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Review

Blood-brain barrier penetrating nanosystems enable synergistic therapy of glioblastoma

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ABSTRACT

Glioblastoma multiforme (GBM) is a highly malignant and formidable central nervous system tumor that lacks effective therapeutic options. Various characteristics of GBM contribute to this plight, which include inter-/extra-tumor heterogeneity, the presence of the blood brain barrier and GBM stem cells. Standard clinical therapy of GBM has multiple limitations including poor efficacy reflecting, in part, the development of multidrug resistance and unexpected side effects. In addressing these challenges, combination therapies have emerged as promising front-runners and nanotechnology, with its rapid advancements and unique advantages, offers the potential to further improve synergistic combination therapies for GBM. In this review, we outline proof-of-concept studies showcasing recent advances in nanosystem-mediated combinational GBM therapies, with an emphasis on the amplified therapeutic effects of monomodal and multimodal synergistic treatments. The examples detailed in this review provide valuable insights to further understand key paradigms of GBM combinational treatment, inspiring

Abbreviations: GBM, glioblastoma multiforme; RT, radiotherapy; TME, tumor microenvironment; TMZ, temozolomide; BBB, blood brain barrier; GSCs, GBM stem cells; EPR, enhanced permeability and retention; GSH, glutathione; ROS, reactive oxygen species; Tf, transferrin; LDLR, low-density lipoprotein related receptor; Ang, Angiopep-2; ApoE, apolipoprotein E; PTX, paclitaxel; DOX, doxorubicin; LM, lomeguatrib; 5FU, 5-fluorouracil; MPEG, methoxy poly ethylene glycol; PCL, poly ε-caprolactone; WGA, wheat germ agglutinin; FA, folic acid; CDDP, cisplatin; C.I., combinatorial index; Tf-J-T, TMZ and JQ1 loaded nanomedicines; MGMT, O6-methylguanine-DNA methyltransferase; Bcl-2, B-cell lymphoma/leukemia-2; ABT, ABT—263; A12, A-1210477; MOMP, mitochondria outer membrane permeabilization; RBCms, red blood cell membranes; PLGA, poly[*d*,*l*-lactide-co-glycolide]; CQ, chloroquine; ETO, etoposide; BCNU, carmustine; RNAi, RNA interference; SiRNA, short interfering RNA; MiRNA, microRNA; RISC, silencing complex; RGD, Arginyl-glycyl-aspartic acid; VEGFR, vascular endothelial growth factor receptor; PAMAM, polyamidoamine; PLK1, polo-like kinase; RAS, renin-angiotensin system; AKT, Phosphoinositide 3-Kinase PI3K-Protein Kinase B; BTICs, brain tumor-initiating cells; PBAE, bioreducible poly beta-amino ester; Robo1, roundabout homolog 1; YAP1, yes-associated protein 1; NKCC1, sodium-potassium-chloride cotransporter; Lex, lexiscan; TLR7/8, toll-like receptor 7 and 8; ICL-161, cellular inhibitor of apoptosis protein inhibitor; Ags, antigens; PDMA, poly2-dimethylaminoethyl methacrylate); PDPA, poly 1-(diisopropylamino ethyl methacrylate); CSssSA, Disulfide-linked glycolipid-like copolymer; Fe_3O_4, iron oxide; CTX, chlorotoxin; RBBP4, retinoblastoma binding protein 4; SNA, spherical nucleic acid; SiRNA-SS-PNIPAM, siRNA-disulfide-polyN-isopropylacrylamide; SiSTAT3, signal transducer and activator of transcription 3 siRNA; HMOX1, heme oxygenase-1; HSSP, HMOX1 specific short peptide; IONPs, iron oxide nanoparticles; SiGPX4, glutathione peroxidase 4 siRNA; Pt, cisplatin1; NOX, nicotinamide adenine dinucleotide phosphate oxidase; NEs, neutrophils; US, ultrasound; CZT, crizotinib; BK, bradykinin; GSCs, stem-like GBM cells; CAR, chimeric antigen receptor; TPZ, pro-drug triapazamine; PDT, photodynamic therapy; SDT, sonodynamic therapy; CDT, chemodynamic therapy; EDT, electrodynamic therapy; MDT, microwave dynamic therapy; PS, photosensitizer; NIR, near-infrared; ICG, indocyanine green; Ce6, chlorin e6; PTD, poly 2,2″-thiodiethylene 3,3″-dithiodipropionate; NE, neutrophil elastase; PGA, polyglutamic acid; ICD, immunogenic cell death; TAAs, tumor-associated antibodies; DAMPs, damage-associated molecular patterns; PpIX, protoporphyrin IX; COS, chitosan oligosaccharide; HA, hyaluronic acid; MBs, microbubbles; PEX, poly-ethyleneimine xanthate; Cu^{2+}, copper ions; PLL-CA, citraconic anhydride grafted poly-lysine; PTT, photothermal therapy; BGT-1, betaine transporter 1; GA, gambogic acid; GBM-PDTCM, GBM patient-derived tumor cell membrane; AuNRs, gold nanorods; HSPA5, heat shock protein A5; AuNS, gold nanosphere; UPR, unfolded protein response; MRI, magnetic resonance imaging; CT, computed tomography; TAMCs, tumor-associated myeloid cells; STING, stimulator of interferon genes; CCL-2, chemokine ligand 2; MMP-2, matrix metalloproteinase 2; 3WJ, three-way junction; TAT, cell penetrating peptide; IKKα, IκB kinase; DCHB, dicysteamine-modified hypocrellin derivative; PF, perfluorcarbon; MPDA, mesoporous polydopamine; TAMs, tumor-associated macrophages; Gox, glucose oxidase; AQ4N, banoxantrone dihydrochloride.

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researchers not only in the development of multifunctional nanosystems for brain drug delivery, but also in the design of more rational and effective GBM combination therapies.

Instruction

Approximately 6.2 per 100,000 people are diagnosed with central nervous system tumors every year worldwide, with an estimated 45.2% having glioblastoma multiform (GBM), which is classified as the most malignant glioma (IV) by the World Health Organization [1–4]. Over the past several decades, tremendous efforts have been devoted to combating GBM, leading to the emergence of multiple therapies, such as surgery, radiotherapy (RT), chemotherapy, gene therapy, phototherapy and immunotherapy [5–8]. While these therapies have somewhat improved anti-GBM therapy, the average median survival of GBM patients remains less than 14.6 months and the 5-year survival rate is still poor at less than 10% [9–11].

These disappointing outcomes are mainly attributable to the inherent properties of GBM, including high infiltration, genetic heterogeneity, unique tumor-promoting microenvironment (TME), tumor stem cells that contribute to GBM initiation, drug resistance and recurrence, as well as the limitations of monotherapies [12–15]. The highly infiltrative nature of GBM makes complete surgical resection impossible and GBM tumor cells are capable of direct migration, both of which lead to high recurrence [16–18]. Gene profile analysis indicates that GBM phenotype can be variable although the genotype is same, while identical phenotypes may differ genetically [19,20]. Concurrently, frequent gene mutations can generate self-renewing tumor cells that respond more aggressively to treatments [17,21]. These genetic factors contribute to the failure of monotherapies since these are unable to repair multiple pathways simultaneously. For example, GBM is generally considered strongly temozolomide (TMZ)-resistant primarily due to multiple activated DNA repair systems [22,23]. In addition, the TME of GBM also presents a significant challenge to treatments as GBM is classified as a “cold tumor”, lacking pre-existing tumor T-cell infiltration and tumor antigens (Ags), while encapsulating high levels of immunosuppressive cells [24–29]. “Cold tumors” also show less immunogenicity and limited efficacy to immune checkpoint inhibitors or other therapies [25]. Furthermore, the blood brain barrier (BBB), composed mainly of endothelial cells with tight junctions, prevents therapeutic compounds from crossing into the central nervous system and tumor sites, limiting drug accumulation in GBM tissues [30,31]. This unsatisfactory treatment landscape is also aggravated by GBM stem cells (GSCs), which play crucial roles in sustained tumor growth, therapeutic resistance, metastasis, recurrence and immune evasion [12,32].

Given the characteristics of GBM that promote self-survival and the inefficiency of mono-drug therapies, consistent efforts have been made to improve GBM therapeutic efficiency. Much research has focused on developing multifunctional nanosystems to conquer these barriers for effective GBM treatment [33–35]. Nanoplatforms can effectively amplify therapeutic effects by increasing drug accumulation at GBM sites utilizing the enhanced permeability and retention (EPR) effect firstly [34,36]. Nanocarriers also can provide protection to otherwise unstable therapeutics, enabling these to exhibit better stability, extended blood circulation time and therapeutic efficacy [37]. There are various types of nanocarriers, generally divided into organic and inorganic materials. While inorganic nanocarriers are more stable and advantageous for imaging in GBM treatments, especially with metal nanomaterials, organic nanocarriers like micelles, polysomes, dendrimers, nanogels and liposomes have enhanced biocompatibility. More importantly, they are more easily fabricated and functionalized, facilitating multiple drug delivery for GBM therapies [38,39]. Functionalization of nanocarrier surfaces with targeting ligands and/or camouflaging with various cell-membrane derived ‘cloaks’ has been proven to significantly improve GBM targeting, enhance BBB penetration, reduce drug resistance and mediate lower side effects [40–43]. Meanwhile, developing degradable and advanced biocompatible nanomaterials, along with biomimetic strategies, could effectively improve the safety profile of nanosystems, further reducing systemic toxicity [44]. Moreover, multiple controlled release strategies of nanocarriers, relying on specific materials or bonds, responsive to the TME and glioma cells with lower pH values, hyperthermia, higher levels of glutathione (GSH) and reactive oxygen species (ROS) have been developed and applied to GBM therapies [45]. Furthermore, combinational GBM therapies have been clinically employed which typically comprise maximal surgical resection followed by RT and adjuvant chemotherapy using TMZ [46,47]. While these prolong survival time by approximately 2.5 months, as noted above, the 5-year survival rate remains poor at less than 10% [48,49]. Importantly, rather than simply optimizing monotherapy, nanosystems combined with multi-drugs or multi-modal treatments might generate superadditive or synergistic therapeutic effects thereby mediating fewer adverse effects and reduced resistance. These strategies could further partly overcome the difficulties in GBM treatments.

Herein, we summarize the recent advances in monomodal and multimodal synergistic therapies that co-deliver two or more therapeutics using various types of promising BBB penetrating nanosystems (Fig. 1). The challenges and prospects of nanosystem-mediated GBM combinational therapy are discussed to emphasize considerations for further rational designs of nanosystems as well as combination strategies.

Strategies of nanosystems for BBB penetrating

Over the years, the BBB has been considered one of the most regulated and exclusive barriers to drug delivery, challenging treatments of brain diseases. To address it, various nanoparticles have been developed to improve BBB penetration [50]. In this section, we summarize the BBB penetrating strategies of nanosystems involved in this review as well as their transport mechanisms. Collectively, BBB penetration mechanisms include passive penetration, receptor-mediated transcytosis, cell membrane camouflaging-mediated penetration and stimuli-mediated BBB disruption (Fig. 1**b**).

Passive BBB penetration, also known as non-specific transcytosis, has two main pathways to cross the BBB. For one thing, small molecules with a mass of less than 400 kDa can enter the brain through the narrow spaces between tight junctions [51]. For another, the EPR effect carries the nanoparticles into cancer tissues, facilitating BBB penetration with efflux proteins present in the BBB [52]. Regarding receptor-mediated BBB penetration, strategies mainly focus on tailored surface functionalization of nanocarriers according to specialized receptors expressed on endothelial cells [53,54]. For example, nanosystems modified with transferrin (Tf) and low-density lipoprotein receptors (LDLR) ligands, such as Angiopep-2 (Ang) and Apolipoprotein E (ApoE), can promote BBB permeability via receptor-mediated mechanisms. Moreover, various vesicle camouflaging strategies have emerged as an effective approach to endow nanoparticles with BBB penetrating abilities by inheriting unique characteristics from their parent cells, including multiple molecular interactions and specific recognition. For example, tumor cell-derived membranes tend to have BBB crossing abilities through reducing expressions of tight junction proteins. Exosomes have the nature of BBB penetration by multiple mechanisms, including receptor-mediated transcytosis, adsorptive-mediated transcytosis and endocytosis. Neutrophils biomimetic nanomedicines can cross the BBB owing to the natural ability of neutrophils to migrate from circulation into injured brain tissues. Bacteria outer membrane also can be used for

elevating BBB penetration due to its invasive characteristics. Furthermore, stimuli-mediated BBB disruption, such as agonists, laser and ultrasound (US), has been widely explored to enhance BBB permeability [50]. The stimuli could temporarily open the BBB, specifically altering the integrity of BBB by reducing the degree of tight junctions, resulting in increased BBB penetration and tumor site accumulation of nanomedicines, enhancing the active transport of drugs. In addition, some studies combine multiple kinds of these strategies for improving BBB penetration. Overall, nanosystems with these strategies can more effectively deliver therapeutics into GBM tissues, finally achieving superior combinational effects.

Monomodal GBM combinational therapy

GBM is a malignant brain tumor characterized by multiple heterogenic alterations that make it highly migratory and invasive as well as promoting drug resistance which makes GBM impossible to cure with monotherapy [20,55]. Depending on the anti-tumor mechanism of the therapeutic agents, drug delivery nanosystems have been designed to simultaneously transport the same type of therapeutics with different functions into the brain for GBM monomodal combinational therapies. This strategy aims to block dual, or multiple interconnected, or non-related pathways involved in GBM occurrence, progression, resistance and apoptosis, thus potentially overcoming current obstacles. Agonists may also be employed together with other anti-GBM therapeutics to achieve improved effects. In this section, we highlight advancements in augmented GBM synergistic treatments mediated by single nanosystems that deliver drugs of the same category but with different mechanisms of action, or agonists with other drugs.

Monomodal combinational chemotherapy

Chemotherapy continues to play a central role in cancer treatment and has achieved some success in managing a limited range of cancers

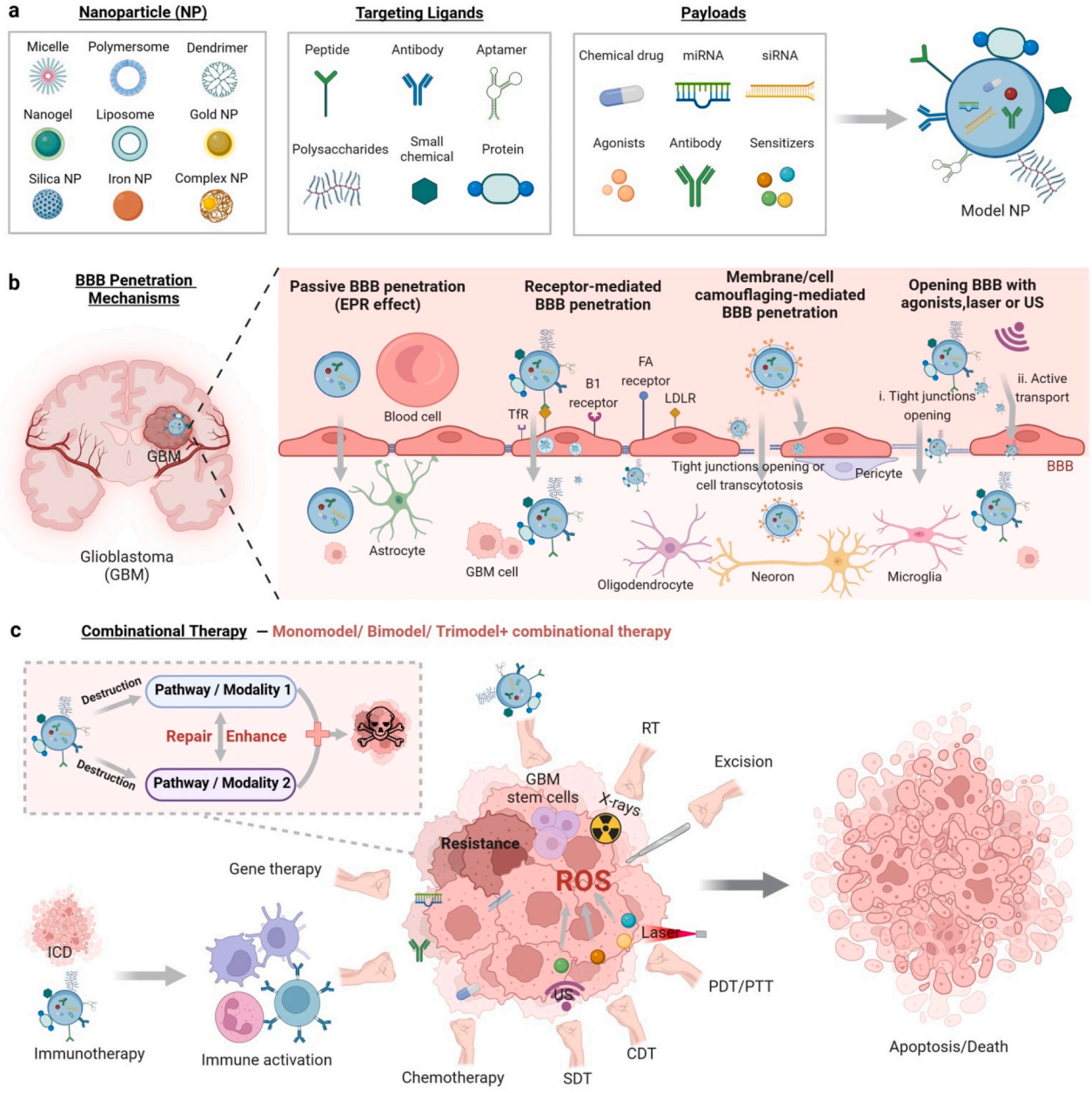

Fig. 1. Schematic overview of nanosystems and mechanisms discussed in this review. (a) Various nanosystems are employed to load multiple therapeutics. (b) BBB penetration mechanisms of multifunctional nanosystems. (c) The schematic of how combinational therapies achieve boosted synergistic effects.

including GBM [56,57]. Various anti-tumor chemical agents are currently applied as the first-line clinical treatment for GBM [47]. However, the effectiveness of GBM chemotherapy is significantly restricted by poor solubility, strong hydrophilicity, limited BBB permeability and substantial systemic toxicity of chemical agents [58,59]. Faced with these bottlenecks, nanocarrier-based chemotherapies with smart modification strategies have shown promise [60]. Additionally, drug resistance, primarily induced by enhanced self-repair mechanisms, and dose-limiting side effects account for the general failure of GBM chemotherapy. In an effort to eliminate systemic toxicity and resistance in chemotherapy, researchers have explored dual or multiple drug delivery systems that combine various anti-tumor agents with different mechanisms of action such as TMZ, paclitaxel (PTX) and doxorubicin (DOX) amongst others, that offer improved GBM therapeutic efficacy with reduced resistance and side effects (Table 1).

Dual drug-based combinational chemotherapy

In the last two decades, dual drug-based synergistic chemotherapies for GBM have been extensively explored and have exhibited enhanced anti-GBM effects compared to monotherapy. Firstly, resistance in cancer cells can be effectively thwarted by the concurrent targeting of different pathways, especially those associated with resistance development, like repair mechanisms [68]. Therapeutics with distinct mechanisms normally have non-overlapping resistance pathways, making it harder for cancer cells to develop resistance simultaneously to both drugs. Dual drug combinations tend to result in reduced toxicity, resistance and side effects because generally lower doses of each drug are needed to mediate anti-GBM effects. Importantly, combining two drugs with distinct mechanisms of action leads to more potent anti-cancer activity through induction of synergy that leverages the strengths of each drug and inhibits tumor progression through multiple targets [69]. With the integration of smart nanosystems, GBM treatment has witnessed remarkable improvement that takes advantage of synergistic therapy. A variety of small molecule chemical drugs was applied in these studies, mainly including TMZ, cisplatin (CDDP), PTX and DOX et al.

TMZ-based dual drug monomodal combinational chemotherapy. TMZ, the first FDA-approved alkylating agent, has been the most commonly employed small-molecule drug in GBM clinical trials since it can penetrate the BBB and specifically inhibit GBM [70]. As a DNA-alkylating drug, TMZ exerts its effects by causing DNA double-strand breaks, cell cycle arrest and eventual cell death through methylating guanine and adenine bases of DNA [71]. In addition, a moderately improved GBM therapeutic effect has been realized after utilizing nanosystems to increase TMZ delivery to GBM sites [72]. However, TMZ treatment is associated with significant bone marrow suppression and intrinsic resistance mechanisms, which limit its effectiveness [73–75]. To address these choke points, researchers have studied the combination of TMZ with other chemical agents. One study by Hammond et al. utilized a transferrin-functionalized nanoparticle (Tf-NP) to simultaneously deliver TMZ and bromodomain inhibitor JQ1 to significantly alleviate bone marrow suppression caused by TMZ and elevate tumor inhibition (Fig. 2**a**) [61]. With strong BBB penetration and active targeting mediated by Tf, increased quantities of TMZ and JQ1 were delivered to the GBM sites as reflected by significantly increased Cy5.5 fluorescence (Fig. 2**b**). The combination of TMZ and JQ1 generated additive cytotoxic effects on GBM cells as combinatorial index (C.I.) values were 0.95 and 0.94 in U87MG and GL261 cells (Fig. 2**c**). Accordingly, a 1.5–2.0-fold decrease in tumor volumes and significantly extended survival time were observed in GBM-bearing mice treated with TMZ and JQ1 loaded nanomedicines (Tf-J-T) (Fig. 2**d**). Notably, immunocompetent mice receiving Tf-J-T were effectively protected from bone marrow suppression in contrast to mice treated with TMZ only. This nanosystem provides a promising platform for dual drugs brain targeted delivery. However, further investigation is needed to assess the long-term bone marrow suppression effects and indicate the combination mechanism of TMZ and JQ1.

Apart from marked bone marrow suppression, intrinsic drug resistance plays a crucial role in reducing the effectiveness of TMZ-based GBM therapies. Notably, this resistance is associated with the O6-methylguanine-DNA methyltransferase (MGMT), a well-known DNA repair protein induced by alkylating agents. It has been reported that GBM cells also exhibit elevated levels of MGMT that undermine the efficacy of TMZ-based treatments [76,77]. To address MGMT-related resistance, there are two main approaches. One is delivering two chemotherapeutics involved in different molecular pathways to kill GBM cells. Our group constructed a multifunctional biomimetic nanosystem (MNPs@TMZ+CDDP) for the co-delivery of TMZ and CDDP, to GBM sites (Fig. 2**e**). The nanosystem was designed utilizing cancer membrane cloaking, which greatly improved blood circulation, advanced biocompatibility, enhanced BBB crossing and increased homologous targeting. In addition, the pH-responsive nanocarrier (acetylated dextran) was used to achieve controlled and precise release of TMZ and CDDP, approximately 80% under acid conditions, which could lead to better anti-GBM effects and less side effects. In orthoptic U87MG and TMZ-resistant $U251^R$ mice models, treatment with MNPs@TMZ+CDDP significantly prolonged the survival of mice up to 3-fold compared to mice treated with mono-drug loaded nanomedicine groups or free drugs. Interestingly, the western blot results showed that the expression levels of MGMT were obviously reduced by TMZ and CDDP co-administration, which contributed to the superior therapeutic efficacy (Fig. 2**f-h**). Moreover, there were no discernible differences in the major organs and blood examination results of mice treated with MNPs@TMZ+CDDP and PBS group, indicating negligible side effects. An alternative to combat MGMT resistance is to inhibit MGMT expression directly [62]. Combination therapies involving MGMT inhibitors, sensitizing tumors to alkylating agents, have gained attention as potential strategies. Recently, our group co-transported TMZ and the MGMT inhibitor lomeguatrib (LM) using an ApoE targeting peptide decorated GBM cell membrane coated nanosystem (AMNPs@TMZ+LM) based on MNPs above. ApoE modification additionally promoted endothelial cell endocytosis and the accumulation of nanomedicines at GBM sites by

Table 1
Summary of chemical drug-based combinational therapies.

Chemotherapeutics	Nanocarrier	Targeted strategies	Responsive release designs	Major applications	Ref
TMZ+JQ1	Liposomal	Tf	/	Overcoming bone marrow suppression	[61]
TMZ+CDDP	Acetalated dextran	Cancer membrane	pH	Overcoming drug resistance	[62]
TMZ+Lomeguatrib (LM)	Acetalated dextran	ApoE and cancer membrane	pH	Overcoming drug resistance	[63]
PTX+Melittin	Lipodisks	Glycopeptide	/	Overcoming drug resistance	[64]
DOX+5-fluorouracil (5FU)	Chitosan-gold NPs	Nucleolin aptamer	pH	Improving GBM therapeutic effects	[65]
A12+ABT	Acetalated dextran	ApoE	pH	Overcoming tumor resistance	[66]
Etoposide+ Carmustine+DOX	methoxy poly (ethylene glycol) (MPEG)-poly (ε-caprolactone) (PCL)	Wheat germ agglutinin (WGA) and folic acid (FA)	pH	Enhancing anti-proliferative activity without detectable side effects	[67]

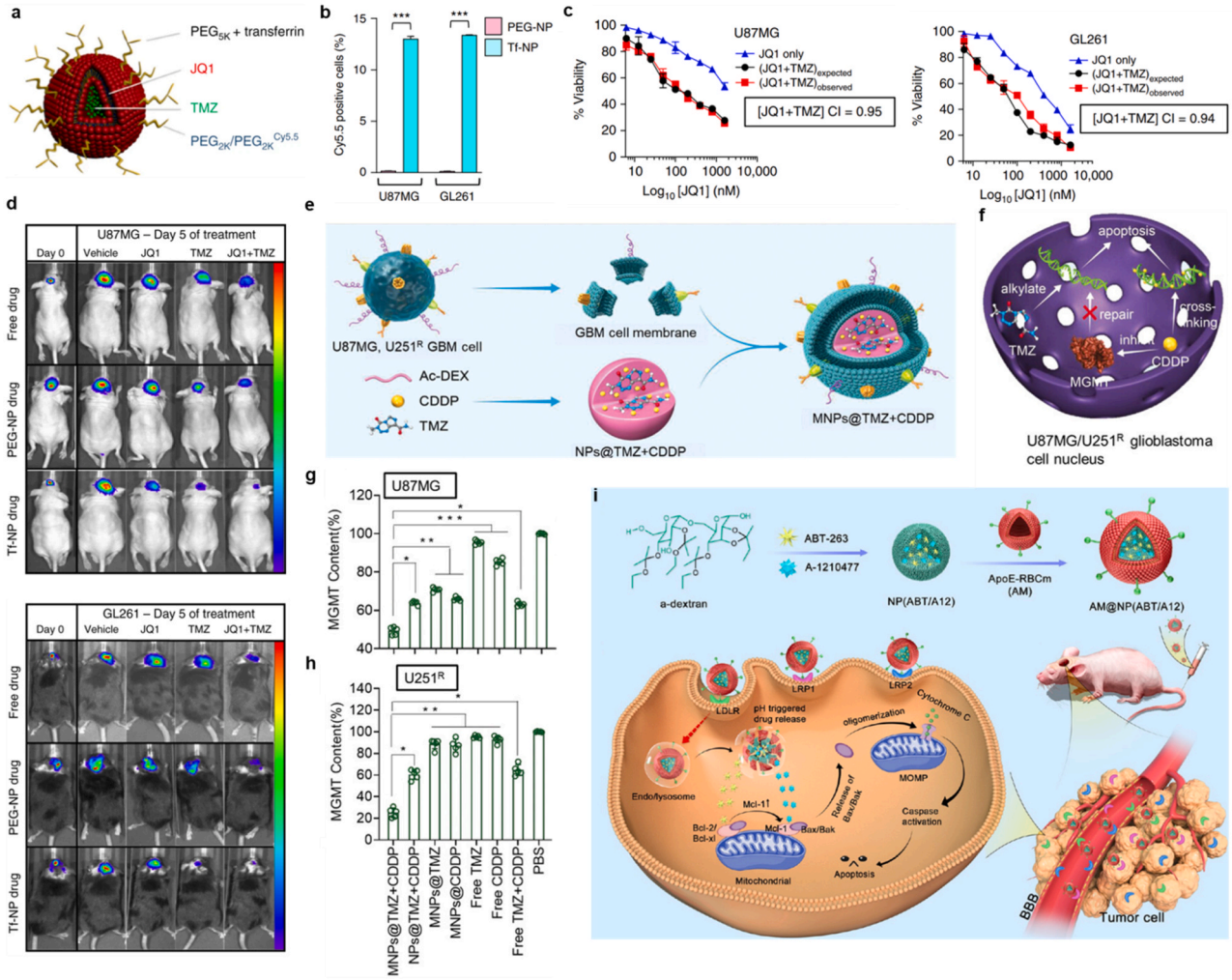

Fig. 2. (a) Liposome schematic depicting JQ1 and TMZ loading. (b) Quantification of cellular Tf-NP uptake in U87MG and GL261 cells. (c) Cell viability and combinational index (C.I.) values of JQ1 and TMZ in U87MG and GL261 cells. (d) Representative bioluminescence images of mice bearing orthotopic U87MG and GL261 after treatment with different nanomedicines, free drugs or vehicles. (e) Schematic of MNPs@TMZ+CDDP with a pH-sensitive acetylated dextran polymeric nanoparticle core and GBM cancer cell membrane shell. (f) Illustration of the mechanism of synergistic action of TMZ and CDDP in the cell nucleus. (g) & (h) MGMT concentration in U87MG and U251^{R} cells after treatment with different nanomedicines, free drugs or vehicle. (i) Illustration of construction of AM@NP(ABT/A12) NPs, which can penetrate BBB by receptor-mediated transportation and synergistically induce tumor cell apoptosis.
(a) (a-d) Printed with permission from Ref [61]. (b) (e-h) Printed with permission from Ref [62]. (c) (i) Printed with permission from Ref [79] .

targeting the LDLR overexpressed by both endothelial cells of the BBB and GBM cells, as evident by the obvious stronger brain Cy5 fluorescence intensity of mice treated with AMNPs@Cy5 than that of MNPs@Cy5 group. More importantly, the expression of MGMT protein was efficiently inhibited by LM, further increasing the sensitivity of U251^{R} and GBM stem cells to TMZ [63]. Although the smart design improved BBB penetration and tumor targeting through both ApoE and cancer membrane coating, the manufacturing process seems too intricate to be further applied in combinational GBM therapy.

These studies reveal that combinational chemotherapy based on smart nanosystems can amplify the therapeutic efficacy of TMZ by reducing MGMT-mediated drug resistance, while concurrently diminishing systematic side effects. Considering the resistance, which is induced by the immunosuppressive TME, is detrimental to TMZ-based therapies, a promising strategy is to pair TMZ with immunotherapies as summarized below. Moreover, further development of more optimized nanosystems could see the emergence of new promising treatments for GBM.

Other dual drug combinational chemotherapy. In addition to TMZ, a diverse array of other effective anti-tumor small-molecule chemical drugs have shown promising inhibitory activity against GBM in combination with other drugs. These drugs work by a variety of mechanisms, including blockade of the cell cycle and up-regulation of apoptosis-related proteins. Similarly, the majority of compounds used in combination produce enhanced therapeutic efficiency by simultaneously targeting two entirely distinct pathways as previously discussed. At the same time, some combined drugs target the same molecular cascade, thereby amplifying their impact and disrupting the specific pathway at multiple points. These therapeutics mainly include PTX, DOX and anti-apoptotic protein-related inhibitors.

PTX was co-delivered with melittin by glycopeptide modified lipodisks for GBM targeted synergistic therapy [64]. Evidence has shown that nanosized lipodisks extended blood circulation time and improved tumor accumulation for several reasons. Firstly, the PEGylated layers reduced degradation and clearance in the circulation. Secondly, discoidal nanoparticles more migrated to the vessel walls and infiltrated tumor tissues. Decoration with the targeting glycopeptide further significantly enhanced nanoparticle BBB penetration and accumulation at tumor sites. As PTX mediates anti-proliferative activity by acting as a tubulin polymerization promotor, tumors frequently recur when PTX resistance develops. The incorporation of melittin as a wide-spectrum antimicrobial peptide further enhances anti-GBM, while melittin

causes severe hemolysis. The PTX/melittin co-loaded lipodisks exerted an in-vitro synergistic effect as indicated by the low CI value (0.45) and in-vivo mediated a10-day longer survival time without causing hemolysis, providing a potential and safe nanosystem for brain drug delivery. However, there is no evidence to explain the mechanism behind the synergistic inhibition of glioma by combining PTX with melittin. Additionally, it is worth considering whether wide-spectrum antimicrobial drugs have the advantage of enhancing GBM combinational efficacy by synergizing with other anti-tumor drugs.

Compared with PTX, DOX more thoroughly removes tumor cells including GBM. The mechanism of action of DOX centers on interfering with DNA replication, topoisomerase II inhibition, cell cycle arrest, and apoptosis induction, setting the stage for effective tumor elimination. Despite its potential, like other single chemotherapeutic agents, DOX encounters the challenges of drug resistance and systemic toxicity. Combining DOX with other agents thus holds promise to mitigate these limitations. In a more recent study, Wang et al. designed a pH-responsive and targeted nanosystem for co-delivery of DOX and 5-fluorourail (5FU) [65]. The nanosystem comprised chitosan-gold nanoparticles functionalized with an aptamer that could specifically recognize and interact with the necleolin receptor, which is overexpressed on the surface of GBM tumor cells. The aptamer modification facilitated the cellular internalization of nanocarriers, which were designed to be pH-responsive, rapidly releasing the co-loaded DOX and 5FU in the slightly acidic environment present in tumor tissues. These smart design elements significantly improved in-vitro therapeutic effect as indicated by the highest induction of tumor cell apoptosis (23.11%) relative to free drugs and significant induction of cell cycle arrest (73.3%). This nanosystem demonstrates to be effective for BBB penetration and brain tumor targeting mediated by aptamer. Although the combination of DOX and 5FU showed enhanced synergistic effects in vitro, the mechanism and the specific limitations of DOX addressed by 5FU are unclear, and in vivo investigations are necessary to further evaluate the therapeutic potential of this combination.

While targeting apoptosis pathways is an effective approach in the battle against GBM, GBM cells often exhibit intrinsic resistance to apoptosis due to overexpression of anti-apoptotic B-cell lymphoma/leukemia-2 (Bcl-2) family proteins, such as Bcl-2, Bcl-xl and Mcl-1 [66,78]. Simultaneously targeting multiple points in the same apoptosis pathway potentially could overcome this kind of drug resistance. To exploit this potential, our team developed a multifunctional biomimetic nanosystem (AM@NP(ABT/A12)) capable of co-delivering ABT-263 (ABT) and A-1210477 (A12) for GBM therapy (Fig. 2**i**) [79]. ABT is a Bcl-2/Bcl-xl specific protein inhibitor, while A12 can directly inhibit the function of Mcl-1. As Mcl-1 inhibits mitochondrial outer membrane permeabilization (MOMP), the release of cytochrome C is prevented, inhibiting apoptotic cascades. Importantly, A12 synergistically enhances the effect of ABT. To optimize the activity of nanoparticles, red blood cell membranes (RBCms) were used as biomimetic camouflaging resulting in significantly improved blood circulation time of nanoparticles. Nanoparticles were also functionalized by surface ApoE modification, which improves BBB penetration and GBM targeting ability through LDLR. To facilitate pH responsive drug release, acetylated dextran was used as the nanoparticle shell. Accordingly, these nanoparticle construction elements enabled the combination of ABT and A12 to show significantly enhanced anti-GBM activity both in vitro and in vivo. U87MG, U251^{R} and CSC-2 cells incubated with AM@NP (ABT/A12) showed higher apoptosis, which contributed to enhanced levels of apoptotic proteins and decreased levels of anti-apoptotic proteins. Furthermore, mice bearing orthotopic GBM and treated with AM@NP(ABT/A12) displayed greatly the smallest tumor volumes and the longest survival time, relative to control formulations. The smart nanosystem also reduced cytotoxicity to normal tissues. Apoptosis-related pathways are complex, suggesting that combining multiple interacting targets, involved in either pro-apoptosis or anti-apoptosis, shows the potential to induce boosted apoptosis of GBM cells, achieving superior synergistic effects.

Collectively, recent research indicates a promising future in dual chemical drug combination strategies for GBM treatment. By skillfully combining agents with distinct or similar mechanisms with smart nanosystems, researchers aim to enhance therapeutic outcomes, overcome resistance and minimize the impact on normal cells, offering the possibility for improved treatments against GBM. As more potent drugs and innovative nanosystems continue to be developed, there is a growing potential to combine multiple other therapeutics for effective combinational chemotherapy.

Triple drug-based combinational chemotherapy

To further improve combinational chemotherapy, several studies have combined two or more anti-tumor chemotherapeutics to target GBM. For example, the combination of three already approved chemotherapy drugs, TMZ, chloroquine (CQ) and sirolimus was found to simultaneously induce marked DNA damage, mitochondrial destruction and lysosome-dependent apoptosis of GBM cells, significantly inhibiting in vivo tumor growth and increasing survival rate of mice bearing GBM8401 xenografts [80]. More importantly, with the empowerment of well-designed nanosystems, anti-GBM triple drug combination therapies could climb higher up the GBM therapy mountain. To realize this potential, Liu et al. adopted biodegradable poly[(d,l)-lactide-co-glycolide] (PLGA) nanofibers to concurrently deliver carmustine (BCNU), irinotecan and CDDP into the cerebral cavity [81]. BCNU is a commercially used drug for malignant brain tumor treatment that effectively improves patient survival by alkylating both DNA and RNA. Irinotecan has shown activity against DNA replication and transcription by inhibiting topoisomerase I. CDDP, as one of the most effective anti-GBM drugs, could interfere with DNA repair and disrupt DNA structure. The combination of these three drugs has the promise to generate a more comprehensive disruption of genetic materials, further effectively inhibiting GBM growth. However, the potential should be weighed against the limited brain accumulation and toxicity to normal tissues. This study demonstrated that the designed nanofibers could simultaneously deliver three different agents in one step, ensuring a sustained and high drug concentration for over 8 weeks in rat models without inflammation reactions. Subsequently, this group observed that the GBM volumes of rats treated with these nanofibers significantly decreased with time, and no tumor regrew, leading to 86.50±48.41-day median survival time [82]. Hence, incorporation of these drugs into nanofibers overcomes the disadvantage associated with these as free drugs.

While triple drug-based monomodal combinational chemotherapies have shown significant advancements in therapeutic efficacy, their broader application still faces certain limitations. Small molecule chemical drugs are inherently toxic and damage normal tissues and cells. Although nanosystems have helped improve these effects by enhancing drug delivery precision, the combination of too many small molecule drugs might, in some cases, exacerbate systemic toxicity. Hence, it is necessary to continue to develop more functional nanosystems with carefully calibrated combinational strategies to circumvent the potential additional side effects. The pursuit of multidrug-based therapies that have a balance between heightened effectiveness and minimal side effects is crucial for advancing GBM treatment.

Monomodal combinational gene therapy

Gene therapy involves using oligonucleotides to specifically target and regulate abnormal genetic expression related to tumor proliferation in GBM cells [83]. Compared with other therapeutic approaches, gene therapy presents exciting potential, as they generally can be expected to be more efficient with minimized systemic cytotoxicity. However, gene therapeutics are inherently unstable, and significant efforts have been devoted to improving stability and hence delivery to target sites. In this regard, employing specially designed gene therapy nanocarriers provides a promising advance in GBM therapy as evidenced by recent

studies [84–86]. However, GBM occurrence is often accompanied by mutations in numerous gene sites involved in tumor growth, metastasis and invasiveness. Therefore, it becomes necessary to simultaneously silence, or stimulate expression, of multiple genes with a designed nanoparticle delivery system (Table 2).

One pivotal GBM gene therapy is based on RNA interference (RNAi) technology, which can regulate targeted gene expression with sequence specific small molecules, like short interfering RNA (siRNA) and microRNA (miRNA) [91]. These specialized siRNAs and miRNAs bind to the targeted RNA to form a silencing complex (RISC), which then inhibits tumor proliferation by matching and marking its targeted mRNA for degradation or ribosomal arrest [92]. In recent years, combining dual siRNAs or miRNAs to enhance anticancer efficacy in GBM has been widely explored [93]. To improve the inherent limitations of RNA, known to be easily degraded by hydrolytic enzymes and possessing the same charge as cell membranes, various nanocarriers have been designed and employed in GBM synergistic gene therapy. Shi et al. developed Arginyl-glycyl-aspartic acid (RGD) modified nanoparticles to transport two distinct siRNAs (vascular endothelial growth factor receptor siRNA (VEGF siRNA) and Bcl-2 siRNA) into GBM cells for targeted gene silencing [87]. The cytocompatibility, BBB penetration and cellular uptake of siRNAs were significantly elevated after being encapsulated into poly(amidoamine) (PAMAM) coated gold nanoparticles that were surface modified with PEG and RGD, which can recognize and interact with overexpressing αvβ3 integrin on GBM cell surface. These targeted nanoparticles resulted in the lowest protein expression levels of both VEGF and Bcl-2 in cell and animal levels, compared to controls, indicating high-efficiency transfection and successful specific gene silencing. Simultaneous knockdown of both VEGF and Bcl-2 genes can synergistically induce the apoptosis of tumor cells, providing a promising way for efficient gene combinational therapy. The combination mechanism behind VEGF and Bcl-2 genes as well as in vivo synergistic efficacy should be further illustrated and investigated. Additionally, improved therapeutic effects could be achieved if the gold nanoparticles in this nanosystem are fully utilized for imaging or other therapies.

Generally, siRNA delivery nanocarriers use positive charges to compress and internalize the negatively changed siRNAs. However, this electrostatic interaction between nanocarriers and siRNAs can be easily disrupted by negatively charged biological macromolecules present in blood, causing the destabilization of siRNA nanomedicines, further leading to short blood circulation time and weakened therapeutic effects [94]. In order to optimize RNAi combinational gene therapy, stability of the siRNA delivery nanosystems must be improved. Our team constructed a siRNAs-encapsulated ROS-responsive nanomedicine (Ang-3I-NM@siRNA) stabilized by triple interactions including electrostatic, hydrogen bond and hydrophobic to co-target Polo-like kinase (PLK1) and VEGFR2 for treating GBM (Fig. 3**a**) [88]. Surface functionalization using Ang promoted significantly increased BBB penetration and nanomedicine accumulation in GBM cells by binding to the LDLR. Accordingly, the designed nanosystem enhanced the delivery of siRNAs into tumor tissues with the triple interactions strategy, effectively protecting siRNAs from degradation during circulation. Of noted, the elimination half-lifetime ($t_{1/2,\beta}$) of 3I-NM@siRNA was 37.5 min, much longer than 2I-NM@siRNA and 1I-NM@siRNA, at 25.4 and 7.8 min, respectively. Further experiments showed that PLK1 and VEGFR2 siRNAs synergistically reduced angiogenesis and potently induced GBM cell apoptosis. It was also demonstrated that Ang-3I-NM@siRNA nanomedicines had superb stability, prolonged blood circulation time, high transfection efficacy, controlled siRNAs release and gene knockdown effects, resulting in effective antitumor effects in vivo, culminating in an extended median survival time of 36 days in the orthotopic U87MG xenograft mouse models. This study provides an innovative nanosystem for delivering unstable gene therapeutics into the brain, and the potential of this system would be further boosted by combining two or more other gene targets with effective synergistic effects.

More recently, anti-miR-21 and anti-miR-124 were co-delivered by this triple-interaction nanosystem to further improve GBM therapy. As reported, miRNAs are capable of regulating more than one mRNA and so may advantageously modulate cancer cell proliferation and apoptosis in comparison to siRNA [95,96]. The combination of anti-miRNA-21 and miRNA-124 can simultaneously repair mutant renin-angiotensin system (RAS) and PTEN pathways, leading to favorable downstream effects on Phosphoinositide 3-Kinase (PI3K)-Protein Kinase B (AKT) signaling in GBM cells. Importantly, both miRNA-21 and miRNA-124 expression levels in U87MG cells were significantly down-regulated after treatment with dual miRNA nanomedicines, leading to potent inhibition of GBM invasion, growth and migration (Fig. 3**b**). In fact, tumor volumes and survival time of U87MG-bearing nude mice treated with Ang-3I-NM@miRNA nanomedicines showed only slight improvement compared to control treatments. Therefore, it is essential to consider whether this combination of these two miRNAs is an effective approach to improve anti-GBM effects.

As described, almost all molecular pathways and gene mutations in GBM are highly complicated, including multisite mutations. Thus, simultaneously targeting multiple genes, at more than two sites for each gene is required to effectively amplify GBM therapeutic effects. It has been shown that multiplexed RNAi therapies targeting four transcription factors (SOX2, OLIG2, SALL2 and POU3F2) concurrently against brain tumor-initiating cells (BTICs) can further improve resistance, limit recurrence and progression of diffuse gliomas. BTICs are difficult to combat due to their genetic heterogeneity and epigenetic aberrations [97]. In recent years, Green et al. designed a bioreducible poly (beta-amino ester) (PBAE) nanosystem to carry siRNAs targeting several anti-GBM genes including Roundabout homolog 1 (Robo1), yes-associated protein 1 (YAP1), sodium-potassium-chloride cotransporter (NKCC1), EGFR and survivin together to greatly improve apoptosis induction in GBM cells [90]. The PBAE is a highly effective siRNA delivery nanoplatform as it can be easily degraded by hydrolysis without non-specific toxicity. Robo1 is a protein related to tumor cell migration. YAP1 can support the growth of GBM cells. NKCC1 is an ion transporter affecting cancer metastasis. EGFR is an oncogene that has an abnormal expression in GBM cells. This study showed that the combination of five anti-tumor siRNAs in the PBAE nanosystem led to simultaneous knockdown of all targeted genes under a relatively low concentration of each siRNA (20 nM) (Fig. 3**c**), which resulted in the

Table 2
Summary of applications of monomodal combinational gene therapy.

Gene therapeutics	Nanocarrier	Targeted strategies	Responsive release designs	Major applications	Ref
EGFR siRNA+Bcl-2 siRNA	PAMAM entrapped gold nanoparticles	RGD	/	Significant gene silencing	[87]
PLK1 siRNA+VEGFR2 siRNA	PEG-P(Gu/Hb)	Ang	ROS	Improving therapeutic effects	[88]
miRNA-21+miRNA-124	PEG-P(Gu/Hb)	Ang	ROS	Further enhancing therapeutic effects	[89]
Robo1 siRNA+YAP1 siRNA+ NKCC1 siRNA+EGFR siRNA+surviving siRNA	(Bioreducible poly (beta-amino ester)) PBAEs	/	/	Enhancing apoptosis of GBM cells	[90]

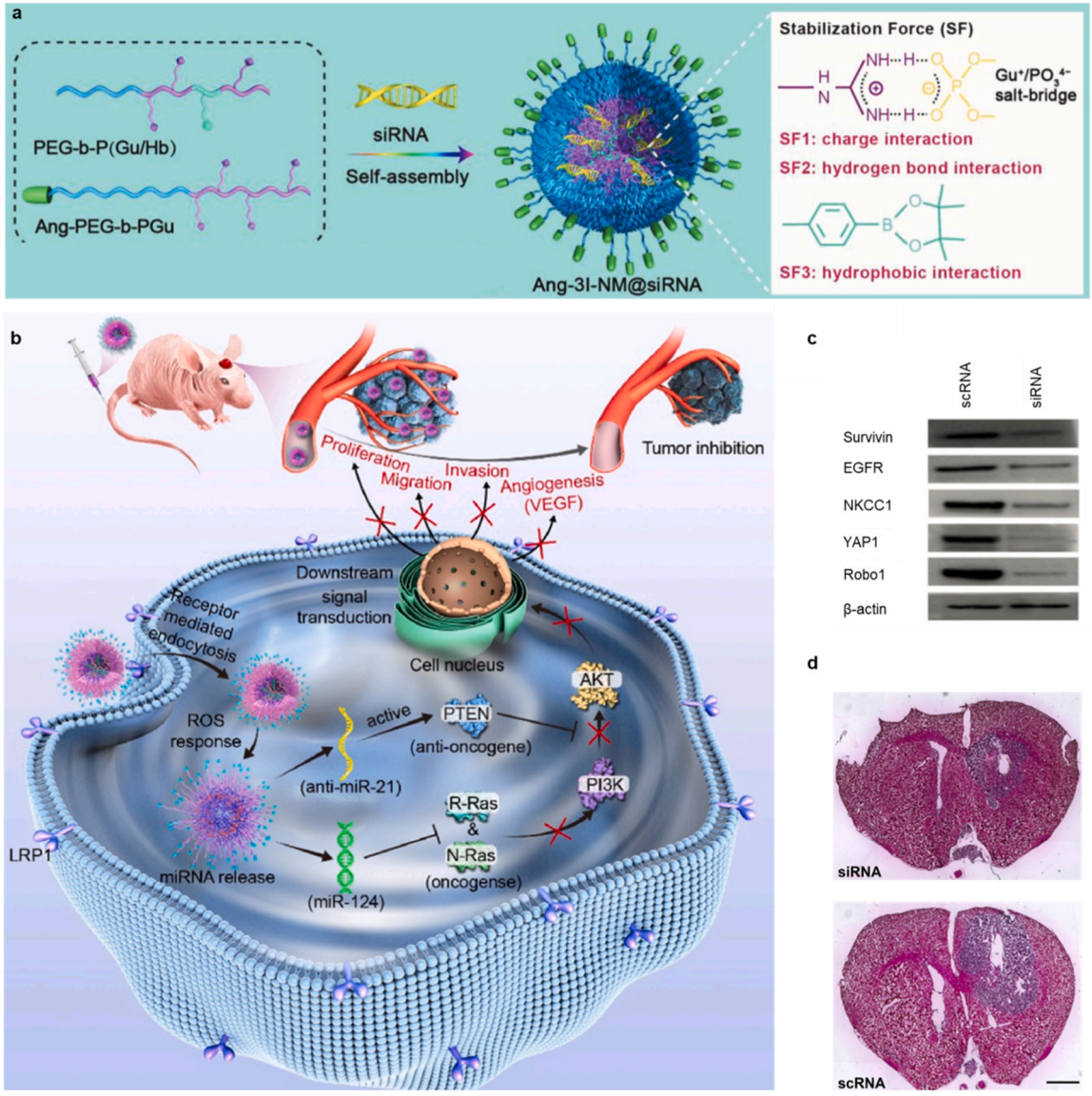

Fig. 3. (a) Schematic depicting the formation of Ang-3I-NM@siRNA nanoparticles stabilized by the three "triple-interaction" forces; electrostatic, hydrogen bond and hydrophobic interaction. (b) Dual miRNAs nanomedicine regulates the proliferation, migration, invasion of U87MG cells and angiogenesis in glioma. (c) Significant downregulation of five targeted genes in primary human GBM cells treated with nanoparticles containing a mixture of five siRNA sequences. (d) Co-delivery of five siRNAs reduced tumor growth in orthotopic GBM-bearing mouse models.
(a) Printed with permission from Ref [88]. (b) Printed with permission from Ref [89]. (c) (c-d) Printed with permission from Ref [90].

highly effective inhibition of GBM cell growth and migration, and a significant 50% reduction in the tumor burden in mouse models compared to the controls (Fig. 3**d**). However, the anti-tumor effects of therapy combining five genes were just enhanced slightly compared with those of single siRNA treatments, and it is important to investigate whether dual-gene or three-gene combined therapy could achieve more effective therapeutic effects, thereby reducing the complexity, risk of off-target effects and resource consumption of this system.

Monomodal combinational gene therapy has revealed enhanced anti-tumor efficacy without notable side effects, providing a new and promising way to manage GBM. Despite the considerable enhancement in gene delivery facilitated by nanosystems, the extent of survival extension remains relatively limited. This could be attributed to factors like off-target effects, delayed therapeutic effects and short-lived impacts of gene therapeutics. To address these limitations and optimize GBM treatment outcomes, gene therapy could be integrated with other distinct treatment modalities.

Agonist modulated monomodal therapy

Currently, nanotechnology based GBM combinational therapy has shown enhanced ability to penetrate the BBB, through passive targeting and further surface modification [34]. Despite these efforts, only less than 1% of targeting functionalized nanomedicines can successfully pass through the BBB and accumulate in GBM cells, which results in unacceptable systemic adverse effects and poor treatment efficacy, hence the BBB is still the greatest bottleneck preventing full realization of GBM treatment [98].

Fortunately, utilizing agonists can improve the BBB permeability of nanoparticles by momentarily opening the BBB through the activation of specific receptors, which in turn increases the intercellular space between endothelial cells (Table 3) [99]. As a result, increased numbers of nanoparticles can arrive and accumulate at GBM sites. Combining with receptor-mediated transcytosis may lead to further boosted BBB penetration. Exploiting this mechanism, our team developed Ang-grafted RBCms camouflaged acetylated dextran nanocarriers to transport DOX

Table 3
Summary of applications of agonist-based monomodal combinational therapies.

Agonists and therapeutics	Nanocarrier	Targeted strategies	Responsive release designs	Major applications	Ref
Lexiscan (Lex)+DOX	Acetalated dextran	Ang	pH	BBB penetration and elevated anti-GBM efficacy	[100]
SC79+PTX	Disulfide-linked glycolipid-like copolymer (CSssSA)	Ang	Redox	Opening BBB and improving therapeutic effects	[101]
R848+ LCL-161	Cyclodextrin-adjuvant nanoconstructs	/	/	Rebuilding the TME and achieving promoted immunotherapy	[105]
R848+CpG	poly(2-dimethylaminoethyl) methacrylate) (PDMA)	/	pH	Potentiating antitumor immunity and combinational therapeutic effects	[106]

and lexiscan (Lex) concurrently to brain tumor sites for agonist-enhanced GBM treatment (Fig. 4**a**) [100]. Lex is an A2A adenosine receptor agonist, which stimulates temporary opening of the BBB enabling functionalized nanoparticles to better penetrate the BBB (Fig. 4**b**). As mentioned above, Ang has a high affinity to the LDLR, facilitating nanoparticle accumulation in brain tumor sites (Fig. 4**b**) and RBCm-decorated nanoparticles further contribute to enhanced therapeutic effects by prolonging blood circulation time and reducing induction of immunogenicity. Collectively, these three design elements allow more nanoparticles to accumulate at brain tumor sites, resulting in greater GBM cell death. Subsequent in-vivo studies demonstrated that the multifunctional biomimetic synergistic nanomedicines notably improved blood circulation, increased BBB penetration (Fig. 4**c**), suppressed tumor growth and lengthened the medium survival time of orthotopic U87MG-bearing nude mice by 10 days relative to non-functionalized control nanoparticles. This study provides an effective nanoplatform for significantly enhancing BBB crossing with agonists for brain disease treatment. More lately, Hu et al. reported that inhibition of the VEGF-mediated signaling pathway greatly impaired BBB function [101]. Hence, they adopted an AKT agonist (SC79) to transiently re-open the BBB by blocking the VEGF-PI3K-AKT signaling pathway, ensuring enhanced delivery of Ang modified PTX loaded nanoparticles (Ang-CSssSA/P). The results demonstrated that treatment with SC79 disrupted BBB integrity by reducing the tight junction proteins, further leading to increased BBB permeability and depressed tumor marker by -2.6-fold when compared to non-SC79 treated groups,

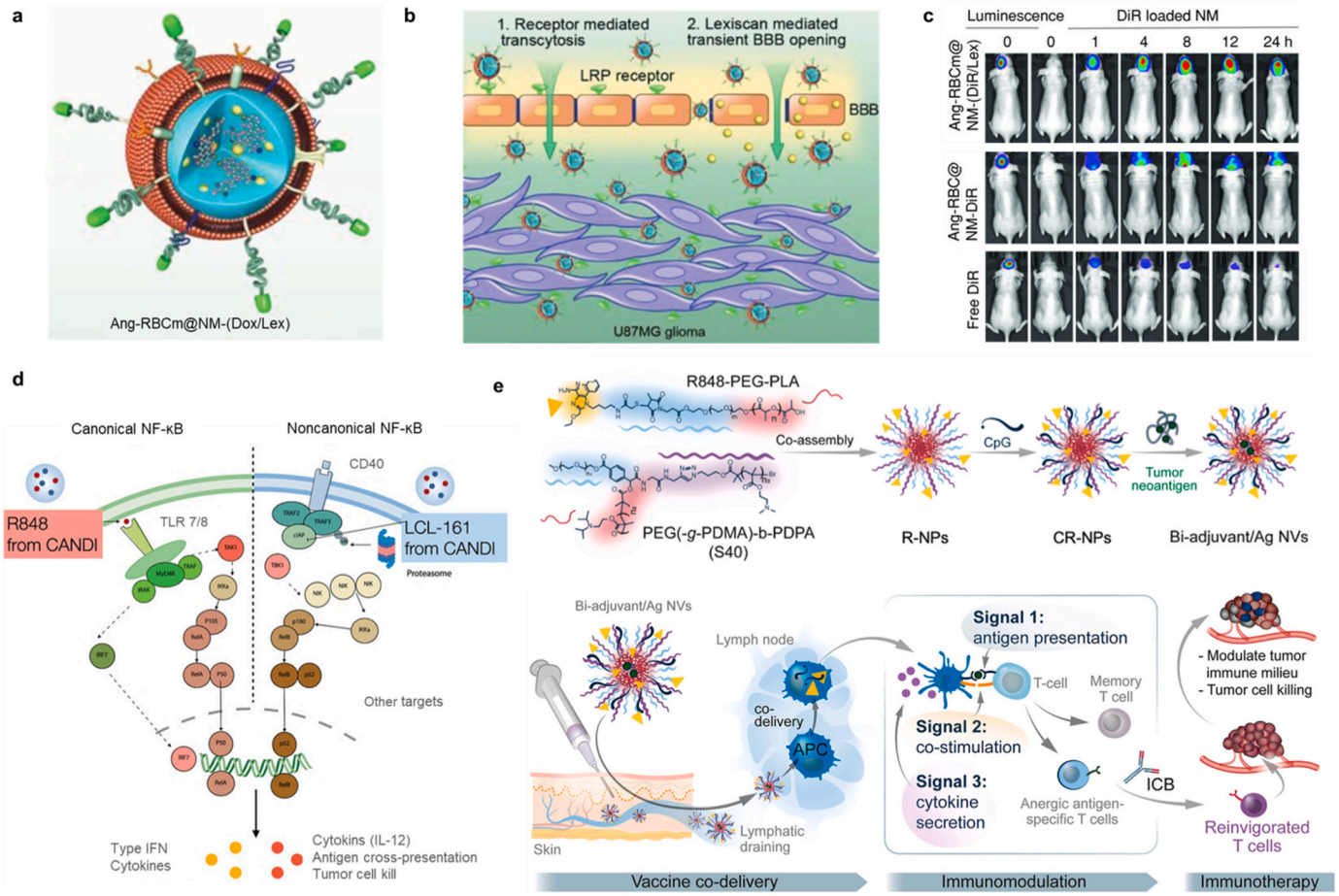

Fig. 4. (a) The structure of Ang-RBCm@NM-(DOX/Lex). (b) Mechanisms of Ang RBCm@NM-(DOX/Lex) traversal across the BBB. (c) Tumor uptake in nude mice bearing orthotopic U87MG-Luc after treatment with different nanomedicine formulations at different time points. (d) The cyclodextrin-based nanomedicines kill GBM cells by inducing the canonical NF-kB pathway resulting from R848 agonism of the TLR7/8 as well as LCL-161 inducing the noncanonical NF-kB pathway in myeloid cells. (e) Schematic illustration of the formation of nanomedicines containing dual agonists and their mechanisms of action for combination GBM immunotherapy.
(a) (a-c) Printed with permission from Ref [100]. (b) (d) Printed with permission from Ref [105] (c) (e) Printed with permission from Ref [106].

resulting in additive anti-tumor efficacy and significantly prolonging median survival time (30 days vs 19 days in non-SC79 nanoparticle treated group) of GBM-bearing mice. This study further confirmed that the combination of receptor-mediated crossing with agonist-mediated BBB opening effectively elevated the synergistic therapeutic effects for GBM. However, it is important to consider the dual roles of agonists, not only in opening the BBB, but also in potentially inhibiting tumor progress, which could further enhance the efficacy against GBM. Additionally, there are concerns regarding the potential damaged effects of opened BBB because this may cause harmful substances to enter the brain. Therefore, careful consideration should be given to the application of agonists for BBB opening, and further investigation to ensure safety.

Beyond temporarily opening the BBB, agonists have been explored as effective and potential therapeutics for improved GBM combination treatment as agonists can effectively trigger immune responses and long-term immunity to suppress tumor proliferation [102–104]. Therefore, the combination of agonists with anti-tumor agents is likely to potentiate GBM suppression (Table 3). Weissleder et al. developed a nanoparticle to stimulate double immune-related pathways by co-loading a Toll-like receptor 7 and 8 (TLR7/8) agonist (R848) with a cellular inhibitor of apoptosis protein inhibitor (ICL-161) for rebuilding the GBM TME [105]. In this study, highly cross-linked cyclodextrin-modified polymeric nanosystems were developed to encapsulate these two drugs. This nanocarrier showed myeloid cell targeting ability and high drug loading efficiency. Interestingly, ICL-161 also can act as an immunomodulator. These dual agents loaded nanomedicines induced higher levels of IL-12 and IFN cytokines and increased T effector cell production more substantially than single agent nanoparticles, since non-canonical and canonical NF-kB signaling pathways were activated simultaneously (Fig. 4**d**). In this way, a highly immune-activated TME was induced, paving a solid path for controlling GBM progression. Accordingly, survival of mice bearing orthotopic GBM treated with the combination nanomedicine compared to mono-drug control groups was significantly improved. Accordingly, after 60 days, 30% of mice treated with the dual-loaded nanomedicine survived, while no mice receiving any other control treatments survived.

Nevertheless, immune therapies based on TLR7/8 agonists have limitations in treating GBM because TLR expression regulation is complicated and can lead to multiple off-target expression patterns. Thus, stimulating multiple TLR pathways at the same time by a combination of two, or more, agonists has the potential to elicit elevated GBM therapeutic effects. Zhu et al. designed ionizable polymeric nanocarriers to co-deliver R848 and TLR9 (CpG) TLR agonists to the brain [106]. To further promote the potential of immunotherapy, the Ags peptide, SIINFEKL, was also encapsulated in this nanocarrier. The ionizable nanosystem was based on cationic poly (2-dimethylaminoethyl) methacrylate) (PDMA) that could optimally load negatively-charged CpG, PEG shields for enhanced biocompatibility and pH-responsiveness of the poly (1-(diisopropylamino) ethyl methacrylate) (PDPA) shell (Fig. 4**e**). This multifunctional nanocarrier resulted in improved pharmacokinetic profile, reduced immunogenicity, decreased systemic toxicity and most importantly, enhanced synergistic immunostimulation. The codelivery of dual agonists and peptide neoAgs potentiated GBM innate and adaptive immune responses (Fig. 4**e**), evident by markedly increased levels of antitumor T cells and induction of robustly immune-activated TME in orthotopic GL261 GBM-bearing mice administered the smart nanovaccines. This platform shows promise in inhibiting the occurrence, progress and recurrence of GBM and achieving personalized immunotherapy by applying heterogeneous tumor Ags to overcome the heterogeneity and specific immunosuppression.

The application of agonists to enhance the BBB permeability of nanoparticles shows great promise in GBM therapy. However, there is still a long way for the further application of agonist-mediated BBB opening regarding safety. Additionally, agonists bolster immune therapy and enhance GBM combination treatments. The therapeutic cooperation of agonists with immune therapeutics, or others, offers an effective approach to finally achieve more optimal therapeutic outcomes.

Bimodal combinational therapy

While evidence has demonstrated that monomodal nanosystem combinational therapy can, to some extent, overcome several challenges associated with GBM treatment, combining different therapeutic modalities should result in even better efficacy, reflecting an additive potentiation afforded by the combination of two distinct patterns of action. Over the years, research in bimodal combinational therapy has experienced explosive growth, providing an effective platform for GBM treatment. The most common forms of bimodal therapy include chemo-gene therapy, chemo-immune therapy, dynamic therapy-based, photothermal therapy-based and radiation-based synergistic therapy.

Chemo-gene therapy

Chemotherapy and gene therapy are indeed the backbone of GBM treatment, but each has certain drawbacks that can shadow their clinical outcomes. For chemotherapy, the development of drug resistance and systematic toxicity are clear limitations [107]. On the other hand, while gene therapy offers high precision and minimal toxicity, it tends to have a slower onset of action and has limited effects when administered ‘naked’ [108]. Considering this situation, substantial efforts have been focused on developing novel strategies that combine chemotherapy and gene therapy, aiming to complement each other and finally elevate therapeutic effects for GBM. Currently, TMZ remains the standard of care for GBM due to its potent antitumor power and it is because of this, that chemo-gene combinational therapies are mainly built around TMZ treatment, with the goal to directly or indirectly limit MGMT mediated drug resistance to TMZ, without generating additional side effects. In this regard, gene therapies have been proven to directly target MGMT expression, silencing its activity to sensitize GBM cells to TMZ. Zhang et al. combined TMZ with MGMT siRNA, which were delivered by an iron oxide (Fe_3O_4) nanosystem featuring an engrafted targeting chlorotoxin (CTX) ligand [109]. These nanocarriers were able to penetrate BBB, actively target and enter GBM cells through CTX receptor-mediated transcytosis. The accumulated siMGMTs, subsequently silenced the MGMT gene, further sensitizing both GBM cells and GBM stem-like cells to TMZ (Fig. 5**a**). The results showed that the survival time of mice administrated with chemo-gene combinational nanomedicines was prolonged 8.8-fold, compared to mice treated with TMZ alone (Fig. 5**b**). Directly targeting the pathways responsible for drug resistance could more straightforwardly and powerfully overcome this challenge, leading to superior therapeutic outcomes.

Additionally, there are many strategies to remodel MGMT expression indirectly by targeting other closely related pathways to MGMT. Our group developed the Ang-PEG-b-PFPMA polymeric nanosystem for the co-delivery of TMZ and retinoblastoma binding protein 4 (RBBP4) siRNA to achieve GBM chemo-RNAi synergistic therapy [110]. The targeted polymeric nanocarriers can effectively load different kinds of drugs, penetrate BBB and actively infiltrate GBM cells. Incorporation of siRBBP4s was shown to effectively downregulate the expression of RBBP4 leading to reduced expression of MGMT, as evidenced by western blot results. Furthermore, the sensitivity of GBM cells to TMZ was significantly improved and siRBBP4/TMZ nanomedicines showed superior synergistic effects as the median survival time of GBM tumor-bearing mice was extended to 40 days from 32 days mediated by treatment with nanocarrier containing solely TMZ. Although this polymersomal nanosystem provides a platform for loading multiple drugs simultaneously, the reduction of MGMT expression shows a discount by indirect regulation.

As mentioned above, positive charged siRNA delivery nanocarriers have certain weaknesses, including easy degradation and cation-

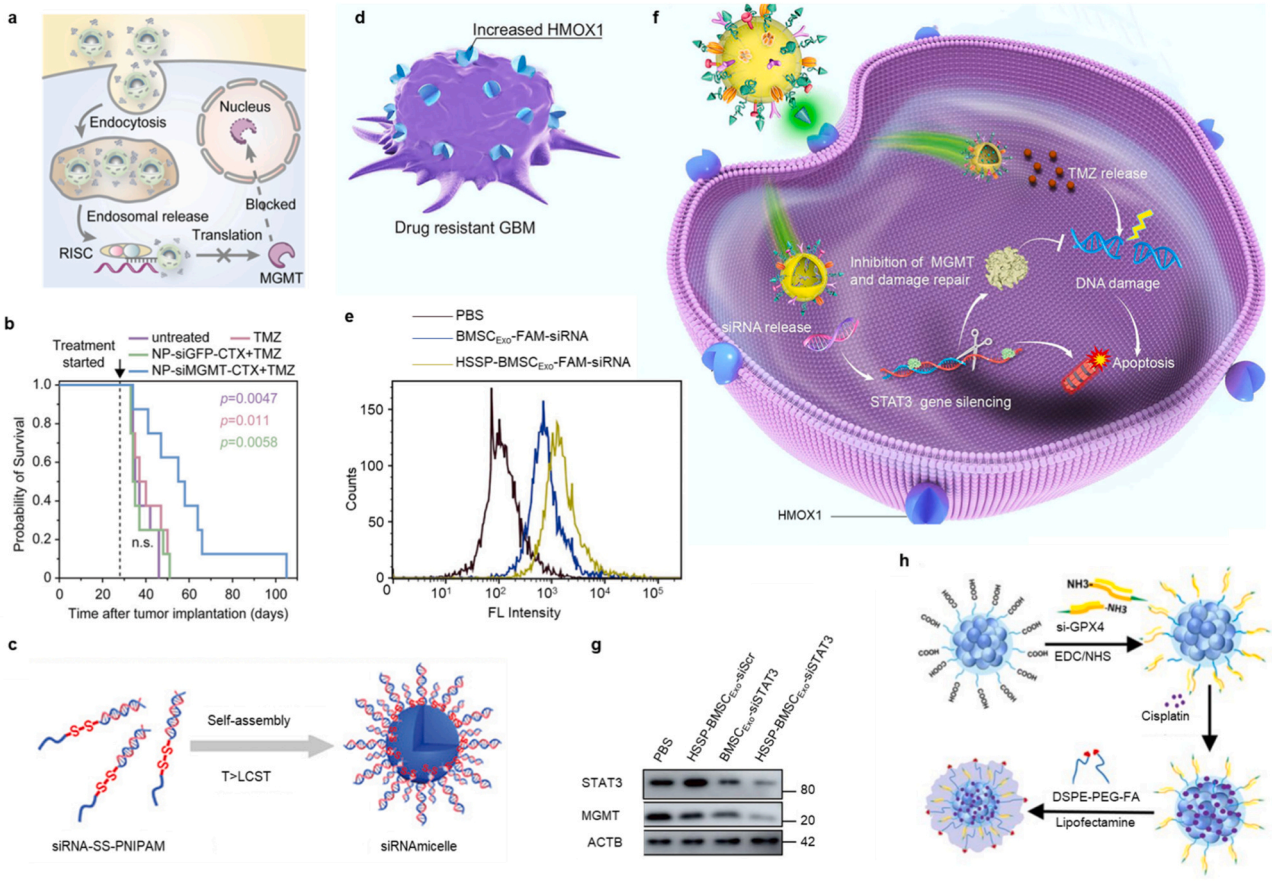

Fig. 5. (a) Schematic illustration of the mechanism of NP-mediated knockdown of MGMT expression. (b) Survival of mice after treatment with the iron oxide (Fe_3O_4) nanosystem, NP-siMGMT-CTX+TMZ or relevant control formulations. (c) Schematic illustration of the formation of siRNA micelles. (d) High HMOX1 receptor expression on the TMZ-resistant GBM cell surface. (e) Flow cytometry of U251^R cells after treatment with HMOX1 decorated nanoparticles. (f) HMOX1 modified nanoparticles penetrate BBB and target TMZ resistant GBM cells, to further achieve siSTAT3/TMZ synergistic therapy. (g) Protein expression in the STAT3-MGMT signaling axis after treatment of siSTAT3/TMZ loaded nanomedicines. (h) Schematic of the fabrication and drug loading process of IONPs.
(a) (a-b) Printed with permission from Ref [109]. (b) (c) Printed with permission from Ref [111]. (c) (d-g) Printed with permission from Ref [112]. (d) (h) Printed with permission from Ref [113].

associated cytotoxicity. To address these challenges and further amplify the synergistic effect on TMZ-resistant GBM, a cation-free polymeric siRNA micellar spherical nucleic acid (SNA) was developed by our team for chemical drug and siRNA co-delivery [111]. This innovative SNA is based on self-assembling siRNA-disulfide-poly(N-isopropylacrylamide) (siRNA-SS-PNIPAM) diblock copolymers, which offer several key advantages, including enhanced RNA stability, no charge-associated toxicity, enhanced BBB penetration and tumor internalization through scavenger receptor-mediated endocytosis and temperature/redox-controlled drug release (Fig. 5**c**). Importantly, when TMZ and signal transducer and activator of transcription 3 siRNA (siSTAT3) were loaded together, the siRNA micelles showed a significant robust combinational therapy effect against TMZ-resistant GBM, as reflected by the weakest tumor signals and longest median survival time (46 days). In addition, histochemical staining, blood analysis and proinflammatory cytokines evaluations showed that these micelles had good biocompatibility. The innovative siRNA micelles showed remarkable synergistic effects against TMZ-resistant GBM, providing a versatile drug co-delivery BBB penetrating system. However, the mechanism regarding siSTAT3 improving TMZ resistance needs further clarification.

Although polymeric nanomaterials have obvious highlights in small-molecule drug and gene therapeutic delivery, systematic toxicity and immune clearance are still major barriers to optimal clinical efficacy. Therefore, nanosystems with better biocompatibility are required to further improve chemo-gene combinational GBM treatment. Biomimetic nanotechnologies provide an exciting potential to achieve this goal. Accordingly, our group changed the polymeric nanocarrier to exosomes derived from bone marrow mesenchymal stem cells to encapsulate TMZ and siRNA for GBM chemo-gene combinational therapy [112]. Firstly, the biomimetic exosomes were found to possess great biocompatibility and low immunogenicity, thus resulting in enhanced blood circulation time. In addition, exosomes were also decorated with heme oxygenase-1 (HMOX1) specific short peptide (HSSP) for targeting TMZ resistant GBM cells that overexpress HMOX1 receptors (Fig. 5**d**). These innovations resulted in greatly improved BBB penetration and cellular uptake by U251^{R}cells compared to non-functionalized carriers (Fig. 5**e**). These studies also showed that down-regulating MGMT expression by reducing STAT3 level by siSTAT3 led to enhanced TMZ sensitivity and apoptosis of TMZ-resistant GBM cells (Fig. 5**f, g**). Co-delivery of TMZ and siSTAT3 also showed significant DNA damage, retarded tumor growth in-vivo and 30-day longer survival of GBM-bearing mice than mono TMZ or siSTAT3 nanomedicine administration groups, without detectable side effects. In recent years, exosomes have emerged as promising natural carriers for delivering various therapeutics in brain disease treatments. Thus, more systems based on different kinds of cell-derived exosomes should be developed and applied to advance GBM combinational therapy.

Undoubtedly, other small-molecule chemical drugs could be successfully combined with gene therapy for GBM synergistic treatment. Using iron oxide nanoparticles (IONPs), for example, Ni et al. successfully co-delivered glutathione peroxidase 4 siRNA (siGPX4) and platinum (Pt) (Fig. 5**h**) [113]. The IONPs were modified with folate to

enable effective targeting and accumulation in GBM cells via receptor-mediated transcytosis. Upon release, Pt initially destroyed nuclear DNA as well as mitochondrial DNA and persistently increased H_2O_2 level by activating nicotinamide adenine dinucleotide phosphate oxidase (NOX), all of which caused GBM cell apoptosis. Release of siGPX4 inhibited GPX4 expression, similarly inducing robust apoptosis of GBM cells. Furthermore, the Fenton reaction was notably induced due to increased iron levels (Fe^{2+}, Fe^{3+}) and abundant H_2O_2-initiated ferroptosis, further synergistically enhancing therapeutic efficacy. Their results demonstrated that the combinational nanomedicines enhanced GBM inhibition through both genetherapy mediated ferroptosis and chemotherapy induced apoptosis, leading to potent anti-GBM activity in U87MG GBM-bearing mice, which was proved by the lowest levels of tumor bioluminescence and longest median survival time (38.3 days).

Collectively, implementation of chemo-gene bimodal combinational therapy has provided significant evidence of its potential to enhance the effectiveness of anti-GBM strategies. This was confirmed by studies assessing TMZ-based treatments by overcoming drug resistance through the direct downregulation of MGMT expression or regulation of MGMT-associated pathways with gene tools. In addition, the use of engineered nanosystems, like non-cation nanoparticles and exosome-based nanocarriers, further amplified anti-GBM efficiency by reducing toxicity. The success of this approach underscores its versatility and opens the door for broader applications. By utilizing the power of other types of small-molecule drugs and gene therapeutics, GBM treatment can be further diversified and optimized.

Chemo-immunotherapy

In recent decades, chemo-immunotherapy has developed into one of the most effective combination strategies against GBM. Initially, the human immune system attempts to eliminate tumor cells during the early stages of tumor formation. However, progressive tumors can evade immune recognition and destruction by reducing immunogenicity and creating an immunosuppressive TME, characterized by the secretion of immune-suppressive cytokines and elevated levels of PD-L1. The immunosuppressive TME not only promotes tumor survival and progression but also contributes to tumor resistance against treatments [26]. In particular, the effects of chemotherapy are seriously hindered by the immunosuppressive TME. On the other hand, immunotherapy kills tumor cells by activating and restoring normal anti-tumor immune responses by immune checkpoint suppression, use of small-molecule inhibitors or vaccines. Proof of concept studies demonstrate that the efficacy of chemotherapy can be significantly improved when the immunosuppressive TME is modulated by immunotherapy [114]. Additionally, chemotherapy sensitizes tumor cells to cytotoxic T cells, further enhancing the potential benefits of immunotherapy and reducing side effects [115].

Immunotherapy mainly comprises immune checkpoint inhibitors and immune cell therapy [116]. Immune checkpoint inhibitors are regarded as one of the most effective approaches for cancer treatment. Countless studies have highlighted the value of immune checkpoint inhibitor therapy, in particular, using PD-1/PD-L1, in combination with chemotherapy drugs in GBM [117]. Li et al. encapsulated PTX and

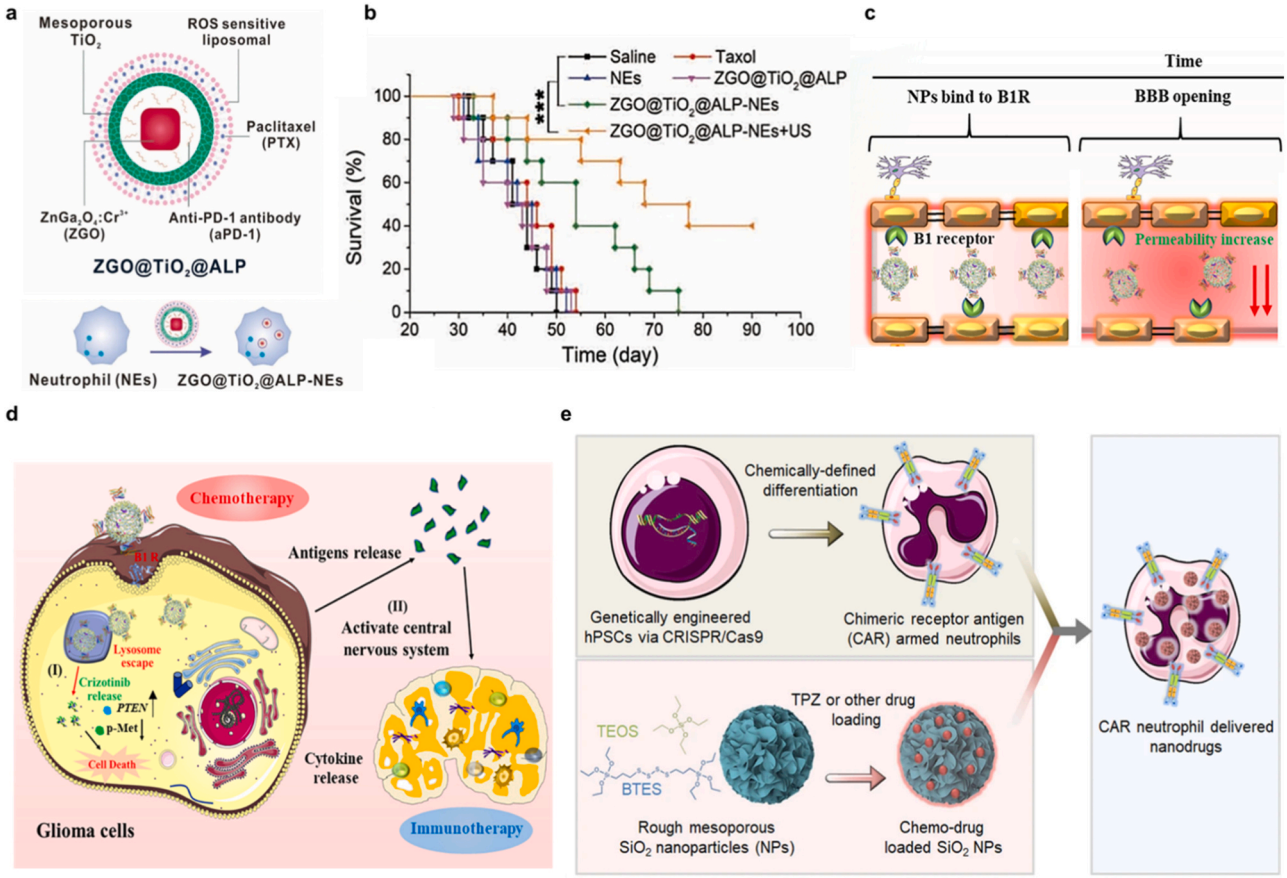

Fig. 6. (a) Composition of ZGO@TiO2@APL nanomedicine loaded with neutrophils. (b) Survival times of GBM-bearing mice treated with ZGO@TiO2@ALP-NEs + US treatment and relevant formulation controls. (c) BK containing nanoparticles transiently open the BBB through binding B1R. (d) Induction of chemo/immunotherapy is achieved by anti-PD-L1 antibodies and CZT co-loaded nanomedicines. (e) Schematic illustration of formulation of CAR-neutrophils containing chemo-drug loaded silica nanoparticles (SiO_2 NPs).
(a) (a-b) Printed with permission from Ref [118]. (b) (c-d) Printed with permission from Ref [119]. (c) (e) Printed with permission from Ref [122].

anti-PD-1 antibody into the nanocarrier (ZGO@TiO2@APL), which was composed of a ZnGa2O4:Cr^{3+} (ZGO) core and a hollow sono-responsive TiO2 shell (Fig. 6**a**) [118]. The nanocarriers were first covered with ROS-sensitive liposomes for controlled drug release and further coated in neutrophils (NEs) to promote BBB penetration and GBM accumulation. In the hyper-ROS tumor environment, the liposome covers detached, exposing the sono-sensitive shells. US irradiation further triggered ROS generation by the shells, resulting in rapid release of PTX and anti-PD-1 antibodies. PTX inhibits proliferation of GBM cells directly and anti-PD-1 antibodies modify the local TME by specifically binding to PD-L1 on the surface of tumor cells. The 90-day survival rate of the GBM bearing mice administrated with these nanomedicines increased to 40%, compared to 0% for mice treated with control formulations, indicating successful induction of long-term immuno--surveillance combined with chemotherapeutic apoptosis (Fig. 6**b**). Anti-PD-L1 antibodies have also been combined with different small-molecule chemical drugs. Sun et al. explored the therapeutic effects of co-loaded magnetic nanomedicines, BK@MTNPs, fabricated to contain anti-PD-L1 antibodies and crizotinib (CZT) [119]. This delivery platform contained bradykinin (BK), which transiently opens the BBB and increases targeting of GBM cells (Fig. 6**c**). CZT is an inhibitor of protein kinase c-Met, a marker for stem-like GBM cells (GSCs). Upregulation of c-Met promotes malignant GBM progression, migration and recurrence. Hence, release of CZT at GBM sites effectively inhibited proliferation of GSCs and induced GBM cell death by reducing the level of c-Met. Anti-PD-L1 antibodies, on the other hand, augmented immune responses by attenuating immunoescape mediated by the TME (Fig. 6**d**), as indicated by noticeable increases in M1 macrophages, cytotoxic T lymphocytes and apoptotic GBM cells. Importantly, promising anti-GBM activity of BK@MTNPs was observed in GBM bearing mice with good extension of survival time, 50% of mice survived on day 60, without significant side effects. This study developed a smart nanosystem that could target and inhibit the proliferation of GSCs, providing a meaningful platform for cancer treatment. GSCs play crucial roles in drug resistance, metastasis and recurrence, so it is important to target them to completely remove GBM.

Additionally, immune checkpoint related chemoimmunotherapy has also been achieved by the delivery of dual small molecule chemical agents using multifunctional nanosystems. For instance, our laboratory encapsulated TMZ and the epigenetic bromodomain inhibitor (OTX015) within the biomimetic nanoparticle, ABNM@TMZ/OTX, for GBM chemoimmunotherapy [120]. This nanosystem exhibited superior blood circulation, effective BBB penetration, active GBM cell uptake and responsive drug release, facilitated by functionalization of ApoE decorated RBC membrane onto pH-sensitive nanocarriers. In vitro and in vivo studies showed that OTX015 effectively activated the immune system by inhibiting PD-1/PD-L1 conjunction and induced immunogenic cell death, thereby amplifying the anti-GBM effects of TMZ, by reducing TMZ drug-resistance through the suppression of cellular DNA repair pathways. In mice treated with ABNM@TMZ/OTX, T cell levels in lymph nodes, tumors, and blood were 3 times higher than the PBS group and twice as high as the ABNM@TMZ and ABNM@OTX groups. Finally, these nanomedicines significantly extended survival of GBM bearing mice and effectively avoided tumor recurrence of GL261 tumor surgically resected mouse models by mediating long-term immune surveillance.

The recent advent of function-engineered immune cells has set a new direction in cell immune therapy [121]. Chimeric antigen receptor (CAR) modification technology has significantly boosted the anti-tumor activity of T cells or natural killer cells. CAR-T cell therapy now stands out among various cell immune therapies mediating precise tumor targeting and higher safety. To genetically engineer human pluripotent stem cells, Bao et al. utilized CRISPR/Cas9 technology with neutrophil-specific signaling domains, as these are more susceptible to gene editing and can readily differentiate into neutrophils, to allow an unlimited supply of CAR-neutrophils (Fig. 6**e**) [122]. At the same time, a chemical pro-drug triapazamine (TPZ) was loaded into mesoporous organic silica nanoparticles, which were subsequently bagged into CAR-neutrophils, which inherit, from natural neutrophils, the ability to traverse biobarriers and thereby induce lower immunogenicity. In the hypoxic TME, hypoxia-activated pro-TPZ is simultaneously dislodged from the nanoparticles and activated, inducing DNA damage in GBM cells. The CAR-neutrophils exhibited a significantly higher BBB penetration ratio of 22%, in contrast to non-neutrophil-leading nanoparticles, which achieved only 6%. Notably, reduction of the immunosuppressive character of the TME by CAR-neutrophils resulted in nearly complete tumor elimination and significantly prolonged the median survival time, 90 days, of GBM bearing mice without generating additional inflammation. Cellular immunotherapy-based combinational therapies have great promise in the clinical application of GBM. By utilizing patients' own immune cells, such as T cells and dendritic cells, the GBM cells are specifically and effectively attacked without triggering immunological rejection. When combined with other therapies, the overall outcomes of GBM patients might be improved.

Immunotherapy has been proven to be a powerful strategy to reverse the immunosuppressive TME in GBM, thereby significantly boosting synergistic effects of chemo-immunotherapy combinations. By integrating immunotherapy with other therapeutic modalities, GBM cells face a dual attack on both external (TME) and internal (drug-action) fronts, leaving little room to evade destruction. This strategy provides an exciting prospect for combating GBM and its potential will be sure to find further realization.

Dynamic therapy-based bimodal therapy

Dynamic therapies, depending on ROS overgeneration in tumor sites through different exogenous and endogenous stimuli, have found appeal in recent years. These include photodynamic therapy (PDT), sonodynamic therapy (SDT), chemodynamic therapy (CDT), electrodynamic therapy (EDT) and microwave dynamic therapy (MDT) [123]. Due to their focused and non-invasive nature, dynamic therapies mediate negligible side effects and have become a novel and promising option for GBM treatment. To further improve anti-GBM efficacy, multiple dynamic therapies have been combined with other treatment modalities, including chemotherapy, gene therapy and immunotherapy.

PDT is the first ROS-based dynamic therapy. The photosensitizer (PS) activated by light at specific wavelengths, plays a central role in PDT treatment [124]. Under laser irradiation, PSs cause oxygen molecules to generate highly toxic ROS, which results in the death of tumor cells through apoptosis or necrosis. As PDT suffers from limited laser brain penetration, PDT has been combined with chemotherapy and immunotherapy to help remedy this disadvantage. For instance, Gao et al. developed DOX-loaded ultra-small $Cu_{2-x}Se$ nanoparticles (CS-D NPs) to induce robust anti-GBM effects [125]. In this nanosystem, $Cu_{2-x}Se$ NPs could penetrate the BBB effectively with the assistance of the US, leading to significantly improved NP accumulation in GBM. Once irradiated by a 1064 nm laser, abundant ROS was generated by $Cu_{2-x}Se$ NPs, which significantly induced apoptosis of GBM cells, synergistically enhancing the chemotherapeutic activity of DOX. The bioluminescence intensities of mice after 16-day treatments with US+PBS+1064, US+CS-D, CS-D+1064 and US+CS-D+1064 were 9.4, 29.8, 34.9 and 44.7-fold larger than those at the day 0, followed by 16-day longer survival time of US+CS-D+1064 group compared with monotherapy groups, suggesting that the combination of PDT and chemotherapy could more effectively suppress GBM growth. Likewise, Shen et al. adopted a clinically approved near-infrared (NIR) dye, indocyanine green (ICG), combined with the small-molecule drugs SN38 or PTX for improved and safer PDT/chemotherapy [126]. The amphiphilic ICG and hydrophobic drugs could be self-assembled into nanoparticles without any excipients, which prevented intramolecular self-aggregation. The cell toxicity studies performed on U251 and U87MG cells showed that these nanomedicines elicited an obvious synergistic killing effect in comparison to

single therapy. More recently, Luo et al. constructed biomimetic nanogels for co-delivering TMZ and ICG to inhibit orthotopic GBM. ApoE-modified RBCm nanogels resulted in prolonged circulation time, good BBB penetration and active GBM targeting by virtue of the low immunogenicity mediated by the biomimetic camouflaging and receptor-mediated transcytosis of ApoE (Fig. 7**a**) [127]. In addition, under near infrared irradiation, ICG and TMZ were released rapidly from the nanogels (over 80%) and generated large amounts of ROS as well as inducing DNA damage, together leading to the death of GBM cells. More importantly, the co-loaded biomimetic nanogels effectively extended median survival time to 69 days in U87MG GBM-bearing mice and 63 days in GBM stem cells-bearing mice respectively, which was significantly longer than approximately 35 days seen in both TMZ monotherapy and ICG monotherapy. The smart designs of nanosystems for BBB penetration and targeted drug delivery lay the foundation for the improved effects. However, to maximize the combinational therapeutic efficacy, the synergistic points behind these PS and chemical drugs should be clearly investigated and understood firstly.

Considering that PDT also can activate the immune system by initiating the release of Ags, combining PDT with standard immunotherapies could yield potent synergistic anti-GBM effects. Sun et al. developed a multifunctional nanosystem to carry the PS (5-ALA) and anti-PDL1 antibodies (aPDL1) into GBM tissues [128]. The nanocarriers were conjugated with the B1R kinin ligand for BBB traversal and active GBM targeting, exploiting the fact that the B1 receptor is over expressed by both endothelial cells and GBM cells. Upon 980 nm laser excitation, the released 5-ALA is converted to protoporphyrin IX, producing ROS, leading to greatly enhanced tumor cell damage. Induction of GBM immunogenic death causes inflammation stimulating the recruitment of T cells to the TME which, in turn, attacks GBM. Moreover, the aPDL1 blocked PDL1 on the surface of GBM cells, resulting in the secretion of various anti-tumor immune Ags as well as recruitment of CD4+ and CD8+ T cells. These events resulted in potent elimination of GBM cells. Additionally, long-term immune memory also led to effective prevention of GBM recurrence (Fig. 7**b**), constituting a tremendous advance in GBM treatment.

SDT is an emerging dynamic therapy which generates toxic levels of ROS by the activation of sonosensitizers by the US. Compared to PDT, US possesses deeper penetration, which may lead to greater potential in GBM treatment. Accordingly, chemotherapy and immunotherapy have recently been combined with SDT against GBM. For example, Zhou et al. combined chemotherapy and SDT, achieved by co-delivery of DOX and the sonosensitizer chlorin e6 (Ce6) [129]. In this study, a novel GSH-responsive poly (2,2″-thiodiethylene 3,3″-dithiodipropionate) (PTD) polymer was developed and engineered for brain penetration by neutrophil elastase (NE)-triggered shrinkability, iRGD-mediated targeting and lexiscan-induced BBB opening. These decorations effectively enhanced nanomedicine uptake in GBM tissues. The high level of GSH in the TME led to GSH-responsive DOX and Ce6 release. $^1O_2$ production increased 12-fold by the US, demonstrating that superior sonodynamic effects can be induced by the US. Finally, treatment with these multifunctional nanomedicines effectively inhibited tumor growth and extended the median survival time to 57 days of GBM-bearing mice, which was significantly longer than that achieved by treatment with single DOX or single Ce6 loaded group. In this study, multiple strategies were employed together for enhancing BBB penetration. Despite these efforts, the observed synergistic effects were modest, possibly attributed to non-specific interactions between DOX and Ce6. In another study, Cai et al. prevented chemoresistance by combining SDT and chemotherapy [130]. The biodegradable and pH-sensitive polyglutamic acid (PGA) polymer was synthesized to encapsulate DOX and then camouflaged with GBM cell membranes. The nanomedicine (MDNPs) displayed good biocompatibility, homologous targeting, BBB penetration and controlled release, enhancing the anti-cancer activity of DOX. The fluorescence intensity at U87MG tumor sites of MDNPs+laser group was over 4-fold greater than that in groups without membrane coating and responsive release. Not only is DOX a small-molecule chemical drug, but it also acted as a sonosensitizer in this study. Extra ROS was generated by DOX upon US stimulation and it induced GBM cell apoptosis by increasing sensitivity to chemotherapy by downregulating heat shock factor 1 expression and P-glycoprotein generation. Studies in orthotopic GBM

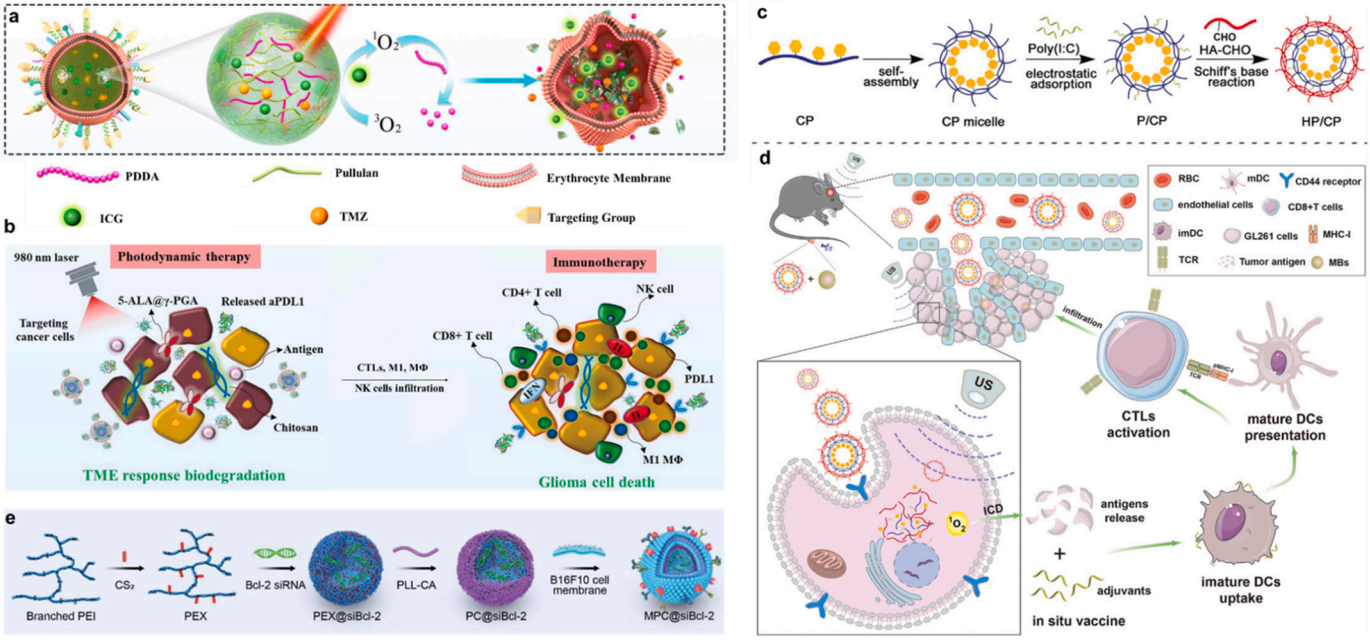

Fig. 7. (a) ApoE-modified erythrocyte membrane-cloaked near infrared-activatable nanogels release loaded TMZ and ICG under NIR irradiation. (b) Schematic illustration of photodynamic therapy and antitumor immune responses induced by ALA and aPDL1 loaded 5-ALA@γ-PGA nanomedicines. (c) Schematic of the formulation of HP/CP nanosonosensitizers. (d) Targeted SDT and immunotherapy of GBM by HP/CP. (e) Schematic illustration of the fabrication of biomimetic MPC@siBcl-2 nanomedicines.
(a) Printed with permission from Ref [127]. (b) Printed with permission from Ref [118]. (c) (c-d) Printed with permission from Ref [131]. (d) (e) Printed with permission from Ref [134].

tumor bearing mice confirmed that these nanomedicines had SDT-enhanced chemotherapeutic efficacy as 80% of the mice treated with MDNPs+laser survived and no mice were alive in other groups on day 23. The authors used one single drug to achieve bimodal synergistic treatment, providing insights into designs of GBM combinational therapies based on single therapeutic.

Similar to PDT, SDT has been shown to activate immunogenic cell death (ICD) in tumor cells, which further induces adaptive immune responses by releasing endogenous tumor-associated antibodies (TAAs) and activating damage-associated molecular patterns (DAMPs). Therefore, combining SDT with immunotherapy is also a potential strategy for GBM treatment. Recently, a sonosensitizer protoporphyrin IX (PpIX) and an immune adjuvant Poly(I:C) were co-grafted onto chitosan oligosaccharide (COS) by electrostatic adsorption and then hyaluronic acid (HA) was cross-linked yielding HP/CP nanomedicine for GBM targeting (Fig. 9**c**) [131]. When HP/CPs were injected together with microbubbles (MBs), tight endothelial cell junctions were precisely disrupted by US, thus non-invasively opening the BBB. Consequently, abundant nanoparticles accumulated in GBM tissues benefiting from HA-mediated active targeting and highly effective BBB penetration. Upon US, PpIX generated significant ROS, simultaneously resulting in mitochondrial and DNA damage. Subsequently, TAAs and Poly(I:C) vaccines were released by dead GBM cells in situ, activating antitumor immune responses thereby transforming GBM from immunogenically "cold" to "hot", further inhibiting GBM growth (Fig. 7**d**). This work showed that sonoimmunotherapy has great potential for BBB opening, GBM suppression and immune system activation.

PDT and SDT depend on external stimuli to trigger sensitive agents to produce ROS and kill GBM cells. Although these dynamic modalities have been applied in a variety of cancer treatments, their therapeutic effects are significantly restricted by the limited tissue penetration of external irritation, especially for GBM. Therefore, ROS-based dynamic therapies that utilize endogenous molecules to stimulate specific chemical agents to produce ROS have been developed in recent years. CDT is an approach that relies on small-molecule agents activated by certain factors in the tumor TME, like H^+, GSH or H_2O_2, to initiate or enhance Fenton or Fenton-liked reactions in tumor tissues, inducing toxic levels of ROS [132]. Numerous studies have demonstrated that metal-based nanomaterials could be employed as Fenton agents for GBM treatment, such as Cu and Fe ions [133]. Combining other therapies with CDT has been a promising way to elevate anti-GBM effects. Our team constructed a metastatic melanoma cell membrane decorated with anti-apoptotic Bcl-2 siRNA loaded nanomedicine for GBM CDT and gene combinational therapy (Fig. 7**e**) [134]. Tumor membrane coating endows these MPC@siBcl-2 nanoparticles with both superior BBB penetration, by momentarily decreasing the tightness of the BBB, and homologous tumor targeting. The nanosystem is based on complexed poly-ethyleneimine xanthate (PEX), which can chelate copper ions (Cu^{2+}) in blood as well as tumor cells with charge-conversional citraconic anhydride grafted poly-lysine (PLL-CA), responsively releasing siRNA in the mild acid environment in GBM cells. Cu^{2+} then induces robust GBM cell apoptosis by generating toxic ·OH by Fenton-like reaction. Meanwhile, silencing of the Bcl-2 gene by Bcl-2 siRNA not only causes direct GBM cell death, but also boosts apoptosis through ROS generation. Collectively, the multifunctional nanomedicines improve anti-GBM effects with increased survival rates of the U87MG GBM-bearing orthotopic mice by inducing cascade ROS generation mediated with CDT and gene therapy, as reflected by the significantly improved median survival time (47 days) compared with single CDT (29 days), free siBcl-2 (22 days) and PBS (21 days). Of noted, in this study, metastatic cell-derived membrane was used to camouflage the nanoparticles to enhance the BBB penetration and tumor targeting, and the nanomaterials also played a role in anti-tumor, showing the potential for inhibiting the tumor brain metastasis and directions for nanocarrier developments.

These studies have explored whether combining dynamic therapies with other therapies leads to amplified ROS generation and synergistic therapy, confirming that combinations of treatment modalities overcome the disadvantages of monotherapy. However, to fully achieve anti-GBM potential, there is a need for continued efforts to develop strategies that enable deeper penetration of dynamic therapies into tumors.

Photothermal therapy-based bimodal therapy

Photothermal therapy (PTT) employs light-absorbing agents (photothermal agents) to generate targeted heat, destroying tumor cells. PTT holds promise in various cancers, including GBM [135]. In recent advancements, PTT has been integrated into bimodal therapy, combined with chemotherapy and precise surgical resection, to achieve synergistic anti-GBM effects. Qian et al. developed super-small zwitterionic micelles to facilitate BBB penetration through both size-dependent penetration and BBB over-expressed (BGT-1) mediated transcytosis [136]. Additionally, conjugating the photochemical sensitizer, IR780, on the UV-crosslinking self-assembly PTX-loaded nanoparticles realized efficient chemo- and PTT combinational therapy for GBM, resulting in about 2 weeks of prolonged median survival time. While this work provides a versatile platform for robust BBB crossing, further improved therapeutic outcomes would be achieved if drugs could be combined more reasonably. Meanwhile, Liu et al. combined the photothermal agent ICG with a heat shock protein inhibitor, gambogic acid (GA), which can elevate the thermal sensitivity of GBM, to achieve more effective combinational therapeutic effects [137]. In this study, brain metastatic breast cancer cell membrane and GBM cell membrane hybrid membrane camouflage strategy was used to enhance BBB penetration and tumor targeting, while also prolonging the blood circulation time. Then, superior antitumor effects were observed in the early-stage GBM bearing mice administrated by hybrid membrane coated GA and ICG loaded nanomedicines (HMGINPs), with the highest temperature (48.8 ℃). However, it should be noted that this study focused on effects in the early-stage GBM. Considering that GBM is often diagnosed at late stages, further studies are needed to verify the effects of inhibiting and eliminating GBM at late stages.

PTT has also been combined with precise surgical resection [138]. Similarly, homotypic membrane was used in this nanosystem for BBB penetration and GBM targeting. However, differently, this membrane was derived from GBM patient-derived tumor cells (GBM-PDTCM), closer to clinical. GBM-PDTCM coated Raman reporter and lipophilic fluorophore loaded gold nanorods (AuNRs) provided the guidance of dual signals for precise surgical resection. After surgery, PTT effects caused the temperature of GBM tissues up to 64.6℃, together resulting in doubling median survival time of mice treated with combinational therapy. This study tried to provide a more personalized therapy. However, it is not easy to produce patient tumor cell-derived membranes on a large scale.

In recent years, an increasing number of studies have explored the combination of PTT with other types of therapies for GBM synergistic treatments. PTT, as an effective method to damage GBM cells, can be improved by utilizing the BBB crossing and tumor targeting nanosystems. To further achieve elevated combinational effects, therapies that interact with PTT could be used. For example, immunotherapy could be combined with PTT as it could overcome the limitation of non-complete tumor elimination associated with PTT.

Radiotherapy-based bimodal therapy

Radiotherapy (RT) is one of the current standard treatments for GBM. RT induces damage to cancer cells through the deposition of energy by irradiation with gamma rays, X-rays or ion beams [139]. However, the median survival of GBM patients remains relatively low, typically less than 2 years, which can be attributed, in part, to sub-optimal radiological doses, necessary to prevent damage to normal tissues. Mason and colleagues have attempted to address this problem by

using gold nanoparticles to achieve a higher and more targeted RT dose in cancerous tissues [140]. Despite these advances, excess toxicity to normal tissues and resistance to radiation induced by even moderate radiation doses still fail to completely eradicate tumors [141]. Given this, researchers have been investigating the combination of RT with other therapies with nanotechnology. The goal is to solve existing problems of RT to synergize the antitumor activities of these two therapies, ultimately realizing highly effective GBM treatment.

Combining PTT with RT enhances therapeutic effects against GBM. To realize this potential, Zhang et al. designed multifunctional nanoplatforms (PES-Au@PDA) co-loaded a heat shock protein A5 (HSPA5) inhibitor (pifithrin-μ, PES) and radiosensitizer (gold nanosphere, AuNS) for synergistic PTT and RT in GBM (Fig. 8**a**) [142]. Upregulation of HSPA5 induces radiation resistance in tumor cells as HSPA5 is involved in repairing protein and DNA damage induced by irradiation and controls the activation of unfolded protein response (UPR) cascades that are used to maintain cellular TME homeostasis. PES is a new HSPA5 inhibitor that can amplify pro-apoptotic UPR cascades and reduce RT resistance. Moreover, PDA was coated on the surface of AuNS, promoting hydrophilicity, biocompatibility, photothermal stability and efficiency of Au nanoparticles. The in vitro and in vivo results showed that this combinational nanosystem remarkably enhanced both RT and PTT efficacy by activating pro-apoptosis UPR cascades and removing RT resistance. Notably, PES-Au@PDA+laser+RT combined therapy resulted in the complete inhibition of orthotopic GBM tumors 6 days after the last treatment. In addition, this nanosystem can also be used for dual modality magnetic resonance imaging (MRI) and computed tomography (CT) imaging, providing an integrated diagnosis and therapeutic platform for GBM management.

Furthermore, it has been reported that tumor radio-resistance is also caused by immunosuppressive tumor-associated myeloid cells (TAMCs). Located within the TME, TAMCs induce a marked upregulation of PD-L1, which contributes to radio-resistance through PD-L1 interactions, limiting the immune system response and reducing sensitivity to radiation. Lesniak et al. designed a lipid nanoparticle modified with anti-PD-L1 antibodies to target TAMCs [143]. A cyclin-dependent kinase inhibitor, dinaciclib, was also loaded in the PD-L1 targeted nanoparticles, attenuating immunosuppressive TAMCs, further promoting anti-GBM immune responses and reducing the radio-resistance in GBM. Accordingly, RT combined with PD-L1-targeted nanomedicine-based immunotherapy led to 30% of mice bearing GL261 and CT2A GBM tumors having long-term survival. Later, this group generated a bridging-lipid nanoparticle to further promote RT and immune synergistic therapy [144]. Stimulator of interferon genes (STING) agonist (siABZI) was encapsulated in this nanocarrier. Together with RT, the immunosuppressive TME was reversed, and anti-tumor immune responses were significantly enhanced, which resulted in potentiated GBM inhibition and long-lasting antitumor immunity. More recently, Zhang et al. made further efforts to overcome RT resistance and high-dose RT damage [145]. This study began with analyzing clinical data, which showed that RT induced basal chemokine ligand 2 (CCL-2) expression in the tumor region, leading to the activation of monocytes. Based on this, the authors designed and synthesized matrix metalloproteinase 2 (MMP-2) peptide and lipoteichoic acid modified liposome (D@MLL) to hitchhike circulating monocytes via targeting mediated by lipoteichoic acid receptor CD14, and these monocytes have the natural ability to penetrate the BBB and accumulate at tumor sites. Then, DOX·HCl was released under triggering by higher levels of MMP-2 at tumor sites, inducing immunogenic cell death of GBMs. The upregulated CCL-2 and M1 polarization, caused by RT, promoted maturation of these monocytes and further T cell activation, thereby achieving high-precision treatments for GBM (Fig. 8**b**). Although the survival of mice treated with these nanomedicines did not significantly prolong, they provide a potential tool for delivering therapeutics across the BBB and a promising way to develop effective combinational therapies for GBM by studying clinical data.

Moreover, to overcome the radio-resistance of GBM, gene-silencing has been combined with RT by Pang et al. [146]. In this study, a broccoli light-up aptamer-included three-way junction (3WJ) scaffold was engineered with siRNA EGFR and miRNA Let-7g on bacteriophage Qβ-based nanoparticles, followed by a cell penetrating peptide (TAT)

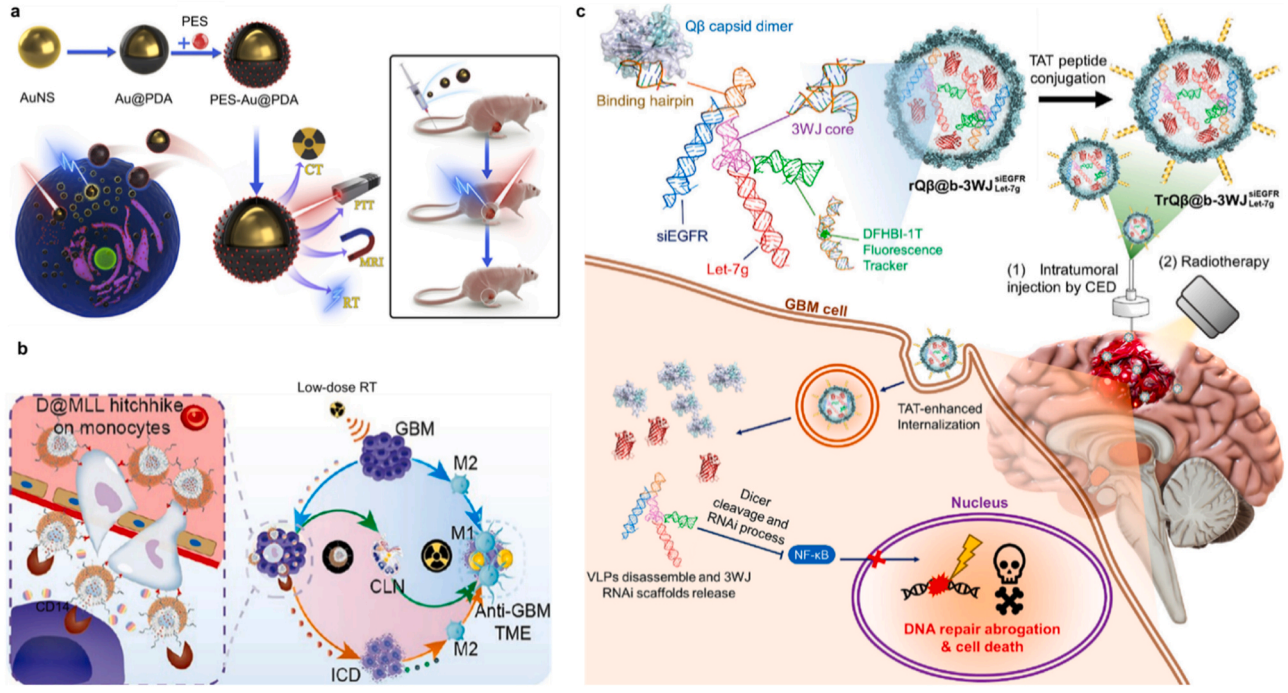

Fig. 8. (a) Schematic of PES-Au@PDA nanoparticles for synergistic GBM photothermal therapy and RT. (b) Illustration of D@MLL hitchhiking on monocytes and activating immune cells for GBM treatment after low-dose RT. (c) Illustration of synergistic anti-GBM activity mediated by RT and TrQβ@b-3WJ$_{Let}$-7g^{siEGFR} through inhibition of DNA repair and increased RNAi process.
(a) Printed with permission from Ref [142]. (b) Printed with permission from Ref [145]. (c) Printed with permission from Ref [146].

conjugated to the surface (TrQβ@b-3WJ$_{Let}$-7g^{siEGFR}) (Fig. 8**c**). These design elements enabled TrQβ@b-3WJ$_{Let}$-7g^{siEGFR} nanomedicines to efficiently knock down EGFR and IκB kinase (IKKα) simultaneously and inactivate NF-γB signaling, thereby inhibiting multiple genes-related to DNA repair for promoting RT. Accordingly, this nanosystem exhibited enhanced RNA stability, good BBB penetration, tumor cell internalization and increased anti-GBM effects by virtue of biological components, 3WJ, TAT modification and dual gene silencing. Impressively, the median survival was extended to over 60 days, while the median survival of mice receiving single irradiation treatment was only 31 days.

These data show that the challenge of radio-resistance can be effectively addressed through combinations of RT with PTT, immunotherapy and gene therapy. As a result, the therapeutic effects against GBM have been significantly amplified. The potency of RT for treating various cancers in clinical practice makes it an ideal partner for other modalities to yield robust anti-tumor effects. Hence, combinations of RT with other therapies are worth continued focus on the treatment of GBM.

Trimodal+ combinational therapy

The complex nature of GBM, with its unstable genetic heterogeneity, unique brain TME and complicated histopathology, seriously undermines the therapeutic potential of all treatments. Although both monomodal and bimodal combinational therapies discussed earlier have shown considerably improved anti-GBM efficacy compared to monotherapy, each of these therapies may still have deficiencies. As a result, there is a growing trend toward exploring trimodal+ combinational therapy that integrates three or more types of therapeutic drugs with smart nanosystems. By combining the therapeutic effects of several types of monotherapies, trimodal+ combinational therapy has the potential to be even more potent than monomodal and bimodal combination. In recent years, trimodal+ combinational therapies have been developed and utilized for GBM treatments, resulting in several remarkable superadditive effects.

As reported, the combination of PTT, PDT and chemotherapy can effectively remove the hypoxia problem in PDT, the short-term therapeutic effect of PTT and drug resistance in chemotherapy. Accordingly, Wang et al. developed a well-designed photo-theranostic agent, constructed by co-loading dicysteamine-modified hypocrellin derivative (DCHB) as a natural PS and an octadecane-grafted TMZ derivative (TMZ-C18) with DSPE-PEG2000-cRGD (Fig. 9**a**) [147]. The resulting nanomedicines (DTRGD NPs) efficiently traversed the BBB, and actively targeted tumor cells to enrich in GBM sites. The DCHB has a high singlet oxygen quantum yield (0.51) and photothermal conversion capability of 33% upon a wide 702–721 nm laser irradiation. TMZ-C18 was easily released following induction of higher temperature after DCHB laser absorption, leading to DNA damage in GBM cells. In mice bearing subcutaneous U87MG tumors, the triple synergistic nanoplatform completely inhibited the tumor growth after 14 days of treatment while tumor volumes in mice receiving control treatments were just slightly suppressed (Fig. 9**b**). In addition, all mice receiving multifunctional nanomedicines were alive at day 60, whereas all mice receiving control treatment had succumbed to tumor by day 60 (Fig. 9**c**). These triple modalities of treatment were combined properly with smart

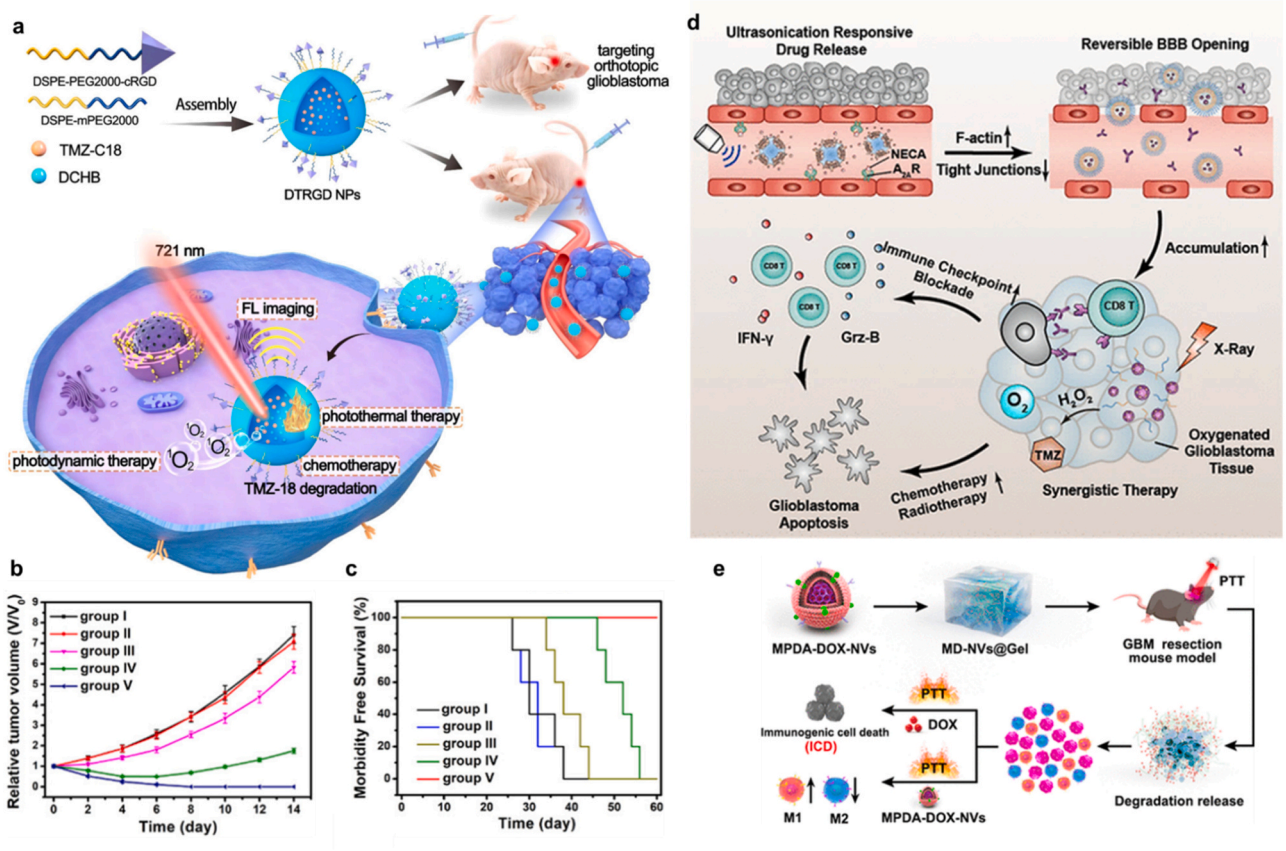

Fig. 9. (a) Schematic illustration of the fabrication of DTRGD NPs and mechanisms of effective anti-GBM chemo/photodynamic/photothermal synergistic therapy by DTRGD NPs. (b) Relative tumor volumes of mice treated with DTRGD NPs or control treatments. (c) Kaplan-Meier survival of GBM-bearing mice administered DTRGD NPs or controls. (b-c); group I: control, group II: Laser only, group III: DTRGD NPs only, group IV: "DTRGD NPs + Laser", group V: "DTRGD NPs + Laser (0.5 W cm^{-2})). (d) Schematic of the novel combined chemo/immuno/radiotherapy strategy proposed by Han et al. Targeted BBB regulating nanoparticles lead to improved GBM chemoradiation and immunotherapy. (e) Depiction of how synergistic PTT/ chemo/immunotherapy is achieved by MPDA-DOX-NVs.
(a) (a-c) Printed with permission from Ref [147]. (b) (d) Printed with permission from Ref [148]. (c) (e) Printed with permission from Ref [149].

nanosystems, effectively addressing the challenges of each modality, finally getting the promising synergistic effects. However, the effects evaluated in subcutaneous models, instead of orthotopic mouse models, may limit the further application of the findings because orthotopic models more closely mimic the TME of GBM.

A novel combined chemo/immuno/radiotherapy cooperative strategy was proposed by Han et al. for enhanced GBM therapeutic efficacy (Fig. 9**d**) [148]. Interestingly, RBC membrane decorated US-responsive nanovesicles (BRN) carrying $A_{2A}R$ agonists and perfluorcarbon (PF) were initially injected firstly to promote responsive drug release and reversible BBB opening following US. Subsequently, manganese dioxide nanoparticles, co-loaded with TMZ and PD-L1 antibodies, were administered to induce DNA damage and specific immune responses. Finally, X-ray therapy was applied to further optimally amplify therapeutic efficacy. BBB opening was demonstrated to be reversible from 2 to 4 h after BRN+US administration, confirmed by the immunofluorescence analysis, ensuring increased nanomedicines accumulate in GBM sites. Accordingly, 94.28% tumor inhibition was achieved in the combinational group due to the synergetic effects. This study demonstrated that separate administration of diverse modalities can help to fully exert the intended function of each therapy, providing a new way of managing GBM multimodal combinational therapy.

Trimodal combinational therapies have also been demonstrated to effectively prevent tumor recurrence after GBM surgical resection. For example, Zhao et al. developed a multifunctional bioresponsive nanogel for localized synergistic photo-chemo-immunotherapy (Fig. 9**e**) [149]. These authors successfully synthesized DOX loaded mesoporous polydopamine (MPDA) nanoparticles based on nanoemulsion assembly technology, followed by surface modification with M1-derived nanovesicles. Final suspension into fibrin gel yielded MPDA-DOX-NVs. After GBM resection and hydrogel cavity-injection, ICD was significantly induced by DOX and PTT, while both M1 vesicles and PTT reprogramed M2-like tumor-associated macrophages (TAMs) to M1-like TAMs, together resulting in marked activation of the immune system. Importantly, GBM resected mice showed that this hydrogel system combined with PTT completely inhibited tumor growth and reduced tumor recurrence, followed by that 71.4% of hydrogel-treated mice became long-term survivors. In this study, it is a promising method for hydrogel cavity-injection after surgery, which means more accumulation of nanomedicines at tumor sites, to achieve better therapeutic effects although M1 vesicle coating could prolong blood circulation and enhance BBB penetration.

In the past year, Mou et al. developed a multifunctional nanosystem for GBM four-modal combinational therapy, combining chemotherapy, PTT, starvation therapy and CDT [150]. The BBB penetration ability, enhanced by lipopolysaccharide-free bacterial outer membrane camouflaging, was 6.67-fold in vitro and 4.09-fold in vivo higher than that of bare nanoparticles. The core of the nanosystem was hollow Cu_9S_8, which can mediate Fenton/Fenton-like CDT and NIR-II PTT therapy. Glucose oxidase (Gox) conjugated onto this core achieved starvation therapy by rapidly depleting endogenous glucose and oxygen in GBM cells, meanwhile also generating H_2O_2 and gluconic acid. The produced H_2O_2 further promoted CDT and PTT, and the latter in turn enhanced Gox activity. Additionally, the loaded banoxantrone dihydrochloride (AQ4N), activated by the hypoxic microenvironment exacerbated by Gox oxygen depletion, further provided chemotherapy accordingly. Despite the smart combination of four distinct antitumor mechanisms and bacterial membrane enhanced BBB penetration, the survival outcomes were only prolonged slightly compared to monotherapies.

These innovative trimodal+ approaches offer the potential of combining different therapeutic modalities to achieve enhanced therapeutic outcomes in GBM treatment. Despite this, they also come with certain challenges, limiting their current applications, let alone the clinical application. Notably, administering multiple therapies simultaneously poses inconvenient time-management, potential cost increases and safety concerns. Ongoing research aimed at understanding the complexities of GBM and the properties of different modalities may overcome these limitations to fully realize the promise of trimodal or even 'extra'-modal combinational therapies for GBM.

Conclusion and future perspectives

GBM is the most malignant type of brain tumor with extraordinarily complicated mechanisms that include various intricate molecular networks, gene mutations, tumor TMEs, multidrug resistance and glioma stem cells, making it challenging to find an effective cure [47]. Recent advances in nanoparticle-based multimodal combination therapy strategies described in this review have shown several unique features in reducing drug resistance and side effects, and effectively improving the GBM therapeutic outcomes, by targeting and attacking multiple sites within tumor. For clinical translation, the optimal combinational therapy depends on tumor stage. Surgical resection combined with adjuvant therapies is still the most potential strategy for early-stage GBM, while more precision therapies, including but not limited to immunotherapy and gene-targeted therapy, could be more effective in inhibiting progress and metastasis in late-stage GBM. Additionally, bimodal combinational therapy might deserve further investigation because of the limited efficacy of monomodal combinational therapies, and hard administration and resource consumption associated with trimodal+ combinational therapies.

To develop more effective combinational therapy for GBM, there are still several obstacles that need to be addressed. One of the key challenges is deepening our understanding of the complex mechanisms of GBM and the distinctive properties of therapeutic molecules. A thorough exploration of the molecular and genetic complexities of GBM may reveal potential vulnerabilities, paving the way for the development of more effective combination therapies. These therapies not only circumvent resistance and reduce side effects but also strategically target multiple weak points within GBM simultaneously. By also understanding the interactions between different therapeutic agents, researchers can formulate combination therapies that fully capitalize on strengths of each therapeutic component while minimizing their weaknesses, aiming to generate a "1+1>2" synergistic effect.

Additionally, the design of nanocarriers with multifunctionality is crucial. These carriers should possess better capability to load multiple kinds of therapeutic agents simultaneously. Subsequently, successfully delivering these drugs into glioma and accumulating at targeted sites is pivotal. Exploring more effective strategies, like innovative ligand or biomimetic modifications, to enhance BBB penetration and active targeting is important for improving GBM treatment efficacy as BBB crossing, glioma cell targeting and organelle targeting are still significant challenges. To further enhance the functionality of nanosystems, strategies for controlled release should be optimized. Achieving precise and optimal release kinetics of each drug at the targeted location is critical for maximizing anti-GBM effects and avoiding undesired interactions or degradation. The design of nanosystems should consider the unique properties and mechanisms of action of each drug, ensuring their coordinated and efficient release. For example, certain therapeutics exert their effects within specific subcellular compartments, such as the mitochondria or nucleus, requiring precise drug release at their intended site of action. Therefore, more responsive nanocarriers that can detect specific GBM environments should be designed. Researchers should also endeavor to utilize external stimuli, such as US and laser, to finely tune drug release in both spatial and temporal dimensions.

Furthermore, the safety of nanomaterials and the appropriate dosage of multiple drugs are critical factors that must be thoroughly considered in GBM treatment. Nanosystems employed in GBM combinational therapies should undergo careful safety evaluation and improvement, and nanocarriers with improved safety profiles should be developed. For instance, while biomimetic nanomedicines have been demonstrated enhanced biocompatibility, a comprehensive safety examination,

particularly genetic materials, should be conducted to ensure and further elevate the safety of biomimetic nanomedicines. Regarding therapeutics in synergistic therapies, finding the right balance between effective dose range, toxicity threshold and potential interactions with other drugs of each drug is essential to optimize synergistic effects against GBM while inducing minimal side effects.

To advance clinical translation, it is crucial to design and develop nanosystems with the capability for scalable production while maintaining excellent qualities. Meanwhile, personalized nanomedicines should be developed, utilizing their advantages of specifically activating immune systems and eliminating tumor cells in patients. Further, theranostic nanomedicines that deliver imaging agents and therapeutics together should be widely explored, facilitating personalized medicines by tailoring treatments based on real-time assessments of tumor responses. While studies have shown promising results in cell and mouse models, there remains a need to develop and use more relevant models that better mimic human GBM in clinical settings. Such models would provide more accurate insights into the potential effectiveness and safety of multimodal combination therapies before transitioning to clinical trials. By addressing these obstacles and continuously refining the approach, there is potential to create increasingly better multimodal synergistic nanosystems that could significantly amplify the treatment outcomes and eventually lead to better management and eradication of GBM.

CRediT authorship contribution statement

Yajing Sun: Writing – original draft, Writing – review & editing, Conceptualization. **Bingyang Shi:** Writing – review & editing, Supervision, Funding acquisition. **Meng Zheng:** Writing – review & editing, Supervision, Conceptualization. **Ming Li:** Writing – review & editing, Supervision, Funding acquisition. **Yan Zou:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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References

[1] K.D. Miller, Q.T. Ostrom, C. Kruchko, N. Patil, T. Tihan, G. Cioffi, H.E. Fuchs, K. A. Waite, A. Jemal, R.L. Siegel, J.S. Barnholtz-Sloan, CA Cancer J. Clin. 71 (2021) 381–406.

[2] C. Horbinski, L.B. Nabors, J. Portnow, J. Baehring, A. Bhatia, O. Bloch, S. Brem, N. Butowski, D.M. Cannon, S. Chao, M.G. Chheda, A.J. Fabiano, P. Forsyth, P. Gigilio, J. Hattangadi-Gluth, M. Holdhoff, L. Junck, T. Kaley, R. Merrell, M. M. Mrugala, S. Nagpal, L.A. Nedzi, K. Nevel, P.L. Nghiemphu, I. Parney, T. R. Patel, K. Peters, V.K. Puduvalli, J. Rockhill, C. Rusthoven, N. Shonka, L. J. Swinnen, S. Weiss, P.Y. Wen, N.E. Willmarth, M.A. Bergman, S. Darlow, J. Natl. Compr. Cancer Netw. 21 (2023) 12–20.

[3] B. Delgado-Martín, M. Medina, Adv. Sci. 7 (2020) 1902971.

[4] T.T. Lah, M. Novak, B. Breznik, Semin. Cancer Bio. 60 (2020) 262–273.

[5] A.M. Molinaro, S. Hervey-Jumper, R.A. Morshed, J. Young, S.J. Han, P. Chunduru, Y. Zhang, J.J. Phillips, A. Shai, M. Lafontaine, J. Crane, A. Chandra, P. Flanigan, A. Jahangiri, G. Cioffi, Q. Ostrom, J.E. Anderson, C. Badve, J. Barnholtz-Sloan, A.E. Sloan, B.J. Erickson, P.A. Decker, M.L. Kosel, D. LaChance, J. Eckel-Passow, R. Jenkins, J. Villanueva-Meyer, T. Rice, M. Wrensch, J.K. Wiencke, N.A. Oberheim Bush, J. Taylor, N. Butowski, M. Prados, J. Clarke, S. Chang, E. Chang, M. Aghi, P. Theodosopoulos, M. McDermott, M.S. Berger, JAMA Oncol. 6 (2020) 495–503.

[6] P.Y. Wen, M. Weller, E.Q. Lee, B.M. Alexander, J.S. Barnholtz-Sloan, F.P. Barthel, T.T. Batchelor, R.S. Bindra, S.M. Chang, E.A. Chiocca, T.F. Cloughesy, J. F. DeGroot, E. Galanis, M.R. Gilbert, M.E. Hegi, C. Horbinski, R.Y. Huang, A. B. Lassman, E. Le Rhun, M. Lim, M.P. Mehta, I.K. Mellinghoff, G. Minniti, D. Nathanson, M. Platten, M. Preusser, P. Roth, M. Sanson, D. Schiff, S.C. Short, M.J.B. Taphoorn, J.C. Tonn, J. Tsang, R.G.W. Verhaak, A. von Deimling, W. Wick, G. Zadeh, D.A. Reardon, K.D. Aldape, M.J. van den Bent, Neuro Oncol. 22 (2020) 1073–1113.

[7] R. Ma, M.J.B. Taphoorn, P. Plaha, J. Neurol. Neurosurg. Psychiatry 92 (2021) 1103–1111.

[8] S.J. Bagley, S. Kothari, R. Rahman, E.Q. Lee, G.P. Dunn, E. Galanis, S.M. Chang, L. B. Nabors, M.S. Ahluwalia, R. Stupp, M.P. Mehta, D.A. Reardon, S.A. Grossman, E.P. Sulman, J.H. Sampson, S. Khagi, M. Weller, T.F. Cloughesy, P.Y. Wen, M. Khasraw, Clin. Cancer Res. 28 (2022) 594–602.

[9] M. Potharaju, A. Mathavan, B. Mangaleswaran, J. Clin. Oncol. 36 (2018) 14047.

[10] T. Hirst, P. McAleavey, T. Flannery, Neuro Oncol. 23 (2021) 19.

[11] R. Rahman, G. Youssef, S. Ventz, R. Redd, J. McDunn, W. Louv, B.M. Alexander, P.Y. Wen, L. Trippa, J. Clin. Oncol. 40 (2022) 2051.

[12] F. Sharifzad, S. Ghavami, J. Verdi, S. Mardpour, M. Mollapour Sisakht, Z. Azizi, A. Taghikhani, M.J. Łos, E. Fakharian, M. Ebrahimi, A.A. Hamidieh, Drug Resist. Updat. 42 (2019) 35–45.

[13] K.J. Wolf, J. Chen, J.D. Coombes, M.K. Aghi, S. Kumar, Nat. Rev. Mater. 4 (2019) 651–668.

[14] A. Shergalis, A. Bankhead, 3rd, U. Luesakul, N. Muangsin, N. Neamati, Pharmacol. Rev. 70 (2018) 412–445.

[15] A.C. Tan, D.M. Ashley, G.Y. López, M. Malinzak, H.S. Friedman, M. Khasraw, CA: Cancer J. Clin. 70 (2020) 299–312.

[16] N. Tatari, S. Khan, J. Livingstone, K. Zhai, D. McKenna, V. Ignatchenko, C. Chokshi, W.D. Gwynne, M. Singh, S. Revill, N. Mikolajewicz, C. Zhu, J. Chan, C. Hawkins, J.-Q. Lu, J.P. Provias, K. Ask, S. Morrissy, S. Brown, T. Weiss, M. Weller, H. Han, J.N. Greenspoon, J. Moffat, C. Venugopal, P.C. Boutros, S. K. Singh, T. Kislinger, Acta Neuropathol. 144 (2022) 1127–1142.

[17] A.M. Knudsen, B. Halle, O. Cédile, M. Burton, C. Baun, H. Thisgaard, A. Anand, C. Hubert, M. Thomassen, S.R. Michaelsen, B.B. Olsen, R.H. Dahlrot, R. Bjerkvig, J.D. Lathia, B.W. Kristensen, Neuro Oncol. 24 (2021) 1074–1087.

[18] K.E. Miller, K.A. Cassady, J.C. Roth, J. Clements, K.M. Schieffer, K. Leraas, A. R. Miller, N. Prasad, J.W. Leavenworth, I.B. Aban, R.J. Whitley, G.Y. Gillespie, E. R. Mardis, J.M. Markert, Clin. Cancer Res. 28 (2022) 498–506.

[19] S. Han, H. Shin, J.-K. Lee, Z. Liu, R. Rabadan, J. Lee, J. Shin, C. Lee, H. Yang, D. Kim, S.H. Kim, J. Kim, J.-W. Oh, D.-S. Kong, J.-I. Lee, H.J. Seol, J.W. Choi, H. J. Kang, D.-H. Nam, Exp. Mol. Med. 51 (2019) 1–11.

[20] T. Kondo, Semin. Cancer Bio. 82 (2022) 176–183.

[21] F. Cheng, C. Eng, Trends Mol. Med. 25 (2019) 461–463.

[22] D.M. Tiek, B. Erdogdu, R. Razaghi, L. Jin, N. Sadowski, C. Alamillo-Ferrer, J. R. Hogg, B.R. Haddad, D.H. Drewry, C.I. Wells, J.E. Pickett, X. Song, A. Goenka, B. Hu, S.A. Goldlust, W.J. Zuercher, M. Pertea, W. Timp, S.-Y. Cheng, R. B. Riggins, Sci. Adv. 8 (2022) 3471.

[23] C. Lu, Y. Wei, X. Wang, Z. Zhang, J. Yin, W. Li, L. Chen, X. Lyu, Z. Shi, W. Yan, Y. You, Mol. Cancer 19 (2020) 28.

[24] Y. Hoogstrate, K. Draaisma, S.A. Ghisai, L. van Hijfte, N. Barin, I. de Heer, W. Coppieters, T.P.P. van den Bosch, A. Bolleboom, Z. Gao, A.J.P.E. Vincent, L. Karim, M. Deckers, M.J.B. Taphoorn, M. Kerkhof, A. Weyerbrock, M. Sanson, A. Hoeben, S. Lukacova, G. Lombardi, S. Leenstra, M. Hanse, R.E.M. Fleischeuer, C. Watts, N. Angelopoulos, T. Gorlia, V. Golfinopoulos, V. Bours, M.J. van den Bent, P.A. Robe, P.J. French, Cancer Cell 41 (2023) 678–692.

[25] Z. Chen, D. Hambardzumyan, Front. Immunol. 9 (2018) 1004.

[26] A. Bikfalvi, C.A. da Costa, T. Avril, J.V. Barnier, L. Bauchet, L. Brisson, P. F. Cartron, H. Castel, E. Chevet, H. Chneiweiss, A. Clavreul, B. Constantin, V. Coronas, T. Daubon, M. Dontenwill, F. Ducray, N. Enz-Werle, D. Figarella-Branger, I. Fournier, J.S. Frenel, M. Gabut, T. Galli, J. Gavard, G. Huberfeld, J. P. Hugnot, A. Idbaih, M.P. Junier, T. Mathivet, P. Menei, D. Meyronet, C. Mirjolet, F. Morin, J. Mosser, E.C. Moyal, V. Rousseau, M. Salzet, M. Sanson, G. Seano, E. Tabouret, A. Tchoghandjian, L. Turchi, F.M. Vallette, S. Vats, M. Verreault, T. Virolle, Trends Cancer 9 (2023) 9–27.

[27] K. Woroniecka, P. Chongsathidkiet, K. Rhodin, H. Kemeny, C. Dechant, S. H. Farber, A.A. Elsamadicy, X. Cui, S. Koyama, C. Jackson, L.J. Hansen, T. M. Johanns, L. Sanchez-Perez, V. Chandramohan, Y.A. Yu, D.D. Bigner, A. Giles, P. Healy, G. Dranoff, K.J. Weinhold, G.P. Dunn, P.E. Fecci, Clin. Cancer Res. 24 (2018) 4175–4186.

[28] D.V. Sawant, H. Yano, M. Chikina, Q. Zhang, M. Liao, C. Liu, D.J. Callahan, Z. Sun, T. Sun, T. Tabib, A. Pennathur, D.B. Corry, J.D. Luketich, R. Lafyatis, W. Chen, A.C. Poholek, T.C. Bruno, C.J. Workman, D.A.A. Vignali, Nat. Immunol. 20 (2019) 724–735.

[29] D. Henrik Heiland, V.M. Ravi, S.P. Behringer, J.H. Frenking, J. Wurm, K. Joseph, N.W.C. Garrelfs, J. Strähle, S. Heynckes, J. Grauvogel, P. Franco, I. Mader,

M. Schneider, A.L. Potthoff, D. Delev, U.G. Hofmann, C. Fung, J. Beck, R. Sankowski, M. Prinz, O. Schnell, Nat. Commun. 10 (2019) 2541.
[30] A. Wong, M. Ye, A. Levy, J. Rothstein, D. Bergles, P. Searson, Front. Neuroeng. 6 (2013) 7.
[31] L. Jena, E. McErlean, H. McCarthy, Drug Deliv. Transl. Re. 10 (2020) 304–318.
[32] B.C. Prager, S. Bhargava, V. Mahadev, C.G. Hubert, J.N. Rich, Trends Cancer 6 (2020) 223–235.
[33] Y. Xing, F. Yasinjan, M. Yang, Y. Du, H. Geng, M. He, Y. Wang, J. Sun, W. Jiang, L. Zhang, B. Guo, K. Fan, Nano Today 52 (2023) 101961.
[34] G.D. Cha, T. Kang, S. Baik, D. Kim, S.H. Choi, T. Hyeon, D.H. Kim, J. Control. Release 328 (2020) 350–367.
[35] I. Khan, M.H. Baig, S. Mahfooz, M.A. Imran, M.I. Khan, J.-J. Dong, J.Y. Cho, M. A. Hatiboglu, Semin. Cancer Bio. 86 (2022) 172–186.
[36] G. Chen, I. Roy, C. Yang, P.N. Prasad, Chem. Rev. 116 (2016) 2826–2885.
[37] P.L. Chariou, O.A. Ortega-Rivera, N.F. Steinmetz, ACS Nano 14 (2020) 2678–2701.
[38] N. Wang, X. Cheng, N. Li, H. Wang, H. Chen, Adv. Health Mater. 8 (2019) 1801002.
[39] N. Zhao, L. Yan, X. Zhao, X. Chen, A. Li, D. Zheng, X. Zhou, X. Dai, F.-J. Xu, Chem. Rev. 119 (2019) 1666–1762.
[40] P. Säälik, P. Lingasamy, K. Toome, I. Mastandrea, L. Rousso-Noori, A. Tobi, L. Simón-Gracia, H. Hunt, P. Paiste, V.R. Kotamraju, G. Bergers, T. Asser, T. Rätsep, E. Ruoslahti, R. Bjerkvig, D. Friedmann-Morvinski, T. Teesalu, J. Control. Release 308 (2019) 109–118.
[41] X.Y. Lim, S.M. Capinpin, N. Bolem, A.S.C. Foo, W.G. Yip, A.P. Kumar, D.B.L. Teh, Bioeng. Transl. Med. 8 (2023) e10483.
[42] Y. Zou, Y. Sun, Y. Wang, D. Zhang, H. Yang, X. Wang, M. Zheng, B. Shi, Nat. Commun. 14 (2023) 4557.
[43] W. Jiang, Q. Li, R. Zhang, J. Li, Q. Lin, J. Li, X. Zhou, X. Yan, K. Fan, Nat. Commun. 14 (2023) 8137.
[44] S. Wang, L. Yang, W. He, M. Zheng, Y. Zou, Small Methods, 2400096.
[45] M. Zhang, W. Hu, C. Cai, Y. Wu, J. Li, S. Dong, Mater. Today Bio. 14 (2022) 100223.
[46] M. Mehta, P. Wen, R. Nishikawa, D. Reardon, K. Peters, Crit. Rev. Oncol. Hematol. 111 (2017) 60–65.
[47] C. McKinnon, M. Nandhabalan, S.A. Murray, P. Plaha, BMJ 374 (2021) n1560.
[48] D. Ghosh, S. Nandi, S. Bhattacharjee, Clin. Transl. Med. 7 (2018) 33.
[49] M. Zhao, D. van Straten, M.L.D. Broekman, V. Préat, R.M. Schiffelers, Theranostics 10 (2020) 1355–1372.
[50] D. Wu, Q. Chen, X. Chen, F. Han, Z. Chen, Y. Wang, Signal Transduct. Target Ther. 8 (2023) 217.
[51] S.B. Hladky, M.A. Barrand, Fluids Barriers CNS 15 (2018) 30.
[52] Y. Shi, R. van der Meel, X. Chen, T. Lammers, Theranostics 10 (2020) 7921–7924.
[53] G.C. Terstappen, A.H. Meyer, R.D. Bell, W. Zhang, Nat. Rev. Drug Discov. 20 (2021) 362–383.
[54] W. Zhang, Q.Y. Liu, A.S. Haqqani, S. Leclerc, Z. Liu, F. Fauteux, E. Baumann, C. E. Delaney, D. Ly, A.T. Star, E. Brunette, C. Sodja, M. Hewitt, J.K. Sandhu, D. B. Stanimirovic, Fluids Barriers CNS 17 (2020) 47.
[55] S. Baek, S.E. Yu, Y.H. Deng, Y.J. Lee, D.G. Lee, S. Kim, S. Yoon, H.-S. Kim, J. Park, C.H. Lee, J.B. Lee, H.J. Kong, S.-G. Kang, Y.M. Shin, H.-J. Sung, Ad. Healthc. Mater. 11 (2022) 2102226.
[56] U. Anand, A. Dey, A.K.S. Chandel, R. Sanyal, A. Mishra, D.K. Pandey, V. De Falco, A. Upadhyay, R. Kandimalla, A. Chaudhary, J.K. Dhanjal, S. Dewanjee, J. Vallamkondu, J.M. Pérez de la Lastra, Genes Dis. 10 (2023) 1367–1401.
[57] J.P. Fisher, D.C. Adamson, Biomedicines 9 (2021) 324.
[58] L.K. Penny, H.M. Wallace, Chem. Soc. Rev. 44 (2015) 8836–8847.
[59] J.J. Marin, M.R. Romero, A.G. Blazquez, E. Herraez, E. Keck, O. Briz, Anticancer Agents Med. Chem. 9 (2009) 162–184.
[60] M.J. Mitchell, M.M. Billingsley, R.M. Haley, M.E. Wechsler, N.A. Peppas, R. Langer, Nat. Rev. Drug Discov. 20 (2021) 101–124.
[61] F.C. Lam, S.W. Morton, J. Wyckoff, T.-L. Vu Han, M.K. Hwang, A. Maffa, E. Balkanska-Sinclair, M.B. Yaffe, S.R. Floyd, P.T. Hammond, Nat. Commun. 9 (2018) 1991.
[62] Y. Zou, Y. Wang, S. Xu, Y. Liu, J. Yin, D.B. Lovejoy, M. Zheng, X.J. Liang, J. B. Park, Y.M. Efremov, I. Ulasov, B. Shi, Adv. Mater. 34 (2022) e2203958.
[63] Y. Wang, Y. Sun, N. Geng, M. Zheng, Y. Zou, B. Shi, Adv. Ther. 5 (2022) 2200095.
[64] H. Wang, S. Wang, R. Wang, X. Wang, K. Jiang, C. Xie, C. Zhan, H. Wang, W. Lu, Nanoscale 11 (2019) 13069–13077.
[65] A. Sathiyaseelan, K. Saravanakumar, A.V.A. Mariadoss, M.-H. Wang, Carbohydr. Polym. 262 (2021) 117907.
[66] I. Kapoor, J. Bodo, B.T. Hill, E.D. Hsi, A. Almasan, Cell Death Dis. 11 (2020) 941.
[67] Y.-C. Kuo, Y.-H. Chang, R. Rajesh, Mater. Sci. Eng. C. 96 (2019) 114–128.
[68] Z.N. Lei, Q. Tian, Q.X. Teng, J.N.D. Wurpel, L. Zeng, Y. Pan, Z.S. Chen, Med Comm. 4 (2023) e265.
[69] R. Pisa, T.M. Kapoor, Nat. Chem. Biol. 16 (2020) 817–825.
[70] S.P. Thyparambil, W.-L. Liao, E. An, A. Bhalkikar, R. Heaton, K.G. Sylvester, X. B. Ling, J. Clin. Oncol. 38 (2020) 2555.
[71] S.Y. Lee, Genes Dis. 3 (2016) 198–210.
[72] R. Jatyan, P. Singh, D.K. Sahel, Y.G. Karthik, A. Mittal, D. Chitkara, J. Control. Release 350 (2022) 494–513.
[73] D. Mcmahon, A. Hussein, H. Mangleburg, A. Nichianain, O. Fitzpatrick, R. A. McLaughlin, M.R. Conroy, S.J. Marks, J. Naidoo, W. Grogan, A.G. Murphy, O. S. Breathnach, B.T. Hennessy, P.G. Morris, J. Clin. Oncol. 40 (2022) 14037.
[74] S. Wu, X. Li, F. Gao, J.F. de Groot, D. Koul, W.K.A. Yung, Neuro Oncol. 23 (2021) 920–931.
[75] R. Stupp, W.P. Mason, M.J. van den Bent, M. Weller, B. Fisher, M.J. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R.C. Janzer, S. K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J.G. Cairncross, E. Eisenhauer, R. O. Mirimanoff, N. Engl. J. Med. 352 (2005) 987–996.
[76] B. Hong, E. Yang, D. Su, J. Ju, X. Cui, Q. Wang, F. Tong, J. Zhao, S. Yang, C. Cheng, L. Xin, M. Xiao, K. Yi, Q. Zhan, Y. Ding, H. Xu, L. Cui, C. Kang, Neuro Oncol. (2023).
[77] J. Zhao, S. Yang, X. Cui, Q. Wang, E. Yang, F. Tong, B. Hong, M. Xiao, L. Xin, C. Xu, Y. Tan, C. Kang, Neuro Oncol. 25 (2023) 857–870.
[78] S.A. Valdés-Rives, D. Casique-Aguirre, L. Germán-Castelán, M.A. Velasco-Velázquez, A. González-Arenas, BioMed. Res. Int. 2017 (2017) 7403747.
[79] W. He, X. Li, M. Morsch, M. Ismail, Y. Liu, F.U. Rehman, D. Zhang, Y. Wang, M. Zheng, R. Chung, Y. Zou, B. Shi, ACS Nano 16 (2022) 6293–6308.
[80] S.P.C. Hsu, J.S. Kuo, H.C. Chiang, H.E. Wang, Y.S. Wang, C.C. Huang, Y.C. Huang, M.S. Chi, M.P. Mehta, K.H. Chi, Oncotarget 9 (2018) 6883–6896.
[81] Y.Y. Tseng, Y.C. Wang, C.H. Su, T.C. Yang, T.M. Chang, Y.C. Kau, S.J. Liu, Colloids Surf. B Biointerfaces 134 (2015) 254–261.
[82] Y.Y. Tseng, C.H. Su, S.T. Yang, Y.C. Huang, W.H. Lee, Y.C. Wang, S.C. Liu, S. J. Liu, Oncotarget 7 (2016) 59902–59916.
[83] A. Kwiatkowska, M.S. Nandhu, P. Behera, E.A. Chiocca, M.S. Viapiano, Cancers 5 (2013) 1271–1305.
[84] Y. Li, Y. Yi, J. Lv, X. Gao, Y. Yu, S.S. Babu, I. Bruno, D. Zhao, B. Xia, W. Peng, J. Zhu, H. Chen, L. Zhang, Q. Cao, K. Chen, Nucleic Acids Res 51 (2023) 6020–6038.
[85] X.Q. Zhou, R. Wang, W. Sun, S. Bonnet, Matter 5 (2022) 2502–2504.
[86] J. Karlsson, K.M. Luly, S.Y. Tzeng, J.J. Green, Adv. Drug Deliv. Rev. 179 (2021) 113999.
[87] L. Kong, Y. Wu, C.S. Alves, X. Shi, Nanomedicine 11 (2016) 3103–3115.
[88] M. Zheng, Y. Liu, Y. Wang, D. Zhang, Y. Zou, W. Ruan, J. Yin, W. Tao, J.B. Park, B. Shi, Adv. Mater. 31 (2019) e1903277.
[89] Y. Liu, M. Zheng, M. Jiao, C. Yan, S. Xu, Q. Du, M. Morsch, J. Yin, B. Shi, Biomaterials 276 (2021) 121036.
[90] K.L. Kozielski, A. Ruiz-Valls, S.Y. Tzeng, H. Guerrero-Cázares, Y. Rui, Y. Li, H. J. Vaughan, M. Gionet-Gonzales, C. Vantucci, J. Kim, P. Schiapparelli, R. Al-Kharboosh, A. Quiñones-Hinojosa, J.J. Green, Biomaterials 209 (2019) 79–87.
[91] N.D. Germain, W.K. Chung, P.D. Sarmiere, Mol. Asp. Med. 91 (2023) 101148.
[92] S. Kumar, L.E. Fry, J.-H. Wang, K.R. Martin, A.W. Hewitt, F.K. Chen, G.-S. Liu, Ppog. Retin. Eye Res. 92 (2023) 101110.
[93] P. Wang, Y. Zhou, A.M. Richards, Theranostics 11 (2021) 8771–8796.
[94] C. Xu, D. Li, Z. Cao, M. Xiong, X. Yang, J. Wang, Nano Lett. 19 (2019) 2688–2693.
[95] V. Baumann, J. Winkler, Future Med. Chem. 6 (2014) 1967–1984.
[96] M.S. Ebert, P.A. Sharp, Cell 149 (2012) 515–524.
[97] G. Fiscon, F. Conte, V. Licursi, S. Nasi, P. Paci, Sci. Rep. 8 (2018) 7769.
[98] D. Rosenblum, N. Joshi, W. Tao, J.M. Karp, D. Peer, Nat. Commun. 9 (2018) 1410.
[99] Y. Zhou, Y. Guo, L. Chen, X. Zhang, W. Wu, Z. Yang, X. Li, Y. Wang, Z. Hu, Z. Wang, Theranostics 12 (2022) 5488–5503.
[100] Y. Zou, Y. Liu, Z. Yang, D. Zhang, Y. Lu, M. Zheng, X. Xue, J. Geng, R. Chung, B. Shi, Adv. Mater. 30 (2018) e1803717.
[101] L. Wen, K. Wang, F. Zhang, Y. Tan, X. Shang, Y. Zhu, X. Zhou, H. Yuan, F. Hu, Biomaterials 237 (2020) 119793.
[102] S. Hosseindoost, S.M. Mousavi, A.R. Dehpour, S.A. Javadi, B. Arjmand, A. Fallah, M. Hadjighassem, Mol. Ther. Oncolytics 26 (2022) 76–87.
[103] F. Yang, Z. He, H. Duan, D. Zhang, J. Li, H. Yang, J.F. Dorsey, W. Zou, S. A. Nabavizadeh, S.J. Bagley, K. Abdullah, S. Brem, L. Zhang, X. Xu, K.T. Byrne, R. H. Vonderheide, Y. Gong, Y. Fan, Nat. Commun. 12 (2021) 3424.
[104] H. Sun, Y. Li, P. Zhang, H. Xing, S. Zhao, Y. Song, D. Wan, J. Yu, Biomark. Res. 10 (2022) 89.
[105] S. Lugani, E.A. Halabi, J. Oh, R.H. Kohler, H.M. Peterson, X.O. Breakefield, E.A. A. Chiocca, M.A. Miller, C.S. Garris, R. Weissleder, Adv. Mater. 35 (2023) 2208782.
[106] T. Su, X. Liu, S. Lin, F. Cheng, G. Zhu, Bioact. Mater. 26 (2023) 169–180.
[107] G. Wei, Y. Wang, G. Yang, Y. Wang, R. Ju, Theranostics 11 (2021) 6370–6392.
[108] T.M. Belete, Biologics 15 (2021) 67–77.
[109] K. Wang, F.M. Kievit, P.A. Chiarelli, Z.R. Stephen, G. Lin, J.R. Silber, R. G. Ellenbogen, M. Zhang, Adv. Funct. Mater. 31 (2021) 2007166.
[110] M. Zheng, C. Yan, Q. Yang, F. Zhu, Q. Du, X. Xia, M. Morsch, A. Lee, J. Yin, Y. Zou, B. Shi, Chem. Phys. Mater. 1 (2022) 203–210.
[111] T. Jiang, Y. Qiao, W. Ruan, D. Zhang, Q. Yang, G. Wang, Q. Chen, F. Zhu, J. Yin, Y. Zou, R. Qian, M. Zheng, B. Shi, Adv. Mater. 33 (2021) e2104779.
[112] F.U. Rehman, Y. Liu, Q. Yang, H. Yang, R. Liu, D. Zhang, P. Muhammad, Y. Liu, S. Hanif, M. Ismail, M. Zheng, B. Shi, J. Control. Release 345 (2022) 696–708.
[113] Y. Zhang, X. Fu, J. Jia, T. Wikerholmen, K. Xi, Y. Kong, J. Wang, H. Chen, Y. Ma, Z. Li, C. Wang, Q. Qi, F. Thorsen, J. Wang, J. Cui, X. Li, S. Ni, ACS Appl. Mater. Inter. 12 (2020) 43408–43421.
[114] M. Bausart, V. Préat, A. Malfanti, J. Exp. Clin. Canc. Res. 41 (2022) 35.
[115] C. Bailly, X. Thuru, B. Quesnel, NAR Cancer 2 (2020) zcaa002.
[116] R.S. Riley, C.H. June, R. Langer, M.J. Mitchell, Nat. Rev. Drug Discov. 18 (2019) 175–196.
[117] J. Moslehi, A.H. Lichtman, A.H. Sharpe, L. Galluzzi, R.N. Kitsis, J. Clin. Invest. 131 (2021) e145186.
[118] Y. Li, X. Teng, Y. Wang, C. Yang, X. Yan, J. Li, Adv. Sci. 8 (2021) e2004381.
[119] W. Wang, M. Zhang, Q. Zhang, M. Mohammadniaei, J. Shen, Y. Sun, J. Control. Release 352 (2022) 399–410.
[120] Y. Liu, W. Wang, D. Zhang, Y. Sun, F. Li, M. Zheng, D.B. Lovejoy, Y. Zou, B. Shi, Exploration 2 (2022) 20210274.

[121] E.W. Weber, M.V. Maus, C.L. Mackall, Cell 181 (2020) 46–62.
[122] Y. Chang, X. Cai, R. Syahirah, Y. Yao, Y. Xu, G. Jin, V.J. Bhute, S. Torregrosa-Allen, B.D. Elzey, Y.-Y. Won, Q. Deng, X.L. Lian, X. Wang, O. Eniola-Adefeso, X. Bao, Nat. Commun. 14 (2023) 2266.
[123] Q. Zhang, Q. Luo, Z. Liu, M. Sun, X. Dong, Chem. Eng. J. 457 (2023) 141225.
[124] C. Donohoe, M.O. Senge, L.G. Arnaut, L.C. Gomes-da-Silva, BBA Rev. Cancer 1872 (2019) 188308.
[125] H. Zhang, T. Wang, H. Liu, F. Ren, W. Qiu, Q. Sun, F. Yan, H. Zheng, Z. Li, M. Gao, Nanoscale 11 (2019) 7600–7608.
[126] S. Hu, C. Dong, J. Wang, K. Liu, Q. Zhou, J. Xiang, Z. Zhou, F. Liu, Y. Shen, J. Control. Release 324 (2020) 250–259.
[127] D. Zhang, S. Tian, Y. Liu, M. Zheng, X. Yang, Y. Zou, B. Shi, L. Luo, Nat. Commun. 13 (2022) 6835.
[128] M. Zhang, X. Jiang, Q. Zhang, T. Zheng, M. Mohammadniaei, W. Wang, J. Shen, Y. Sun, Adv. Funct. Mater. 31 (2021) 2102274.
[129] H. Wu, X. Gao, Y. Luo, J. Yu, G. Long, Z. Jiang, J. Zhou, Adv. Sci. 9 (2022) 2203894.
[130] H. Chen, S. Zhang, Q. Fang, H. He, J. Ren, D. Sun, J. Lai, A. Ma, Z. Chen, L. Liu, R. Liang, L. Cai, ACS Nano 17 (2023) 421–436.
[131] D. Lin, Z. Wang, W. Long, M. Xu, A. Liu, Y. Gao, Z. Wen, C. Liu, J. He, Y. Cheng, S. Jiang, J. Chen, Q. Liu, L. Zhang, R. You, L. Yin, Y. Guan, Adv. Funct. Mater. 33 (2023) 2209219.
[132] C. Jia, Y. Guo, F.G. Wu, Small 18 (2022) e2103868.
[133] J. Xin, C. Deng, O. Aras, M. Zhou, C. Wu, F. An, J. Nanobiotechnology 19 (2021) 192.
[134] D. Zhang, Y. Sun, S. Wang, Y. Zou, M. Zheng, B. Shi, Adv. Funct. Mater. 32 (2022) 2209239.
[135] M. Overchuk, R.A. Weersink, B.C. Wilson, G. Zheng, ACS Nano 17 (2023) 7979–8003.
[136] K. Wang, B. Zhao, Y. Ao, J. Zhu, C. Zhao, W. Wang, Y. Zou, D. Huang, Y. Zhong, W. Chen, H. Qian, J. Control Release 364 (2023) 261–271.
[137] S. Chi, L. Zhang, H. Cheng, Y. Chang, Y. Zhao, X. Wang, Z. Liu, Angew 62 (2023) e202304419.
[138] H. Zhang, S. Guan, T. Wei, T. Wang, J. Zhang, Y. You, Z. Wang, Z. Dai, J. Am. Chem. Soc. 145 (2023) 5930–5940.
[139] W. Roa, P.M. Brasher, G. Bauman, M. Anthes, E. Bruera, A. Chan, B. Fisher, D. Fulton, S. Gulavita, C. Hao, S. Husain, A. Murtha, K. Petruk, D. Stewart, P. Tai, R. Urtasun, J.G. Cairncross, P. Forsyth, J. Clin. Oncol. 22 (2004) 1583–1588.
[140] K. Haume, S. Rosa, S. Grellet, M.A. Śmiałek, K.T. Butterworth, A.V. Solov'yov, K. M. Prise, J. Golding, N.J. Mason, Cancer Nanotechnol. 7 (2016) 8.
[141] F. Meyer, I. Bairati, W. Xu, A.K. Azad, G. Liu, J. Clin. Oncol. 29 (2011) 5507.
[142] H. Zhu, X. Cao, X. Cai, Y. Tian, D. Wang, J. Qi, Z. Teng, G. Lu, Q. Ni, S. Wang, L. Zhang, Biomaterials 232 (2020) 119677.
[143] P. Zhang, J. Miska, C. Lee-Chang, A. Rashidi, W.K. Panek, S. An, M. Zannikou, A. Lopez-Rosas, Y. Han, T. Xiao, K.C. Pituch, D. Kanojia, I.V. Balyasnikova, M. S. Lesniak, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 23714–23723.
[144] P. Zhang, A. Rashidi, J. Zhao, C. Silvers, H. Wang, B. Castro, A. Ellingwood, Y. Han, A. Lopez-Rosas, M. Zannikou, C. Dmello, R. Levine, T. Xiao, A. Cordero, A. M. Sonabend, I.V. Balyasnikova, C. Lee-Chang, J. Miska, M.S. Lesniak, Nat. Commun. 14 (2023) 1610.
[145] J. Kuang, Z.Y. Rao, D.W. Zheng, D. Kuang, Q.X. Huang, T. Pan, H. Li, X. Zeng, X. Z. Zhang, ACS Nano 17 (2023) 13333–13347.
[146] H.H. Pang, C.Y. Huang, P.Y. Chen, N.S. Li, Y.P. Hsu, J.K. Wu, H.F. Fan, K.C. Wei, H.W. Yang, ACS Nano 17 (2023) 10407–10422.
[147] C. Zhang, J. Wu, W. Liu, X. Zheng, W. Zhang, C.S. Lee, P. Wang, ACS Appl. Bio. Mater. 3 (2020) 3817–3826.
[148] L. Meng, C. Wang, Y. Lu, G. Sheng, L. Yang, Z. Wu, H. Xu, C. Han, Y. Lu, F. Han, ACS Appl. Mater. Inter. 13 (2021) 11657–11671.
[149] R. Zhang, Y. Ye, J. Wu, J. Gao, W. Huang, H. Qin, H. Tian, M. Han, B. Zhao, Z. Sun, X. Chen, X. Dong, K. Liu, C. Liu, Y. Tu, S. Zhao, ACS Appl. Mater. Inter. 15 (2023) 17627–17640.
[150] Y. He, Y. Pan, X. Zhao, L. Ye, L. Liu, W. Wang, M. Li, D. Chen, Y. Cai, X. Mou, Chem. Eng. J. 471 (2023) 144410.