Chapter 2

On TRAIL for malignant glioma therapy?

Jos MA Kuijlen¹, Edwin Bremer², Jan Jakob A Mooij³, Wilfred FA den Dunnen³ and Wijnand Helfrich⁴

¹Department of Neurosurgery, University Medical Center Groningen, The Netherlands
²Department of Molecular Internal Medicine, Medical Clinic and Polyclinic II, University of Wuerzburg, Germany
³Department of Pathology and Medical Biology, University Medical Center Groningen, The Netherlands
⁴Department of Surgery, Surgical Research Laboratories, University Medical Center Groningen, The Netherlands

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Abstract

Glioblastoma Multiforma (GBM) is a devastating cancer with a median survival of around 15 months. Significant advances in treatment have not been reached yet, even with a host of new therapeutics under investigation. Therefore, the quest for a cure for GBM remains as intense as ever. Of particular interest for GBM therapy is the selective induction of apoptosis using the pro-apoptotic Tumor Necrosis Apoptosis Inducing Ligand (TRAIL). TRAIL signals apoptosis via its two agonistic receptors TRAIL-R1 and TRAIL-R2. TRAIL is normally present as homotrimeric transmembrane protein, but can also be processed into a soluble trimeric form (sTRAIL). Recombinant sTRAIL has strong tumoricidal activity towards GBM cells, with no or minimal toxicity towards normal human cells. Unfortunately, GBM is a very heterogeneous tumour, with multiple genetically aberrant clones within one tumour. Consequently, any single agent therapy is likely to be not effective enough. However, the anti-GBM activity of TRAIL can be synergistically enhanced by a variety of conventional and novel targeted therapies, making TRAIL an ideal candidate for combinatorial strategies. Here we will, after briefly detailing the biology of TRAIL/TRAIL-receptor signalling, focus on the promises and pitfalls of recombinant TRAIL as a therapeutic agent alone and in combinatorial therapeutic approaches for GBM.
Introduction

Glioblastoma multiforme (GBM) is the most frequent and aggressive type of tumour to develop from neuro-epithelial tissue. GBMs are very heterogeneous with multiple clones that contain varied genetic imbalances within one tumour, making it a very hard cancer to treat successfully. Even with improved surgical techniques and post-operative radiotherapy, the mean overall survival time of patients with GBM after neurosurgical debulking and radiotherapy is still limited to approximately 12 months. Importantly, most chemotherapeutics have no real beneficial effect on patient survival [1,2,3,4]. The only positive exception is the alkylating agent Temozolomide (TMZ), which in combination with radiotherapy prolongs survival by 2 to 3 months and doubles the number of long-term survivors [5]. However, it is painfully obvious that the treatment options of the clinician are at the moment ineffective for GBM. Therefore, development of new and more potent therapies is urgently needed.

In recent years, a variety of cancer-specific molecular aberrations have been identified and subsequently exploited as potential targets for the treatment of patients with GBM therapy. A particularly promising novel therapeutic approach for GBM is the reactivation of apoptosis using members of the Tumour Necrosis Factor (TNF) family, of which the TNF-related Apoptosis-Inducing Ligand (TRAIL) holds the greatest appeal. TRAIL is an effector molecule involved in immune surveillance by various T cell subpopulations and NK-cells. TRAIL is important for the elimination of virally-infected and cancer cells [6,7,8]. Apoptotic activity of TRAIL towards normal cells appears very limited, if present at all. By now a recombinant version of TRAIL has advanced into clinical trials for Chronic Lymphocytic Leukemia, with promising preliminary data on tolerability and beneficial therapeutic activity. Both the organized way of getting rid of malignant cells by apoptosis in combination with the lack of neuro or systemic toxicity makes TRAIL an interesting molecule to treat GBM. In this review, we first detail TRAIL-TRAIL receptor biology after which the potential of TRAIL-based therapeutics for the treatment of GBM will be discussed.
**TRAIL/TRAIL-receptor biology**

TRAIL is normally expressed on both normal and tumour cells as a non-covalent homotrimeric type-II transmembrane protein (memTRAIL). In addition, a soluble form of TRAIL (sTRAIL) can be generated due to alternative mRNA splicing [9] or proteolytic cleavage of the extracellular domain of memTRAIL [10,11] and thereby still retaining tumour-selective pro-apoptotic activity [12,13].

TRAIL has an intricate receptor system comprising 4 distinct membrane receptors, designated TRAIL-R1, TRAIL-R2, TRAIL-R3 and TRAIL-R4. Of these receptors, only TRAIL-R1 and TRAIL-2 transmit the apoptotic signal. These two receptors belong to a subgroup of the TNF receptor family, the so-called Death Receptors, and contain the hallmark intracellular death domain (DD). This DD is critical for apoptotic signalling by Death Receptors.

TRAIL activates the extrinsic pathway of apoptosis by binding to TRAIL-R1 and/or TRAIL-R2 (Figure 1), whereupon the adaptor protein FADD and initiator caspase-8 are recruited to the DD of these receptors. Assembly of this so-called Death Inducing Signalling Complex (DISC) leads to the sequential activation of initiator and effector caspases, and ultimately results in apoptotic cell death.

In certain cells, the execution of apoptosis by TRAIL further relies on an amplification loop via the intrinsic mitochondrial pathway of apoptosis. The mitochondrial pathway of apoptosis is a stress-activated pathway, e.g. upon radiation, and hinges on the depolarization of the mitochondria, leading to release of a variety of pro-apoptotic factors into the cytosol (Figure 2). Ultimately, this also triggers effector caspase activation and apoptotic cell death. This mitochondrial release of pro-apoptotic factors is tightly controlled by the Bcl-2 family of pro- and anti-apoptotic proteins [14]. In the case of TRAIL-receptor signalling the Bcl-2 Homology (BH3) only protein Bid is cleaved into a truncated form (tBid) by active caspase-8. Truncated Bid subsequently activates the mitochondrial pathway.

TRAIL-R3 is a GPI-linked receptor that lacks an intracellular domain, whereas TRAIL-R4 only has a truncated and non-functional DD. The latter two receptors are thought to function as decoy receptors that modulate TRAIL-sensitivity, however the mechanism underlying this decoy function is not yet elucidated. Evidence suggests that TRAILR3 binds and sequesters TRAIL in lipid membrane microdomains. TRAIL-R4 appears to form hetero-trimers with TRAIL-R2, whereby TRAIL-R2 mediated apoptotic signalling is disrupted. TRAIL-R4 might activate Nuclear Factor kappa B (NFkB), although conflicting evidence concerning activation of NFkB exists [15,16]. Of note, TRAIL also interacts with the soluble protein Osteoprotegerin (OPG), although the exact consequence of this interaction remains to be clarified.
TRAIL-(receptor) expression in human tissues

Although data on the expression of TRAIL in normal human tissues is limited, diffuse expression of TRAIL has been detected on liver cells, bile ducts, tubuli contorti of the kidney, cardiomyocytes, lung epithelia, Leydig cells, normal odontogenic epithelium, megakaryocytic cells and erythroid cells[17,18,19,20]. In contrast, no or weak expression of TRAIL was in colon, glomeruli, Henle’s loop, germ and Sertoli cells of the testis, endothelia in several organs, smooth muscle cells in lung, spleen and in follicular cells in the thyroid gland[21,22]. With regard to the brain, expression of TRAIL has been detected on mRNA levels in normal brain tissue (not specified if this was neuronal or glial tissue) as well as protein level (glial cells of the cerebellum) [23,22]. One study was unable to confirm these findings [24].

All of the TRAIL receptors are expressed on many normal tissues. For instance, TRAIL-R1 is expressed on heart myocytes, colon, lung epithelium, Leydig cells, ovaries and in the brain on astrocytes[25,17,24]. TRAIL-R2 is detected on heart myocytes, colon, lung epithelium, Leydig cells, ovaries, oligodendrocytes and on neurons [25,17,24]. Vascular brain endothelium appears to be negative for TRAIL-R1 and weakly positive for TRAIL-R2 [17]. With regard to the decoy receptors, TRAIL-R4 and TRAIL-R3 have been detected on oligodendrocytes and neurons[24].

TRAIL-receptor expression in glioblastoma

TRAIL-R1 and TRAIL-R2 are ubiquitously expressed on a variety of tumour types [26,25,27,17,21,28]. Importantly, TRAIL-R1 and TRAIL-R2 are also expressed in the tumour tissue from astrocytoma grade II and glioblastoma patients[23]. In a study on 62 primary GBM tumour specimens, TRAIL-R1 and TRAIL-R2 were expressed in 75% and 95% of the tumours respectively. Of note, a statistically significant positive association was identified between agonistic TRAIL receptor expression and survival [29]. Interestingly and perhaps counter intuitively, highly malignant tumours actually express a higher amount of TRAIL receptors in comparison to less malignant tumours or normal tissue. In general TRAIL-R2 is more frequently expressed on tumour cells than TRAIL-R1. Several studies in GBM cell lines were unable to correlate TRAIL-sensitivity to the expression levels of the agonistic TRAIL receptors TRAIL-R1 or TRAIL-R2 nor to the expression levels of the decoy receptors TRAIL-R3 and TRAIL-R4 [30,31].

Recombinant TRAIL and TRAIL-R specific antibodies for tumour therapy

TRAIL and agonistic antibodies directed at the TRAIL death receptors TRAIL-R1 and/or TRAIL-R1 hold a prominent place as potential anti-cancer drugs[32,33,34]. Indeed, many
tumour types are sensitive to apoptotic elimination by TRAIL, whereas normal human cell types are resistant. A variety of sTRAIL preparations has shown promising tumoricidal activity in vitro and in vivo. Importantly, loco-regional application of TRAIL in an intracranial GBM xenograft model of the cell line U87MG revealed strong tumoricidal activity towards pre-established xenografts, with long-term survival of >100 days in treated mice compared to ~36 days survival in non-treated mice.

These preclinical studies have illustrated the promise of TRAIL as a therapeutic reagent in vivo with no or minimal toxicity. Indeed, a recombinant trimeric form of TRAIL is being explored in an ongoing multi-centre clinical trial for B-Chronic Lymphocytic Leukemia patients. Preliminary reports indicate that the main effect of single agent TRAIL treatment in CLL patients is the induction of stable disease and a number of partial responses at higher doses of 8 mg/kg[35,32]. Importantly, no significant side-effects have been reported so far, thus corroborating the apparent safety of sTRAIL treatment in humans.

In addition, a number of agonistic antibodies (HGS-ETR1, HGS-ETR2, HGS-TR2J, LBY135, CS-1008, AMG 655) that selectively target TRAIL-R1 or TRAIL-R2 have been developed. All of these antibodies have potent tumoricidal activity in vitro and in vivo and appear to have a low toxicity profile in early phase clinical studies [36,33,37,38,39]. An obvious difference between these TRAIL-receptor-selective mAbs and TRAIL is the fact that TRAIL interacts with both of its agonistic receptors. This might provide TRAIL either with a wider therapeutic spectrum or a narrow and more unpredictable therapeutic window, especially in light of its additional interaction with decoy TRAIL receptors.

It is interesting to note that several groups have pursued the design of sTRAIL variants that show selectivity for TRAIL-R1 or TRAIL-R2[40,41,42,43]. Although the precise fine-specificity of some of these variants remains a matter of debate, the use of TRAIL-receptor selective variants for the treatment of a specific tumour type may prove valuable. For instance, CLL appears to be preferentially sensitive to TRAIL-R1 apoptotic signalling, whereas certain solid tumours appear to preferentially signal via TRAIL-R2. Rational integration of TRAIL-receptor selective sTRAIL variants may in those cases help to optimize efficacy.

Importantly, as will be described in more detail below, normal cells can be sensitized to sTRAIL by certain other anti-cancer drugs. These side effects are likely due to a sensitizing effect by the co-administered drug on normal cells for the ubiquitous priming of TRAILR1 by sTRAIL trimers, since sTRAIL trimers are fully capable of TRAILR1 activation. In contrast, TRAILR2 is not/minimally activated by homotrimeric sTRAIL. Therefore, it seems a reasonable assumption that TRAILR1 signalling is the main culprit behind potential side effects of sTRAIL trimers. Thus, the rational design and use of TRAIL-R2 selective sTRAIL variants may help to optimize therapeutic efficacy, while minimizing the occurrence of toxic side-effects.
TRAIL-resistance in GBM

The available preliminary data indicate that activation of apoptotic TRAIL-receptor signalling using sTRAIL or agonistic TRAILR antibodies may indeed prove beneficial to cancer patients and certainly warrant further evaluation of this reagent in clinical trials. However, intrinsic and/or acquired resistance to TRAIL-receptor signalling is likely to pose a significant hurdle to clinical efficacy. Indeed, almost half of tumour cell lines analyzed have intrinsic resistance to TRAIL-receptor signalling, which also holds true for GBM cell lines. Resistance of GBM to TRAIL may be due to a variety of reasons, including high decoy receptor expression, low expression levels of critical mediators of TRAIL-signalling, such as caspase-8 and FADD [30], or high expression of inhibitors of apoptosis such as cellular-Flice Inhibitory Protein (cFLIP)[44-46]. Interestingly, a recent report indicates that non-genetic naturally occurring differences in the levels or states of anti- or pro-apoptotic proteins are the primary causes of cell-to-cell variability in timing and likelihood of apoptotic cell death in cell lines [47]. Of note, TRAIL-resistance seems to be even more pronounced when assessing TRAIL activity towards primary patient material. Indeed, TRAIL sensitivity in GBM cell lines does not correlate well with activity towards primary GBM cells. In fact, TRAIL resistance in primary GBM cells appears rather widespread, thus questioning the ultimate clinical benefit of TRAIL as single agent therapy.

Overcoming TRAIL-resistance by combination therapy with radiation/chemotherapy

Intrinsic or acquired resistance to TRAIL can often be overcome by combination of TRAIL-based agents with chemotherapeutics, radiation or other novel therapeutic drugs. Preliminary clinical data also highlights the rationale of this approach, with 2 complete and 2 partial responses upon co-treatment of a small group of non Hodgkin lymphoma (NHL) patients with TRAIL and the anti-CD20 antibody rituximab [48]. These clinical observations are corroborated by recent in vitro data indicating that combined treatment of cells with rituximab and TRAIL or an agonistic TRAILR1 antibody synergistically induced apoptosis[49,50]. Thus, the presence of in vitro synergy may be a useful indicator for potential clinical benefit in combinatorial strategies.

Both radiotherapy and chemotherapy have been studied in combination with TRAIL in preclinical studies in a variety of tumour types [51-62]. With regard to GBM, positive results on tumour regression were obtained after combination therapy. This synergy may be due to various points of crosstalk between TRAIL and chemo/radiation (for overview see figure 3, pg 184) including upregulation of agonistic TRAIL receptors by irradiation [56-58] and chemotherapy [59]. Of note, upregulation of TRAIL-R2 by chemotherapeutics in TRAIL resistant GBM cell lines appears to be p53 dependent, with upregulation of TRAILR2 only occurring in p53wt but not p53mut cells[60]. In contrast, others have found no effect on the level of receptor expression after irradiation or chemotherapy [51,61]. As p53 mutations
are mainly associated with secondary GBM’s while EGFR amplification and mutation is strongly associated with “de novo” GBM it should be considered that bypassing resistance, for both GBM subgroup tumors, requires different targeting strategies.

Another possible point of synergy is down-regulation of the anti-apoptotic proteins cFLIP and phosphoprotein enriched in Diabetes/Astrocytes (PED/PEA-15) that both competitively inhibit caspase-8 activation in the DISC[63].

Systemic in vivo administration of TRAIL with cisplatin synergistically suppressed both tumour formation and growth of established subcutaneous human glioblastoma xenografts in nude mice and also significantly extended the survival of mice bearing intracerebral xenografts compared to single agent treated mice [59]. In another study, the efficacy of intra-cerebral infused TRAIL was significantly enhanced by co-treatment with TMZ in a U87MG intracranial xenograft model [62]. As TMZ is nowadays part of the standardized treatment schedule of patients with GBM it will be in the future used in combination with several other drugs for example TRAIL.

**Overcoming TRAIL-resistance by combination therapy with new ‘smart’ drugs**

Preclinical studies have also evaluated the combination of sTRAIL with a variety of novel therapeutic approaches for potential synergistic pro-apoptotic activity (for overview see figure 3, pg 184). The results of all these studies clearly demonstrate the added benefit of combination therapy on TRAIL-mediated cytotoxicity. Of particular interest for GBM is the combination treatment of cells with TRAIL and proteasome inhibitor Bortezomib. Bortezomib inhibits the Ubiquitin-proteasome pathway (UPP), which controls the timely removal and degradation of the majority of cellular proteins[64]. An important feature of bortezomib is the differential response of normal and cancer cells to treatment[65]. Both normal and cancer cells are growth-arrested in the G2/M phase of the cell cycle. However, whereas cancer cells die by apoptosis, normal cells resume division after treatment. Bortezomib has been shown to potently augment the apoptotic activity of other therapeutics, including TRAIL[66]. Notably, primary TRAIL-resistant GBM cells were highly sensitive to combination treatment with bortezomib and TRAIL[63].

Another interesting candidate is the antibiotic rapamycin, which inhibits the pro-survival Akt-mTOR pathway by inhibiting mTOR. Akt pro-survival signalling is often up-regulated in glioblastoma and therapeutic inhibition appears warranted. Importantly, rapamycin sensitizes cells to TRAIL-mediated apoptotic signalling. The Akt-mTOR pathway is causally linked to PTEN status of glioblastoma cells, which may be used to enable the identification of a subset of patients that would benefit from rapamycin-TRAIL combination therapy[67]. Also X-linked inhibitory apoptotic proteins (XIAP) antagonists are used in combination with TRAIL. Clinical studies with antisense oligonucleotide targeting
X-linked IAP’s (XIAP) are ongoing[68].

As described above, the intrinsic mitochondrial pathway of apoptosis is regulated by the balance between pro- and anti-apoptotic members of the Bcl-2 family[14]. In GBM, anti-apoptotic proteins such as Bcl-2 are frequently overexpressed, leading to cell survival. Selective inhibition of these anti-apoptotic proteins has been successfully pursued using the small molecule ABT-737, a mimetic for Bcl-2 and Bcl-xL[69]. ABT-737 has shown prominent activity towards various different types of tumour. Recently, ABT-737 was also shown to markedly prolong survival in an intracranial xenograft GBM model[70]. Moreover, ABT-737 synergistically enhanced the activity of sTRAIL as well as standard chemotherapeutic drugs in GBM cells.

Another approach of particular interest in modulating TRAIL-sensitivity is the specific up or down-regulation of microRNAs (miRs). MiRs are small (20-22 nucleotide) non-coding RNAs that degrade or inhibit translation of mRNA by binding to recognition sequences on the mRNA sequence. One miR can modulate a number of genes and as such function as a master regulator. In the case of apoptosis signalling for instance, several miRs have been shown to imprint an apoptosis resistant phenotype on tumour cells. Several miRs have been reported to modulate apoptotic signalling by TRAIL and other TNF-family members. In GBM, a specific miR (miR21) has been reported as highly overexpressed in >90% of tumours analyzed. Interestingly, inhibition of miR21 significantly blocked GBM outgrowth, while co-treatment of anti-miR21 therapy with neural stem cells expressing sTRAIL resulted in synergistic inhibition of tumour growth in vivo.

An important consideration to make regarding all of these combinatorial strategies is the possible sensitization of normal cells. For instance, synergistic pro-apoptotic anti-cancer activity upon combination of sTRAIL with proteasome inhibition yielded a therapeutic window in hepatoma cells, but was also associated with enhanced toxicity towards hepatocytes[71]. In addition, hepatocytes were strongly sensitized to Fas upon initial priming with TRAIL[72]. Hepatocytes indeed appear the most TRAIL-sensitive type of cell, with aggregated TRAIL preparations strongly reducing hepatocyte viability[73]. Therefore it is apparent that purely homogenous sTRAIL as well as the rational design of non-toxic combinatorial strategies is required for effective anti-cancer strategy in humans.

Target cell-restricted delivery and optimal activation of TRAIL apoptotic signalling

From a conceptual point of view, the efficacy of sTRAIL is likely to be hampered by several factors, including rapid clearance from the circulation by the kidney. Indeed, sTRAIL has an approximate half-life of only 30 min in primates and a similar pharmacokinetic profile in humans in a phase I clinical trial [32, 74]. Together with the ubiquitous expression of TRAIL receptors in the human body this may severely limit tumour accretion.
Moreover, many tumours express higher levels of TRAILR2 compared to TRAILR1, whereas TRAILR2 signalling is only poorly activated by sTRAIL[75].

We and others have attempted to overcome these drawbacks by fusing sTRAIL to an antibody derivative, such as an antibody fragment. The resultant trimeric molecule will be ~180 kDa and likely has a longer circulation half-life, since renal clearance should be impeded at these higher molecular weights. The antibody targeting domain of the fusion protein will ensure better tumour-accretion and retention (for schematic see figure 4, pg 185) [76-s80]. Importantly, antibody fragment-mediated binding to a cell surface-expressed target antigen converts the sTRAIL into membrane-bound TRAIL that efficiently signals apoptosis via TRAILR1 but also TRAILR2 in a mono- and/or bi/multi-cellular manner[81,82]. In this way also neighbouring tumour cells devoid of target antigen can be effectively eliminated by the so-called bystander effect[83]. The promise of this approach has been shown preclinically in vitro and in vivo for both solid tumours and leukaemia[76,77,78,79].

Of particular interest for GBM is the targeted delivery of sTRAIL to the Epidermal Growth Factor Receptor (EGFR) using EGFR-blocking antibody fragment scFv425. Binding of this blocking antibody fragment to EGFR inhibited EGFR-mitogenic signalling, while the sTRAIL domain at the same time efficiently activated TRAILR-apoptotic signalling (for schematic see figure 5, pg 186)[78]. Obviously this bifurcate strategy of inhibition of tumourigenic EGFR signalling and simultaneous activation of apoptotic signalling is of great appeal for GBM. Moreover, dual EGFR-inhibition by further combination with EGFR tyrosine kinase inhibitor Iressa synergistically enhanced apoptosis by scFv425:sTRAIL. Based on the available data, we further attempted to exploit a reportedly TRAILR1 selective mutant for targeted therapy to EGFR-positive tumour cells. This EGFR-targeted sTRAIL mutant showed a significantly higher activity on ~50% of the cell lines analyzed, whereas it lacked activity towards normal human hepatocytes. However, in our experiments we identified residual binding as well as signalling capacity for TRAILR2[76]. Although the sTRAIL mutant may not be TRAIL-receptor selective, the augmented activity upon targeted delivery to EGFR indicates that the targeted delivery of rationally designed sTRAIL mutants may help to optimize TRAIL-based therapy.

**Gene delivery strategies for TRAIL-GBM therapy**

As described above, sTRAIL has a rather poor half-life and is likely to be poorly delivered to the tumour. This holds particularly true for GBM cells in the infiltrating zone, where the Blood Brain Barrier still functions and will hamper tumour accumulation of sTRAIL. Several groups have attempted to circumvent these problems by using gene therapeutic approaches. A particularly interesting approach is the transduction of neural stem cells with sTRAIL. Neural stem cells exhibit extensive tropism for GBM and have been shown
to migrate towards outgrowing microsatellites. Thus, secretion of sTRAIL by these cells will ensure GBM localized production. Various preclinical studies have revealed a potent anti-GBM effect of TRAIL-transduced neural stem cells. Of note, combinatorial strategies with these neural stem cells and temozolomide synergistically inhibited GBM outgrowth. In an analogous fashion, the use of human umbilical cord blood-derived mesenchymal stem cells (UCB-MSC) transduced with sTRAIL resulted in prolonged survival of GBM bearing mice. The advantage of these cells over neural stem cells may lie in the ease of isolation and expansion compared to neural stem cells. In addition, ethical problems may be less of an issue for the latter cell type. Next to the use of cell-based strategies, direct TRAIL gene delivery to the tumour using e.g. adenoviruses or Adenovirus associated vectors (AAV) has also resulted in promising preclinical activity in vivo.

**Delivery strategy for TRAIL-based GBM therapy**

An important issue for any GBM-targeted therapeutic strategy is the selective delivery to the vicinity of the tumour. Animal bio-distribution studies with radio-iodinated rhTRAIL (\(^{125}\)I-rhTRAIL) have demonstrated that intravenous injection of TRAIL does not yield detectable levels of TRAIL in the brain. Therefore, local delivery strategies such as Convection enhanced delivery (CED) seem more appropriate. CED uses positive pressure infusion to achieve loco-regional delivery of therapeutic agents through an intra-cerebral catheter\[84,85\]. In animal models, CED can achieve locally high and effective concentrations. By now CED has progressed into phase III clinical studies for immunotoxin delivery \[86,87\], results of which are likely to yield insight into the feasibility of using CED on a routine basis in GBM patients and its potential applicability for TRAIL-based therapy.
Conclusions and perspectives

The ample preclinical data on GBM cell lines and primary GBM tissue, as well as the notable absence of TRAIL-related toxicity in phase I clinical trials, clearly highlight that TRAIL receptor-targeted strategies hold great appeal for future cancer and more specifically GBM therapy. However, it is also evident from the available literature that GBM is unlikely to be sufficiently responsive to single agent therapy with TRAIL-receptor targeted strategies. Indeed, when taking into account the inherent heterogeneity of GBM it seems most prudent to examine the feasibility of combinatorial strategies that on the one hand sensitize GMB cells to apoptosis, and on the other hand induce apoptosis using TRAIL or agonistic TRAIL-receptor antibodies. As highlighted in this review, TRAIL can be combined with a variety of different conventional and novel therapeutic strategies to yield synergistic pro-apoptotic activity. Of particular appeal in our opinion is the use of dual purpose TRAIL-based molecules, such as the EGFR-targeted TRAIL fusion protein scFv425:sTRAIL. This fusion protein simultaneously blocks EGFR-mitogenic signalling; thereby sensitizing tumour cells to apoptosis, and induces apoptosis via TRAIL-receptor signalling. This fusion protein efficiently activates apoptosis and shows promising in vivo activity. Obviously, further rational combination with other therapeutic strategies may help to optimize anti-GBM activity.

An important aspect in considering GBM therapy is the observation that, as in many other types of tumour, a so-called ‘stem cell’ population can be identified in GBM. These Glioblastoma Stem Cells (GSCs) can re-grow into original glioblastoma in xenograft nude mouse models and express neural stem cell markers, such as CD133. Importantly, GSCs are particularly refractory to radiotherapy and chemotherapy due to e.g. over-expression of multidrug resistance pumps and over-expression of aldehyde dehydrogenase. A recent report identified, in 2 primary patient-derived GSC cultures, that these cells were also refractory to sTRAIL treatment, partly due to selective down-regulation of caspase-8. Whether or not the sTRAIL used in this study was also capable of efficiently activating TRAIL-R2 and whether for instance target cell-restricted activation of TRAIL using scFv:sTRAIL could restore sensitivity of these cells to apoptotic elimination TRAIL is not clear and warrants further investigation. Regardless, this study does serve to illustrate the heterogeneity of GBM, with certain subpopulations that may be (more) refractory to TRAIL-treatment and further illustrates the need for combinatorial therapeutic approaches. Indeed, in a study with the Bcl-2 mimetic ABT-737 the GSC subpopulation of cells was more resistant to treatment than the non-GSC population. This resistance was likely due to over-expression of the anti-apoptotic Bcl-2 family member Mcl-1, already known to confer resistance to ABT-737 in other tumour cell types[88]. Therefore, effective treatment regimes have to include the GSC subpopulation and capitalize on synergistic and complementary activities of the individual reagents.

As reported above, the specific modulation of miRs may be of particular interest, since miR modulation influences the expression of a number of genes and as such can function as a
master regulator. Recent efforts in this field have also helped identify several miR families that are involved in ‘stem cell-ness’, including let-7 and miR-200. Therefore, rational integration of therapeutic miR modulation with TRAIL (and conventional) therapy may prove an elegant way of shifting the intrinsic cellular balance of normal GBM cells and GBM stem cells towards apoptotic elimination. In a related fashion, the use of small inhibitory RNA to selectively down-regulate an important anti-apoptotic gene, such as cFLIP, may be applied to sensitize GBM for TRAIL-based strategies. The use of siRNA has to date been limited by the question of selective delivery to target cells. However, in a recent seminal paper the use of antibody fragment-targeted anti-HIV siRNA proved successful in curing HIV-infected mice. A similar approach may be adapted to GBM. Indeed, GBM is one of the few cancers reported to express a tumour-specific antigen, the EGFR variant III, for which the MR1-1 antibody fragment is available. Thus, GBM seems an ideal candidate to test the applicability of this novel scFv-siRNA approach in cancer.

Obviously, the application of such rational combinatorial strategies critically depends on the proper identification of specific cancer-related aberrancies in each individual patient/tumour as well as the ability to monitor biological response via e.g. downstream pathway components. Therefore, further development of reliable, cost-effective and high-throughput diagnostic tools will be required to enable the successful application of such patient-tailored therapeutic approaches. Such molecular profiling for GBM is still in its infancy but has gained attraction in recent years with several useful markers available, including EGFRvIII[89].

In conclusion, the vast body of evidence from preclinical data indicates that the rational design of combinatorial TRAIL-based approaches with conventional as well as novel therapeutics may ultimately help to combat GBM and improve patient survival for this devastating disease.
The extrinsic apoptosis pathway triggers apoptosis independently of p53 in response to pro-apoptotic ligands, such as Apo2L/TRAIL. These ligands activate specific pro-apoptotic receptors, such as TRAIL-R1 and TRAIL-R2. TRAIL-R1 can induce apoptosis after binding non-cross-linked and cross-linked sTRAIL. TRAIL-R2 can only be activated by cross-linked sTRAIL. Death receptor binding leads to the recruitment of the adaptor FADD and initiator procaspase-8 and 10 to rapidly form the DISC. Procaspase-8 and 10 are cleaved into its activated configuration caspase-8 and 10. Caspase-8 and 10 in turn activate the effector caspase-3, 6 and 7, so triggering apoptosis. cFLIP, cellular FLICE-inhibitory protein; DISC, death-inducing signalling complex; FADD, Fas-associated death domain; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.
The intrinsic apoptosis pathway is triggered in response to DNA damage and other types of severe cell stress and involves the release of pro-apoptotic factors from the mitochondria. The p53 oncogene protein activates the pro-apoptotic BCL2 family proteins BAX and BAK which leads to the release of cytochrome c. Cytochrome c activates the apoptotic protease caspase 9. Caspase 9, in turn, activates downstream caspases, including caspase 3, 6, and 7, leading to apoptosis. SMAC/DIABLO, directly interacts with inhibitor of apoptosis (IAP) proteins, preventing them from attenuating apoptosis. Anti-apoptotic members of the BCL2 family regulate the mitochondria-initiated caspase activation pathway by preventing the release of cytochrome c (BAK: BCL2 homologous antagonist/killer; BAX: BCL2-associated protein; BCL2: B-cell chronic lymphocytic leukemia/lymphoma 2; IAP: inhibitor of apoptosis protein; SMAC: second mitochondria-derived activator of caspase; DIABLO: direct IAP binding protein with low pl).
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