INTRODUCTION

Glioblastoma (GBM) is a devastating primary brain cancer that has a poor prognosis for patients due to limited treatment options. One of the main reasons for poor treatment efficacy is due to chemoresistance. The major chemotherapeutic drug used for GBM is temozolomide (TMZ), and TMZ-resistance is a major reason for tumor recurrence following standard-of-care therapies [surgical resection, radiation therapy, chemotherapy, followed by an anti-angiogenic antibody against vascular endothelial growth factor (VEGF), also known as bevacizumab or Avastin]. The poor efficacy of therapy, and a short interval between remission and recurrence, is thought to be due to the resistance of a small fraction of tumorigenic cells, which are often attributed to cancer stem cells, in their response to treatment[1]. There is compelling experimental evidence that suggests that the cancer stem cells present are therapy-resistant glioblastoma stem cells, which subsequently leads to tumor recurrence and subsequent metastasis[1-5]. Common gene mutations associated with GBM include, epidermal growth factor receptor (EGFR)[6], IDH1[7], PDGFRA[8-10], HDM2[11-13], PIK3CA[14,15], TERT[16], PIK3R1[10,15], PTEN[17,18], TP53[19], CDKN2A[20,21], NF1[22], ATRX[23,24], and RB[25]. Many of these have been investigated regarding therapeutic targets, however efficacy results have been unfruitful in substantially increasing overall survival (OS). There are other genes, proteins and pathways of interest that may provide more promise. The purpose of this review is to identify novel therapeutic approaches to target genes and pathways associated with GBM chemoresistance. In our group, we have developed and characterized two potential therapeutic approaches in pre-clinical GBM xenograft models. One involves a small molecule called OKN-007, which effects the transforming growth factor β1 (TGF-β1) pathway, and is currently in clinical trials for adult GBM. The other is a monoclonal antibody against a novel target, identified by bioinformatics, called ELTD1 or ADGRL4, which is currently being translated for subsequent human trials. In addition to these therapeutic approaches that address chemoresistance in GBM, we will also discuss recent promising therapeutic target developments by other investigators. A summary of the pathways, signaling molecules or tumor environments that can be targeted with therapeutic approaches to decrease tumor drug resistance is presented in Table 1.

THERAPEUTIC OPTIONS FOR TMZ-CHEMORESISTANCE IN GBM

OKN-007 targeting of the TGF-β1 pathway

TGF-β signaling drives the regulation of proliferation, differentiation and survival/or apoptosis of several cells, including glioma cells[26]. TGF-β acts through explicit receptors that activate multiple intracellular pathways, resulting in the phosphorylation of receptor-regulated Smad2/3 proteins that are associated with the common mediator, Smad4[26-28]. This complex translocates to the nucleus, and subsequently binds to DNA and regulates the transcription of several genes[26]. In addition, TGF-β-activated kinase-1 is an element of TGF-β signaling, and activates mitogen-activated protein kinase (MAPK) cascades[26]. Negative regulation of TGF-β/Smad signaling often occurs through the inhibitory Smad6/7 signaling path[26,29,30]. Although the genetic alterations in TGF-β genes related to signaling are relatively infrequent in gliomas, the altered expression of those genes is a frequent event[26]. The increased expression of TGF-β1-3 correlates with the degree of malignancy in human gliomas[26,31]. TGF-β may contribute to tumor pathogenesis in several means, such as, via the direct support of tumor growth[26,32], via maintaining self-renewal of glioma initiating stem cells[26,33,34], and by inhibiting anti-tumor immunity[26,35]. Glioma initiating cells are thought to be dedifferentiated cells that maintain many stem cell-like properties, and play a role in tumor initiation, as well as contributing to tumor recurrence[26]. TGF-β1,2 stimulates the expression of VEGF, the plasminogen activator inhibitor, and some metalloproteinases that are implicated in vascular remodeling, angiogenesis and degradation of the extracellular matrix[26,36-38]. Inhibitors of TGF-β signaling have been found to reduce the proliferation and subsequent invasion of gliomas in animal models, and could provide a path forward for developing promising anti-tumor therapeutics[39,40].

There is a differential expression of TGF-β1 in GBM tumors[41]. Specifically, it has been reported that there was a significant relationship between TGF-β1 expression and OS and progression free survival in newly

Table 1. Summary of pathways, signaling molecules, or tumor environments that can be targeted with therapeutic approaches in order to help combat tumor drug resistance

Pathway/target	Therapeutic approaches	Ref.
TGF-β	OKN-007 (TGFβ pathway inhibitor)	[40]
PI3K	BKM120 (PI3K inhibitor)	[55]
	GDC-0941 (PI3K inhibitor)	[53]
NFκB	Parthenolide (NFκB inhibitor)	[61]
c-MET	Endothelial cell-specific knock-out of MET	[63]
Notch	miR-139-5p (oncogene inhibitor)	[64]
	DAPT, MRK-003, GSI-18 (GSIs)	[66,67,71]
	GW280164X, INCB3619 (ASIs)	[72]
	Antibodies against ELTD1	[74,75]
EGFR	Combined therapies targeting EGFR (gefitinib) and mTOR (sirolimus, everolimus)	[82-84]
TME	Targeting macrophages and monocytes	[91]

TGF-β: transforming growth factor β; PI3K: phosphoinositide 3-kinase; NFκB: nuclear factor-κB; EGFR: epidermal growth factor receptor; TME: tumor microenvironment; GSIs: γ-secretase inhibitors; ASIs: α-secretase inhibitors

diagnosed GBM[41]. It has been found that dysregulated TGF-β signaling leads to a cascade of events that contribute to oncogenesis[42,43], which includes decreased apoptosis[42,44], up-regulated proliferation[42,45], immune surveillance evasion[42,46], and an epithelial-to-mesenchymal transition (EMT)[42,47].

We have previously found that OKN-007 increases TMZ sensitivity and also suppresses TMZ-resistant GBM tumor growth[40]. OKN-007 seems to elicit its effect on GBM tumors by inhibiting tumorigenic TGF-β1, mainly by affecting the extracellular matrix [Figure 1][40]. When combined with TMZ, OKN-007 was found to significantly increase percent survival [Figure 2A], decrease tumor volumes [Figure 2B-F], and normalize tumor blood vasculature *in vivo* compared to untreated tumors in a human GBM G55 orthotopic xenograft model[40]. It is known that TGF-β1 plays a major role in TMZ-resistance[48,49], and we believe that OKN-007 may actually be affecting TMZ-resistance by targeting TGF-β1.

When we obtained RNA-seq data for TMZ-resistant LN18 human GBM cells, and compared the combined TMZ + OKN treatment group to TMZ-treatment alone, we found an interesting downregulated gene, SUMO2[48], that seems to be directly associated with treatment resistance. It was previously found that overexpression of SUMO, which occurs in conditions such as brain ischemia and hypoxia, could increase cell survival, whereas in contrast, the knockdown of SUMO expression has been shown to be toxic to cells and is associated with TGFβ1 in resistant glioma cells[48]. In particular, SUMOylation has been found to regulate TGF-β1/Smad4 signaling in-resistant glioma cells[49].

Targeting the phosphoinositide 3-kinase pathway

Phosphatidylinositol-3 kinases, PI3Ks, comprise of a lipid kinase family that are characterized by their ability to phosphorylate the inositol ring 3'-OH group in inositol phospholipids, which leads to the generation of a second messenger phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P 3)[50,51]. Subsequently, receptor protein tyrosine kinase activation results in PI (3,4,5)P 3 and PI (3,4)P 2 production by PI3K at the inner side of the cellular plasma membrane[51,52]. protein kinase B (Akt) then interacts with these phospholipids, resulting in its translocation to the inner membrane, where it then becomes phosphorylated and activated by PDK1 and PDK2[51,52]. Activated Akt is known to modulate the function of numerous substrates that are involved in the regulation of cellular growth, cell cycle progression, and cell survival[51]. More recently it has been shown that several components of the PI3K/Akt signaling pathway are commonly altered in human cancers[51]. It is widely known that cancer treatments by chemotherapy and γ-irradiation kills target cells primarily by the induction of apoptosis[51]. Unfortunately, resistance to therapy commonly occurs, and is a major clinical problem that needs to be solved. Failure to activate apoptosis is characteristic as an important mechanism of drug resistance in tumor cells[51]. As cell survival signals are

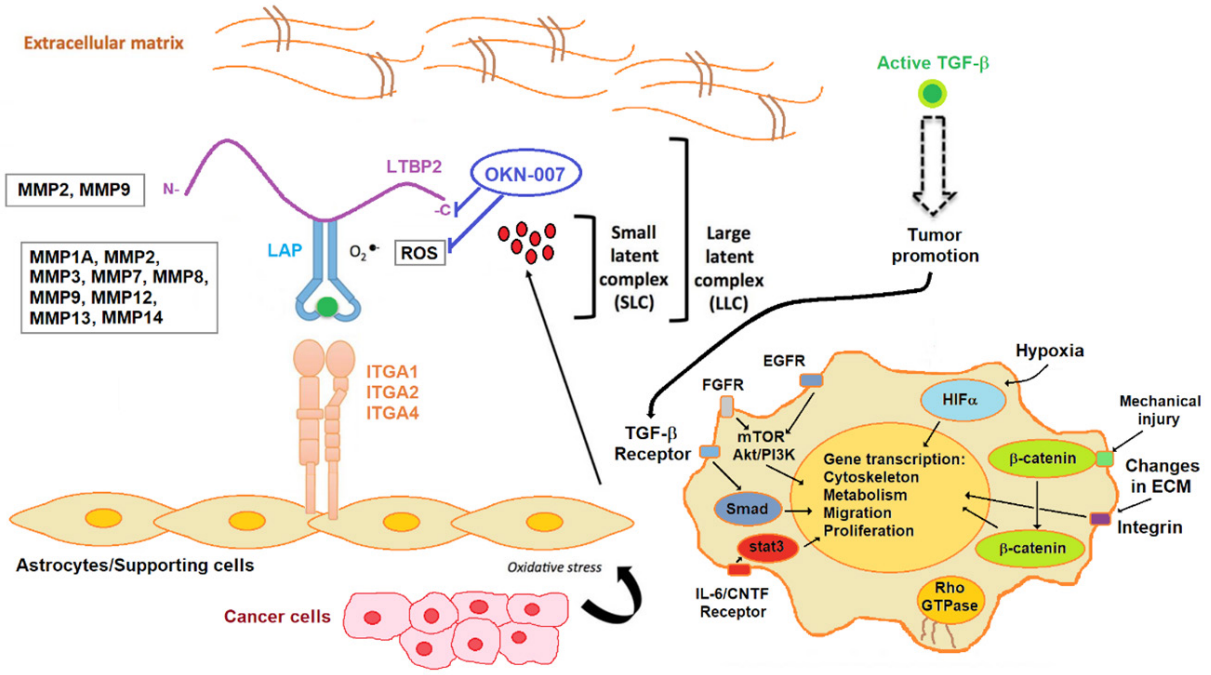

Figure 1. Stromal activators of transforming growth factor-β (TGF-β) in the tumor microenvironment. MMP2 and MMP9 proteolytically cleave latent TGF-β binding protein (LTBP), thereby releasing latent TGF-β from the extracellular matrix. MMP1A, MMP2, MMP3, MMp7, MMP8, MMP9, MMP12, MMP13 and MMP14 activate latent TGF-β via proteolytic cleavage of the latency-associated peptide (LAP), while integrins expressed on astrocytes (ITGA1, 2 and 4) bind to the large latent complex (LLC) and activate latent TGF-β through MMP-dependent cleavage of LAP. Integrins (ITGA1, 2 and 4) bind to the LLC and induce conformational changes in the latent complex via contractile action from activated astrocytes. Reactive oxygen species (ROS) produced by activated astrocytes via the induction of oxidative stress from adjacent cancer cells can lead to the oxidation of the LAP domain and induce allosteric changes that release mature TGF-β from LAP. The mature (active) form of TGF-β can then bind to its cognate receptor and exert its tumor promoting and tumor suppressive properties. Dashed arrow indicates recruitment of the mature TGF-β protein to its cognate receptor. Other tumor-associated pathways/signaling molecules include fibroblast growth factor receptor (FGFR), EGFR, mammalian target of rapamycin (mTOR)/Akt/PI3K, HIFα (hypoxia inducible factor α), β-catenin, and stat3 (signal transducer and activator of transcription 3) (via the IL-6/CNTF receptor). Modified from Costanza *et al*.[104] (2017). Based on microarray and RT-PCR data from the rat F98 glioma model, comparing untreated to OKN-007-treated tumor tissue, OKN-007 is thought to act on LTBP[40], as well as ROS[105]. LTBP2, MMP1A, MMP2, MMP3, MMP7, MMP8, MMP9, MMP12, MMP13 and MMP14, were all found to be downregulated in microarray and/or RT-PCR data from the F98 glioma study[40]. Modified with permission from Dr. Towner, which was originally published in Towner *et al*.[40] (2019)

known to be induced by several receptors mediated by PI3K/Akt, it is anticipated that this pathway may substantially contribute to the generation of resistant phenotypes[51].

It has also been established that GBM is also characterized by overt activity of the PI3K signaling pathway[53]. The activity of the PI3K-Akt signaling pathway is correlated with higher cell survival and motility, as well as chemotherapeutic resistance[53]. Inhibition of the PI3K pathway has been shown to sensitize human glioma cells to alkylating drugs[54]. For instance, PI3K inhibitors such as BKM120 have revealed decreased proliferation and increased apoptosis in not only tumor cell lines[55], and tumor xenograft models[55], but also cancer patients with PI3K activating mutations[55].

It has been previously shown that following TMZ treatment and within TMZ resistant GBM biopsies, there was a distinct activation pattern of the PI3K signaling cascade, further indicating that this pathway is involved in chemoresistance[54]. This pathway was also found to be activated in GBM cell lines[54]. The PI3K pathway seems to play a crucial role in resistance to alkylating agents, and should therefore be considered as a potential drug target for chemosensitization[54]. As an example, the highly specific PI3K inhibitor GDC-0941, was found to reduce chemoresistance to TMZ and enhance radiosensitization in GBM cell lines[53].

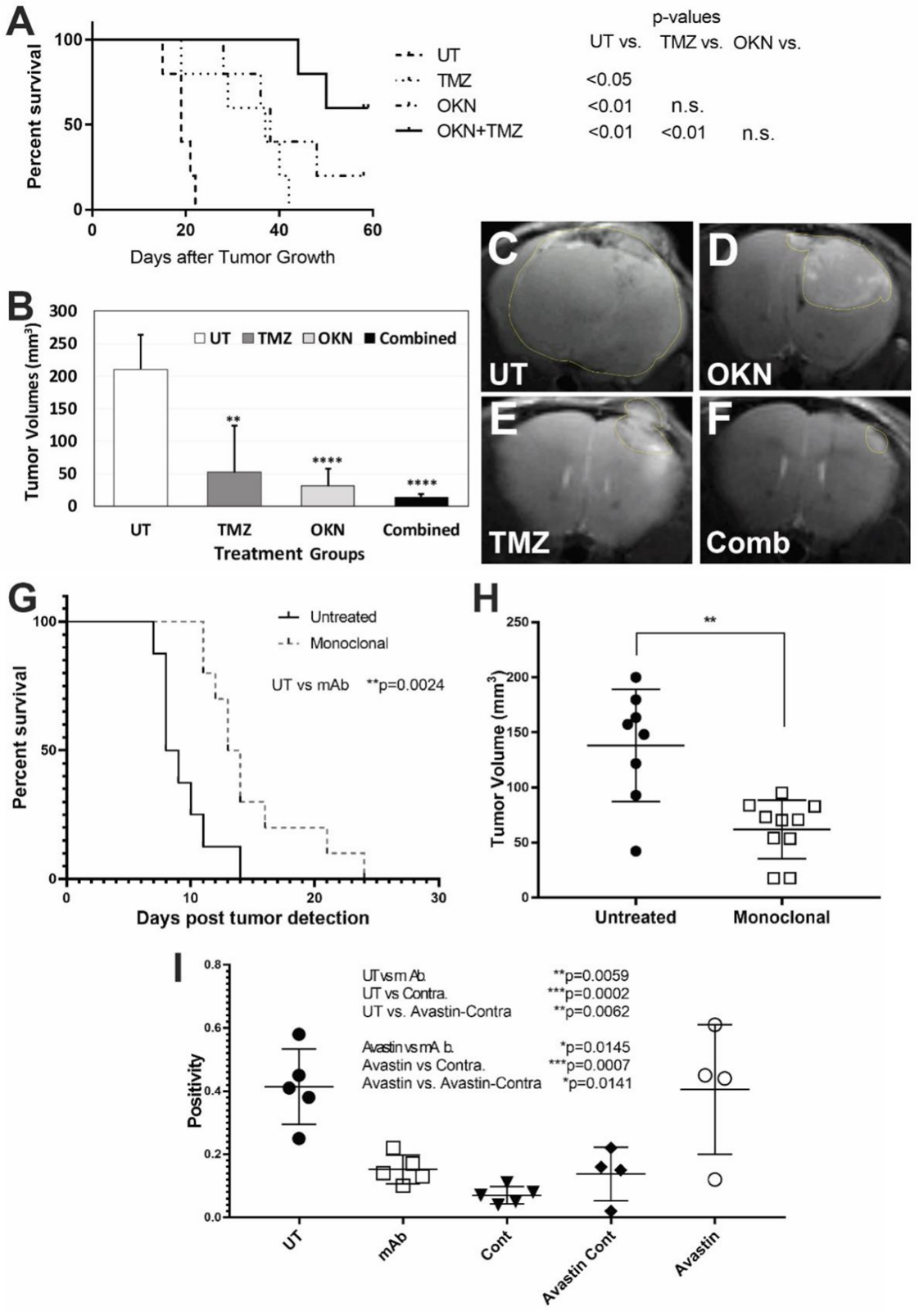

Figure 2. Targeting either the transforming growth factor β1 pathway or ELTD1 in pre-clinical studies for glioblastoma multiforme (GBM). OKlahoma Nitrone 007 (OKN-007) is able to significantly increase animal survival (A) or decrease tumor volumes (B) following combined OKN-007 and TMZ treatments in an orthotopic G55 GBM xenograft model. Examples of MR images from untreated (UT) (C), OKN-007- (OKN) (D), TMZ- (E), or combined (OKN-007 + TMZ) (F) treatments; a monoclonal antibody (mAb) against ELTD1 significantly increased animal survival (G) and decreased tumor volumes (H) in an orthotopic G55 GBM xenograft model; (I) Notch 1 levels were significantly decreased with a mAb against ELTD1 in a G55 GBM model. This figure was obtained from modified data with the permission of Dr. Towner, as reported in Towner *et al*.[40] and Zalles *et al*.[74]