The microtubule-destabilizing agent AUS_001 is an attractive candidate for glioblastoma therapy

Abstract No: 3114

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Background

Microtubules are a well-established target for cancer treatment, however there is a need to identify microtubule targeting agents (MTAs) with decreased toxicity and the ability to also retain efficacy in drug-resistant tumors. The novel MTA, AUS_001 has been shown to impede the growth of 15 established glioma cell lines with a half-maximal inhibitory concentration in the range of 0.04-0.246uM.

Aim

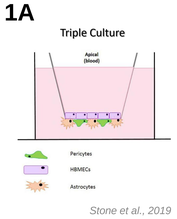
The goal of the current study was to explore the potential of AUS_001 in GBM treatment.

Results

1. AUS_001 crosses the Blood Brain Barrier

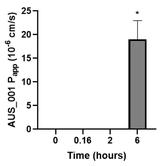
Figure 1. (A) Brain Microvascular Endothelial Cells (BMECs) (purple) are cultured on the luminal side of the transwell and Pericytes and Astrocytes (orange and green) are cultured on the abluminal side of the transwell to form a barrier that mimics an *in vivo* model. **(B)** Evaluation of the trans endothelial electrical resistance (TEER) indicates that the BMECs' co-culture possesses reasonable barrier tightness (TEER >150+40 Ωxcm^2) on Day 5 upon system activation. BMECs treated with 1uM AUS_001 for 6h exhibited strikingly decreased TEER across 2 independent biological replicates. Drug passage through the membrane was confirmed using LC-MS and the apparent permeability coefficient (Papp) was calculated based on the permeation rate and compound concentration.

1A



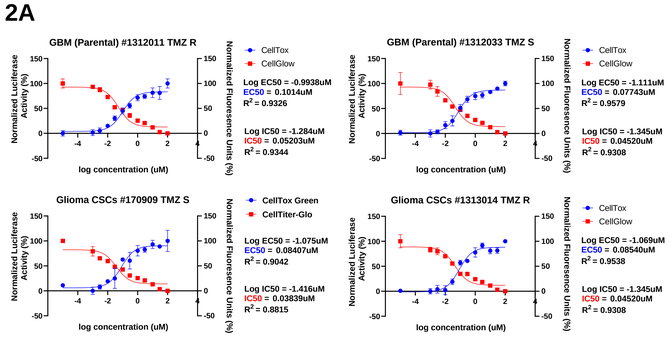
Stone et al., 2019

1B



2. AUS_001 efficiently prevents the growth of primary patient-derived glioma cells in 2D and 3D cultures

2A



2B

Drug	IC50 (uM)		
	BNA99	BNB04	BNB18
AUS_001	14.95	10.89	1.329
TMZ	NR	MR	R

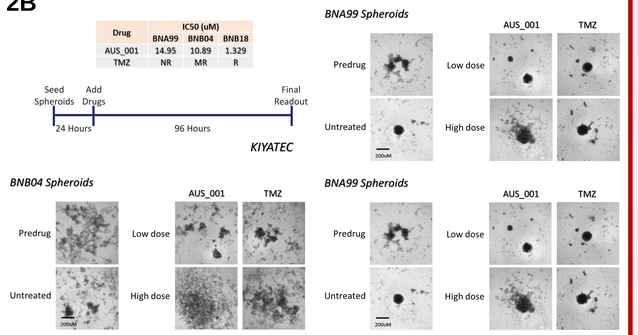
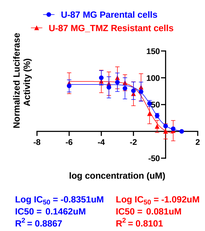


Figure 2. (A) Adherent 2D cells were grown as a monolayer in Human Glioblastoma (GBM) Cancer Stem Cells culture media with serum. Cell Tox Green Cytotoxicity assay was used to assess cell death multiplexed with CellTiter-Glo 2D Viability Assay. **(B)** Representative brightfield images of patient-derived spheroids prior and upon drug treatment. Drugs were dosed from 0.005uM to 100uM and response was measured via CellTiter-Glo 3D. IC50s indicate varying sensitivity for AUS_001 and TMZ across 3 different samples. NR; Non-responder, MR; Moderate responder, R; Responder.

3. Acquired Temozolomide resistance does not confer sensitivity to AUS_001

3A



3B

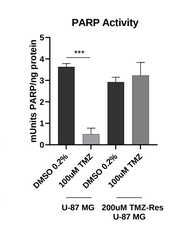
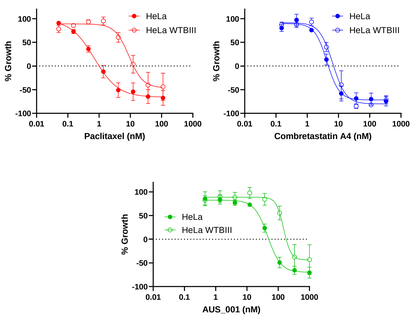


Figure 3. (A) Cell viability and **(B)** PARP activity were monitored upon AUS_001 treatment in GBM TMZ-resistant cells cultured for 90 days under the presence of 200uM TMZ vs. the parental cells.

4. βIII-tubulin overexpression confers limited resistance to AUS_001



	GI_{50} (mean ± SEM; nM)		Relative Resistance due to βIII tubulin
	HeLa	HeLa WTβIII	(GI_{50} WTβIII)/(GI_{50} HeLa)
Paclitaxel	0.4 ± 0.1	5.9 ± 1.5	14.8
CA-4	2.8 ± 0.3	4.2 ± 0.2	1.5
AUS_001	24.3 ± 4.3	110.9 ± 18.2	4.6

Figure 4. HeLa parental cells compared to an isogenic line stably expressing the βIII-isotype of tubulin (HeLa WTβIII). The sulforhodamine B assay was used to determine the concentration-dependent effects of AUS_001, Paclitaxel and Combretastatin A-4 on the proliferation of cancer cells over a 48h period. The concentration that inhibited cellular proliferation by 50% (GI50) was calculated.

Conclusions

AUS_001 has the potential to circumvent significant limitations of clinically approved MTAs, including brain penetration, drug resistance and peripheral neuropathy, making it a promising approach for the treatment of glioblastoma

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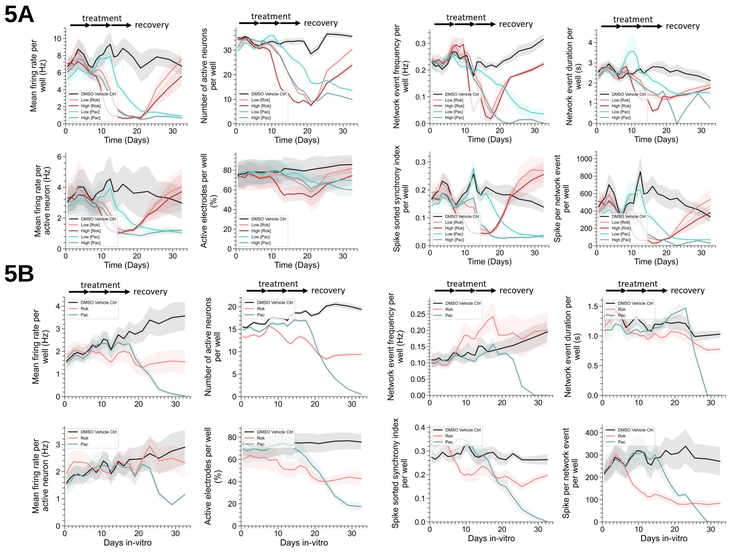
5. hPSC-derived cortical and midbrain neurons fully recover upon AUS_001 removal

5A



5B

Figure 5. Electrical activity and functionality assessment of mature human Pluripotent Stem Cell (hPSC)-derived midbrain and cortical neurons. **(A)** Midbrain and **(B)** cortical neurons have been maintained in culture for 192 days and 137 days, respectively, prior to the initiation of drug treatments. Dose escalation occurred every 5-6 days depending on the feeding schedule. MEA Recordings were taken daily during drug exposure, and at least twice a week during recovery, by a Maestro pro MEA system (Axion Biosystems). MEA Recordings were single-cell spike sorted using Plexon Offline Sorter version 4.5 (Plexon, Inc) to isolate individual neurons and analyzed with Neural Metric Tool.

Midbrain Neurons

Low AUS_001: 0.1uM → 2uM → 8uM
High AUS_001: 1uM → 4uM → 16uM

Low Paclitaxel: 0.1uM → 2uM → 8uM
High Paclitaxel: 1uM → 4uM → 16uM

Cortical Neurons

Low AUS_001: 0.1uM → 1uM → 2uM

Low Paclitaxel: 0.1uM → 2uM → 8uM