In vivo efficacy of VAL-083 in the treatment of MGMT-positive glioblastoma multiforme (GBM)

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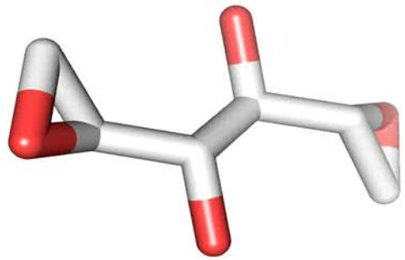
ABSTRACT: Median survival for patients with recurrent GBM is <6 months. Front-line systemic therapy is temozolomide (TMZ) but resistance due to O6-methylguanine-DNA-methyltransferase (MGMT) activity is implicated in poor outcomes. Dianhydrogalactitol (VAL-083) is a structurally unique bi-functional DNA alkylating agent that crosses the blood-brain barrier and accumulates in brain tumor tissue. VAL-083 has demonstrated *in vitro* activity against pediatric and adult GBM cell lines, as well as GBM cancer stem cells. VAL-083 has furthermore been shown to overcome MGMT-induced drug resistance *in vitro*, and previous clinical trials suggest that VAL-083 has anti-tumor activity against a range of tumors, including GBM. **Taken together, these results propose that VAL-083 may be a therapeutic option for MGMT-positive GBM patients who have a high likelihood of TMZ-resistance.**

The purpose of this study was to evaluate the activity of VAL-083 in *in vivo* models of drug-resistant GBM. Rag2 mice bearing intracranial human GBM xenograft tumors of either MGMT-positive and TMZ-resistant origin (BT74), or MGMT-negative and TMZ-sensitive origin (U251) were treated.

VAL-083 Overview

USAN Name ***dianhydrogalactitol***

Molecular Structure

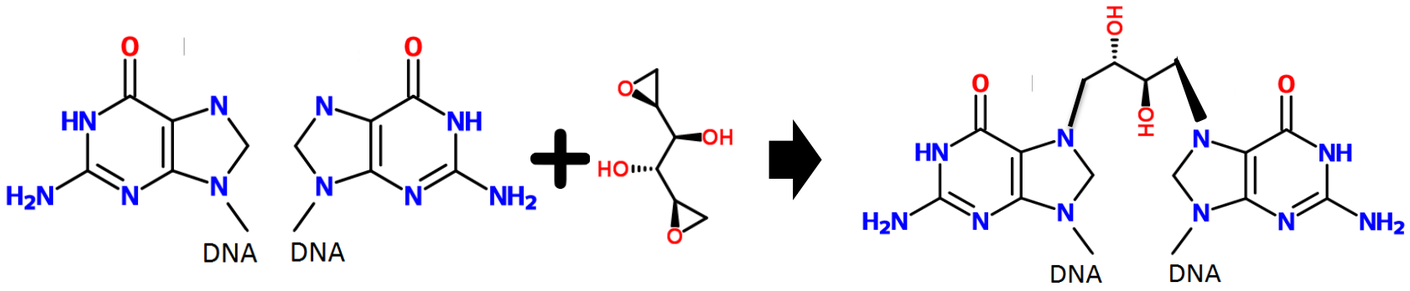


Chemical Formula $C_6H_{10}O_4$

Molecular Weight **146.1 g/mol**

Proposed anticancer mechanism

VAL-083 forms intra-strand DNA cross links @ N7 position of guanine leading to double-strand DNA breaks, apoptosis and cell death



VAL-083:
Clinical History

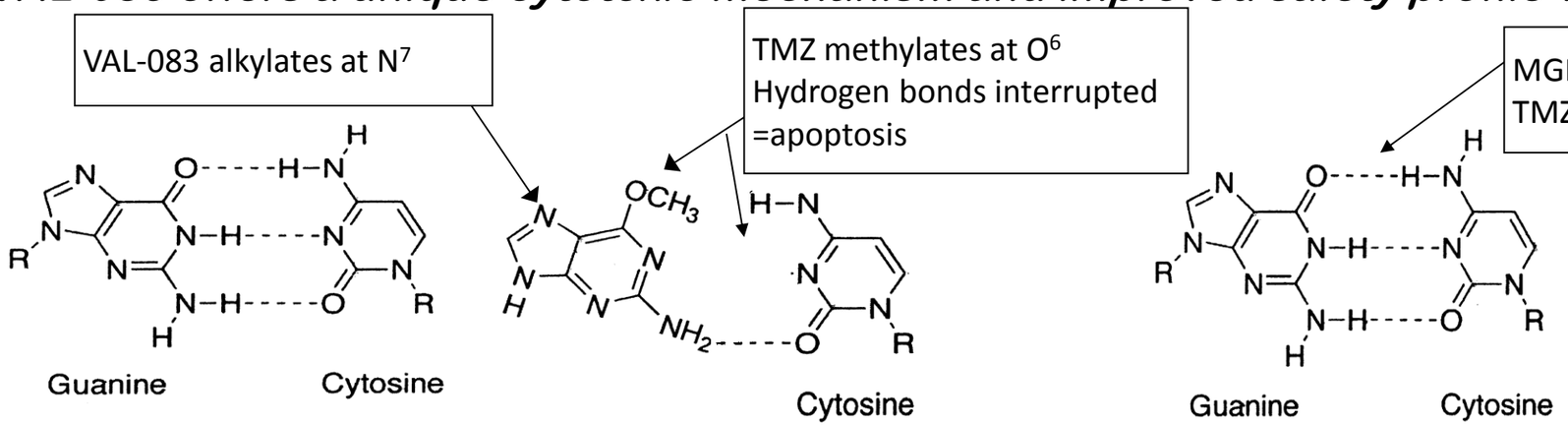
- Studied extensively in 1970s & 80s by National Cancer Institutes in USA
- Approved for CML and Lung Cancer in China
- Demonstrated clinical activity against a range of tumor types, including GBM
- Historical outcomes are comparable to modern standard-of-care
- Active against nitrosourea (BCNU) failed tumors

Chemotherapy	Comparative Therapy Survival		Median Survival Benefit vs. XRT
	Radiation	Radiation + Chemotherapy	
Temodar™	12.1 months	58 weeks (14.6 months)	2.5 months
VAL-083	8.3 months	67 weeks (16.7 months)	8.0 months

Reported Radiation + Chemo Survival w/ other chemotherapeutic agents in GBM	
Carmustine™ (BCNU)	40-50 weeks
Lormustine™ (CCNU)	52 weeks
Nimustine™ (ACNU)	35 weeks
Avastin™	No reported benefit to survival

VAL-083:
Differentiation from temozolomide

VAL-083 offers a unique cytotoxic mechanism and improved safety profile versus temozolomide



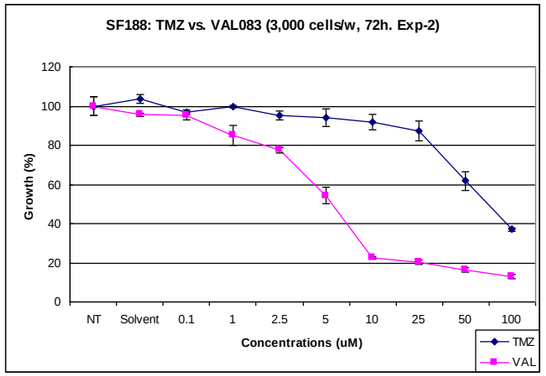
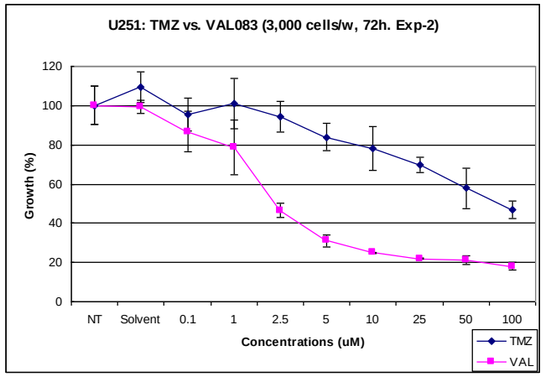
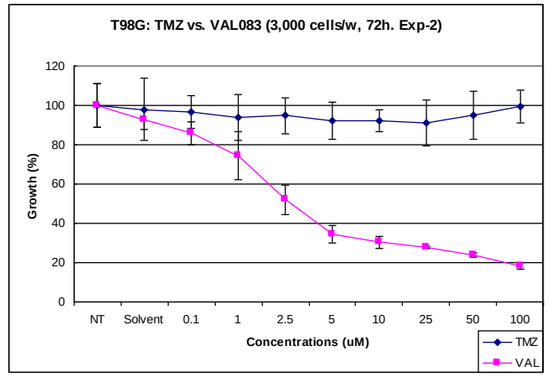
MGMT enzyme DOES NOT repair VAL-083 alkylation

	VAL-083	temozolomide
Cross Blood Brain Barrier?	**YES**	**YES**
Cytotoxic mechanism	**Bifunctional cross-link via guanine N7**	**Monofunctional methylation via guanine O6**
Subject to resistance by MGMT repair enzyme	**NO**	**YES**
Dose Limiting Toxicity	**Hematologic**	**Hematologic**
Recovery	**Within 7-8 days**	**Within 14 days**
Other severe toxicities reported (>2%)	**n/a**	**Nausea, vomiting, fatigue, asthenia, neuropathy**

VAL-083:
Differentiation from temozolomide

In vitro data summary

TMZ resistance and MGMT status in GBM cell lines SF188 (pediatric), U251 (adult), and T98G (adult)

GBM Cell Line	SF188	U251	T98G
MGMT Promoter Methylation Status	methylated	methylated	unmethylated
In vitro activity			

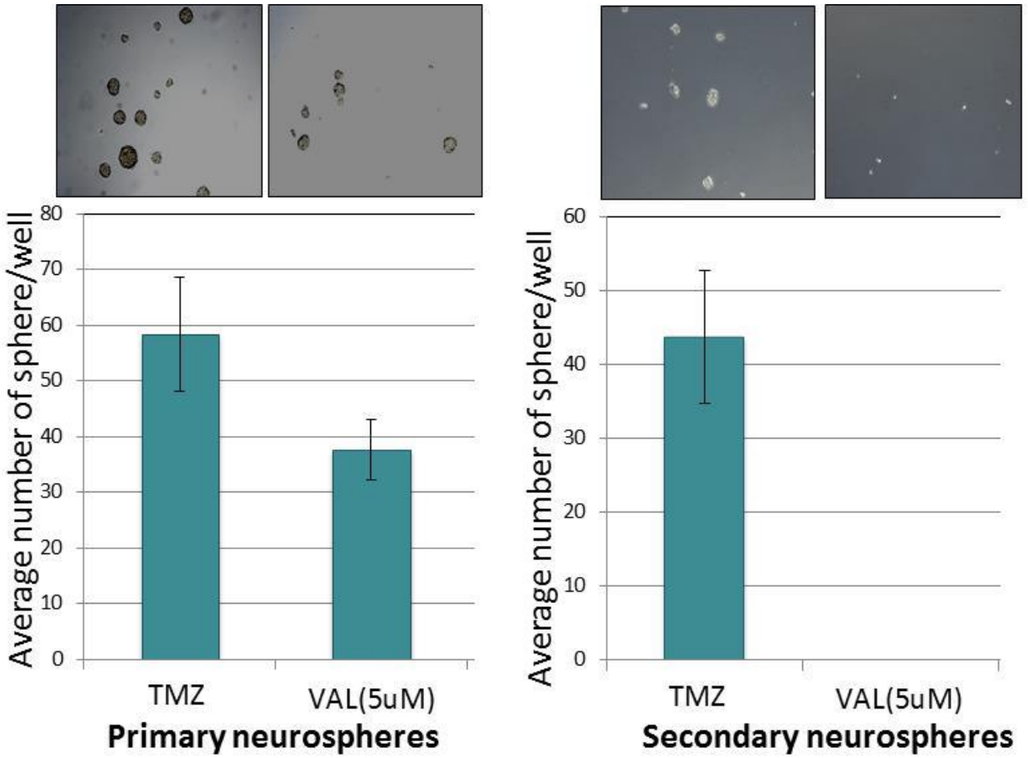
- VAL-083 is more potent than temozolomide against GBM cell lines *in vitro*
- VAL-083 maintains cytotoxic activity independent of MGMT expression, whereas temozolomide activity is inhibited by MGMT

VAL-083:
Differentiation from temozolomide

In vitro data summary

VAL-083 inhibited the growth of Brain Tumor Initiating cells (BTIC) (BT74, GBM4 and GBM8) by 80-100% in neurosphere growth assays, with minimal effect on normal human neural stem cells. Temozolomide was ineffective against BTICs.

BTICs are thought to give rise to relapse as they are resistant to conventional therapies.



VAL-083:
Differentiation from temozolomide

In vivo study

***In vivo* mouse model of VAL-083 efficacy in comparison to TMZ in xenograft GBM models of U251(TMZ-sensitive) and BT74 (TMZ-resistant).**

Treatment protocol for testing the efficacy of VAL-083 in comparison to temozolomide (TMZ) in the treatment of female Rag2 mice bearing intracranial human GBM xenograft tumors of U251 (TMZ-sensitive) or BT74 (TMZ-resistant) origin. On day 0, 7.5 x 10^4 cells were implanted intracranially. On day 18, treatment was initiated. Disease progression is evaluated by overall survival, clinical observations and body weight measurements.

Group	Dose (mg/kg)		No. mice	Admin. route	Volume (uL/20g)	Schedule
	U251 (TMZ-senstitive)	BT74 (TMZ-resistant)				
1 - Control	n/a	n/a	10	n/a	n/a	n/a
2 - TMZ	30	30	10	i.p.	200	Q7D X 3
3 - VAL-083	3	3	10	i.p.	200	M, W, F X 3
4 - VAL-083	4	3.5	10	i.p.	200	M, W, F X 3
5 - VAL-083	5	4	10	i.p.	200	M, W, F X 3

VAL-083:
Differentiation from temozolomide

In vivo study

RESULTS TO DATE

U251 ***(TMZ-sensitive)***	**As expected, U251 was sensitive to both TMZ and VAL-083. Treatment with either compound resulted in a statistically significant survival benefit compared to control.**
BT74 ***(TMZ-resistant)***	**Study Ongoing**

- Full data to be presented / published upon study completion

VAL-083:
Translational Research

VAL-083 Clinical Study

VAL-083 is currently undergoing a Phase I/II clinical trial in refractory glioblastoma

- ***Three clinical sites in USA (UCSF, SCRI, FCA)***
- ***Clinicaltrials.gov identifier: NCT01478178***

Modern side-effect management has enabled higher dosing in comparison to previous NCI studies

Comparator	NCI Regimen	DelMar Regimen
Single Dose	25 mg/m^2	25 mg/m^2
Acute cycle	125 mg/m^2 *(5 days)*	150 mg/m^2 *(3 days)*
Overall 33-day cycle	125 mg/m^2	300 mg/m^2

VAL-083:
Conclusions

- ***VAL-083 has a cytotoxic mechanism that is different from TMZ***
- ***VAL-083 is effective in various GBM cell lines, and the activity is independent of MGMT***
- ***VAL-083 inhibits neurosphere formation and growth of brain tumor initiating cells BT74, GBM4 and GBM8***
- ***VAL-083 has a favorable safety profile in GBM patients***
- ***VAL-083 is currently in clinical trial for the treatment of refractory GBM***
- ***VAL-083 is safe and well tolerated at doses tested to date (up to 40mg/m^2), delivering more drug to the tumor more often in comparison to historical NCI dosing regimens***

1. *Eagan R.T. et al. JAMA; 1979; 241: (19): 2046-50*
2. *Stupp et al. N Eng J Med; 2005; 352 (10): 987-96*
3. *Dunn S.E et al. Cancer Research: April 15, 2012; 72 (8); Supplement 1.*
4. *Dunn S.E. et al. AACR 2012*