Regulation of cancer stem cells and immunotherapy of glioblastoma (Review)

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Abstract. Glioblastoma (GB) is one of the most adverse diagnoses in oncology. Complex current treatment results in a median survival of 15 months. Resistance to treatment is associated with the presence of cancer stem cells (CSCs). The present review aimed to analyze the mechanisms of CSC plasticity, showing the particular role of β-catenin in regulating vital functions of CSCs, and to describe the molecular mechanisms of Wnt-independent increase of β-catenin levels, which is influenced by the local microenvironment of CSCs. The present review also analyzed the reasons for the low effectiveness of using medication in the regulation of CSCs, and proposed the development of immunotherapy scenarios with tumor cell vaccines, containing heterogenous cancer cells able of producing a multidirectional antineoplastic immune response. Additionally, the possibility of managing lymphopenia by transplanting hematopoietic stem cells from a healthy sibling and using clofazimine or other repurposed drugs that reduce β-catenin concentration in CSCs was discussed in the present study.

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1. Introduction

Glioblastoma (GB) is a primary glial tumor of astrocytic type, grade IV of malignancy according to the World Health Organization (WHO) classification (1). Compared with other cancer types, the incidence of GB is relatively low, varying from 3 to 5 cases per 100,000 individuals in North America, Europe and Central Asia (2). The large majority of GB cases occur sporadically, and the tumor is found in all age groups, but is more frequently detected during the second half of life in men of the so-called 'non-Hispanic type' (3).

Current treatment protocols for GB include neurosurgery, high-dose radiation and chemotherapy (CT), most frequently using such cytostatics as temozolomide (TMZ) and lomustine, as well as the targeted antitumor agent, bevacizumab (4). The effect of treatment is insufficient, with a median relapse-free survival of 4-8 months, and an overall survival of 15 months (5). A total of ~25% of patients are able to survive for 24 months following diagnosis, which makes GB one of the most dismal diseases in oncology.

Resistance to treatment is associated with the heterogeneous nature of the cancer stem cell (CSC) population that dominates in the GB cell hierarchy (6). High β-catenin content is the most important characteristic of functionally active CSCs (7), due to their interaction with the local immunosuppressive microenvironment, thus activating the intracellular Wnt signaling pathway (8), PI3K/AKT/mTOR (9) and other mechanisms (10) that are responsible for plasticity and tumor progression.

Cytostatic drugs fail to effectively destroy CSCs in the body of a patient (11), and attempts to regulate CSCs by combining TMZ, immune checkpoint inhibitors (12) and molecular-targeted drugs (13) have not been unequivocally successful; thus, there is an urgent need to supplement the existing GB treatment protocols with more effective methods of regulating the functional activity of this cell type.

It could be hypothesized that a combination of drugs that inhibit the synthesis and biological activity of β-catenin with immunotherapy may destabilize the interaction between CSCs and the tumor microenvironment; block the mechanisms of

CSC phenotypic heterogeneity and plasticity; enhance the antiglioma effect of cytostatics; hinder tumor progression; and prolong the life of a patient.

The present study aimed to investigate the possibility of regulating GB CSCs by combining antagonists of the Wnt/β-catenin signaling pathway with immunotherapy.

2. Principles of GB treatment

Eliminating as many cancer cells (CCs) as possible is the key principle of GB treatment. Surgery is the method of choice for treating patients with GB (14), since it provides one-stage elimination of numerous CCs, reduces intracranial pressure, relieves brain compression, and improves the overall condition of the patient (15). However, the applicability of surgical treatment is limited. The severity of the condition of the patient, a functional status <70 on the Karnofsky scale, or subtentorial, thalamic, multifocal or bihemispheric localization of the tumor often render it impossible to treat the condition surgically without a direct risk to the life of the patient, and make a strong case for the refusal of surgery in patients with newly diagnosed GB (16,17). Moreover, brain tissue infiltration by CCs, even with the use of the most modern surgical technologies, does not allow the radical removal of the tumor without causing severe and irreparable neurological damage to the patient (18,19).

Cytoreduction effectiveness increases significantly when surgery is combined with radiation (20). Radiation therapy damages DNA, induces ageing processes and destroys GB cells (21). Intraoperative irradiation (22) can be used, as well as remote γ-therapy (23), which can be supplemented with brachytherapy (24), proton therapy (25) and neutron-capture therapy (26). The life expectancy of patients with GB is associated with the dose of radiation received, which may reach ≥60 Gy (27), while dose escalation can result in radiation-induced brain necrosis, impaired cerebral circulation, reactive gliosis, sclerosis, cyst formation and development of psychoorganic syndrome (28).

The majority of cases exhibit tumor relapse in 4-8 months after its removal, with ≤30% of patients undergoing reoperation (29), which improves their overall condition and quality of life, reduces their dependence on corticosteroids, and improves the effects of adjuvant CT.

Reoperation requires special skill, as fluid-filled cystic cavities that appear after radiotherapy can be misleading in terms of correctly identifying the necessary extent of resection; thus, careful planning of the surgery is necessary regarding clear anatomical landmarks (such as sulci, ventricles and dura mater boundaries), which is not always possible (30). It is partly for this reason that <10% of patients who are reoperated due to GB progression or recurrence undergo a third surgery, and only 2% of patients undergo ≥3 reoperations (17).

CT is recommended for all patients with GB. CT is the main method of destroying CCs, and it is used in combination with radiotherapy for treating a newly diagnosed GB to prolong remission after standard chemoradiation therapy and to prolong the life of patients with tumor recurrence (6). The drug of choice in CT is a cytostatic alkylating antineoplastic agent called TMZ, which is a tetrazine derivative.

Based on the potent cytotoxic activity of TMZ against glioma and carcinoma cells, the current stage of neuro-oncology development is termed the 'temozolomide era' (31). TMZ passes through the blood-brain barrier (BBB), easily penetrates into the cerebrospinal fluid, and, once in the bloodstream, undergoes a chemical transformation into monomethyltriazenoimidazolcarboxamide, the effect of which is attributed to the alkylation of guanine at O6 and N7 positions, with the subsequent triggering of aberrant reduction of methyl residues.

Patients are recommended a TMZ dosage of 75 mg/m^2 in combination with radiotherapy, followed by 6-8 cycles of TMZ with an increased dose of ≤150-200 and ≤400 mg/m^2. The maximum tolerated dose per treatment cycle is 1,000 mg/m^2. TMZ is usually employed in combination with lomustine (32) and bevacizumab, an inhibitor of endothelial growth factor in blood vessels.

Lomustine is an alkylating antineoplastic agent that is a nitrosourea derivative and a second-line CT drug. In addition to DNA alkylation, its antineoplastic effect is achieved via inhibition of genome repair enzymes, DNA matrix damage and suppression of some key enzymatic processes in CCs in the late G_1 and early S phases of the cell cycle (32). The recommended dose of lomustine in adults is 130 mg/m^2 when administered once orally every 6 weeks, and the total dose for all treatment regimens should not exceed 1,000 mg/m^2. Similar to TMZ, further doses of lomustine should be determined based on the therapy effectiveness and hematological response of the patient to the previous dose.

Previous attempts to enhance the antineoplastic treatment effect by combining TMZ or lomustine with the poly(ADP-ribose) polymerase inhibitor, olaparib (33), the histone deacetylase inhibitor, vorinostat (34) and other blockers of DNA repair enzymes (35) have not yet been successful. Procarbazine, vincristine, paclitaxel and platinum-based agents and other cytostatics have no significant advantages over TMZ and lomustine, and their administration has a detrimental effect on hematological status, increases toxicity and does not prolong the life of patients with GB (36). Electromagnetic therapy, which has been widely used in the last decade, expands the possibilities of cytostatics (28), but does not have any strategic advantages.

Thus, cytoreduction, cytotoxic and cytostatic principles define the current paradigm of GB treatment, which is able to ensure an average survival of patients of 15 months, with an average cost of treatment of 62,602$ (37). The main reason for such a cost is CT, the effectiveness of which is rather low despite the large number of antineoplastic agents available. The effect of CT is limited by the BBB permeability (28), but attempts to administer drugs into the removed GB bed, intrathecal/intraventricular administration of CT, or encasing cytostatics into nanocapsules for their targeted delivery to the brain by using monoclonal antibodies have not yet been successful (11), which is usually explained by the phenotypic plasticity (6) and particular properties of CSCs (38).

3. CC plasticity and resistance to treatment

Traditionally, the plasticity of CCs has been defined as their ability to transition into an undifferentiated state and resist the

effects of CT (39). A decisive step in understanding GB plasticity was made by Verhaak *et al* (40), who described proneural, neural, classical and mesenchymal tumor subtypes, showing the possibility of their transformation during treatment. It is noteworthy that the proneural subtype, identified in this study, was the most proliferative, while the mesenchymal subtype of the tumor was poorly proliferative and highly resistant to CT. Subsequently, high genome heterogeneity in the structure of these tumor subtypes (41) was revealed, while the critically low degree of DNA methylation, which was inherent to the mesenchymal GB subtype, predetermined the main trend of the problem development.

Brennan *et al* (42) further described six classes of DNA methylation in GB cells, with the highest methylation status assigned to isocitrate dehydrogenase *(IDH)* mutant tumors as the most differentiated and least plastic ones (42). The *IDH* mutation resulted in an excess of 2-hydroxyglutarate in CCs, which was accompanied by hypermethylation of the promoter regions of the O^6-methylguanine-DNA methyltransferase (*MGMT)* gene, which provided direct DNA repair (43). Reducing the degree of genome methylation made CCs more plastic (44). In light of this, in 2016, the WHO selected *IDH* mutations as the main criterion for systematization of gliomas, distinguishing *IDH*-wild-type and mutant types of GB (45).

Theoretically, *IDH*-wild-type GB cells have great replicative freedom and can use a wide arsenal of mechanisms for repairing single- and double-stranded DNA breaks, including homologous recombination and nonhomologous end joining (43-45). *IDH*-wild-type GB was divided into seven additional classes on the basis of genome methylation (46), which allowed Neftel *et al* (47) to describe four types of phenotypic states inherent in CCs: i) Neural progenitor-like cells with amplification of the *CDK4* gene; ii) oligodendroglial progenitor-like cells with amplification of the *PDGFRA* gene; iii) astrocyte-like cells with amplification of the *EGFR* gene; and iv) mesenchymal-like cells with mutation of the *NF1* gene.

Such taxonomy makes the phenotypic plasticity of CCs directly dependent on their local microenvironment (48-50), which not only predetermines the C phenotype, but also appears to be able to switch reproduction programs on and off depending on external conditions (51-53). In light of this, it can be presumed that the majority of phenotypic plasticity is exhibited by CCs with a hypomethylated genome, which are almost insusceptible to CT.

It is commonly considered that cells of this type, which are called CSCs, were described in 1997 by Bonnet and Dick (54) in their study on the hierarchical structure of the cell population in acute myeloid leukemia. However, as early as in 1877, Julius Friedrich Conheim, a student of Rudolf Virchow, indicated the presence of neoplastic elements with embryonic characteristics among the cells of gastric, breast and other aggressive tumors (55).

It is highly probable that CSCs are transformed descendants of normal neural stem cells (NSCs) that inhabit the subventricular zone and other germinative centers of the human brain. This is indicated by: i) The identity of >60% of proteins in the NSC proteome and CSCs of GB (56); ii) complex attractive-permissive interactions between cells of these types (57); iii) the CSC microenvironment, including clones of astrocytic and oligodendroglial progenitor cells that are transcriptomically similar to NSCs of the brain of a patient (58); and iv) the presence of NSC-like elements carrying the same mutations as differentiated CCs at all stages of gliomagenesis (59).

Normal NSCs and CSCs have a number of similar immunocytochemical markers on the cell surface, among which the CD133 antigen (prominin-1) is considered to be the main marker of GB stem cells (13). However, in addition to NSCs, this glycoprotein is present in hematopoietic stem cells (HSCs), endothelial progenitor cells, as well as in kidneys, trachea, salivary and mammary glands, placenta, digestive tract, testes, and other normal cells and tissues (60).

The role of CD133 antigen in the neoplastic process is not fully understood, but its direct association with cancer is clear. This marker is present in CSCs of lung cancer (61), colorectal carcinoma (62) and breast cancer (63). CSCs of the $CD133^+$ immunophenotype rank the highest in the hierarchy of GB cells (64) and are characterized by their tumorigenicity and high proliferative activity. However, the presence of differentiated non-tumorigenic $CD133^+$ cells, progenitor-like $CD133^+$ cells with limited proliferative potential, and CSCs that are tumorigenic and negative to this marker (65) but immunopositive to CD56, SRY-box transcription factor (SOX)2, SOX9, CD15, A2B5 and other antigens (64), allows to assert that the CSC phenomenon is not directly associated with cells of one certain immunophenotype.

This interpretation explains the failure of previous attempts to increase the effect of treating invasive gliomas by combining TMZ or lomustine with monoclonal antibodies against different CSC antigens (64,65). Probably, at the initial stage of a neoplastic process, mutations forming the primary stem lineage occur specifically in NSCs, which have the highest proliferation rate among all the cells of the nervous system. The proliferation of mutant cells leads to an increase in cell mass and competition for oxygen, thus triggering mechanisms that produce new generations of NSCs capable of arbitrarily switching between anaerobic and aerobic types of metabolism by regulating the level of lipid and glutamine utilization, which proliferate or remain in a quiescent state (66).

The functional activity of such cells is determined by the local microenvironment, which activates a number of molecular mechanisms, leading to the proliferation of CCs that have adapted to certain local microconditions. Perivascular localization is a characteristic of proneural CSCs, which are mesenchymal CSCs that are extracted from hypoxic zones and areas of necrosis (67), where local microconditions are unfavorable. The proneural type of GB is characterized by proliferating CSCs with a glycolytic type of metabolism and a low level of lipid utilization, whereas the mesenchymal type of GB is characterized by CSCs that are predominantly in the state of proliferative quiescence and are able to switch between glycolysis and aerobic respiration as well as exhibit a high level of lipid metabolism.

In fact, proneural and mesenchymal tumor subtypes reflect two possible states of CSCs, namely proliferation and survival. The transition to the survival state occurs under the influence of hypoxia (68), cytostatics (69), irradiation (70,71) and anti-angiogenic therapy (72). It can be assumed that the reverse transition, triggering tumor relapse, is also precipitated by the influence of the local microenvironment, which

activates numerous molecular mechanisms in CSCs (73), with the canonical Wnt signaling pathway playing a particular role among those mechanisms.

4. Wnt signaling pathway and the microenvironment of CSCs

The canonical Wnt signaling pathway is the most important intracellular signaling pathway, regulating embryogenesis and differentiation of normal stem cells (74). In GB pathogenesis, this pathway controls the balance between symmetric and asymmetric CSC divisions, which predetermines the aggressiveness and fatality of the tumor (75). The central link of this mechanism is β-catenin, which even in the absence of signaling is bound by a multiprotein 'destructive complex' represented by the adenomatous polyposis coli (APC), AXIN1/2, CK1 and glycogen synthase kinase (GSK)-3β proteins. GSK-3β protein kinase, which is activated by the 'destructive complex', phosphorylates β-catenin, which loses its functionality and undergoes degradation in the proteasome (74).

The Wnt signaling pathway is activated by one of 19 secreted Wnt-proteins interacting with Frizzled and low-density lipoprotein receptor-related protein (LPR)4/5 family receptors of the CSC surface, which activates the intracellular Disheveled protein and triggers a cascade of intermolecular interactions, leading to the blockade of the 'destructive complex' and accumulation of β-catenin in the CSC cytoplasm, which enters the nucleus, activates T-cell factor (TCF), and triggers the expression of pluripotency genes (76-78), inluding *MYC, CCND1*, cellular communication network factor 4 (*CCN4* also known as *WISP1*) and *PPARG*.

Mutations of the proline, glutamate and leucine rich protein 1 (*PELP-1*) gene, that is a potential co-activator of the canonical Wnt signaling pathway, are found in the majority of patients with GB, while mutations of the *APC* gene are identified in 14.5% of cases, and mutations of the *FAT1* gene, a negative regulator of Wnt signaling, are detected in 57% of patients (77-79). Mutations of other components are rare (80). A high content of β-catenin is one of the main characteristics of $CD133^+$ CSCs (81,82), which indicates the involvement of other mechanisms that cause its accumulation in this type of cells.

One of the mechanisms previously described (83) is the activation of the PI3K/AKT/mTOR signaling pathway, which enables both the stimulation of PI3K (13) and the immediate activation of intracellular AKT, which directly phosphorylates GSK-3β and increases β-catenin content (12). In turn, mTOR creates two multiprotein complexes, namely mTORC1 and mTORC2, which antagonistically regulate each other's activity: The first one decreases the β-catenin content in CSCs (84), while the second one increases it (85).

The increase in β-catenin content activates telomerases, thus leading to telomere lengthening, immortalization, stabilization of the CSC genome, and survival of CSCs under chemoradiotherapy (CRT) (86-88). The β-catenin content in CSCs increases due to semaphorins. a particular class of secreted and membrane proteins produced by neuroblasts and neurons (89). The synthesis of β-catenin in CSCs is induced by TGF-β secreted by CCs and immunosuppressive microglia cells (90). Through the SMAD and death-associated protein 6 (DAXX) signaling pathways, this cytokine activates the PI3K/AKT/mTOR axis (91), leading to β-catenin accumulation in CSCs, which indicates the strategic role of the microenvironment in CSC regulation.

Thus, β-catenin is one of the most significant regulators of CSC proliferation, with the local microenvironment being the main regulator of the intracellular β-catenin content in CSCs. The microenvironment regulates CSCs by triggering two divergent processes, namely autophagy and epithelial-mesenchymal transition (EMT) (73). The inflammatory microenvironment triggers micro- and macro-autophagy, which degrades intracellular proteins (92), thus providing energy-independent utilization of β-catenin, critically reducing CSC activity, and destabilizing the interaction with the extracellular matrix, which allows CSCs to migrate, and survive hypoxia, metabolic acidosis and CRT.

On the contrary, an immunosuppressive microenvironment (93) produces Wnt3a and Wnt5 ligands that activate the Wnt signaling pathway, with WISP1 as one of the target genes (94), the protein product of which increases the synthesis of other Wnt ligands, as well as that of IL-10 and IL-35, thus suppressing autophagy, and leading to an increase in β-catenin content and enhanced interaction with the extracellular matrix. In turn, the activation of AKT, which is mediated by α6β1 and other components of the integrin signaling pathway (95), activates the production of Wnt ligands, immunosuppressive cytokines, programmed cell death (PD)-1 and cytotoxic T-lymphocyte-associated protein 4 (94), which creates an 'autocrine loop', leading to increased immunosuppression, niche development and proliferation of CSCs (96).

Thus, the plasticity of CCs is caused by the influence of an immunosuppressive microenvironment, contributing to the increase of β-catenin content, which provides the transition from survival to proliferation in CSCs. Numerous attempts have been made to regulate these processes using targeted therapy.

5. Targeted therapy and CSC plasticity

Differentially activated signaling pathways differ for proneural and mesenchymal types of CSCs (97). In the former, the main role belongs to the signaling pathways of tyrosine kinase (TRK) β-receptor of platelet-derived growth factor (PDGF) and Notch; in the latter, the TGF-β, NF-κB and glycolysis signaling pathways are dominant. Pharmacologists have paid close attention to these molecular mechanisms; however, important breakthroughs have yet to emerge.

PDGF has become one of the priority targets for the inhibition of CSC proliferation. However, imatinib, an inhibitor of the TRK activity of PDGF from a new class of targeted cytostatics, failed trials in patients with GB (98). The multikinase inhibitor, sorafenib, was marginally effective when delivered directly to the tumor and only together with alternating electromagnetic field therapy (99), while sunitinib showed no effect at all in patients with GB (100).

Proliferation suppression of CSCs via the TRK-signaling domain of epidermal growth factor receptor (EGFR) in patients with GB has been attempted and failed. The peptide vaccine, rindopepimut, against the EGFRvIII antigen failed to meet expectations in combination with TMZ and bevacizumab (101).

Depatuxizumab, a monoclonal antibody against EGFR, loaded with the monomethyl auristatin F and displaying antimitotic effects, has demonstrated modest results both in combination with TMZ (102) and without it (103).

Onartuzumab, a monoclonal antibody directed against TRK-receptor of hepatocyte growth factor (HGF), was not successful in treating patients with GB in combination with TMZ and bevacizumab (104). The multikinase inhibitor cabozantinib, by blocking HGF signaling among other mechanisms, was ineffective in treating patients with GB (105). Only bevacizumab, by inhibiting TRK-receptor of vascular endothelial growth factor (VEGF) (106), in combination with TMZ, increased the life expectancy of patients with GB by 4-6 months, which is a remarkable achievement for targeted therapy.

Poor efficiency of TRK receptor inhibitors has spawned numerous unsuccessful attempts to target downstream components of the PI3K/AKT/mTOR signaling pathway. Buparlisib, a PI3K-kinase inhibitor, proved to be no more advantageous than monotherapy with TMZ (107), carboplatin and lomustine (108). The inhibitors of AKT kinase (perifosine) (109) and mTOR kinase (temsirolimus) failed to meet expectations both in combination with TMZ and without it (110).

The BRAF-V600E mutation is found in 20% of tumors, and it involves the substitution of valine-600 for glutamic acid, permanently activating the RAS/RAF/MEK/ERK signaling pathway. However, the inhibitors of the mutant RAF protein kinase, sorafenib (99) and vemurafenib (111), have not exhibited any effect in patients with GB.

The Notch signaling pathway is the oldest reported mechanism regulating the processes of stem cell differentiation (112). Activation of this pathway occurs when one of four Delta-like ligands or Jag1-2 ligands, binds to the membrane of one cell, directly interacts with one of four Notch receptors on the membrane of the cell, receiving the signal that triggers a cascade of intermolecular interactions, thus leading to the activation of target genes. Suppression of these mechanisms can block the proliferative functions of CSCs, but none of the Notch-signaling inhibitors has shown any effect in GB (113).

Attempts to affect CSCs via pharmacological suppression of the EMT-related mechanisms were also ineffective. The inhibitors of TGF-β, trabedersen (114) and galunisertib (115), did not produce a significant increase in survival rates when combined with TMZ, procarbazine, lomustine and vincristine.

Theories on a possible pharmacological regulation of CSCs via the PI3K/AKT/NF-κB axis are inconsistent (116). There have been some reports on the apoptosis of GB cells under the influence of this target (117) and the ability of cannabidiol to inhibit NF-κB activity (118), but this method has not been used for the regulation of CSCs in the complex treatment of GB.

Therefore, attempts of regulating CSC with the help of targeted therapy have been shown to be practically ineffective, but certain prospects are associated with the suppression of the Wnt/β-catenin signaling pathway.

6. β-catenin inhibitors and CT

β-catenin is a central factor that ensures the transition of CSCs from survival to proliferation mode. The strategy of counteracting this transition is based on (78) inhibiting the Wnt-ligand bind to the Frizzled receptor complex, suppressing the antagonists of the β-catenin destruction complex, and blocking the interaction between β-catenin and TCF/LEF. Most inhibitors of the Wnt signaling pathway have not yet undergone clinical trials; in this regard, repurposed drugs are of particular interest (Fig. 1 and Table I).

Wnt-inhibitory activity has been described for a number of agents from the group of non-steroidal anti-inflammatory drugs. Aspirin is one of the most popular types of medication from this group; its pharmacological effect is based on the inhibition of cyclooxygenase enzymes that reduce the levels of β-catenin in CCs, inhibit the expression of Wnt-target genes, enhance the cytotoxic effect of TMZ and bevacizumab (119), and prevent the development of colorectal cancer and a number of Wnt-dependent neoplasms (Fig. 1A).

Celecoxib, another non-steroidal anti-inflammatory agent, reactivates GSK-3β, eliminates the effects of the activation of the 'β-catenin degradation complex', inhibits TCF at a dose of 1,200-1,600 mg/day, and suppresses cyclooxygenase 2 and carboanhydrase, which impairs the adaptation of CCs to hypoxia (120) and enhances the cytotoxic effect of TMZ. In light of this, the administration of celecoxib preoperatively and before the end of CRT (121) has been described as a low-risk and justifiable procedure, but the ability of celecoxib to inhibit platelet aggregation combined with the hematological toxicity of TMZ raises some doubts concerning such claims (Fig. 1B).

The ability to inhibit the intracellular Wnt/β-catenin signaling pathway has also been observed in other drugs of this group. Indomethacin (Fig. 1C) is an indoleacetic acid derivative and cyclooxygenase 2 inhibitor, which disrupts the formation of the β-catenin/TCF complex with DNA and inhibits gene expression (122). Sulindac, a non-steroidal anti-inflammatory drug (Fig. 1D) from the group of acetic acid derivatives (123), enhances the degradation of β-catenin and prevents its translocation to the nucleus, thus inhibiting the expression of Wnt-target genes in CCs.

Despite the antiglioma potential of cyclooxygenase inhibitors, there are practically no data concerning their effect on CSCs. It should not be dismissed that inhibition of cyclooxygenase enzymes (124) may suppress the synthesis of Wnt-ligands by microglia cells, fibroblasts and endothelial cells from the microenvironment of CSCs. However, these drugs are not the only ones to hinder the activity of the Wnt signaling pathway in CSCs.

The antibiotic tigecycline (Fig. 1E) stimulates *AXIN1 β* gene expression and reduces β-catenin levels in CCs (125). The antiparasitic drug niclosamide (Fig. 1F) causes LRP6 degradation, reduces β-catenin content in the nucleus, and inhibits TCF/LEF factor activity (126). Pyrvinium pamoate (Fig. 1G) regulates *MGMT* gene expression in GB cells, reactivates GSK-3β and increases the sensitivity of GB cells to TMZ (127). Ivermectin (Fig. 1H) binds to the telomere maintenance 2 (TELO2) protein, which regulates PI3K activity and decreases β-catenin content in CCs (128).

Particular interest should be paid to a drug from the riminophenazine group called clofazimine (CFZ), N,5-bis-(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-2-phenazinamine, which was synthesized by Vincent Barry in 1957. CFZ was originally used as an antimycobacterial agent with proven bactericidal activity against Hansen's bacillus

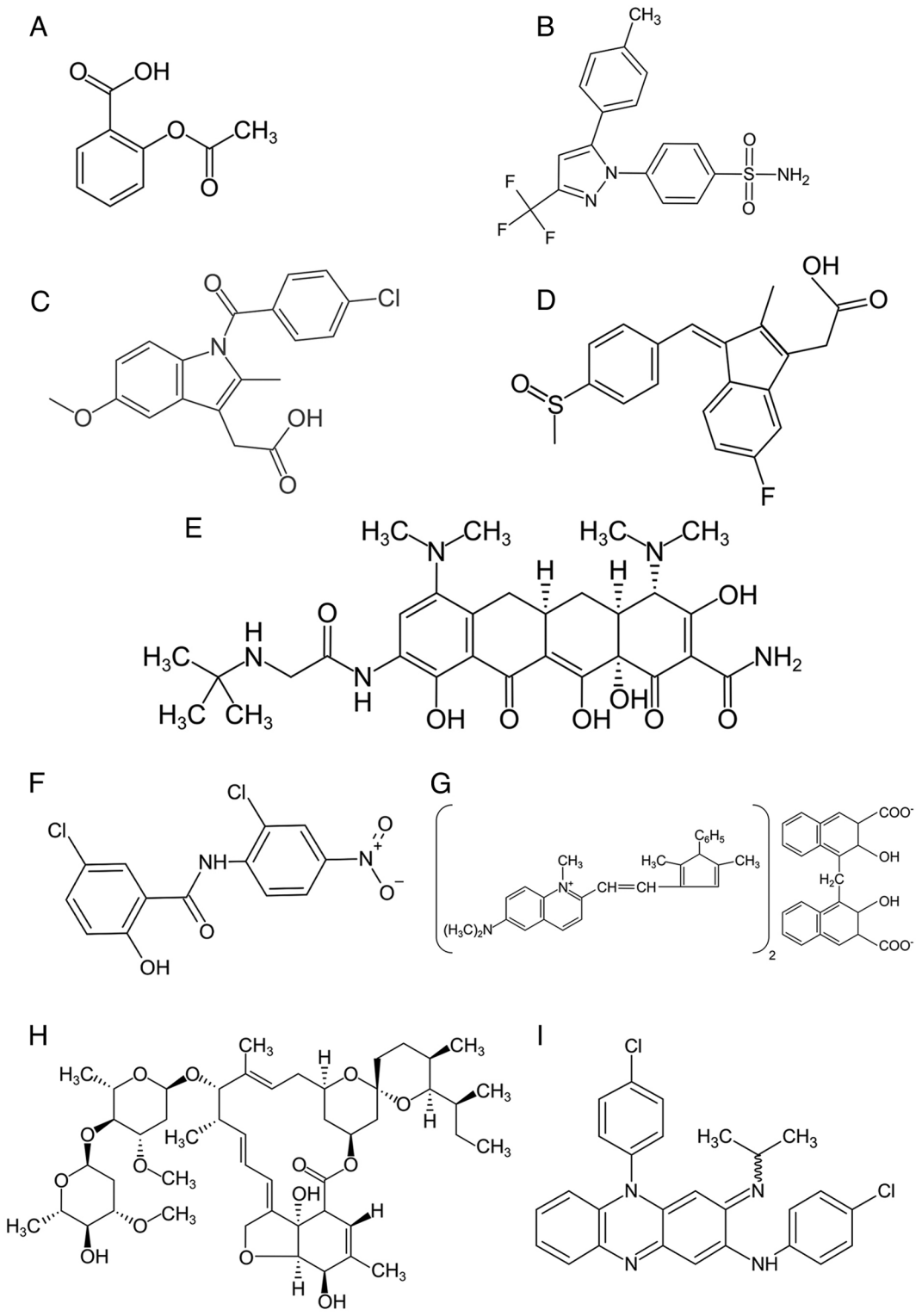

Figure 1. Repurposed drugs targeting the Wnt/β-catenin signaling pathway: (A) Aspirin (acetylsalicylic acid), (B) celecoxib, (C) indomethacin, (D) sulindac, (E) tigecycline, (F) niclosamide, (G) pyrvinium pamoate, (H) ivermectin and (I) clofazimine.

(Fig. 1I). CFZ inhibits mycobacterial growth, promotes the formation of reactive oxygen species, interacts with phospholipids of cell membranes, and disrupts the ionic equilibrium and energy metabolism of bacterial cells (129). Its anti-inflammatory properties combined with its ability to induce the release of prostaglandins and inhibit phospholipase A2 made it applicable in the complex treatment of erythema nodosum leprosum (130).

The antineoplastic properties of CFZ against triple-negative breast cancer are associated with the inhibited expression of Wnt signaling pathway-related genes, reduction of the cytoplasmic β-catenin level, and triggering of apoptosis due to cell cycle arrest in the G_2/M-phase in CCs (131). The antineoplastic effect of CFZ against CCs of different cell lines from colorectal adenocarcinoma and ovarian cancer is exhibited by

Table I. Repurposed drugs targeting the Wnt/β-catenin signaling pathway.

Name of the drug	Mechanism of Wnt signaling inhibition	Results of preclinical/ clinical studies	(Refs.)
Aspirin	-Prostaglandin E2/cyclooxygenase-dependent -Inactivation of PP2A and phosphorylation of β-catenin -Cross-talk between other pathways (such as NF-κB)	-Suppression of proliferation in almost any Wnt-dependent cancer -Reduction of tumor formation in the FAP mouse model, reduction of β-catenin levels in the tumor -Retrospective studies, especially for the prevention of colon cancer -Recommended for the prevention of CRC in individuals 50 to 69 years of age	(119)
Celecoxib	-Prostaglandin E2/cyclooxygenase-dependent -Promotes proteasomal degradation of TCF1 and TCF4 -Cross-linking of the c-Met/AKT pathway promoting GSK-3β phosphorylation	-Violation of proliferation in CRC, hepatoma, osteosarcoma and GB Decreased $CD133^+$ colon cancer stem cells -Inhibition of β-catenin-positive precancerous lesions in the colon of mice and in a model of colon cancer in rats -Reduction of polyps in patients with FAP after 6 months of treatment	(120,121)
Indomethacin	-PGE2/COX-dependent -Degradation of β-catenin through transcription inhibition -Impaired formation of the β-catenin/TCF4 complex	-Growth suppression of CRC cell lines -Decreased tumor burden in chemically-induced colon cancer	(122)
Sulindac	-PGE2/COX-dependent -Inactivation of PP2A and β-catenin phosphorylation	Growth suppression of colorectal cancer cell lines	(122)
Tigecycline	-Decrease in the content of β-catenin in the cytoplasm of CCs -Increased synthesis of AXIN1	-Suppression of the growth of cervical cancer cells -Inhibition of growth of cervical cancer xenografts	(125)
Niclosamide	-Promotion of FZD 1 endocytosis -Inhibition of DVL2 -LPR6 degradation	-Antiproliferative activity against osteosarcoma, CRC, breast cancer, lung cancer, hepatoma and GB. -Reduces the levels of β-catenin in mouse models of colorectal and basal breast cancer	(126)
Pyrvinium pamoate	-Activation of the isoform of casein kinase 1α, part of the Wnt pathway destruction complex	-Suppresses tumor growth in a colon cancer model	(127)
Ivermectin	-Deactivation of β-catenin by reducing C-terminal phosphorylation due to overactivation of phosphatases PP2A and PP1	-Antiproliferative against colon cancer (including stem cells) and lung cancer -Reduced tumor growth in colon cancer xenograft models	(128)

Table I. Continued.

Name of the drug	Mechanism of Wnt signaling inhibition	Results of preclinical/ clinical studies	(Refs.)
Clofazimine	-Participation in the inhibition of the TCF transcription complex -Cross-talk between other pathways	-Suppression of the growth of squamous hepatocellular cancer and lung cancer -Suppression of the growth of glioblastoma, lung and breast cancer -Combination and monotherapy for hepatocellular carcinoma with moderate positive results	(129)

PP, protein phosphatase; GB, glioblastoma; CRC, colorectal cancer; TCF, transcription factor; CC, colon cancer; FZD1, Frizzled class receptor 1; DVL2, dishevelled segment polarity protein 2; LRP, low-density lipoprotein receptor-related protein.

the IC_{50} of the drug, in the range of 2-10 μm/l, while against different cell lines of human GB this value is 20-40 μm/l, which may be associated with both Wnt inhibition and other metabolic effects of the drug (132).

CFZ partially penetrates into the cerebrospinal fluid through the intact BBB, but by being a lipophilic substance, the drug accumulates well in adipose tissue and in monocytes, and is captured by macrophages (133). Phagocytosis of CFZ crystals is not accompanied by obvious toxic manifestations, it suppresses NF-κB, enhances the synthesis of IL-1 receptor antagonist, and induces the M2 activation of macrophages (134). CFZ, captured and transported by monocytes, accumulates in the lungs, liver, and spleen (135), and partially in the bone marrow. Such findings suggest that CFZ may be a promising medical agent to be delivered into a tumor with the help of mononuclear cells, which constitute an important part of the CSC microenvironment (136).

There is a reasonable assumption that the need for specific transport through the BBB could decrease the antiglioma potential of the drug. However, the antiglioma Wnt-inhibitory effect has been described for valproic acid derivatives, phenothiazine neuroleptics, olanzapine, amisulpiride and other drugs (28), which pass through the BBB with ease. Nevertheless (120), since 2005, >100 studies on the combination of CT with different drugs have failed. Identifying the reasons of such outcomes is directly dependent on the need to regulate the CSC microenvironment, which is, the main factor contributing to the lower antioglioma potential of pharmacological agents. However, the development of practical approaches to solving this problem requires rethinking a number of its important theoretical aspects.

7. CSC microenvironment, immunotherapy and immunodeficiency

Since the 1950s, the brain has been considered to be an immune privileged organ. The microenvironment of CSCs was associated only with resident microglia cells, which were considered to originate from cells of the embryonic yolk sac and not to interact with the immune system after the BBB closure (137), and mononuclear cells and other bone marrow immunocytes were not considered to interact with microglia in any way. However, numerous data suggest otherwise.

It has been demonstrated that microglial cells can enter deep cervical lymph nodes and interact with T and B lymphocytes. Each T cell can recognize several hundred fragments of a single antigen on the antigen-presenting cell membrane together with class I and class II major histocompatibility complex molecules. At the same time, B cells can bind intact antigens with their native structure, indicating an active informational interaction between bone marrow cells and microglia. Simultaneously, immunocytes are able to penetrate the brain through the tumor bloodstream, vascular plexuses, cranial microchannels and sinuses, and cerebrospinal and interstitial fluid (138), and kill cells containing the presented antigen. Such findings indicate an important role of the immune system in the formation of the CSC microenvironment.

Experimental studies conducted in the last decade have expanded the concept with regard to the participation of immunocytes in GB pathogenesis. Tumor development in the brain is accompanied by migration and homing of red bone marrow cells to the tumor nidus (139). To date, >80 chemoattractants have been described to draw bone marrow cells to the tumor nidus through different types of receptors, including the recognition of stromal cell factor (SDF)1 or chemokine C-X-C motif ligand 12 (CXCL12), a chemokine of the CXC subfamily that binds to the CXCR4 receptor on the membrane of $CD45^+$ cells in the bone marrow, inducing their migration to the tumor (140). Numerous studies (141,142) suggest that the involvement of bone marrow cells in the neoplastic process enriches the population of immunosuppressive tumor microglia, and is accompanied by a stronger resistance of CCs to cytostatics.

Production of numerous immunosuppressive cytokines by neoplastic cells determines the microglia polarization vector, with a significant proportion of CCs being completely removed during surgery or destroyed by CRT. It is safe to assume that vaccination with dead tumor tissue can enhance the antineoplastic immune response, and the production of exosome-containing microRNAs and other antitumor

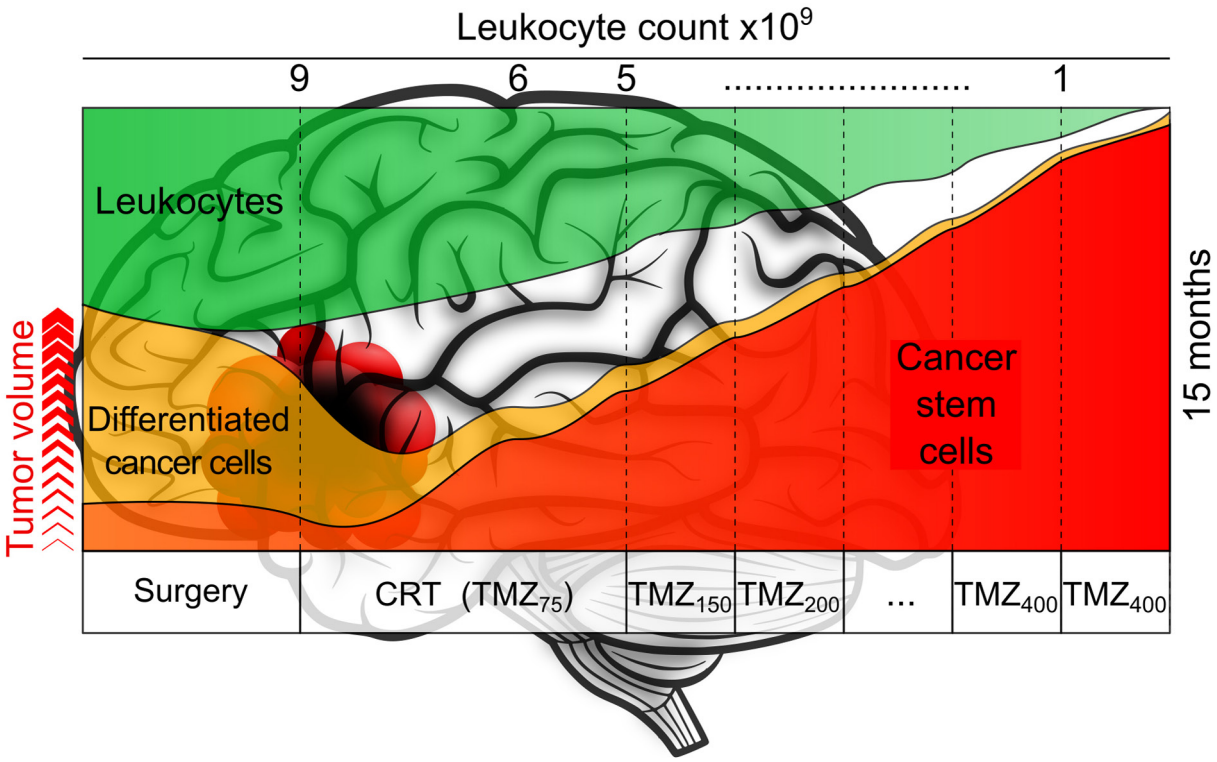

Figure 2. Association between the level of leukocytes (green color) after multiple cycles of chemotherapy and aggressiveness of GB. The red color indicates cancer stem cells of GB. The yellow color indicates the differentiated cells of GB. GB, glioblastoma; CRT, chemoradiotherapy; TMZ, temozolomide.

factors that can significantly alter the properties of the CSC microenvironment, resulting in increased effectiveness of CT. However, the local microenvironment in tumor recurrence is formed in conditions of overall immunodeficiency that results from the debilitating effect of PD-ligands, glucocorticosteroids, radiation and CT (143), and this fact predetermines its characteristics.

In fact, the exhausted phenotype of immunocytes is developed due to the GB cells producing PD-L1 and 2, CTLA-4 and other proteins that inhibit T-cell activity receptors (144). The emergence of a new class of drugs that prevent PD-ligands from binding to T-cell receptors has attracted interest (145), but the effectiveness of nivolumab and other immune checkpoint inhibitors in the complex treatment of GB was revealed to be low (146), thus suggesting the existence of other causes of systemic immunosuppression.

Corticosteroids in the complex treatment of GB are used to counteract cerebral oedema, but their use (147) is associated with inhibition of lymphocyte proliferation, suppression of migration and interaction of macrophages with T and B lymphocytes, inhibition of interferon gamma (IFN-γ) release from macrophages, and reduced antibody formation. The use of corticosteroids leads to increased blood glucose levels and dexamethasone-induced leukocytosis (148), which exhausts the red bone marrow (149) and reduces overall survival.

The maximum possible reduction of the number of CCs in brain tissue is achieved by active use of chemoradiation therapy. According to a previous study, >30 fractions of γ-therapy allow lymphocytes to accumulate an average radiation dose of 2.3 Gy, and the number of $CD4^+$ cells in the organism decreases by half and remains low for >1 year (150), which is time a patient may not have. It is likely that higher radiation doses, which are often used in treatment regimens such as in the case of radiosurgery, may be accompanied by accumulation of a significantly higher radiation dose in immunocytes, which induces a bystander effect in the bone marrow and causes the death of a significant number of immune cells.

In turn, cytostatics suppress hematopoiesis and immunopoiesis, contributing to the development of clinically significant thrombocytopenia and leukopenia, which is generally considered the only criterion (151) limiting TMZ dose escalation. Notably, a neutrophil count ≥40% below the norm is a criterion for a satisfactory prognosis in *IDH*-wild-type GB (152), while reaching grades 3 and 4 neutropenia is considered a positive prognosis for CT outcome, with a neutrophil count of $<1\text{-}10^9$ cells/l, warranting dose reduction during the subsequent treatment stage.

In light of this, the completion of the main regimen of GB treatment results in the immunodeficiency of the patient, while tumor relapse is accompanied by the recruitment of immunocytes with an 'exhausted phenotype' and the formation of an immunosuppressive CSC microenvironment, which supports tumor growth, promotes further involvement of the bone marrow in the neoplastic process and prevents immunotherapy from fulfilling its antineoplastic potential completely (Fig. 2).

8. HSCs and immunotherapy

Reprogramming of HSCs in the bone marrow during tumor growth largely contributes to the process of immunosuppression. Participation of normal stem cells in the tumor process became a focus of attention after the publication of the study

by Aboody *et al* (152), which showed the migration of NSCs to glioma cells. Snyder *et al* (153) reported the existence of an association between GB cells and normal stem cells of other types, in particular with HSCs. HSCs have been demonstrated to have high mobility towards GB cells (154) and, when injected into the bloodstream of an animal with glioma, they migrate to the tumor nidus, where they accumulate in the blood vessels of the tumor (155), spread throughout the invasion area, and penetrate into the necrotic zones of neoplastic tissue.

While migrating into a tumor, HSCs are capable of interacting with CCs and can exchange a fluorescent stain, which becomes inextricably connected with intracellular proteins in the process of staining (156), and this fact indicates the exchange of information between stem cells and CCs. The reported mechanisms of such exchange (157) include the fusion of stem cells and CCs, horizontal transfer of information through tights intercellular junctions with areas of partial membrane fusion and cytoplasm unification (158), and the production of exosomes and other microvesicles containing fragments of DNA, microRNA and proteins (159) into the external environment. All this is accompanied by the reprogramming of interacting cells and their development of new properties.

The significance of reprogramming of HSCs in GB pathogenesis has barely been studied, but there are reasons to consider that it is the most important factor of the immune system inactivation in the tumor process (160). Bone marrow stem cells are known to be a heterogeneous mixture of subpopulations, having cell elements with different degrees of maturity, lifespan, gene expression profiles and epigenetic programs of differentiation (161). Notably, a part of HSCs, reprogrammed by the tumor, are able to gain advantage over other HSC clones in bone marrow (162), which can be accompanied by the expansion of mutant immunocytes that are tumor tolerant (163).

Theoretically speaking, this is the most important issue predetermining the final effect of all existing glioma immunotherapy methods, the scenarios of which are based on the use of mononuclear $CD45^+$ cells recruited from the bone marrow into the systemic bloodstream when granulocytic (G-CSF) or granulocytic-macrophage (GM-CSF) colony-stimulating factors are administered to patients. According to experimental data (164,165), the introduction of G-CSF into the organism of experimental animals with injected glial brain tumor fills the tumor tissue with markers of anti-inflammatory microglia (166), while the subsequent introduction of bacterial lipopolysaccharide and IFN-γ (167) promotes the inflammatory M1 activation of macrophages. An even greater antiglioma effect is achieved when HSCs of a healthy sibling are transplanted into the organism of an animal with glioma (161).

This fact directly indicates the essential role of HSCs in the formation of the CSC microenvironment. Reprogramming of HSCs during their interaction with CSCs and severe leukopenia from chemoradiation therapy form a vicious circle, creating conditions for β-catenin accumulation in CSCs and enhancing the lethal potential of this cell type. In this regard, β-catenin content in tumors is an important criterion of immunotherapy effectiveness, and the existing protocols of immunotherapy should be supplemented with drugs that reduce the level of β-catenin (Fig. 3). At the same time, the construction of immunotherapy scenarios using HSCs of a healthy relative or autologous HSCs prepared before the disease, suggests positive future prospects for the complex treatment of gliomas.

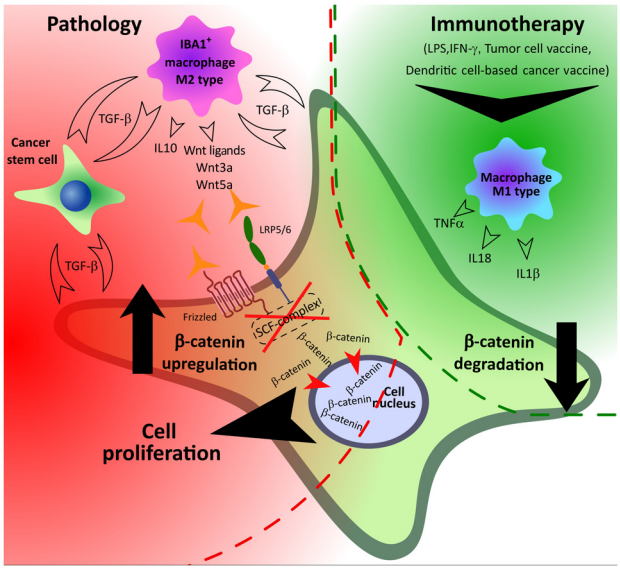

Figure 3. Scheme of the relationship between β-catenin activity and immunotherapy. LRP, low-density lipoprotein receptor-related protein; LPS, lipopolysaccharide.

The existing algorithms of adaptive cell-based immunotherapy of gliomas are still far from reaching this goal. Adaptive immunotherapy involves the use of autologous immunocytes, the source of which is a 'leucoconcentrate' of mononuclear cells expressing the leukocyte common antigen $CD45^+$, with subsequent separation of T-lymphocytes *ex vivo,* their additional activation by IL-2 and the targeting of GB cells with proteins against key antigens, including IL13Rα2, HER2 and EGFRvIII. The disadvantages of such therapy are clear, since administration of G-CSF to the patient and stimulation with antigens of Bacillus Calmette-Guérin, *Staphylococcus aureus* or other suppurative microflora may lead to increased brain oedema, which is a life-threatening condition.

An alternative strategy involves the stimulation of the immunocytes of a patient *ex vivo* with antigens of suppurative microflora and CCs; however, after CT, the bone marrow of the patient is exhausted, and it is practically impossible to accumulate the required number of immunocytes during one stage of leucopheresis. At the same time, repeated administration of G-CSF to the patient further exhausts the bone marrow and can promote the formation of an immunosuppressive environment of CSCs.

Attempts have been made to use genetically modified T cells equipped with chimeric antigen receptors (CARs), in which sections of antigen-recognition domains, consisting of monoclonal antibodies, are connected to sections of intracellular T-lymphocyte signaling domains. The main problem with this technology is the lack of absolutely perfect target antigens that are completely tumor-specific, since the antigens IL13Rα2, HER2, EGFRvIII, B7-H3 and CSPG4, which are traditionally used as targets for CAR-T cell creation, are not specific to CSCs, but much less homogeneously expressed in tumors.

Attempts to create multivalent CAR-T cells (167) targeted against the synNotch receptor and other molecular targets in CSCs have not yet produced the desired effect, since local immunosuppression remains the main problem limiting the antitumor potential of CAR-T cells. Attempts to combine CAR-T cells with microRNAs that locally inhibit immunosuppressive genes (168) have not yet exhibited any specific benefits and are associated with toxicity and BBB penetrability problems (169). Attempts to increase the efficiency of the targeted delivery of CAR-T cells by binding them to chlorotoxin, a peptide particularly tropic to CCs (170) that is found in the venom of the scorpion *Leiurus quinquestriatus*, as well as to combine CAR-T therapy with intratumoral delivery of IL-12 (171) have not been successful either.

The main disadvantage of adaptive immunotherapy methods is the use of autologous exhausted immunocytes. Exhaustion is a state of T-cell dysfunction that occurs in cases of chronic infections and cancer, and is characterized by decreased effector function and self-renewal capacity, sustained expression of inhibitory receptors and a transcriptional state (171) distinct from that of functionally active effector cells or memory T cells. Exhaustion prevents optimal control of infections and tumors, but serves as a mechanism to protect cells from death when they are hyperstimulated by tumor antigens.

It can be assumed that a high proliferation rate and a dynamic change of the antigen spectrum of GB cells are aggression factors that inactivate the immune system. Attempts to solve this problem using only immune checkpoint inhibitors have not been successful thus far; in this regard, the use of allogeneic cytotoxic lymphocytes derived from a healthy donor has been reported (172), which requires increasing doses of dexamethasone, revealing the typical consequences of such therapy. However, this approach deserves special attention, particularly taking into account the possibility of regulating the local microenvironment of CSCs by using a combination of CAR-T cell technology with oncolytic adenoviruses (173) and immune checkpoint inhibitors (174).

According to a previous study (175), CAR-T cell therapy poses serious problems in the treatment of central nervous system tumors, which emphasizes the problem of the hostile immunosuppressive microenvironment of CSCs. However, in addition to CAR-T cells, attempts to use CAR-T natural killer cells or CAR macrophages and to generate active antineoplastic immunity in patients with GB are of great interest.

Active immunotherapy involves the development of antineoplastic immunity in a patient with GB by vaccination with tumor cell vaccines or incubation of immunocytes with CC lysates *ex vivo*, with the subsequent return to the patient in the form of a dendritic cell vaccine (176). The use of tumor cell vaccines has numerous advantages, including the fact that systematic vaccination activates CD8-lymphocytes, the processes of antigen presentation by macrophages, synthesis of pro-inflammatory cytokines and modification of the tumor microenvironment (177), which increases the survival rate of patients with both newly diagnosed and relapsed GB (178).

Autologous or allogeneic CSCs are used as antigens to create tumor cell vaccines, which can stimulate an immune response considering the heterogeneity of the neoplastic cell population (179), which is particularly relevant for GB. In light of this, a tumor cell vaccine should include a combination of dead CSCs (for example, after repeated freezing and thawing of proneural-type CSCs derived from the first removed tumor) and CSCs of mesenchymal phenotype obtained by sequential irradiation of autologous CCs, as well as CSC derivatives from other patients. This approach allows not only the destruction of CCs, but can also significantly increase antineoplastic immunity (180) and allow the formation of local criss-cross intratumor interactions between $CD4^+$ lymphocytes and other T cells (181), thus leading to increased production of proinflammatory cytokines and modifying the microenvironment of CSCs.

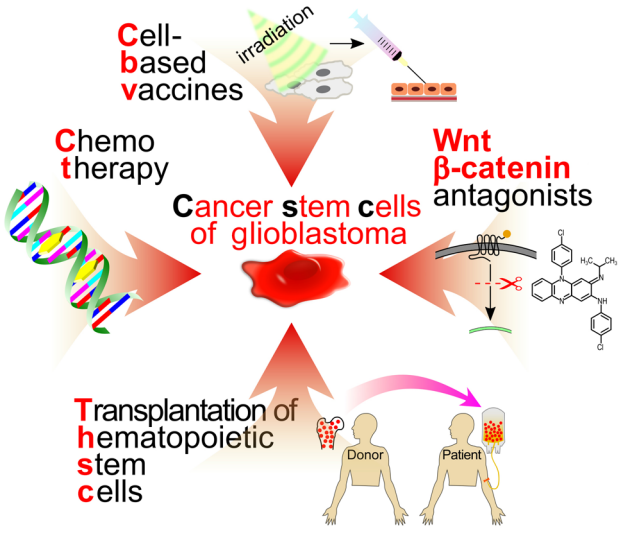

Figure 4. Hypothetical scheme for personalized therapy of glioblastoma using inhibitors of the Wnt/β-catenin signaling pathway and immunotherapy.

It is possible to create vaccines (182) using live genetically modified CCs producing GM-CSF. Antigen-specific antineoplastic vaccines producing GM-CSF have been used for tumor treatment for >20 years, and their use predominantly reveals the problem of leucopenia and immunodeficiency, actually limiting the antiglioma potential of immunotherapy. A possible method to solve this issue is the combination of a tumor cell vaccine with transplantation of HSCs from a haploidentical donor, ideally a sibling.

The common disadvantages of using tumor cell vaccines are characteristic of the immunotherapy method with dendritic cell vaccines. Dendritic cells are professional antigen-presenting cells with high functional plasticity, which originate from HSCs and show immunostimulatory or immunosuppressive potential depending on the sequence and combination of microenvironmental stimuli. The technology for the preparation of dendritic cell vaccines includes the administration of G-CSF or GM-CSF to the patient in order to recruit immunocytes into the bloodstream, subsequent isolation of a fraction of mononuclear cells containing a sufficient number of $CD34^+$ HSCs, *ex vivo* stimulation with CC antigens combined with IL-2 or IL-4, multiplication in the presence of pro-inflammatory cytokines, and return to the patient (182). Antigens for the creation of dendritic cell vaccines include CSCs, CC lysates, produced CCs and CSCs, exosomes, glioma-associated peptides, and DNA and RNA fragments (183), but their efficacy in the treatment of GB is relatively low.

Despite increased individual survival periods of >23 months that have been reported (184), the overall situation has not changed even after combining dendritic cell vaccines with immune checkpoint inhibitors and other immunotherapy methods (185). Therapy with dendritic cell vaccines results in general immunostimulatory effects in the form of increased levels of immunosuppressive cytokines and local infiltration of the tumor stroma by immunocytes. However, the main challenge of therapy with dendritic cell vaccines is the problem of immunosuppression, the solution of which is possible only after creating such vaccines on the basis of healthy HSCs or developing methods of restoring the patient's own HSCs.

It is the condition of HSCs that predetermines the final effectiveness of almost all immunotherapy methods, but the official ClinicalTrails website describes only one study (namely NCT00014573) suggesting the use of immunotherapy methods while supporting HSC transplantation in treatment-refractory brain tumors. The use of autologous HSCs or stem cells of a sibling, and pharmacological regulation of β-catenin level in HSCs together with immunotherapy has not been discussed to date.

9. Conclusion

CSCs present a major challenge in GB treatment. Destruction of these CCs with irradiation and cytostatics appears to be impossible, and requires certain adjustments of existing GB treatment strategies towards regulation of CSCs rather than destruction of CCs. Numerous attempts to solve this problem with the help of targeted medication have not been successful thus far, which is usually explained by the plasticity of CSCs, with β-catenin being the central link in the system of intracellular signaling pathways, regulating the plasticity of this cell type.

Pharmacological regulation of the β-catenin level in CSCs using repurposed drugs opens new horizons for the regulation of the proliferative potential of this cell type. The antileprosy drug CFZ has the ability to inhibit the intracellular Wnt/β-catenin signaling pathway. It is characterized by its good tolerability and has demonstrated antineoplastic activity against CSCs of a number of aggressive carcinomas, while its antiglioma potential is virtually unexplored. The ability of CFZ to accumulate in monocytes makes it very promising to be used as a drug for targeted delivery to the tumor nidus, due to the transportation potential of this cell type (Fig. 4).

Particular attention should be paid to the fact that the β-catenin level in CSCs directly increases under the influence of an immunosuppressive microenvironment, which is formed with participation of microgliocytes and monocytes of the exhausted phenotype, caused by radiation, cytostatics and the reprogramming effect of the tumor on bone marrow HSCs. Indeed, all existing GB treatment protocols lead to immunodeficiency, which actually limits the therapeutic potential of all drugs and technologies, since it is almost impossible to reduce the β-catenin level without suppressing Wnt ligand production by the local microenvironment of HSCs.

The use of CFZ and other repurposed drugs with demonstrated Wnt-inhibitory activity can reduce the level of β-catenin in CSCs. In turn, immunotherapy can regulate the microenvironment of CSCs, resulting in a decrease in Wnt-ligand synthesis and breaking the aforementioned vicious circle, which would allow to create a technology of CSC management as part of the complex immunotherapy of GB. One of the most important tasks in the creation of such technology is developing immunotherapy scenarios with the use of tumor cell vaccines containing a heterogeneous composition of autologous and allogeneic, irradiated and non-irradiated CCs, which will allow to induce a multidirectional antineoplastic immune response. This should be enhanced by the transplantation of healthy sibling HSCs and the administration of CFZ or other drugs, thus reducing the β-catenin content in CSCs. Pharmacological regulation of β-catenin content in CSCs and cellular immunotherapy should be used together, since they are two sides of the same coin.

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Authors' contributions

AK, OP and IB conceived the study and searched for relevant articles. AK and IB wrote the original draft, and reviewed and edited the final manuscript. Data authentication is not applicable. All authors have read and agreed to the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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