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Current approaches in enhancing TRAIL therapies in glioblastoma

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Abstract

Glioblastoma (GBM) is the most prevalent, aggressive, primary brain cancer in adults and continues to pose major medical challenges due in part to its high rate of recurrence. Extensive research is underway to discover new therapies that target GBM cells and prevent the inevitable recurrence in patients. The pro-apoptotic protein tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has attracted attention as an ideal anticancer agent due to its ability to selectively kill cancer cells with minimal toxicity in normal cells. Although initial clinical evaluations of TRAIL therapies in several cancers were promising, later stages of clinical trial results indicated that TRAIL and TRAIL-based therapies failed to demonstrate robust efficacies due to poor pharmacokinetics, resulting in insufficient concentrations of TRAIL at the therapeutic site. However, recent studies have developed novel ways to prolong TRAIL bioavailability at the tumor site and efficiently deliver TRAIL and TRAIL-based therapies using cellular and nanoparticle vehicles as drug loading cargos. Additionally, novel techniques have been developed to address monotherapy resistance, including modulating biomarkers associated with TRAIL resistance in GBM cells. This review highlights the promising work to overcome the challenges of TRAIL-based therapies with the aim to facilitate improved TRAIL efficacy against GBM.

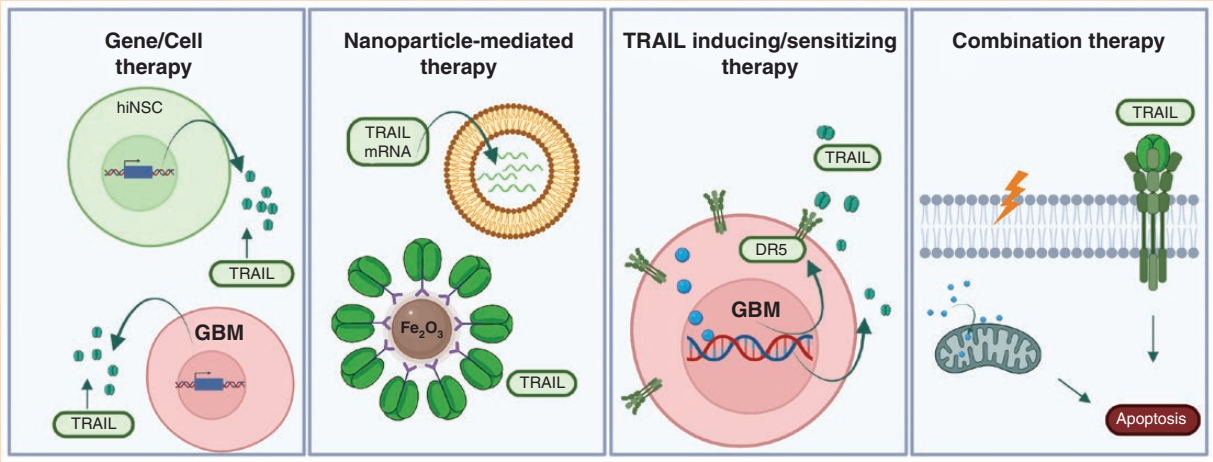
Keywords:

gene and cell therapy | glioblastoma | nanoparticle-mediated delivery | TRAIL-sensitizing agents | tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)

Glioblastoma (GBM) is the most common primary brain cancer in adults and is associated with a median survival beyond 5 years of less than 10%,[1] despite current standard-of-care treatment of maximal surgical resection followed by radiotherapy and chemotherapy.[2] The infiltrative nature of GBM renders surgical resections incomplete, and the genetically heterogeneous nature of GBM commonly results in resistance to therapies.[3] Consequently, rapid recurrence is common, and 90% of GBM patients ultimately develop tumor recurrence.[4,5] Despite promising results in the preclinical and early clinical phases in the last 3 decades, GBM remains virtually incurable.[6]

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising tumoricidal agent. TRAIL is a naturally occurring protein in the TNF super family. Through binding to death receptors 4 and 5 (DR4 and DR5) on cancer cells, the complex selectively kills cancer cells by activating extrinsic apoptotic pathway without damaging normal cells, offering potential for targeted therapy.[7] In addition, apoptosis is a desirable mechanism of inducing cancer cell death as it induces apoptosis in cancer cells without eliciting robust immune responses.[8] Using purified or recombinant TRAIL as therapeutic agent, TRAIL has been shown to be tumoricidal in a broad range of GBM cells in culture across a variety of doses.[9] Using orthotopic xenograft models, repeated systemic infusion of TRAIL suppressed tumor progression in in vivo models of colon carcinoma and mammary adenocarcinoma.[10,11] Based on the strength of these preclinical results, TRAIL recombinant

Graphical Abstract



protein and TRAIL mimetic antibodies (Abs) advanced into clinical testing for several types of cancers.

However, the results were not as promising as anticipated due to TRAIL's short half-life[12] and TRAIL resistance, necessitating additional studies to enhance TRAIL and TRAIL-based therapies.[13,14] Methods to promote sustained TRAIL release at the tumor site, such as with cell or nanoparticle (NP) delivery vehicles, have been extensively studied in GBM and have generated promising preclinical in vitro and in vivo results. Additionally, novel sensitizers have been investigated to induce or elevate expression of DRs to circumvent adaptive resistance. Furthermore, more mutations associated with TRAIL resistance have been discovered that are potential targets for future therapies to enhance the cytotoxic effect of TRAIL.[15] Here, we will highlight the current understanding of the mechanism behind TRAIL apoptosis and explore techniques to enhance the efficacy of TRAIL against GBM.

Apoptosis Signaling Pathways

Programmed cell death is a normal, dynamic, and heavily regulated physiological event in cells to eliminate defective cells and maintain homeostasis.[16] Abnormal regulation in programmed cell death signaling pathways result in uncontrolled cell proliferation, which can lead to cancer and neurodegenerative diseases.[17] Apoptosis has been well characterized in its role at controlling abnormal cell proliferation and is essential to prevent several diseases including cancer.[18] Cellular apoptosis can be achieved via intrinsic or extrinsic pathways facilitated by various signal transducers (Figure 1).[19]

Intrinsic apoptosis is often mediated by cellular stress, DNA damage, and cell cycle checkpoint defects, which signal the mitochondria to release pro-apoptotic factors to initiate the cell death process.[20] In GBM cells, these signals first activate dysregulated p53 that further mediates the activation of PUMA, NOXA, and BH3-only proteins such as BAD, BID, and BIM. Activated PUMA, NOXA, and BH3-only proteins then activate pro-apoptotic molecules BAX and BAK, which permeabilize the mitochondria and result in the release of cytochrome c in the cytosol.[21] BCL-2, BCL-XL, and MCL-1 can block the activation of BAX and BAK and inhibit apoptosis. Cytochrome c further assembles into an apoptosome by interacting with the adaptor protein, apoptotic peptidase activating factor 1 (APAF-1), deoxyadenosine triphosphate (dATP), and pro-caspase 9. The apoptosome then cleaves and activates proteolytic enzymes caspase 9, which then further activates effector caspases and ultimately results in apoptosis by cleaving proteins in the cytoplasm and nucleus.[22] SMAC/DIABLO also are released from the mitochondria and are responsible for inactivating inhibitors of apoptotic proteins (IAPs) such as X-linked inhibitor of apoptosis protein (XIAP) and survivin.[23]

Extrinsic apoptosis is induced when the membrane-bound full-length intracellular death domain containing death receptors 4 and 5 (DR4 or TRAIL-R1; and DR5 or TRAIL-R2) are activated via both membrane-bound and -soluble TRAIL proteins, which are endogenous, pro-apoptotic cytokines expressed and secreted by most cells.[24] TRAIL, a member of TNF superfamily, is a type II transmembrane protein and is also expressed and released in soluble form in various types of tissues and on the surface of immune cells, such as natural killer cells and cytotoxic T cells.[19] When TRAIL binds and interacts with DR4 or 5, the complex undergoes homotrimerization, and the intracellular domain of the DR forms a death-inducing signaling complex (DISC) by recruiting an adaptor molecule, Fas-associated death domain (FADD), and pro-caspase 8. In several cell types, cleaved the activated caspase 8 further cleaves effector caspases, such as pro-caspase 3, resulting in apoptosis. Interestingly, there is crosstalk between the intrinsic and extrinsic apoptotic pathways, where caspase 8 from the extrinsic pathway cleaves the pro-apoptotic molecule BH3-domain interacting protein (BID) to truncated

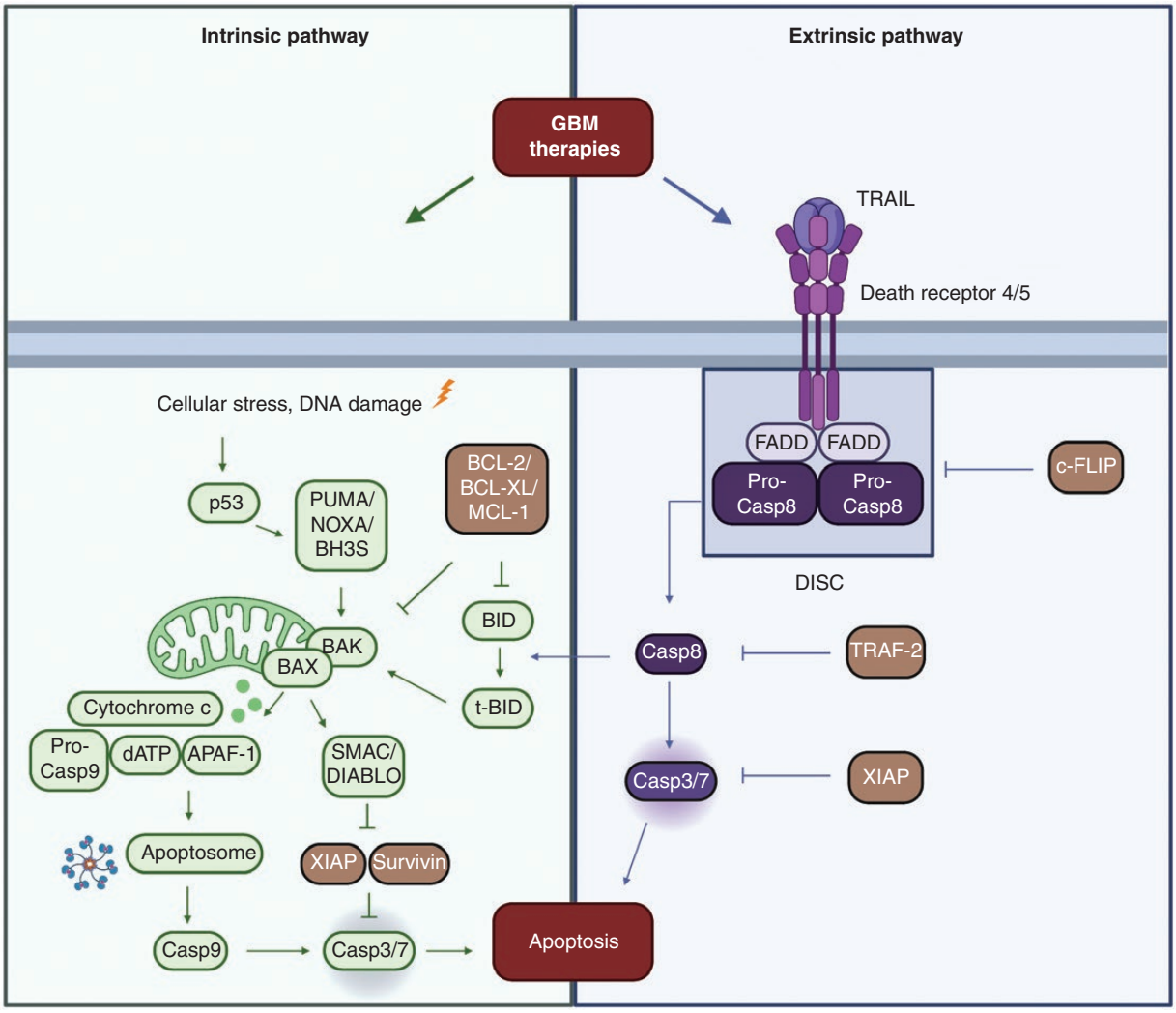


Figure 1. Apoptosis signaling pathways. The intrinsic pathway is induced by cellular stress, DNA damage, and cell cycle checkpoint defects activating often dysregulated p53, which activates PUMA, NOXA, and BH3-only proteins. Activated PUMA, NOXA, and BH3-only proteins further mediate the activation of pro-apoptotic molecules BAX and BAK in the mitochondria, resulting in cytochrome c release in the cytosol. BCL-2, BCL-XL, and MCL-1 can block BAX and BAK from permeabilizing the mitochondria and inhibit apoptosis. Interaction of cytochrome c, APAF-1, pro-caspase 9, and dATP results in the formation of an apoptosome, which cleaves and activates proteolytic enzymes caspases 3 and 7 and induces cellular apoptosis. Released from the mitochondria, SMAC/DIABLO proteins inactivate IAPs to proceed with apoptosis. The extrinsic pathway is activated when TRAIL binds to DR4 and DR5, leading to the formation of the death-inducing signaling complex (DISC) by recruiting an adaptor molecule, FADD, and pro-caspase 8. c-FLIP can block the formation of DISC and inhibit apoptosis. Cleaved caspase 8 further cleaves effector caspases 3 and 7, which induce apoptosis. Cleaved caspase 8 from extrinsic pathway can also truncate BID and initiate mitochondrial-associated intrinsic apoptosis. However, TRAF-2 can inactivate cleaved caspase 8 by ubiquitinating and inhibiting induction of both intrinsic and extrinsic apoptosis.

Bit (t-BID), which translocates to mitochondria and subsequently activates BAK and BAX.[20] Cells may have decoy death receptors TRAIL-R3 and TRAIL-R4, but they lack cytoplasmic death domains and are not implicated in TRAIL-induced apoptosis.[25] Instead, because TRAIL-R3 and -R4 can still bind TRAIL without inducing apoptosis, they may reduce the concentration of TRAIL available to bind to DR4 and DR5, effectively reducing TRAIL-induced apoptosis.[7]

TRAIL-induced apoptosis is tightly regulated; there are several negative regulators present at distinct stages of the pathway that can inhibit cell death.[26,27] At the DISC stage, anti-apoptotic protein cellular FLICE-like inhibitory protein (c-FLIP) can suppress the stimulation of caspase 8 and prevent DISC formation—activating the pro-survival signaling pathway instead. Receptor clustering and DISC formation are vital to initiate TRAIL-induced apoptosis, and growing evidence has indicated that DR-mediated signal transductions are most optimized when DRs cluster into lipid rafts.[13] Elevation of c-FLIP is associated with TRAIL resistance in many cancers, including GBM.[9] Loss or downregulation of c-FLIP via siRNA was reported to overcome TRAIL resistance in carcinoma cells and

Table 1. Clinical Trials of TRAIL and TRAIL-Based Therapies[19,34] (www.clinicaltrials.gov)

Drug	Adjuvant Therapy	Type of Cancer	Phase	Status and Notable Conclusions (If Available)	Identifier
AMG951 Recombinant human TRAIL	Bevacizumab, Carboplatin, Paclitaxel	NSCLC	Phase II	Completed	NCT00508625
Dulanermin Recombinant human TRAIL	FOLFIRI, Bevacizumab, Cetuximab, Irinotecan	Colorectal cancer	Phase I	Completed	NCT00671372
	FOLFOX, Bevacizumab	Metastatic colorectal cancer	Phase I	Completed	NCT00873756
	Rituximab	B-cell non-Hodgkin's lymphomas	Phase II	Terminated	NCT00400764
	N/A	NSCLC stage IV	Phase III	Unknown	NCT03083743
SCB-313 Recombinant human TRAIL-trimer fusion protein	N/A	NSCLC, malignant pleural effusions	Phase I	Completed	NCT03869697
	N/A	Peritoneal malignancies	Phase I	Completed	NCT0343674
Mapatumumab TRAIL-R1 agonistic monoclonal antibody	Sorafenib	Advanced hepatocellular carcinoma	Phase I	Completed	NCT00712855
	Sorafenib	Advanced hepatocellular carcinoma	Phase II	Completed The addition of mapatumumab to sorafenib did not improve either primary end point (time to progression) or efficacy end points.[35]	NCT01258608
	Bortezomib	Relapsed or refractory multiple myeloma	Phase II	Completed	NCT00315757
	Carboplatin, Paclitaxel	Advanced NSCLC	Phase II	Completed	NCT00583830
	Cisplatin, radiotherapy	Advanced cervical cancer	Phase II	Completed	NCT01088347
TRM-1 or HGS-ETR1 TRAIL-R1 agonistic monoclonal antibody	N/A	Relapsed or refractory non-Hodgkin's lymphoma	Phased II	Completed	NCT00094848
	N/A	Carcinoma, NSCLC	Phase II	Completed	NCT0092924
Tigatuzumab TRAIL-R2 agonistic monoclonal antibody	Carboplatin, Paclitaxel	Metastatic NSCLC	Phase II	Completed Tigatuzumab was well tolerated, but it did not improve efficacy.	NCT00991796
	Abraxane	Metastatic TNBC	Phase II	Completed Unsatisfactory results[36]	NCT01307891
	Gemcitabine	Pancreatic cancer	Phase II	Completed	NCT00521404
	Sorafenib	Advanced hepatocellular carcinoma	Phase II	Completed Sorafenib alone vs. combined with tigatuzumab did not meet its primary efficacy end point (time to progression). However, sorafenib with tigatuzumab was well-tolerated.[37]	NCT01033240
Conatumumab TRAIL-R2 agonistic monoclonal antibody	Birinapant	Relapsed epithelial ovarian cancer, relapsed primary peritoneal cancer, relapsed fallopian tube cancer	Phase I	Completed	NCT01940172
	Panitumumab	Metastatic colorectal cancer	Phase II	Completed	NCT00630786
	FOLFOX6 or Ganitumab or Bevacizumab	Advanced solid tumors	Phase II	Completed	NCT01327612
	Gemcitabine hydrochloride, Capecitabine, Radiation	Pancreatic cancer	Phase II	Withdrawn due to agent availability	NCT01017822

Table 1. Continued

Drug	Adjuvant Therapy	Type of Cancer	Phase	Status and Notable Conclusions (If Available)	Identifier
AMG655 TRAIL-R2 agonistic monoclonal antibody	Vorinostat or Bortezomib	Relapsed or refractory low-grade lymphoma	Phase I	Completed	NCT00791011
	AMG479	Solid tumors	Phase II	Completed	NCT00819169
	Doxorubicin	Unresectable sarcoma	Phase II	Completed	NCT00626704
	N/A	NSCLC	Phase II	Completed	NCT00534027
	Modified FOLFOX6, Bevacizumab	Metastatic colorectal cancer	Phase II	Completed	NCT00625651
	FOLFIRI, AMG479	KRAS-mutant metastatic colorectal carcinoma	Phase II	Completed	NCT00813605
	AMG479 or Gemcitabine	Metastatic pancreatic cancer	Phase II	Completed	NCT00630552
DS-8273a TRAIL-R2 agonistic antibody	Nivolumab	Advanced colorectal cancer	Phase I	Terminated due to business decision	NCT02991196
	Nivolumab	Melanoma stage III or IV	Phase I	Completed	NCT02983006
	N/A	Advanced solid tumors or lymphomas	Phase I	Completed	NCT02076451
Gen1029 Multivalent antibody	N/A	Colorectal cancer, NSCLC, TNBC, renal cell carcinoma, gastric cancer, pancreatic cancer	Phase II	Terminated due to sponsor decision	NCT03576131
MSC-TRAIL TRAIL-secreting stem cell therapy	Pemetrexed, Cisplatin	NSCLC, adenocarcinoma of lung	Phase II	Recruiting	NCT03298763
CPT Recombinant human TRAIL	Thalidomide	Relapsed and refractory multiple myeloma	Phase II	CPT is well tolerated; wide safe dosage ranges as single-agent CPT (5–15 mg/kg for 5 consecutive days). CPT at 10 mg/kg for 5 consecutive days every cycle in combination with thalidomide and dexamethasone will be conducted in the next trial.[38]	ChiCTRONC-1200206

NSCLC, nonsmall cell lung cancer.

TRAIL-resistant melanoma cells.[28,29] Moreover, ectopic expression of c-FLIP in TRAIL-sensitive melanoma cells inhibited TRAIL-mediated apoptosis.[30] Tumor necrosis factor receptor-associated factor 2 (TRAF-2) is an adaptor protein and a component of TNF superfamily signaling complexes that can ubiquitinate and suppress the cleaved caspase 8 by inhibiting apoptosis by obstructing the cleavage process of effector protein caspase 3 and by truncating BID, which inhibits the communication between intrinsic and extrinsic pathways.[31] Overexpression of TRAF-2 promotes activation of survival signaling, leading to progression in GBM cell lines. Furthermore, high expression of TRAF-2 was detected in GBM patient tissues and can predict poorer prognosis in GBM patients.[32] XIAP is another negative regulator that also inhibits effector caspase 3 from inducing apoptosis. Upregulation of XIAP can promote tumor progression and is associated with resistance to chemotherapy and radiation.[33] Thus, dysregulation in these negative regulators can result in GBM progression as well as GBM cells acquiring TRAIL resistance. These negative regulators present opportunities for therapies to optimize TRAIL-induced apoptosis.

Recombinant TRAIL and TRAIL Receptor Agonists in Human Clinical Trials

TRAIL attracted attention for clinical application due to its specificity to cancer cells. Abnormal regulation in the Janus kinase (JAK) and tumor suppressor gene *p53* is known to influence the upregulation of TRAIL receptors (DR4 and DR5) in cancer cells.[34] However, the mechanism is still being elucidated as several reports indicate that the levels of DR4 and DR5 receptor expression may not correlate to TRAIL sensitivity.[35,36] Besides its selectivity to tumor cells, the nontoxic profile observed in the preclinical testing led to the investigation of TRAIL-based therapies in clinical trials for various types of cancers, including GBM (Table 1).[13,14,37]

In these clinical trials, treatments focused on using the soluble human TRAIL recombinant protein or soluble TRAIL agonistic monoclonal Abs. Dulanermin is a soluble human recombinant TRAIL protein with affinity for both DR4 and

DR5 that was under phase II clinical trials for B-cell lymphoma and phase III clinical trials for nonsmall cell lung cancer (NSCLC). In phase III clinical trials, dulanermin combined with chemotherapy (vinorelbine and cisplatin) improved primary end point (progression-free survival) and secondary end point (objective response rate), but overall survival was not significant compared with the control group that received placebo with chemotherapy.[38] However, this underwhelming effect on survival could be attributed to dulanermin's limited exposure to cancer cells since its half-life was under an hour in humans.[13,39] Circulating permuted TRAIL (CPT) is another form of soluble human recombinant TRAIL that has entered phase II clinical trials. In the preclinical testing, CPT was more potent at inducing apoptosis than dulanermin.[40] However, the results from phase II of the clinical trials suggested that CPT only induced partial responses in patients, though the treatment was well tolerated in the majority of patients.[41,42] However, 3 of the patients experienced severe side effects from CPT and one of them experienced CPT-mediated liver toxicity.[41,43] Thus, although phase I studies of human recombinant TRAIL in several forms indicated promising results, early phase II trials failed to demonstrate robust clinical efficacy in patients.[14] Likewise, many agonistic monoclonal Abs-targeting DR4 and DR5 separately were also evaluated in the clinic for safety, toxicity, and antitumoral activity. However, these monoclonal Abs failed to demonstrate therapeutic efficacy in early phase II clinical trials but were well tolerated in patients.

There are likely multiple factors contributing to TRAIL's lack of significant efficacy in clinical trials, including TRAIL's short half-life, poor tumor-targeting capability, receptor clustering, and resistance.[19] TRAIL is rapidly cleared from the serum due to its short half-life (<1 hour in humans), which makes it difficult to achieve the sustained levels of TRAIL necessary for efficacy.[44] Due to its rapid clearance from serum, the soluble human recombinant TRAIL protein dulanermin fails to reach therapeutic concentrations at the tumor site when administered intravenously (NCT0053830, NCT01258608), requiring effective therapeutic carriers. Additionally, effective DR4 and DR5 aggregation by cross-linking is crucial to exert extrinsic apoptotic signal transduction by DR4 and DR5 agonists. Exogenous agents can be administered to facilitate the receptor cross-linking in vitro,[45] but the presence of Fcγ receptors (FcγRs) on the surface of infiltrating immune cells in vivo must interact with agonists to aid with receptor clustering and induce agonistic activity. Clinical trial data from DR5 agonistic Ab AMG655 suggests that patients with the high-affinity allele of FcγRIIa exhibited a stronger treatment response.[14,45] Thus, DR4 and DR5 agonists may not generate consistent, robust efficacy across all patients, especially in GBM with its notoriously immunosuppressive microenvironment and lack of FcγRs available for cross-linking.[46] Finally, monotherapy resistance is extremely prevalent and remains one of the biggest challenges in treating GBM. Since nearly 70% of cancer cells are inherently resistant to TRAIL or can acquire resistance to TRAIL,[47] it is important to explore other therapeutic agents that simultaneously target both the intrinsic and extrinsic apoptotic pathways to fully exploit the potential of TRAIL-based therapies for clinical applications.

Although recombinant TRAIL protein and TRAIL Ab mimetics are not being evaluated for GBM, TRAIL inducing, the selective dopamine receptor D2 (DRD2) antagonist and mitochondrial caseinolytic protease P (ClpP) agonist ONC201 has demonstrated encouraging early signs of efficacy in 17 patients with aggressive and recurrent GBM in early phase II trials.[15,37] ONC201 will be discussed in more detail (see "TRAIL-sensitizing agents").

Gene and Cell Therapy

One method of addressing TRAIL's short circulating half-life through the delivery of TRAIL via viral or nonviral vehicles to tumor sites.[48] mRNA-based therapies offer rapid, transient, local expression by cancer cells of TRAIL or another antitumor protein, and adaptive convertibility without mutagenesis.[49,50] Although clinical trials heavily utilize viral vectors to deliver genes into the cells, viral vectors are not the ideal delivery vehicle due to their immunogenicity and low delivery capacity.[19] Recent nonviral vectors can mitigate these disadvantages. A recent study revealed synergistic efficacy in vitro of synthetic mRNA-based gene therapy comprised of *PTEN*-mRNA (m-PTEN) and *TRAIL*-mRNA (m-TRAIL) against GBM. PTEN sensitizes cancer cells to TRAIL, and nearly 40% of glioma patients have mutation in PTEN gene. PTEN functions as a central negative regulator of the phosphoinositide 3-kinase–protein kinase B–mammalian target of rapamycin [mTOR] (PI3K–AKT–mTOR) pathway which controls the intrinsic apoptosis. Combining m-PTEN and m-TRAIL via intracerebral injection significantly suppressed the tumor growth and prolonged survival in a mouse model.[50] Nevertheless, the blood–brain barrier (BBB) poses a hurdle as it contributes to insufficient therapeutic delivery at the tumor site.[51] To bolster drug delivery efficiency, NP-mediated gene therapy has been evolving to mitigate these distinct challenges (see "Nanoparticles").

Another method for increasing the concentration of TRAIL at the tumor site is with stem cells, which can act as carriers for antitumor therapeutics due to their innate capability to home to both solid and invasive tumor foci.[52] Previous studies have reported that human adipose-derived stem cells, mesenchymal stem cells (MSCs), and human-induced neural stem cells (hiNSCs) can not only migrate to GBM tumor regions and secrete biotherapeutics,[53] but they can also proliferate and persist in the brain for sufficient time to provide sustained delivery of their cytotoxic payloads.[54,55] When transduced to constitutively secrete TRAIL by viral transduction, hiNSCs can home to GBM and brain metastasis foci efficiently and induce tumor killing when administered via intracerebroventricular administration.[56,57] Although there are allogenic stem cells that are "off-the-shelf," stem cells can also be induced from an individual patient's fibroblasts to generate personalized stem cells to avoid potential immunogenicity.[52,55] Additionally, 1 study showed that hiNSC therapy can be combined with radiation, demonstrating that this therapy can be combined with standard-of-care radiation.[57] Moreover, TRAIL-secreting hiNSCs can be encapsulated in the biodegradable hemostatic agent FLOSEAL to promote sustained release

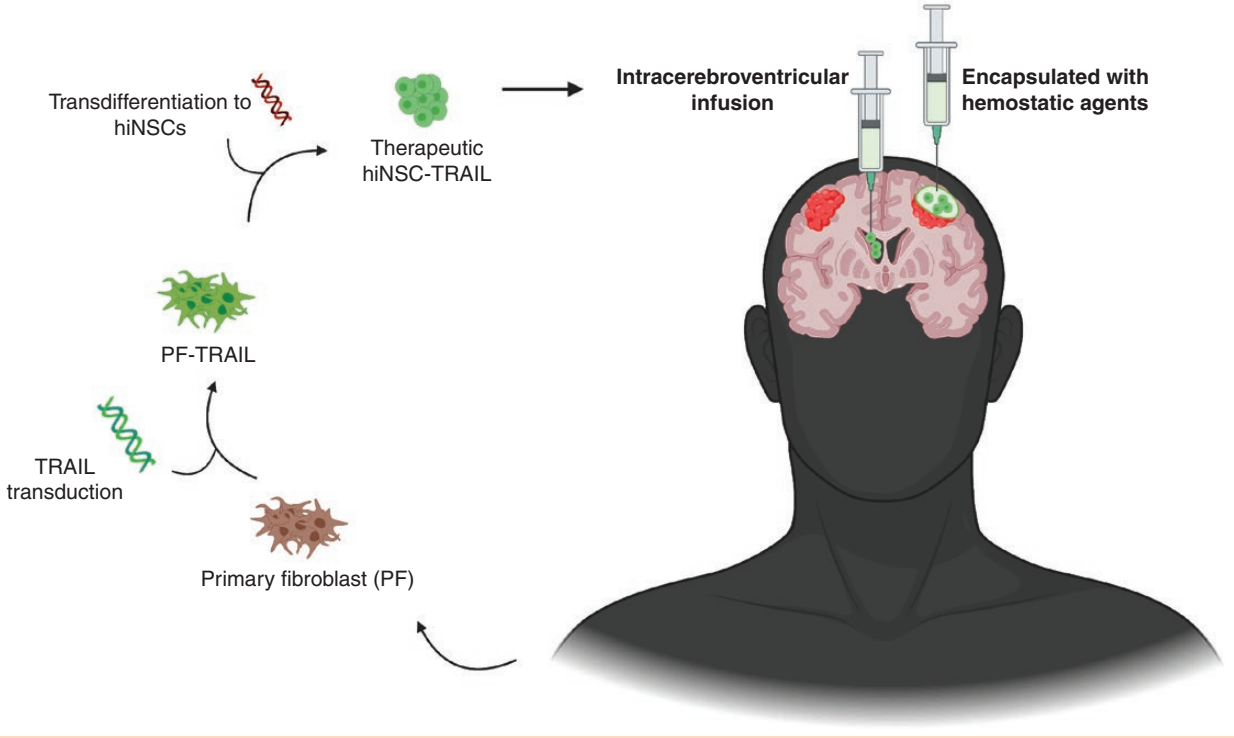


Figure 2. An illustration for transdifferentiation of patients derived primary fibroblasts (PFs) to human-induced neural stem cells (hiNSCs). PFs from patient skin biopsies are transduced to constitutively express TRAIL. TRAIL-secreting PFs are then directly differentiated into hiNSCs. Ultimately, patient-derived hiNSC-TRAIL could be administered via intracerebroventricular infusion or encapsulated with hemostatic agents such as FLOSEAL and implemented upon surgical resection.

of TRAIL to prevent tumor recurrence with minimal immune clearance in vivo (Figure 2). FLOSEAL-encapsulated, TRAIL-secreting hiNSCs persisted in the brain for 95 days in a murine recurrent GBM model, and the mice survived 30–60 days longer than the control mice that were treated with nonencapsulated TRAIL-secreting hiNSCs.[58] Hence, patient-derived tumor-homing stem cells that deliver TRAIL to tumor sites hold potential as drug delivery vehicles.

While gene and cell therapy strategies can increase the concentration of TRAIL at the tumor site, TRAIL resistance remains a significant challenge in improving treatment durability in GBM.[15] Due to GBM tumor heterogeneity, a subpopulation of tumor cells can develop TRAIL resistance.[59] Therefore, it is ideal to explore other agents to combine with TRAIL therapies. One option is to combine TRAIL with agents that target molecular markers implicated in aberrant cancer cell proliferation, such as AMP-activated protease kinase (AMPK). Recently, it was reported that combining TRAIL-secreting MSCs (MSC-TRAIL) with the AMPK inhibitor compound C significantly enhanced TRAIL-induced cell death in GBM cells lines by modulating pro- and anti-apoptotic gene expression patterns.[54] Additionally, combining MSC-TRAIL with AMPK inhibitor decreased tumor burden over single-agent therapies in U87 glioma-bearing mice.[54] Another option is to combine TRAIL with sensitizing agents that target crosstalk pathways with TRAIL for potential synergistic tumor suppression and improved survival (see "TRAIL-sensitizing agents" and "TRAIL-based therapies with standard-of-care"). Redjal et al. reported that a low dose of cisplatin increases DR4 and DR5 expression on GBM cells and sensitizes them to TRAIL-induced apoptosis.[60] They further demonstrated that combining TRAIL-secreting stem cells (SC-TRAIL) with a low dose of cisplatin decreased GBM tumor recurrence and increased survival in GBM-bearing mice.[60] Due to the novelty of TRAIL-based gene/cell therapies, the literature regarding use with small molecule drugs/chemotherapies in GBM is currently limited. Hence, utilization of gene/cell therapy with additional inhibitors/activators to mitigate TRAIL resistance should be further investigated in in vivo models of GBM.

Nanoparticles

Insufficient TRAIL accumulation at the tumor site due to BBB is another challenge in the development of treatments for GBM.[51] NPs can encapsulate drugs with low BBB permeability and facilitate safe and effective therapeutic transport across the BBB. NPs can be synthesized by inorganic, organic, or polymeric materials, and they range in size from 1 to 100 nm (Figure 3).[61] Iron oxide NPs conjugated to TRAIL (NP-TRAIL) induces apoptosis efficiently in glioma cells and glioma stem cells and had a synergistic effect with radiation in vitro. Sufficient accumulation of NP-TRAIL

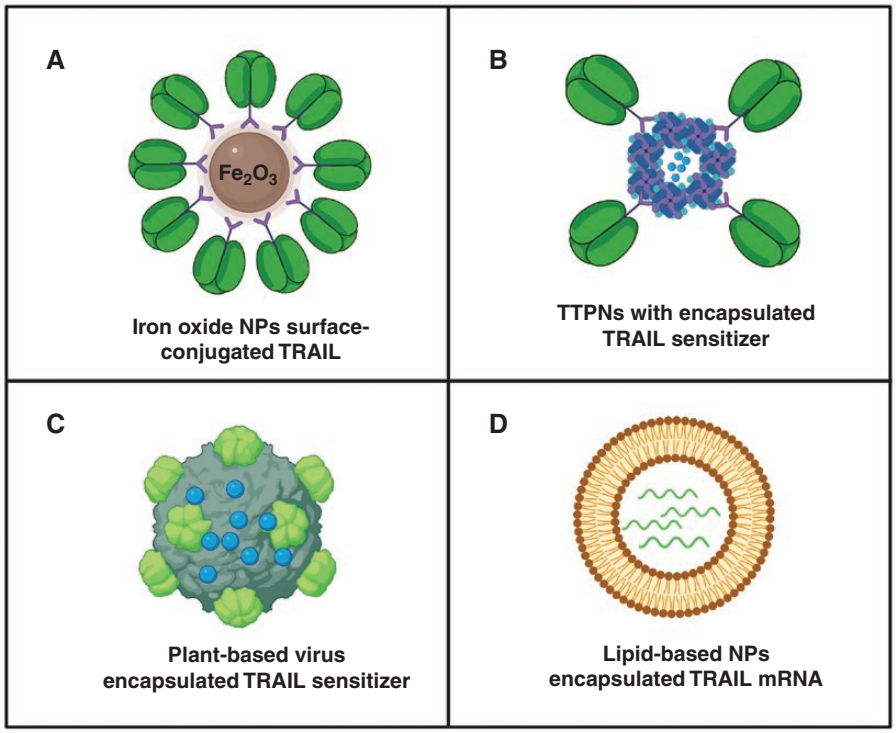


Figure 3. Illustration for nanoparticles (NPs) mediated TRAIL-based therapies. NPs can safely and effectively deliver drugs, proteins, and mRNA by (A) conjugating TRAIL onto the surface of iron oxide NPs, (B) loading TRAIL into plant-based cow pea mosaic virus (CMPV), (C) conjugating trimeric TRAIL onto ferritin-based nanocages, and (D) loading TRAIL mRNA transcripts into lipid-based NPs.

for a therapeutic effect was also observed in glioma xenografts in vivo compared with control NP group, which did not exhibit apoptotic effect on tumor cells. Furthermore, NP-TRAIL-treated group of mice had significant tumor reduction and prolonged survival without signs of toxicity.[62] In another study, iron oxide NPs coated with chitosan–polyethylene glycol–polyethyleneimine copolymer and chlorotoxin (CTX)-mediated TRAIL gene delivery transfected GBM cells, secreted sufficient quantities of TRAIL, and promoted apoptosis in vitro. Mice with GBM flank tumors were administered NP-TRAIL-CTX systemically, and tumor volume was significantly reduced.[63] However, survival data of the treated mice was not discussed. Additionally, because this study used a flank tumor model, BBB penetration was not studied. Therefore, future studies should focus on a brain tumor-bearing model to fully investigate and elucidate the potential of clinical application by assessing both tumor reduction and survival outcome.

Researchers have also utilized NPs that are biologics such as virus-based NPs as alternatives to synthetic NPs to deliver therapeutics for safety in humans.[64] A recent study developed a novel, biocompatible plant-based viral NP (CPMV) to carry mitoxantrone, an agent with poor BBB penetration but has shown to sensitize TRAIL in GBM cells. Mitoxantrone-loaded CPMV NPs were internalized by GBM cells and demonstrated tumor kill both alone and in combination with TRAIL in vitro.[65] Another method of increasing the efficacy of TRAIL is to present TRAIL in a nanocage. The inability of TRAIL to form its native homotrimeric complex structure can prevent it from properly activating its receptors, which can result in low efficacy. TRAIL can be presented to its receptors in its native structure by incorporating cellular iron storage proteins, ferritin nanocages, and the trimeric TRAIL-presenting nanocage (TTPN).[66] Recently, a novel nanocage was developed that not only carried trimeric TRAIL that possess enhanced half-life, but also simultaneously encapsulated TRAIL-sensitizing agents. These nanocages effectively induced TRAIL-resistant cells to internalize the nanocages via receptor-mediated endocytosis in vitro and a TRAIL-resistant xenograft mouse model of colorectal adenocarcinoma, inducing a significant tumor kill.[47] Although this therapeutic delivery method has not been used in GBM studies, the ability of NPs to effectively deliver TRAIL and encapsulate TRAIL-sensitizing agents has the potential to increase the efficacy of TRAIL-based therapies in GBM preclinical models. Hence, further investigations should be conducted to further characterize the BBB penetration of NP including TTPN.

TRAIL-Sensitizing Agents

TRAIL resistance is a prevalent barrier and a crucial setback in bolstering TRAIL-based therapies toward GBM. Nearly 70% of tumor cells have acquired resistance to TRAL, leading to disappointing clinical results.[47,67] One method to

combat TRAIL resistance is by modulating TRAIL and its receptors to upregulate pharmacologically. Although TRAIL receptor expression level does not correlate with TRAIL sensitivity,[35] previous studies suggest that increasing TRAIL receptors can overcome TRAIL resistance.[68–70] ONC201 is a novel small molecule drug of imipridone drug that is currently being evaluated in phase II clinical trials against several cancers including GBM. Imipridones are a class of drugs that selectively target cancer cells via G-protein-coupled receptors and the mitochondrial ClpP and induce cell death.[71] ONC201 exhibited encouraging efficacies in preclinical in vitro and in vivo models of GBM by inducing TRAIL and DR5 expression in GBM cells with limited toxicity in normal cells. Furthermore, ONC201 upregulated TRAIL in temozolomide-resistant GBM cell lines and potently induced cytotoxicity in bevacizumab, radiation, and temozolomide-resistant GBM human patient samples.[72,73] Currently, ONC201 is the only TRAIL-based treatment under clinical evaluation for GBM. The results of ONC201 have been encouraging in aggressive H3 K27M subset of glioma in both adult and children. 6/14 patients diagnosed with a H3 K27M glioma and who received ONC201 exhibited tumor regression, including a patient whose tumor volume was reduced by 96% (NCT02525692, NCT03416530) (atriumhealth.org).

Despite its promising results in clinical trials, the underlying mechanism of action for ONC201 is poorly elucidated.[74] Reports state that ONC201 acts as a selective antagonist against dopamine (DRD2) receptors and inhibits the phosphorylation of ERK/AKT pathway, subsequently resulting in a decrease in GBM cell survival. Additionally, the inhibition of ERK/AKT pathway leads to dephosphorylation of FOXO3A wherein FOXO3A translocates into the nucleus and activates TRAIL expression. The BBB permeable ONC201 also acts as a direct activator of a serine protease, ClpP.[75] When ClpP, located in the mitochondrial matrix, is either activated or inhibited, antitumoral activity is achieved via dysregulation of mitochondrial proteostasis, disrupting the oxidative phosphorylation.[76] Disruption in the oxidative phosphorylation subsequently inhibits the GBM cell survival and activates the transcription factors ATF4 and CHOP, resulting in DR5 expression (Figure 4).[73]

Current studies focus on TRAIL-sensitizing compounds that demonstrated synergistic treatment efficacy with TRAIL in various GBM cell lines.[77,78] Moursin, isolated from the root bark of Morus alba, synergizes with TRAIL in GBM cell lines in vitro by upregulating DR5 and significantly downregulating IAPs survivin and XIAP.[79] Upregulation of these IAPs is associated with chemoresistance and radioresistance in the clinical setting against several cancers.[33,80] Therefore, future studies should investigate whether moursin and TRAIL can have a synergistic cytotoxic effect in murine GBM models. Inhibiting IAPs and anti-apoptotic proteins such as BCL-2 demonstrates synergistic tumor kill with TRAIL proteins in GBM cell lines. Furthermore, poly ADP ribose polymerase 1 (PARP-1) inhibitor, PJ34 was shown to synergize with TRAIL in GBM cells by restoring sensitivity in the extrinsic apoptotic pathway via BAX (pro-apoptotic protein)-dependent manner.[81] In vivo, combining PARP-1 inhibitor with TRAIL significantly reduced tumor growth compared with single

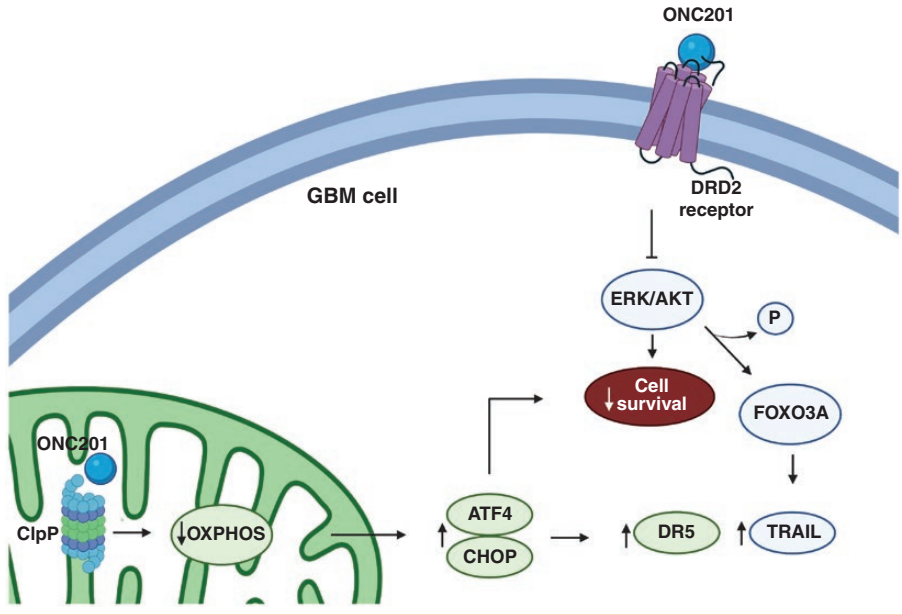


Figure 4. Proposed mechanisms for ONC201 in GBM (modified from www.chimerix.com). ONC201 selectively antagonizes dopamine (DRD2) receptors and inhibits the phosphorylation of ERK/AKT pathway, which results in a decrease in GBM cell cancer cell survival. Additionally, the inhibition of ERK/AKT pathway leads to dephosphorylation of FOXO3A. Dephosphorylated FOXO3A then translocates into the nucleus and upregulates TRAIL expression. BBB permeable ONC201 also selectively activates a mitochondrial protease, ClpP. Activated ClpP leads to mitochondrial proteolysis, disrupting the oxidative phosphorylation. Ultimately, the disruption in the oxidative phosphorylation inhibits the GBM cell survival and activates the transcription factors ATF4 and CHOP, resulting in DR5 upregulation. GBM, glioblastoma.

Table 2. Current Approaches in Enhancing TRAIL Therapies in GBM

Approach	Treatment	Preclinical		Clinical
		In Vitro	In Vivo	
Cell therapy	MSC-TRAIL and compound C	X	X	
Cell therapy	iNSC-TRAIL	X	X	
Cell therapy	Hemostatic agent FLOSEAL-encapsulated iNSC-TRAIL	X	X	
Cell therapy	TRAIL-secreting stem cells (SC-TRAIL) with cisplatin	X	X	
Gene therapy	PTEN-mRNA and TRAIL-mRNA	X	X	
Gene therapy	Tumor-targeted RGD–PEG–PEI and brain-targeted micelle CDX–PEG–PLA with Paclitaxel	X	X	
Gene therapy/ nanoparticle	Iron Oxide NP coated with chitosan–polyethylene glycol–polyethyleneimine copolymer and chlorotoxin	X		
Cell therapy/ nanoparticle	TRAIL overexpressing adipose-derived stem cells	X	X	
Nanoparticle	Co-delivery of TRAIL gene with paclitaxel	X	X	
Nanoparticle	Plant virus-based NP with mitoxantrone	X		
Nanoparticle	TRAIL-conjugated NP	X	X	
TRAIL sensitizer	ONC201			Phase III (Recruiting)
TRAIL sensitizer	Morusin	X		
TRAIL sensitizer	Celastrol	X		
TRAIL sensitizer	Pseudomonas exotoxin targeting IL13Ra2 and EGFR	X		
TRAIL sensitizer	IAP and Bcl-2 antagonists	X		
TRAIL sensitizer	Tanshinone IIA	X		
TRAIL sensitizer	SGI-1776	X		
TRAIL sensitizer	2,5-Dimethyl-celecoxib (DMC)	X		
TRAIL sensitizer	Chaetocin	X	X	
TRAIL sensitizer	ABT-737	X	X	
TRAIL sensitizer	Paclitaxel	X	X	
TRAIL sensitizer	Salinomycin	X	X	
TRAIL sensitizer	Carbenoxolone	X		
TRAIL sensitizer	MS275	X		
TRAIL sensitizer	Temozolomide	X	X	
TRAIL sensitizer	PARP inhibitors (Olaparib, PJ34)	X	X	
TRAIL sensitizer	Bortezomib	X	X	
TRAIL sensitizer	Synthetic iron(II)-polypyridyl complexes	X	X	
TRAIL sensitizer	Quinacrine and matrix-metalloproteinase (MMP)-sensitive and arg-gly-asp-ser (RGDS) peptide functionalized poly (ethylene-glycol) (PEG) particles	X		
TRAIL sensitizer/ chemotherapy	Mitoxantrone	X		

GBM, glioblastoma.

agents.[81] Another study reported that celastrol, a pleiotropic compound in traditional Chinese medicine, sensitized GBM cell lines to TRAIL by upregulating DR5 at both transcriptomic and proteomic levels.[82] The DNA-damaging chemotherapeutic agent mitoxantrone was also shown to synergize with TRAIL in vitro by upregulating both DR4 and DR5 and by altering pro- and anti-apoptotic gene expressions without causing toxicity in nonmalignant cells.[78]

Additional studies for TRAIL-sensitizing agents and selective inhibitors in models of GBM are summarized in Table 2. Although these agents induce DR expression and alter pro- and anti-apoptotic proteins, the underlying mechanisms of the drugs are poorly understood. However, given the promising in vitro results, additional in vivo studies for these drugs are necessary to evaluate their potential to combat the frequent obstacle of resistance in GBM.

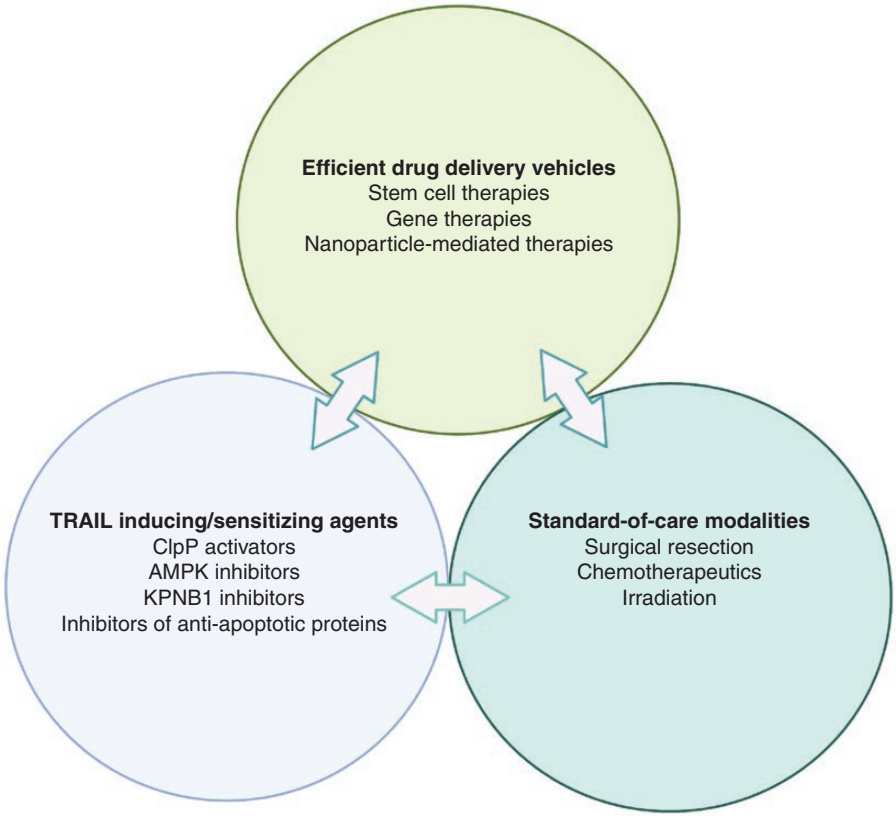


Figure 5. Combination therapy approaches to augment treatment durability in GBM to overcome the challenges associated with TRAIL and TRAIL-based therapies in clinical application. GBM, glioblastoma.

TRAIL-Based Therapies With Standard-of-Care

To yield synergistic efficacy and maximize the effect of TRAIL therapy, recent approaches have focused on combining TRAIL therapies with existing standard-of-care treatments. TRAIL is a worthy candidate to combine with standard-of-care treatments because it activates different pathways than many existing chemotherapies and radiation.[83] Most chemotherapies rely on the activity of the tumor suppressor p53, but this protein can become inactivated after initial treatment, decreasing the effectiveness of following rounds of chemotherapy.[84] In addition, disruption in p53-mediated apoptosis can result in chemoresistance and tumor progression.[85] Since TRAIL-mediated apoptosis does not require p53 activation, TRAIL can be used in conjunction with other chemotherapies to reduce the risk of treatment-resistant tumors.[84]

Previous studies have demonstrated that combining standard-of-care therapies with TRAIL can rescue sensitivity in TRAIL-resistant GBM cell lines by restoring the extrinsic apoptotic pathway.[60,86] A recent study revealed that TRAIL synergized with radiation in vitro by augmenting DR5 expression in resistant GBM cell lines.[86] Although GBM cancer stem cells are resistant to single agents, combining TRAIL with radiation achieved enhanced tumor kill by upregulating DR5 expression and downregulating c-FLIP expression, which is associated with TRAIL resistance.[86] In another study, temozolomide sensitized glioma stem-like cells to TRAIL by upregulating casitas B-lineage lymphoma (c-Cbl) protein while subsequently inhibiting c-FLIP expression in vitro. Convection-enhanced delivery of TRAIL with systemic administration of temozolomide has also been shown to prolong survival in GBM xenograft rats.[87] Cisplatin restored TRAIL-mediated apoptosis by inducing DR5 upregulation and c-FLIP downregulation in TRAIL-resistant GBM-derived neurospheres and demonstrated synergistic tumor kill with TRAIL in vitro.[88] Lastly, the combination of paclitaxel and TRAIL also augmented TRAIL-mediated apoptosis by upregulating DR4 and caspases 8 and 3 without inducing mitochondrial-related tumoricidal activity in GBM cells.[89] TRAIL-based therapies appear to be compatible with standard-of-care treatments. Futures studies should further investigate the combination of TRAIL-based therapies with standard-of-care treatments in in vivo orthotopic models to better understand potential synergistic tumoricidal activity, recurrence suppression, and survival outcomes.

Conclusion and Perspective

GBM is the most aggressive, incurable primary brain cancer in adults, and efficacious treatments are urgently needed to prolong survival outcome in patients.[1] TRAIL-mediated therapies hold immense potential for treating GBM because of their selectivity toward cancer cells to induce apoptosis without harming normal cells.[90] Nevertheless, TRAIL-based therapies are associated with limitations due to poor pharmacokinetics and resistance. Hence, innovative approaches in administering TRAIL and TRAIL-based therapies should be urgently explored in GBM. Here, a compilation of strategies is presented to overcome these challenges. Gene/cell therapies and NP-mediated therapies can promote continuous TRAIL release at the tumor site to circumvent poor pharmacokinetics such as short serum half-life. Development of TRAIL resistance in GBM is almost inevitable, and thus combining multiple therapies to target heterogeneous GBM is vital for durable tumor kill (Figure 5).[91] For example, patients could receive maximal surgical resection and TRAIL-secreting personalized stem cells encapsulated with biodegradable hemostatic agents such as FLOSEAL to prevent tumor recurrence.[58,92] In addition, TRAIL sensitizers could minimize the effects of TRAIL resistance. For patients who are ineligible for surgery, chemotherapy, and radiation could be accompanied by administration of either TRAIL proteins encapsulated NPs or TRAIL genes encapsulating NPs to target both intrinsic and extrinsic apoptotic pathways. Future studies of these combination approaches should vigorously assess in vitro, ex vivo, and in vivo orthotopic models of GBM to better understand their clinical potential. Finally, a better understanding of the mechanisms of TRAIL resistance in GBM will be important to maximize the potential of TRAIL-based therapies for the treatment of patients with GBM.

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Conflict of interest statement

We affirm that our manuscript has not been submitted for publication elsewhere, and there are no undeclared competing financial interests. We disclose that Dr. Hingtgen has ownership interest as CSO of Falcon Therapeutics. No potential conflicts of interest are disclosed by the other authors.

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