Targeting Glioblastoma through Nano- and Micro-particle-Mediated Immune Modulation

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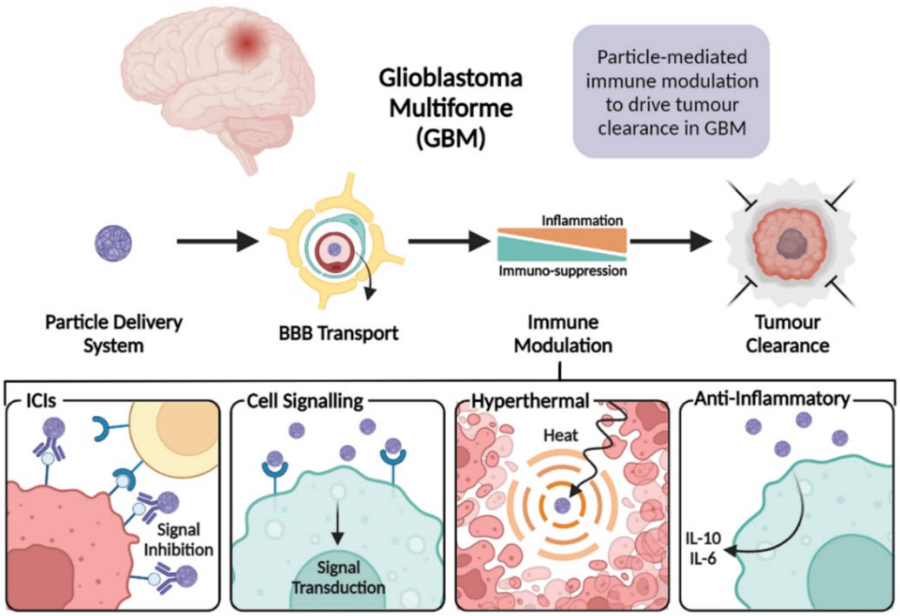
Abstract

Glioblastoma Multiforme (GBM) is a multifaceted and complex disease, which has experienced no changes in treatment for nearly two decades and has a 5-year survival rate of only 5.4%. Alongside challenges in delivering chemotherapeutic agents across the blood brain barrier (BBB) to the tumour, the immune microenvironment is also heavily influenced by tumour signalling. Immunosuppression is a major aspect of GBM; however, evidence remains conflicted as to whether pro-inflammatory or anti-inflammatory therapies are the key to improving GBM treatment. To address both of these issues, particle delivery systems can be designed to overcome BBB transport while delivering a wide variety of immune-stimulatory molecules to investigate their effect on GBM. This review explores literature from the past 3 years that combines particle delivery systems alongside immunotherapy for the effective treatment of GBM.

Graphical Abstract



Key Words

Glioblastoma Multiform; Blood Brain Barrier; Temozolomide; Tumour-Associated Macrophages; T regulatory cells; particle delivery; Immune Checkpoint Inhibitor; cytokines and cell signalling; short interfering RNA (siRNA); hyperthermal therapy

1. Introduction

1.1 Glioblastoma multiforme

Glioblastoma multiform (GBM) is the most common type of primary adult brain tumour and suffers from extremely poor prognoses. The current treatment regime for GBM is much like other cancers; surgical resection followed by radiotherapy and chemotherapy. Currently, the first-line chemotherapeutic approved for use in GBM is Temozolomide (TMZ), although other chemotherapies can be used following disease recurrence, such as Bevacizumab or a combination of procarbazine, lomustine and vincristine (PCV).[1] While patient survival showed some improvement with the introduction of TMZ in 2005, there have been no significant developments in GBM treatment since then and current estimates suggest a 5-year survival rate of just 5.4%.[1–4] Various factors contribute to these poor survival rates; the tumour itself has very diffuse edges (*Fig. 1*), meaning it is not possible to surgically remove the tumour in its entirety without risking the removal of healthy brain tissue. The

brain is also protected by the blood-brain barrier (BBB), which only allows small and lipophilic molecules to pass through without requiring specific transport channels. As a result, the BBB prevents the repurposing of chemotherapeutics with high success rates in other forms of cancer for GBM treatment. Additionally, GBM is a very heterogeneous disease; gene expression, tumour cell origin, and the tumour microenvironment can all contribute to variation within the disease.[5–7]

To analyse GBM heterogeneity, four distinct subclasses of GBM have been identified based on transcriptional profiles; classical, mesenchymal, proneural and neural, each with differing prognoses.[5,6,8,9] Classical GBM is classified by amplification of chromosome 7 and epidermal growth factor receptor (EGFR), and upregulation of neural stem cell and precursor signalling pathways, including Notch and Sonic hedgehog signalling.[5] Unexpectedly, classical GBM shows little to no alteration in TP53 expression, despite TP53 aberrations being the most common genetic mutation in GBM.[5] Mesenchymal GBM upregulates mesenchymal markers and markers associated with the epithelial-to-mesenchymal transition (EMT), and commonly harbours neurofibromin 1 (NF1) and phosphatase and tensin homolog (PTEN) mutations.[5] Increased immune infiltration is also seen in mesenchymal GBM, likely as a result of NF1 downregulation.[10–12] Proneural GBM is associated with high expression of oligodendrocytic development genes, and frequent dysregulation and mutation in TP53, platelet-derived growth factor receptor A (PDGFRA) and isocitrate dehydrogenase 1 (IDH1) genes.[5] Neural GBM is classified by expression of neuronal markers, including NEFL and GABRA2, although does show some increase in oligodendrocyte and astrocytic marker expression.[5] Prognosis is best for proneural GBM as it shows a median survival time of 40 months, whereas mesenchymal GBM has the poorest prognosis with a median survival of only 15 months.[8] One important aspect to consider in regards to these results is that IDH1/2 mutations are often associated with improved survival, mutations that are most commonly associated with proneural GBM.[5,13] Additionally, research suggests that mesenchymal tumours arise from proneural tumours, an event most frequently seen at disease recurrence, which results in treatment resistance and poorer prognoses.[8,14,15]

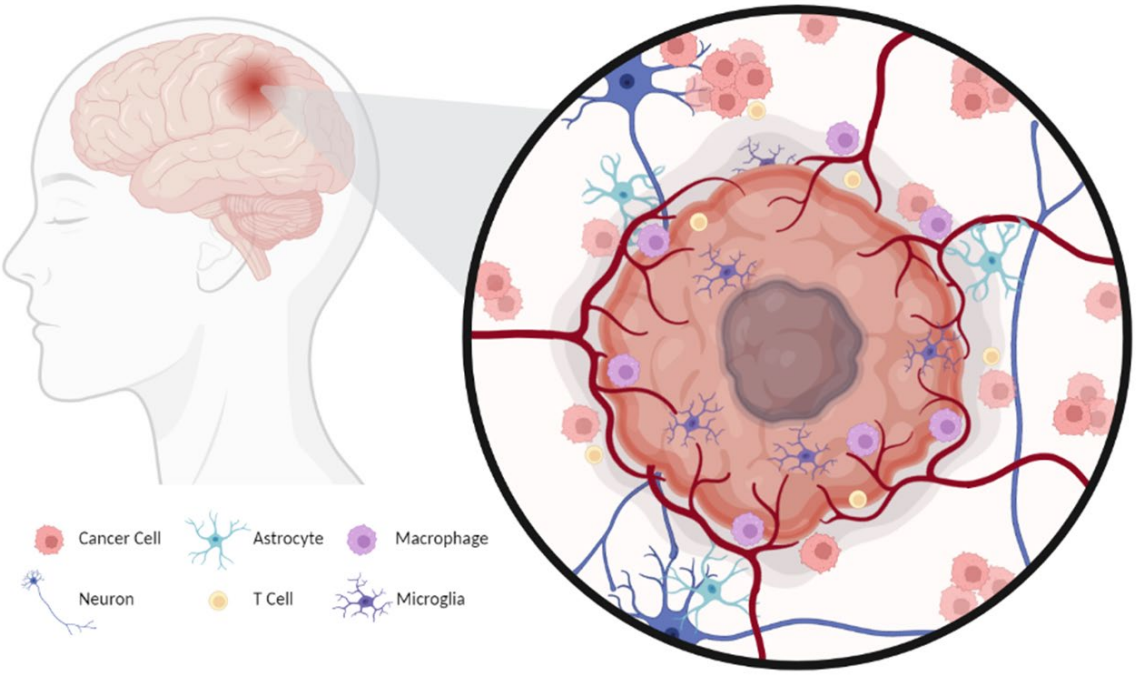


Figure 1. Glioblastoma Tumour Microenvironment. GBM tumours have a very heterogeneous microenvironment. Cancer cells drive recruitment of macrophages, microglia, astrocytes, and T cells to support tumour growth and promote angiogenesis, while inhibiting inflammatory immune responses. Additionally, a hypoxic core develops within the tumour, while cancer cells at the tumour periphery infiltrate into surrounding healthy normoxic tissues, creating diffuse edges to the tumour. *Created with BioRender.com*

As mentioned previously, the immune microenvironment also contributes to tumour heterogeneity, both between GBM subtypes and within a single tumour (*Fig.1*).[5,10–12,16] Given that immune cells can contribute to 30% of tumour mass, and are heavily involved in cancer cell regulation, they are a crucial aspect to study if we are to improve GBM treatment.[17–19]

1.2 Immune Regulation in GBM

The immune cell population in GBM is very heterogeneous, both in terms of different cell populations and expression of traditional inflammatory and anti-inflammatory markers, it is generally agreed that GBM is a 'cold' and immunosuppressive tumour (*Fig. 2*).[20–23] Various factors contribute to the immunosuppressive environment in GBM including the actions of immunosuppressive cytokines released by tumour cells, microglia and tumour-associated macrophages (TAMs).[21] TAMs expressing the cytokine transforming growth factor-beta 1 (TGF-β1) can drive tumour invasiveness, as well as acting alongside IL-10 to downregulate Major histocompatibility complex (MHC) expression in microglia and tumour cells.[24,25] Reductions in MHC expression prevent successful antigen presentation, increase programmed death-ligand 1 (PD-L1) expression to cause immune cell death,

and recruit T regulatory cells (Tregs).[26–29] In addition, the hypoxic tumour microenvironment in GBM drives inflammatory gene expression through activation of the signal transducer and activator of transcription 3 (STAT3) pathway, which influences the activity of a number of different immune cell populations. Overall, immunosuppression maintained by the tumour and innate immune system prevents activation of the adaptive immune system. Increased expression of STAT3 and increased numbers of CD163 positive TAMs have both been linked to poor prognosis in GBM, supporting the hypothesis that immunosuppression leads to poorer disease outcomes.[10,21,22,30,31]

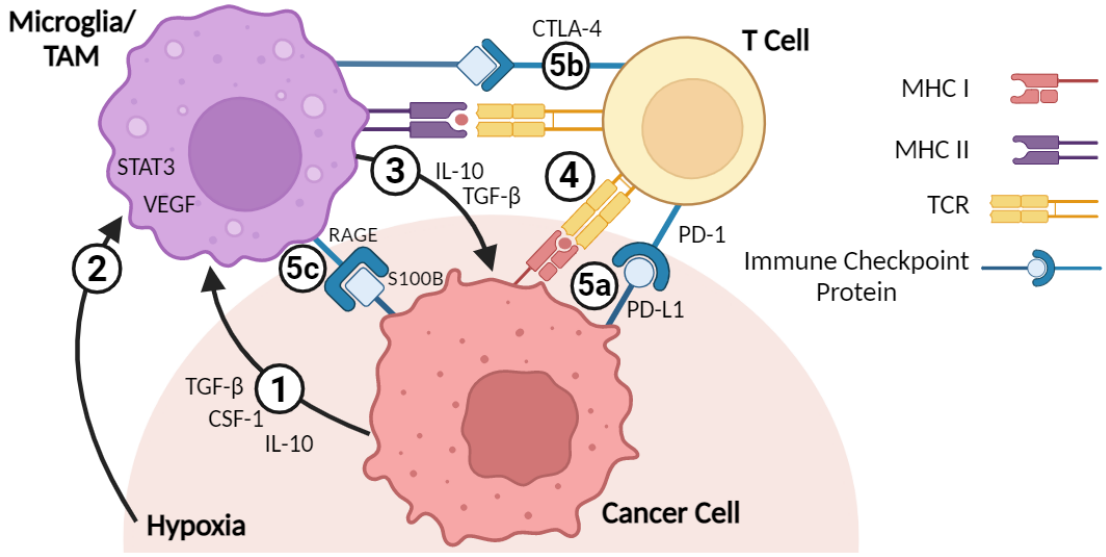


Figure 2. Immune Signalling in Glioblastoma. Highlighted are the key pathways in the crosstalk between cancer cells and the immune system, covered in this review. Cancer cells release chemokine colony stimulating factor 1 (CSF-1) to recruit tumour-associated macrophages (TAMs) and microglia to the tumour site, and anti-inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor-beta (TGF-β) to suppress the immune system once recruited (**1**). Additionally, hypoxia in the tumour microenvironment drives increased vascular endothelial growth factor (VEGF) expression and upregulation of the signal transducer and activator of transcription 3 (STAT3) pathway in microglia and TAMs (**2**). Overall, this drives release of IL-10 and TGF-β from microglia and TAMs (**3**), promoting cancer cell invasiveness and downregulating major histocompatibility complex (MHC) expression, reducing MHC-I/T cell receptor (TCR) interactions and antigen presentation between MHC-II receptors and TCRs (**4**). Immune checkpoint proteins, including programmed death-ligand 1 (PD-L1) (**5a**), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (**5b**) and receptor for advanced glycation end products (RAGE) (**5c**), are also used to suppress the immune system. PD-L1 overexpression by cancer cells drives T helper cell apoptosis and T regulatory cell immunosuppressive functions. Binding between RAGE and S100B inhibits TAM and microglial production of immunostimulatory cytokines, while binding to CLTA-4 on T cells downregulates T cell responses. *Created with BioRender.com*

Nevertheless, despite suggestions that immunotherapy should succeed in treating GBM given the detrimental effects of immunosuppression, no benefits to survival have been observed in recurrent GBM patients.[32] This is potentially due to many cytokines playing dual roles in both inflammation and immune suppression, or because the immune system is so severely inhibited during GBM such that immunotherapies are simply not enough to reverse this.[22] Additionally, excessive immune cell infiltration has also been shown to be detrimental; tumours containing IDH1/2 mutations show reduced recruitment of immune cells and improved survival, while mesenchymal GBM tumours show increased infiltration compared to other disease subtypes and have poorer prognoses.[8,10,33] However, given that mesenchymal tumours also have increased EMT markers, known drivers of tumour invasiveness, this may provide an alternative explanation for poorer outcomes.[5,10] It has been hypothesised that increased immune infiltration increases brain volume, which within an enclosed space like the brain increases pressure to dangerous levels.[22] For immunotherapy to be successful, a balance between driving inflammation and immune cell recruitment must be found. Suggestions have been made that immunotherapy can be tailored to tackle cancers with varying immune statuses – from immunosuppressed to high immune infiltration.[34] For example, inhibition of anti-inflammatory signalling molecules like interleukin 10 (IL-10) and TGF-β is suggested for immunosuppressed tumours.[34]

However, in order to target the immune microenvironment in GBM, treatments must be designed to successfully reach the brain.

1.3 Current Treatment Delivery Routes

Designing drugs to ensure successful delivery to the target site is a complex feat, even without the added challenge in central nervous system (CNS) diseases of crossing the BBB. Ideally, for both patient compliance and economic reasons, drugs are designed to be taken orally (*Fig. 3*) – this enables patients to easily take medication at home without the need for medical professional to administer them. However, oral delivery subjects drugs to hepatic first-pass metabolism, which either limits drug bioavailability, or is exploited for the development of prodrugs that only become active following metabolism. Intravenous delivery enables drugs to avoid first-pass metabolism, potentially simplifying drug design, but often requires professional administration (*Fig. 3*). For both oral and intravenous delivery, drugs must be designed to cross the BBB. Currently, most chemotherapies used in GBM patients are given using these two types of delivery; TMZ, lomustine, and procarbazine are given orally, while Bevacizumab and vincristine are administered intravenously.[2,35]

Interestingly, delivery routes that avoid the BBB are possible. In GBM, given that standard treatment often involves surgical resections, it is possible to implant therapeutics directly within the resection site. One such therapy, Gliadel® wafers, are biodegradable polymeric 14 mm discs loaded with chemotherapeutic carmustine (BCNU), which are able to be implanted directly at the tumour site.[36,37] Unfortunately, as surgical resection is not always possible, implanting therapeutics within the brain cannot be the primary method of drug delivery.[38] Intrathecal delivery involves injection of drugs directly into the cerebrospinal fluid (CSF), a highly invasive procedure currently limited to use for pain management and specific chemotherapies for cancers that metastasise to the CSF.[39] Given the invasiveness of this procedure, it is best to find other alternative drug delivery routes. Intranasal delivery can allow uptake directly into the blood vessels abundant in the nose, but can also bypass the BBB by allowing transport through the olfactory and trigeminal nerves directly to the brain (*Fig. 3*).[40–42] This is a considerably less invasive technique, although often requires additional modifications to improve uptake efficiency, such as the use of ultrasound or additives to increase mucosal membrane permeability.[41–43]

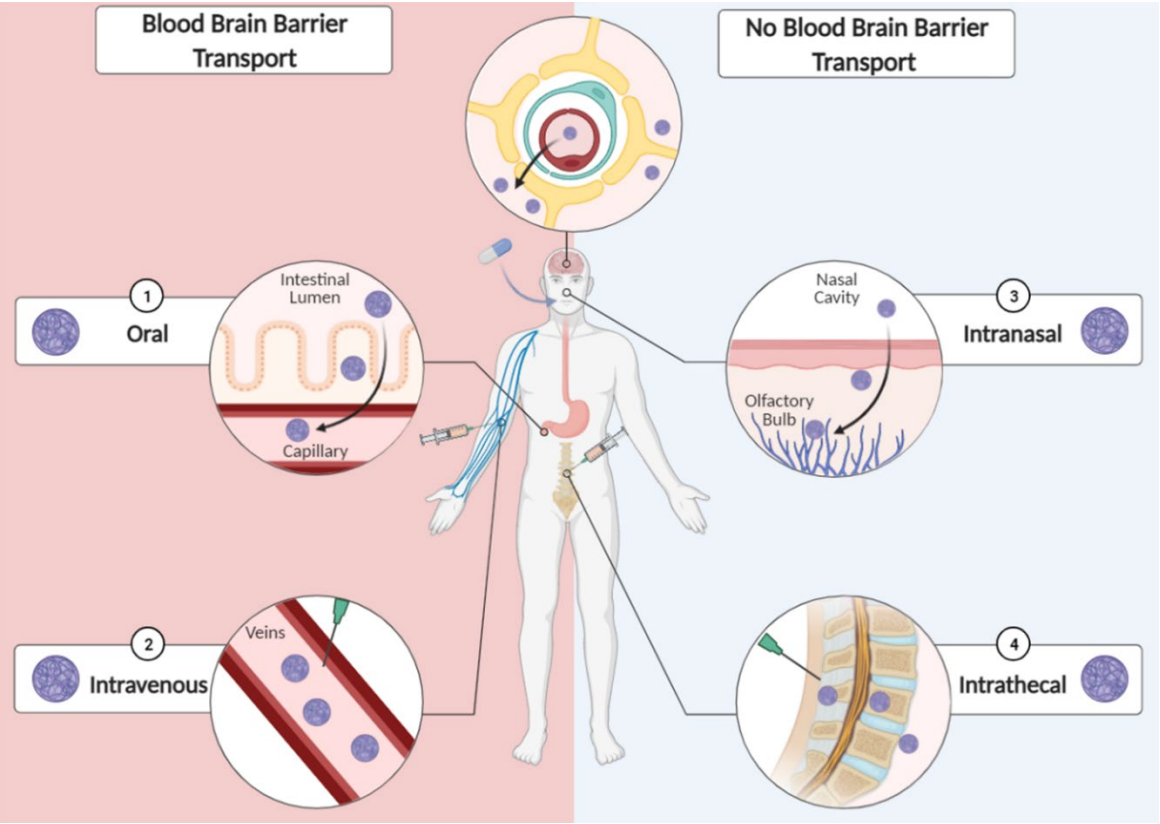


Figure 3. Administration Routes to the Brain. Particle delivery systems can be administered through a variety of routes, depending on their ability to cross the blood brain barrier (BBB). The BBB is formed by pericytes coating the blood vessels in the brain, which are in turn surrounded by astrocyte end feet, preventing molecules from diffusing across the barrier unless small and lipophilic. If particle delivery systems are small and lipophilic, usually between 10-100 nm, or have been functionalised to allow active transport across the BBB, they can be delivered orally (**1**) or intravenously (**2**) and be able to reach the brain. If this is not possible, intranasal delivery (**3**) is an alternative route to reach the brain via the olfactory nerves, avoiding the BBB. For intranasal delivery, particle diameter can be between 100-700 nm. Intrathecal delivery (**4**) involves injection of particles directly into the cerebrospinal fluid in the spinal cord, meaning that particle size and design is not limited, but this is a highly invasive procedure. *Adapted from "Drug Delivery in Rheumatoid Arthritis", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates*

Alternatively, drug administration and targeting can be improved through the development of particle delivery systems, allowing particle modification to improve delivery without the need to alter the drugs themselves.[44]

1.4 Particle Delivery Systems

Generation of nano- and micro-scale particles has been a promising topic of research for many years now. These particles exploit the ability to encapsulate compounds within a protective shell, which is often made of polymeric materials, but lipid membranes such as liposomes, micelles, and extracellular vesicles can also be used (*Fig. 4*).[44] These shells can increase the half-life and bioavailability of compounds, reduce metabolic clearance, and allow surface modifications to improve cell-specific targeting to reduce harmful side effects, without the need to modify the original compound and risk reducing efficacy (*Fig. 4)*.[44,45] Additionally, encapsulation provides a method for the delivery of drugs with limited solubility, which would otherwise have low bioavailability.[44,45] Currently, more than 30 therapies exploiting particle delivery systems have been approved by the Food and Drug Administration (FDA).[45–47]

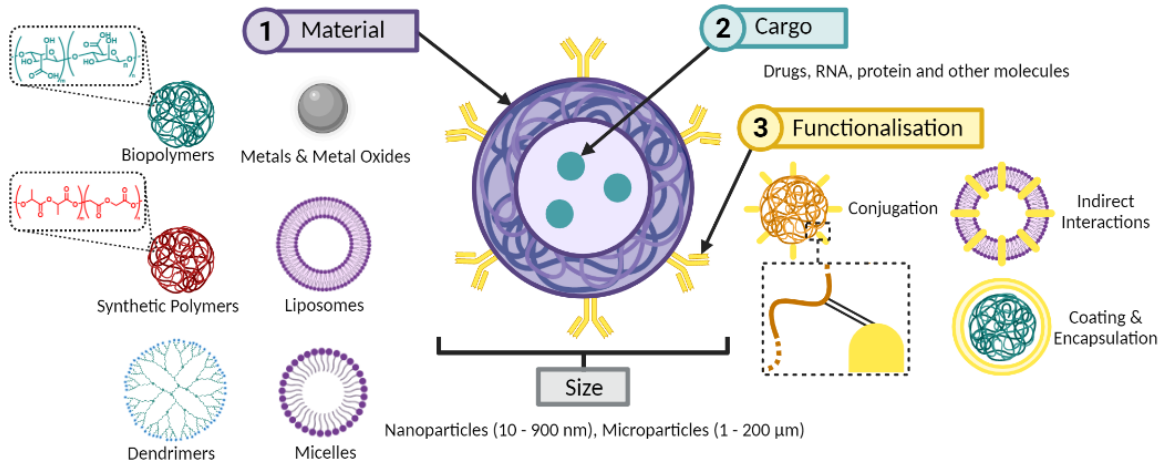


Figure 4. Particle Delivery Systems. Particle delivery systems can be either nanoparticles or microparticles, depending on the size of the system. They are commonly comprised of a polymeric or lipid matrix (**1**), which enables encapsulation of a cargo. Either this can involve direct interaction of the cargo with the matrix, or encapsulation of the cargo within a hollow, or fluid, particle core (**2**). Particle functionalisation involves proteins, sugars, or other molecules being attached to the surface of the particle material (**3**). Attachment can be through direct conjugation between the material and the functionalising molecule, or indirect interactions, such as embedding proteins within a liposome membrane. Particles can also be functionalised by coating the surface in another class of material, or by encapsulating the particle within a membrane. Particles do not have to contain cargo or functionalisation to be used therapeutically, depending on the material used. *Created with BioRender.com*

In terms of GBM, the use of a particle delivery system may provide a potential solution to crossing the BBB without needing to modify existing drugs to accomplish this, or enable drugs to be delivered via alternative routes, such as intranasally (*Fig. 3*). While generation of a successful particle delivery

system remains complex – with some designs unable to cross the BBB, or suffering from low cellular uptake – steps can be taken to improve bioavailability and cell-specific targeting.[48] Altering the size, choice of materials used, surface charge, and functionalising particle surfaces with proteins can improve transport across the BBB (*Fig. 3*).[49] Overall, this generates a highly tuneable system that is considerably easier to create than modifying existing drugs to be able to cross the BBB. Thereby enabling the use of chemotherapeutics that have been successful in other solid tumours, but that have not yet been exploited for GBM due to transport difficulties. Even in terms of the existing GBM chemotherapeutic temozolomide, particle encapsulation has been shown to improve the efficiency of BBB transport as well as reducing toxicity to surrounding tissues.[50] Additionally, particles themselves can even be tailored for use as a therapy, with the emergence of photothermal and magnetic hyperthermia therapies.[51,52]

1.5 Model Systems

In order to investigate particle delivery systems within GBM, different model systems are used with varying advantages and disadvantages.[53,54] Here we summarise the key models referenced in this review. To simulate the bulk of the tumour itself, primary patient-derived cells provide the closest disease model, and while they are sometimes used, established GBM cell lines such as U87 cells are more commonly exploited.[54–56]. *In vitro* assays allow culturing of particles with cells or spheroids and are ideal for investigating the initial stages of particle delivery and cellular effects; toxicity, particle uptake, and cargo release and effects. More complex *in vitro* systems have also been developed, such as *in vitro* BBB models.[57] *In vivo* models include genetically engineered mice in which oncogene overexpression or loss of tumour suppressors are used to drive GBM development, but xenograft models using GBM cell lines are often favoured, predominantly using mouse models, although zebrafish models are becoming more popular.[53,54,58–61] Ideally, an orthotopic xenograft model is best to recreate GBM; cancer cells are implanted in the native tumour tissue, in this case the brain. However, xenograft models using human cell lines require the use of immuno-compromised mice, and for studies investigating the immune microenvironment it is necessary to use mouse-derived GBM models where the tumour cells can be implanted into syngeneic hosts. This ensures the tumour and immune microenvironment mimic the actual disease as closely as possible, as well as enabling testing of particle delivery systems' ability to cross the BBB. Alternatively, subcutaneous allografts are also used, but while these enable investigation of immune responses, they do not resemble GBM tumours as closely.[62] Delivery of particle-based therapies to *in vivo* models is similar to delivery to patients, with intravenous delivery being the predominant choice (*Fig. 4*). Intranasal delivery is an interesting

approach to deliver particles without the need to cross the BBB, which is directly translatable to patient treatment. On the other hand, intracranial or intratumoral injections are also used, allowing investigation of the particles in the tumour environment with an immune system.

Previous reviews have focused on the role of nanoparticles in visualising GBM, the use of immunotherapy and nanoparticles as separate methods for treating GBM, and the role novel particle design in immunotherapy without the ability to cross the BBB.[63–65] This review investigates recent literature from the past 3 years combining particle technologies and immunotherapy together to create BBB-permeable systems for GBM immune modulation. Key sections will focus on different types of immunotherapy, including checkpoint inhibitors, immune signalling pathways, and strategies for using heat to drive immune responses. Finally, some of the more novel approaches to driving the immune system towards GBM tumour clearance will be considered.

Particle Delivery System Material	Immune Checkpoint Inhibitors	Cytokines and Cell Signalling	Photodynamic Therapy	Magnetic Hyperthermia	Anti-Inflammatory	Novel Approaches
Polymeric	(Galstyan et al. 2019)	(F. Zhang et al. 2019; Gregory et al. 2020; Kadiyala et al. 2021)			(I. Zhang et al. 2020; Karakaş et al. 2019)	(Qiao et al. 2021)
Dendrimers		(Sharma et al. 2020)				
Proteins		(Rizzuto et al. 2021; Voth et al. 2020)				
Liposomes/Micelles	(P. Zhang et al. 2019)	(Azambuja et al. 2020)	(Shibata et al. 2019)			(Sunil et al. 2021; Pinton et al. 2020)
Nucleic Acids		(Gao et al. 2021)				
Gold			(Liu et al. 2019)			(Saxena et al. 2019)
Metal Oxides	(Meng et al. 2021)			(Grauer et al. 2019; Persano et al. 2021; Chauhan et al. 2021)		
Other		(Bielecki et al. 2021; Turan et al. 2019)	(M. Zhang et al. 2021)			

Table 1. Recent literature on GBM immune microenvironment-modulating particle delivery systems.

2.1 Immune Checkpoint Inhibitors

A promising therapy for many cancers are immune checkpoint inhibitors (ICIs). The immune system is naturally regulated by set checkpoints to ensure that T cells do not attack the body's own cells. If a T cell binds to an immune checkpoint protein on another cell, this turns off signals in the T cell to attack it. Unfortunately, cancers hijack this mechanism by highly upregulating immune checkpoint proteins, such as PD-L1 and CTLA-4, inhibiting clearance by the immune system (*Fig. 2*). ICIs are designed to overcome this by preventing binding between immune checkpoint proteins during cell-cell interactions, enabling immune activation and clearance.[66] Currently, the only FDA approved ICIs are antibodies against CTLA-4, PD-1 or PD-L1.[67]

ICIs have been trialled for GBM, however success has been severely limited, especially in trials using monotherapies as opposed to combination therapy.[68,69] One possible explanation is that GBM patients express high levels of checkpoint proteins alongside depletion of tumour infiltrating lymphocytes – causing a degree of immunosuppression that cannot be overcome through conventional checkpoint inhibitor therapies.[69,70] However, the high levels of checkpoint protein expression in GBM suggest that if immunosuppression is overcome, ICIs have the potential to be successful.

Through conjugation of nanoparticles to immune checkpoint inhibitors, three main benefits can be achieved; improved delivery of ICIs to the brain, targeted delivery of nanoparticles to the tumour, and increased localised concentration of ICIs. Improved delivery of ICIs is observed when conjugated to a poly(β-malic acid) nanoparticle, or when un-conjugated but injected alongside nanoparticles able to disrupt the BBB and increase accumulation at the tumour site.[71,72] Targeting of the particles themselves can also be improved through conjugation to ICIs, given that GBM tumours express high levels of checkpoint proteins, enabling delivery of additional therapeutics alongside ICIs within a single particle. One study trialled this with a lipid nanoparticle functionalised with PD-L1 antibodies encapsulating the cyclin-dependent kinase (CDK) inhibitor dinaciclib, ensuring the cargo was only delivered to immunosuppressive cells to alleviate the immunosuppression.[73] Unfortunately, this therapy was not shown to cross the BBB, so it is unclear how much PD-L1 functionalisation is able to improve nanoparticle targeting.[73] Finally, as each of these ICI-focused studies gave a reduction in tumour volume and induction of an anti-tumour immune response, it is possible that the improved accumulation of ICIs in the brain was enough to overcome the immunosuppression, unlike in patient trials where ICIs are not used in combination with particle delivery systems.[71–73]

ICIs are a promising avenue for stimulating the immune system to treat cancer and have shown high success rates in certain solid tumours.[74] However, they carry the risk of over-activating the immune

system and driving immune-related adverse events (irAEs) that manifest as autoimmune disorders, including colitis, encephalitis, myocarditis and even the development of type I diabetes.[66,75] The severity of autoimmune reactions varies between both patients and the type of ICI given, with approximately 10-20% of all patients given ICIs experiencing severe high grade irAEs. Additionally, roughly 40% of patients developed chronic irAEs, regardless of initial irAE severity, following anti-PD-1 therapy for melanoma.[76] The use of particle delivery systems to ensure selective ICI targeting may reduce the overall risk of developing autoimmunity, however, if used for the treatment of GBM this would not remove the risk of neurological irAEs.

2.2 Cytokines and Cell Signalling

2.2.1 *General Inflammation Pathways*

One clear method to drive inflammation is to target existing signalling pathways, using particle therapies to deliver proteins and antibodies to improve their bioavailability and stability. This can involve antibodies that specifically bind to tumour cells without checkpoint inhibition; such as using anti-EGFR antibodies to bind cancer cells to drive antibody-dependent cell-mediated cytotoxicity (ADCC).[57] Rizzuto *et al.* have shown that mAb-conjugated ferritin nanoparticles can cross an *in vitro* model of the BBB, but this delivery system has not yet been trialled in an *in vivo* model with a functional immune system to confirm whether ADCC is possible in an immunosuppressed GBM model.[57] Alternatively, simple delivery of cytokines can also be used to activate inflammation, such as delivery of chemokine CCL21 to recruit lymphocytes and dendritic cells to the tumour site.[62] However, delivery of cytokines relies on being able to ensure activity is limited to the target site – meaning therapies either need to be directly administered to the tumour, or particles need to be designed to encapsulate the protein cargo and only release it once within the tumour. Voth *et al.* demonstrated that it is possible to encapsulate CCL21 within a vault-protein nanoparticle (*Fig. 5*), however cytokine release was not designed to be limited to the tumour site, leading to the use of intratumoral injection.[62] Given the location of GBM, direct administration is potentially a highly invasive process, and systemic delivery risks off-target inflammation, thus alternative approaches are more commonly favoured.

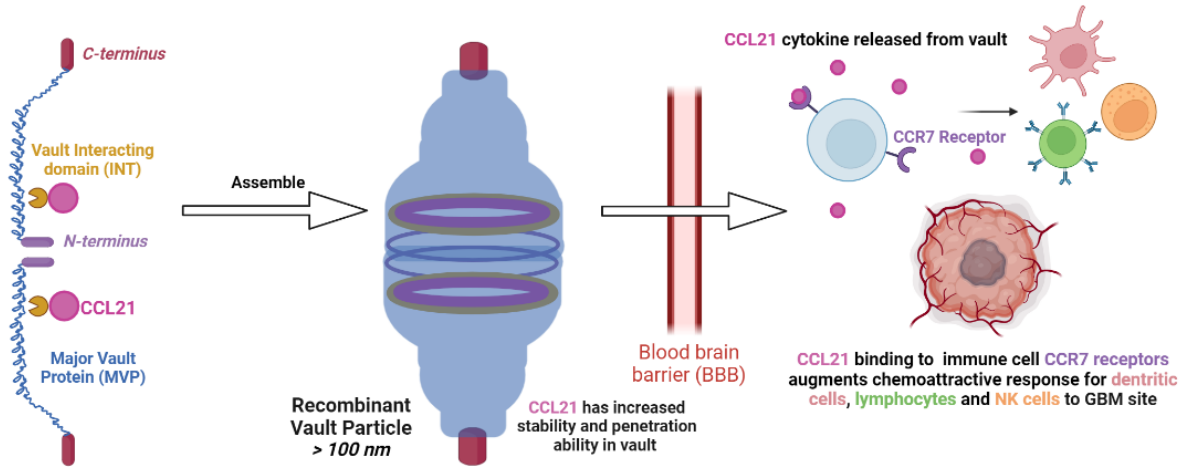


Figure 5. Vault Particle Protein delivery of CCL21 cytokine. Assembly of Vault Protein from multiple major vault proteins (MVP) encapsulating cytokine CCL21 conjugated to vault interacting domain (INT), stable transportation and release of CCL21 at higher concentration gradients at GBM site. CCL21 interaction with CCR7 receptor induces chemoattractive response for dendritic cells, lymphocytes, and NK cells. Created with BioRender.com.

Therapeutic delivery of nanoparticles encapsulating RNA is a common solution to activating inflammation without the need for bulky proteins, while providing a protective coating to the nucleic acids to prevent clearance before reaching the target cells (*Fig. 6*). Nanoparticles are commonly made using cationic materials such as poly(β-amino ester) (PbAE) to better incorporate the negatively charged RNA. PbAE encapsulation reduces leakage, as the cargo is released upon nanoparticle degradation following endocytosis within the target cell itself (*Fig. 7*).[77,78] The RNA delivered can vary between simple mRNAs for transcription factors that promote inflammatory gene expression, or short interfering RNA (siRNA) to downregulate anti-inflammatory signalling and alleviate immunosuppression.[77–79]

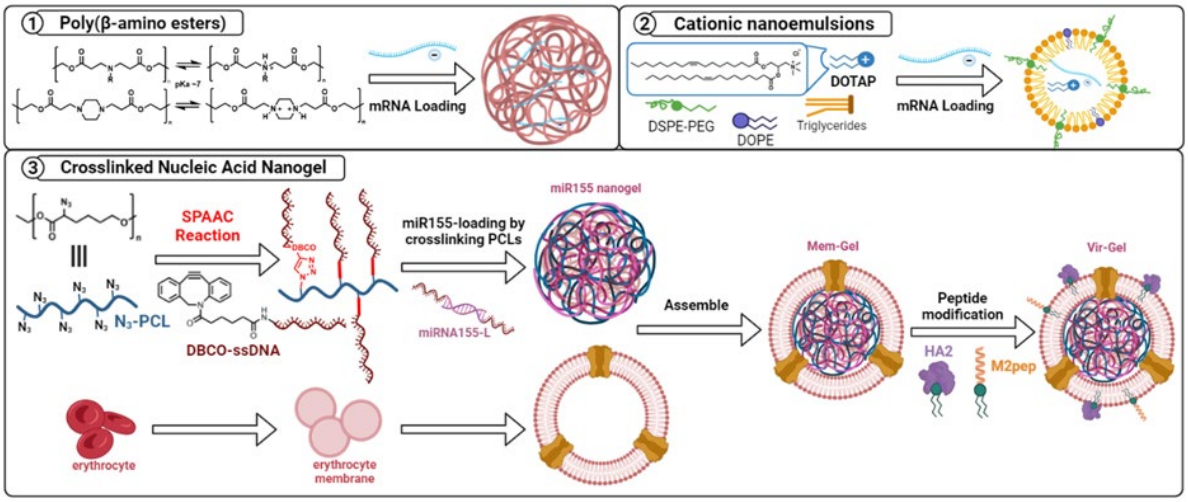


Figure 6. RNA Delivery systems. Nanoparticle delivery vehicles for the transportation of RNA to a desired target. Nanoparticles composed of **Poly (β-amino esters)** (**1**) contain a tertiary amine with pKa ~ 7 that can be protonated under physiological conditions; these particles can accommodate negatively charged mRNA until endocytosis into the target cell. **Cationic nanoemulsions** (**2**) composed of: DSPE-PEG, DOPE, and triglycerides form nanoparticles and in the presence of amphiphilic positively charged tertiary amines attract and hold negatively charged mRNA until release at a target site. Azide (N_3) containing polycaprolactone (PCL) conjugated to short ssDNA via SPAAC click chemistry gives a **cross-linked nucleic acid nanogel** (**3**) which assembles in the presence of miRNA155-L containing end-caps complementary to the PCL ssDNA. The gel can be encapsulated within an erythrocyte membrane and modified with HA2 surface protein to enhance endosomal escape and M2pep for specific targeting of microglia. Created with BioRender.com

One key consideration for intracellular cargo delivery (*Fig. 7*) is to ensure that the cargo is not degraded through the endosome-lysosome pathway following particle uptake. A solution to this is to modify the surface of particles to aid endosomal escape.[79–81] One study has approached this by taking advantage of viral mechanisms for endosomal escape to create a highly specialised nanoparticle.[79] In this study (*Fig. 6*), miRNA was cross-linked with a complementary DNA-grafted polycaprolactone brush to create a nanogel particle coated by an erythrocyte membrane to improve circulation time, with influenza virus protein HA2 and microglial targeting peptide M2pep conjugated to the surface.[79] HA2 enables endosomal escape while M2pep ensures the particles are only targeted toward and engulfed by microglial cells, ensuring specific cargo delivery. An alternative system developed by the Karathanasis lab uses silica nanoparticles functionalised with primary and secondary amines to drive endosomal escape.[80,81] However, rather than using RNA to drive inflammation, cyclic diguanylate monophosphate (cdGMP) is delivered to activate stimulator of interferon genes (STING), a protein normally responsible for responding to infection by intracellular pathogens and capable of activating a strong immune response.[80]

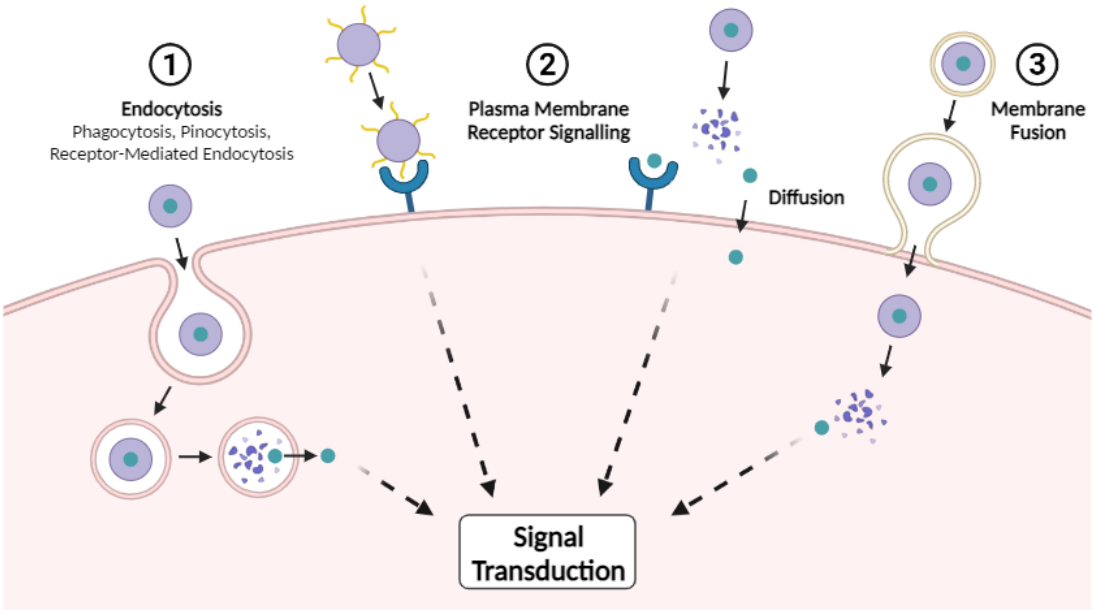


Figure 7. Cellular Uptake of Particle Delivery Systems. To exert a cellular effect, particle delivery systems can operate in a variety of ways. Endocytosis of particles (**1**) enables delivery of cargo intracellularly, but risks the cargo being degraded in the endosomal-lysosomal pathway unless able to escape the endosome. If the particle delivery system is designed to result in interactions with plasma membrane receptors (**2**), this can be done through surface functionalisation with the corresponding ligand, or through lysis of the particle in the extracellular space to release cargo able to bind the target receptor. Alternatively, the delivery system may degrade and release a molecule able to diffuse across the plasma membrane to reach an intracellular target. Finally, functionalisation of a particle by containing it within a cell membrane (**3**) could allow particle delivery through membrane fusions, allowing the particle material or any cargo to impact signalling from within the cell. *Created with BioRender.com*

Broad activation of common inflammatory pathways can be useful to overcome immunosuppression; however, excessive inflammation is also detrimental. Damage of healthy tissues, chronic inflammation, and even increased infiltration of immune cells within a confined space like the skull could be dangerous. Considering the unique immune microenvironment present in GBM, targeted inhibition of immune pathways known to be upregulated in GBM may be a more suitable approach.

2.2.2 Glioblastoma-Specific Signalling

A variety of immune regulatory pathways are overexpressed in GBM, and have been reviewed in various publications.[82–85] These pathways provide interesting therapeutic targets for potentially

driving tumour clearance. Here we discuss some of the pathways targeted through the use of particle delivery systems.

STAT3 is upregulated in many cancers, and has been known for some time to be overexpressed in GBM patients, with increased levels of activated STAT3 correlating with poorer prognoses.[86] Given that STAT3 is involved in many pro-tumoral functions; including angiogenesis, proliferation, invasion, metastasis, and immune suppression; this makes it an ideal target for anti-cancer therapies.[87] Small molecule inhibitors have been trialled in intracranial animal models of GBM, however studies were either unsuccessful due to poor molecule permeability, or were promising but relied on direct intracranial injections for successful drug delivery, an unfavourable route for clinical therapeutics.[88,89]. Hence, the Castro lab have developed a nanoparticle to improve delivery of STAT3 siRNA to downregulate STAT3 signalling and drive tumour clearance.[90,91] A synthetic protein nanoparticle, made of human serum albumin (HSA) and oligo(ethylene glycol) loaded with STAT3 siRNA and cell-penetrating peptide iRGD, was shown to be: capable of crossing the BBB; infiltrating the tumour; inhibiting tumour growth; and significantly improving survival.[90] This highlights how promising STAT3 therapies could be for GBM when particle delivery systems are employed to improve BBB permeability.

Long non-coding RNA (lncRNA) LSINCT5 expression has also been observed in other cancers, with higher expression levels correlating to reduced survival rates.[92] LSINCT5 has been hypothesised to function by sequestering miRNA molecules, and has been linked to repression of miRNA-451 expression in glioma cells, which can increase tumour cell survival and migration.[93–95] From this, Jin *et al*. hypothesised that delivering siRNA against LSINCT5 (siLSINCT5) within a poly(amidoamine) (PAMAM) dendrimer nanoparticle would exhibit anti-tumour properties.[96] Particles were also conjugated to anti-NKG2A antibodies (aNKG2A) - another immune checkpoint protein - to counteract the immunosuppressive microenvironment, as well as cell-penetrating peptide tLyp-1 to improve tumour-specific targeting.[96] Particles were able to cross the BBB, inhibit LSINCT5 expression, and drive an anti-tumour immune response. However, no comparison was made between the effects of aNKG2A-tLyp-1 nanoparticles and aNKG2A-tLyp-1-siLSINCT5 nanoparticles, preventing conclusions as to whether the successes seen are due to the combination therapy, or predominantly reliant on the presence of NKG2A antibodies.[96]

Defects in mitochondrial function are found across several diseases, including cancers, and can lead to alterations in metabolism, proliferation, apoptosis, and even immune responses.[97,98] Furthermore, it has been observed that the activation states of TAMs and microglia are regulated by mitochondrial metabolism; high expression levels of the mitochondrial transport protein (TSPO) are

observed in TAMs and are linked to poorer prognoses in GBM patients.[99–101] However, it is still debated as to what specific role TSPO plays in immune regulation, with conflicting evidence as to whether TSPO promotes a pro- or anti- inflammatory phenotype in TAMs, overall indicating a complex relationship between TSPO and immune regulation.[99] Nevertheless, as TSPO expression is known to result in poorer GBM prognoses this makes it an attractive target for therapies.[101] In one study, Sharma *et al*. developed a PAMAM dendrimer nanoparticle conjugated to TSPO ligand DPA, improving the normally poor delivery of mitochondrial-targeting compounds.[102,103] It was shown that DPA delivery upregulated an anti-tumour response, with specific targeting towards TAMs in an *in vivo* orthotopic model of GBM.[102] TAM-specific targeting was achieved through the use of the PAMAM dendrimer, with this and previous studies indicating that PAMAM nanoparticles can be used to ensure TAM-specific drug delivery.[102,104,105] This research highlights that even without full understanding of GBM-associated immune regulators like TSPO, it is possible to target them to drive an anti-tumour response.[102]

Stimulation of general inflammation and GBM-associated pathways provides the opportunity to drive a robust immune response, while avoiding the risks of stimulating autoimmunity through the removal of immune checkpoints like ICIs. However, care will still need to be taken to ensure excessive inflammation is limited, and that inflammation is limited to the target site. GBM specific approaches, which often rely on downregulating anti-inflammatory pathways rather than upregulating inflammation, may provide more control over other general approaches. Additionally, by pairing immune manipulation with particle delivery systems, the risks of off-target inflammation can be limited, creating a promising avenue for reversing GBM immunosuppression.

2.3 Hyperthermal Therapy

Hyperthermal therapy is the process of heating a tissue to 40-45 °C to induce cell death, as well as promoting pro-inflammatory immune responses and sensitisation towards chemo- and radio-therapy.[106,107] This process is largely thought to be driven by heat shock factor 1 (HSF-1), a temperature-sensitive transcription factor that regulates expression of various cytokines and other heat shock proteins.[107,108] The exact mechanisms surrounding heat-related immune activation are not fully understood, but research has shown that temperatures from 38-45°C alter immune cells to increase infiltration and cytokine release, drive antigen presenting cell maturation towards inflammatory phenotypes, and increase CD4+ T cell differentiation.[106,107] Alongside changes to immune cells, hyperthermal therapy also affects cancer cells; temperatures above 41°C drive apoptosis, and even necrosis, due to increased cellular stress, reduced DNA damage repair and

replication, and promotion of 'eat me' signals.[106] Overall, this increases immune-mediated tumour clearance via upregulation of inflammation alongside increased exposure of tumour antigens due to cancer cell death.[106–108]

Hyperthermal therapy has been considered as a potential treatment for cancer for many years, with a noticeable spike in research reported in PubMed between the mid-80s to early 90s. However, at the time this approach was limited by ongoing issues that were not overcome, including: invasive monitoring; difficulties heating tumours deeper within the body; and a lack of tumour-specific targeting.[109,110] Questions have also been raised since as to the rationale behind hyperthermal therapy, and the validity of clinical trials performed at the time.[111] Nevertheless, recent advancements in particle therapies have led to improvements in the field of hyperthermia, as well as the development of two branches of hyperthermal therapy; photodynamic and photothermal therapy, and magnetic hyperthermia.[112–114]

2.3.1 Photodynamic and Photothermal Therapy

Photodynamic and photothermal therapies rely on molecules that can be photoactivated by a specific band of light, usually a near infrared (IR) light source, to specifically target abnormal cells (*Fig. 8*). Photodynamic therapy involves photosensitiser drugs which generate reactive oxygen species (ROS) when exposed to near IR light through a series of photochemical reactions, resulting in oxidative stress in cancer cells.[115] Similarly, in photothermal therapy, a photothermal agent converts the near IR light source to vibrational energy and generate heat leading to apoptosis in target cells at the site of interest.[115] Together, these effects drive tumour cell death and acute inflammation, an appealing outcome for the treatment of glioblastoma.[116,117] In addition to this, near infrared light appears able to penetrate through intact skin and skull of mice and humans, suggesting that it has potential for use in glioblastoma treatment.[118,119] Delivery of photosensitive drugs conjugated to nanoparticles, and even the creation of photosensitive nanoparticles, are an attractive approach to ensure targeted delivery. Recent research has shown that indocyanine green conjugated to liposomal nanoparticles can accumulate in tumours and drive a strong immune response by inducing heat-shock protein 70 (HSP70).[120] In terms of photosensitive nanoparticles, a collaboration between Danish and Chinese researchers has developed bradykinin-conjugated aggregation-induced-emission (AIE) luminogen nanoparticles; using bradykinin to improve tumour permeation, while AIE-active luminogens form the bulk of the particle and act as a photo-inducible agent able to drive ROS production.[121] These studies show promising results and not just in the resulting immune responses; both nanoparticle systems were delivered intravenously to an orthotopic glioma model, highlighting the ability of the nanoparticles to cross the BBB and localise in the tumour.[120,121]

Researchers at Duke University, USA have developed a photothermal nanotherapy using spiked gold 'nanostar' particles.[122] Unlike traditional photodynamic therapies that rely on the production of reactive oxygen species (ROS) and heat, these gold nanoparticles only produce heat, but are delivered alongside anti-PD-L1 antibodies to enable a stronger immune response. Interestingly, following hyperthermal treatment and long-term survival, mice rechallenged with tumour cells displayed immunological memory.[122] Unfortunately, this was trialled in a subcutaneous glioma model, so it is unclear if the gold nanoparticles and antibodies can cross the BBB, and whether they would specifically accumulate at the tumour site.[122]

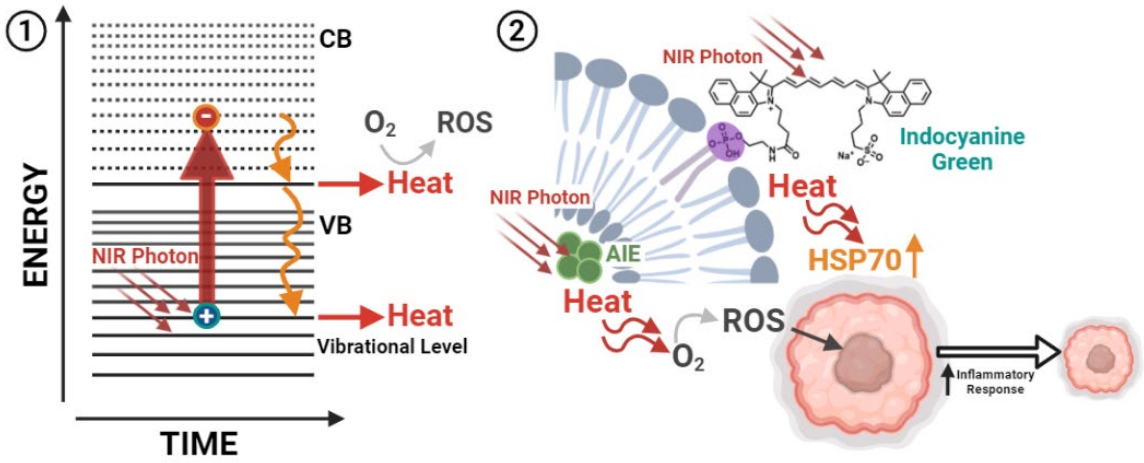


Figure 8. Photothermal effect. (**1**) Jablonski diagram illustrating the photothermal effect. NIR photon excites an energy transition of electrons in the valence band (VB) to an excited vibrational level in the conductive band (CB), leaving behind positively charged "holes". Shortly after, electrostatic interactions between excited electrons and "holes" recombine, releasing the absorbed energy as lattice vibrations and heat. The localised heat energy produced can then cause thermal degradation with oxygen to produce ROS. (**2**) Depiction of photothermally active molecule indocyanine green undergoing photothermal therapy and photosensitiser aggregation-induced emission (AIE) molecules in photodynamic therapy absorbing NIR light and producing heat and ROS to increase inflammatory response in GBM to drive tumour regression. Created with BioRender.com

2.3.2 Magnetic Hyperthermia

Magnetic hyperthermia relies on the use of magnetic particles (often made of superparamagnetic iron oxide), which produce heat when exposed to an alternating magnetic field, killing tumour cells and driving a localised immune response.[112,114] Beneficially for glioblastoma, alternating magnetic fields are deeply penetrating, and can non-invasively drive magnetic hyperthermia in brain tumours.[51]

Recent research has shown that it is possible to drive an inflammatory immune response through magnetic hyperthermia; however, these studies have used either *in vitro* models, which do not replicate the complexity of the tumour microenvironment, or non-orthotopic *in vivo* models, making it unclear whether particles could successfully cross the BBB.[55,123] Nevertheless, it is possible that not all particles need to be able to cross the BBB. In the case of magnetic hyperthermia, metal nanoparticles are difficult to clear from the body and cannot biodegrade like other nanoparticle materials – but they can perform multiple rounds of therapy if they are embedded at the tumour site.[123,124] As standard GBM treatment already involves initial surgical resection, long-term nanoparticle therapies could also be implanted at this stage.

Promisingly, a small pre-trial has been conducted in six recurrent glioblastoma patients, whereby superparamagnetic iron oxide nanoparticles were implanted directly into the tumour resection site before treatment with an alternating magnetic field.[124] The therapy induced a strong pro-inflammatory immune response across all patients, with increased T cell and macrophage infiltration, cytokine expression, and tumour necrosis.[124] However, cerebral oedema formed around the nanoparticles following treatment, with two-thirds of patients requiring surgical removal of the particles. Additionally, while median overall survival for patients treated at their first recurrence was 23.9 months, medial overall survival of the cohort was only 8 months.[124] Significant inflammation to the degree observed here may prove to be too unregulated to clear tumours safely, potentially highlighting the need for more controlled induction of inflammation for GBM treatment.

Hyperthermal therapy enables a strong immune response, and the ability to ensure localised treatment through a two-point system of particle localisation and targeted irradiation. Some issues remain for the development of thermo-stable materials for photodynamic and photothermal therapy, and the degree of heat and immune activation suitable to drive inflammation without significant side effects.[115,124]

2.4 Anti-inflammatory Approaches

Interestingly, approaches to reduce inflammation are also being trialled in GBM.[125,126] GBM is largely agreed to be immunosuppressive, however several factors suggest that simply trying to increase inflammation may not be the correct approach.[20–23] Variation in immune microenvironments between GBM subtypes, and even down to individual mutations, suggest that more immunosuppressed tumours show improved survival.[5,8,10,33] In order to reduce inflammation, rather than through the use of existing drugs, recent research has focused on the

construction of nano- and micro-particles that have anti-inflammatory properties; either algal extracts or dendritic polyglycerol sulfate (dPGS) formed into micro- and nano-particles.[125,126] While both approaches showed reductions in tumour growth in cancer cell culture models, this may be due to additional functions of algal extracts and dPGS beyond reducing inflammation, such as anti-proliferative effects.[125,126] Additionally, neither approach has been trialled in an *in vivo* model with a functional immune system, which may respond differently to anti-inflammatory signalling than GBM cancer cells themselves.[125,126]

Some debate remains on the effects of immune suppression versus activation in GBM; immune mediators with traditionally inflammatory functions and others with both inflammatory and anti-inflammatory roles can be seen in GBM.[82–84] Additionally, mesenchymal GBM has increased immune infiltration and poorer prognoses, while tumours with IDH1 mutations show reduced immune infiltration and improved prognoses.[8,10,11,33] Nevertheless, anti-inflammatory treatment approaches are unlikely to be the best solution. GBM cancer cells purposefully drive immunosuppression to enable immune escape and tumour survival, while immune cell recruitment signalling pathways - driven by traditionally 'inflammatory' cytokines and chemokines – increase the number of anti-inflammatory cells, such as Tregs and myeloid-derived precursor cells.[127,128] Furthering immunosuppression is likely to be more beneficial to the tumour in the long term, unless careful therapeutic design enables selective inhibition of detrimental inflammatory pathways, such as angiogenesis and increased cellular invasion, without promoting further immunosuppression.[83,84]

3. Novel Approaches (Case Studies)

Finally, several novel approaches to tackling specific challenges in treating GBM through immune-mediated means have been conducted (*Fig. 9*).

3.1 Hypoxia and Antigen Presentation

A key aspect of solid tumours is the presence of hypoxia, which in the case of GBM also contributes to polarising TAMs towards immune-suppressive M2 phenotypes.[30] Additionally, hypoxia can limit the effects of certain therapies, such as photodynamic therapy, due to their reliance on the presence of oxygen to generate ROS.[116,117,129] A novel approach to solve these problems is the development of light-responsive antigen-capturing oxygen generators (LAGs), used to form micelles loaded with the anti-cancer drug Nutlin-3a.[129] Together, this particle delivery system is able to: drive oxygen production from hydrogen peroxide present in hypoxic environments, reversing hypoxia; release an

anti-cancer drug in response to light, driving cancer cell death; and capture antigens released by dying cancer cells, promoting antigen presentation (*Fig. 9a*).[129]

This therapy poses an interesting and multi-faceted approach to driving tumour clearance; however, there are some challenges if this is to become a successful clinical therapy. In this paper, the LAG micelles have only been trialled in a spheroid model of GBM that avoids two key issues: BBB transport and light depth penetration.[129] As the delivery system is a 100-150 nm diameter micelle, it is likely that it would successfully reach the brain via an intranasal delivery route, though may require additional surface modification to improve delivery that would further complicate particle design.[129–131] LAGs are currently activated using a 630 nm wavelength, which has some considerable variation in reported depth penetration through cranial tissue ranging from 0.4-3.0 cm.[132–134] For the treatment of GBM, it is unlikely that this degree of depth penetration would be sufficient to reach the tumour mass, suggesting that surgical implantation of fibre optics during tumour resection may be required to achieve LAG activation. Intracranial implantation of fibre optics is an incredibly rare procedure in humans, and is instead limited to *in vivo* murine models.[135,136] To avoid this, if the particle delivery system was modifiable to respond to alternative wavelengths of light – although debate remains on which wavelength of light has the highest depth penetration through cranial tissue – it may be possible to drive LAG activation without invasive surgeries.[137,138]

3.2 Macrophage-Specific Targeting

As discussed in Section 1.2, TAMs are crucial to tumour microenvironment regulation and can comprise up to 30% of the tumour volume.[17–19,21] This makes them a promising target for anti-GBM particle therapies, but requires some additional modification of particle delivery systems to ensure TAM- and microglial-specific targeting. One approach investigated the effect of lipid nanoparticle size and surface charge on uptake into tumour cells, TAMs and microglia, and T cells.[139] It was observed that particles with a positive surface charge and 100 nm diameter showed the greatest uptake in macrophages.[139] Promisingly for translation to clinic, while small, positively charged particles may struggle to cross the BBB, the added positive charge will not hinder intranasal uptake.[139,140] Another approach has been to modify dendrimer-based nanoparticles with the addition of sugar moieties to increase cellular uptake, taking advantage of the increased expression of sugar transporters on TAMs and microglia during GBM.[141] Conjugation of glucose moieties onto dendrimers was found to be the most successful approach to increase uptake of nanoparticles into TAMs and microglia, while conjugation with galactose drove increased tumour cell uptake (*Fig. 9b*).[141] Together, these approaches highlight the modifiable nature of particle delivery systems to enable immune cell-specific targeting in GBM. Further research can now focus on screening different

immune-stimulating compounds to use alongside these particle delivery systems for TAM-specific targeting in GBM treatment.

3.3 Targeting TMZ-Resistant Cells

One major issue in treating GBM is chemotherapeutic resistance, where expression of DNA repair protein O^6-methylguanine DNA methyltransferase (MGMT) is highly upregulated following repeated courses of TMZ, leading to resistance.[142] This has led to one recent paper using a particle delivery system to overcome TMZ resistance through targeted Zoledronate (ZOL) delivery (*Fig. 9c*).[143] ZOL has a different mechanism of action compared to TMZ and inhibits the post-translational modification of proteins, enabling ZOL to effectively stimulate apoptosis in MGMT-overexpressing cells.[144,145] Additionally, ZOL shows increased sensitivity and toxicity towards macrophages, which Qiao *et al.* hypothesised would enable clearance of tumour cells and TAMs, alleviating immunosuppression.[143,146] Poly(propylene glycol dithiodipropionate) nanoparticles were developed to encapsulate ZOL, which were coated in the cell membrane of BV2 microglial cells.[143] Microglial cell membrane coating facilitated nanoparticle recruitment via chemoattractants CX3CL1 and CSF-1, factors abundant in GBM tumours.[84,143] Once recruited to the tumour, the nanoparticles release ZOL in the high glutathione environment, due to the nanoparticle polymer composition.[143] Overall, it was found that nanoparticles were actively recruited to the tumour, and resulted in apoptosis of GBM cells as well as TAMs, increasing the proportion of pro-inflammatory M1 phenotype TAMs.[143] This therapy is a promising avenue for treating TMZ-resistant patients and appears to be at a promising stage to begin pre-clinical trials.

3.4 Glial Scarring

An additional aspect of GBM is its aggressive invasiveness; it is almost impossible to entirely remove GBM tumours through surgical resection alone due to the extensive spread of cancer cells into surrounding healthy brain tissue.[147] This leads to tumour recurrence at both adjacent and distant sites within the brain.[147] One unusual approach to tackling this problem was to hijack the natural formation of glial scar tissue in response to injury in order to 'wall-in' tumour cells, whereby the presence of chondroitin sulfate proteoglycans (CSPGs) within scar tissue repels tumour cells from passing through it.[148] By functionalising the surface of gold nanoparticles with poly(ethylene glycol) (PEG) and peptides derived from zymosan, a known stimulant of reactive gliosis and glial scarring, Saxena *et al*., were able to generate a pro-inflammatory nanoparticle capable of stimulating glial scarring.[148,149] Additionally, when nanoparticles were delivered intravenously to tumour-bearing rats, scar tissue developed around the tumour site, with tumours found to be significantly smaller with reduced growth (*Fig. 9d*).[148] Overall these results suggest that generation of a physical barrier

through upregulated inflammation may be able to reduce GBM invasiveness. Further understanding of targeting mechanisms to ensure scar tissue development is limited to the tumour periphery would be required before translation to human trials. Monitoring animals over a longer period is also required to establish whether containment was successful and that tumours do not develop at distant sites post-scarring. Additionally, as this system appears to limit tumour size via a physical barrier, studies employing this particle system alongside additional therapeutics to inhibit tumour proliferation at the cellular level may be able to produce further tumour regression. Overall, this study highlights an interesting concept to limit GBM tumour growth and invasion, but requires considerable further research and understanding prior to translation to clinic.

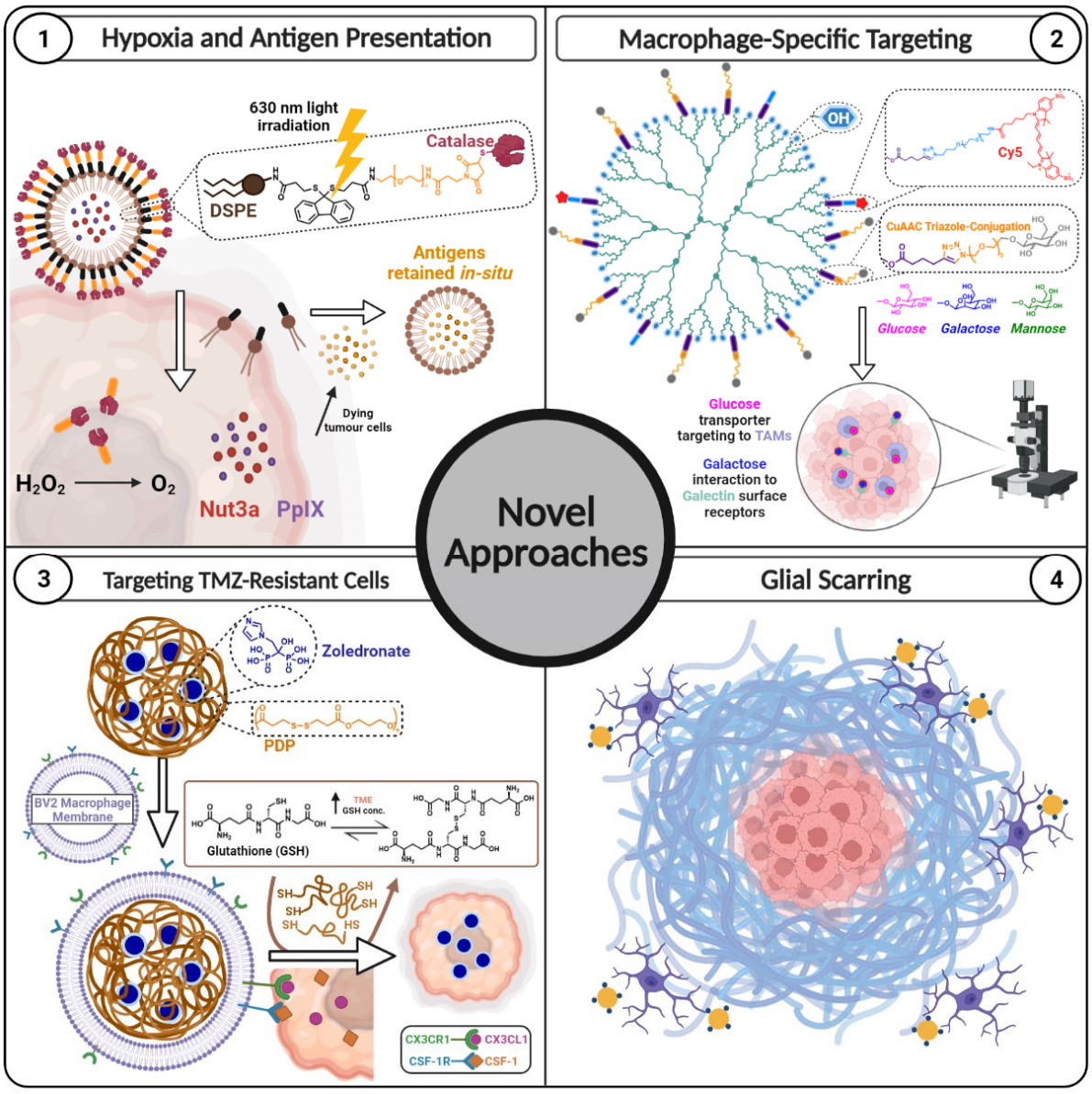


Figure 9. Case Studies. (1) *DSPE-based nanoparticle fused to catalase held together by a photocleavable thioketal linker cleaved at the GBM tumour site by irradiation at 630 nm, uncoupling the enzyme catalase that can alleviate the hypoxic conditions deep within the GBM by oxidising H_2O_2 into O_2 meanwhile releasing a payload of drugs: Nutlin-3a and Protoporphyrin IX (PpIX).* ***(2)*** *Dendrimer nanoparticle labelled with Cy5 and bound to sugars through CuAAC chemistry to target defined sites in GBM. Ubiquitous hydroxyl (OH) groups target macrophages. Glucose can interact with glucose transporters in TAMs and galactose interacts with galectin surface receptors in tumour cancer cells, leading to accumulation of respective nanoparticles at these sites, imaged by confocal microscopy.* ***(3)*** *Zoledronate (ZOL) specific delivery to GBM via encapsulation into disulfide-based poly(propylene glycol dithiopropionate) (PDP) nanoparticles enclosed in a BV2 macrophage membrane containing chemoattractant receptors CX3CR1 and CSF-1R which promote delivery to GBM site. High concentrations of glutathione (GSH) in the tumour microenvironment (TME) drives disulfide exchange and decomposition of the nanoparticle, releasing ZOL into tumour.* ***(4)*** *Zymosan peptide-functionalised gold particles stimulate reactive gliosis, causing the formation of CSPG-containing scar tissue around the tumour mass, restricting tumour growth and invasiveness. Created with BioRender.com*

4. Conclusions and Future Perspectives

GBM is a disease defined by its complex immune microenvironment, which despite being an obstacle to treating GBM, may be the key to developing successful treatments in the future. Conflicting evidence exists as to whether driving inflammatory or anti-inflammatory immune responses would be beneficial for GBM treatment; lower grade and IDH-mutant tumours were found to have more immunosuppression but improved prognoses, while other immunosuppressive factors like CD163 and hypoxia showed reductions in survival.[5,13,22,31] One explanation is the dual role of many cytokines in both inflammation and immunosuppression; pro-inflammatory cytokine interferon gamma (IFN-γ) and can be beneficial to treat tumours by increasing MHC expression and cancer cell apoptosis, but also drives PD-L1 expression, benefiting the tumour.[22] Additionally, excessive inflammation is detrimental for any tissue, healthy or cancerous. This is even more critical within the brain; both in terms of destroying healthy cells that cannot regenerate, and as the skull creates a confined space where inflammation and immune cell infiltration increasing brain volume can lead to dangerous levels of pressure.[22] For immunotherapies to successfully treat GBM, a carefully balanced immune response able to alleviate immunosuppression while avoiding excessive inflammation is required.

For the future treatment of GBM, reversing immunosuppression rather than driving general inflammation is likely to be the safest and most successful approach. This would prevent the tumour from using immunosuppressive pathways to drive proliferation and immune escape, without excessive inflammation and oedema that risks further damage. The recent success of ICIs in colorectal cancer further promotes the use of therapies that enable the immune system to drive tumour clearance.[150] However, as ICIs have had limited success in GBM, possibly due to the sheer degree of immunosuppression present in GBM, incorporating an additional element to promote mild inflammation may be the solution. Particle delivery systems may be the solution required to create this type of multi-faceted approach. Based on the research discussed in this review, a particle delivery system loaded with a mild immune-stimulant and surface functionalised with an ICI to improve particle-to-tumour targeting and to block excessive checkpoint protein expression would be ideal. Using a polymer-based material for the bulk of the particle would enable ICI conjugation directly to the surface, as well as enabling the exploitation different polymer properties to control the conditions that drive polymer degradation. For example, the development of PDP nanoparticles that decompose in high glutathione environments.[143] To drive mild inflammation, it would be difficult to achieve intracellular delivery of mRNA alongside ICI conjugation within the same particle, so small molecule drugs, such as toll-like receptor (TLR) agonists, could be encapsulated to stimulate

inflammation.[151,152] Alternatively, use of hyperthermia is also able to prime the immune system towards inflammation. While hyperthermal therapy appears to drive too strong a response to be safe in GBM, fever-mimicking hyperthermia between 37-40°C may be enough to improve ICI efficacy without excessive damage.[106,107] By creating a particle with a superparamagnetic iron oxide core, coated with polymer, and surface functionalised with an ICI, it may be possible to limit toxicity with particle targeting, promote mild inflammation following alternating magnetic field exposure, and limit GBM immunosuppression to enable immune-driven tumour clearance.

Overall, combining immunotherapies with particle delivery systems holds considerable promise for the future of GBM treatment.

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