Poster# 1843/15

Dianhydrogalactitol (VAL-083) for the Treatment of Glioblastoma Multiforme (GBM): Impact of Glucose Transporters for Crossing the Blood Brain Barrier (BBB)

John M. Langlands[1], Sarath Kanekal[1], Anne Steino[1], Armando Mendez,[2] Zoltan Karman[3], Beata Martonne Toth[3], Krisztina Heredi-Szabo[3], Dennis Brown[1]

[1] Kintara Therapeutics, Inc. Menlo Park, CA, [2] U. of Miami Leonard School of Medicine, Miami, FL, [3] Solvo Biotechnology, a Charles River Company, Szeged, Hungary

BACKGROUND

Glucose transporters at the blood brain barrier (BBB) maintain the continuous high glucose and energy demands of the brain. These transporters have been targets for the creation of therapeutic molecules as a means to cross the BBB. The BBB has been a major limitation for the successful development of treatments for brain tumors. To date, only a limited number of lipophilic small chemical entities, which can cross the BBB, have been approved for the treatment of GBM. These include the nitrosoureas such as carmustine (BCNU) and lomustine (CCNU) and temozolomide (TMZ).

VAL-083 is active against several GBM cell lines and animal models and acts as a DNA targeting agent rapidly forming cross-links at the N7 position of guanine, resulting in lethal DNA double strand breaks and cancer cell death[1]. It is active in GBM patients, both newly diagnosed and recurrent. Furthermore, its activity is independent of the DNA repair enzyme MGMT which limits the effectiveness of O6 guanine alkylating agents such as the nitrosoureas and TMZ[2,3].

Recent clinical studies have shown VAL-083 is active in patients with newly diagnosed GBM when administered in combination with radiation therapy or as adjuvant therapy, and also when administered to patients with recurrent GBM.[4, 5, 6]

Clinical pharmacology studies have demonstrated that VAL-083 accumulates in cerebrospinal fluid (CSF) and brain tissue[7]:

- ^{3}H-Dianhydrogalactitol (DAG) appears in the CSF rapidly after intravenous administration, with peak levels at approximately 1-2 hrs, thereafter being eliminated slowly with a half-life of approximately 20 hrs in CSF.
- Unchanged VAL-083 accounted for 6-30% of total radioactivity in CSF.
- Most brain tissues accumulated VAL-083 to a greater extent than intact white matter.

A recent clinical study in newly diagnosed GBM, demonstrated that levels of VAL-083 in CSF were at least as high as in plasma when measured 2 hours after the end of infusion of drug Table 1[5].

Table 1 Concentration of VAL-083 in plasma and CSF from newly diagnosed GBM patients

Dose of VAL-083 mg/m^2/day	N	VAL-083 in plasma 2 hr post dose (ng/mL)	VAL-083 in CSF 2 hr post dose (ng/mL)	Ratio at 2hr CSF / Plasma
20	1	110.0 (-)	154 (-)	1.40
30	6	107.84 (16.65)	127.09 (26.24)	1.24 (0.35)
40	3	169.7 (41.9)	189.67 (69.89)	1.13 (0.41)

Plasma and CSF collected on day 3 of cycle 1 of treatment with VAL-083. Values are mean (SD)

In contrast to VAL-083, the level of temozolomide in CSF is only approximately 20% of that observed in plasma[8].

REFERENCES

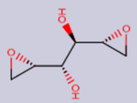
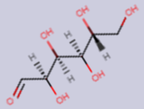
1: Zhai B, *et al.* (2018) Cell Death and Disease. 9:1016
2: Zhai B, *et al.* (2017) Cancer Res. 77(13), abst #248
3: Fouse S, *et al.* (2014) Neuro Oncol. 16(Supl 5), ET-18
4: O'Brien, B *et al* (2021) Neuro-Oncol 23(supl 6), vi 65
5: Chen, Z *et al* (2021) Neuro-Oncol 23(supl 6), vi 63-64
6: Guo, C *et al* (2019) Glioma 2(4), 167-173
7: Eckhardt, S *et al* (1977) Cancer Treat Rep 61(5), 841-847
8: Ostermann, S *et al* (2004) Clin Can Res 10(11), 3728-3736
9: Hitchcock S *et al* (2006) J Med Chem 49(26), 7559-7583
10: Pajouhesh, H and Lenz G (2005) NeuroRX 2(4), 541-553

VAL-083 is a di-epoxide substituted hexitol sugar (galactitol). It has chemical properties similar to D-glucose.

For drugs to penetrate the BBB and thus have activity in the brain and central nervous system, a polar surface area of less than 90 Å^2 is usually needed.[9]

The ability for drugs to penetrate across brain capillaries is, in part, associated with the number of hydrogen bond acceptors (N or O in molecule), optimal if ≤5.[10]

Table 2 Properties of VAL-083 (dianhydrogalactitol) and D-Glucose

	VAL-083 (Dianhydrogalactitol)	D-glucose
Structure		
Molecular weight	146.1	180.16
Polar surface area	62.22 Å^2	118.22 Å^2
Water solubility	Highly soluble, 1000 mg/mL	Soluble, 261 mg/mL
Log P	-1.4	-3.6

OBJECTIVES OF STUDIES

Clinical studies have demonstrated that VAL-083 is taken up into CSF and brain tissue and is available to exert its therapeutic effect.

Studies were undertaken to determine if VAL-083 is taken up into CSF or brain tissue through one or more glucose transport mechanisms.

- Is VAL-083 a potential substrate for sodium dependent glucose transporters, SGLT1 and SGLT2 in HEK293 cells?
- Is VAL-083 a potential substrate for GLUT1 transporter in Caco-2 cells?

SGLT Transporter Assays

Human SGLT1 and SGLT2 transporters were expressed in HEK293 cells and cultured in DMEM, high glucose with GLUTAMAX (Gibco 31966-021) medium. Cells were seeded to 96-well plates. 24 hours after seeding the cells were washed with glucose-free Krebs-Henseleit buffer (pH 7.4) at 37°C prior the experiment. Cells were incubated with reference non-metabolized probe α-methyl D-glucopyranoside (AMG; 1 μM) or VAL-083 (3 and 15 μM). Cells were treated with VAL-083 for 2 or 20 minutes, in the presence of a specific inhibitor (SGLT1 inihibitor: phloridzin; SGLT2 inhibitor: dapagliflozin). The amount of VAL-083 accumulated in cells was measured using LCMS/MS and expressed as pmol VAL-083/mg protein. AMG accumulation was monitored using liquid scintillation. VAL-083 did not accumulate in these cells under these conditions.

GLUT1 Transporter Assays

Caco-2 cells were cultured in in DMEM, high glucose with GLUTAMAX (Gibco 31966-021) medium. Cells were seeded on 24-well plates and cultured for 4 days at which culture time GLUT1 expression was demonstrated by qPCR. Cells were washed with glucose- and sodium-free medium and incubated with assay buffer for 15 min at 37°C. Cells were incubated with D-glucose (10 or 100 μM) or VAL-083 (3 and 15 μM). Cells were incubated with D-glucose or VAL-083 for up to 60 minutes, in the presence of a specific GLUT-1 inhibitor (BAY876; 10 μM). The amount of VAL-083 accumulated in cells was measured using LCMS/MS and expressed as pmol VAL-083/mg protein.

While D-glucose accumulated in Caco-2 cells, it was not inhibited by BAY876, suggesting uptake independent of GLUT1. VAL-083 did not accumulate in these cells under these conditions.

Table 3 VAL-083 accumulation in the SGLT2 uptake transporter substrate assay. Values are mean (SD), n=3

	Treatment	Accumulation in HEK293-SGLT1-Fin (pmol/mg prot)	Accumulation in HEK293-Mock-Fin (pmol/mg prot)	Active Transport (pmol/mg prot)	Fold Accum.
SGLT1	15 μM VAL-083	22.86 ± 1.56	20.81 ±1.71	2.05 ± 2.32	1.10
	15 μM VAL-083 + Phloridzin	24.63 ± 1.96	23.89 ± 1.31	0.74 ± 2.36	1.03
	3 μM VAL-083	2.35± 0.27	2.54 ± 0.37	-0.19 ± 0.46	0.93
	3 μM VAL-083 + Phloridzin	3.06 ± 0.10	2.73 ± 0.24	0.34 ± 0.26	1.12
	1 μM AMG	153.1 ± 4.64	5.05 ± 0.99	148.1 ± 4.74	30.33
	AMG + Phloridzin	5.77 ± 2.05	4.74 ± 1.70	1.03 ± 2.67	1.22
	Treatment	Accumulation in HEK293-SGLT2-LV (pmol/mg prot)	Accumulation in HEK293-Mock-LV (pmol/mg prot)	Active Transport (pmol/mg prot)	Fold Accum.
SGLT2	15 μM VAL-083	26.60 ± 2.32	30.50 ± 5.53	-3.90 ± 5.99	0.87
	15 μM VAL-083 + Dapa	23.36 ± 3.86	28.59 ± 5.45	-5.23 ± 6.68	0.82
	3 μM VAL-083	2.79 ± 0.13	2.07 ± 0.65	0.72 ± 0.66	1.35
	3 μM VAL-083 + Dapa	2.73 ± 0.39	3.16 ± 0.39	-0.43 ± 0.55	0.86
	1 μM AMG	53.49 ± 2.78	2.57 ± 0.24	50.89 ± 2.79	20.73
	AMG + Dapa	5.58 ± 1.51	2.23 ± 0.43	3.34 ± 1.57	2.5

SGLT1 inhibitor phloridzin (100 μM); SGLT2 inihbitor dapagliflozin (Dapa) 0.3 μM; all incubations 20 minutes except AMG + Dapa (10 min)

CONCLUSIONS AND FUTURE DIRECTIONS

- VAL-083 does not appear to be an in vitro substrate of SGLT1 and SGLT2 SLC transporters.
- Studies need to confirm if other GLUT transporters are involved in update of VAL-083 across BBB
- Transport via passive diffusion or transport via water (as drug is highly soluble) is possible.
- Update across the BBB may be driven, at least in part, by the low PSA (< 120)
- Uptake across the BBB may be assisted by number of proton acceptors (N or O) ≤5, VAL-083 has 4.

➢ Further studies will continue to explore possible mechanisms which may be involved in the uptake of VAL-083 into the CNS, including active transport mechanisms and passive diffusion processes.