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Abstract

Glioblastoma (GBM) is the most frequent and the most aggressive primary cancer of the brain in adults. Despite aggressive therapeutic interventions, the median overall survival is below 18 months after initial diagnosis. The current standard of care of newly diagnosed GBM includes concurrent administration of temozolomide (TMZ) and radiotherapy followed by adjuvant TMZ. Since 2005, TMZ remained the first-line chemotherapy in treating GBM patients with its ability to cross the blood-brain barrier. However, after initial efficacy, GBM cells acquire resistance to TMZ and other chemotherapeutic agents *via* multiple mechanisms, including the expression of ATP-binding cassette (ABC) efflux proteins. These transporters are involved in normal physiological functions, i.e., physiological cholesterol transport and elimination of toxins, but also it plays a role in pathological conditions, i.e., chemotherapies drug resistance. In humans, each ABC protein has specific tissue's locations and specific functions. In this review, we highlight the role of ABC proteins superfamily members ABCB1, ABCC1 and ABCG2 in the resistance of GBM cells to chemotherapy.

Keywords: Glioblastoma, ABC transporters, multidrug resistance, chemotherapies, P-glycoprotein, the clinical role of ABC proteins

Introduction

Glioblastoma (GBM) is the most common and the deadliest primary brain cancer in adults with a median overall below 18 months after initial diagnosis (1). The current standard of care in newly diagnosed GBM patients, established in 2005, relies on concurrent administration of temozolomide (TMZ) and radiotherapy regimen followed by adjuvant TMZ alone (2). Despite remarkable efforts in the neuro-oncology field to develop new treatments, TMZ remains today the standard first-line chemotherapy in GBM patients 'treatment (1, 2). TMZ is an alkylating agent with a small molecular weight (194.15 g/mol) that readily passes the blood-brain barrier (BBB) (3).

The essential and complex organ, which is the human brain, is separated from the BBB's blood. The BBB is a specificity of the central nervous system (CNS) blood vessels. The BBB isolates the brain from the blood for protection purposes (4). Indeed, it prevents potentially toxic molecules circulating in the bloodstream to access brain cells while ensuring the supply of nutrients to maintain homeostasis (5). Highly specialized brain capillary endothelial cells (ECs) form an essential part of the BBB. In addition to ECs, various cells contribute to the biophysical structure of BBB described in Figure 1. To date, five mechanisms are known to regulate the exchanges of molecules from blood to brain and vice versa. Passive diffusion of molecules through the BBB can occur paracellularly for very low molecular weight molecules (*e.g.,* inorganic ions, water, gazes) and transcellularly for lipophilic compounds. An active transport can also occur either by : (i) transcytosis for some proteins (*e.g.,* leptin, insulin, transferrin) or (ii) carrier-mediated proteins belonging to two major transporter superfamilies for small molecules. The solute carrier (SLC) superfamily contains more than 400 transporters that allow exchanges of small molecules through the BBB while ATP-binding cassette (ABC) transporters limit CNS penetration of small molecules by effluxing substrates from the brain ECs directly into the bloodstream **(Figure 1)** (4, 6, 7).

GBM cells acquire resistance to anticancer drugs via multiple mechanisms without being exhaustive: (i) acquisition of mutation in DNA repair genes, (ii) activation of alternative signalling pathways, (iii) immune escape, (iv) invasive switch from angiogenic growth and, (v) multidrug resistance mechanism (MDR) (8, 9) (10). MDR phenotype is observed in many types of cancers and induces : (i) increased efflux of drugs outside tumor cells and, (ii) reduced influx of drugs inside tumor cells (11). ABC transporters are efflux pump proteins involved in MDR. To date, 49 members of ABC proteins have been identified to be involved in different biological mechanisms within the human body, and are classified in seven subfamilies; ABCA (12 proteins), ABCB (11 proteins), ABCC (13 proteins), ABCD (4 proteins), ABCE (1 protein), ABCF (3 proteins), and ABCG (5 proteins) (12).

ABC transporters are expressed in various tissues such as the liver and the intestine and have a distinct role in absorption, distribution, and excretion of drugs. Some ABC transporters are predominantly expressed in ECs of the BBB (13). Indeed, ABCB1 and ABCG2 are expressed in ECs of the BBB, while others (ABCC, ABCG2) can be found in other cells such as astrocytes and neurons (5, 13, 14). In humans, each ABC protein is expressed in specific locations and exhibit specific functions. i.e. ABCA subfamily members are expressed mainly in the CNS while ABCB subfamily members are mainly expressed in the BBB and liver (15). ABC proteins were studied in several types of cancers (16). In GBM, three proteins (ABCB1, ABCC1 and ABCG2) were extensively studied and were shown to impact GBM cells biology. In this review, we highlight the role of ABC protein family mainly (ABCB1, ABCC1 and ABCG2) in resistance of GBM cells to chemotherapy.

Structure and Functions of ABC Proteins

Structure of ABC Proteins

The BBB was described for the first time in the 20th century when an intravenous injection of Evans blue significantly stained all tissue except the CNS while a direct intrathecal injection could stain only the brain tissue. This staining pattern highlighted the possibility of a barrier, termed the BBB, that prevents the dye to reach the brain tissue (4). The BBB's integrity is preserved throughout life to maintain homeostasis and regulate the influx and efflux of nutrients/metabolites between the blood and the brain (17). ABC transporters, within the BBB, play a pivotal role in brain protection by eliminating harmful agents.

The ABC transporters' primary function is to actively transport their substrates, ranging from low molecular weight molecules to polypeptides, outside cells. Despite the large number of ABC transporters, they share structural similarities. In general, a typical ABC protein includes two functional units called transmembrane domain (TMD) and nucleotide-binding domains (NBD) (Figure). The NBDs are the ATPs binding units, and they contain the Walker A motif – a phosphate-binding structure-, Walker B motif, and Walker C motif. C motif is specific for ABC proteins while Walker A and B are present in all ATP dependent proteins. The TMDs include six or ten transmembrane helices depending on ABC superfamily members. There are two types of transmembrane helices: the inward part (open to the cytoplasm) and the outward part (open to the extracellular environment). These helices determine the direction of transport of the ABC transporters i.e. importer or exporter (14, 18).

Functions of ABC proteins

Several physiological functions are reported for each ABC subfamilies. However, ABC proteins' main function is to actively transport cytotoxic xenobiotics and endobiotics against their concentration gradient (18). According to their location, they transport many substrates including anions, metal ions, peptides and lipophilic compounds (13). ABCA subfamily is mainly responsible for lipids and cholesterol

transport while ABCB, ABCC and ABCG subfamily members are mainly associated with drug resistance and elimination of xenobiotics. Genetic variants in ABC proteins are linked to genetic disorders *e.g.* a pathogenic variant in the ABCD2 is responsible for 95% of cases of X-linked adrenoleukodystrophy (ALD) disorder(19). **(Table)** summarizes the physiological functions of each ABC subfamilies.

Mechanisms of action of ABC transporters

ABC proteins hydrolyze ATP to efflux chemical agents against their concentration gradient. The active transport cycle starts with binding a substrate, *e.g.* xenobiotic to a high-affinity structure formed by the TMDs and two ATP molecules binding NBDs. A conformational contribution forms the ATP binding sites at the NBDs residues from each NBD monomer. As a result, a conformational change in TMDs occurs from either outward to inward (importer) or *vice versa* (exporter) allowing the NBD units to form a dimer. The NBD dimer induces a major conformational change on the TMDs, allowing the xenobiotic to be translocated across the plasma membrane. The hydrolysis of ATP allows the NBD dimers to be dissociated and again inducing the TMDs conformational change, resulting in the xenobiotic to be released. A final step of restoration of the open NBD-dimer conformation then takes place **(Figure 2)** (13, 14, 18, 20, 21).

ABC transporters in glioblastoma

Our review used public data obtained from PubMed and Google Scholar. Original published articles were obtained using the keywords (ABC transporters, glioblastoma, TMZ, and chemotherapy). 151 articles appeared in the results from the search engines. Another step was carried out to exclude the duplicated and review articles. Following the removal of duplicated article and review articles, 91 abstracts were reviewed (abstract review), and only articles that studied ABC transporters in glioblastoma were selected. Full texts were obtained for all 36 selected articles using access from https://universiteparissud.focus.universite-paris-saclay.fr/ and

https://insermbiblio.inist.fr/ using personal access. **(Figure)** summarizes the methodology used in the reviewing processes.

ABCB1 (MDR1, P-Glycoprotein)

ABCB1 which is also known as MDR1 and P-glycoprotein (P-GP) was identified by Victor Ling in 1976 (22) making it the first studied protein among all ABC proteins. ABCB1 protein is a 170 kDa glycoprotein highly expressed in endocrine tissues, liver, gallbladder and brain, and it is usually co-expressed with the ABCG2 protein (23). The physiological impact of ABCB1 protein was accidentally identified in 1994 by Schinkel, Smit (24), who found that a homozygous knockout of ABCB1 in laboratory mice induced a 100-fold increase in susceptibility to antiparasitic medications (25).

In humans, ABCB1 protein is encoded by the *ABCB1* gene. An update was published in 2011 to illustrate the role of *ABCB1* genetic polymorphisms, which accounts for more than 65 exon related single-nucleotide polymorphisms (SNPs) (26). These SNPs could be responsible for the differences in drug response and toxicity in several types of cancers (15). In brain, ABCB1 is localized in the luminal membrane of ECs of the BBB (13). It has an essential role protecting the brain from a possible brain uptake of toxic molecules or metabolic substances with a wide range of known substrates including TMZ (27).

Schaich et al., investigated the role of three different SNPs of ABCB1 in GBM patients treated with TMZ. He showed that the rs1128503 SNP in *MDR1* exon 12 is an independent predictive biomarker of response to TMZ. Patients with GBM exhibiting the homozygous allele (C/C allele) have better survival compared to their heterozygous variant counterparts (28). However, more recently, another large clinical cohort analyzed the clinical impact of four SNPs variants (rs2229109, rs1128503, rs2032582 and rs1045642) in patients with newly diagnosed GBM patients treated with the standard of care. They did not find any clinical value of the SNPs investigated in a large Swedish

cohort, hence could not validate the results obtained from Schaich study (29). One pilot clinical trial tried to evaluate ABCB1 protein among glioma patients. They measured the uptake of (^{11}C) N-desmethyl-loperamide ((^{11}C)dLop) using positron emission tomography (PET) imaging as a marker of ABCB1 activity. The clinical study aimed to recruit ten patients, however, only two registered patients are available in the clinical trial database, suggesting that early termination of the trial was due to the lack of patients that fits the inclusion criteria of the study(30).

Several studies have investigated the role of ABCB1 in the context of TMZ treatment in GBM preclinical models. ABCB1 downregulation was associated with increased efficacy of TMZ in U87 cell lines (31, 32). Two recent studies showed that that downregulation of ABCB1 also increases efficacy of TMZ *in vitro* and *in vivo* in GBM preclinical models (33, 34). Furthermore, an in *vivo* study reported a higher concentration of irinotecan in the brain of *Mdr1a* (-/-) mice *versus* wild-type when both exposed to the same dose of irinotecan (35). The antitumor efficacy of TMZ against three intracranial tumor GBM models was significantly enhanced when *Abcb1a/b* and *Abcg2* were genetically deficient or pharmacologically inhibited (27, 36). ABCB1 expression can be altered by several compounds including carbonic anhydrase XII (CAXII), Bone morphogenetic protein 7 (BMP7) and TMZ (33, 37, 38). Tso *et al* found that BMP7 sensitizes GBM stem cells to clinically relevant doses of TMZ (33) while Riganti and Salaroglio have found that GBM exposure to TMZ downregulates the expression of ABCB1 (37). A recent study showed that CAXII could also reduce ABCB1 activity and sensitize GBM cells to TMZ (38). **(Table)** summarizes the xenobiotic that alters the function of ABCB1, ABCG2 and ABCC1 transporters in GBM.

ABCC1 (MRP1)

The multidrug resistance-associated protein 1 (MRP1) is encoded by the *ABCC1* gene. It was described for the first time by Cole, Bhardwaj (39). ABCC1 protein is a 180-

190 kDa protein and is ubiquitously expressed in many tissues in humans. It is highly expressed in intestine, kidney and testis, while a lower expression is detected in the lung, colon and brain (40). ABCC1 protein has a wide range of substrates including anticancer drugs tested in GBM cell lines. *i.e.* vinca alkaloids (vincristine and vinblastine) and topoisomerase inhibitors (mitoxantrone) (41, 42). Many genetic alterations were detected in ABCC1 gene, and most of them are SNPs in non-coding sequences and introns. A complete list of all ABCC1 SNPs can be obtained from available public database accessible from the national center for biotechnology information.

In the literature, ABCC1 inhibitors including KIAP –an anti-apoptotic protein– reduce ABCC1 activity and sensitize U251 GBM cell line to TMZ (43). Two in *vitro* studies found that ABCC1 inhibition sensitizes cells to vincristine and etoposide but not to TMZ (42). Furthermore, a study showed that MK571, an inhibitor of ABCC1 and ABCC4, increased the anti-tumor efficacy of vincristine and etoposide in primary GBM cell lines (21). On the other hand, the overexpression of both ABCB1 and ABCG1 in GBM cell lines (U87 and U251) is associated with resistance to TMZ (44).

ABCG2, Breast Cancer Resistance Protein (BCRP)

The ABCG2 protein was the first MRP-associated protein to be discovered. This 72 kDa protein was first identified in 1998 after being cloned from a human breast cancer cell line, which led to its alias, BCRP (45). It is highly expressed in the small intestine, colon, rectum, placenta, and smooth muscles in humans while a lower expression is detected in adrenal and thyroid glands, lung and cerebral cortex (46). In isolated brain microvessels and cortex biopsies from 12 patients with epilepsy or glioma, the expression of ABCG2 protein was 1.6 folds the expression of ABCB1 (47). ABCG2 protein is a ABC half transporter, therefore it requires the dimerization of two NBDs to function as a drug efflux pump (48). Many SNPs were identified in the *ABCG2* gene. The

frequency of SNPs in ABCG2 gene is highly variable among ethnic groups, potentially associated with heterogeneous drug responses among these groups (49)

In 2017, a study enrolling 50 caucasian GBM patients found a correlation between expression of 8 different proteins (ABCG2, XIAP, MGMT, MSH2, pATM, pTp53, pAKT, Nestin) including ABCG2 and they reported a correlation between ABCG2 and the poor prognosis among GBM patients treated with the TMZ (50). To study the role of ABCG2 protein in *vitro*, they used GBM stem cells (GSC) and noticed an enhanced efficacy of TMZ following the inhibition of ABCG2. Therefore, they considered ABCG2 a promising therapeutic target in GBMs (50). However, another study reported that ABCG2 knockdown results in the upregulation of other drug transporters (ABCB1 and ABCC3) when treated with TMZ (51), suggesting that there might compensate mechanisms between transporters.

Several studies reported the importance of ABCG2 in drug response in GBM. An in *vivo* study reported that ABCG2 knockout in mice is associated with a better overall survival compared to wild-type mice when treated with dasatinib, a Src inhibitor (52). Another study showed that melatonin enhanced ABCG2 promoter methylation and sensitized GBM cell lines to mitoxantrone, doxorubicin and TMZ (53). Consistently with this study, the overexpression of ABCG2 in human GBM cell lines is found to be associated with mitoxantrone resistance (54). It was also reported that dual knockout of *ABCB1* and *ABCG2* improves efficacy of TMZ therapy in spontaneous GBM mouse models (55). Finally, TMZ exposure, in U87 and T89G cells, was found to increase ABCB1 and ABCG2 mRNA expression. Therefore, exposure to TMZ itself could modulate the levels of ABC proteins and could induce TMZ resistance among patients (56).

Clinical Value

To date, several strategies are developed to overcome the ABC transporters mediated MDR. These strategies are summarized in (**Figure**) and they vary from

partial/complete inhibition to bypass approaches: (i) nanocarriers technologies, (ii) antibody-drug conjugates -ADC-, and (iii) ultrasound-mediated BBB opening (UMBO).

Using partial or complete inhibitors of ABC transporters can be combined with their substrates to enhance their CNS penetration and anticancer activity. Variety of modulators were tested to suppress activity of efflux proteins mainly (ABCB1 and ABCG2) and few were successful enough to reach clinical trials. In GBM, a limited number of clinical trials were initiated to modulate ABC proteins. To date, these trials failed to show significant clinical benefit, which could be related to a few reasons. Firstly, the study design was not optimal *i.e.*, in the early clinical trials, the patients were not stratified based on high expression of ABC proteins, and a precise evaluation of the role of ABCB1 and ABCG2 transporters in patients could not conducted. Secondly, modulators of ABC proteins could change the pharmacokinetics of other drugs reducing their anti-tumor properties. Thirdly, the dose that was selected to inhibit the ABC protein was not sufficient or a higher dose could not be applied safely in patients.

The selection of cancer cell line is a crucial step in *in vitro* studies dedicated to GBM. Many commercial human cells lines are available for GBM. However, a study from our laboratory has tested ABC proteins expression in GBM patient-derived cell lines (PDCL) and their parental tumors (57). The study showed that PDCLs recapitulated better ABC gene expression pattern of human GBM compared to commercial cell lines and can thus be considered a better model to test the biology of ABC proteins in GBM. In addition, we found that fetal bovine serum that is usually added to cell culture medium for commercial GBM cell lines modulates resistance to TMZ. Recently, a study highlighted the importance of using low passage number PDCL and serum-free medium when studying the role of ABC transporters in *vitro*. The high passaging number of commercial GBM cell lines could change the expression level of ABC protein and could lead to conclusions irrelevant to newly diagnosed human tumor (57-59).

Furthermore, sophisticated strategies using nanocarriers (nanocapsules, liposomes, micelles, dendrimers) for ABC protein substrates were developed to allow these substrates to enter via endocytosis, improving drug half-live and drug protection (60). One clinical study investigated pegylated liposomal doxorubicin's efficacy when administered with TMZ and radiotherapy in newly diagnosed GBM patients. This study showed that the pegylated liposomal doxorubicin form is safe and tolerable however, no meaningful efficacy was observed from either the prolongation of TMZ therapy or the addition of liposomal doxorubicin (NCT00944801) (61). Another study evaluated the safety and the pharmacokinetics of a liposomal form of irinotecan, the study confirmed the safety of liposomal form of irinotecan. However, the efficacy still under investigation (62). Therefore, the nanocarrier forms' utilisation could be effective tools in future clinical studies (60).

Another strategy consists in the use of antibody-drug conjugates (ADCs) against a variety of targets in GBM and this approach is currently under investigation (63). ADCs are a newly developed biopharmaceutical compounds that allow the targeting of tumour cells while sparing healthy cells. This method is based on the use of an antibody to carry the substrate when binding to its ligand. In 2017, a published clinical trial showed a promising efficacy of depatuxizumab mafodotin (ABT-414), an ADC specific for the activated form of EGFR to selectively deliver a cytotoxic, in *epidermal growth factor receptor* (*EGFR*)-amplified recurrent GBM patients with manageable adverse effects (64). Another clinical trial was designed to evaluate its efficacy in newly diagnosed GBM patients (NCT02573324).

Finally, UMBO was used in pre-clinical models to bypass BBB efflux transporters and increase the brain's penetration of a wide variety of therapeutics. Low-intensity pulsed ultrasound can be delivered to the brain to induce a safe oscillation of intravenously injected microbubbles within blood vessels (65). Oscillation of these

microbubbles opens the BBB by reversibly disrupting the tight junctions between ECs. A range of drugs have been tested for use with UMBO for treating gliomas and include TMZ, carmustine, irinotecan, carboplatin, doxorubicin, and drug-loaded liposomes (7, 66). A new study showed that UMBO could decrease the expression of ABCB1 protein in cerebral vessels without affecting the integrity of other proteins (67). UMBO has recently moved to clinical trials where its clinical safety was confirmed: Sonocloud-induced UMBO was found to be safe and tolerable among GBM patients (68). Two other phase 1 and phase 2 clinical trials are currently in progress to evaluate UMBO's efficacy in combination with carboplatin in patients with recurrent GBM (NCT03744026).

Conclusion

GBM is an aggressive primary brain tumour with dismal prognosis. Over the last 15 years, no new drugs were found to be superior to TMZ. The long-term limited efficacy of TMZ is explained, at least partly, by the effect of MDR proteins (ABCB, ABCC1 and ABCG2). Accumulating evidence are rising to connect the effect of chemotherapies and ABC proteins. The clinical role of ABC proteins is still under investigation and the failure of previous clinical trials raised several questions regarding the strategies to overcome MDR in GBM. A few clinical recommendations are currently being reported in the literature regarding future clinical trials. Firstly, all drugs that are going to be used in the trials should be tested against the major ABC proteins (ABCB, ABCC1 and ABCG2). A wide range of in *vitro* and *in vivo* models could allow a precise testing of the novel drugs (58). Secondly, newly available non-invasive diagnostic imaging approaches *i.e.* PET scanning have the potential to determine whether ABCB1 or other transporters are functioning to reduce drug accumulation and whether inhibition can change drug uptake in solid tumours (69, 70). Furthermore, dual ABC inhibitors with a high specificity could be developed. Indeed, for example, the ABCB1 specific inhibitor zosuquidar enhanced sunitinib brain concentration in mice, but not to the same level as the dual

inhibitions of ABCB1 and ABCG2 (71). Following these recommendations could lead to the design of clinical trials that might successfully demonstrate the therapeutic potential of ABC protein inhibition in GBM treatment.

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Authors' contributions

MA performed the literature analysis, drawing of the figures, writing of the manuscript and final approval of the final version. AI participated in the original concept of the article, reviewing, and editing of the figures, reviewing the manuscript and final approval of the manuscript. MV and XD participated in reviewing the figures, reviewing the manuscript and final approval of the manuscript.

Conflicts of interest

No potential conflicts of interest were disclosed

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Table 1: Analysis of published studies that show the effects of ABC transporters on chemotherapeutic agents used in GBM.

Effect	**ABC Transporter involved**	**Drug involved**	**Reference**
High dose of cyclosporine[1] doubles the plasma concentration of etoposide among glioma patients	**ABCB1**	Cyclosporine	(72)
Nimodipine[2] enhances sensitivity to procarbazine in viability tests in vitro using PDCLs obtained from glioblastoma patients	**ABCB1 and ABCG2**	Nimodipine	(73)
Paclitaxel in combination with valspodar[3] significantly decreases the tumour volume of U-118 MG tumors compared to the control and paclitaxel groups in mice	**ABCB1**	Valspodar	(74)
Vincristine exposure induces an elevated expression of ABCG1 in rats' brain. This effect could lead to the assumption that ABCB1 is partially responsible for the observed resistance of a relapsing tumours.	**ABCB1**	Vincristine	(75)
Overexpression of ABCG2 in human GBM cell lines is associated with mitoxantrone resistance.	**ABCG2**	Mitoxantrone	(54)
GBM cell lines overexpressing ABCB1 exhibit high resistance to carmustine, carboplatin and etoposide	**ABCB1**	Carmustine, carboplatin and Etoposide	(76)
Elacridar[4] sensitizes GBM cell lines to dasatinib. Homozygous knockout of ABCG2 in mice results in a better overall survival compared to the wild type when treated with dasatininb	**ABCB1 and ABCG2**	Elacridar	(52)

Similar brain-to-plasma concentration was observed for sunitinib in both ABCB1 and ABCG2 knockout mice model and with elacridar treatment in mice. However, mild effect was observed with the zosuquidar[5] and no effect with KO143[6]	**ABCB1 and ABCG2**	Elacridar, KO143 and Zosuquindar	(71)
Inhibition of ABCB1 and ABCG2 with ABT-888 improves the efficacy of TMZ therapy in GBM patients.	**ABCB1 and ABCG2**	ABT-888	(55)
Mdr1-/- mice show a higher concentration of irinotecan compared to mdr1a+/+ mice when both exposed to the same dose of irinotecan.	**ABCB1**	Irinotecan	(35)
MRP1 inhibition enhanced Vincristine and Etoposide but not TMZ chemotherapeutic effect however the combined inhibition of MRP1 and P glycoprotein (P-gp) using Reversan[7] increased TMZ response in GBM PDCLs	**ABCC1**	MK571 and Etoposide	(21)
Inhibition of ABCB1 and ABCG2 with verapamil and KO143 increases the efficacy of TMZ when combined with MGMT inhibitors.	**ABCB1 and ABCG2**	Verapamil and KO143	(77)
Melatonin enhances ABCG2 promoter methylation hence sensitizes GBM cell lines to mitoxantrone, doxorubicin, TMZ	**ABCG2**	Melatonin	(53)
Limited drug delivery into brain tumors may significantly limit the efficacy of rucaparib[8] combined with TMZ in GBM	**ABCB1 and ABCG2**	Rucaparib	(78)
AZD2461[9] has a limited brain permeability in *vivo* due to its efflux by ABCB1 protein.	**ABCB1**	AZD2461	(36)
Downregulation of ABCB1 and ABCG2 by Bone morphogenetic protein 7 sanitize the GBM stem cells to the clinically relevant dose of TMZ.	**ABCB1 and ABCG2**	BMP7	(33)
ABCE1 downregulation enhance the efficacy of TMZ in GBM cells (U87 and A172).	**ABCE1**	TMZ	(34)
ABCG2 knockdown in several GBM cell lines resulted in upregulation of other drug transporters ABCB1 and ABCC3 when treated with TMZ.	**ABCG2**	TMZ.	(51)
KIAP -anti apoptotic protein- sensitizes U251 cells to TMZ through reduction of the ABCC1 expression	**ABCC1**	TMZ	(43)
The study was not conclusive. Only 7% of the 125 cases studied showed detectable MDR1 expression, suggesting that ABCB1 was not a major contributor to drug resistance in the selected cohort	**ABCB1**	TMZ	(79)

Histone-lysine N-methyltransferase (EZH2) enzyme silencing decreases the ABCB1, ABCC1 and ABCG2 mRNA and protein levels, which would lead to reduce efflux pump activity	**ABCC1, ABCB1 and ABCG2**	EZH2	(80)
TMZ treatment upregulate the expression of ABCC3 compared to control mice. ABCC3 protect natural killers from TMZ. A GL261 syngeneic mouse model was used in this study.	**ABCC3**	TMZ	(81)
LRIG1, human EGFR inhibitor, reversed MDR in GBM cell lines (U87 and U251) by inhibiting EGFR and secondary ABCB1 and ABCG2	**ABCB1 and ABCG2**	TMZ	(82)
CDK6 knockdown in GBM cell line (U251) resulted in significant downregulation of MDR1, MRP which enhanced the TMZ response	**ABCB1, ABCC1**	TMZ	(83)
The antitumor efficacy of TMZ against three different intracranial tumor models was significantly enhanced by a homozygous knockout of Abcb1a/b and Abcg2 genes.	**ABCB1 and ABCG2**	TMZ	(27)
The single nucleotide polymorphism (SNPs) in the MDR1 gene exon12 C1236T is an independent predictive factor for prediction of the TMZ treatment in GBM patients.	**ABCB1**	TMZ	(28)
Overexpression of MDR and MRP in GBM cells (U87, U251, U373) is associated with a high resistance to TMZ.	**ABCB1 and ABCC1**	TMZ	(44)
Activated EGFR kinase enhanced the ability of GBM cells (U87 and T98G) to resist TMZ through the upregulation of MDR1	**ABCB1**	TMZ	(56)
Loss and gain of function for MDR1 showed an enhanced and reduced efficacy of TMZ in GBM cell lines (U87 and T98G)	**ABCB1**	TMZ	(32)
Inhibition of ABCG2 enhanced the efficacy of TMZ and is considered a promising therapeutic target in GBMs	**ABCG2**	TMZ	(50)
TMZ downregulate the expression of ABCB1 in GBM stem cells	**ABCB1**	TMZ	(37)
MDR1 and ABCG2 is responsible for the resistant of recurrent GBM to TMZ. Following a TMZ exposure in U87 and T89G cells 8 folds expression MDR1 and 4 folds for ABCG2 in the cells compared to naive cells was recorded using real time PCR.	**ABCB1 and ABCG2**	TMZ	(84)
Multiple inhibition of the MDR1 protein showed no enhanced associated with an enhanced sensitivity of TMZ in GBM cell line (T98G)	**ABCB1, ABCB1**	TMZ	(42)
Downregulation of p-glycoprotein enhance the efficacy of TMZ in GBM U87 cell line	**ABCB1**	TMZ	(31)
ABCA13 overexpression is associated with a decreased progression free survival in univariate and multivariate analyses in GBM patients.	**ABCA13**	TMZ	(57)

Carbonic anhydrase XII (CAXII) sensitizes primary GBM cells to TMZ by reduction of ABCB1 protein activity.	**ABCB1**	TMZ	(38)

ABC Subfamily	ABC proteins	Physiological functions	Reference

1 Immunosuppressant medication with ABCB1 blocking activity
2 Calcium channel blockers with ABCB1 blocking activity
3 An experimental cancer drug with ABCB1 inhibition properties. It is a derivative of cyclosporine.
4 An experimental small molecule that has a dual ABCB1 and ABCG2 inhibition
5 A potent ABCB1 inhibitor, has reached clinical trials.
6 Experimental drugs with ABCG2 inhibition activity.
7 It is an experimental drug with ABCC1 and ABCB1 inhibition.
8,9 Poly (ADP ribose) polymerase (PARP) inhibitors

Table 2: Identified subfamilies of ABC transporters and their physiological functions.

ABCA	**12**	• Lipid and cholesterol transport, ABCA2 is involved in drug resistance	(20)
ABCB	**11**	• Elimination of toxins • Inhibition of apoptosis • Volume dependent Cl^-channel regulator • Phospholipid translocation (can translocate short-chain phospholipids) • Maintenance of plasma membrane cholesterol esterification • Drug resistance	(20, 85)
ABCC	**13**	• Anion efflux. • Drug resistance • Nucleoside transport	(20)
ABCD	**4**	• Mainly expressed in peroxisomes. • ABCD2 fatty acid transport and a major modifier locus for clinical diversity in X-linked ALD	(20)
ABCE/ABCF	**1 ABCE** **3 ABCF**	• Along with ABCE1, ABCF members have ATP-binding domains, but no transmembrane domains, making transporter function unlikely • Mainly regulate protein synthesis or expression	(20)
ABCG	**5**	• Transport of diverse drug substrates, sterols, and lipids • ABCG4 is expressed in macrophages • Drug resistance	(20)

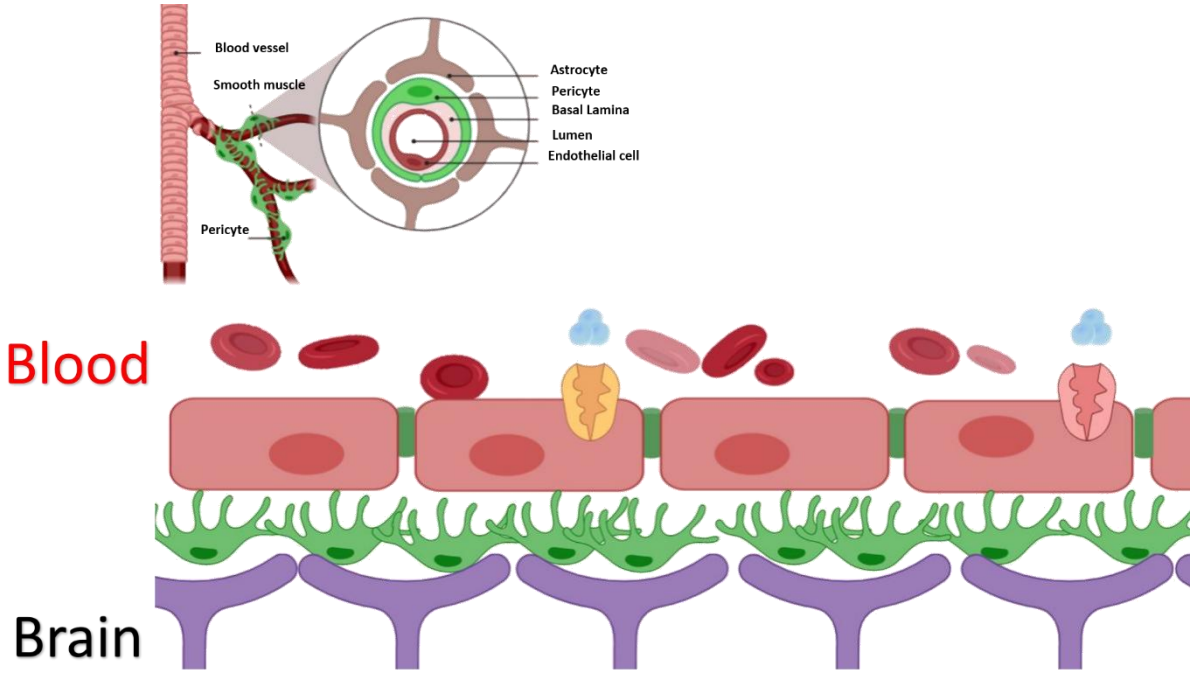

Figure 1: The blood-brain barrier (BBB) is formed of different types of cells tightly knit together. Highly specialized endothelial cells (ECs) surround blood vessels and form part of the BBB. In addition to brain ECs various cells contribute to the structure of BBB. Pericytes (represented in green) are attached to endothelium cells via gap junctions whilst astrocytes end feet (represented in purple) surround endothelial cells of the BBB, providing structural and functional support to these cells. Five mechanisms are known to regulate the entry of molecules to the brain. The efflux pumps pathway is considered a mechanism of active transport through the BBB (7).

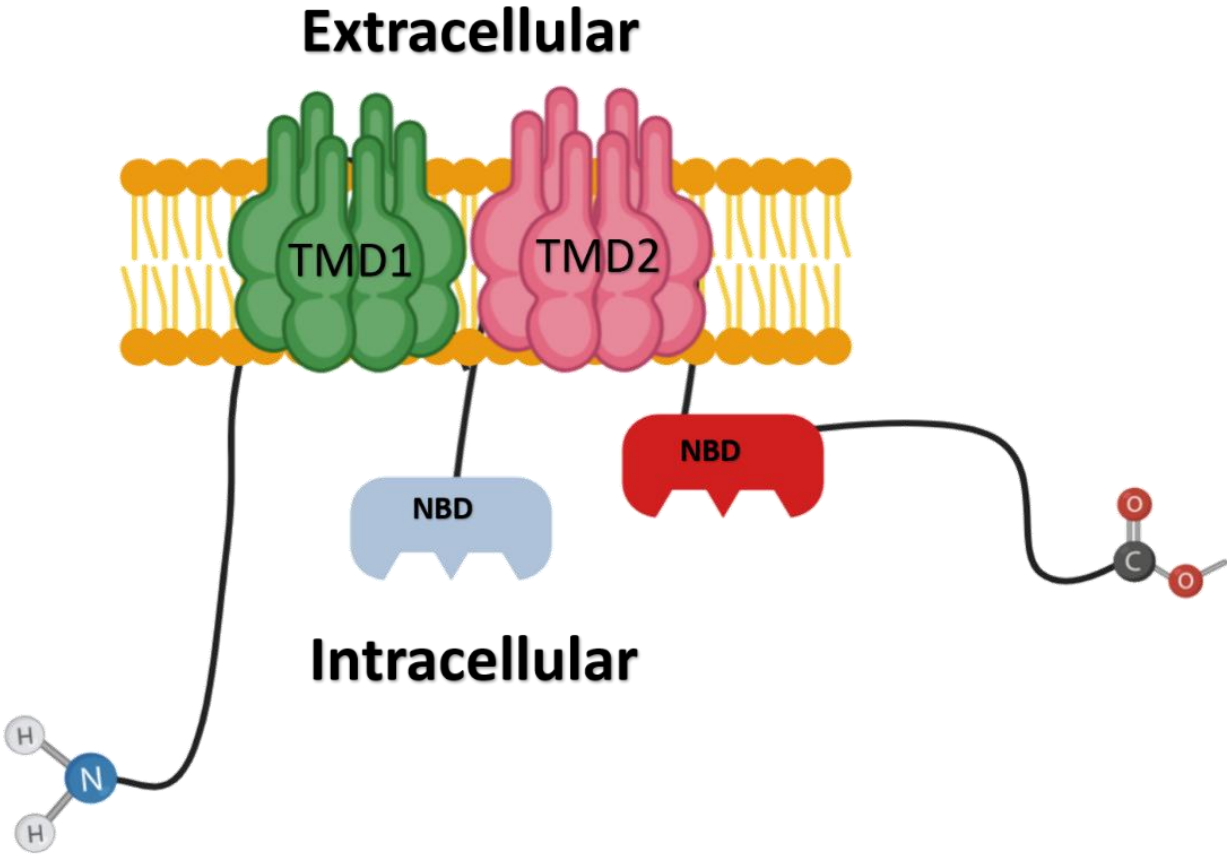

Figure 2: A full ABC transporter consists of four building units. The first two building units called TMDs are formed by six transmembrane segments. TMD1 and TMD2 are colored in green and pale red, respectively. The two other building units are called NBDs, NBD1 (pale blue) and NBD2 (red). ABC half transporters have only one TMD and one NBD and need to dimerize to become a functional protein. Additionally, some other ABC transporters have an additional TMD unit that is conjugated to the N-terminus of the protein and called "Long" ABC transporter (86).

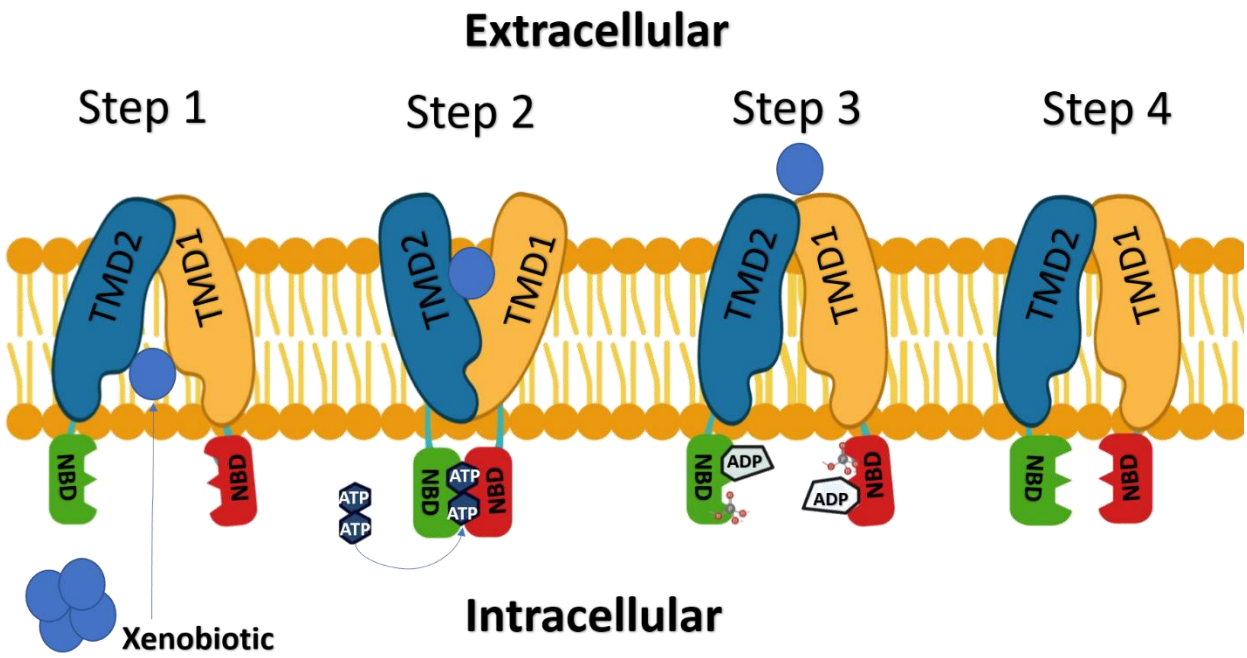

Figure 2: ABC transporters are transmembrane proteins capable of actively transporting a xenobiotic from the intracellular to the extracellular compartments. This active transport requires the hydrolysis of ATP to provide the energy necessary for the transport. The active transport cycle starts with the binding of the xenobiotic to a high-affinity structure formed by the TMDs (step 1). As a result, a conformational change makes the NBD units more exposed for ATP binding. The NBD dimer induces a major conformational change on the TMDs allowing the xenobiotic to be translocated (step 2). The hydrolysis of ATP allows the NBD dimers to be dissociated and again induces a TMDs conformational change (step 3). A final step of restoration of the open NBD-dimer conformation then takes place (step 4) (14, 18).

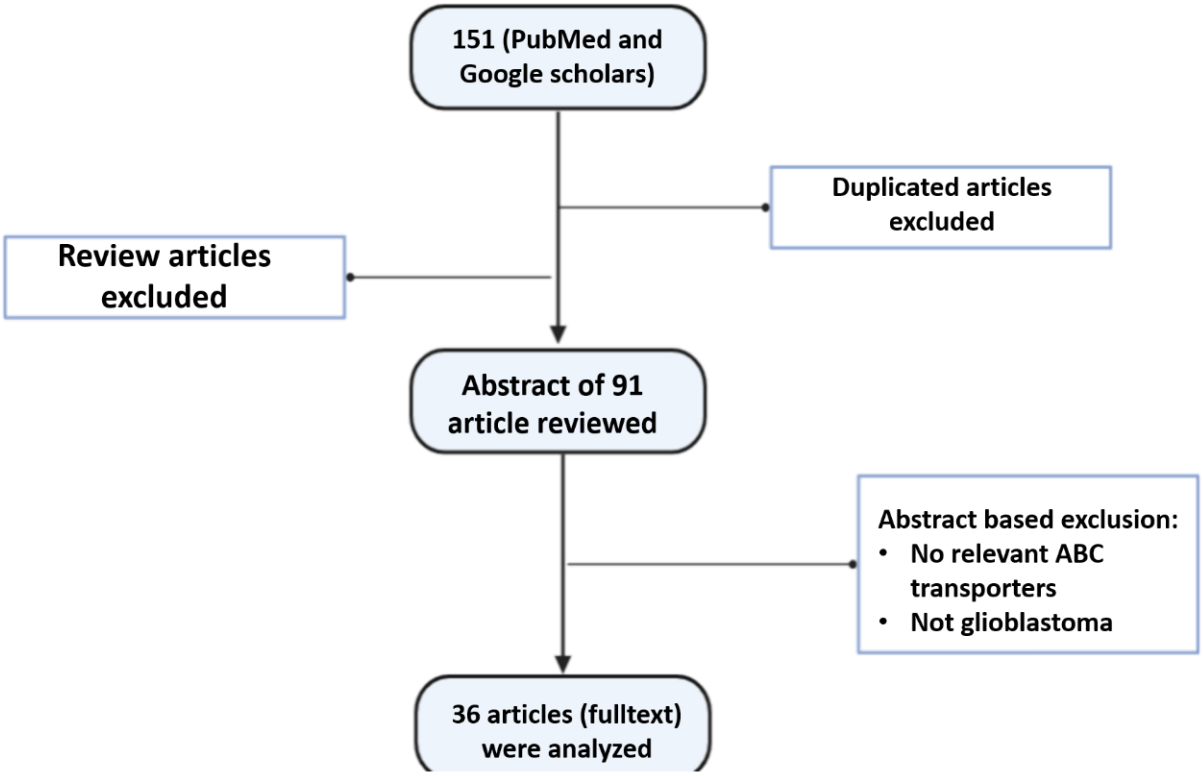

Figure 4: Represents the methodology of this literature review. The key words (ABC transporters, glioblastoma, TMZ, and chemotherapy) were used in PubMed and Google scholar search engines. 151 articles appeared in the results, then a few steps were carried out to exclude the duplicated and review articles. 91 abstracts were then reviewed and from the abstract, only articles that studied ABC transporters or GBM were selected. Full texts were obtained for all 36 selected articles.

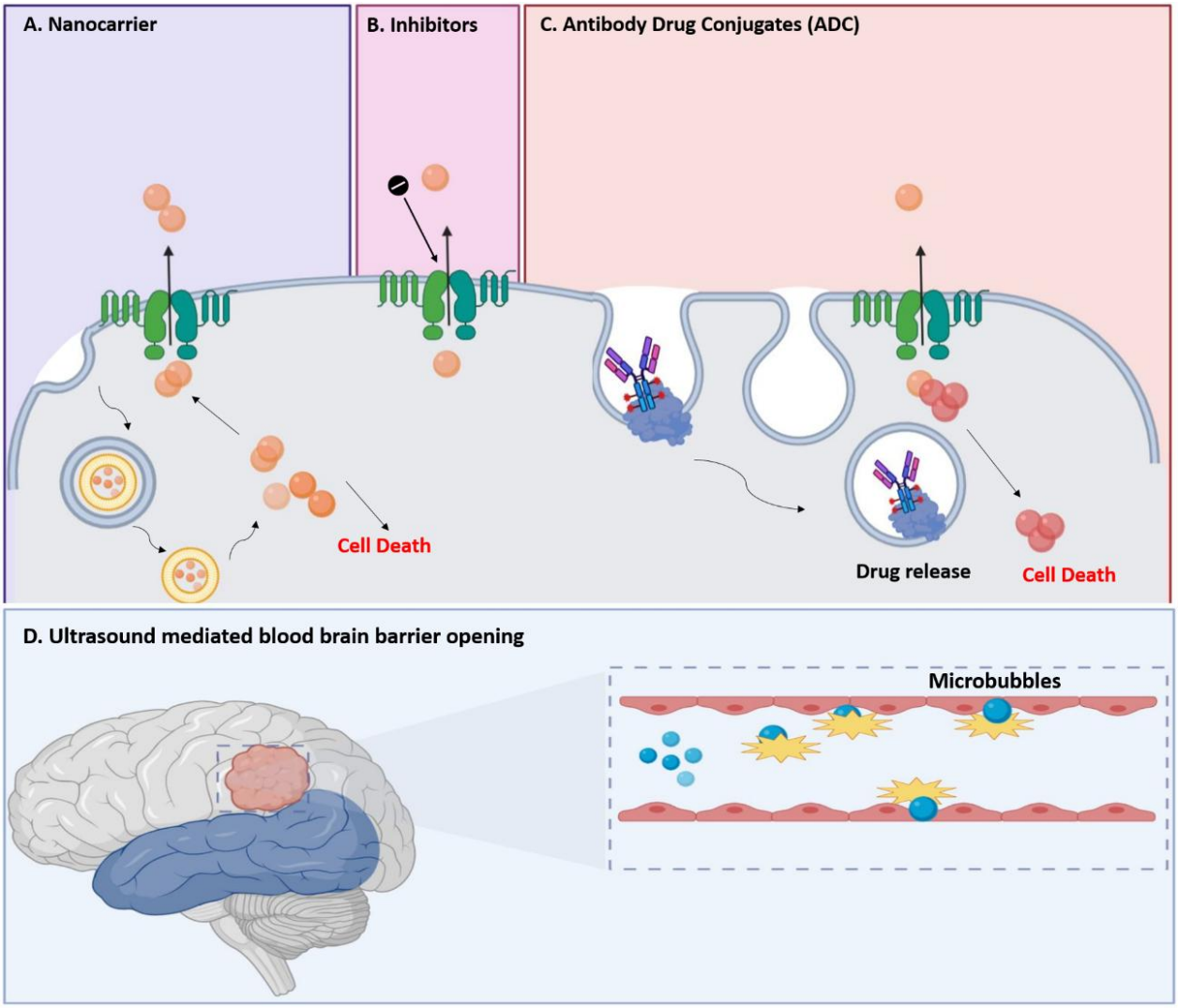

Figure 5: Summary of methods that are being developed to overcome ABC transporters. Panel A: represent the development of nanocarriers that allow the drug to enter via endocytosis. Panel B shows another method by using a partial or complete antagonist that can be administered in combination with the efflux pumps substrates and as a result enhance the activity of the substrates. Panel C shows the antibody drug conjugates approach that relies on an antibody to carry the substrates when binding to its ligand. Panel D represents the combined effect of using microbubbles and low intensity ultrasound to open the BBB. These four methods have been used in vitro and in vivo to develop strategies to overcome ABC efflux pumps (87).

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