Dianhydrogalactitol (VAL-083) has the potential to overcome major challenges in the treatment of diffuse intrinsic pontine glioma (DIPG)

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#EXTH-09

BACKGROUND

Despite decades of clinical trials, children with diffuse intrinsic pontine gliomas (DIPG) continue to have a very poor prognosis and dismal survival. DIPG is inoperable and standard treatment is radiation alone. Major obstacles to the successful treatment of DIPG include:

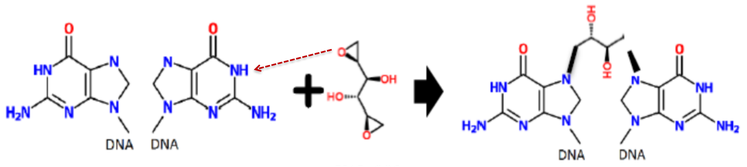
1) Intact blood-brain barrier impeding drug penetration

2) Inherent tumor cell resistance mechanisms to conventional chemotherapeutics

3) Lack of drug-induced potentiation of radiotherapy

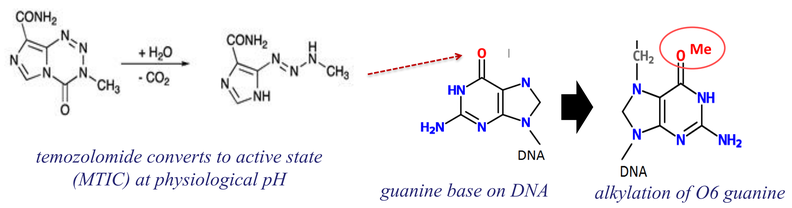
VAL-083 is a novel bi-functional DNA targeting agent that readily ***crosses the blood-brain barrier and accumulates in brain tumor tissue***. In prior NCI-sponsored clinical trials, VAL-083 was well-tolerated and demonstrated activity against pediatric brain tumors, including pediatric high-grade glioma and medulloblastoma[1,2]. VAL-083 ***overcomes MGMT-related chemoresistance*** (Figure 1) and is equally active against GBM cancer stem cells and non-stem cells and ***potentiates the effect of radiation in adult GBM cells***, *in vitro*.[3]

Mechanism of VAL-083 via crosslinks at N7 of guanine



2 guanine bases on DNA *VAL-083* *VAL-083 – interstrand crosslink (ICL)*

Mechanism of temozolomide via alkylation at O6 of guanine



temozolomide converts to active state (MTIC) at physiological pH *guanine base on DNA* *alkylation of O6 guanine*

Figure 1. *The N7-targeting mechanism of action of VAL-083 differs from those of O6-alkylating agents such as temozolomide and nitrosoureas.*

VAL-083 combination with Wee1 kinase inhibitor AZD1775

Wee1 regulates the G2/M checkpoint in the cell cycle. Wee1 activation results in G2 arrest and allows DNA damage to be repaired prior to cell cycle progression. **AZD1775** is a Wee1 inhibitor that allows a cancer cell with DNA damage to progress past the G2 checkpoint and into premature mitosis leading to cancer cell death.[4,5]

VAL-083 is a novel bi-functional DNA targeting agent that rapidly induces interstrand cross-links at N7-guanine, leading to DNA double-strand breaks (DSBs) and cell cycle arrest in S/G2 phase.[1,2]

AZD1775 has been shown to enhance the effect of other DNA-damaging agents and we hypothesized that AZD1775-treatment would sensitize cancer cells to VAL-083-induced DNA damage (see Figure 2).[6] Combined with VAL-083's ability to overcome MGMT-related chemoresistance and ability to cross the blood-brain barrier, we hypothesized that VAL-083 as single agent or as part of a combination therapy with AZD1775 could potentially offer a treatment alternative against DIPG.

SUGGESTED MECHANISM OF ACTION

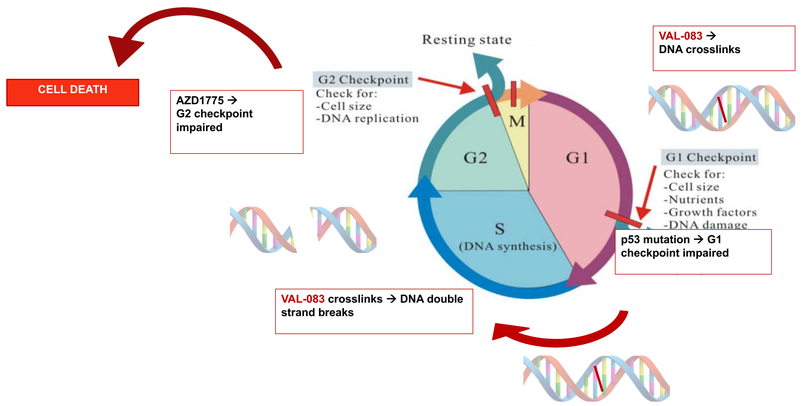


FIGURE 2. VAL-083 induces interstrand crosslinks leading to double-strand breaks and S/G2 cell cycle arrest. AZD1775 allows cells with DNA damage to escape the G2 checkpoint leading to premature mitosis and cell death.

RESULTS – *in vivo*

VAL-083 as single agent and in combination with Wee1 inhibitor AZD1775 significantly increased survival in a DIPG *in vivo* model.

In vivo activity of VAL-083 as single agent and in combination with AZD1775 was assessed in an orthotopic engraftment model of pediatric DIPG (SF8628). The study showed that combined treatment with VAL-083 and AZD1775 conferred significantly greater survival benefit to mice with engrafted DIPG tumors compared to control as well as single agent treatment with AZD1775. A significant survival benefit in this SF8628 PDX DIPG model was observed with VAL-083 (3 mg/kg) as single agent and as part of a combination with Wee1 inhibitor AZD1775 (60 mg/kg). The median survival for mice treated with VAL-083 alone was 54.5 days and for the VAL-083/AZD1775 combination it was 62 days, compared to 44 days for control and 47 days for mice treated with AZD1775 alone.

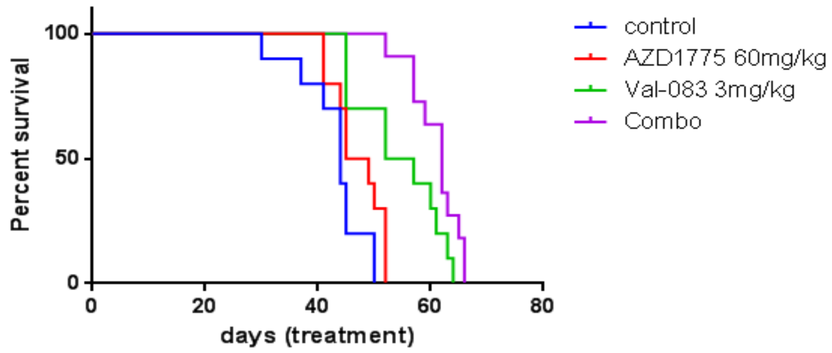


FIGURE 3. *Kaplan-Meier survival plot for animals bearing PDX DIPG tumors of SF8628 origin. Table 1 to the right shows the median survival and corresponding p-values for the four groups.*

TABLE 1: Median survival of mice with engrafted DIPG tumors of SF8628 origin

Group	control	AZD1775 60mg/kg	VAL-083 3mg/kg	VAL-083 /AZD1775
Median survival	44	47	54.5	62

	p-value
VAL-083 vs. control	0.0004
AZD1775 vs. control	0.0839
VAL-083 vs. AZD1775	0.0101
VAL-083/AZD1775 vs. control	<0.0001
VAL-083/AZD1775 vs. VAL-083	0.0401
VAL-083/AZD1775 vs. AZD1775	<0.0001

RESULTS - *in vitro*

VAL-083 was active against DIPG cell lines and pediatric GBM cell line, independent of histone mutation status, *in vitro*

VAL-083 inhibited proliferation/growth of three biopsy-derived pediatric DIPG cell lines with varying genomic profiles, resulting in IC_{50} values ranging between 1 and 10 μM (Figure 4 and Table 2).

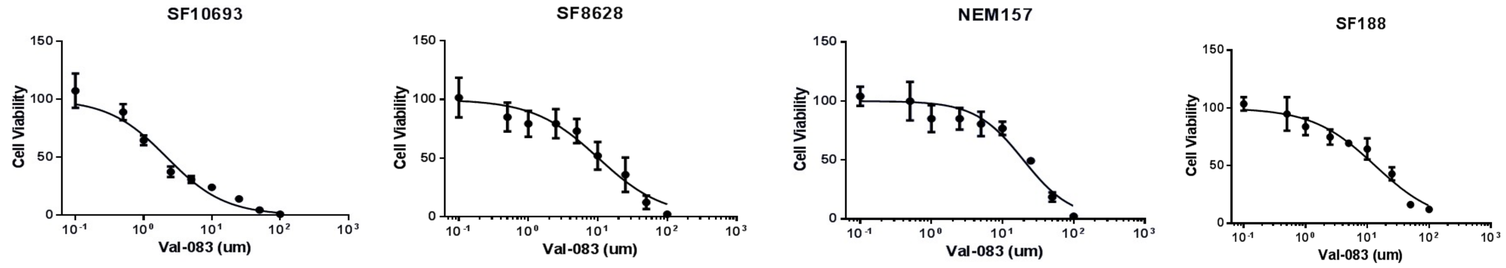


FIGURE 4. *VAL-083 inhibited proliferation/growth of three biopsy-derived pediatric DIPG cell lines with varying genomic profiles. Proliferation/viability was quantified using CellTiter-Glo® Luminescent Cell Viability Assay Kit after 3 days of treatment. DIPG derived cell lines SF8628 and NEM157 (H3.3K27), as well as SF10693 (H3.1K27M) and pediatric glioblastoma cell lines SF188 (H3.3K27 wildtype) were treated with increasing concentrations of single agent VAL-083 alone and in combination with AZD1775.*

TABLE 2: IC_{50} values for VAL-083 in DIPG tumor cell lines SF10693, SF8628, NEM157 and pediatric GBM cell line SF188. N=3

Cell line	SF10693	SF8628	NEM157	SF188
Tumor type	DIPG	DIPG	DIPG	GBM
Histone mutations	H3.1 K27M	H3.3 K27M	H3.3 K27M	WT
p53	WT	WT	Mutant	Mutant
VAL-083 IC_{50}	1 μM	5 μM	10 μM	10 μM

VAL-083 displayed synergy with Wee1 inhibitor AZD1775 in DIPG and pediatric GBM cell lines

VAL-083 exhibited synergistic activity in cell lines SF8628 and SF188 and additive effect in NEM157 with CI values ranging from 0.405 to 2.066 with < 1 indicating synergy.

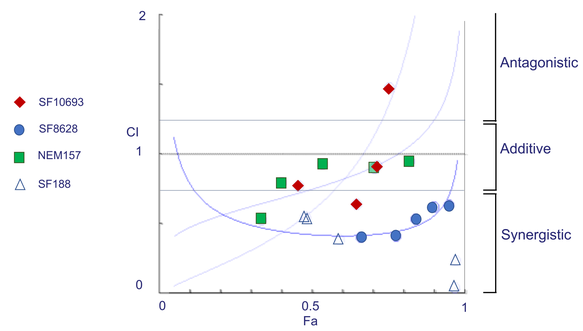


FIGURE 5. *DIPG cell lines SF10693, SF8628 and NEM157 and pediatric GBM cell line SF188 were treated with VAL-083 and AZD1775 and proliferation/viability was quantified using CellTiter-Glo® after 3 days of concomitant treatment. The data was analyzed using the Chou-Talalay method,* which allows the quantitative determination of drug interactions by calculating a combination index (CI).[7] *N=3.*

CONCLUSIONS &FUTURE DIRECTIONS

Our present study highlights that the combination of VAL-083 and Wee1 inhibitor AZD1775 might be a promising new therapeutic strategy for children with DIPG.

- **VAL-083 as single agent significantly increases median survival in DIPG *in vivo* compared to control and to AZD1775 alone;**
- **VAL-083 in combination with AZD1775 further increases survival in DIPG *in vivo*;**
- **VAL-083 is active against DIPG cell lines with varying genetic profiles including p53 and H3.3/H3.1 K27M mutations;**
- **VAL-083 is synergistic with AZD1775 against DIPG and pediatric GBM cell lines.**

Ongoing studies will continue to assess the *in vivo* activity of VAL-083 with AZD1775 as well as explore the underlying mechanism of action of the combination strategy.

References:

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