A guide through conventional and modern cancer treatment modalities: A specific focus on glioblastoma cancer therapy (Review)

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Abstract. Cancer still ranks as one of the top causes of morbidity and mortality despite recent improvements in standard chemotherapy, radiotherapy, and surgery. This underlines some of the difficulties in creating successful therapeutic strategies, but it also highlights the shortcomings of conventional methods. In order to enhance the standard treatment of cancer patients, biology-driven therapies are emerging towards more specific and effective clinical options. In the present review, both conventional and novel methods for cancer treatment were addressed, with a particular focus on Glioblastoma multiforme (GBM) therapies. GBM is one of the most challenging cancers for conventional treatments, and survival rates of patients remain very low. In the present review, focus was addressed on employed chemo- and radiotherapies along with developing novel targeted and immunotherapies assessed in clinical trials on patients with GBM or yet to be evaluated clinically. It was aimed to evaluate efficiency of treatments in suppressing GBMs, roadblocks and challenges. A brief discussion of a few promising delivery methods for targeted drug and gene therapy for cancer was also provided. Increment advancements in this field emphasizes the significance of combining different treatment strategies for improved survival and quality of patients' lives.

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1. Introduction

Among the top leading causes of death in the world, cancer represents a major public health concern accounting for more than 19 million new cases and nearly 10 million death cases worldwide in 2020, according to the International Agency for Research on Cancer (https://gco.iarc.fr/today/home accessed on Dec 25th 2021). The severity of cancer arises from the capacity of cancerous cells, harboring oncogenic mutations, to divide chaotically, clump to form tumors, destroy surrounding tissues and metastatically invade distant organs. The burden of cancer is thus a major problem in developing countries and aging populations. Fighting cancer thus necessitates i) a thorough understanding of cancer biology and driving intrinsic and extrinsic stimulators such as lifestyle and environmental factors, ii) alleviated awareness of preventive measures, iii) early detection and iv) efficient and targeted treatments.

Glioblastoma multiforme (GBM) is a grade IV malignant glioma and is the most frequent and aggressive brain tumor associated with a very poor patient prognosis (1). According to the Central Brain Tumor Registry of the United States, GBM was reported as the most commonly occurring malignant brain tumor from 2014-2018. Among brain and other central nervous system (CNS) tumors, GBM accounts for 14.3% of all tumors and 49.1% of malignant tumors (2). Due to its high tumor cell proliferative nature and neovascularization, GBM can infiltrate crucial structures in the brain, thus increasing the possibility of tumor recurrence even after conventional treatment (3). In 2007, the World Health Organization (WHO) reported that the median survival rate of patients diagnosed with GBM is significantly low, ranging between 1 to 2 years (4). This low survival rate suggests that more efficient treatment methods are needed to increase the lifespan of the patients.

Massive research has been conducted in the field of cancer therapies since the discovery of X-rays in 1896 and the

emergence of radiotherapy as a main source for cancer treatment (5). Chemotherapy, which involves the use of cytotoxic drugs that target proliferating cells, is the second conventional modality widely used for cancer treatment. Both radio- and chemotherapy are considered as a 'two-edged sword'. This is because these treatments are often associated with damage or genetic modifications to normal tissues, nonspecific drug distribution, and multidrug resistance (MDR). Progress in these standard treatments is built on evidence-based medicine where clinical achievements are evaluated from results of randomized trials.

The drawbacks of conventional therapies necessitate the identification of specific genetic modifications and molecular signaling events responsible for tumor cell formation. This paved the way for new therapeutic interventions that specifically target tumors, providing an advantage over toxicity and lack-of-specificity associated with conventional therapies. Immunotherapy and targeted drug- and gene therapy are emerging as the treatment breakthroughs for cancer therapy. These modalities aim to target the patient's own immune system or genes to eliminate cancer cells. With few exceptions, these interventions are not yet part of standard therapy despite their advantages and the progress made in the field (6).

Though cancer is a genetic disorder, cancer cells hold genetic and phenotypic heterogeneity between individuals and from one cell to another within the same tumor. In the line of this within-tumor heterogeneity, combination therapy is now a plausible therapeutic approach where cancer management relies on more than one method. Improved clinical outcomes of standard cancer control interventions and increased investment in translatable immunotherapy and targeted therapy research would augment progress against cancer. Consequently, treatment advancements would help managing treatment-related adverse effects and hurdles, allowing for an improved quality of life for cancer patient survivals. In the present review, both conventional and new cancer therapy strategies were reviewed within the context of GBM (Fig. 1), and recent treatment breakthroughs were illustrated in the light of the corresponding potentials.

2. Radiotherapy (RT)

Since the discovery of X-rays in 1896, RT has emerged as a main source for cancer treatment, either alone or in combination with surgery and other medical treatments (5). RT aims to deliver the optimal isodose of radiation to the target tumor volume with almost no radiation on organs at risk (OAR). Radiation acts mainly as a tumor initiating agent at low doses resulting in DNA modifications, whereas radiation of higher doses relevant to RT is considered a tumor promoter (7). For almost a century, several technological innovations were implemented in RT to achieve high delivery doses in tumors without exceeding OAR tolerance dose and thus translated into medical benefit. In addition, randomized trials aided in improving the use of RT and altering clinical practice (8). Starting in the 1990s, technological research benefited from the computer assisted technology and CT-based simulations to deliver radiation to a 3D conformal approach allowing for an improved dose distribution (9). Later, high-precision modern era of RT was made possible with stereotactic RT used to target mobile tumor using image-guided technology (10) in addition to an image-guided RT approach which accounts for changes in patient anatomy and tumor shape (11,12). It is worth noting that, in certain cases, patients with GBM having a highly immunosuppressive tumor environment do not efficiently respond to traditional RT. For this reason, other viable therapies, such as Proton Beam Treatment (PBT), have emerged to substitute for traditional treatments. After the invention of particle accelerators, proton therapy was made possible with remarkable developments reported in the past 20 years (13). This radiation aims to reduce the volume of OAR and irradiated normal tissues thus avoiding radiation-induced secondary cancers (8). In a study conducted by Lee *et al* (14) in 2021, combining PBT and Tumor Treating Fields (TTFields), which uses anti-mitotic alternating low and intermediate electric field frequency, showed that PTB sensitizes GBM cells to TTFields. The study provided evidence that this combined therapy could limit cell migration and metastasis by downregulating the NFkB, MAPK and PI3K/AKT signaling pathways (14). These technologies paved the way for personalized therapies where different RT machines are used according to the special types of tumors and/or patients conditions (15). Given technological advancements, and owing to its cost effective (16) and curative treatment (17), RT is considered a conservative cancer therapy that yields improved patient care over the past decades.

RT has long been the standard adjuvant approach for GBM. Among the multimodal therapeutic strategies used to treat GBM, RT remains the primary treatment approach for patients with unresectable GBM (18). Randomized trials by Walker *et al* (19) showed a dose-dependent relationship with improved survival rates in patients with proven malignant gliomas. The median survival of these patients increased from 18 to 28 weeks upon the administration of no radiation vs. 50 Gy, respectively. Additionally, the median life span of these patients doubled when employed with 55 Gy and increased by 2.3 times at 60 Gy when compared with those who did not receive RT. Doses of 60 Gy or 60 Gy, plus a booster dose of 10 Gy, to whole brain were associated with survival rates of ~35 and 31 weeks respectively in patients with GBM (20). While the administration of doses below 60 Gy had relatively poorer outcome, increased radiation doses through standard fractionation increased the risk of normal brain injury (21). In this regard, the European Organization for Research and Treatment of Cancer recommends a total dose of 60 Gy in 30 fractions delivered to patients with GBM in a single-phase technique with a unique gross tumor volume. This comprises the T1 contrast enhancement region plus a margin of 20 to 30 mm (22).

In general, and for most cancer types, second cancer is one of the most serious sequelae of RT. RT-associated second cancer can be also influenced by environmental factors, lifestyle and genetic susceptibility of the host (23). Second neoplasms may develop at various organs depending on the sites of first neoplasms and the degree of risk differs depending on the organ (24). Additionally, one of the hallmarks of GBM is tumor heterogeneity and its capability for local relapse (25). Its intertumor and/or intratumor heterogeneity feature renders it to resist standard treatments including RT. Therefore, to improve the overall survival rate of patients with GBM and decrease the treatment toxicity, radiation oncology should

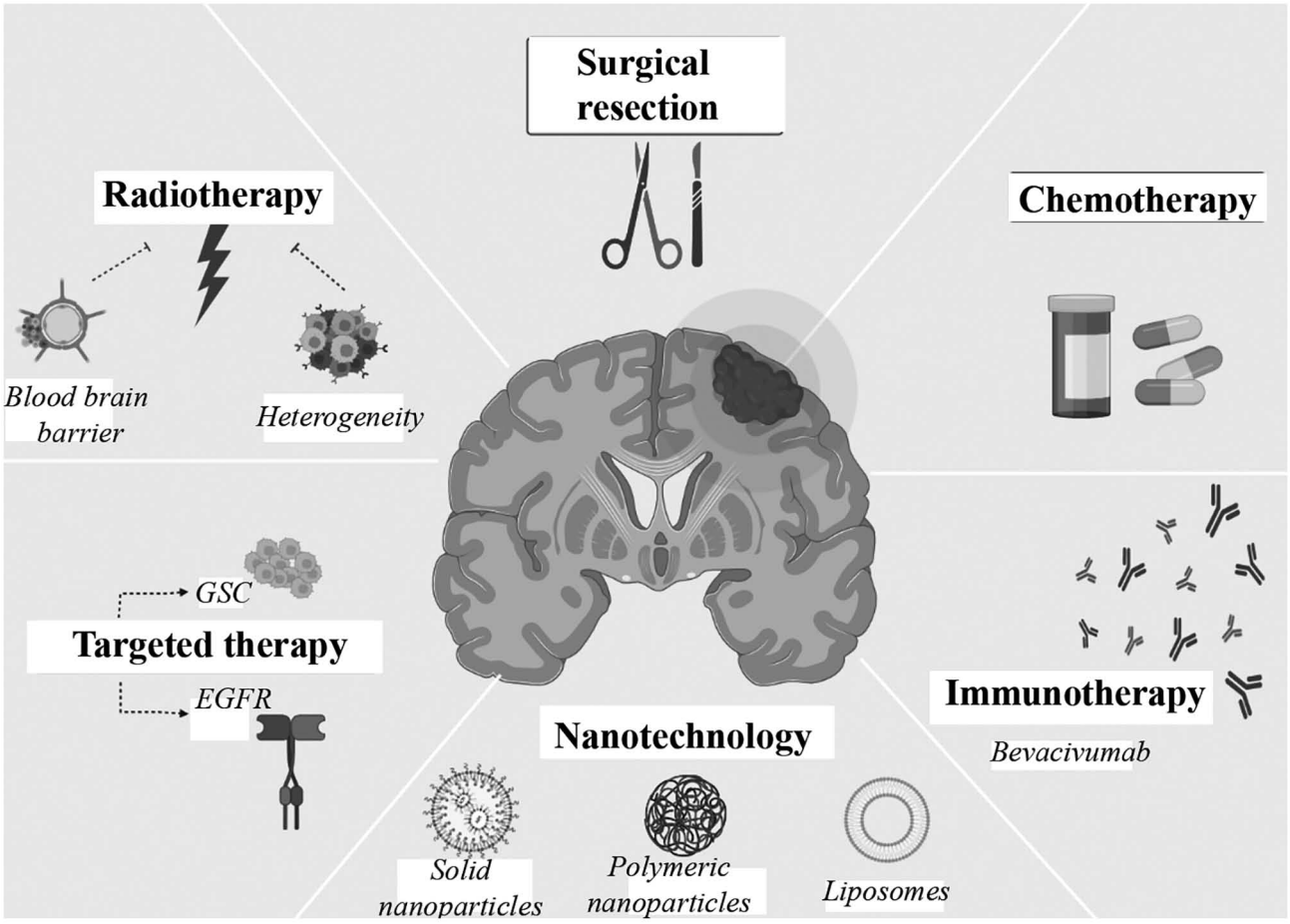

Figure 1. An illustration of both conventional and new cancer therapy strategies within the context of glioblastoma multiforme.

improve the mode of delivery, define the target in an improved way and optimize the dose of radiation (26). Consequently, there is an urge for mechanism-based studies of radiation carcinogenesis, in addition to epidemiological studies (lifestyle and physiological and heritable factors), to jointly allow for long-lasting cancer-preventing measures.

3. Chemotherapy

Chemotherapy is considered the primary treatment for cancer therapy where drugs are used to destroy cancer cells. Chemotherapeutic drugs target growing, and dividing cells thus they have a greater effect on tumor cells. However, they can still damage healthy cells. Planning for chemotherapy course treatment depends on the age and health of a patient, in addition to the type, size and location of a tumor, and whether it has spread. Accordingly, chemotherapy is used in different ways: i) it could be the primary and the only treatment as in the case of cancers of the blood or lymphatic system, ii) it could be used to shrink tumors by slowing cancer growth prior to other cancer treatments or eliminate any remaining cancer cells after surgery or RT (27,28).

There are numerous drugs used in chemotherapy to treat cancer. Chemotherapeutic drugs can either damage the genetic material of a cell affecting its growth or inhibit certain chemicals or enzymes that the cell needs in order to divide. Depending on their mode of action, chemotherapy drugs are classified into four main groups; i) alkylating agents that are the most common chemotherapeutic drugs used today (damage the DNA of tumor cells inhibiting cell division), ii) plant alkaloids (topoisomerase inhibitors and mitotic inhibitors that inhibit cell division by interfering with topoisomerases and inhibiting enzymes the cells for replication), iii) antimetabolites (mimic structures in the DNA of tumor cells incorporating into the cellular metabolism and change the function of enzymes disrupting cell division), and iv) antitumor antibiotics (uncoil DNA strands of cancer cells and prevent cell replication) (6,29,30).

Among the chemotherapeutic drugs used to treat GBM is Temozolomide (TMZ). TMZ, a DNA alkylating agent known to induce cell cycle arrest at G2/M, is approved by the US Food and Drug Administration (FDA) for use in the treatment of newly diagnosed adult GBM patients in 2005 (31). This lipophilic agent is the current gold standard treatment for GBM due to its ability to easily penetrate the blood brain barrier (BBB) and to effectively target cancerous cells (32). In a study conducted by Malmström *et al* (33), TMZ chemotherapy was found to be a potential alternative to RT in elderly and frail patients with GBM. The overall survival rate was significantly improved in elderly patients who received TMZ alone (32 weeks in overall patients) compared with those who received 60 Gy standard RT (24 weeks in overall patients). A Surveillance, Epidemiology, and End Results-based study evaluated the efficacy of chemotherapy for 25,698 patients

diagnosed with GBM between 2004 and 2015 (34). In this study, the median overall survival of the chemotherapy cohort was ~4.3 times higher than the cohort with no chemotherapy. The survival of elderly patients improved by chemotherapy, however, younger patients benefited more. Tumor recurrence in patients with GBM confers a dismal prognosis associated with low progression free survival rate despite the treatment with conventional methods (35). However, chemotherapy remains the most common treatment option for GBM. Akin to TMZ, carmustine is another alkylating agent used to treat patients with GBM. A meta-analysis evaluating the efficacy of carmustine in newly diagnosed and recurrent patients with GBM showed that this nitrosourea derivative contributes favorably to both groups (36).

There are several reasons that limit the efficacy of chemotherapeutic treatments. These include mutational accumulations leading to tumor heterogeneity, drug resistance, BBB, and low selectivity, in addition to adverse and side effects (37). Chemotherapy may be associated with numerous severe side effects that include immediate signs of toxicity and late signs of chronic toxicity. Their intensity can be mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening or disabling (grade 4), according to WHO classification. For instance, high doses of alkylating agents can damage the bone marrow and increase the risk of developing leukemia. High doses of antitumor antibiotics on the other hand can damage the heart. Plant alkaloids are also accompanied with side effects where topoisomerase inhibitors increase the chances of developing a second cancer, while mitotic inhibitors can cause nerve damage when delivered at high doses. Common adverse events associated with TMZ treatment include nervous system, hematological and vascular disorder (38). Carmustine-based chemotherapy causes nausea, vomiting and hematotoxicity in patients with GBM and more dreaded side effects such as pulmonary fibrosis and severe bone marrow suppression (35,36). Consequently, chemotherapeutic strategies are now combined with RT or targeted modalities to increase its efficacy or lessen its side effects.

4. Chemoradiation: combined conventional treatments

Treatment of primary tumors with conventional fractionated RT in numerous cancer sites is associated with local failure rate, while chemotherapeutic treatment of several common solid tumors shows a relatively poor efficacy. Therefore, the combination of RT with chemotherapeutic cytotoxic agents started to gain attention and the first combined chemoradiation therapy took place in the seventies (39). Since then, chemoradiotherapy has been considered a standard modality for the treatment of locally advanced solid tumors in a plenty of clinical examples. This combined therapy aims to achieve spatial cooperation and to enhance the local response, thus improving the survival rate.

Chemotherapy can be administered prior to (neoadjuvant chemotherapy) or during (concurrent chemoradiation) or after (adjuvant chemotherapy) RT. Neoadjuvant chemotherapy aims to increase tumor shrinkage reducing the tumor volume to be irradiated and thus sparing normal tissues (40,41). Neoadjuvant chemotherapy has shown, clinically, meaningful survival benefits to several cancer types. In this context, it improves the survival rate and decreases distant metastasis in non-small cell lung cancer (NSCLC) (42). This method is particularly efficient in non-rapid distant metastatic tumors such as advanced pancreatic tumor (43). It also offers an advantage in organ preservation specifically in laryngeal-hypopharyngeal tumor (44). In addition to its role in improving the pathological response, neoadjuvant chemotherapy increases chances of breast conservation in earlier stage breast cancer patients (45). However, this was not the case with GBM. In a randomized trial, Malmström *et al* (46) in 2017 showed that neoadjuvant TMZ treatment prior to standard RT did not have any significant survival benefit in GBM patients ≥60 years of age, since the median overall survival was 15.3 months for neoadjuvant TMZ vs. 18.1 months for standard RT. Notably, in the same study, a substantial survival benefit was observed for the anaplastic astrocytoma subgroup.

Concurrent chemotherapy has a major role in treating solid tumors and is usually administered with fractionated RT. It offers enhancement in local control rate and organ preservation as well as suppression of distant micro-metastasis (47). Adjuvant chemotherapy on the other hand aims to improve drug delivery after decreasing the tumor burden and can be efficient against occult metastasis, as in the case of locally advanced nasopharyngeal carcinoma where patients are at risk of distant metastasis (47). Both concurrent and adjuvant treatments demonstrated effectiveness in patients newly diagnosed with GBM. In a previous randomized, multicenter phase III trial in patients with GBM younger than 70 years, the risk of death decreased by 37% upon receiving concurrent treatment of TMZ with RT, followed by adjuvant therapy with TMZ four weeks after the initial treatment. The survival benefit of the cohort who received chemotherapy and RT was 2.5 months with a progression free survival rate of 10.7% compared with RT alone (1.5%) (48). Poor prognosis, tolerance to treatment, existing conditions and the toxic effects of conventional therapies have made it difficult to manage GBM in elderly patients (49). In this regard, the impact of short course RT with TMZ was assessed in newly diagnosed elderly patients with GBM. This new chemoradiation strategy conferred survival advantage with a 37.8% overall survival rate at 12 months as compared with 22.2% with short course RT alone (50). Proton beam therapy (PBT) concurrent with chemotherapy was also evaluated for effective treatment against GBM. In a study conducted by Mizumoto *et al* (51) in 2016, the safety and efficacy of PBT administrated with concurrent chemotherapy, using either TMZ or nimustine hydrochloride (ACNU), was examined on carefully selected GBM patients with similar characteristics. The study reported 21.1 months of median survival with no significant difference between the TMZ and ACNU groups, suggesting that the proton therapy concurrent with TMZ or ACNU is tolerable and promising (51). Chemoradiation has shown potential survival benefits in patients with GBM. However, the challenge remains to optimize the clinical outcomes by reducing the toxic effects, minimizing death rates, and finding the optimal combination of RT with the cytotoxic agents.

5. Targeted therapy

Targeted cancer therapies have been developed to specifically target cancer cells, leaving normal cells unaffected. Similar

to chemotherapy, the targeted approach utilizes compounds that inhibit cancer metastasis and growth. Nonetheless, targeted treatment focuses on specific tumorigenic proteins rather than a broad range of targets (52-54). The two major players in targeted therapy are the small molecule inhibitors and monoclonal antibodies. The former works by penetrating cells, inactivating enzymes, and thus interfering with the tumor cell growth. Therapeutic monoclonal antibodies, however, recognize targets outside the cell and control downstream cellular processes such as cell cycle progression and cell death (55).

Cancer cells need to overcome low nutrient- and oxygen-availability to grow, divide, and infiltrate into surrounding tissues. Tumors are thus dependent on angiogenesis by which they form new blood vessels from a pre-existing vasculature (neovascularization), or undergo vasculogenesis; *de novo* formation of vessels from hematopoietic precursor cells (56,57). Since the process of angiogenesis is regulated by vascular endothelial growth factor (VEGF) signaling, and considering the defining feature of GBM in neovascularization, anti-angiogenic agents that target VEGF, or its receptors are considered as potential therapeutic options (56,58). Expression of VEGF and VEGF receptors (VEGFR) predicts the aggressiveness of gliomas and is robustly expressed in malignant gliomas such as GBM (58). The US FDA and the National Medical Products Administration of China approved 89 small molecule targeted antitumor drugs such as kinases, proteasomes, epigenetic regulatory proteins and DNA damage repair enzymes by December of 2020 (59). Cediranib (AZD2171) and sunitinib are two receptor tyrosine kinase inhibitors actively targeting all three VEGFRs (VEGFR-1, VEGFR-2 and VEGFR-3). Sorafenib and cabozantinib (XL184) target only the VEGFR-2 from the VEGFR family (59). These drugs are evaluated on patients with recurrent GBM. Phase II clinical trials of sunitinib and sorafenib demonstrated limited activity for patients with recurrent GBM (NCT00923117 and NCT00597493). Cediranib on the other hand had an encouraging phase II clinical trial (NCT00305656) with six-month progression-free survival of 25.8% (60) which led to phase III trial (NCT00777153) and was evaluated in combination with chemotherapeutic lomustine.

Bevacizumab (BEV), also called Avastin, is a highly studied humanized therapeutic monoclonal antibody. The FDA approved the use of BEV to treat multiple cancers such as colon, lung, kidney and cervix. In May 2009, the FDA approved the use of BEV as a single agent treatment for patients with recurrent GBM and had failed to respond to other treatment options (61). Since then, it has become the standard of care for this group of patients. This recombinant immunoglobulin (Ig) G1 monoclonal antibody works by neutralizing VEGF-A from the circulation. Receptor signaling thus decreases within endothelial cells leading to tumor vasculature regression (62,63). Another anti-VEGF candidate is aflibercept. This recombinant fusion protein has the second Ig domain of VEGFR-1 and the third Ig domain of VEGFR-2, fused to the constant region (Fc) of human IgG1. Aflibercept targets not only VEGF-A but also VEGF-B and placental growth factor (64). Despite having broader targets than BEV, a single-arm phase II clinical study (NCT00369590) revealed that a single agent aflibercept had minimal activity in patients with recurrent GBM with a considerably low six month progression-free survival rate (7.7%) mainly due to toxicity issues (65).

It is well known that ionizing radiation eradicates tumors, mainly, through DNA damage and that tumor radiosensitivity depends on the DNA damage repair mechanisms of tumor cells. Elements of DNA damage repair are thus targets for cancer therapy in attempts to intensify the therapeutic effects of RT (66). Olaparib is a small molecule inhibitor of poly(ADP-ribose) polymerase which is an important protein in DNA repair pathways. Olaparib was revealed to be an effective radio-sensitizing agent in GBM and other cancer cell lines and preclinical glioma models (66,67). In a recent phase I trial OPARATIC (NCT01390571), Olaparib was shown to penetrate core and margin regions of GBM at radio-sensitizing concentrations and was reported to be safely administered with continuous low-dose of chemotherapeutic TMZ (68).

Targeted gene therapy. In the last three decades, gene therapy has emerged as a promising tool of genome editing that can potentially aid in the treatment of various diseases such as heart failure, neurodegeneration and cancer (69). Gene therapy implicates the correction of a defective gene by introducing a functional and healthy version thus compensating for the abnormal/missing gene (70). Advancements in this field paved the way for considering gene therapy as an adjuvant treatment to conventional chemo- and RT, allowing for a reduced dose of standard modalities in combinatorial approaches.

Several different strategies are currently employed for targeting tumors using gene therapy. One of the common strategies is the delivery of different gene types expressing tumor suppressor genes to compensate for the defective or missing gene, or expressing suicide genes, tumor antigens and growth factors in order to induce apoptosis or improve tumor sensitivity to conventional therapies. Plenty of tumor suppressor genes have been evaluated for gene therapy such as *Rb* and *PTEN* (71), and *p53* was introduced to treat non-small cell lung carcinoma (72). TNF-related apoptosis inducing ligand and Interleukin-24 (IL-24) are examples of apoptosis inducers that induce apoptosis in various tumor cells with minimal effect on normal cells (73,74).

Another strategy is the gene interference using RNA interference (RNAi) approach to block the expression of an oncogene and, in numerous cases, sensitize tumor cells to RT (75). *In vivo* and *in vitro* studies showed that tumor cells treated with antisense RNA targeting c-*myc* gene and K-*ras* suppress tumor growth in melanoma and pancreatic cancer cells respectively (76,77). A platform for the treatment of GBM has also been developed using RNAi technology including small interfering (si)RNA, microRNA (miRNA), short hairpin (sh)RNA and long non-coding (lnc)RNAs. siRNA is used in several studies to silence the target proteins that are overexpressed in the GBM (78). Danhier *et al* (79) in 2015 targeted galectin-1 (gal-1) which is involved in the development and progression of chemoresistance in GBM. Using anti-gal-1 and anti-EGFR siRNA administrated via nanocapsules with TMZ, researchers reported that the median survival rate of mice bearing glioma cell increases (79). Another study using anti-CD73 siRNA reported tumor volume suppression by 60% in C6 glioma rats (80). A first-in-human early phase I clinical trial (NCT03020017) examined the safety and efficiency of

NU-0129 drug, which is an RNAi-based spherical nucleic acids (SNAs), in patients with recurrent glioblastoma. SNAs consist of gold nanoparticle cores covalently conjugated with siRNA oligonucleotides specific for the GBM oncogene Bcl2Like12 (siBcl2L12). Results showed successful enrichment of gold particle in tumor-associated endothelium, macrophages and tumor cells, along with decreased expression of tumor-associated Bcl2L12. The study demonstrated the potential of SNA nanoconjugates as a brain-penetrant precision medicine strategy for the systemic management of GBM (81).

GBM cells display abnormal pattern of miRNAs that may result in increased expression of oncogenes or decreased expression of tumor suppressor genes, thus affecting various downstream signaling pathways (78) [reviewed in (82)]. The glioma angiogenesis, for instance, is significantly influenced by miR-26a, which is found in glioma tissues. Additionally, serum exosomes of patients with GBM showed overexpressed miR-21 (83). According to a previous study conducted by Møller *et al* (84), the microenvironment of GBMs was found to overexpress 256 miRNAs while underexpressing 95 miRNAs when compared with the normal brain. Numerous miRNAs are thus considered as possible biomarker for early diagnosis and prognosis of GBM, and investigated for gene therapy (83,85). Transfection of miR-34a, which is a miRNA normally downregulated in GBM tumor microenvironment (TME), restores normal physiological conditions by inhibiting cell proliferation and invasion, and suppressing tumoral survival in glioma cell lines and glioma xenograft model (86). miR-21 and miR-10b, however, are usually upregulated in GBM cells and exhibit oncogenic properties. In an *in vitro* model, the inhibition of these miRNAs with synthetic anti-miRNA counterparts prevents cell cycle progression (87).

shRNAs are artificially generated RNA molecules and contain hair-pin-like structures intended to silence target mRNAs. Viel *et al* (88) in 2013 used shRNA to target O^6-Methylguanine-DNA-methyltransferase (MGMT), a DNA repair enzyme. Treatment of GBM xenografts with anti-MGMT shRNA and TMZ results in a significant decrease in tumor size (88). Another study showed that knocking down cyclin D1 by shRNA results in the inhibition of cell proliferation and migration and induces apoptosis in GBM cell lines (89). Moreover, Song *et al* (90) in 2016 used Sox2-shRNA to downregulate Sox2, a gene associated with stemness of CD133 positive GBM cells. The aforementioned study reported that Sox2-shRNA abrogates tumor initiation and drug resistance of these cells, thus, suggesting that RNAi technology could help decreasing GBM cell resistant to anti-GBM treatments.

GBM cells also express aberrant expressions of lncRNAs associated with pluripotency and tumorigenesis. Akin to miRNAs, several lncRNAs are downregulated while others are upregulated in GBM cells compared with normal human brain tissue (78). Thus, numerous lncRNAs are suggested as biomarkers for GBM tumorigenesis and chemoresistance. For instance, Zhang *et al* (91) in 2019 provided evidence that knockdown of lncSBF2-AS1 decreases chemoresistance of GBM cell lines to TMZ. These expression patterns of lncRNA revealed a relationship between tumor histopathological differentiation and malignancy grade that could be effectively used to treat GBM (91).

Achieving an effective delivery to the intra-cerebral tumors is the main difficulty in treating GBM. Due to the degradation of therapeutic active molecules, the development and marketing of various nucleic acid-based therapies into clinical trials have been hampered by the uncertainty of effective delivery, therapeutic efficacy and toxicity profile. As enormous research is conducted to investigate the safety and efficacy of gene therapy, delivery tools are thus acquiring a great research focus for more efficient GBM treatment (discussed in 'Novel Approaches' section).

Targeting glioblastoma stem cells. In addition to the highly invasive nature of glioblastomas and the presence of BBB, the presence of cancer stem cells is another factor that limits clinical options and treatments of GBMs (92). Glioblastoma stem cells (GSCs), which are self-autonomous units, play a significant role in tumor initiation and growth as well as therapeutic resistance (93). Their prolonged proliferation and ability to metastasize and suppress anti-inflammatory responses are among the most significant mechanisms by which GSCs contribute to tumor malignancy (94). Enriched understanding of GCS biology paved the way for *numerous* studies that are now being conducted to target GSC. In the present review, major aspects of GSCs recognized as hot target for *improved* GBM treatment were discussed.

GSCs are affected by differentiated glioblastoma cells (DGC) due to a reciprocal signaling pathway. DGCs express brain-derived neurotrophic factor (BDNF), whereas GSCs express the BDNF receptor NTRK2 which accelerates GSC tumor growth (94). It is thus important to understand the reciprocal signaling between GSCs and DGCs for improved targeted cancer treatment. BDNF induces nerve growth factor (VGF) expression in GSCs through the NTRK2-PI3K-AKT signaling pathway. VGF promotes GSC growth and self-renewal and further secretion of BDNF by DGC for its survival. Thus, BDNF-NTRK2-VGF paracrine signaling pathway enhances the cooperation between DGC and GSC in promoting tumor growth (94). Several studies have thus proposed NTRK and VGF as potential therapeutic targets for GBM treatment (94,95).

CD133 positive GSCs play a significant role in maintaining and proliferating GBM (96). Eliminating CD133 positive stem cells is a method that has proven to increase the survival rate in patients with GBM. Depleting CD133 can be achieved by numerous methods (97). Knocking down BMI1 gene, an oncogene that is involved in the regulation of self-renewal and differentiation of stem cells, may destroy CD133 stem cells (98). Targeting these stem cells is thus another promising target for GBM therapy. Another GCS target is the mitochondria that plays an important role in tumorigenesis and tumor progression, particularly through oxidative phosphorylation (OXPHOS), which is the major source of ATP in numerous types of cancers (99). Among GBM cells, GSCs depend on OXPHOS which requires mitochondrial translation (100). Blocking mitochondrial translation by a bacterial antibiotic quinupristin/dalfopristin (Q/D) does not only suppress the growth of the GSCs, but also dysregulates cell cycle and promotes apoptosis (100). These findings propose

that inhibiting mitochondrial translation may be investigated to therapeutically inhibit GSC growth and that Q/D may be considered for the treatment of GBM.

One promising investigational drug is salinomycin that preferentially eliminates GSCs and other varieties of CSCs. Salinomycin and its derivatives are currently extensively investigated as an anti-CSCs treatment for numerous types of malignancies, however, clinical trials evaluating the potential efficacy of salinomycin in glioblastoma have not yet been published (101). Collectively, these findings suggested that targeting GSCs represents a promising strategy for improved treatment of GBM.

To ensure targeted therapy success, the following key steps should be taken into consideration: identification of targets, identification and development of target-specific small molecules and antibodies followed by pre-clinical studies and clinical trials. Current major challenges in this field include the development of drug resistance and low efficiency to drugs. To date, except for BEV, targeted therapies in GBM patients have not provided significant survival benefits (59,102).

6. Immunotherapy

Considered the 'fifth pillar' of cancer therapy following surgery, chemotherapy, RT, and targeted therapy, immunotherapy revolutionized the field of oncology (103). Immune cells of the adaptive and innate immune systems can modulate tumor progression and are thus the cellular underpinnings of immunotherapy. Therefore, cancer immunotherapy employs the immune system by boosting natural defenses to eliminate malignant cells (104). Since 1868, several categories of immunotherapies were developed, including cancer vaccines, cytokine therapies, adoptive cell therapies, and immune checkpoint inhibitors (104). These forms showed promising clinical responses in numerous aggressive tumors such as NSCLC, melanoma, renal cancer, and several hematological malignancies. Nevertheless, to date, no immunotherapies have been approved by the FDA for GBM, bringing this type of tumor to the forefront of immunotherapy research (105). Immunotherapy strategies in clinical phases II and III are summarized in Table I.

Cancer vaccines. To prevent tumor growth and eradicate tumor cells in patients with cancer, cancer vaccines are intensively studied as a promising therapeutic approach. This method activates the humoral and cellular immunity of patients with cancer (106). To achieve a favorable clinical efficacy, a critical step of cancer vaccine design is antigen selection. An ideal antigen for a cancer vaccine should be specifically expressed and present on all cancer cells-but not in normal cells-, highly immunogenic and essential for cancer cell survival (107). Consequently, the two major classes of antigens employed in cancer vaccine design include tumor-associated antigens (TAA) and tumor-specific antigens (TSA). TAAs are abnormally overexpressed self-antigens derived from tumor cells. However, they may be expressed as well in a subset of normal cells at certain level (108). Differentiation antigens, such as Melan A and CD19, and overexpressed antigens such as HER2 and TROP2 are examples of TAAs (109). Utilizing these antigens may potentially induce autoimmunity against normal tissues and possibly skip T cell recognition, since these antigens are typically deleted from the immune repertoire by central and peripheral tolerance mechanisms (107). TSAs, on the other hand, are exclusively expressed by cancer cells and are not present in normal host cells. This reduces the risk of autoimmune destruction and strongly activates high-affinity antibodies and T cells against the non-self-antigens (110). TSAs include onco-viral antigens, such as E6 and E7 of the human papillomavirus, private neoantigens, and shared neoantigens such as KRAS and p53 (109). To destroy tumor cells, cancer vaccines employ the following mechanism of action. After antigen delivery, tumor antigens are taken up and processed by antigen-presenting cells (APCs) such as dendritic cells (DC). DCs present the antigens to major histocompatibility complex (MHC) class I and II molecules (104). Next, these cells migrate to the vaccine draining lymph nodes (primary site for T cell priming), to recruit and activate immune cells by presenting relevant antigens on MHC I and MHC II to $CD8^+$ and $CD4^+$ T cells, respectively (106). Activated T lymphocytes differentiate into memory T cells and effector T cells. Effector T cells migrate to the TME, recognize tumor cells, and destroy them through exploiting multiple mechanisms. Cytotoxic T cells can eliminate cancer cells by releasing cytotoxic particles such as perforin and granzymes. Furthermore, they can induce cancer cell apoptosis through direct cell to cell-mediated interactions. Additionally, mediators such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) induce cytotoxicity (111). Besides T cells, B lymphocytes and cells of the innate immune system such as natural killer (NK) cells and macrophages promote tumor eradication (111). Peptide-based, DC-based and personalized vaccines are being explored as potential vaccine therapies in GBM (112,113).

Multiple DC based vaccines are under investigation for the treatment of GBM. One notable DC vaccine is the DCVax®-L developed by Northwest Biotherapeutics. Newly diagnosed patients with GBM in phase I and II clinical trials who received the vaccine and the standard of care treatment had an overall survival of 36 months and almost 24 months of progression free survival with no major side effects (114). An ongoing randomized, placebo-controlled phase III clinical trial (NCT00045968) involves 348 eligible newly diagnosed GBM participants. The primary objective of the study is to compare the overall survival between patients receiving the vaccine and the control group receiving the standard of care treatment. The treatment cohort will receive two intradermal injections per treatment of DCVax®-L vaccine at days 0, 10, 20 and at weeks 8, 16, 32, 48, 72, 96 and 120.

Another cancer vaccine that represents a silver lining for GBM immunotherapy is ICT-107, an autologous DC vaccine. Immune cells are pulsed with six synthetic peptide epitopes that recognize and target glioma TAA and stem cell-associated antigens such as HER-2, MAGE-1, AIM-2, TRP2, gp100, and IL13Rα2 expressed in 83% of tumors (115). In an open-label, single-institution, single-arm phase I clinical trial, newly diagnosed patients with GBM who previously received a conventional treatment prior to vaccination had promising responses to ICT-107 (116). The trial achieved its primary endpoint of immunogenicity. The vaccine was well tolerated and efficacious with a median overall survival of 38.4 months in newly diagnosed patients with GBM (116). The

Table I. Summary of immunotherapy strategies in clinical phases II and III.

Therapeutic agent	Therapeutic strategy	Recruitment status	Status
ADCTA	ADCTA vaccine with Bevacizumab as a standard therapy vs. standard therapy alone	Recruiting	Phase III NCT04277221
Dendritic cell immunization	Immunization with DCs after finalizing radiotherapy and concomitant temozolomide	Recruiting	Phase II and III NCT03548571
Ipilimumab and Nivolumab	Ipilimumab and Nivolumab vs. Temozolomide	Recruiting	Phase II and III NCT04396860
Temozolomide	Temozolomide Plus Radiation Therapy Combined with Nivolumab or Placebo	Active, not recruiting	Phase III NCT02667587

ADCTA, Autologous Dendritic Cell/Tumor Antigen.

aforementioned study was followed by a randomized, double blinded, placebo-controlled phase II trial (NCT01280552). Similarly, the vaccine was well tolerated, however, there was no significant difference in the overall survival when compared with the control group (115). DC vaccines thus offer a favorable immunotherapeutic strategy to treat patients with GBM.

The concept of personalized vaccination in glioblastoma gained interest after two phase I clinical trials that used multi-epitope-based personalized vaccine to target neoantigens only (NCT02287428) (117) or both neoantigens and unmutated tumor-specific antigens (NCT02149225) (118). Keskin *et al* (117) in 2019 compared whole-exome sequencing data from the surgically removed glioblastoma and matched normal cells in order to discover neoantigens. By identifying the coding mutations for each patient, they chose a pool of 7-20 peptides comprising actionable neoepitopes anticipated to bind to the HLA class I molecules with high affinity. Results reported neoantigen-specific $CD4^+$ and $CD8^+$ T cell responses migrating into an intracranial glioblastoma tumor with increased number of infiltrating T cells (117). On the other hand, and in order to maximize the amount of actionable epitopes, Hilf *et al* (118) in 2019 targeted unmutated tumor-specific antigens in addition to neoantigens. For each patient, unmutated antigens were chosen from a common pool of HLA-bound peptides that were specific to glioblastoma and based on unique HLA immunopeptidome data and pre-vaccination T cell reactivity of patients. Vaccination plan involved an actively personalized vaccine of 9 unmutated peptides (APVAC1) followed by a 20-peptide pool that targeted neoantigens (APVAC2). Researchers reported results similar to those aforementioned by Keskin *et al*, with 29 month median OS (118). Another phase II clinical trial is testing personalized cancer vaccine AV-GBM-1 (developed by AIVITA Biomedical Inc.) based on autologous DCs full of autologous tumor neoantigens (NCT03400917). These neoantigens are derived from tumor-initiating cells following standard surgical resection. The aforementioned study reported significantly improved progression-free survival (PFS) in 57 participants (119). These studies along with others demonstrated the positive effect of immunization using vaccines in patients with GBM.

Cytokine therapies. Cytokine therapy is considered an important immunotherapeutic approach to activate the immune system of cancer patients, recognize cancer cells and destroy them (120). As molecular messengers of the innate and adaptive immunity, these regulators enable cells of the immune system to communicate over short distances in paracrine and autocrine manners (120). To date, only two cytokines are approved by the FDA to treat selected malignancies (121). Interleukin 2 (IL-2) is approved for metastatic renal cell carcinoma and metastatic melanoma treatment, whereas Interferon-α (IFN-α) is approved to treat hairy cell leukemia, follicular melanoma, non-Hodgkin lymphoma and AIDS-related Kaposi's sarcoma (122). Cytokine therapies stimulate the function, survival and proliferation of NK and T cells that mediate immune responses against tumors. Despite their antitumor activity in murine models and clinical treatment of selected human cancers, several issues are associated with this therapeutic approach (120,123). These limitations include the short half-life of most cytokines *in vivo*, narrow therapeutic windows, severe toxicity at therapeutic doses and lack of efficacy (122,123). While certain cytokines support the growth and invasiveness of glioma cells, others may decrease or inhibit their growth (124). Among the cytokines studied, IL-2, 12, 15 and 21, as well as type 1 interferons, IFN-γ and granulocyte macrophage colony stimulating factor affect various populations of the immune cells with antitumor properties (125). In GBM, cytokines such as transforming growth factor-β (TGF-β), IFN-α, IFN-γ and IL-12 have an antitumor activity decreasing the size of the tumor, inhibiting GBM cell growth and tumorigenesis at the early stages (124).

In a multicenter phase I dose escalation clinical trial (NCT02026271), 31 patients with recurrent high grade glioma were treated with Ad-RTS-hIL-12, an inducible adenoviral vector that encodes the hIL-12 p70 transgene put under the control of the RTS gene switch (126). The oral administration of 20 mg of the hIL-12 activator veledimex (VDX) showed an improved safety and tolerability profile, less severe cytokine severe syndrome, increased but tolerable IFN-γ and tumor infiltrating lymphocyte generation and improved median overall survival of 12.7 months (126). The efficacy and safety of a single tumoral injection of Ad-RTS-hIL-12 with VDX in combination with the FDA-approved antibody cemiplimab completed its phase II trial targeting patients with recurrent or progressive GBM (NCT04006119). Preliminary data revealed a tolerated outcome of the combined treatment with elevated

levels of serum cytokine and a significant increase in circulating cytotoxic T cells (127).

The immunomodulating effects of interferons offer another possible therapeutic potential in treating malignant gliomas. IFN-γ, for instance, has an array of antitumor properties such as inducing programmed cell death, inhibiting glioma proliferation and angiogenesis, and enhancing tumor immunogenicity (128). Thus, researchers consider it as an immunotherapeutic option. Furthermore, a phase I open-label trial evaluated the combination of INF-β and conventional therapy with TMZ. Out of the 23 enrolled patients, 16 were newly diagnosed with high-grade glioma and 7 with recurrent high-grade glioma. Newly diagnosed patients received RT, intravenous INF-β and TMZ, and had a median overall survival time of 17.1 months. The study outcome emphasized that the combination therapy caused minimal toxicity (129).

The role of multiple cytokines remians under study in regards to their clinical effectiveness to treat gliomas. The safest and most effective dosages of the cytokines are being delineated with some considered as a promising adjunct to other therapies (130).

Adoptive cell therapy (ACT). ACT is an immunotherapeutic approach that developed rapidly in the recent years and is considered a promising and effective anticancer treatment. This treatment method involves the isolation of autologous T lymphocytes with antitumor activity of a cancer patient, ex vivo expansion, and the adoptive transfer of the amplified tumor-resident or engineered T cells back to patients (131,132). There are three categories of ACT used to modulate the immune system and target cancer cells: i) ACT with tumor infiltrating lymphocytes (TIL), ii) ACT with genetically modified peripheral blood T cells such as T cell receptor (TCR), and iii) chimeric antigen receptor (CAR) gene therapies (133). TIL are genetically unmodified killer lymphocytes that infiltrate the TME (134). These cells, however, can lose their tumor eliminating ability due to immunosuppressive factors of the TME. Accordingly, the number of specific TIL should be abundant to enhance the efficacy of TIL therapy before intravenous adoptive transfer into the patient (134). T cells equipped with a TCR can recognize peptides presented on MHC molecules and target cancer cells. Nevertheless, and to overcome the limitation of antigen presentation, CAR-modified T cells recognize various types of antigens regardless of their presentation on MHC molecules (135). The aforementioned categories have different mechanisms of action to eliminate cancer cells.

Among the three aforementioned ACT approaches, CAR-T cell gene therapy gained ample interest in eradicating cancer cells. These modified T cells can recognize and bind surface expressed antigens through the single-chain variable fragment recognition domain. After recognition, T cells mediate tumor elimination via three different mechanisms: the perforin and granzyme axis, cytokine secretion and Fas and Fas ligand axis (136). Currently, 24 clinical trials are studying the effectiveness of CAR-T cell therapy on GBM (Accessed through Clinicaltrials.gov on July 1, 2022). Amongst these, one is in the early phase I study, 21 in phase I study and 2 studies in phase II clinical trial. Since its discovery, multiple CARs were developed to target GBM antigens including, but not limited to, IL13Rα2, EGFRvIII, HER2 and CD70 (137).

To assess the feasibility, safety, anti-glioblastoma activity and the persistence of T cells in patients, an open-label phase I dose-escalation study tested the activity of HER2-specific CAR-modified virus-specific T cells (VSTs) (NCT01109095). 17 HER2 positive patients with progressive recurrent GBM received the treatment. The infusion of VSTs was safe and well tolerated with no dose-limiting effects and the median overall survival was 24.5 months from diagnosis. It is worth mentioning that although T cells were present in the peripheral blood, HER2-CAR VSTs did not expand (138). Another clinical study utilized a retroviral vector containing the CAR that recognizes EGFRvIII tumor antigen (NCT01454596). A total of 18 patients with malignant gliomas expressing EGFRvIII received immunotherapy to evaluate the safety and feasibility of the T cells expressing anti-EGFRvIII CAR. One more study evaluated the clinical benefit of CAR-engineered autologous primary human $CD8^+$ T lymphocytes against IL13 receptor α2 (IL-13Rα2) in 3 patients with recurrent GBM (NCT00730613). IL-13Rα2 is reported to be overexpressed in more than 50% of patients with GBM. In this first human pilot study, the intracranial administration of IL-13Rα2 specific CAR-T cells exhibited anti-glioma activity which was promising for CAR-T cell immunotherapy (139).

The outcomes of CAR-T cell immunotherapy is promising as it displays efficacy with minimal toxicity. Nonetheless, limitations such as tumor heterogeneity and the heterogonous expression of antigens as well as the function of T lymphocytes at the sites of the tumor render it difficult to eradicate the tumor (137).

Immune checkpoint-blocking antibodies. Using monoclonal antibodies, researchers are able to target cancer cells by utilizing immune checkpoint inhibitors. These monoclonal antibodies are involved mainly to free T lymphocytes from the negative regulation of several immune checkpoint proteins, which are in turn involved in helping cancer cells evade the immune system (140,141). Cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD-L1) are highly studied targets for inhibition (104). CTLA-4 and PD-1 are proteins expressed on cytotoxic T cells. The former interacts with cluster differential 80 (CD80) and induces T cell immunosuppression. Cluster differential 28 (CD28) protein, however, can also interact with CD80 and induce T cell activation. Similar to CTLA-4, once PD-1 binds to PD-L1, a protein expressed on tumor cells and APCs, it negatively regulates the T cell activation (142). Accordingly, and to surpass the negative regulation, immune checkpoint inhibitors are developed. The FDA approved several immune checkpoint inhibitor antibodies for several cancer types including, but not limited to, melanoma, renal cell carcinoma, NSCLC, small cell lung cancer and head and neck squamous cell cancer (143). Ipilimumab is used to target CTLA-4, pembrolizumab, nivolumab and cemiplimab for PD-1, and atezolizumab, avelumab and durvalumab for PD-L1 (143).

In patients with GBM, immune checkpoint inhibitors have so far demonstrated limited activity in regard to efficacy, safety and toxicity. In a randomized, open-label multi-institution pilot study, the immunotherapeutic activity of pembrolizumab, an anti-PD-1 monoclonal antibody, was examined on 35 patients

with recurrent surgically respectable GBM (NCT02337686). The aforementioned study included 16 patients for neoadjuvant therapy and 19 in the adjuvant group to receive only the monoclonal antibody. Overall, pembrolizumab was well tolerated in the neoadjuvant group with improved overall and progression-free survival. The neoadjuvant arm had a median overall survival of 13.7 months, whereas the adjuvant arm had 7.5 months of median overall survival. Notably, PD-1 blockade alters the gene expression profile of the patients with a transcriptional increase in INF-γ genes, increased T cell expression and enhanced T cell clonal expansion (144).

Following the evaluation of nivolumab safety and tolerability in phase I cohorts of CheckMate 143 with recurrent GBM (145), a phase III clinical trial was conducted. In this open-label, multicenter, randomized phase III trial, the PD-1 blocking monoclonal antibody, nivolumab, was compared with bevacizumab to determine its survival benefit. A total of 369 patients were randomized to receive nivolumab or bevacizumab after standard radiation and TMZ therapy. The CheckMate 143 phase III clinical trial (NCT02017717) did not meet its primary endpoint. The median overall survival was almost similar with nivolumab and bevacizumab, 9.8 months vs. 10.0 months respectively. Likewise, the median progression free survival and treatment-related grade 3/4 adverse events were similar among both groups (146).

The activity of a CTLA-4 blocker, ipilimumab, was investigated alone or in combination with other immune checkpoint inhibitors such as nivolumab. In NCT02311920 phase I trial, patients with newly diagnosed GBM received ipilimumab, nivolumab, or the combination after standard chemoradiotherapy with adjuvant TMZ. The aforementioned study reported that the treatments were safe and tolerated, with no grade 5 adverse events (147). In patients with recurrent GBM, a recent study showed that the intratumoral and intracavitary administration of ipilimumab and nivolumab combination is under phase I trial (NCT03233152) with median overall survival of 71 weeks (148).

Glioma cells have often different mechanisms to escape immune surveillance. This is achieved by activating immune checkpoint ligands. Accordingly, ligand inhibition is considered a promising immunotherapeutic approach to treating GBM (137).

7. Novel approaches

Due to their high proliferation rate and remarkable neovascularization, GBMs can infiltrate the basic structures of the brain. Clinical options and drug delivery are limited due to the presence of BBB and certain highly integrated physiological systems in the brain (149,150). Thus, targeted delivery of gene therapy acquired a great awareness since safety and efficiency are two major factors of this treatment modality. Viral and non-viral vectors have been designed to allow for optimized gene delivery and expression. Retrovirus, adenovirus, herpes simplex virus and adeno-associated virus are common viral vectors used in cancer gene therapy. Despite the gene transfer efficiency supported by these vectors, toxicity and immunogenicity of viral proteins are two big concerns for this delivery mode (71). Thus, vector development is giving increased focus on non-viral vectors for targeted gene delivery. This includes the use of molecular conjugates or liposomes that can evade clearance by the immune system. However, these delivery methods are limited with short term gene expression for molecular conjugates and the lack cell-specific targeting in the case of liposomes (71,151). Targeted gene therapy was also evaluated using nano-based carriers and their applications are currently in development stage Phases I-III for various types of cancer (151). In the present review, nanotechnology and oncolytic viruses as therapeutic delivery tools to limit the toxicity driven by combined chemoradiotherapy were discussed.

Nanoparticles can be classified based on the type of colloidal drug carriers from which they are constructed. These colloidal drug carriers include liposomes, polymeric nanoparticles, solid lipid nanoparticles (92) (nanotechnology used in GBM clinical trials summarized in Table II). Specifically, liposomes are similar in structure to cell membrane. This lipophilic characteristic enables such molecules to cross the BBB. Nanotechnology can be used in two main ways: i) Combining RT with nanoparticles loaded with cytotoxic drugs or ii) developing nanoparticles that can be co-loaded with cytotoxic drugs and therapeutic radioisotopes (64Cu, 131I and 177Lu). It is thus considered that the chemoradiotherapy nanomedicine will be progressively applied in clinic in the following years (152). This breakthrough enables for future studies combining nanoparticles and monoclonal antibodies to improve the efficiency of immunotherapy by designing a new novel combined treatment nano-immunotarget therapy (92).

On the other hand, oncolytic viruses are vectors of targeted gene therapy and could be considered as a promising strategy to treat GBM due to their ability to overcome BBB (oncolytic viruses used in GBM clinical trials summarized in Table III). Research conducted by Staquicini *et al* (3) examined the delivery of two different genes: Cytotoxic TNF and theranostic Herpes simplex virus thymidine kinase (HSVtk), delivered by an adeno-associated virus and phage (AAVP) to treat GBM. Both AAVP constructs exhibited tumor-associated neovasculature and induced cell death after treatment (3). Despite its promising outcomes in treating GBM-targeted therapy, oncolytic viruses possess numerous limitations such as poor transduction efficiency, poor penetrance after intertumoral injections and lack of viral receptors for viral vectors. Thus, further studies should be performed to enhance the transduction efficiency of the transgene by vectors into tumor areas.

Despite the significant pre-clinical progress of enhanced targeting and expression of a transgene, the translatability of gene therapy still faces several challenges; the safety of vectors and their specificity, regulation of transgene expression and long-term integration in the host. Improved understanding of vector biology for optimized gene delivery is a key element for the implementation of this technique. The US FDA applies regulatory considerations for the development of gene therapy products, while clinical trial designs are restricted with unique features of gene therapy products and safety measures [reviewed in (153)]. Until today, the FDA approved 20 cellular and gene therapy products and only recently, the first cell-based gene therapy for patients with multiple myeloma (ABECMA) has been approved (154). Future research is focusing on optimizing vector design to minimize toxicity and exploit gene targeting in GBM treatment. This paves the way not only for

Table II. Summary of nanotechnology used in glioblastoma multiforme clinical trials.

Therapeutic agent	Therapeutic strategy	Recruitment status	Status
Polysiloxane Gd-Chelates based nanoparticles (AGuIX)	AGuIX Nanoparticles with Radiotherapy Plus Concomitant Temozolomide	Recruiting	Phase I and II (randomized) NCT04881032
NU-0129 based on Spherical Nucleic Acid (SNA) platform	NU-0129 IV plus standard of care tumor resection within 8-48 h.	Completed	Early Phase I NCT03020017
ABI-009 (nab-Rapamycin)	ABI-009 (alone)	Active, not recruiting	Phase II NCT03463265
	ABI-009 + bevacizumab		
	ABI-009 + temozolomide		
	ABI-009 + lomustine		
	ABI-009 + temozolomide + radiotherapy		
	ABI-009 + marizomib		
Nanoliposomal CPT-11 (NL CPT-11)	NL CPT-11 in patients with recurrent high-grade gliomas: glioblastoma multiforme (GBM), gliosarcoma (GS), anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic mixed oligoastrocytoma (AMO), or malignant astrocytoma NOS (not otherwise specified	Completed	Phase I NCT00734682

Table III. Summary of oncolytic viruses used in glioblastoma multiforme clinical trials.

Therapeutic agent	Therapeutic strategy	Recruitment status	Status
DNX-2440 virus	Injected during stereotactic biopsy	Recruiting	Phase I NCT03714334
DNX-2401 virus	Single intratumoral injection of DNX-2401 associated with Interferon-gamma (IFN-γ)	Completed	Phase I NCT02197169
H-1 parvovirus (H-1PV)	Administered either intratumoral or intravenously, with tumor resection after 10 days with a subsequent administration of H-1PV into the walls of the resection cavity	Completed	Phase I and II NCT01301430
rQNestin (a virus modified from herpes simplex virus)	Treatment with rQNestin34.5v.2 and Immunomodulation With Cyclophosphamide	Recruiting	Phase I NCT03152318
DNX-2401 (a conditionally Replicative Adenovirus)	DNX-2401 with Pembrolizumab (KEYTRUDA®)	Completed	Phase II NCT02798406
DNX2401	Combination of DNX-2401 with a short course of Temozolomide	Completed	Phase I NCT01956734
DNX-2401	DNX-2401 with therapeutic conventional surgery	Recruiting	Phase I NCT03896568
G207 (a virus modified from herpes simplex virus	Combination G207 with a single low dose of radiation	Recruiting	Phase I NCT03911388
reovirus (REOLYSIN®)	Injected intralesionally	Completed	Phase I NCT00528684

the development of new cancer therapies, but also for the inception of gene therapy-based vaccines and permanent cures.

8. Discussion

Cancer remains one of the most dreaded diseases of the 21th century and is still spreading with increasing incidences affecting all ages and populations. Owing to the improvements in lifestyle, early detection and cancer treatment, cancer death rate scored 31% drop in overall mortality in the US following a peak in 1991 through 2018 (155). Cancer patients profited from continuous therapeutic innovations over the past few decades, endowing increased survival and improved quality of life. In the present review, cancer therapies between conventional strategies and novel concepts were presented, with particular focus on GBM cancer treatment (Fig. 1).

With the accelerated progress in designing new methods for cancer treatments, chemotherapy and RT continue to be the main cancer weapons despite cytotoxicity of normal tissues. Improved survival and quality of life provided by these standard modalities could possibly outweigh their adverse effects. For instance, chemotherapy shows a favorable success rate treating locally advanced and irresectable pancreatic cancer thus offering a chance for secondary resection (156). Moreover, combined chemotherapy and RT noticeably provides an improved control of loco-regional tumors, thus improving the outcome after treatment and prolonging survival in patients with locally advanced solid tumors (157). The evolution of chemoradiotherapy relies on evidence-based medicine grounded on randomized trials to optimize the use of chemoradiation and direct clinical practices. Thus, medical oncology societies are urged to re-evaluate the use of cytotoxic drugs with severe side effects, and update clinical practices to achieve spatial cooperation and to enhance the local response. Currently, surgical resection of the tumor, followed by RT and TMZ is the typical treatment used for GBM (158). Cancer management relies nowadays on more than one therapeutic modality and combination therapy in providing improved anticancer effects and improved after-treatment outcome. Multimodal tactics provide the advantage of targeting multiple pathways, minimizing non-specificity and MDR caused by chemotherapy, and improving the therapeutic ratio of radiation using RT.

Improved comprehension of the biology of cancer facilitated the development of novel therapeutic concepts derived from biological types of procedures and thus opened new horizons to enhance the standard treatment of patients with advanced cancers. Increment advancements in this field introduced immunotherapy and targeted drug therapy as mild approaches for cancer treatment with the advantage of mild and well-tolerated side effects. The aim of these approaches is to provide a specific method of treatment that is made-to-measure the molecular properties of a tumor and the integrity of the immune system of the patient. Well understanding of basic tumor immunology helped designing monoclonal antibodies, checkpoint inhibitors and cancer vaccines or cancer immunotherapy. While numerous targeted therapies are being investigated and early phase clinical vaccine studies show promising results, no ground-breaking outcomes are achieved yet for GBM treatment. It is thus considered that immune diagnosis, monitoring and follow-up will be the new doors opened for cancer immunotherapy (159).

Animal models of glioblastoma. The enhanced understanding of the GBM biology discussed, was in part, possible due to an expansion of murine preclinical GBM models. Current models include glioblastoma cell-line xenografts, patient-derived xenografts (PDX) and genetically engineered mouse (GEM) models [reviewed in (160)]. PDX are anticipated to serve as effective preclinical models in translational research, compared with glioblastoma cell-line xenografts, since they retain the genetic and histological characteristics of the parent tumor. However, they do not entirely reflect the antitumor immunity of the host. On the other hand, GBM GEM models allow for the identification of specific genomic changes involved in tumor initiation and development and can provide information about the consequences brought on by certain mutations. GEM models can be used to examine how the microenvironment affects tumor biology and are thus helpful for evaluating therapeutic plans as well (160,161). Although mice have historically been the best organism for simulating the genetic and physiological characteristics of cancer, its translational potential has been constrained by the substantial differences between mice and humans. Therefore, and in order to bridge the translational gap between murine animal studies and human clinical trials, investigations in large-animal models of several cancer types have evolved. It is reasoned that anatomy and physiology of large animals has an improved applicability to human disease and could thus reliably recapitulate human GBM. For instance, GBM can develop spontaneously in canines, however, this event is relatively rare and it is thus difficult to reproduce experiments (162,163). On the other hand, several reproducible models of glioma have been successfully developed in pigs, evolving as the most promising preclinical large-animal models (164-167). Xenograft and genetically engineered porcine models are thus being studied as prospective research tools as a promising transitional step between murine models and human clinical trials (163).

Challenges. Drug resistance and normal tissue damage are not the only factors limiting GBM cancer treatment. The BBB and the plasticity and heterogeneity of both GBM and TME are challenges hindering successful GBM treatment. In GBM, the integrity and function of the BBB is impaired resulting in a resistant barrier known as Blood Brain Tumor Barrier (BBTB). BBTB is characterized by overexpression of efflux transporters thus restricting drug delivery to the brain (168-171). Additionally, the multiple cellular states and the existence of 'cancer stem cell likes' add to the complexity of the heterogeneous cancer cell populations within the same tumor (172,173). To overcome these challenges, the genetic, epigenetic and molecular profile of each patient should be evaluated.

In conclusion, despite its adverse side effects, chemotherapy remains the first line therapy for cancer, solely or in combination with RT and surgery. However, late sequelae of these modalities are of a big concern particularly in regard of young cancer patients who are expected to live longer. Thus, improved survival health and quality of life for cancer survivors are a public health priority. Improved understanding of the molecular aspects of a tumor initiated the emergence of biology-driven therapeutic means and made personalized-cancer therapy possible. Doctors can customize cancer treatment to a tumor of a patient thanks to personalized or precision medicine. With the use of precision medicine, clinicians can select the treatment that is most likely to be effective based on the exact genetics of that patient's particular cancer rather than using a 'passe-partout' strategy. Precision medicine is beginning to be utilized more frequently to treat cancer patients, thanks to developments resulting in quicker and less expensive gene sequencing. It is also advised that physicians examine the structure of BBB to evaluate the ability of a drug to cross the BBB before enrolling patients in any clinical trials. Along with advanced technologies in conventional methods, immunotherapy and targeted therapy provide a hope for improved survival and quality of life for the patients. Finally, the effectiveness of the GBM treatment may be

improved by using a combination therapy approach that targets both tumor cells and cells in the surrounding TME. While healthcare is a huge market, it is worth to note that changing the routine of cancer therapy is not easy. It is therefore more challenging now for an oncologist to navigate through different therapeutic options (both single and combinatorial) to select the optimal treatment whilst ethics are to be respected.

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Authors' contributions

MES conceived the review, provided resources, finalized the writing and edited the manuscript. RN and HD co-wrote the first draft of the manuscript. HB and AB wrote large sections of the review and helped with the research.

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Competing interests

The authors declare that they have no competing interests.

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