF gene compared to those only under the influence of cytomegavirus promoter. (Boado, Zhang, Zhang, Wang, &amp; Pardridge, 2008)</td>
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<td>Plasmid DNA encoding [glucuronidase was encapsulated in pegylated immunoliposomes conjugated to 8D3 mAb. The gene expression was confined to the brain tissues when the transgene was under the influence of a glial fibrillary acidic protein (GFAP) promoter similar to a Simian virus 40 promoter. (N. Shi, Zhang, Zhu, Boado, &amp; Pardridge, 2001)</td>
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<td></td>
<td>Peptide-bound gold nanoparticles disrupted amyloid-β aggregates. The retro-enantio THR delivered quantum dots to the brain parenchyma. (Prades et al., 2012; Prades et al., 2015)</td>
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<td>Variants of the 8D3 anti-TIR with reduced affinity fused with IL-1 receptor antagonist IL-1RA. Male C57B/L mice i.v., injected with IgG1M–IL–1RA showed 22 to 69-fold greater brain content of lower affinity variants vs. 8D3. Reverse of mechanical hyperalgesia also observed. (Webster et al., 2017)</td>
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<td>High (anti-TIR/A–BACE1) and low (anti-TIRD/BACE1) affinity bispecific antibodies ani TIR and β-amyloid cleaving enzyme-1 (BACE1). Mouse model of PD i.v., injected, high-affinity binding to TIR caused a dose-dependent reduction of brain TIR levels and lysosomal degradation enzyme-1. (Bien-Ly et al., 2014)</td>
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<td>Monovalent binding anti-TIR antibody, “Brain Shuttle” antibody for AD. Enhanced RMT compared to bivalent Ab. Increased destruction of β-amyloid plaques in mouse model of AD. Changes in binding mode attenuated peripheral effects. (Di Paolo &amp; Przedborski, 2013; Weber et al., 2018)</td>
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<td>CTIRMAb complexed with single chain Fv (ScFv) antibody: cTIRMAb-ScFv. Bi-functional binding to TIR and Aβi, accumulation in mouse brain &gt;33ID/g. Mouse model of Alzheimer’s disease (AD) showed 40–60% reduction in Aβi fibrils. (Boado, Lu, Hui, &amp; Pardridge, 2010; Sumbrria, Hui, Lu, Boado, &amp; Pardridge, 2013)</td>
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<td>CTIRMAb complexed with erythropoietin (EPO): cTIRMAb-EPO. cTIRMAb complexed with glial-derived neurotrophic factor (GDNF): cTIRMAb-GDNF. Mouse model of PD i.v., treated for 3 weeks showed &gt;300% and &gt;250% increase in striatal TH, respectively and improvements in behavioral testing. (A. Fu et al., 2010; Q.-H. Zhou, Boado, Hui, Lu, &amp; Pardridge, 2011)</td>
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<td>Liposomes decorated with Tf-poly-L-arginine loaded with imaging agents or β-gal expressing plasmid. 4% of injected dose of imaging agents reached the brain 24 h after i.v., injection. Greater β-gal compared to injection of naked DNA. (G. Sharma et al., 2013)</td>
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<td></td>
<td>Insulin receptor (HIR)</td>
<td>Anti-insulin antibody (29B4) Human serum albumin–PEG3400–maleimide–29B4 transported loperamide across the BBB. Induced significant antiincoercitive effects in the tail-flick test in CD-1 mice after intravenous injection. (Ulbrich, Knoebloch, &amp; Kreuter, 2011)</td>
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<td>Murine 83–14 mAb to HIR Carmustine loaded SLNs grafted to 83–14 monoclonal antibody stimulated endocytosis into the endothelial cells with enhanced permeability across the BBB. (Xuo &amp; Shi-Huang, 2013)</td>
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<td>Diblock copolymer poly(dimethylsiloxane)-block-poly (2-methyl-2-oxazoline) polymersomes conjugated to antibody 83–14 demonstrated hCMEC/D3 cellular binding and uptake. The efficacy and potency of BDNF fused to HIRMAb was like that of chimeric HIRMAb and free BDNF, respectively. Residence time in the blood also increased. (Dieu, Wu, Palivan, Balazsdrumanian, &amp; Huwyler, 2014)</td>
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<td>Human anti-IR antibody (HIRMAb) complexed with iduronidase: HIRMAb-IDUA, Valanafusp, ACT-181. In a Phase II trial, 11 children with mucopolysaccharidosis type I (MPS1), a lysosomal storage disease, showed evidences of cognitive and somatic stabilization. (Boado, Zhang, Zhang, &amp; Pardridge, 2007)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIRMAb complexed with N-sulfogalcosamine sulfotransferase (SGSH): HIRMAb-SGSH. 72–83% reduction in lysosomal glyco-aminoglycans in mucopolysaccharidosis type III (MPSIIIa) fibroblasts. In Rhesus monkeys, i.v., injected, brain uptake of ~1% ID/100 g. Reduction in brain heparan sulfate in MPSIIIa mouse. (Boado, Lu, Hui, &amp; Pardridge, 2014b, Boado, Lu, Hui, &amp; Pardridge, 2018)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIRMAb complexed with arylsulfatase (ASA): HIRMAb-ASA. In Rhesus monkeys i.v., injected, brain uptake of 1.1 and 0.32% ID/100 g in gray and white matter, respectively, HIRMAb-ASA observed in all parts of brain. (Boado, Lu, Hui, Sumbrria, &amp; Pardridge, 2013)</td>
<td></td>
</tr>
</tbody>
</table>

(continued on next page)
Here, we will discuss nanomaterials (and lent candidates for targeting therapeutics to the brain (Ahmadi et al., 2020) possess special physicochemical characteristics rendering them excellent candidates for overcoming the limitation of crossing the BBB. Indeed, nanomaterials technology approaches have emerged as promising strategies to overcome the barrier posed by the blood-brain barrier (BBB) (Golden & Pollack, 2003).

### 4.2. Nanotechnology for crossing therapeutics over the BBB

Transcytosis, which helps in the movement of molecules across the BBB, is a vital process for endothelial cells because it prevents the accumulation of noxious substances in the brain. However, some of the drugs cannot reach or sufficiently maintain their therapeutic concentration in the brain such as relatively lipophilic compounds that would be predicted to permeate the endothelial lining of the brain microvasculature (Golden & Pollack, 2003).

<table>
<thead>
<tr>
<th>Target/Transport mechanism</th>
<th>Ligands/Antibodies/Peptides</th>
<th>Major outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIRMAb fused to paraoxonase (PON)-1:</td>
<td>Fusion protein detected in brain in Rhesus monkeys after i.v. injection. Brain uptake in Rhesus monkeys approximately 3% ID/100 g tissue. No toxicity observed during a 6-month treatment study.</td>
<td>(Teramoto, Tanaka, Lee, &amp; Endo, 2008)</td>
<td></td>
</tr>
<tr>
<td>HIRMAb-PON1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIRMAb fused to iduronate 2-sulfatase (IDS):</td>
<td>In parkinsonian monkeys twice a week 3-mo i.v., injections of HIRMAb-GDNF did not improve parkinsonian motor symptoms and induced a dose-dependent hypersensitivity reaction</td>
<td>(Ohshima-Hosoyama et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>HIRMAb-IDS.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIRMAb fused to GDNF: HIRMAb-GDNF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose-6-phosphate</td>
<td>131I-phosphorylated β-glucuronidase was transported across the BBB with a high brain influx rate after intravenous injection. Approximately 62% was found in the brain parenchyma which was significantly reduced with an injection of the non-labeled β-glucuronidase or mannose-6-phosphate</td>
<td>(Ohshima-Hosoyama et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL) receptors</td>
<td>Paclitaxel-loaded nanoparticles linked to peptide 22 increased uptake in BECs and C6 glioma cells. An elevated transport ratio of paclitaxel was noted across the BBB.</td>
<td>(B. Zhang et al., 2013a)</td>
<td></td>
</tr>
<tr>
<td>Nanoparticles decorated with apolipoprotein A (ApoE).</td>
<td>ApoE-modified nanoparticles cross BBB in brain capillary endothelial cells</td>
<td>(Wagner et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Lentivirus vector encoding amyloid β-degrading enzyme neprilysin fused to ApoB transport domain.</td>
<td>Mouse model of AD showed reduced Aβ and plaques levels.</td>
<td>(Karthik et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Angiopep-2 combined with antitumor drug paclitaxel: (ANG1005, GRN1005).</td>
<td>Phase I study in recurrent malignant glioma patients showed brain delivery of drug with therapeutic activity.</td>
<td>(Drappatz et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Lentiviral IDS fused to ApoEII (IDS:ApoEII) used in stem cell therapy.</td>
<td>MPSII mice showed normalization of brain pathology and behavior, including correction of astrogliosis and lysosomal swelling.</td>
<td>(Gleitz et al., 2018)</td>
<td></td>
</tr>
<tr>
<td>Sulphamidase fused to secretion signal peptide of iduronate-2-sulphatase (IDS) and ApoB-binding domain.</td>
<td>Single i.v., injection on MPSIIA mice showed efficient BBB transcytosis and restoration of sulphamidase activity in the brain.</td>
<td>(Sorrentino et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Lactoferrin receptor</td>
<td>Radiolabel complex loaded in lactoferrin-conjugated PEGylated liposomes exhibited a three-fold increase in vitro uptake and two-fold higher in vivo brain uptake compared to pegylated liposomes</td>
<td>(F.-Y. J.Huang et al., 2013)</td>
<td></td>
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<tr>
<td>Lactoferrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single domain llama antibodies (FC5, FC44)</td>
<td>BBB transcytosis of FC5 is dependent on clathrin-coated endocytic vesicles and on the recognition of α(2,3)-sialylglycoprotein receptor on human endothelial cells. MS based methods showed that systemic administration in rats produces highly facilitated BBB transport of FC5.</td>
<td>(Abulrob, Spong, En Henegouwen, &amp; Stanimirovic, 2005)</td>
<td></td>
</tr>
<tr>
<td>Single domain FC5 antibody.</td>
<td>After i.v., injection in rats &gt;tenfold higher accumulation of BBB-mGluR1 in brain, and suppression of thermal hyperalgesia.</td>
<td>(Haqqani et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Single domain FC5 antibody.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bispecific antibody FC5-mGluR1 (BBB-mGluR1)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Brain parenchyma. This efflux pump activity also inhibits absorptive transcytosis, which helps in the movement of molecules across the BBB (Misra, Ganesh, Shahwala, & Shah, 2003; W. Zhang et al., 2021). Efflux transport is unidirectional and removes harmful substances and xenobiotics from the brain to the blood. As a result, most ionized, watersoluble molecules more than 180 Da, as well as compounds that are substrates of its efflux system, are filtered out (Krol et al., 2013). This process is vital for endothelial cells because it prevents the accumulation of noxious substances in the brain. However, some of the drugs cannot reach or sufficiently maintain their therapeutic concentration in the brain such as relatively lipophilic compounds that would be predicted to permeate the endothelial lining of the brain microvasculature (Golden & Pollack, 2003).

### 4.2. Nanotechnology for crossing therapeutics over the BBB

The development of novel methods to overcome drug delivery limitations due to the BBB is of utmost importance in modern medicine (Tapeinos, Battaglini, & Ciofani, 2017). Nanotechnology is the control and manipulation of materials in the size range of 1–100 nm to produce and develop novel applications (Stern & Johnson, 2008). Several nanotechnology approaches have emerged as promising strategies to overcome the limitation of crossing the BBB. Indeed, nanomaterials possess special physicochemical characteristics rendering them excellent candidates for targeting therapeutics to the brain (Ahmadi et al., 2020; Teixeira et al., 2020). Here, we will discuss nanomaterials (and their characteristics) most relevant to potential applicability for the treatment of AD. Table 7 summarizes the significance of nanomaterials in the design and development of therapeutic agents against the diagnosis and progression of AD. The effectiveness of a treatment can be increased by surface modification of nanoparticles and rational design of nanocarriers for crossing the BBB.

### 4.3. Nanomaterials for the modulation of autophagy in AD

Autophagy modulation has emerged as a promising therapeutic strategy for the treatment of various proteinopathy disorders such as AD. NPs can be used to modulate autophagy in target cells in two different ways: direct induction of autophagy as foreign particles or as carriers of autophagy modulating drugs.

NPs frequently end up in the lysosomes and may themselves induce autophagy inside the treated cells. This phenomenon is a popular subject of ongoing research and has been observed for both inorganic and organic NPs, including metal NPs, carbon nanotubes and quantum dots (Wei, Rosenkranz, Luo, Lan, & Cai, 2019). The direct induction of autophagy by NPs depends on their size, shape and chemical composition (Cui et al., 2018). Since inorganic NPs cannot be digested by the lysosomal enzymes, they might accumulate inside the cells and eventually block the autophagy flux, which may even result in cell death. In this regard, biodegradable organic NPs (e.g., liposomes and micelles) show some advantages as they usually do not accumulate inside the cells (Y. Li et al., 2021b). Several nanomaterials have been evaluated for their...
effects on autophagy signaling (Fig. 7). For example, Xue et al. demonstrated the ability of low concentrations of single-walled carbon nanotubes (SWNT) to upregulate autophagy and reverse defects in the autophagic degradation of Aβ aggregates within astroglial cells in the brains of an AD mouse model (Xue et al., 2014). In AD, autophagy-related vesicular compartments accumulate within dystrophic neurites, indicating the defective clearance of autophagic substrates by lysosomes. In several LSDs, characterized by impaired neuronal autophagy, glial cells are essential to the clearance of abnormally aggregated proteins and neuroprotection. The aforementioned study demonstrated that autophagy was significantly impaired in primary glia of AD-related amyloidosis mice but that autophagic dysfunction and autophagic substrate clearance could be reversed by SWNT (Xue et al., 2014). The ability of SWNT to induce autophagy and promote lysosomal clearance of autophagic substrates in AD models represents a promising area of research, requiring further in-depth mechanistic and clinical studies to identify and validate its therapeutic applications.

The activity of lysosomal enzymes, and thereby autophagosome maturation, depends on the intraluminal pH of lysosomes. The lysosomal pH is maintained within low acidic ranges by the vacular ATPase (v-ATPase) pump, which is located on the membrane of lysosomes and pumps H+ ions into the lysosomal lumen (Mindell, 2012). Failure of lysosomal acidification has been demonstrated in several AD (Colacurcio & Nixon, 2016). In a familial form of AD, due to mutations in Presenilin-1 (PS1), the lysosomal pH is unusually high and the function of lysosomes is impaired (Lee et al., 2010). Impaired lysosomal acidification has also been reported in animal models of AD (Torres et al., 2012; D. S. Yang et al., 2011). Interestingly, dysregulation in the expression levels of v-ATPase subunits can be detected in brain samples of sporadic AD patients (Ginsberg et al., 2010). Based on these reports, strategies aimed at maintaining or promoting lysosomal acidification look promising for the treatment of AD. In this regard, considering NPs typically end up in the lysosomes, several studies have used different types of acidic NPs and were able to drastically reduce the intraluminal lysosomal pH (Baltazar et al., 2012). Lee et al. showed that knocking out PS1 results in lysosome alkalization and impaired calcium homeostasis in cells. Interestingly, the authors used acidic NPs, composed of poly (DL-lactide-glycolide) (PLGA), and were able to restore normal lysosomal function and autophagy (Lee et al., 2015). Lipotoxicity (chronic exposure to high levels of fatty acids) has been demonstrated to decrease lysosome acidity and impair autophagy flux (S. E. Choi et al., 2009; González-Rodríguez et al., 2014; M. Park, Sabetski, Kwan Chan, Turdi, & Sweeney, 2015). Using photoactivated acidifying NPs, autophagy flux was rescued in a cell model of lipotoxicity (Trudeau et al., 2016). Moreover, impaired lysosomal function in a series of toxin and genetic cellular models of NDDs could be restored by poly(DL-lactide-glycolide) (PLGA) acidic NPs (Irinopoulos et al., 2016; Bourdoux et al., 2016).

Another way NPs can modulate autophagy is by using them as nanocarriers for the delivery of autophagy modulating compounds. This approach aims to improve drug delivery and release in cells of interest, and has been used to increase the efficacy of autophagy modulating compounds (e.g., chloroquine diphosphate) in advanced cases of human malignancies (Z. Shi et al., 2018). In one study, for example, the nanocomposites were covalently bound to BECN1 and the generated polymer-BECN1 (P-Becn) NPs enhanced autophagy and cytotoxicity in a breast cancer cell line (Y. Wang et al., 2015). Others used an emulsion method to make ferritin-binding nano erastin and rapamycin nanospheres, and demonstrated that these NPs were able to dramatically increase autophagy flux in treated cancer cells (Y. Li et al., 2019). While most of the identified anti-amyloidogenic materials successfully inhibit protein aggregation, they are incompetent in clearing intraneuronal protein aggregates. To overcome this limitation, Dey et al. designed a biopolymer micelle (15-30 nm hydrodynamic size) that could clear protein aggregates from inside the cells via upregulated autophagy mechanisms (Dey, Jana, & Jana, 2018). The polymer consisted of a poly-aspartic acid backbone that was conjugated to fatty amines (e.g., arginine) as well as primary amines to facilitate polymer

### Table 6

Some of the receptor-mediated transcytosis (RMT)-based brain drug delivery.

<table>
<thead>
<tr>
<th>Receptor Ligand</th>
<th>Vehicle</th>
<th>Therapeutic</th>
<th>Molecule</th>
<th>Target</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferin Receptor (TIR)</td>
<td>Gold Nanoparticle</td>
<td>Transferinn (TF)</td>
<td>Photodynamic pro-drug</td>
<td>Human glioblastoma cell line/ Human glioma cancer cell line (Dixit et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Immunoliposome</td>
<td>Transferrin receptor antibody (Ox26)</td>
<td>Oxautilatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maleimide-Polyethylene glycol-Poly lactic acid Nanoparticles</td>
<td>Ox26</td>
<td>α Coborotoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transferrin-cell penetrating peptide-stereically stabilized liposome</td>
<td>TF</td>
<td>Coumarin-6 Doxorubicin 1,10-510 diocdateyl-3,3,30,30-tetramethylindotricarbocyanine iodide</td>
<td>Globlastoma (Aguilera et al., 2019)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe3O4 NP</td>
<td>TF</td>
<td>–</td>
<td>BBB (Qiao et al., 2012)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanostructured Lipid Carrier</td>
<td>TF</td>
<td>Curcumin</td>
<td>Alzheimer Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin Receptor (IR)</td>
<td>–</td>
<td>Insulin</td>
<td>Hematoencephalic Barrier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surfactant micelle</td>
<td>Insulin</td>
<td>Haloperidol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>Human Serum Albumin Nanoparticle</td>
<td>Anti-insulin receptor monoclonal antibody (29B4)</td>
<td>Loperamide</td>
<td>BBB (Boado, Lu, et al., 2014)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6** Some of the receptor-mediated transcytosis (RMT)-based brain drug delivery.
Frequently used nanomaterials for drug delivery through BBB.

<table>
<thead>
<tr>
<th>Type of nanomaterials</th>
<th>Examples</th>
<th>Intrinsic BBB permeability</th>
<th>Approaches to enhance BBB permeability</th>
<th>Drug delivery for AD therapy</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Viral vectors         | 1. Retroviral  
2. Lentiviral  
3. Adenoviral  
4. Adeno-associated viruses (AAV)  
5. Herpes simplex viruses  
6. Sendai viral | transient disruption of brain endothelial tight junctions | 1. AAV Capsid Engineering  
1–1. Rational design of AAV capsids  
1–2. Directed evolution of AAV capsids  
1–3. Computationally designed ancestral capsid  
2. Approaches without AAV engineering  
2–1. Polyoloid AAV vectors.  
2–2. Small molecules.  
2–4. antibodies | 1. long-term therapeutic effects  
2. Targeting expression | (W. Chen et al., 2021; Fischell & Fishman, 2021; H. Fu & McCarty, 2016; Ittner, Klugmann, & Ke, 2019; C. Li & Samulski, 2020; T.-E. Park et al., 2019; D. Wang, Tai, & Gao, 2019) |
| Polymers nanospheres | 1. Nature  
1–1. human serum albumin (HAS)  
1–2. Gelatin (GE)  
1–3. Chitosan (CS)  
1–4. Alginic acid | 1. Tight junction modulation of the BBB.  
2. cationic polymer (such as chitosan) through the BBB via endocytosis and transcytosis | Surface modification:  
1. surfactants  
2. antibodies  
3. transferrin | 1. increased modiﬁcation of drug in the brain  
2. low toxicity  
3. Decreased amyloid-beta (Aβ) plaques and related inﬂammation characteristics.  
4. Increased activity of superoxide dismutase | (Barbara et al., 2017; Ediriweera, Chen, Verbury, Thurecht, & Vine, 2021; Q. Lu et al., 2018; Shakeri et al., 2020; Y. Zeng et al., 2020; W. Zhang et al., 2021) |
| Liposomes            | 1. cationic lipid:  
1,2-dioleoyl-3-trimethylammonium propane (DOTAP)  
2. neutral:  
2–1. lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE)  
2–2. cholesterol  
2–3. poly(ethylene glycol) (PEG) lipid  
1,2-distearyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] | 1. transport lipid-mediated free diffusion  
2. lipid-mediated endocytosis  
3. Via the intranasal route | Surface modiﬁcation:  
1. lactoferrin  
2. glucose  
3. peptides  
4. transferrin  
5. polymer | 1. high drug loading capacity  
2. enhance brain-targeting and cellular internalization  
3. signiﬁcant binding of the liposomes to amyloid-β monomers  
4. disrupted amyloid-β peptide ﬁbrillation  
2. enhanced biocompatibility  
3. drug-release kinetics  
4. targeted drug delivery | (S. Arora, Layek, & Singh, 2020a; S. Arora, Sharma, & Singh, 2020b; Bilal, Barani, Sabir, Rahdar, & Kyzas, 2020; Canovi et al., 2011; Fonseca-Santos, Gremião, & Chorilli, 2015; Y. Li, Martin, et al., 2020a; Wechsler, Vela Ramirez, & Peppas, 2019) |
| Dendrimers           | 1. poly(aminoamine) (PAMAM)  
2. poly etheryhydroxyamine (PEHAM)  
3. poly-(propyleneimine) (PPII) dendrimers  
4. Poly-l-lysine (PLL)  
5. Carbosilane Dendrimers  
6. phosphorous (PPH)  
7. Janus Dendrimers | Disruption of tight endothelial cell connections | 1. Transferrin  
2. Insulin  
3. Endothelial growth factor receptors  
4. Low-density lipoprotein  
5. Folic acid  
6. amino acids  
7. peptides  
8. Aptamers  
9. monoclonal antibodies | 1. disrupted amyloid-β peptide ﬁbrillation  
2. enhanced biocompatibility  
3. drug-release kinetics  
4. targeted drug delivery | (Srinageshwar et al., 2017; Wechsler et al., 2019; Y. Zeng et al., 2020; Zhu, Liu, & Pang, 2019) |
| Micelles             | 1. Block copolymer micelles  
1–1. poly(styrene)-poly(acrylic acid)  
1–2. poly(ethylene glycol)-b-poly(lactic acid)  
1–3. distearyl-sn-glycero-3-phosphoethanolamine-N-methoxy poly(ethylene glycol) | Endocytosis pathways of endothelial cell | 1. brain-targeted drug delivery  
2. high drug loading | | (Kanazawa, 2015; Shiraiishi, Wang, Kokuryo, Aoki, & Yokoyama, 2017; Teleau, Chircov, Grumezescu, Volceanov, & Teleau, 2018; |
micellization, increase microcell uptake of the micelles, and upregulate autophagy. Upregulated autophagy plays a vital role in the clearance of amyloid protein aggregates via lysosomal degradation. The reduced negative charge and lipophilic properties of micelles increase their intake ability. The absorbed micelles are transported to the perinuclear regions, where protein aggregates exist predominantly. Finally, polymer micelles clear toxic aggregated protein from the cell via autophagy and promote cell survival (Debnath et al., 2018).

5. Future prospects

The impairments of autophagy process in AD have been reported by several studies (D.-S. Yang et al., 2008). Different groups also suggested the enhancement of autophagy as a viable therapeutic modality for AD (Ulamek-Koziot et al., 2016). However, few autophagy specific modulators have been developed for clinical applications so far. Autophagy is a process regulated by diverse upstream signaling pathways. In addition, more than 30 autophagy-related (ATG) genes are involved directly in the process of the formation and maturation of the autophagosome. So, the fine regulation of autophagy is quite possible by targeting different regulatory or effector genes involved in this process. Indeed, various core autophagy genes have been proposed to be suitable as therapeutic targets and several startup companies are currently investigating these targets in preclinical models (Boland et al., 2008). However, development of new drugs is expensive and time consuming. One strategy to circumvent these pitfalls is the repurposing of approved drugs. In these strategies the drugs which are developed to target other biologic process (e.g., growth and survival) are evaluated for their ability to enhance autophagy flux (L. Zhang et al., 2019a). Fortunately, many of these drugs have been evaluated so far for their effects on autophagy process. Also, there are established libraries of approved drugs which could be used for high throughput screening to find undefined autophagy modulators (Shibuya et al., 2015). It looks that the repurposing is the best current strategy to target autophagy for therapeutic purposes until the time that new generation of drugs targeting the core autophagy genes are available in clinic (Kocak et al., 2021).

6. Concluding remarks

The prevalence of AD and therefore the need for effective treatment is increasing as the global population keeps aging. Indeed, AD is the most prevalent NDDs and its etiology is multifactorial and characterized by several pathogenic hallmarks that are associated with the progression and the development of the disease. One of the most important physiological pathways downregulated in AD is autophagy. Autophagy has emerged as a target for treatment in various diseases. For example, several compounds have been developed to modulate autophagy to improve the chemotherapy response or control cancer progression and metastasis.

It has been recognized that traditional drug discovery processes (e.g., single target - single drug) are insufficient to answer the unmet need for effective therapeutics for the treatment of complex multifactorial diseases, such as AD. On the other hand, combination therapies targeting several specific targets may increase drug toxicity due to drug-drug interactions. One modern approach, DML, is currently
gaining significant interest in medicinal chemistry. Identifying a single
drug molecule able to simultaneously modulate several targets/condi-
tions involved in the pathogenesis of a complex disease will be a
major and unique milestone in drug discovery. In addition, a
polypharmacological approach may increase therapeutic potential of a
drug through synergistic effects, which could lead to reducing the
dose and, hence, the adverse effects associated with that particular
drug. There are numerous examples of the identification of poly-
pharmacological patterns in already approved drugs, including statins
and metformin. These clinical data further sparked the search for and
discovery of novel polypharmacological moieties and scaffolds via
screening of registered drugs. This so-called repurposing of drugs that
have been approved for other indications is attractive to the pharma-
ceutical industry, as this approach avoids long and expensive drug dis-
covery and development processes. Considering diseases are driven by
various mechanisms while keeping in mind that at the same time cer-
tain molecular and cellular processes contribute to several diseases,
careful evaluation of specific but overlapping disease pathological
pathways may provide selective strategies for drug repurposing. Be-
because targeting autophagy has proven promising in cancers and defec-
tive autophagy contributes to AD, it only makes sense to consider
medications that have been used to modulate autophagy in cancer for
the treatment of AD. In the current review, we provided a comprehen-
sive review on applications of drugs that induce autophagy in different
models and evaluated their potential in AD.

Fig. 7. Two main mechanisms through which nanoparticles may affect the autophagy pathway. Maintaining or promoting lysosomal acidification: Nanoparticles frequently end up in the lysosomes and may themselves induce autophagy inside the treated cells. This pathway can be utilized for maintaining or promoting lysosomal acidification using acidic nanoparticles for the treatment of NDDs. Delivery of autophagy modulating compounds: Nanocarriers can be utilized for the delivery of autophagy modulating compounds.
targeting therapeutics to the brain. Importantly, nanomedicine has been tailored and transformed for both diagnostic and therapeutic purposes, which would directly benefit management of AD. The implementation of multipurpose ‘smart’ NPs with multi-therapeutic capacities is a promising approach and would provide scientists with numerous options for the type of material/formulation, the function(s) of loaded therapeutic agent, stimuli triggers, and active/passive targeting. While some challenges still need to be carefully considered (e.g., large-scale reproducible production, biodegradability, and long-term toxicity of nanomaterials), these smart nanotechnologies could ultimately promote the development of personalized medicine products.

**Declaration of Competing Interest**

The authors declare that there is no conflict of interest regarding the publication of this article.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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