A Novel BBB-permeable Chemotherapeutic Agent for the Treatment of Glioblastoma

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Abstract

Background: Standard of care for Glioblastoma (GBM) patients is surgical tumor resection, followed by radiation and chemotherapy with temozolomide (TMZ). Unfortunately, 60% of newly diagnosed GBM patients express high levels of the DNA repair enzyme MGMT and are TMZ-insensitive, and all patients eventually become refractory to treatment. The blood-brain barrier (BBB) remains an obstacle to adequate delivery of chemotherapeutic agents to brain tumors. Lipophilic drugs such as TMZ and lomustine (CCNU) are able to cross the BBB via passive diffusion; however, their non-selective transport also causes toxicity to critical systemic organs, such as the bone marrow, leading to dose-limiting toxicity. BBB-permeable chemotherapeutic agents that are efficacious in TMZ-insensitive and refractory patients are needed.

The large amino acid transporter 1 (LAT1) is highly expressed on the BBB and in GBM, where it is associated with poor prognosis, but is undetectable in normal brain tissue. Targeting LAT1 transport, while avoiding interaction with other closely related transporters expressed on normal tissue such as LAT2, will target brain tumors and avoid uptake into healthy tissue.

Objectives: We report the generation of a novel molecule (QBS10072S) that combines a potent cytotoxic domain with the structural features of a selective LAT1 substrate.

Results: QBS10072S is 50-fold more selective for LAT1 vs. LAT2, and demonstrates significant in vitro cytotoxicity to LAT1-expressing GBM cell lines and minimal cytotoxicity to normal human astrocytes (LAT1-negative). Unlike TMZ, QBS10072S is cytotoxic to cells with both high and low MGMT expression. In orthotopic GBM xenografts, QBS10072S causes significant delay in tumor growth and increase in mouse survival vs. vehicle or TMZ. QBS10072S is well tolerated, with no myelosuppression in mice dosed up to three times a week and no toxicity to BBB or normal brain cells.

Conclusions: In summary, QBS10072S is a novel BBB-permeable chemotherapeutic agent with the potential to treat TMZ-insensitive and recurrent GBM.

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References

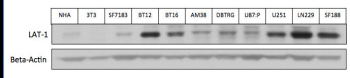
Haining Z, Kawai N, Miyake K et al. Relation of LAT1/4F2hc expression with pathological grade, proliferation and angiogenesis in human gliomas. BMC Clinical Pathology 2012 12:4

Methods

Transporter recognition element for selective LAT1+ cell entry — **Potent N-mustard cytotoxic element for rapid cell killing**

MW <400

QBS10072S was generated by attaching a nitrogen mustard chemotherapeutic agent to a LAT1-specific transporter recognition element.



LAT1 is not expressed in normal human astrocytes (NHA) and is expressed variably in a panel of brain tumor cell lines.

In vitro work- Cells were treated for 6 days in media containing 10% serum with the conditions shown. Viable cells were detected by a WST-1 Cell Viability assay (Roche).

In Vivo work- Nude mice were obtained from Simonsen Inc. QBS'72S is dosed by IV administration weekly by teil vein injection at the doses shown. 300,000 U251:FL cells were injected in 3uL volume 5mm lateral to the bregma. Mice were imaged twice weekly using a Xenogen Lumina imaging station. Mice were monitored daily and sacrificed at the first sign of neurological deterioration or >15% weight loss, per institutional guidelines.

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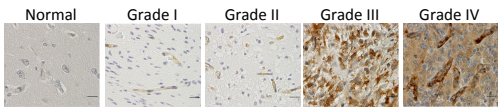
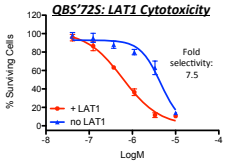
Results



LAT1 expression correlates with grade in glioma (Haining et al, 2012)

QBS'72S: LAT1 Cytotoxicity

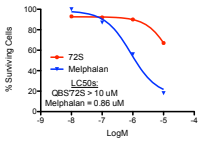


LAT1 & LAT2 Competition

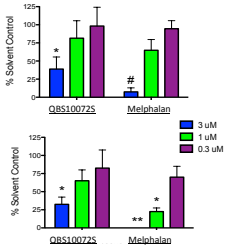
Compound	Competition IC_{50} (uM)	
	LAT1	LAT2
72S	21	1000
Melphalan	170	390

LPCK cells transduced with either LAT1 or LAT2. QD'72s shows increased potency in the presence of LAT1 and is selective for LAT1 >LAT2.

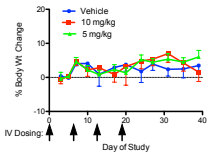
Normal Human Astrocytes *in vitro* cytotoxicity



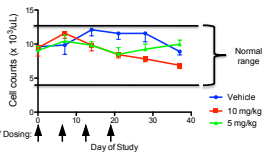
Human Bone Marrow Progenitor Cells *in vitro* Colony Forming Assays



% Body Weight Change



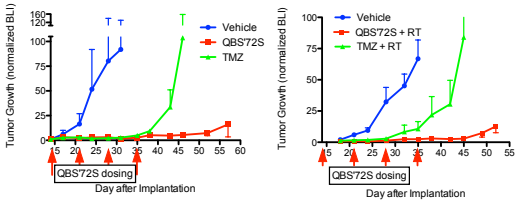
White Blood Cell Counts



Nude mice were treated with four weekly doses IP of QD'72S and monitored for body weight and WBC counts, with no negative effects seen.

Cell Line		Cytotoxicity (LC_{50}, uM) QBS'72S	TMZ
TMZ-sensitive	U251	7.4	44
	LN229	6.7	30
	8MGBA	6.8	40
TMZ-resistant	G55	11	> 100
	42MGBA	15	> 100

QBS'72S shows potency in vitro against both TMZ-sensitive and Insensitive GBM cell lines.

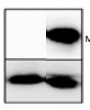


U251 GBM intracranial xenografts showed decreased tumor growth rate (above) and increased median survival (below) compared to temozolomide.

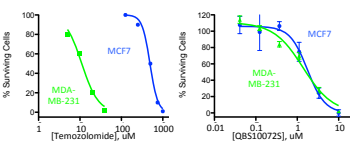
Treatment	Days
Vehicle	31
TMZ	45
QBS'72S	> 55

Treatment	Days
Vehicle	35
TMZ + RT	48
72S + RT	> 60

Protein Expression



In vitro Cytotoxicity



Breast cancer cell lines MDA-MB-231 (MGMT-deficient) and MCF7 (MGMT-expressing) show differential sensitivity to temozolomide, but similar high sensitivity to QBS'72S.