Repurposing drugs for glioblastoma: from bench to bedside

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Abstract

Glioblastoma multiforme is the most common, aggressive and lethal type of brain tumour. It is a stage IV cancer disease with a poor prognosis, as the current therapeutic options (surgery, radiotherapy and chemotherapy) are not able to eradicate tumour cells. The approach to treat glioblastoma has not suffered major changes over the last decade and temozolomide (TMZ) remains the mainstay for chemotherapy. However, resistance mechanisms to TMZ and other chemotherapeutic agents are becoming more frequent. The lack of effective options is a reality that may be counterbalanced by repositioning known and commonly used drugs for other diseases. This approach takes into consideration the available pharmacokinetic, pharmacodynamic, toxicity and safety data, and allows a much faster and less expensive drug and product development process.

In this review, an extensive literature search is conducted aiming to list drugs with repurposing usage, based on their preferential damage in glioblastoma cells through various mechanisms. Some of these drugs have already entered clinical trials, exhibiting favourable outcomes, which sparks their potential application in glioblastoma treatment.
Repurposing drugs for glioblastoma: from bench to bedside

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Abstract
Glioblastoma multiforme is the most common, aggressive and lethal type of brain tumour. It is a stage IV cancer disease with a poor prognosis, as the current therapeutic options (surgery, radiotherapy and chemotherapy) are not able to eradicate tumour cells. The approach to treat glioblastoma has not suffered major changes over the last decade and temozolomide (TMZ) remains the mainstay for chemotherapy. However, resistance mechanisms to TMZ and other chemotherapeutic agents are becoming more frequent. The lack of effective options is a reality that may be counterbalanced by repositioning known and commonly used drugs for other diseases. This approach takes into consideration the available pharmacokinetic, pharmacodynamic, toxicity and safety data, and allows a much faster and less expensive drug and product development process.

In this review, an extensive literature search is conducted aiming to list drugs with repurposing usage, based on their preferential damage in glioblastoma cells through various mechanisms. Some of these drugs have already entered clinical trials, exhibiting favourable outcomes, which sparks their potential application in glioblastoma treatment.

Keywords:
Drug repurposing; Glioblastoma Multiforme; Pharmaceutical Industry; Clinical Trial

1. Drug repurposing
Nowadays, the process of drug research and development in the field of cancer is increasingly challenging, mainly due to the limited success with current therapies and the constant need to find safer and more effective medicines. [1] The strategy of drug repurposing has emerged as an attractive method to tackle this challenge, with the opportunity of reusing drugs already approved for other indications (drug repositioning), setting new drug combinations or making changes in the formulation of the original drug. [2]

From our knowledge, many reasons may promote this repurposing of drugs that:

- although without safety concerns, were not effective enough for a specific indication during late stage clinical trials;
- lost market exclusivity with the entry of generic drugs industry;
- were stalled in development or discontinued in the market for commercial reasons;
- despite they have already entered small or emerging markets, have not yet been launched in the largest markets in the world, i.e. United States & Europe (also known as geographic drug repositioning).
- are in development process for a particular indication, but whose mechanism of action is found to be useful to another disease as well, being such drug explored to both indications simultaneously. [3, 4]

Thanks to the high quality molecular data and the most recent advancement in technologies, especially in biology and bioinformatics, the drug repurposing activity has grown up exponentially. [1, 5] In fact, this has become a comprehensive area that has been explored on the basis of known drugs, targets, pathways, disease biomarkers and side effects. From here, it is possible to apply target-based, knowledge-based, signature-based, pathway- or network-based, and targeted-mechanism-based methods capable of focusing on different orientations according to the available information, thereby developing drug-repurposing studies. [6, 7]

1.1 Pharmaceutical industry and drug repurposing’s impact on market access

De novo drug discovery & development is a complex, long-standing procedure comprising identification and optimization of lead compounds, as well as pre-clinical and clinical studies. [1] The overall average time from the initial experiments to drug registration and marketing is about 10-17 years, requiring a strong regulatory support and huge investments. [1, 8] Such facts have sparked a growing interest for drug repurposing, since pharmacokinetic, pharmacodynamic and toxicity data are usually known from previous preclinical and clinical studies, so that drug development costs can be significantly reduced. [9] Obviously, with drug
repurposing, research & development timelines can decrease by up to 3-5 years, and promoting a higher probability of success when compared to de novo drug discovery. [10] This represents a win-win situation for both marketing authorization holders and patients. On one hand, it promotes a major hope for the pipeline growth of pharmaceutical industries, thus increasing the competitiveness of the market; on the other hand, this should reduce the sales prices of the medicines, with major impact in the area of oncology, where drugs are extremely expensive and not always accessible to many patients.

Over the last years, a successful drug repurposing has been observed in different therapeutic areas. Some well-known examples include aspirin, sildenafil, erythromycin, minoxidil and thalidomide. [1, 8, 11] Depending on the nature of the disease, side effects are more or less difficult to tolerate, which may influence patient compliance with repurposed drugs. In the case of thalidomide, its use to treat nausea (original indication) was banned due to the potential teratogenicity, but the same risk is now acceptable if thalidomide is used in patients with a life-threatening condition, such as multiple myeloma (new indication). Overall, considering the aggressive side effects of chemotherapy, it is expected that the repurposing of anticancer drugs will be more difficult than that of any other drug. [11, 12]

2. Glioblastoma treatment: state of the art

Recently, the World Health Organization has reclassified Central Nervous System (CNS) tumours, by taking into consideration histological and molecular parameters. In this new classification, WHO groups all CNS tumours in 17 categories. [13] Glioblastoma Multiforme (GBM), now referred solely as glioblastoma (for glioblastoma subtype classification, please see [14]), is deeply imbedded in the scientific community within the diffuse astrocytic tumour category. In fact, it comprises a group of grade IV lesions caused predominantly by astrocytic cell differentiation, presenting nuclear atypia, cellular pleomorphism, diffuse growth pattern, mitotic activity, microvascular proliferation and with or without necrosis. [14]

Glioblastoma is considered the most common, aggressive and lethal type of brain tumour, and is associated with a poor prognosis due to its location and noteworthy diffuse infiltrative characteristics on neighbouring brain structures. [15] About 50% of all primary malignant brain tumours are diagnosed as GBM, affecting 3 to 4 people per 100 000 Europeans, North Americans and Australians. [15, 16] Glioblastoma affects mostly older adults, in a wide range of age. Patients with primary glioblastoma are, on average, diagnosed when 59 years old. However, secondary glioblastoma is usually diagnosed in younger individuals, with a mean age of 43 years. [17] Primary glioblastoma is more common in male individuals, with a male-to-female ratio of 1.63:1. On the contrary, secondary glioblastoma is more common in women,
with a male-to-female ratio of 0.96:1. [18] These tumours are rare in children and in adolescents and do not commonly show significant morphological differences between younger or adult patient groups, although tumour proliferation in children and adolescents occurs more rapidly. [19]

Glioblastoma proliferation and tissue invasion is generally limited to the central nervous system organs, with the spinal cord being a rare location for development. The presence of tumour cells in blood and metastasis is not common in individuals who have not undergone brain surgery. [20, 21] This tumour usually develops within the white matter and deeper grey matter of the brain. However, while primary glioblastoma displays a widespread distribution, affecting temporal, parietal, frontal and occipital lobes, secondary glioblastoma usually develops within the frontal lobe, the preferential location of diffuse and anaplastic astrocytoma and oligodendroglioma development, which suggests these tumours are in the genesis of secondary glioblastoma. [22, 23] The invasive properties of glioblastoma are probably responsible for the tumour recurrence after therapy. The cancer stem cells escape surgical resection and do not receive lethal radiotherapy or chemotherapy doses, the latter due to the low drug bioavailability promoted by the blood brain barrier. [24]

Despite the intensive scientific study of the disease, approved therapy options lack the ability to completely remove all tumour cells. Glioblastoma treatment includes surgical tumour resection, radiotherapy and chemotherapy. However, rapid tumour growth and proliferation of cancer stem cells and drug resistance mechanisms are responsible for a poor treatment outcome. Consequently, glioblastoma has also a reduced prognosis. The overall survival time for a patient diagnosed with primary glioblastoma and submitted to surgery, radiotherapy and chemotherapy is only of 15 months, vs. 9.9 months if no chemotherapeutic agent is used. In case of the secondary glioblastoma, the mean survival time is higher, with 31 months for the triple therapy vs. 24 months for surgery and radiotherapy. [25, 26]

Standard therapy includes a triple approach with safe surgical resection followed by ionizing radiation therapy and temozolomide per os, as well as adjuvant chemotherapy also with temozolomide. [27]

Chemotherapy regimens are limited in drug variety and mostly in efficacy, with only carmustine, temozolomide and bevacizumab being approved by Food Drug and Administration (FDA) and European Medicines Agency (EMA) (Figure 1). It is expected that approximately 70% of the patients with glioblastoma that are treated, will experience disease progression within the first year after treatment. [28] A more recent approach to treat glioblastoma was approved in 2011, with a medical device that uses electrical fields to block cellular division and to cause cancer cell death, without significantly affecting healthy brain cells. Optune™,
formerly known as NovoTF-100A system, as an adjuvant therapy for non-recurrent glioblastoma, has shown a longer progression free survival (7.1 vs. 4 months) and overall survival (20.5 vs. 15.6 months), when compared to temozolomide alone. [29] In recurrent tumours, this technology improved the life quality of patients, but did not prolong progression-free survival nor overall survival. [30]

Figure 1. Evolution of glioblastoma treatment approaches over the last few years.

3. Drug repositioning in Glioblastoma

An extensive literature search with relevant information on drug repurposing in glioblastoma was carried out, using the following keywords: “glioblastoma”, “GBM”, "repositioning", “repurposing”, "re-profiling”, “re-tasking”, "off-label", “switching”, “anticancer” and “anti-cancer”. A total of 2110 articles, published until December 31, 2017 were found using a worldwide database (Web of Science). Only articles regarding FDA or EMA approved drugs with available abstract in English were considered in the analysis. Moreover, antineoplastic agents were discarded, due to their intrinsic anticancer mechanism. Simultaneously, the search was conducted for clinical trials (CTs) in glioblastoma using the ClinicalTrials.gov database, published until December 31, 2017. CTs using photodynamic therapy, radiotherapy, with only antineoplastic agents and with herbal compounds or extracts were not taken into consideration.

Table 1 presents 76 drugs that have shown potential activity to treat glioblastoma, as well as the corresponding proposed mechanism of action in tumour cells and consequent outcomes. The stage of the drug development for a possible repositioning was also evaluated. In addition, information about IC \textsubscript{50} values and effective concentrations in GBM cell lines can be found in Supplementary Table S.1.

Table I – List of licensed drugs showing potential to be repurposed as anticancer drugs in GBM treatment, through \textit{in vitro/in vivo} studies and clinical trials.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary indication</th>
<th>Supposed mechanism of action in GBM</th>
<th>GBM outcomes</th>
<th>Combinati on</th>
<th>Drug Development Process</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>Malaria</td>
<td>Induction of autophagy/ reduction of cell proliferation/ inhibition of MMP-2 activity and cell invasion/ inhibition of TGF-β secretion and signaling pathway</td>
<td>A  B  C  D  E  F</td>
<td>2A  3</td>
<td>[31, 32]</td>
<td></td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td></td>
<td>Induction of autophagy</td>
<td></td>
<td>1A  3</td>
<td>[32]</td>
<td></td>
</tr>
<tr>
<td>Mefloquine</td>
<td></td>
<td>Inhibition of proliferation and induction of cell cycle arrest in G2/M phase through enhancement in p21WAF1/CIP1 and p53 expression/ induction of autophagy</td>
<td>+  +  +  +  0/3  3</td>
<td>[32, 33]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinacrine</td>
<td>Giardiasis</td>
<td>Enhancement of TRAIL-induced apoptosis/ induction of autophagy</td>
<td>+  +</td>
<td>1A/3  2</td>
<td>[32, 34]</td>
<td></td>
</tr>
<tr>
<td>Pyrvinium pamoate</td>
<td>Nematode infections</td>
<td>Specific targeting of CD133+/ inhibition of Glioblastoma-initiating cells self-renewal (by inhibition of Wnt/β-catenin signaling)</td>
<td>+  +</td>
<td>0  2</td>
<td>[35]</td>
<td></td>
</tr>
<tr>
<td>Mebendazole</td>
<td></td>
<td>Disruption of microtubule formation</td>
<td>+  0</td>
<td>2  2</td>
<td>[36]</td>
<td></td>
</tr>
<tr>
<td>Aciclovir</td>
<td>Viral infections</td>
<td>Inhibition of the cell cycle in both the G1 and S phases/ inhibition of cell growth</td>
<td></td>
<td>1/3  2/3  3</td>
<td>[37]</td>
<td></td>
</tr>
<tr>
<td>Valganciclovir</td>
<td></td>
<td>Unknown</td>
<td>+  3</td>
<td>4  4</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td>Lopinavir</td>
<td></td>
<td>Inhibition of cell proliferation and tumor growth/ blockage of blood flow to the tumor</td>
<td>+  +</td>
<td>0/3  3</td>
<td>[39, 40]</td>
<td></td>
</tr>
<tr>
<td>Ritonavir</td>
<td></td>
<td>Inhibition of chymotrypsin-like activity of the proteasome/ induction of cell cycle arrest in the G1 phase followed by apoptosis/ reduction of tumor growth/ blockage of blood flow to the tumor</td>
<td>+  +  +  +  0/3  2/3</td>
<td>[39, 41]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nelfinavir</td>
<td></td>
<td>Induction of cell death by triggering the ER stress response/ Reduction of VEGF/HIF-1α expression and angiogenesis</td>
<td>+  +</td>
<td>2A  3</td>
<td>[42]</td>
<td></td>
</tr>
<tr>
<td>Atazanavir</td>
<td></td>
<td>Induction of cell death by triggering the ER stress response</td>
<td>+  +</td>
<td>0  2</td>
<td>[42]</td>
<td></td>
</tr>
<tr>
<td>Ribavirin</td>
<td></td>
<td>Modulation of oncogenic pathways (eIF4E, ERK and EZH2)/ Inhibition of cell proliferation/ induction of cell cycle arrest and cell death processes/ Impairment of cell migration and adhesion capacities</td>
<td>+  +  +  +  +  +  2A  2</td>
<td>[43]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Fungal infections</td>
<td>Repression of AKT1-MTOR signaling, induction of autophagy, and inhibition of cell proliferation</td>
<td>+  +</td>
<td>0  2/3  3</td>
<td>[44]</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>Increase in Bax/Bcl-2 ratio with the consequent induction of apoptosis</td>
<td>+  +</td>
<td>2  0</td>
<td>[45, 46]</td>
<td></td>
</tr>
<tr>
<td>Salinomycin</td>
<td>Bacterial infections</td>
<td>Inhibition of oxidative phosphorylation in mitochondria</td>
<td>+  +</td>
<td>0  2</td>
<td>[47]</td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td></td>
<td>Inhibition of tumor growth/ induction of autophagy cell death through the activation of caspase-3</td>
<td>+  +  +  +  0  0/3</td>
<td>[48]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td></td>
<td>Reduction of MMP-2 activity and of cell invasiveness</td>
<td>+  +  +  +  +  +  +  2  2</td>
<td>[49]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td></td>
<td>Induction of cell-cycle arrest and inhibition of glioma growth by regulating miRNA-199b-5p-HES1-AKT pathway</td>
<td>+  +  +  +  +  +  0  2</td>
<td>[50]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
<td>Inhibition of ALDH, probably undermining GSCs function</td>
<td>+  +  +  +  0  0  0</td>
<td>[51]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Schizophrenia, Psychotic disorders</td>
<td>Inhibition of CcO complex IV activity bearing the COX4-1 regulatory subunit, leading to the inhibition of growth and proliferation of chemoresistant cells/ Induction of cell cycle arrest/ Induction of autophagic cell death by inhibiting the</td>
<td>+  +  +  +  +  +  +  +  +  +  +  0  2</td>
<td>[52, 53]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>Pathway/Function</td>
<td>Effect on MGMT</td>
<td>Effect on CD44</td>
<td>Effect on Sox1</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td><strong>PI3K/AKT/mTOR pathway</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thioridazine</td>
<td>Induction of autophagy and apoptosis (probably through G protein-coupled receptors)/ upregulation of AMPK activity</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[54, 55]</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>Induction of apoptosis</td>
<td>+</td>
<td>2A</td>
<td>0</td>
<td>[55]</td>
<td></td>
</tr>
<tr>
<td>Perphenazine</td>
<td>Induction of apoptosis/ inhibition of dopaminergic-driven SVZ cell contributions to tumor growth/ inhibition of cell proliferation mediated by decrease in pAKT levels</td>
<td>+</td>
<td>+</td>
<td>2A</td>
<td>0</td>
<td>[55-57]</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Inhibition of proliferation, migration and anchorage-independent growth/ induction of apoptosis, necrosis and/or cytosis</td>
<td>+</td>
<td>2/3</td>
<td>0/3</td>
<td>[58]</td>
<td></td>
</tr>
<tr>
<td>Penfluridol</td>
<td>Suppression of tumor growth by Akt-mediated inhibition of the transcription factor GLU1</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[59]</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Inhibition of the Wnt/β-catenin signaling pathway: promotion of differentiation of GSCs into oligodendrocyte-like cells/ inhibition of TMZ-resistant tumors generated from GSCs</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>1</td>
<td>[60]</td>
</tr>
<tr>
<td>Lithium</td>
<td>Psychotic disorders: Inhibition of GSK-3 activation, which blocks cell migration</td>
<td>+</td>
<td>2/3</td>
<td>3</td>
<td>[61, 62]</td>
<td></td>
</tr>
<tr>
<td>Donepezil</td>
<td>Alzheimer’s disease: Increase in cell mitosis duration or induced cell mitotic arrest</td>
<td>+</td>
<td>2</td>
<td>3</td>
<td>[63, 64]</td>
<td></td>
</tr>
<tr>
<td>Memantine</td>
<td>Inhibition of proliferation and induction of autophagy mediated by NMDAR1</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>3</td>
<td>[65]</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>Activation of p-c-Jun and subsequent increase in cytochrome c release from mitochondria, as well as caspase-3-like activation, leading to apoptosis</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[66]</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Induction of apoptosis: AMPAR binding, thus inducing transmembrane Ca²⁺ influx causing mitochondrial Ca²⁺ overload/ inhibition of cell proliferation mediated by decrease in pAKT levels</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[57, 66, 67]</td>
</tr>
<tr>
<td>Sertraline</td>
<td>Psychiatric disorders: Inhibition of cell proliferation mediated by decrease in pAKT levels</td>
<td>+</td>
<td>0/3</td>
<td>3</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>Inhibition of serum-induced ruffle formation, cell migration, and invasion of tumor cells by suppressing both FAK and Akt/mTOR signaling pathways</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>Psychiatric disorders: Impairment of cell survival: induction of autophagy by coordinately elevating the level of cAMP/ inhibition of PI3K/Akt/mTOR signaling/ reduction of clonogenicity/ Inhibition of mitochondrial activity/ Reduction of CD44, Nestin and Sox1 expression</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/3</td>
<td>1</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Inhibition of mitochondrial activity/ Reduction of CD44 expression/ Inhibition of cell proliferation</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[71, 72]</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>Activation of p-c-Jun and subsequent increase in Cyt c release and caspase-3-like activation, leading to apoptosis</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[68]</td>
</tr>
<tr>
<td>Doxepin</td>
<td>Psychiatric disorders: Inhibition of cell proliferation</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[72]</td>
<td></td>
</tr>
<tr>
<td>Citalopram, Escitalopram</td>
<td>Inhibition of cell proliferation/ induction of apoptosis</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[72, 73]</td>
<td></td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>Epilepsy: Decrease in MGMT protein and mRNA expression levels in cells/ inhibition of cell growth and proliferation/ inhibition of HDAC</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>3/4</td>
<td>[74]</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Reduction of PON2 expression in cells, thus increasing ROS production and inhibition of cell proliferation</td>
<td>+</td>
<td>+</td>
<td>2A/3</td>
<td>0/3</td>
<td>[75, 76]</td>
</tr>
</tbody>
</table>

Notes: + indicates a positive effect, - indicates a negative effect, 0 indicates no effect.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Disease</th>
<th>Effect</th>
<th>+</th>
<th>+</th>
<th>0</th>
<th>0</th>
<th>2/3</th>
<th>3</th>
<th>[Ref]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol</td>
<td>Anesthesia</td>
<td>Suppression of proliferation and invasion of cells by upregulating microRNA-218 expression/ Induction of apoptosis/ Reduction of MMP-2 protein expression levels</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>Disulfiram</td>
<td>Chronic alcoholism</td>
<td>Suppression of cell growth and self-renewal/ inhibition the expression of PLK1/ inhibition of both ALDH and NFKB pathway/ induction of ROS/ inhibition of MGMT/ impairment of DNA repair pathways/ inhibition of 20S proteasome activity, which is dependent on complex formation with copper</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2/3</td>
<td>3</td>
<td>[78, 79]</td>
<td></td>
</tr>
<tr>
<td>Dimethyl fumarate</td>
<td>Multiple Sclerosis</td>
<td>Induction of autophagy and activation of the extrinsic apoptosis pathway/ Reduction of invasiveness of tumor cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1A/3</td>
<td>3</td>
<td>[80]</td>
<td></td>
</tr>
<tr>
<td>Digitoxin</td>
<td>Cardiac failure/ atrial fibrillation</td>
<td>Sensitization effect on TRAIL-mediated apoptosis/ Suppression of HIF-1α accumulation/ reduction of hypoxia-induced activation of ERK ½ pathway/ reduction of CD133 and VEGF expression</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2A</td>
<td>2</td>
<td>[81, 82]</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Hyperlipidemia</td>
<td>Inhibition of cell proliferation/ inhibition of cell migration and invasion/ Downregulation of expression of membrane type 1 MMP via p38 MAPK pathway/ induction of apoptosis y caspase-8- caspase-3 signaling pathway</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/1A</td>
<td>0/3</td>
<td>[83-85]</td>
<td></td>
</tr>
<tr>
<td>Lovastatin</td>
<td>Hyperlipidemia</td>
<td>Inhibition of cell proliferation/ inhibition of the mevalonic acid and MAPK pathways, thereby inducing apoptosis/ Increased G0–G1 cell arrest</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/3</td>
<td>2</td>
<td>[83, 86, 87]</td>
<td></td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Hyperlipidemia</td>
<td>Inhibition of cell proliferation and migration/ induction of apoptosis via suppression of Ras/ERK and Ras/Akt pathways/ increase of caspase-3 activity/ downregulated the PI3K/Akt pathway</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[83, 86, 88]</td>
<td></td>
</tr>
<tr>
<td>Mevatatin, fluvastatin</td>
<td>Hyperlipidemia</td>
<td>Inhibition of cell proliferation/ induction of apoptosis via suppression of Ras/ERK and Ras/Akt pathways</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[83, 86]</td>
<td></td>
</tr>
<tr>
<td>Cerivastatin</td>
<td>Hypertension/ angina pectoris</td>
<td>Down-regulation of FAK phosphorylation, thus inhibiting cell adhesion and invasion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/3</td>
<td>2</td>
<td>[83, 89]</td>
<td></td>
</tr>
<tr>
<td>Pitavastatin</td>
<td>Hypertension/ angina pectoris</td>
<td>Inhibition of NFKB resulting in autophagic death/ Suppression of tumor cell MDR-1 protein/ inhibition of cell proliferation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/3</td>
<td>-1/2</td>
<td>[83, 90, 91]</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>Hypertension/ angina pectoris</td>
<td>Induction of apoptosis by increasing the Bax/Bcl-2 ratio</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>[92]</td>
<td></td>
</tr>
<tr>
<td>Mibebradil</td>
<td>Hypertension/ angina pectoris</td>
<td>Inhibition of tumor growth/ Inhibition of cell cycle/ Inhibition of prosurvival AKT/mTOR pathways and stimulation of proapoptotic survivin and BAX pathways/ Decreased expression of oncogenes and increased expression of tumor suppressor genes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>2/3</td>
<td>[93, 94]</td>
<td></td>
</tr>
<tr>
<td>Losartan</td>
<td>Hypertension/ angina pectoris</td>
<td>Reduction of cell proliferation and tumor growth/ decrease in the number of capillary vessels/ Induction of apoptosis/ decreased levels of the proangiogenic factors VEGF, PDGF and FGF</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>1</td>
<td>[95, 96]</td>
<td></td>
</tr>
<tr>
<td>Captopril</td>
<td>Hypertension/ angina pectoris</td>
<td>Inhibition of MMP-2 and MMP-9/ inhibition of angiogenesis and tumor invasion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/3</td>
<td>0/3</td>
<td>[97]</td>
<td></td>
</tr>
<tr>
<td>Carvedilol</td>
<td>Hypertension/ angina pectoris</td>
<td>Induction of mitochondrial damage (mitochondrial swelling, crista damage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0/3</td>
<td>2</td>
<td>[98]</td>
<td></td>
</tr>
</tbody>
</table>
and formation of myelin figures), with the consequent apoptosis/ inhibitory effect on growth factor receptors (PDGFR and β-ADR)

<table>
<thead>
<tr>
<th>Blood (1)</th>
<th>Ticlopidine</th>
<th>Stroke/ myocardial infarction</th>
<th>Exhibition of a synergistic effect with imipramine in impairing cell survival: induction of autophagy by coordinately elevating the level of cAMP</th>
<th>+</th>
<th>+</th>
<th>3</th>
<th>2</th>
<th>[69]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>Induction of autophagy, apoptosis and cell death (activation of AMPK, Redd1; downregulation of AKT-mTOR signaling pathway)/ inhibition of CLIC1 activity which induces G1 cell arrest</td>
<td>+</td>
<td>+</td>
<td>2/3</td>
<td>3</td>
<td>[99, 100]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repaglinide</td>
<td>Inhibition of proliferation and migration of tumor cells/ reduction of Bcl-2, Beclin-1 and PD-L1 expression/ induction of apoptosis and autophagy/ Blockage of immune checkpoint signaling</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[101]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>Inhibition of β catenin expression/ reduction of cell viability and proliferation/ induction of apoptosis</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>[102, 103]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>Inhibition of cell proliferation by causing G2/M arrest and apoptosis</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[104, 105]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciglitazone</td>
<td>Loss of mitochondrial membrane potential along with the increase in ROS</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
<td>[105]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenformin</td>
<td>Inhibition of tumor growth/ inhibition of cell self-renewal/ reduction of cell stemness and mesenchymal markers/ increased expression of miR-124, 137 and let-7/ induction of apoptosis</td>
<td>+</td>
<td>+</td>
<td>2/3</td>
<td>2</td>
<td>[106]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Inhibition of cystine uptake via the system x−cystine–glutamate transporter, leading to the depletion of intracellular GSH and the consequent compromised cellular redox defense which stymied tumor growth</td>
<td>+</td>
<td>+</td>
<td>2A</td>
<td>2/3</td>
<td>[107]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aprepitant</td>
<td>Increased blockage in phosphorylation of Akt due to NK-1 signaling: induction of apoptosis</td>
<td>+</td>
<td></td>
<td>2/3</td>
<td>3</td>
<td>[108]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Inhibition of GSK-3β: attenuation of cell proliferation and migration by blocking histamine that promotes tumor angiogenesis/ Reduction of expression levels of endogenous receptors needed to adhesion and migration cell processes</td>
<td>+</td>
<td>+</td>
<td>1/3</td>
<td>2/3</td>
<td>[109, 110]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td>Hormone replacement therapy</td>
<td>Induction of JNK-dependent apoptosis</td>
<td>+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>[111]</td>
<td></td>
</tr>
<tr>
<td>Sildenafil</td>
<td>Erectile dysfunction/ pulmonary arterial hypertensio n</td>
<td>Increase in BBTB permeability, via cGMP signaling (only for phosphodiesterase type 5-negative GBMs)</td>
<td>+</td>
<td></td>
<td>0/3</td>
<td>1/3</td>
<td>[112]</td>
<td></td>
</tr>
<tr>
<td>Auranofin</td>
<td>Rheumatoid arthritis</td>
<td>Inhibition of cathepsin B expression, which probably may inhibit glioblastoma growth itself/ inhibition of thioredoxin</td>
<td>+</td>
<td>+</td>
<td>3</td>
<td>3</td>
<td>[113]</td>
<td></td>
</tr>
<tr>
<td>GBM: glioblastoma multiforme; MMP: matrix metalloproteinase; TGF-β: transforming growth factor-beta; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; ER: endoplasmic reticulum; VEGF: vascular endothelial growth factor; HIF: hypoxia-inducible factor; eIF4E: eukaryotic translation initiation factor 4E; ERK: extracellular signal-regulated kinase; EZH2: enhancer of zeste homolog 2; Akt: protein kinase B; mTOR: mammalian target of rapamycin; ALDH: aldehyde dehydrogenase; CcO: cytochrome c oxidase; P13K: phosphatidylinositol-3-kinase; AMPK: adenosine monophosphate (AMP)-activated protein kinase; SVZ: subventricular zone; TMZ: temozolomide; GSC: glioblastoma stem cells; GSK: glycogen synthase kinase; NMDAR: N-methyl-D-aspartate receptor; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor; FAK: focal adhesion kinase; cAMP: cyclic AMP; MGMT: O6-methylguanine-DNA methyltransferase; mRNA: messenger ribonucleic acid; HDAC: histone deacetylases; PON: paraoxonase; ROS: reactive oxygen species; PLK: polo-like kinase; NFkB: nuclear factor kappa B; DNA: deoxyribonucleic acid; MAPK: mitogen-activated protein kinase; MDR: multidrug resistance; PDFG: platelet-derived growth factor; FGF: fibroblast growth factor; β-ADR: beta adrenergic receptor; CLIC: chloride intracellular channel; PD-L1: programmed death-ligand 1; GSH: glutathione; JNK: c-Jun N-terminal kinase; BBTB: blood-brain tumor barrier; cGMP: cyclic guanosine monophosphate; MIF: macrophage migration inhibitory factor; IKK: IkB kinase; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor.Key for “GBM outcomes” column: A) Apoptosis induction; B) Autophagy induction; C) Energy metabolism interference; D) Cell cycle blockage; E) Growth-inhibitory effects and differentiation; F) Others. Key for “Combination” column: 0) alone; 1) combined with TMZ; 1A) combined with TMZ and radiotherapy; 2) alone and combined with TMZ; 2A) alone or combined with TMZ and radiotherapy; 3) other combinations. Key for “Drug development process” column: “-1” in silico studies; “0” in vitro studies; “1” in vivo studies; “2” in vitro and in vivo studies; “3” clinical trials; “4” retrospective studies.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Considering the Anatomical Therapeutic Chemical (ATC) classification, active substances were divided in 10 out of 14 main groups, which indicates a significant diversity in the approved mechanisms of action. However, drugs acting on the nervous system represent 33% of all potential repurposed drugs, probably due to their intrinsic mechanism of action and their ability of easily cross the blood brain barrier. Other representative classes include anti-infectives (18%), drugs acting on cardiovascular system (17%), on the alimentary tract and metabolism (12%) and antiparasitics (8%) (Figure 2).

![Figure 2. Repurposed drugs in glioblastoma per pharmacotherapeutic group.](image)

Considering the compiled data and proposed mechanisms of action in glioblastoma cells for each drug, 5 different outcomes have been selected, as well as an additional group for less common consequences. The heatmap represented in Figure 3 plots the impact of each outcome for all 10 pharmacotherapeutic classes, and the 76 hit drugs. Relative frequency analysis indicates that antiparasitics, anti-infectives, drugs acting on nervous system, cardiovascular system, alimentary tract and metabolism display a huge variety of outcomes, whereas drugs acting on the remaining systems tend to be more selective. This may be ascribed to the diversity between the number of drugs within each class. Autophagy of cancer cells is the most common outcome of antiparasitic drugs. A similar behaviour is observed for tumour growth and cell proliferation inhibition caused by substances acting on nervous and cardiovascular system and for the induction of apoptosis in drugs acting on the alimentary tract and metabolism.
Figure 3. Heatmap displaying relative frequencies of GBM outcomes within each pharmacotherapeutic class.

If we consider the bulk repositioned drugs instead of drugs within each pharmacotherapeutic class, a different scenario is obtained, as can be seen in Figure 4. It is clear that drugs acting on nervous and cardiovascular systems display greater expression in the analysis, revealing the greatest action on growth inhibition and apoptosis induction. In turn, dermatological drugs and drugs acting on genito urinary, musculo-skeletal and respiratory systems have lower impact on glioblastoma outcomes, in relation to the other ones. Part of this evidence can be explained by the greater number of repositioned drugs belonging to nervous and cardiovascular systems, in addition to the anti-infective drugs. The latter, although representing the second largest slice in repositioning for glioblastoma (see Figure 2), are associated to a higher diversity of outcomes, and do not stand out in any specific mechanism.
Figure 4. Heatmap displaying relative frequencies of GBM outcomes, by pharmacotherapeutic class, in relation to the total drugs considered for repositioning.

With regard to our research on clinical trials with drug repositioning for glioblastoma, major class corresponds to active substances acting on the nervous system (31%), followed by anti-infective agents (21%), drugs acting on the alimentary tract and metabolism (14%), on the cardiovascular system (10%) and antiparasitics (10%). Nervous system drugs represent the most studied pharmacotherapeutic class, with several *in vitro* and *in vivo* promising results, in addition to the 36% of those drugs that already entered in CTs (*Figure 5*). This suggests some drugs from this class have a higher treatment potential than the remaining classes, and are closer of being approved for glioblastoma therapy. Other classes that are being targeted are antiparasitics, anti-infective agents and active substances acting on the alimentary tract and metabolism. For drugs acting on musculo-skeletal system, the only two substances that showed promising anti-glioblastoma activity have reached CT stage. However, drugs acting on respiratory system and on blood seem to have less potential for repositioning, since there are no CTs being conducted so far.
Figure 5. Number of clinical trials with repurposed drugs in GBM, per pharmacotherapeutic group.

4. Overview

Due to the specific location of GBM, compounds are required to have good blood brain barrier penetration and site accumulation characteristics. Also, central nervous system drugs, such as antipsychotics and antidepressants, are more likely to be successful in GBM treatment. Sodium valproate has been commonly used to treat epilepsy events for decades, and clinical results of the drug in combination with TMZ support the previous in vitro and in vivo anticancer potential. [53] Olanzapine is an antipsychotic drug that has shown in vitro and in vivo anticancer properties against GBM. Additionally, it displays an antiemetic activity in chemotherapy induced nausea and vomiting, making it a promising agent against glioblastoma. [119] Disulfiram, an aldehyde dehydrogenase inhibitor, has been suggested as a potential adjuvant in cancer treatment. The combination of disulfiram and copper decreases cell proliferation and self-renewal, and successfully overcomes TMZ resistance, thus being one of the strongest candidates for repurposing. However, non-central nervous drugs have also been receiving attention by the scientific community over the last years. The antimalarial chloroquine was intensively studied as an anticancer drug and increased the median survival in several oncological patients. Metformin is an antidiabetic agent that has demonstrated several anticancer activities against GBM, including apoptosis and autophagy induction, cell proliferation, angiogenesis and migration inhibition. Moreover, it has reversed TMZ resistance in vitro and in vivo. These favorable outcomes should be faced as a source of motivation for additional research, since they may be part of a novel paradigm in cancer treatment.

5. Concluding remarks
Drug repurposing is a growing endeavour, which has been gathering support both from the pharmaceutical industry and academia considering that it saves both money and time relative to the traditional drug development process.

Drugs described in this mini-review have shown promising results against glioblastoma and are able to interfere preferentially with tumour cell pathways \textit{in vivo} or \textit{in vitro}, alone or combined with temozolomide. Moreover, they present themselves not only as a much safer option when compared to antineoplastic and immunomodulating agents but, in most cases, effective in common therapeutic concentrations. Nevertheless, the drug permeability across the blood brain barrier and the high heterogeneity of glioblastoma are still major hurdles that cannot be neglected. This may explain why drugs with activity on the central nervous system, which are also being subjected to a higher number of clinical trials, are preferred for repurposing over those directed at other physiological systems.

Despite the anti-glioblastoma activity of these drugs, more information is still required to allow a successful translation to clinical practice, as molecular mechanisms of action of these drugs are not fully elucidated.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at DOI

Conflict of interests

The authors declare no conflict of interests.

6. References


[72] D. Rundle-Thiele, R. Head, L. Cosgrove, J.H. Martin, Repurposing some older drugs that cross the blood–brain barrier and have potential anticancer activity to provide new treatment options for glioblastoma, British Journal of Clinical Pharmacology, 81 (2016) 199-209.


- Repurposing saves money and time relative to traditional drug development process.
- Glioblastoma drug repurposing has been found in different therapeutic areas.
- Central nervous system drugs exhibit the marker anti-glioblastoma activity.
- Mechanisms of action in glioblastoma varies within repurposed therapeutic classes.
- Favourable clinical trials outcomes support the potential of repurposed drugs.
Declarations of interest: none.