LAT1, a novel pharmacological target for the treatment of glioblastoma.

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Abstract

The L-Type Amino Acid transporter, LAT1 (SLC7A5), has a crucial role in mediating amino acid uptake into the cells, thus modulating cell growth and proliferation as well as other intracellular functions. Different studies have reported a central role of LAT1 in glioblastoma development and progression, suggesting that the modulation of its activity could be a novel therapeutic strategy. LAT1 also has an important role in the peripheral immune system, by regulating the activation status of several immune cells through modulation of the mechanistic target of rapamycin kinase. In glioblastoma (GBM), the blood-brain barrier is disrupted, which allows the recruitment of peripheral immune cells to the tumour site. These cells, together with resident microglia, contribute to cancer growth and progression. Currently, little is known about the function of LAT1 in the reprogramming of the immune component of the tumour microenvironment in the context of GBM. In this article, we review the available data on the role of LAT1 in the regulation of GBM biology, including its potential role in the tumour microenvironment, particularly in infiltrating-peripheral immune cells and resident microglial cells. In addition, we review the available data on the main pharmacological inhibitors of LAT1, aiming to evaluate their possible role as novel therapeutics for GBM.

Keywords

Glioblastoma; LAT1; microglia; LAT1-inhibitor JPH203; amino acid; pharmacotherapy.

1. Introduction

Transporters are membrane-spanning proteins that facilitate the movement of substrates across the biological membranes. They regulate the cellular uptake and/or the efflux of different molecules, including ions, nutrients such as glucose, amino acids and lipids, and neurotransmitters. These transport processes help to maintain cellular homeostasis and cell function. In contrast to normal cells, tumour cells require an increased supply of nutrients and amino acids to maintain the high rate of protein synthesis and cell metabolism linked to the so-called "cancer-associated pathways". These pathways are involved in the rapid growth, proliferation and survival of tumour cells. Given that not all the required nutrients are able to cross the membrane, their availability in the tumour cells strictly depends on the activity of different and specific transporters. Among these, the Large-Type amino acid transporter 1 (LAT1) allows the transport of large, neutral essential amino acids (EAAs) within the cells, thus critically sustaining cell growth [1], [2]. LAT1 expression is consistently upregulated in several human cancers [3], and LAT1 has been characterized as a key regulator of GBM cell growth [4]. However, considering the emerging role of LAT1 in the regulation of the immune response, a possibility exists that inhibition of this transporter may result in significant anti-tumour effects, not only by direct targeting of the tumour cells but also by regulating the inflammatory microenvironment. This review will cover the evidence on the role, expression, and modulation of LAT1 in the context of GBM, focusing on the direct effects of LAT1 in the malignant cells and on its potential role in the GBM microenvironment. The main pharmacological inhibitors used to block/modulate LAT1 activity will be reviewed, together with a critical evaluation of their possible role as novel therapeutics for GBM.

2. The L-Type Amino Acid Transporter 1 (LAT1): molecular and structural properties

Transporters can be divided into two main different classes: the ATP-binding cassette (ABC) transporters and the solute carriers (SLCs). The first class utilizes ATP hydrolysis as an energy source

to actively transport molecules across the cell membranes, whereas the SLCs work as uni- or bi-directional transporters without directly using ATP. Many transporters can be organ-specific or organelle-specific and recognise chemically different substrates. They can function as influx or efflux transporters. The SLC superfamily is primarily involved in the uptake of small molecules and nutrients into the cells. These carriers can act as facilitated transporters, exchangers or ion-coupled transporters [5]. In total 65 families of SLCs have been identified, including almost 400 proteins. They interact with a wide range of substrates, such as hydrophilic compounds and amino acids [6].

Among the SLC superfamily, the LAT transporters are known for their ability to exchanging large, neutral L-amino acids within the cells, thus allowing both the uptake and extrusion of various EAAs at the same time [1], [2]. LAT transporters include four principal members (LAT1-4), of which LAT1 has been extensively characterized as an antiporter. LAT1, also known as SLC7A5, is ubiquitously expressed, with the highest levels detected in the brain (particularly at the blood brain barrier [BBB]), spleen, bone marrow, testis, and placenta [5]. The functional LAT1 transporter is constituted by heterodimers between LAT1 and the CD98 protein. LAT1 consists of a light chain (SLC7), responsible of the amino acidic transport, and a heavy chain (the glycoprotein 4F2hc, also known as CD98 or SLC3A2), which is fundamental for the correct localization of the transporter at the plasma membrane [7]. In the functional heterodimers, the main role of CD98 is to stabilize and anchor the transporter to the plasma membrane, although it also modulates substrate affinity. Indeed, the heterodimers show different affinities for the different amino acids that can be transported. LAT1/CD98 can import large branched-chain and aromatic neutral amino acids whilst extruding intracellular amino acids, such as L-glutamine (L-Gln), with a 1:1 stoichiometry and in a Na^+/pH independent manner [8]. In general, the main substrates of the LAT1/CD98 complex are the amino acids L-Phenylalanine (L-Phe), L-Tyrosine (L-Tyr), L-Leucine (L-Leu), L-Isoleucine (L-Ile), L-Histidine (L-His), L-Tryptophan (L-Trp), L-Valine and L-Methionine (L-Met) [9]. In addition, the transporter is involved in the uptake of L-3,4-dihydroxyphenylalanine (L-DOPA), gabapentin, and

the thyroid hormones, T3 and T4 [10], [11], [12], [13], [14], [15]. Even if the LAT1/CD98 complex has a broad substrate selectivity, the uptake of different amino acids shows complex kinetic parameters, which are further influenced by the local concentration gradient of each substrate [16].

Structurally, LAT1 has 12 transmembrane domains (TMs) organized in two main layers with both N- and C- terminals facing the intracellular compartment [7]. Moreover, a structure named LeuT fold, shared with prokaryotic transporters and consisting of 5 + 5 inverted repeat structure allows the symmetrical conformation of the protein. The LeuT fold is commonly present in different transporters and it is usually involved in the structural changes that are essential for the correct functioning of the transporter [17].

The interaction between LAT1 and CD98 takes place at the membrane level, and in the intra- and extra- cellular space. In this regard, it has been shown that isolated-LAT1 alone is not able to transport its substrates into the cells, indicating that CD98 is also essential for the transport activity of the complex. Of note, the interaction between LAT1 and CD98 is limited to one side of LAT1, in proximity to the highly conserved domains [18]. In particular, the main site of interaction is the disulphide bond between two specific cysteine residues, Cys211 and Cys164, on CD98 and LAT1 respectively (Fig. 1A-B) [13], [18]. In addition, polar interactions between the two proteins have been characterized on the extracellular side, in particular on conserved domains of CD98 (Fig. 1B) [19]. Moreover, a study showed the role of hydrophobic residues in the binding of CD98 to LAT2 [20], implying that a similar interaction might occur between CD98 and LAT1. Other important residues on LAT1 protein have been identified while characterizing its interaction with the non-selective LAT inhibitor 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH). In particular, 4 principal residues have been identified in the LAT1 binding pocket that are crucial for BCH binding, *i.e.* Phe252, Gly255, Ser66 and Gly65 (Fig. 1C) [18]. In this regard, the binding of BCH is allowed by the hydrophobic interaction of the drug primarily with Phe252, while there are also hydrogen bonds mainly with TM1 and TM6 of LAT1 [18].

Based on structural analysis, the proposed working model of LAT1/CD98 includes three main conformational states: inward-open, outward-occluded and outward-open, depending on the position and rearrangements of the core domain [19]. A recent *in silico* model suggested that the conformational rearrangements of the heterodimers take place only in a portion of the transporter. This model suggests that the α-helices of LAT1 protein may be the structures involved in the transition from inward to outward conformations, thus favouring substrate binding for the amino acidic exchange from the cells, or the uptake from the extracellular space [21]. The transition between inward and outward, due to the intracellular amino acid binding, might allow the rotation of the core domain. In this model, the core domain is specifically comprised of two main TM domains of LAT1, *i.e.* TM1 and TM6 which represent the gating elements. After the closure of the inward gate, it may be possible that the rotation of both TM1 and TM6 supports the binding of the amino acid in the extracellular space as well as the transition to the outward state. Indeed, with two rotations, TM1 and TM6 allow the release of the substrate, bound during the inward stage, into the extracellular space. The outward-open conformation favours the binding of ligands present in the extracellular space. On the other hand, the binding of the amino acidic substrate to the substrate pocket in the outward conformation allows changes into the outward-occluded structure [19]. Alternatively, a different access mechanism has been described, which is primarily based on the LeuT fold structure. In this model, the transporter presents an initial outward-open conformation (Fig. 1D) [10]. The binding of the amino acid in the extracellular matrix determines the transition to the inward-bound stage, favouring the release of the substrate into the cells. At this point, the inward-unbound gate can interact and bind intracellular amino acids (for example L-Gln) at higher concentrations into the cellular compartment. After the binding, an additional reorganization determines the switch from the inward to the outward state, thus allowing the extrusion of the amino acid from the cells into the extracellular matrix. Indeed, the LAT1-mediated transport involves the efflux of L-Gln, which is normally

available in the cells through the SLC1A5 (also known as Alanine-Serine-Cysteine Transporter 2, ASCT2) transporter activity [9], [22].

Another molecule that can stabilise and promote the correct functioning of LAT1/CD98 is cholesterol or cholesteryl hemisuccinate (CHS). Evidence suggest that lack of cellular cholesterol can affect the V_{max} (velocity of the enzyme-catalysed reaction at infinite concentration of substrate, V_{max}) of the LAT1-mediated transport into the cells. Moreover, cholesterol/CHS is required for the purification of the LAT1/CD98 complex. This suggests that cholesterol/CHS can also have a role in the stabilization of the complex at the membrane level [11]. A recent 3D structure of the LAT1/CD98 complex demonstrated that the addition of cholesterol to proteoliposomes containing LAT1/CD98 heterodimers is necessary to activate the L-Leu uptake [18]. Models for the putative working mechanism of the LAT1/CD98 heterodimers have been obtained through computational analysis [18]. These models have allowed a better understanding of the role of cholesterol and other putative accessory molecules. Computational analysis together with biochemical approaches confirm that cholesterol can effectively stimulate the activity of LAT1, specifically increasing the antiport of the amino acid L-His [23]. Cholesterol seems to mainly regulate the internal affinity of the transporter, promoting conformational changes in the heterodimer and favouring the inward open structure [23], and computational analysis suggests that the cholesterol-binding site can be proximal to an ATP binding site [23]. Furthermore, the addition of intraliposomal ATP to cholesterol can significantly increase the activity of LAT1/CD98, which suggests that ATP might have a role in increasing the transport rate, even if it does not influence substrate affinity and it is not fundamental for LAT1 activity [23]. Finally, no evidence of LAT1 enrichment in lipid rafts has been reported so far, even if CD98 seems to be enriched in lipid rafts upon virus infection in HeLa cells [24]. This enrichment could suggest that in certain circumstances, the LAT1/CD98 heterodimers might be recruited to act specifically in lipid rafts.

3. LAT1 in glioblastoma

Several studies have reported a significant increase in the expression levels of LAT1 in brain tumours, particularly in gliomas [4], [25], [26]. GBM is the commonest primary malignant brain tumour in adults and has a very poor prognosis (median overall survival 8-12 months) and fewer than 5% of patients survive for more than 5 years [27].

Histologically, GBM is a diffuse, infiltrative glioma of astrocytic lineage [28]. The majority of GBM are primary de novo tumours, but they can also develop from malignant transformation of a pre-existing low-grade glioma [29]. GBM harbour different genetic mutations, which largely determine the tumour prognosis. Mutations in the isocitrate dehydrogenase (IDH) gene are relevant genomic biomarkers in gliomas. Most GBM are IDH-wild type, and those that are IDH-mutant (~10%) have usually developed from pre-existing lower grade gliomas [30]. According to the '*5TH Edition of the WHO classification of CNS tumors*' (2021) gliomas can be genetically characterized by three major genomic alterations: IDH mutations, 1p/19q co-deletion and TERT promoter mutations [28]. TERT mutations are commonly observed in GBM and oligodendroglioma, but some GBM are negative for all three genetic alterations. Other relevant genomic variants found in gliomas include mutations of platelet derived growth factor receptor alpha (PDGFRA), over-expression of neuron-related genes, such as the epidermal growth factor receptors (EGFRs), variation in the phosphatase and tensin homolog (PTEN) and phosphatidyl inositol 3-kinase (PI3K) genes [31]. Based on the combination of these genomic biomarkers, The Cancer Genome Atlas (TCGA) reported four different classes of GBMs, namely, proneural, neural, classical and mesenchymal subtypes [31], which have different prognoses and that have the potential to personalize the pharmacological approach to GBM treatment.

Interestingly, all types of GBM consist of tumour cells derived from the astrocyte lineage growing in a heterogeneous tumour microenvironment. The latter includes different cellular types scattered throughout the tumour microenvironment, such as a mixture of immune cells, stromal cells, vascular endothelial cells and pericytes [32]. The immune component of GBM is highly complex with different types of myeloid cells, including resident microglia, bone-marrow derived macrophages (also known

as BMDMs), myeloid-derived suppressor cells (MDSCs), neutrophils and dendritic cells (DCs) [33]. The main component is composed of infiltrating microglia/macrophages, also known as glioma-associated microglia/macrophages (GAMs), which contribute to tumour growth and progression [34]. In general, GAMs represent almost 30-40% of the tumour mass of GBM [35]. Interestingly, cancer cells usually influence and reprogram the immune component of the tumour, in order to support their own survival, growth and functions [36], [37], [38]. GBM released factors direct GAMs' activation towards an immunosuppressive phenotype, by blocking the release of pro-inflammatory factors, essential for the anti-tumour immunity. Consequently, reprogrammed GAMs can sustain tumour growth. Accordingly, an inverse correlation between the number of GAMs and the overall survival of patients with recurrent GBM has been reported [39]. GAMs contribute to the recruitment of T cells and other peripheral immune cells at the tumour site, which might possibly block the cancer cells. Emerging evidence suggests that GAMs may exert different functions in different regions of the tumour, based on their origin (whether they are BMDM or microglial cells). However, their specific role in the GBM immune microenvironment has not been completely elucidated. For example, IDH-mutant GBMs generally present a reduced number of myeloid cells compared to IDH-wild type tumours [40]. The pro-inflammatory activation of these immune cells may promote anti-tumour effects and improve GBM prognosis [41].

3.1 LAT1 functions in GBM cancer cells

Under physiological conditions, LAT1 is primarily expressed in microvessels at the blood-brain-barrier (BBB). The latter is an interface that assures the optimal composition of brain interstitial fluid to sustain neuronal functions. The BBB is predominantly formed by endothelial cells and astrocytes that have two highly specialised basement membranes, the so-called "neurovascular unit" (NVU) (Fig. 2). The endothelium at the BBB is in constant contact with all the other CNS cellular components, *i.e.* pericytes, astrocytes, microglia, neurons through the NVU. Thus, the BBB regulates the uptake of energy substrates from the blood into the brain parenchyma, limiting the passage of

potentially harmful substances in the CNS [42]. Preclinical models suggest that LAT1 is located both at the luminal and abluminal membrane of the endothelial cells [43], [44], where it mediates the CNS uptake of nutrients as well as drugs [4], [17]. LAT1 is involved in transport across the BBB of the prodrug L-4-Chlorokynurenine (4-Cl-Kyn, also known as AV-101) [45], a novel drug candidate in clinical development for the treatment of major depression. Preclinical studies reported ketamine-like antidepressant effects of AV-101 in mice, without the side effects normally observed with ketamine [46]. 4-Cl-Kyn can readily cross the BBB through LAT1 [45] and once in the CNS, 4-Cl-Kyn can be converted to its active form by astrocytic enzymes, acting as a glutamate *N*-methyl-d-aspartate receptor antagonist [47]. Despite this, recent clinical trials have reported that 4-Cl-Kyn is poorly concentrated in the CNS and thus not effective, due to the insufficient brain concentration of its active metabolite, 7-chlorokynurenic acid (7-Cl-Kyna) [48].

In the context of GBM, studies have identified a significant over-expression and increased activity of LAT1 in the tumour cells, where it is emerging as a key regulator of their growth. LAT1 over-expression is associated with the glioma grade and prognosis [49]. In fact, both LAT1 and CD98 are upregulated in high grade rather than in low-grade gliomas and, more importantly, the expression of LAT1, but not of CD98, is associated with the malignant proliferation and tumour progression [49]. LAT1 is mainly expressed and located in the tumour core, particularly in the vascular endothelium and in the tumour cytoplasm [49], [50]. LAT1 is strongly upregulated at the capillary level at the brain-tumour interface [51], where the BBB is more permeable [4]. The increased activity of LAT1 is important functionally to the glioma cells due to its capacity to pump amino acids into the cells that can then act and support the regulation of signalling pathways, such as the mechanistic target of rapamycin (mTOR) kinase. Indeed, evidence in peripheral cancers reveals that the increased L-Leu influx, driven by LAT1 upregulation in malignant cells, support the activity of the mTOR pathway, which functions as a nutrient sensor and promotes tumour growth [52], [53]. In line with this, increased expression of LAT1 at the vascular endothelium suggests a predominant role in

angiogenesis and a potential role in the regulation of immune cell infiltration [4]. Furthermore, LAT1 is widely located at the plasma membrane and on the astrocytic processes of the cancer cells. The areas surrounding the tumour, consisting mostly of normal tissue, comprises infiltrating tumour cells with an intense LAT1 staining. This could suggest that the invasion of the normal brain tissue by the cancer cells may be dependent on LAT1 activity [26], [54].

Multiple studies have underlined the relevance of LAT1 overexpression in GBM, based on data derived from using different LAT1 substrates as tracers, such as L-Phe, L-DOPA, L-Tyr and L-Met. This approach has enabled the visualization of the GBM tumour mass through positron emission tomography (PET) or the development of possible anticancer strategies; in patients, xenograft models of human GBM and *in vitro* [55], [56], [57], [58], [59], [60], [61], [62], [63], [64], [65], [66], [67], [68], [69].The PET technique allows investigation of the tumour mass via the tracing of radiolabelled molecules specifically through the tumour. At the tumour margin the different tracers are specifically transported by LAT1 both *in vitro* [56], [57], [58], [60], [63], [64], [65], [66], [67], [68] and in GBM xenograft experimental models [58], [59] as well as in patients [59]. For example, the uptake of L-methyl-11C-methionine (^{11}C-Met), through LAT1, correlates to the GBM cell density in the active tumours [56]. PET studies have also shown a lack of direct correlation between ^{11}C-Met uptake and the percentage of LAT1 positive vessels in GBMs treated with anti-angiogenic agents [64]. Other tracers have also demonstrated LAT1 transport, including 6-^{18}F-fluoro-L-3,4-dihydroxy-phenylalanine (^{18}F-DOPA) [58], [68], O-(2-fluoroethyl)-l-tyrosine (FET) [60], O-(2-[^{18}F]-fluoroethyl)-l-tyrosine ([^{18}F]FET) [59], [62], [66], [68], 4-borono-2-[^{18}F]-fluoro-phenylalanine ([^{18}F]FBPA) [57], (S)-2-amino-3-[3-(2-18F-fluoroethoxy)-4-iodophenyl]-2-methylpropanoic acid (^{18}F-FIMP) [65], and 2-[^{18}F]-2-fluoroethyl-L-phenylalanine (2-[^{18}F]FELP) [66]. All these studies strongly support the use of LAT1-designed probes to visualize the tumour mass and to monitor changes in cell metabolism. Indeed, the uptake of these tracers was specifically linked to the metabolic re-arrangement of the GBM cells overexpressing the transporter. LAT1 expression levels

also correlate with ^{3}H-L-DOPA and ^{18}F-DOPA uptake both in human GBM cell lines and in patient tumour biopsies [58]. In addition, a recent preclinical *in vivo* study suggests that the use of 2-[^{18}F]FELP can discriminate between GBM tumour tissue and radiation necrosis [66], which is an ongoing clinical dilemma since they have a similar appearance on structural magnetic resonance imaging (MRI). Therefore the use of the 2-[^{18}F]FELP as a tracer could facilitate the differential diagnosis and allow for a better monitoring of these patients after treatment [66].

3.2 LAT1 functions in the GBM microenvironment

Both LAT1 and SLC1A5 are important regulators of the activity of different immune cells. LAT1 is expressed in T-cells, macrophages and other peripheral immune cells with several studies showing a critical role in the regulation of the immune system [70], [71], [72], [73], [74], [75], [76], [77]. LAT1 is primarily involved in the activation of immune responses to pathogens and in chronic inflammatory diseases. As summarized in Table 1, LAT1-null T cells, both $CD4^{+}$ and $CD8^{+}$, display reduced rates of proliferation and impaired differentiation into effector T cells and $CD4^{+}$ T regulatory cells (Tregs) [76]. Moreover, in the presence of pro-inflammatory stimuli, pharmacological or genetic inhibition of LAT1 can reduce T cell activation and the release of pro-inflammatory cytokines [78]. Similarly, pro-inflammatory stimulation of human monocyte-derived macrophages (hMDMs) is associated with increased expression and function of the LAT1 transporter, followed by increased amino acid influx, particularly L-Leu. The augmented availability of L-Leu within the cells can promote the activation of the mTOR kinase within the complex 1 (so called mTORC1), thus facilitating a metabolic switch and cell activation [79]. Increased influx of L-Leu, via upregulation of LAT1, has also been linked to the activity of mTORC1 and cell activation in natural killer cells [80]. Finally, the inflammatory stimulation of B-lymphocytes is also associated with the overexpression of LAT1 that leads to an increase in L-Leu influx and mTORC1 activation. In contrast, inhibition of the LAT1 transporter in B-lymphocytes results in the disruption of the mTORC1 pathway, inhibition of cell differentiation, reduced production of immunoglobulin G and inflammatory cytokines [74].

LAT1 may also be important in the recruitment and the activation of immune cells in the GBM microenvironment (for a schematic diagram, Fig. 3). Interestingly, a direct correlation between hypoxia in the tumour microenvironment and LAT1 overexpression, via hypoxia-inducible factor 2α, has reported in malignant cells [81]. In the immunosuppressive microenvironment, GBM cells and GAMs compete for the uptake of amino acids and nutrients. In this scenario, the overexpression of different nutrient transporters, including LAT1, seems to restore a correct influx of nutrients and substrates in GAMs, increasing the anabolic pathways to prevent GBM progression [82].

A number of studies have reported that LAT1 is an important transporter involved in differentiation and activation of peripheral macrophages. However, less is known about LAT1 expression and function in microglial cells. These cells are the specific myeloid cells of the brain parenchyma and are the main cells involved in immunity within the CNS.

Even if microglia and BMDMs maintain some common regulatory factors and phenotypic markers, they have different characteristics and functions in the brain parenchyma [38]. Microglia are derived from myeloid progenitors developed in the yolk sac, from where they migrate to the CNS at the embryonic stage [83]. This means that microglial cells are different from peripheral macrophages which is required due to their highly specialised role in the regulation of a number of neuronal functions and to help maintain normal homeostasis in the CNS [84], [85]. LAT1 is expressed in murine microglial cells [86], [87], [88], [89], as well as in mouse primary astrocytes [86], [89], [90] and primary neurons [86], [89]. However, single cell RNA-sequencing (RNA-seq) data has shown strong LAT1 expression in human microglial cells [91]. Interestingly, no significant modulation in LAT1 expression and function has been reported at the BBB in an astrocytic model of lipopolysaccharide (LPS)-induced inflammation compared to control conditions. Indeed, in this model, the functional LAT1-mediated uptake of L-Leu was not influenced by the inflammatory process [90].

LAT1-mediated uptake of L-Leu has been observed in mouse microglia, *i.e.,* in BV2 cells [86]. Comparing the cellular uptake into mouse primary neurons, astrocytes and microglia, the uptake seems to be less efficient in the microglial cells. Nevertheless, the activity of the LAT1 transporter in microglia is present and effective in increasing the cellular uptake of different drugs, particularly anti-inflammatory drugs [89]. Specifically, it has been reported that adding LAT1 chemical moieties in a prodrug approach leads to inhibition of the L-Leu uptake into the cells. Furthermore, all the anti-inflammatory pro-drugs, once delivered to microglia through LAT1, can inhibit the prostaglandin E_2 release, a marker of LPS-activated BV2 microglial cells [89].

Notably, LAT1 expression and function have been characterized using primarily mouse models of microglia, however, human microglia have important differences compared to the mouse or rat model [92]. We made use of a novel human microglial cell line, the C20 cells [93], [94], to begin to investigate the role of LAT1 in human microglial cells. In preliminary experiments, we observed that the C20 microglial cells express LAT1, but the expression level was not significantly modified by exposure to pro-inflammatory cytokines. Consistently, we observed a relevant uptake of different amino acids, L-Phe and L-kynurenine (L-Kyn), under basal conditions (*unpublished observations*). L-Phe is one of the primary amino acids transported by LAT1, whilst L-Kyn is a tryptophan metabolite and LAT1 substrate, which could influence microglial activity [95]. L-Kyn seems to have crucial immunomodulatory effects in T cells, as reported below. On C20 microglial cells, we observed a major transport of both L-Phe and L- Kyn through LAT1 under basal conditions. Yet, the uptake of both amino acids was not modulated by the pro-inflammatory stimulus (*unpublished observations*). Microglial cells represent a major component of the GBM microenvironment and the role of LAT1 in microglial cells in the context of GBM warrants further investigation.

Interestingly, a direct correlation between LAT1 and the indoleamine 2,3-dioxygenase (IDO) enzyme has been shown in human corneal endothelial cells [71]. IDO is an enzyme that can degrade L-Trp into L-Kyn. The latter can function as an aryl hydrocarbon receptor (AHR) ligand [96], a relevant

signalling pathway in the modulation of the differentiation of activated $CD4^+$ T cells into immunosuppressive regulatory T cells. It has been shown that only activated T cells, by T cell receptor (TCR) and/or inflammatory stimuli, have the ability to transport L-Kyn into the cells, via the activity of LAT1 leading to the downstream activation of the AHR signalling pathway [96]. IDO activity is also upregulated in a number of cancer types, including in the periphery and in the CNS [97]. In fact, L-Kyn can favour brain tumour progression, by limiting the anti-tumour activity of the immune cells and promoting cancer cell survival and migration [98]. Upregulation of both LAT1 and IDO has been reported in gliomaspheres obtained from biopsies of GBM patients leading to increased intracellular uptake of tryptophan via LAT1 [99]. Accordingly, lower levels of tryptophan have observed in the tumour microenvironment, with consequences on the modulation of cell metabolism at this level [99]. Indeed, one of the mechanisms by which GBM cells can proliferate and escape the immune surveillance is to convert tryptophan into L-Kyn instead of serotonin. This process is mediated by both IDO and Tryptophan 2,3-dioxygenase (TDO) enzymes in GBM cells that leads to a block in anti-cancer immune responses, thus favouring and boosting immune tolerance [100]. In this context, in vitro GBM cells treated with L-Kyn displayed activation of phosphoinositide-3-kinase-protein kinase/ protein kinase B (PI3K/Akt) signalling pathway, which results in an inhibition of the protein phosphatase PP2A. This, in turn, causes an increase in growth and cell proliferation. Therefore, it seems that there is a direct correlation between increase in methionine and L-Kyn uptake into the GBM cells and the upregulation of oncogenic signalling, thus inducing cancer cell survival and proliferation [100].

4. LAT1 pharmacological inhibitors

Despite the high interest in the modulation of LAT1 activity as a pharmacological target in cancer, only a few compounds are currently available for testing. Different compounds derived from L-Phe, L-Tyr, L-Leu and aromatic amino acids have been studied for their high affinity for LAT1. In addition, gabapentin and thyroid hormones (T3 and T4) have been tested as LAT1 inhibitors. These

studies have improved understanding of the structural and molecular characteristics required for the design of novel LAT1 inhibitor drugs [3]. The best characterized examples of LAT1 pharmacological inhibitors are BCH and the *O*-[(5-Amino-2-phenyl-7-benzoxazolyl)methyl]-3,5-dichloro-L-tyrosine dihydrochloride (KYT-0353, also known as JPH203).

BCH is a non-selective competitive L-system inhibitor, which targets all the large amino acid transporters. This drug was shown to inhibit the uptake of different amino acid (L-Leu, L-Phe, and L-alanine) in rat mammary gland cells [101]. However, this compound does not distinguish if the observed pharmacological effects are mediated by LAT1 and/or by the inhibition of other transporters. In addition, the rat mammary tissue expresses other BCH sensitive transporters, *i.e.* the $B^{0,+}$ system [101]. Interestingly, BCH reduced the cell growth of different types of cancers *in vitro*, including the epidermoid carcinoma, osteosarcoma, and glioma [102]. The antiproliferative effects of BCH were associated with increased G1 cell cycle arrest of the cancer cells, due to the indirect inhibition of the mTORC1 pathway [103]. In addition, BCH induced apoptosis of cancer cells, as indicated by the activation of caspase-3 and caspase-7-dependent pathways [102]. Consequently, the drug significantly reduced the volume of the tumour mass *in vivo*, in xenograft models of cholangiocarcinoma and oesophageal cancer [103], [104]. Knocking down LAT1, with specific short hairpin RNA, was associated with a reduction of the number of Ki-67 expressing cells and reduced tumour growth in a xenograft model of castration-resistant prostate tumour, thus supporting the hypothesis that the inhibitory effect of BCH was due to LAT1 inhibition [105]. In line with these findings there is a reported correlation between LAT1 over-expression and other markers, such as Ki-67 and CD34, which normally indicates a poor outcome in patients with biliary tract cancer after surgery [104]. Additionally, a significant correlation between the proliferation marker Ki-67 and LAT1 has been discovered in case of atypical adenomatous hyperplasia (AAH) of the lung. In fact, significant higher levels of LAT1 protein were found in AAH with high Ki-67 labelling index, compared to LAT1 negative AAH [106].

In addition to data on peripheral cancers, BCH effects have also been investigated using *in vitro* models of human glioma [25]. In this case, BCH was used on different glioma cell lines and L-Leu uptake as well as inhibition of cell growth were studied. BCH treatment dramatically reduced the uptake of L-Leu in LAT1 positive glioma cell lines and, more importantly, blocked cell growth in a dose dependent manner. Interestingly, when used on normal human astrocytes, BCH did not exert any cytotoxic effect, suggesting that the over-expression of LAT1 may be a relevant mechanism underlying BHC cytotoxicity in cancer cells [25]. Considering its inhibitory effects on tumour growth, BCH has been used as a "positive control" to develop and compare other types of therapeutics, as for example, a derivate of thyroid hormone, 3-iodo-L-tyrosine characterized as a LAT1 inhibitor. Both BCH and 3-iodo-L-tyrosine were able to reduce GBM cell proliferation *in vitro*. The latter was not able to compete with L-Leu mediated transport, whereas BCH treatments caused a marked reduction in L-Leu uptake [107].

The lack of selectivity of BCH towards different transporters, as well as its low efficacy at low doses, may limit its clinical use. Therefore, a more suitable inhibitor for *in vivo* testing has been recently patented and developed. The compound is named KYT-0353 and acts specifically on LAT1, without any significant inhibitory effects on the other L-system transporters [108]. KYT-0353, also known as JPH203, is a competitive tyrosine analogue inhibitor, blocking the uptake of several substrates through LAT1, although the exact molecular mechanisms remain unknown. This drug was developed from the combination of two other drugs, and was first tested in a model of human colorectal adenocarcinoma for its effects in blocking the L-Leu uptake into the cells [109]. A strong inhibition of L-Leu uptake was observed in cells treated with JPH203, together with significant growth inhibitory effects, both *in vitro* and *in vivo* [109]. Later, the inhibitory activity of JPH203 was confirmed in different types of tumours, such as thyroid cancer [110], [111], colorectal cancer [109], [112], oral and renal cell carcinoma [113], [114]. Interestingly, a marked inhibitory effect of JPH203 was also observed in certain CNS cancers, *e.g.,* medulloblastoma [115]. Particularly, it seems that

both the JPH203 and its bio-converted acetylated form (N-acetyl-JPH203, NAc-JPH203) are substrates for organic anion transporters [115], [116]. Uptake of the drug through these transporters can be an important mechanism for the absorption and distribution of the drug in either forms, acetylated or not [116], [117]. Even if preclinical models suggest that the uptake of NAc-JPH203 can be due to other types of transporters in the process of hepatic elimination, the inhibitory action of JPH203 remains selective for the LAT1 transporter, without affecting LAT2 activity [116]. Moreover, the JPH203 inhibitory effect on LAT1 is sufficient to disrupt the amino acid homeostasis in the tumour microenvironment. JPH203 can block the mTORC1 pathway, reducing the survival and proliferation of two different types of medulloblastoma cell lines. Treatment of malignant cells with this LAT1 inhibitor can significantly inhibit proliferation, induce apoptosis , leading to an efficient and complete anticancer effect [115].

In preliminary experiments, JPH203, tested in a wide range of concentrations (1 nM - 10 μM), did not significantly affect C20 microglial viability both under basal and inflammatory conditions (*unpublished observation*). This suggests that the upregulation of the LAT1 transporter, while promoting cancer cell growth, is also a necessary condition to observe the relevant cytotoxic effects of LAT1 inhibitors. Consistently, JPH203 significantly inhibited the uptake of L-Phe and L-Kyn, but did not affect microglial IL-6 release (*unpublished observation*).

4.1 Relevance of LAT1 in clinical therapy for GBM: pharmacological inhibitors and other strategies

Data from one Phase 1 clinical trial testing the safety of JPH203 in humans (Clinical trial registration: UMIN000016546) [118] were recently published [119]. The study enrolled Japanese patients with solid tumours, including colorectal, pancreatic and biliary tract cancer (BTC). The latter are resistant and aggressive types of cancers that seem to respond to JPH203 treatment. The study enrolled 17 patients, who had failed standard oncological treatments. The study provides limited data on the

follow-up of these patients due to poor cancer prognosis, and the heterogeneity of both cancer types and previous treatments are confounding factors in this trial. One patient with BTC achieved a partial response receiving 12 mg/m^2 JPH203, the lowest dose tested, and continued JPH203 treatment for two years with beneficial effects. Thus, the clinical trial showed antitumor effects of JPH203, plus it defined the safe and tolerated doses in humans. The drug is acetylated by the N-acetyltransferase 2 (NAT2), which converts JPH203 to its inactive metabolite Nac-JPH203. NAT2 is a highly polymorphic phase 2 enzyme, thus its genotype/phenotype appears to be relevant in determining the efficacy and the toxicity of JPH203 treatments. A phase II clinical trial is currently ongoing to further evaluate the response to JPH203 in patients with advanced BTCs and the clinical outcome in relation to the acetylator phenotype (UMIN000034080) [119, 120]. The clinical development of JPH203 is in its infancy and warrants further investigation, including in brain tumours.

Other indirect pharmacological approaches for the treatment of GBM are based on the delivery of cytotoxic drugs into the malignant cells, via the LAT1 transporter (Fig. 3). Several studies have explored this possibility, using liposomes or nanoparticles enriched with LAT1 substrates with some promising results in GBM models [121], [122], [123]. Since the LAT1 transporter is highly expressed in GBM, the delivery of anticancer drugs was followed by significant inhibition of the tumour growth. In line with data on JPH203, the upregulation of LAT1 in GBM cancer cells can be used to specifically induce cytotoxicity and apoptosis in malignant cells, while preserving normal astrocytes. One study showed that using L-Phe coupled nanoparticles loaded with doxorubicin (a chemotherapy drug), it is possible to induce cancer cell cytotoxicity *ex vivo* [121]. Indeed, the uptake of these nanoparticles by the C6 glioma cell line was mediated by LAT1, given that nanoparticles without L-Phe coupling were not internalized. Once internalized, the release of doxorubicin into the glioma cells induces cytotoxicity, and is a promising approach to treat brain tumours [121]. Similarly, the same cell line was exposed to glutamate modified docetaxel-loaded liposomes [122]. *In vivo* results confirmed that these modified liposomes can significantly penetrate the BBB in comparison to

unmodified ones displaying higher cytotoxicity [122]. In line with previous studies, these results also suggest that the glutamate modified liposomes could effectively penetrate the BBB, thus accumulating into the brain via LAT1.

Another interesting example of the crucial role of LAT1 in GBM, and how it can be used for different therapeutic approaches, is the drug delivery of nanomedicines into a mouse model of GBM, using various drugs carried by liposomes coated with L-DOPA functionalized amphiphile (Amphi-DOPA) [123]. The antitumor agent that has been tested is WP1066, a STAT3 inhibitor, in a liposomal formulation of Amphi-DOPA. The drug was administered in combination with DC-targeting liposomes containing a mannose-mimicking shikimoyl moiety directed to mannose receptors, which are overexpressed in DCs. DC-based immunotherapy is often combined with conventional targeted therapy to enhance the antitumor effects [123]. The *in vitro* and *in vivo* results support the finding that, by targeting LAT1 transporter, there is an effective uptake of the drug-loaded liposomes in the GBM cells followed by targeted release of the cytotoxic drug in the tumour cells. The study reported increased overall survival in the GBM mouse model, thus suggesting the potential use of this LAT1-mediated strategy in GBM patients [123]. Finally, another pre-clinical approach has indirectly underlined the importance of LAT1 in the process of cancer metastasis. Targeting ribozyme controlled HSVtk gene (human herpes simplex virus thymidine kinase type 1 gene) via overexpression of a small non-coding RNA (miR-145) has been reported. This causes a significant downregulation in different "metastasis related genes" that includes LAT1 [124]. This strategy allowed for the downregulation of the expression of these metastasis related genes both *in vitro* and *in vivo* including in a human GBM xenograft model. In addition, concomitant inhibition of both migration and invasion of normal brain parenchyma was observed in these experimental models of GBM.

The overexpression of LAT1 has used to develop a specific GBM radiotherapy, namely boron neutron capture therapy (BNCT). The anticancer effects of BNTC are mediated by the boron irradiation of

the cancer cells with neutron beams [125]. The intracellular accumulation of boron in the GBM cells is assured by the use of boronophenylalanine (BPA) or similar derivatives. These are combined molecules of boron and L-Phe or other LAT1 substrates that can be accumulated into the cells via LAT1 uptake [55], [58], [61], [69]. BPA uptake was specifically due to the system L transporters, since it was inhibited by pre-treatment with both L-Phe and LATs' pharmacological inhibition [55]. In addition, a BPA analog, the 4-borono-2-18-F-fluoro-phenylalanine (^{18}F-FBPA) has been studied and compared to ^{11}C-Met on different human glioblastoma cell lines. The ^{18}F-FBPA is the most specific substrate for LAT transporters, whereas ^{14}C-Met uptake is also sustained by other amino acid transporters, *i.e.* system ASC and system A [57]. Additionally, *in vitro* BPA uptake is more effective in hypoxic conditions in GBM cells [61]. A recent study showed that this uptake is specifically due to LAT1 overexpression and over-activity in GBM cells [69]. Thus, the upregulation of LAT1 in GBM cells may be key for using BNCT as a therapeutic strategy.

The existing research into LAT1 has been focused on GBM cells. LAT1 is overexpressed in GBM cells, and this upregulation allows direct inhibition and/or targeted delivery via liposomes/nanoparticles to specifically block this transporter, leading to beneficial effects against the tumour itself. The microenvironment is also emerging as an additional target for antitumor therapies. In the context of GBM, considering that GAMs express LAT1, it may be possible that LAT1 is involved in the modulation of GAM biology as well. In this regard, it has been shown that microglial cells can efficiently uptake synthetized nanoparticles coated with L-DOPA through LAT1 [87], and this process was not associated with increased inflammation or cytotoxicity into the microglial cells. These results further support the approaches of targeting microglial cells, or macrophages, within the tumour microenvironment by liposomes or nanoparticles coated with LAT1 substrates. These data were obtained using a murine microglial model (N9 cell line) and not in the context of the GBM tumour. However, this approach can potentially be exploited to regulate the activity of GAMs within the tumours and redirect their activation towards an anti-tumour response. One possible limitation

could be the higher expression of LAT1 in the malignant cells in comparison to GAMs, although this aspect needs further investigations.

It is possible that the microglial LAT1 is modulated in its activity and/or expression by the GBM cells, through some "feedback signals". The modulation of this transporter in peripheral immune cells could be consistent with the idea that LAT1 would be crucial in the activation of these immune cells in the presence of malignant cells. Therefore, LAT1-mediated strategies for treating GBM should include investigation of the effects on GAMs, to determine whether this can establish a pattern of activation that favours their anti-tumour activity.

5. Research gaps and future prospective

LAT1 has a clear role in mediating cell growth and proliferation in the context of different tumours, including GBM. However, some research gaps remain and need to be addressed in order to further expand current knowledge on this transporter and foster the development of novel and more effective inhibitors and/or anti-tumour strategies via targeting of this transporter. For example, the LAT1 protein conformation and its regulatory residues have been studied primarily with structural analysis. It might be possible that there are additional key residues in the LAT1 protein, which can facilitate the characterization of novel strategies for modulating LAT1 functions. Novel drugs might be discovered by targeting different sites of interaction in the functional LAT1/CD98 heterodimers. Considering the central role of LAT1 in GBM progression, future studies should include the characterization of the structural functioning of this transporter in the context of GBM, including the testing of novel specific inhibitors in this tumour type with studies aiming to target both the malignant cells and the infiltrating immune cells, particularly GAMs.

In the context of CNS tumours, LAT1 has been currently studied in relation to its overexpression linked to the glioma grade and to its function in supporting cancer cell growth. Only a limited number of studies have reported comprehensive results on the efficacy of LAT1 inhibitors in pre-clinical

models of GBM. Indeed, even if different molecules and drugs are being discovered as LAT1 inhibitors, these drugs need to be tested in more complex pre-clinical models and in specific clinical setting to obtain robust data for their clinical use, particularly since to date, there has been only one clinical trial in humans on the safety of JPH203 in solid organ tumours. BCNT may provide a novel and effective strategy to inhibit GBM cell growth via LAT1, however data are limited to *in vitro* models of human GBM, with only one study testing BCNT on GBM xenografts. More complex and relevant pathological models are needed to further characterize the BCNT approach.

Of note, pharmacokinetics data on LAT1 inhibitors are limited to the clinical trial using JPH203 in solid tumours. There are no published data on the BBB permeability of this inhibitor, which would help to predict its efficacy in the treatment of CNS pathologies. Considering the impact of the BBB on drug brain distribution, the dose and the treatment schedule of JPH203 for brain tumours is likely to be different.. Moreover, the relevance of LAT1 in the infiltrating immune cells has not been investigated so far in the context of GBM. Given that the disruption of the BBB in cases of GBM allows the recruitment of peripheral cells and that these, together with resident microglia, can favour cancer growth, additional studies on LAT1 transporter in these cell types are necessary. These studies will allow for a better understanding of the role of LAT1 in the GBM microenvironment and disease progression. In this regard, most studies have investigated the direct role of LAT1 in GBM cancer cells, showing that inhibition of this transporter, particularly because it becomes overexpressed in malignant cells, can modulate the amino acids supply and consequentially the growth of the tumour cells. Little is known about the role that LAT1 may have in reprogramming the immune component of the GBM tumour. In line with RNAseq data, we confirmed that LAT1 is expressed in human microglial cells *in vitro*. Our preliminary data suggest that the expression of LAT1 is not influenced by pro-inflammatory stimulation. These studies should be further expanded by testing, for example, the effects of GBM-released factors on human microglial cell activation, to mimic the conditions occurring *in vivo* during the development of GBM. In addition, it is necessary to expand the

knowledge on the expression, function and regulation of LAT1 in the GBM associated immune cells. The link between LAT1 and mTOR-dependent activation of immune cells suggest that modulation of LAT1 in immune cells could be a winning strategy for brain cancer treatment.

Figure Legends:

Figure 1: Structure and mechanism of LAT1

(**A**) A schematic representation of the functional LAT1/CD98 heterodimer. LAT1 (SLC7A5) is an antiporter that heterodimerize with CD98 (SLC3A2) via disulphide bonds. (**B**) Side on view of LAT1/CD98 heterodimer. LAT1 is represented in red and CD98 in green. Generated from PDB 6irt [19]. (**C**) Important residues at LAT1 binding pocket with BCH bound. BCH 2D chemical structure is shown in the Figure. Generated from PDB 6irt [19]. (**D**) Mechanism of LAT1 mediated transport via the alternative access model.

Figure 2: LAT1 at the BBB

Schematic representation of LAT1 at the BBB level, showing its expression in the different cell types. The neurovascular unit (NVU) is mainly composed by endothelial cells, pericytes, astrocytes, basement membranes and the extracellular matrix. The NVU regulates the influx of nutrients to the CNS and represents a protective barriers. Cells within the NVU are in contact with parenchymal microglia and neurons, thus regulating their functions. LAT1 in endothelial cells favours the uptake of EAAs from the micro blood vessels to the brain, thus assuring an optimal composition of brain interstitial fluid. LAT1 is also expressed in astrocytes and microglia. Examples of amino acids (AAs), transported through LAT1 into the CNS, and drugs, that can interact/inhibit LAT1 activity, have been reported. Chemical structure of BCH, 4-Cl-Kyn and JPH203 have been reported. BCH and JPH203 structures were taken from PubChem Compound Summary (for CID 115288, 2-Aminobicyclo[2.2.1]heptane-2-carboxylic acid: https://pubchem.ncbi.nlm.nih.gov/compound/2-Aminobicyclo_2.2.1_heptane-2-carboxylic-acid; for CID 122553374, JPH203 Dihydrochloride: https://pubchem.ncbi.nlm.nih.gov/compound/JPH203-Dihydrochloride).

Figure 3: LAT1 as a pharmacological target in GBM

Schematic representation of the LAT1 role in GBM. Possible modulation of LAT1 activity in cancer cells, through LAT1 inhibitor JPH203 and LAT1-mediated influx of anticancer drugs, and in GAMs, are reported. The chemical structure of JPH203 is also presented in the Figure.

Declaration of Interests

The authors declare no conflicts of interests.

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List of abbreviations

L-Kyn, L-Chlorokynurenine; 7-Cl-Kyna, 7-chlorokynurenic acid; AAH, atypical adenomatous hyperplasia; ABC, ATP-binding cassette; AHR, aryl hydrocarbon receptor; Akt, Protein kinase B (PKB), also known as Akt; ATP, Adenosine triphosphate; BBB, blood brain barrier; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; BMDMs, bone-marrow derived macrophages; BNCT, boron neutron capture therapy; BPA, boronophenylalanine; CD, cluster of differentiation; CHS, cholesteryl hemisuccinate; CNS, central nervous system; DCs, dendritic cells; EAAs, essential amino acids; EGFRs, epidermal growth factor receptors; GAMs, glioma-associated microglia/macrophages; GBM, glioblastoma; hMDMs, human monocyte-derived macrophages; HSVtk gene, human herpes simplex virus thymidine kinase type 1 gene; IDH, isocitrate dehydrogenase; IDO, indoleamine 2,3-dioxygenase; Ki-67, also known as Ki-67 or MKI67 Marker Of Proliferation Ki-67; KYT-0353, also known as JPH203 O-[(5-Amino-2-phenyl-7-benzoxazolyl)methyl]-3,5-dichloro-L-tyrosine dihydrochloride; LAT1, Large-Type amino acid transporter 1; L-DOPA, L-3,4-dihydroxyphenylalanine;L-Gln, L-glutamine; L-His, L-Histidine; L-Leu, L-Leucine; L-Met, L-Methionine; L-Phe, L-Phenylalanine; LPS, Lipopolysaccharide; L-Trp, L-Tryptophan; L-Tyr, L-Tyrosine; mTOR, mechanistic target of rapamycin kinase; NAT2, N-acetyltransferase 2; NVU, neurovascular unit; PDGFRA, platelet derived growth factor receptor alpha; PET, positron emission tomography; PTEN, phosphatase and tensin homolog; RNA-seq, RNA-sequencing; SLCs, solute carriers; STAT3, Signal transducer and activator of transcription 3; TCR, T cell receptor; TDO, Tryptophan 2,3-dioxygenase; TMs, transmembrane domains; Tregs, T regulatory cells

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Graphical Abstract

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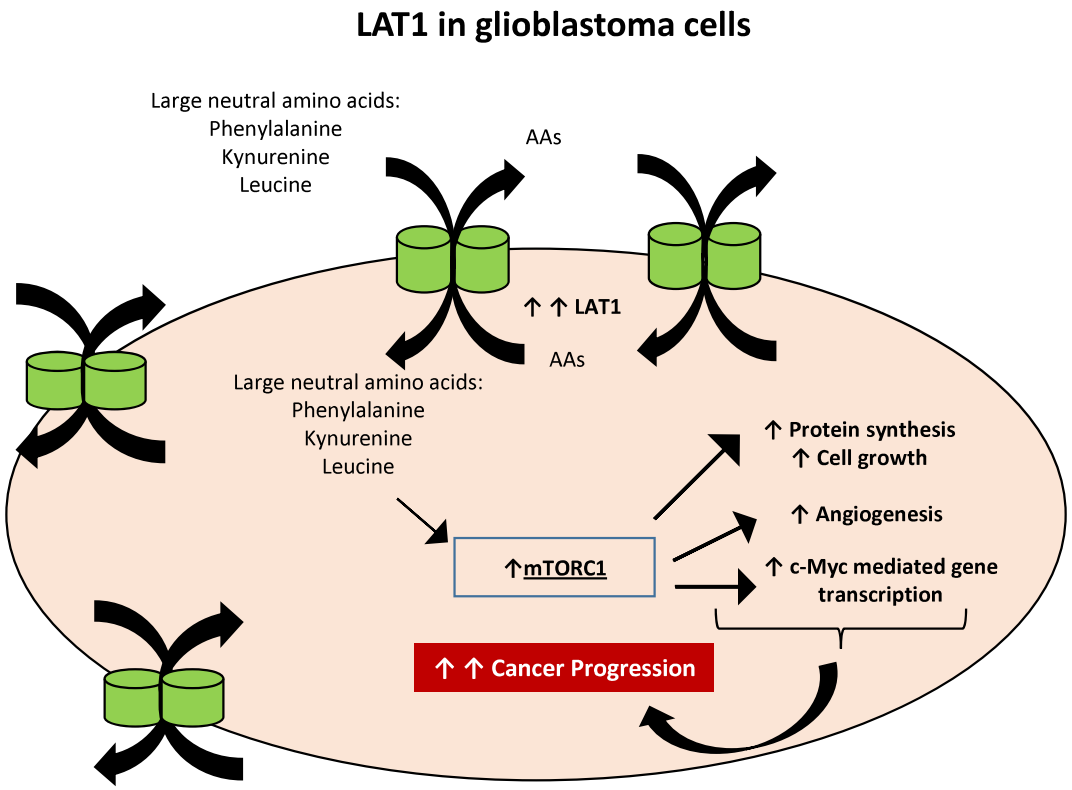

Authors' contributions:

NC carried out the literature review and wrote the first draft of the manuscript. NC carried out preliminary experiments on the human microglial C20 cell line. DD supervised the experiments, data analysis and the literature review. CDR contributed to data analysis and manuscript editing. CDR, MDJ and DD contributed to design, writing and revision of the manuscript. All authors read and approved the final manuscript.

Declarations of interest

None.

The authors declare that the research was carried out in the absence of any commercial or financial relation that could be taken as a potential conflict of interest.

Table 1: LAT1 in the peripheral immune system.

Immune cell types	LAT1 modulation and tracer/aa used for activity evaluation	Treatments	Effects	Refs
T cells ($CD4^+$ and $CD8^+$)	LAT1-null T cells (L-Leu)	To generate CTLs, T cells were activated with TCR activation or anti-CD3 antibody. To generate TH1, TH17 and Treg cells, CD8+ T cells were depleted from lymph node using CD8 depletion kit and treated with cytokines (IL-12 and IL-2 for TH1; IL-6, IL-1β, TGF-B and FICZ for TH17; TGF-β1 and IL-2 for Tregs). CD4 cells and APCs were cultured in the presence of anti-CD3 and anti-CD28 and cytokines	- ↓rate of proliferation - impaired differentiation into effector T cells and $CD4^+$ T regulatory cells (Tregs) - inhibition of mTORC1 activation - impaired c-Myc expression	**[76]**
T cells	Pharmacological (JPH203) or genetic inhibition of LAT1	anti-CD3 and anti-CD28 antibodies	- ↓ T cell activation - ↓ NFκB activity - ↓pro-inflammatory cytokine production and release - ↑ DNA damage-inducible transcript 3	**[78]**
T cells		inflammatory stimulation (TCR stimulation with CD3 monoclonal antibody and anti-CD28)	- ↑ LAT1 activity - ↑ L-Kyn influx	**[96]**
hMDMs	LAT1 knock down	pro-inflammatory stimulus (piperine, LPS treatments)	In response to pro-inflammatory stimulus: - ↑ expression and function of the LAT1 transporter - ↑ mTORC1 activity	**[79]**

Immune cell types	LAT1 modulation and tracer/aa used for activity evaluation	Treatments	Effects	Refs
			- ↑ hMDMs inflammatory responses After LAT1 knock-down: - impaired the activation of mTORC1, metabolic switch and cell activation	
NK cells		pro-inflammatory stimulus (IL-2, IL-12, IL-18, LPS treatments)	- ↑ LAT1 expression and activity - ↑ L-Leu influx - ↑ mTORC1 activation	**[80]**
B lymphocytes	Pharmacological inhibition of LAT1 (BCH)	pro-inflammatory stimulus (CpG stimulation)	In response to pro-inflammatory stimulus - ↑ LAT1 expression and activity - ↑ L-Leu influx - ↑ mTORC1 activation After LAT1 inhibition: - ↓ mTORC1 activity - ↓ cell differentiation - ↓ production of immunoglobulin G (IgG) and inflammatory cytokines	**[74]**
T cells and human corneal endothelial cells (interaction used as a model of corneal transplantation)		pro-inflammatory stimulus (IFNγ+TNFα treatments)	In human corneal endothelial cells: - ↑ LAT1 activity - ↑ IDO expression and activity - ↑ degradation of L-Trp into L-Kyn - ↑ extracellular concentrations of L-Kyn, In human T cells: - ↑ apoptosis (in response to ↑ L-Kyn)	**[71]**

Abbreviations: hMDMs, human monocyte-derived macrophages; LAT1, L-Type Amino Acid Transporter 1; LPS, the bacterial endotoxin lipopolysaccharide; mTORC1, the mechanistic target of rapamycin kinase within the complex 1; NK, natural killer cells; TCR, T-cell receptor; CTLs, Cytotoxic T lymphocytes; CD8 cytotoxic T cell; CD8, CD8-positive cytotoxic T cells; CD4, CD4-positive cytotoxic T cells; APCs, Antigen-Presenting Cells; CD3, cluster of differentiation 3; CD28, cluster of differentiation 28; Tregs, CD4+ T regulatory cells; TH1, T helper type 1 (Th1) cells; TH17, T helper 17 cells (Th17); TGF, transforming growth factor; FICZ, 6-formylindolo[3,2-b]carbazole; L-Kyn, L-Kynurenine; IL-, interleukin; IFNγ, interferon-γ; TNFα, tumour necrosis factor α; IDO, indoleamine 2,3-dioxygenase; L-Trp, L-Tryptophan; JPH203, *O*-[(5-Amino-2-phenyl-7-benzoxazolyl)methyl]-3,5-dichloro-L-tyrosine dihydrochloride; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid.

Figure

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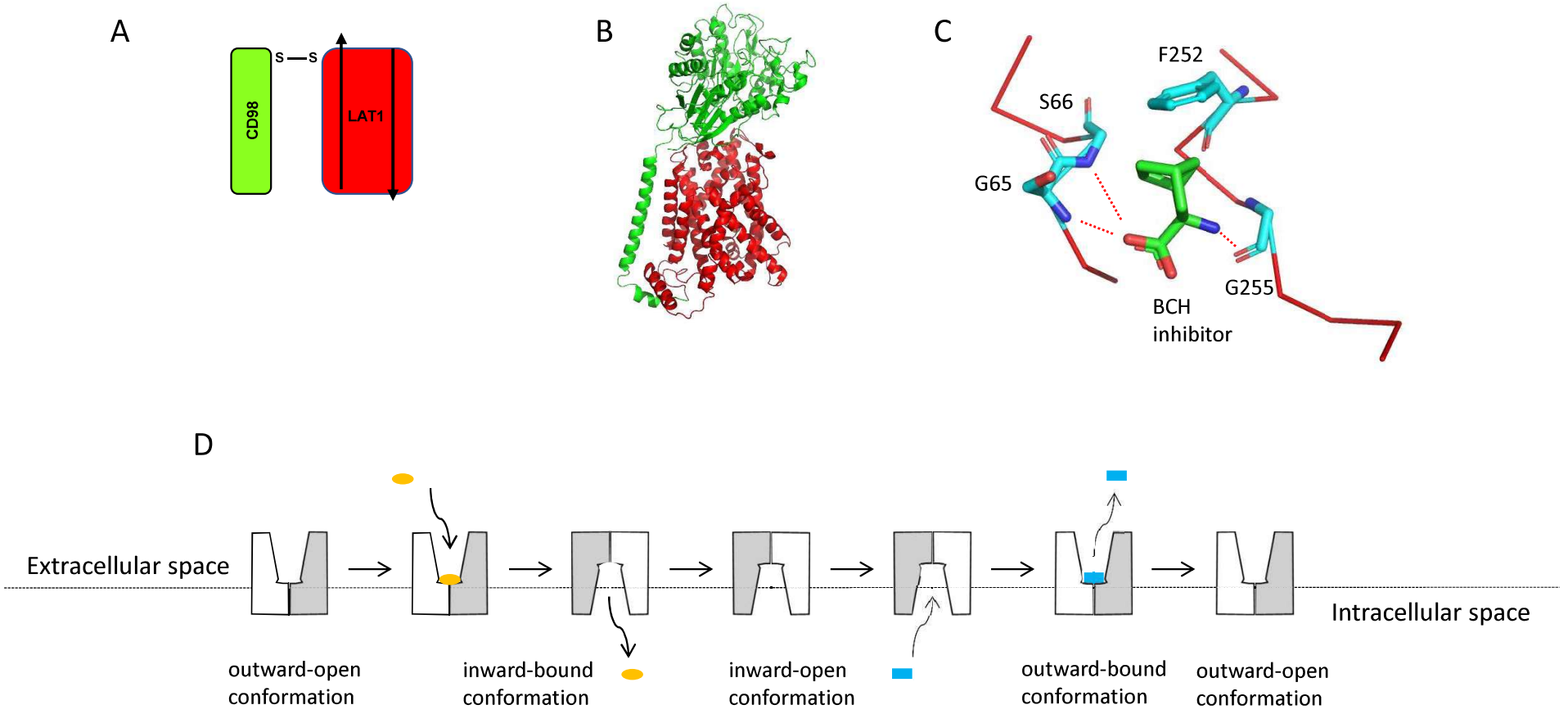
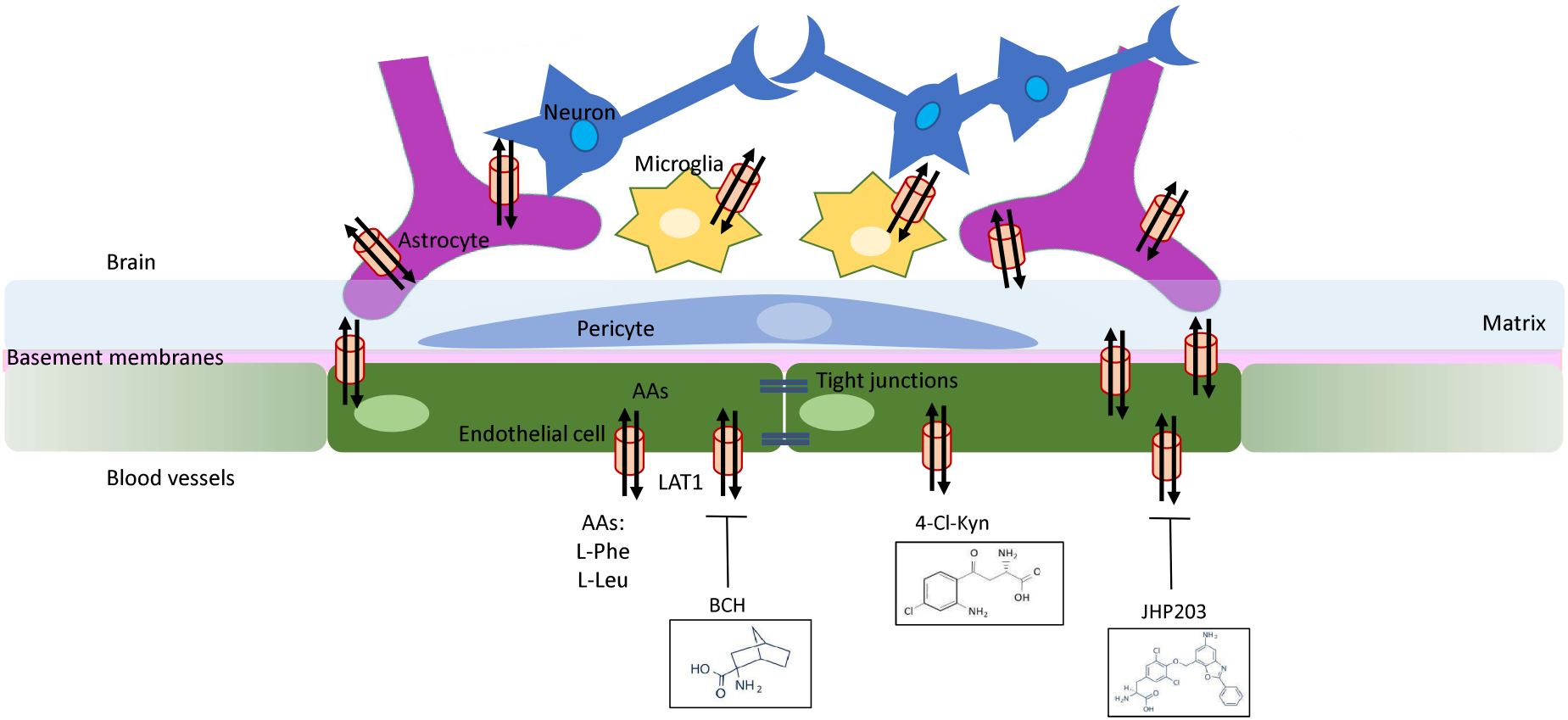


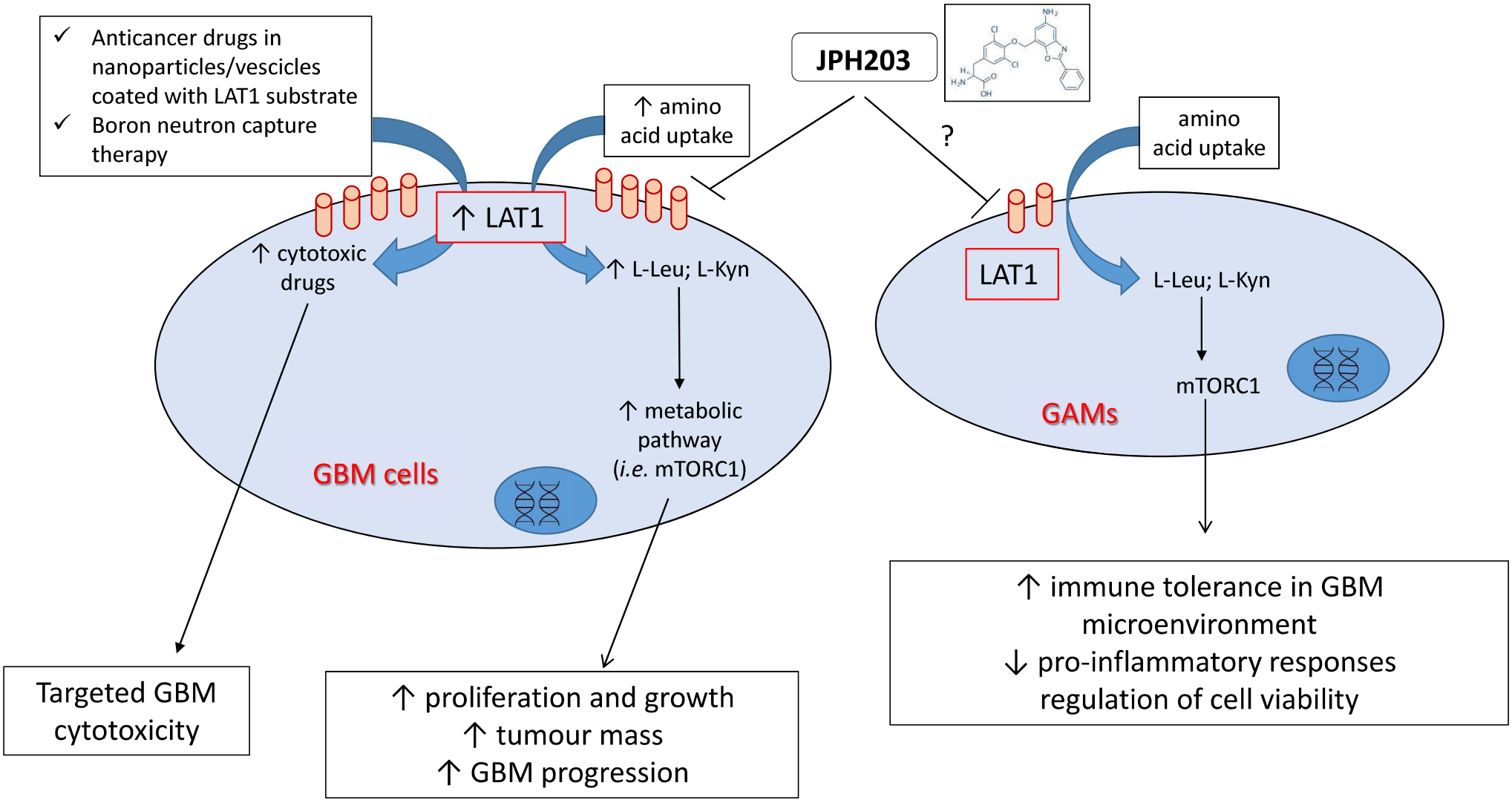
Structure and mechanism of LAT1

Fig. 1



LAT1 at the BBB

Fig. 2



LAT1 as pharmacological target in GBM

Fig. 3