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Review

An Overview of Nanotherapeutic Drug Delivery Options for the Management of Glioblastoma

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Abstract: Glioblastoma is the most common primary, malignant brain tumor that remains uniformly lethal in nearly all cases as a result of extreme cellular heterogeneity, treatment resistance, and recurrence. A major hurdle in therapeutic delivery to brain tumors is the blood–brain barrier (BBB), which is the tightly regulated vascular barrier between the brain parenchyma and systemic circulation that prevents distribution of otherwise beneficial chemotherapeutics to central nervous system tumors. To overcome the obstacle of drug delivery beyond the BBB, nanoparticle formulations have come to the forefront, having demonstrated success in preclinical observations, but have not translated well into the clinical setting. In summary, this review article discusses brain tumors and challenges for drug delivery caused by the BBB, explores the benefits of nanoparticle formulations for brain tumor delivery, describes the characteristics these formulations possess that make them attractive therapeutic strategies, and provides preclinical examples that implement nanoparticles within glioma treatment regimens. Additionally, we explore the pitfalls associated with clinical translation and conclude with remarks geared toward overcoming these issues.

Keywords: nanoparticle; glioma; glioblastoma; nanotherapy; cancer therapy

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1. Introduction

Glioblastomas (GBM) are aggressive World Health Organization (WHO) Grade IV brain tumors with a historically low median survival of 14–16 months [1,2]. While rare overall, GBM accounts for >49% of primary malignant brain tumors, resulting in a 5-year survival rate of 6.8%, the lowest relative survival rate of all malignant brain and central nervous system tumors [3]. Age plays a significant role in GBM incidence, with the median age at diagnosis being 65 years [3]. Furthermore, GBM is 1.6 times more likely to form in males than females [3,4]. While the roles of sex and race in treating GBM are still contested, recent work shows that females have a survival advantage and that patient survival can be significantly stratified by patient demographics [5–7].

The standard of care for GBM was last significantly altered in 2005, at the advent of Stupp's protocol, where temozolomide (TMZ) given concomitantly with radiotherapy increased median survival by 2.5 months and O-6-methylguanine DNA methyltransferase (MGMT) promoter methylation was found to extend TMZ response further [8,9]. The current standard of care treatment for GBM is maximal resection followed by adjuvant chemo-radiotherapy with TMZ [10], which has proven to lack complete therapeutic efficacy due to extensive inter-/intra-tumoral heterogeneity and aggressive biology. Despite extensive efforts to characterize GBM through genomic, epigenomic, transcriptomic, and

metabolomic approaches, the clinical significance of GBM subclassification remains elusive [11–16]. Currently, the main criteria for defining a brain tumor as glioma are chromosome 1p/19q co-deletion and IDH gene mutational status [17]. However, these criteria are in constant evolution. Recognizing the importance of sex-specific differences in human cancer, it is becoming increasingly evident that a patient's sex correlates with prognosis and treatment response beyond hormonal influence [7]. A patient's sex confers different metabolic dependencies, rates of necrosis, and enrichment of apoptotic pathways [7,18,19]. Other challenges in treating GBM stem from extensive tumor heterogeneity, GBM stem cell contributions, tumor radio- and chemo-resistance, and a complex tumor microenvironment [20–22]. All these factors are further confounded by the selectively permeable blood–brain barrier (BBB), which greatly limits chemotherapeutic distribution, passively and actively, to tumors.

CNS vasculature is highly regulated by a specialized endothelium critical for maintaining nutrient and cellular homeostasis. Surrounded by a milieu of support cells including pericytes, microglia, and astrocytic end feet embedded in the abluminal basement membrane, these endothelial cells held together by contiguous tight junction complexes form the BBB [23]. The BBB is the CNS's master regulator of molecular/cellular transport and represents an enormous challenge for targeted drug delivery in GBM [24,25]. Oncogenic drugs, antibodies, antibody–drug conjugates, and hydrophobic molecules must transverse the endothelial cell luminal and abluminal plasma membranes to reach target sites. In the case of lipophilic molecules that easily cross the lipid bilayer, an extensive network of transmembrane efflux transporters actively extrude drugs back into the capillary lumen further limiting the efficacy of targeted therapies [26,27]. For these reasons, the oncogenic pharmacopeia has shown success in vitro but has largely failed to translate into clinical significance. These challenges highlight the importance of developing targeted approaches for increased drug delivery in GBM.

Nanoparticle (NP) formulations offer unique opportunities for novel therapeutic approaches to cancer treatment and for this reason have been extensively studied in numerous settings [28,29]. One particularly attractive feature of NPs is the ability to leverage their surface modifiable features for targeted drug delivery. As highlighted previously, such approaches may be advantageous in improving treatment of GBM due to inherent limitations in transport from the BBB. Exploratory attempts at improving BBB uptake via NP modification frequently employ increasing hydrophobicity for non-energy dependent transport, developing a receptor-specific targeting ligand for energy-dependent cellular transcytosis and/or combining NP technologies with BBB disruptive physico-chemical methods, such as transcranial focused ultrasound (FUS) [28,30]. In addition to potential transport improvements, NPs offer flexibility in combining multiple therapeutic agents for co-delivery, offer improved drug pharmacokinetics compared with free drug formulations, and can increase the relative safety of a given drug dosage [31]. Because of this wide variety of options for designing targeted NP agents for improving anti-cancer efficacy, there is clear and sustained motivation for the continued preclinical investigation of NP formulations in the treatment of GBM. A graphical representation of these important concepts related to NPs is displayed in Figure 1. Clinical trials involving NP formulations for GBM treatment in humans have also been conducted, as briefly summarized in Table 1. These trials are included to highlight the relevance and importance of successfully translating preclinical NP technologies, but a detailed discussion of these trials will not be included here as it is beyond the scope of this article.

Several articles have previously reviewed and discussed NP formulations for GBM treatment and/or improved BBB uptake of drugs (see references [28–31]). This review aims to provide an update on these topics, with specific attention given to comparing single agent versus multimodal therapies and the incorporation of immunotherapy into NP formulations for GBM treatment.

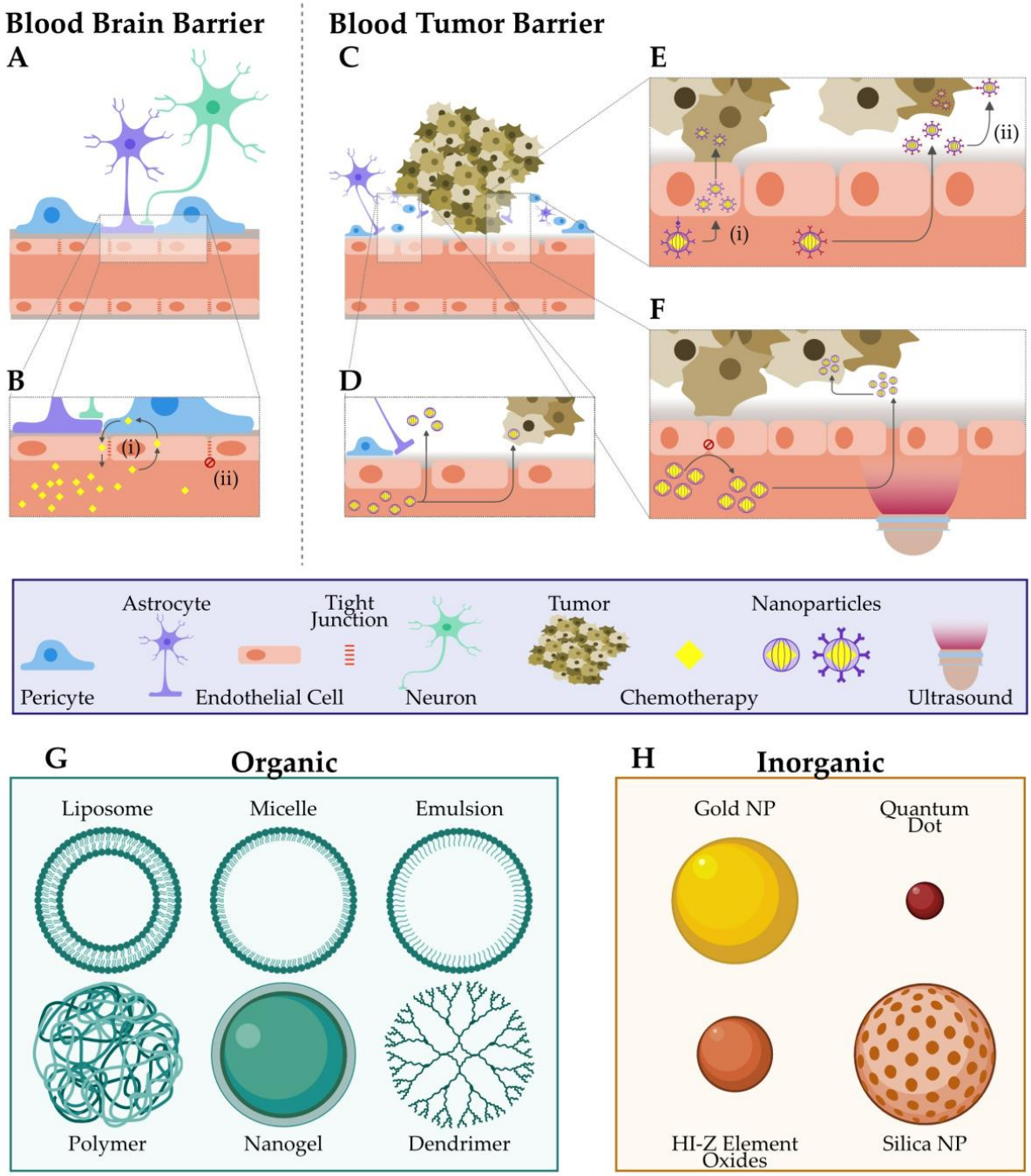

Figure 1. Nanoparticle formulations improve drug delivery beyond the blood–tumor barrier. The BBB (**A**) is a multi-cellular, physio-chemical barrier preventing the distribution of most conventional chemotherapeutics (**B**) to the brain through both active efflux (**Bi**) and passive restriction imposed the contiguous tight junction proteins between endothelial cells (**Bii**). During tumor development, tumor cells disrupt the BBB creating fenestrated, leaky tumor-associated vasculature termed the BTB (**C**). The disrupted vasculature allows for the passive accumulation of nanoparticles driven through the enhanced permeability and retention effect (**D**). Nanoparticle surface modifications can allow for the targeting of cell surface receptors on either the endothelium (**Ei**) or tumor cells themselves (**Eii**) allowing for receptor-mediated transcytosis across the vascular endothelium and/or tumor-specific targeting. There is a multitude of physically disruptive methods to further increase nanoparticle drug delivery, the most popular of late being transcranial low intensity focused ultrasound (**F**). (**G**,**H**) Illustrations of commonly formulated organic and inorganic nanoparticles seen in translational treatments.

Table 1. Clinical trials implementing nanoparticles over the past decade.

Trial ID	Trial Title	Year	Trial Location	Trial Phase	Primary/Recurrent	Aims/Basis	Summary
NCT04881032	AGuIX Nanoparticles With Radiotherapy Plus Concomitant Temozolomide in the Treatment of Newly Diagnosed Glioblastoma (NANO-GBM)	2021	France	I/II	Primary	AGuIX has been shown to penetrate and sensitize tumors to radiation. The aim is to assess the optimal dose of AGuIX for radiotherapy in primary GBM.	Trial still in active recruiting phase
NCT03566199	MTX110 by Convection-Enhanced Delivery in Treating Participants With Newly-Diagnosed Diffuse Intrinsic Pontine Glioma (PNOC015)	2018	United States	I/II	Primary	A water-soluble form of Panobinostat utilizing convection to cross the BBB to treat malignant brain tumors including DIPG.	First trial results posted on 25 February 2022
NCT03020017	NU-0129 in Treating Patients With Recurrent Glioblastoma or Gliosarcoma Undergoing Surgery	2017	United States	I	Recurrent	Spherical nucleic acids adhered to gold nanoparticles cross the BBB and enter tumor tissue where to target the bcl2L12 gene in GBM.	First trial results posted on 26 August 2022
NCT02340156	Phase II Study of Combined Temozolomide and SGT-53 for Treatment of Recurrent Glioblastoma	2015	United States	II	Recurrent	A liposome carrying WT P53 that crosses the BBB and delivers the functional gene to the GBM tumors for TMZ sensitization.	Terminated
NCT00734682	A Phase I Trial of Nanoliposomal CPT-11 (NL CPT-11) in Patients With Recurrent High-Grade Gliomas	2008	United States	I	Recurrent	Utilizes convection enhanced delivery to bypass the BBB and deliver drugs to brain tumor tissue.	Last results updated on 7 January 2015
NCT02766699	A Study to Evaluate the Safety, Tolerability and Immunogenicity of EGFR(V)-EDV-Dox in Subjects With Recurrent Glioblastoma Multiforme (GBM) (CerebralEDV)	2016	United States	I	Recurrent	The aim is to determine the utility of EDV nanocell delivered EGFR immunotherapeutics to tumor tissue.	Update posted 29 August 2019

2. Enhancing GBM Treatment Targeting with Nanoparticles

2.1. Improving BBB Uptake and Bioavailability

The spectrum of available options for improved brain and CNS uptake of therapeutic drugs has been extensively reviewed previously and can be broadly subdivided into invasive and non-invasive strategies [32]. Invasive strategies include intrathecal delivery, deep brain stimulation, and direct brain injection or grafting [32,33]. In general, recent interest has focused on non-invasive delivery methods, which include NP-based delivery systems, intranasal delivery, transcranial focused ultrasound (FUS), and biologic/receptor-mediated transport [32,34]. Of particular importance for this review, FUS, biologic mechanisms, and receptor-mediated transport methods offer attractive pathways for enhancing NP-based GBM treatments. Receptor targets that have been studied previously include the transferrin receptor, lactoferrin receptor, LDL receptor, and CD98 heavy chain, among several others [32]. As examples of specific to NPs targeting receptors present on the BBB, glutathione-targeted liposomes and anti-transferrin receptor antibody conjugated liposomes have been examined in preclinical settings with some successful results [35,36]. Other receptor targets of NPs and NP characteristics for GBM treatment, such as composition, size, shape, and charge, have been reviewed recently by Hersh and colleagues and will not be discussed in detail [30]. Further examples will be discussed in the following sections when relevant.

A recent area of focus for improving NP delivery to GBM has been the use of transcranial FUS to selectively open a target region of the BBB or blood–brain–tumor barrier [37]. As was recently reviewed by Jo et al., FUS-mediated BBB opening has facilitated improved transport and accumulation of a variety of polymeric, lipid, and inorganic NPs into tumor and/or brain parenchyma in preclinical GBM models [38]. Questions regarding optimal FUS parameters still remain, as recent experimental evidence has demonstrated BBB uptake of solutes is dependent on factors such as cavitation dose, nanobubble incorporation, and timing of FUS relative to solute dosing, among others [39].

Taken together, a multitude of methodologies have been explored to improve BBB uptake and bioavailability of therapeutics for a variety of CNS diseases, including GBM. NP formulations can be combined or leveraged in many of these methods and have shown promise in several of the above-mentioned studies. Future work should make a point to emphasize how their NP formulation attempts to address inherent transport limitations of the BBB and blood–brain–tumor barrier in GBM models.

2.2. Nanoparticle Surface-Modifications and GBM Cellular Specificity

NP surfaces—as well as their coronae—are highly versatile and provide a valuable degree of manipulation commonly used for improving therapeutic delivery [40]. The electric charge of a nanoparticle's surface has been shown to impact the fate of the particle. Surfaces with a slight negative charge improve overall blood circulation of the nanoparticle while a more positive charge favors cellular internalization, commonly via the mononuclear phagocyte system, albeit at the cost of causing more systemic cytotoxicity [40–42]. Additionally, conjugation or surface modifications with molecules can actively improve cellular targeting and specificity by acting as unique ligands. Regarding GBM models commonly used in the literature, such as U87MG, the overexpression of certain receptors on these cancerous cells poses as specific targets compared to the rest of the brain parenchyma [43]. Roda et al., have created an excellent review for the current preclinical NP surface modifications being studied within GBM modeling [44]. This section will largely discuss the popular targeting methods for GBM models and a handful of novel approaches with translational promise.

One of the most popular receptor targets for GBM models is the overexpression of $\alpha_v\beta3$ integrin receptors [45,46]. Integrin receptors in GBM and other various solid malignancies are related to promotion of angiogenesis as well as cellular proliferation [46]. The peptide sequence arginylglycylaspartic acid (RGD),and similar non-peptide moieties act as a ligand for the $\alpha_v\beta3$ integrin receptor and, consequently, a unique ligand for GBM

tumor specificity [45]. Antonow et al., provides an example study of formulating lipid-nanocapsules (LNCs) containing doxorubicin that are non-covalently surface functionalized with RGD to improve in vitro internalization of the NPs in U87MG cells. Compared to the human breast cancer cell line, MCF-7, which does not overexpress $\alpha_v\beta3$ integrins, surface functionalization with RGD had a significant effect on reducing cellular viability for U87MG cells. Cytotoxicity of empty LNCs functionalized with RGD was only seen in U87MG cells after a 24 h MTT assay which is proposed by the authors to be related to RGD signaling inducing apoptotic pathways via procaspase-3 [45]. An in vivo study with RGD moieties was performed by Liu and colleagues, where they evaluated a variant of the RGD peptide, internalizing RGD (iRGD), which contains a cryptic C-end Rule motif that has been shown to bind to neuropilin-1 and further improve tissue uptake of NPs in GBM models. The particles formulated in this study were chitosan surface-modified poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with carmustine, an alkylating agent similar to temozolomide, along with the sensitizing agent, O^6-benzylguanine. In F98 intracranially injected glioma-bearing nude mice, Liu et al., reported both a prolonged median survival (49 vs. 34.5 days) and prolonged start of neurological decline (17 vs. 11 days) in mice treated with NPs conjugated with iRGD compared to those treated with unconjugated NPs. Additionally, DiR-labeled NPs conjugated with iRGD showed a 1.96-fold increase in fluorescence of the tumor regions 2 h post injection compared to DiR-labeled unconjugated NPs [47].

As previously mentioned in Section 2.1, Transferrin (Tf) and Lactoferrin (Lf) are known to help improve BBB uptake of nanoparticles due to abundant receptor expression on endothelial cells. Interestingly, both ligands can potentially be used to further improve nanoparticle GBM internalization since Tf and Lf receptors are frequently overexpressed in documented GBM cell models [41,48,49]. Ramalho et al., demonstrated that conjugating Tf on PLGA nanoparticles to encapsulate asiatic acid favorably separated the half maximal inhibitory concentrations between U87MG cells and immortalized human astrocyte cells [50]. It should be noted that the surface of nanoparticles can be functionalized with more than one ligand at a time. This provides an option to overcome specific delivery hurdles, such as the BBB or blood–brain–tumor barrier, while still having tumoral specificity and enhanced efficacy [44]. Qi and colleagues formulated liposomes that were PEGylated and dual-functionalized with Lf and RGD to carry docetaxel, a taxane chemotherapeutic with poor water solubility. DiR-loaded liposomal formulations that were dual-functionalized showed 3.35-fold increase in fluorescence at the tumor site of orthotopic U87MG mouse models 24 h post-tail vein injection compared to unconjugated liposomes. Likewise, the median survival of the mouse models was prolonged in dual-functionalized, docetaxel-loaded liposomes (32 days) compared to unconjugated docetaxel-loaded liposomes (21.5 days) [51].

Having the ability to modify the surface of nanoparticles with ligands significantly increases the therapeutic possibilities of many drugs or molecules where delivery is a primary challenge. Surface modifications also alter the biodistribution of a substance which can be used to mitigate adverse events seen with undesirable dosing to off-target organs [44]. As the cellular surfaces of malignancies and other physiologic barriers are further characterized, new targets will be identified that may improve specificity. One example of this is the discovery of a small peptide called chlorotoxin which has been shown to have high affinity for malignant cells, including GBM [52–54]. There is also the possibility of creating nanoparticles that are inherently cytotoxic to the target of choice. Prabhakar et al., functionalized the surface of mesoporous silca nanoparticles (MSNs) with the polymer polyethyleneimine (PEI). These particles were selectively cytotoxic to glial stem cells, a possible target to mitigate GBM recurrence, with minimal toxicity to surrounding brain parenchymal cell types both in vitro and in vivo [55].

3. Therapy Implementation with Nanoparticles

3.1. Single-Agent Nanotherapies in GBM

While the use of monotherapy is uncommon clinically for the treatment of primary brain malignancies, preclinical evaluation of chemotherapeutics is what identifies the molecular pathways for potential interactions with other modalities as well as the shortcomings or hurdles present for a given chemotherapeutic. Chemotherapeutics that are administered in their free form commonly have difficulties with solubility, circulation duration, and unwanted toxicity at higher doses due to systemic biodistribution [56]. Treating brain malignancies is often accompanied by the additional challenge of bypassing the BBB when the given treatment is not innately BBB-penetrant [57]. In fact, a large number of the clinically available lipophilic chemotherapeutics have affinity for the efflux transporters found at the BBB [58]. Nanoparticle delivery systems have the potential to alleviate many of the challenges faced with reaching the required chemotherapeutic doses to improve treatment efficacy.

Temozolomide, the gold standard alkylating agent for GBM treatment, has demonstrated its excellent bioavailability even with oral administration and is capable of freely traversing the BBB [59,60]. However, temozolomide's excellent bioavailability and high volume of distribution also come at the cost of being able to easily accumulate within healthy tissue leading to systemic toxicity when given at higher doses. Nanoparticle vehicles pose a solution providing GBM specificity for temozolomide as well as further enhancing BBB penetrance. De et al., developed niosomes, a bilayer nanoparticle similar to liposomes, conjugated with chlorotoxin and loaded with temozolomide. Pharmacokinetic studies performed in rats compared the organ distribution of the loaded nanoparticles after injection to oral administration of free temozolomide. The niosome group of rats had a 3.04-fold increase in temozolomide in the brain as well as 1.97-fold and 1.55-fold decrease of the chemotherapeutic in the liver and kidneys, respectively, indicating improved pharmacokinetic specificity and decreased accumulation in healthy, off-target organs [53]. Miller and colleagues attempted to improve biodistribution and selectivity of temozolomide; however, they loaded the chemotherapeutic within pH-responsive polymeric micelles conjugated with a platelet-derived growth factor (PDGF) peptide to improve GBM specificity. Within U87 orthotopic mouse models 24 h post-injection, tumors retained micelles conjugated with PDGF peptide 40% more than micelles that were unconjugated and had no changes in overall excretion pattern [61]. Both aforementioned studies describe methodologies to further lower the overall dose of temozolomide required to achieve therapeutic levels within the brain.

Paclitaxel, a microtubule stabilizing agent, has been shown to exhibit nearly 1400-fold increased potency in glioma cell lines compared to temozolomide. However, paclitaxel by itself struggles to cross the BBB and has been ineffective in in vivo glioma models [62]. Currently, a Phase 1/2 clinical trial is recruiting patients for evaluating the ability of ultrasound to temporarily disrupt the BBB and improve drug accumulation of carboplatin and paclitaxel in patients with recurrent glioblastoma [63]. Another approach that can effectively improve paclitaxel brain accumulation is loading the chemotherapeutic within nanoparticles. Lei et al., created d-alpha-tocopherol polyethylene glycol 1000 succinate (TPGS)-surfaced PLGA nanoparticles containing paclitaxel. After intravenous injection, the clinically available formulation of paclitaxel, Taxol®, had negligible levels in the brain 24 h post-injection while the paclitaxel-loaded nanoparticles showed >800% accumulation within the brain 96 h post-injection [64]. Though this work was not performed in mouse models of GBM, it demonstrates another viable strategy for increased drug delivery via nanoparticle formulations to a tumor of the CNS.

Topoisomerase inhibitors have shown limited improvement of outcome in GBM clinical trials, yet they are still frequently studied within preclinical GBM models [65]. The prominent interest in these chemotherapeutics relies on the fact that the tumor suppressor gene p53, which is commonly mutated in GBM, has been shown to alter expression of topoisomerases [66,67]. Examples of topoisomerase inhibitors include camptothecin and

etoposide, which target topoisomerases I and II, respectively. Both of these drugs are hydrophobic and often have poor parenchymal distribution within the brain [68]. Studies using polymeric nanoparticles composed of PLGA or poly(lactic acid) (PLA) polymers have been able to favorably improve release kinetics and enhance the GBM selectivity of topoisomerase inhibitors [69,70]. Householder et al., provide an example of camptothecin loaded into PLGA nanoparticles without surface modifications reducing tumor growth and improving the median survival of orthotopic GL261 GBM mouse models to 36.5 days compared to free camptothecin with a median survival of 32 days [71].

The anthracycline chemotherapeutic, doxorubicin, has excellent cytotoxicity in a multitude of cancers, including GBM [72]. Doxorubicin damages cells by the production of reactive oxygen species, inhibiting topoisomerase II, and by forming DNA adducts [73,74]. Like most chemotherapeutics, high doses of doxorubicin are required to achieve therapeutic levels within the brain [72]. An unfortunate limiting factor to doxorubicin's clinical use is its cumulative, dose-dependent cardiotoxicity [75]. As with the other chemotherapeutics discussed above, the potency, selectivity, and systemic toxicity of doxorubicin can all be favorably altered with the implementation of nanoparticle systems. Chai et al., accomplished reduced cardiotoxicity and statistically significant prolonged median survival in orthotopic U87 GBM mouse models treated with doxorubicin-loaded nanoparticles derived from red blood cell membranes. Brain targeting and GBM specificity were accomplished with conjugation of streptavidin and a peptide with affinity to brain endothelial nicotinic acetylcholine receptors [76]. Notably, the use of cellular membranes for nanoparticles is intended to reduce immunogenicity and further enhance biocompatibility compared to synthetically derived vehicles [76,77]. Another novel nanoparticle that was created for doxorubicin and evaluated with preclinical GBM models was described by Pandey et al. The authors loaded doxorubicin in nanoparticles composed of silk fibroin coated with Tween-80. While the studies were conducted in vitro, the nanoparticles provide a method to allow sustained drug release as well as enhanced cytotoxicity compared to free doxorubicin as shown in C-6 and LN-229 GBM cell lines [78].

Fullerenes and their nano-conjugates represent another intriguing route for increased distribution beyond the BBB. Hsieh and colleagues created a series of water-soluble fullerene derivatives with varying degrees of brain penetration and innate cytotoxicity. They demonstrated that delivery of two of their eight fullerene derivatives was able to reduce tumor growth in a zebrafish model of GBM [79]. Another group explored the use of a fullerene-based nanoplatform for utility in positron emission tomography with implications in brain tumor imaging [80], while others have explored their functionality in enhanced delivery of gadolinium [81]. These and other carbon-based NPs potentially hold excellent theragnostic capabilities.

A summary table of single-agent chemotherapeutic nanoparticles is presented in Table 2.

Table 2. Overview of single-agent chemotherapeutic treatments using nanoparticles in pre-clincal GBM models.

Agent Used	NP Type	Surface-Modifications	GBM Cell Model(s)	NP Key Results	Reference
Doxorubicin	Lipid-core Nanocapsules	RGD	- In vitro U87MG cells	Increased cellular uptake specifically in U87MG cells.	[45]
	Multifunctionalized Liposomes	mApoE Peptide and Chlorotoxin	- In vitro U87-MG cells	Tunneling nanotubes can preferentially transfer NPs between cells.	[54]
	PLGA NPs coated with RBC-derived cell membranes	Streptavidin and CDX Peptide	- In vitro U87-MG cells - In vivo U87 orthotopic glioma-bearing mice	Increased doxorubicin specificity, BBB-uptake, and median survival of mice.	[76]
	Small extracellular vesicles derived from U87	cRGDyC	- In vitro U87 cells	Significantly increased internalization and targetability of U87 cells.	[77]
	Silk Fibroin NPs coated with Tween-80	Apolipoprotein E/B adsorbed in Tween-80	- In vitro rat C6 and human LN-229 origin cells	Improved drug release and cytotoxicity in non-cytotoxic NPs.	[78]
	Au-NPs within degradable gelatin surface	RRGD and Octarginine	- In vitro C6 tumor spheroids - In vivo orthotopic C6 glioma-bearing mice	Enhanced colocalization in neovessels with increased penetration.	[82]
Paclitaxel	Polymeric (PLGA) NPs	TPGS	- In vitro C6 cells - In vivo non-GBM mice	Enhanced drug release, brain accumulation, and GBM selectivity.	[64]
	Solid Lipid NPs	iRGD	- In vitro U87-MG 2D and 3D tumor models	Improved tumor penetration, targeting, and cytotoxicity.	[83]
	Polymeric (PLGA-PEG) NPs	PAMAM	- In vitro U87 and BBB models - In vivo non-GBM mice - In vivo orthotopic U87MG xenograft mice	Improved cytotoxicity and increased BBB uptake in vitro. Enhanced brain uptake in healthy mice and improved survival in GBM mice.	[84]
	Polymeric (PLGA) NPs	Vimentin Antibody M08	- In vitro C6 cells	Enhanced GBM specificity and targeted cytotoxicity.	[85]
Docetaxel	PEGylated Liposomes	Lf and RGD	- In vitro U87-MG BBB model - In vivo orthotopic U87-MG glioma-bearing mice	Improved transport across BBB for in vitro model and prolonged median survival in mice.	[51]
Camptothecin	Polymeric (PLA-HPG) NPs	Adenosine	- In vitro U87 cells - In vivo orthotopic U87 glioma-bearing mice	Improved in vitro cytotoxicity, increased brain uptake in vivo, and failed to improve mouse survival.	[69]
	Polymeric (PLGA) NPs	None	- In vivo orthotopic GL261 glioma-bearing mice	Improved tolerability of drug and enhanced median survival in mice.	[71]

Table 2. *Cont.*

Agent Used	NP Type	Surface-Modifications	GBM Cell Model(s)	NP Key Results	Reference
Etoposide	PEGylated Polymeric (PLGA) NPs	None	- In vitro C6 and F98 murine cells	Improved in vitro cytotoxicity.	[70]
Temozolomide	Niosomes	Chlorotoxin	- In vitro U-373 MG - In vivo non-GBM mice and rat models	Improved selectivity and cytotoxicity in vitro. Enhanced brain and decreased liver/kidney biodistribution.	[53]
	Micelles	PDGF Peptide	- In vitro U87 and LN229 cells - In vivo orthotopic U87 glioma-bearing mice	Enhanced selective cytotoxicity to GBM cell models and improved brain biodistribution.	[61]
Asiatic Acid	Polymeric (PLGA) NPs	Tf	- In vitro U87 cells	Prolonged release of drug and increased selective internalization in GBM cells.	[50]
azide-terminated survivin ligand (az-TM)	Polymeric (PA) NPs	az-TM	- In vitro U118MG and U251MG cells	Selective binding to survivin expressing GBM cells and enhanced cytotoxicity via increased apoptosis.	[86]
Curcumin	4th Generation PAMAM Dendrimers	None	- In vitro U87, F98, and GL261 cells	Enhanced curcumin delivery and were toxic to the cancer cells tested.	[87]
Methotrexate	MnO_2 NPs	Opca	- In vitro GL261 BBB Model - In vivo orthotopic GL261 glioma-bearing mice	Enhanced in vitro uptake in glioma BBB model. Improved brain biodistribution in GBM mice models.	[88]
Oncocalyxone A	Iron oxide NPs coated with HES	None	- In vitro SNB-19 cells - In vivo non-GBM zebrafish model	Enhanced compound cytotoxicity along with no in vivo deaths during acute toxicity assay.	[89]
Pitavastatin	Silica coated Polymeric (F127/T1307) NPs	cRGDfV	- In vitro HSJD-GBM-001	Enhanced uptake and specific cytotoxicity in glioma cells compared to healthy BBB cells.	[90]

Abbreviations: ApoE—apolipoprotein E; HES—hydroxyethyl starch; HPG—hyperbranched polyglycerol; Lf—lactoferrin, NPs—nanoparticles; PA—propargyl acrylate; PAMAM—poly(amidoamine); PEG—polyethylene glycol; PLA—poly(lactic acid); PLGA—poly(lactic-co-glycolic acid); RGD (cRGDfV, cRGDyC, iRGD, RRGD)—variant of arginylglycylaspartic acid; Tf—transferrin; TPGS—d-α-tocopherol polyethylene glycol 1000 succinate.

3.2. Nanoparticle-Based Immunotherapy in GBM

Leveraging the immune system as a component of cancer care has greatly improved outcomes in several aggressive cancers [91,92]. In particular, the introduction of immune checkpoint inhibitors (ICIs) and chimeric antigen receptor (CAR) T-cell therapies has proven especially promising, improving overall and progression-free survival rates in melanoma and non-small cell lung cancers, among others [92–94]. Historically, the CNS was considered an immune-privileged site, but recent evidence suggests that it instead has a unique immune surveillance capable of mounting an adaptive immune response [95,96]. Attempts to leverage this potential for anti-tumor immune responses have faced challenges in inducing robust immune responses, primarily hindered by BBB transport limitations, immunosuppressive tumor microenvironments, lack of stable tumor specific antigens, and inherent immunosuppressive effects of standard GBM treatments [97]. To address some of these challenges, a variety of NPs have been employed as active delivery agents for immunotherapy of GBM, as Tang et al., recently reviewed [98]. A few recent examples are discussed in further detail below.

Incorporation of ICIs with NP therapeutics is becoming an active area of preclinical study in GBM. Meng and colleagues demonstrated the use of PEGylated, for which TMZ-loaded manganese oxide NPs co-delivered with anti-PD-L1 antibodies induced specific anti-tumor immune responses when dosed after the FUS-nanovesicle induced BBB opening [99]. In another example of NP-based ICI therapy, Tian et al., utilized peptide-targeted extracellular vesicle nanocarriers loaded with siRNA against PD-L1 and found that these peptide-targeted NPs accumulated in GBM stroma after radiotherapy and increased myeloid cell recruitment, increased CD8+ T cell activation, and prolonged survival [100]. Alternatively, other researchers have focused on different immunologic targets in GBM. Alghamri et al., recently explored a synthetic protein NP formulation that encapsulated a CXCR4–CXCL12 signaling inhibitor that, when combined with radiotherapy, induced immunogenic cell death and prevented tumor growth from secondary tumor rechallenge in about 60% of mice [101]. Since the CXCL12-CXCR4 signaling pathway is associated with tumor progression in GBM, this result is a promising new molecular target that was further improved through presentation in a NP formulation. Other components of immune system signaling have also been employed in various NP formulations. Zhao et al., developed cRGD-peptide targeted, cell membrane-encapsulated $CaCO_3$ NPs containing IL-12 mRNA that were designed to stimulate T cell responses after ultrasound treatment [102]. They found that this combination increased IL-12 and IFN-γ levels within tumor tissue, increased the number of CD8+ cells within tumor stroma, and extended mouse survival compared with negative controls. Results such as these serve as a proof of concept for delivery of other immunostimulatory effectors via NPs that warrant investigation. Recent interest in combining nanoparticles with selective targeting agents for GBM has motivated some researchers to utilize bacteria that have an innate ability to cross the BBB, as was recently studied by Sun et al. [103]. Their recent article developed glucose-polymer conjugated, indocyanine green-loaded silicon nanoparticles (SiNPs) encapsulated within *S. typhimurium* and *E. coli* strains that were combined with IR laser exposure to induce photothermal-immunotherapy. They found that compared with the SiNPs or bacteria carriers, the combination under IR induced effective photothermal therapy against both the carrier bacteria and GBM cells, increased anti-tumor immune cell responses via increased recruitment and antigen uptake/presentation, and extended mouse survival. This "Trojan-horse" style of transport warrants further investigation with other formulations, though bacteria-based methods are still limited to preclinical settings only.

An additional area of potential NP-based immunotherapy is via cell-mediated combination treatments. Chimeric antigen receptor (CAR) T cell-based therapies have been exceptionally successful in treating multiple blood cancers and as a result have fostered strong interest in solid tumor oncology, including neuro oncology [104]. Recognizing the broad potential of both CAR T cell therapy and NP-based treatments, several researchers

have developed combination therapies aimed at leveraging the strengths of these individual components. Kim et al., created a mutant IL-13-targeted CAR T cell-mediated NP drug delivery system for GBM that aimed to enhance delivery of NPs to GBM in mice [105]. Their work demonstrated that the TQM-13 (IL-13 mutant) CAR T cells had high affinity for IL13Rα2-expressing glioblastoma cells, increased cytotoxicity in vitro when NPs were loaded with doxorubicin, and significantly increased NP accumulation in vivo GBM models compared with NPs alone. More recently, Chang et al., utilized a complex design system that incorporates anti-GBM chlorotoxin (CLTX)-CAR constructs with neutrophil-specific signaling domains into human pluripotent stem cells (hSPCs) that were differentiated into CAR-expressing neutrophils [106]. They successfully differentiated these CAR-expressing neutrophils and were able to encapsulate SiO_2 NPs loaded with tirapazamine into the CAR-neutrophils. Their results indicate that, at the appropriate dosing frequency, NP-loaded CAR-neutrophil treated groups had improved mouse survival and decreased tumor burden in comparison to NP-only or CAR-neutrophil-only groups. In conjunction, these studies provide supportive and encouraging evidence that cell-mediated combinations with NP formulations have the potential to further expand the benefits of NP-based formulations alone, but further investigation is needed to determine the most efficacious and safest combination therapy.

Regardless of the exact strategies used, NP-based immunotherapy treatments for GBM remain an active area of investigation. Many questions remain unanswered, including both optimization of individual formulations and their timing relative to standard of care therapy. For example, Cloughesy et al., demonstrated from clinical data that neoadjuvant anti-PD-L1 antibodies conferred a survival benefit in GBM patients in recurrent GBM when compared with adjuvant administration [107]. Nonetheless, this promising area of preclinical research remains an important focal point and deserves the current level of interest it has attained.

3.3. Multi-Modal Nanotherapeutics in GBM

In general, most forms of primary brain malignancies are treated with maximally safe resection in combination with chemotherapy and/or radiotherapy [10]. Combining different modalities of treatment is an effective method of introducing favorable synergisms between treatments while reducing adverse events seen with more aggressive single-modality treatment options [108,109]. Likewise, the implementation of nanoparticles provides an additional level of customizability that can further improve current multi-modal treatments available. This section will provide and discuss examples in the literature that utilize multiple agents or treatment modalities involving nanoparticles for the management of primary brain tumors.

3.3.1. Nanoparticles for Co-Delivery of Agents and Invoking Synergism

While temozolomide-induced cytotoxicity is the gold standard chemotherapeutic regiment for GBM, resistance to alkylating agents is common [47]. Upregulation of c-MET has been shown to be associated with resistance to alkylating agents as well as promotion of GBM metastasis [110]. To help enhance the therapeutic efficacy of temozolomide, Pang and colleagues used novel virus-like nanoparticles with interfering c-MET RNA complexes produced within *Escherichia coli*. These nanoparticles were surface-modified with apolipoprotein E and a cell-penetrating peptide to enhance BBB traversing and improve cellular internalization. In orthotopic U87 glioma-bearing mice, tail vein injection of the nanoparticles followed by oral administration of temozolomide 12 h after each pretreatment improved median survival to 42 days compared to 25 days in mice given only oral temozolomide [110].

Lakkadwala and colleagues demonstrate how co-delivery of the chemotherapeutics doxorubicin and erolotinib can be accomplished in liposomes conjugated with transferrin and a cell-penetrating peptide, penetratin, to synergistically treat U87 gliomas in a 3D BBB in vitro model as well as in vivo [111]; however, both chemotherapeutics strug-

gle to successfully accumulate in the brain parenchyma since they are substrates of the p-glycoprotein efflux transporter [112]. Erlotinib has the additional problem of having decreased bioavailability due to its innate hydrophobicity [113]. Compared to free drugs when administered in orthotopic U87 glioma-bearing mice, Lakkadwala et al., showed that the dual-functionalized liposomal form of doxorubicin and erlotinib resulted in a 5.9-fold decrease in tumor size after treatment. Additionally, accumulation of doxorubicin and erlotinib within brain tissue was 12-fold and 3.3-fold higher, respectively, when loaded in the dual-functionalized liposomes [111].

Another example of enhancing co-delivery of chemotherapeutics for GBM is demonstrated by Hettiarachchi et al., where both epirubicin and temozolomide were conjugated on carbon dots along with transferrin to create a triple conjugated nanoparticle [114]. Compared to most of the other carbon-based nanoparticles, carbon dots have demonstrated minimal cytotoxicity in vivo and have the added benefit of being less than 10 nm in size which allows for increased permeability across the BBB along with potentially bypassing the endothelial tight junctions [114–116]. While the work done by Hettiarachchi and colleagues was only in vitro, they successfully created triple-conjugated carbon dots with an average size less than 4 nm and demonstrated synergistic cytotoxicity compared to single-chemotherapeutic conjugation in the pediatric glioblastoma cell line, SJGBM2 [114].

3.3.2. Nanoparticles to Enhance Radiotherapy and Implement Additional Treatment Modalities

A tissue's response to radiation, i.e., its inherent radiosensitivity, can be enhanced through the use of other agents or certain chemotherapeutics [108]. Improving the specificity and accumulation of these radiosensitizers with the implementation of nanoparticles poses as another method for enhancing both current and emerging primary brain malignancy treatment options. Though some variants of gold nanoparticles have recently shown favorable toxicity in GBM cell models due to cytoskeletal alterations [117,118], nanoparticles containing high-z-atom elements are more commonly used as radiosensitizers to improve energy deposition [119,120].

Fangshi et al., created intricate mesoporous silica nanoparticles surfaced with LRP-ligand angiopep-2 and a nitrous oxide donor. These nanoparticles contained $NaGdF_4$:Eu^{3+} nanocrystals and banoxantrone, a selectively hypoxic chemotherapy pro-drug. In orthotopic U87MG glioma-bearing mice, treatment with the nanoparticles along with low-dose radiotherapy reduced tumor burden and prolonged the median survival to 46 days compared to 23 days in mice given low-dose radiotherapy alone [121]. This preclinical trial provides a novel and promising approach in overcoming the hypoxic hurdle that often reduces the radiotherapeutic efficacy seen in gliomas.

One proposed reason for the high recurrence of GBMs is the presence of tumor-associated myeloid cells (TAMCs). These cells are believed to promote immunotherapeutic resistance and have been shown to comprise up to 50% of the tumor's mass [122]. Zhang et al., demonstrated that lipid nanoparticles surface-functionalized with an anti-programmed death-ligand 1 (anti-PD-L1) antibody can selectively target these TAMCs. Loading the nanoparticles with the cyclin-dependent kinase inhibitor, dinaciclib, resulted in dose-dependent cytotoxicity of TAMCs that was not observed in GL261 glioma cells in vitro. Additionally, the authors identified that radiotherapy increased the prevalence of TAMCs that had higher surface expression of PD-L1. Within orthotopic CT2A glioma-bearing mice, 7 days of intracranial cannula administration of the developed nanoparticles along with four doses of 2 Gy irradiation resulted in extending the median-survival of the glioma-bearing mice to 34 days compared to 20 days in the control group. Interestingly, 30% of the treated mice had long-term survival. While the capabilities of the nanoparticles crossing the BBB were not evaluated, this paper reveals that TAMCs are a unique immunotherapeutic target that can synergistically enhance current GBM radiotherapy regiments [123].

Chiang and colleagues demonstrate the implementation of a strategy that they call targeting sensitization-enhanced radiotherapy (TSER) to treat preclinical GBM models. The

TSER treatment approach included the intracranial injection of a radiosensitizer, GoldenDisk (GD), along with a sonosensitizer, 5-aminolevulinic acid (5-ALA). followed by ultrasound and radiotherapy to a dose of 2 Gy 24 h post-injection. The radiosensitizer GD created by the authors is a spherical, silica-coated gold nanoparticle surfaced with hyaluronic acid to selectively target CD44 receptor overexpression seen in GBM cell lines. 5-ALA was used as a sonosensitizer for Sonodynamic therapy (SDT) due to its increased uptake in GBM cells and the possibility of synergism between SDT and radiotherapy. Within GL261-derived orthotopic GBM nude mouse models, TSER treatment resulted in no sign of tumor progression for the first month post-treatment and had a 1.53- and 1.25-fold increase in median survival compared to mice only irradiated to single-fractionated doses of 2 Gy and 10 Gy, respectively [124]. This intricate GBM treatment displays how radiosensitization can significantly surpass the effects of higher-dose radiotherapy and maintain a safer therapeutic profile.

3.3.3. Magnetic Nanoparticles and Their Theragnostic Potential for GBM

Magnetic nanoparticles are an ever-expanding utility within the biomedical field, finding uses as contrast agents for imaging and drug delivery vehicles and even uses for hyperthermic treatments [125]. The most commonly used magnetic nanoparticles are iron oxide magnetic nanoparticles (IOMNPs) which generally consist of a biocompatible material surrounded by an iron oxide core [126]. The type of biocompatible material used, such as lipids or polymers, can impact the size of particles, their stability, drug loading capabilities, and their ability to undergo surface modifications [127]. While tumor imaging methods are beyond the scope of this paper, the diagnostic capabilities of paramagnetic molecules, such as iron oxide, should be acknowledged [49,128]. Regarding therapeutic applications, IOMNPs can be formulated such that drugs or other molecules are co-encapsulated with iron oxide. An external magnetic field can then be used to target a tissue of interest and transiently enhance the delivery of the nanoparticle to the given area [129]. This approach can be used in addition to the receptor-targeting methods that were discussed in Section 2.2 to further enhance cellular specificity and further reduce accumulation of nanoparticles in off-target sites. Induction of tissue hyperthermia can also be done via localization of magnetic nanoparticles followed by irradiation with electromagnetic waves which has been shown to enhance treatments including chemotherapy and radiotherapy [89,130]. Consequently, magnetic nanoparticles are a versatile tool that can be implemented into current GBM treatments and potentially provide further synergisms between other treatment modalities.

Chen et al., recently studied the chemo-photothermal effects on U87 GBM cells with their formulation of IOMNPs and cisplatin co-encapsulated inside PLGA polymeric nanoparticles that were surfaced-modified with hyaluronic acid. In vivo studies were conducted on subcutaneously implanted U87 nude mouse models. Use of a magnetic field on the nanoparticles at the tumor site resulted in a 6.8-fold increase tumor-targeting efficacy during biodistribution studies. Addition of near-infrared radiation on top of magnetic targeting shortly after tail vein injection of the nanoparticles resulted in a prolonged median survival of 42 days compared to 32 days for the control group, 35 days for the nanoparticle injection only as well as the cisplatin only groups, and 39 days for nanoparticle injection with magnetic targeting only. Overall, the addition of a magnetic field with near-infrared radiation resulted in the best treatment outcomes with regard to survival and tumor burden [125].

A summary of these multimodal nanoparticle treatments is detailed in Table 3.

Table 3. Overview of multimodal treatments using nanoparticles in pre-clinical GBM models.

Treatments Used	NP Type	Surface-Modifications	GBM Cell Model(s)	NP Key Results	Reference
Doxorubicin and alpha bisabolol	Lipid-core Nanocapsules	RGD	- In vitro U87MG and U138MG Cells - In vivo non-GBM chicken chorioallantoic membrane assay	Exhibited cytotoxicity in temozolomide-resistant cells in vitro with anti-angiogenic activity in vivo.	[131]
Doxorubicin and Erlotinib	Liposomes	Tf and PFVYL1	- In vitro U87 cells and brain tumor model	Enhanced lethality towards in vitro GBM cells and increased uptake across brain tumor model.	[41]
		Tf and Penatratin	- In vitro U87 cells and brain tumor model - In vivo orthotopic U87 glioma-bearing mice	Enhanced transport to GBM cells with in vitro brain tumor model. NPs enhanced brain tissue penetration, reduced tumor burden, and prolonged median survival in vivo.	[111]
Epirubicin and Temozolomide	Carbon Dots	Tf	- In vitro SJGBM2, CHLA266, CHLA200, and U87 cells.	Synergistically enhanced chemotherapetutic cytotoxicity.	[114]
Carmustine and O6-benzylguanine	Polymeric (PLGA + Chitosan) NPs	iRGD	- In vitro F98, C6, and U87 cells and tumor spheroids - In vivo orthotopic F98 glioma-bearing mice	Enhanced tumor penetration and antitumor activity in vitro. Decreased tumor burden and prolonged median survival in vivo.	[47]
		None	- In vitro F98 and C6 cells - In vivo orthotopic F98 glioma-bearing rats	Enhanced in vitro uptake of drugs. Decreased tumor burden while prolonging median survival in vivo.	[132]
Temozolomide and siRNA for c-MET	Virus-like particles containing siRNA	Cell-Penetrating Peptide and ApoEP	- In vitro U87 cells - In vivo orthotopic U87 glioma-bearing mice	Enhanced cell lethality and reduced treatment resistance in vitro. Crossed the BBB and improved median survival when used with temozolomide in vivo.	[110]

Table 3. *Cont.*

Treatments Used	NP Type	Surface-Modifications	GBM Cell Model(s)	NP Key Results	Reference
AQ4N, NO donor, Gd nanocrystals, and RT	Mesoporous silica NPs	LRP ligand angiopep-2 and NO donor	- In vitro U87MG/U251 cells and BBB model - In vivo orthotopic U87MG glioma-bearing mice	Crossed the BBB and selectively targeted GBM cells both in vitro and in vivo. Demonstrated synergism with RT and tumor hypoxia prolonging mouse survival.	[121]
Dinaciclib and RT	LNP	Anti-PD-L1 antibody	- In vitro GL261 cells and associated TAMCs - In vivo orthotopic GL261 glioma-bearing mice	Enhanced cytotoxicity specific to TAMCs rather than GL261 cells in vitro. NPs with RT significantly improved overall survival in vivo.	[123]
Gold, 5-ALA, SDT, and RT	Silica-coated Gold NPs	Hyaluronic Acid	- In vitro GBM8401 cells - In vivo orthotopic GL261 glioma-bearing mice	Demonstrated selective cytotoxicity to GBM cells in vitro. Novel treatment regiment exhibited the best survival outcomes in vivo.	[124]
Cisplatin and PTT	Polymeric (PLGA) Magnetic NPs	Hyaluronic Acid	- In vitro U87 cells - In vivo SC xenograft of U87 cells in mice	NPs with PTT enhanced in vitro cytotoxicity. Magnetic guidance along with chemo-PT therapy provided the lowest tumor growth rate and longest survival time in vivo.	[125]
Spiropyran and Photodynamic Therapy	Photoresponsive gold-decorated polymer NPs	Folate	In vitro C6 cells	Conjugation enhanced cell lethality during photodynamic treatment with increased ROS production after UV irradiation.	[133]
Atorvastatin and Curcumin	usNLCs	Hyaluronic acid, cRGDfK, $H_7k(R_2)_2$ peptide, and Folic acid	- In vitro U-87 MG cells - In vivo orthotopic U-87 MG glioma-bearing mice	Enhanced GBM selectivity and cytotoxicity in vitro as well as brain biodistribution in vivo. Reduced tumor growth in vivo.	[134]

Abbreviations: 5-ALA—5-aminolevulinic acid; ApoEP—apolipoprotein E peptide; AQ4N—banoxantrone; BBB—blood–brain barrier; Gd—gadolinium; LNP—lipid nanoparticle; LRP—lipoprotein receptor-related peptide; RGD (cRGD, iRGD)—variant of arginylglycylaspartic acid; NO—nitric oxide; NPs—nanoparticles; PD-L1—programmed death-ligand 1; PLGA—poly(lactic-co-glycolic acid); PTT—photothermal therapy; ROS—reactive oxygen species; RT—radiotherapy; SC—subcutaneous; SDT—sonodynamic therapy; siRNA—small interfering RNA; TAMCs—tumor-associated myeloid cells; Tf—transferrin; usNLCs—ultra-small nanostructured lipid carriers.

4. Discussion

The use of nanoparticle formulation systems being evaluated in both preclinical and clinical settings is expanding; however, there are several pitfalls, shortcomings, and barriers that prevent their ultimate clinical translation. One limitation observed in the literature was the inconsistent evaluation of the innate cytotoxicity of nanoparticle formulations. Not only should this be evaluated in the GBM models, but cytotoxicity to the brain parenchyma is also a significant factor to consider. NPs inherently cytotoxic to healthy brain tissue pose increased risk for unwanted off-target effects. Additionally, the possibility of hemolysis—which is more significant with NPs that have a positive surface charge—was rarely evaluated and can impact biocompatibility of the delivery system [111,135].

Assessing the ability for NPs to cross the BBB is essential to developing GBM treatments with translational promise and presents another potential pitfall in preclinical study. Intracranial injections of NPs are an invasive method that neglects many of the safety considerations inherent to the procedure limiting translational potential. Evaluation of BBB transport can be conducted either through in vitro monolayer models or in vivo biodistribution methods [40,46,75,80,128]. Likewise, the use of heterotopic in vivo models for GBMs bypasses the biggest treatment hurdle for brain malignancies. While the use of orthotopic GBM models may compromise the BBB, these models, as well as transgenic models, are far more relevant for translational significance when compared with subcutaneous xenograft models, in general [129].

In addition to the type of model used, the selection of the cell line(s) used for experimentation is an important consideration and can frequently be overlooked in preclinical experiments. For example, U87-MG cells are frequently employed in glioma models, and while these are a perfectly acceptable cellular model to use, only using one cell lineage can severely limit the generalizability and translational relevance of results. It is prudent to strongly consider the use of more than one cell line to account for inherent variations in genetics and interactions between the cell lines and normal brain parenchyma/vasculature. Similarly, the selection of which host organism to use should be carefully considered, particularly when incorporating immunotherapeutic components to the treatment plan. For example, using immune-competent versus immunodeficient rodent models is crucial for interpreting the effects of an immunotherapy intervention and its ultimate translatability.

One final common pitfall that can be encountered further downstream is the selection of nanoparticle formulation components. Certain nanoparticle designs can be very difficult to produce at larger scales required for ultimate translational testing and should be considered when undertaking the initiation of a new design. Likewise, the overall stability and persistent quality of nanoparticle formulations should be taken into consideration in the initial experimentation and design.

Overall, nanoparticle formulations present an enormous number of opportunities for potentially improving GBM treatment. Many variations have been investigated extensively, as discussed in this review, with a wide variety of designs, settings, and results in the preclinical setting. By addressing the potential pitfalls discussed and applying the lessons learned from previous experiments, researchers can effectively explore future work to maximize translational relevance of results and ultimately increase the potential for improving patient outcomes.

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