# Dianhydrogalactitol (VAL-083) exhibit strong efficacy in GBM tumors with different epigenetic background and treatment history

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## ABSTRACT #5231

**Introduction:** Despite aggressive treatment for glioblastoma (GBM) including surgery, radiation and temozolomide (TMZ), tumor recurrence is inevitable and median survival is only 14 months. Second-line treatment with bevacizumab has failed to improve survival. Unmethylated promoter status for $O^6$-methylguanine-DNA-methyltransferase (MGMT) is a validated biomarker for TMZ-resistance responsible for recurrent/refractory disease.

**VAL-083** is a novel bi-functional DNA targeting agent that readily ***crosses the blood-brain barrier and accumulates in brain tumor tissue***[1]. VAL-083 induces DNA interstrand crosslinks at N7-guanine, leading to double-strand breaks and cancer cell-death in GBM cells[2,3]. VAL-083 ***overcomes MGMT-related resistance mechanisms*** 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*[4]. VAL-083 is currently in Phase II clinical trials for the treatment of MGMT promoter unmethylated GBM, both recurrent and treatment-naïve (NCT02717962, NCT03050736).

**VAL-083 induces interstrand crosslinks at $N^7$ of guanine**

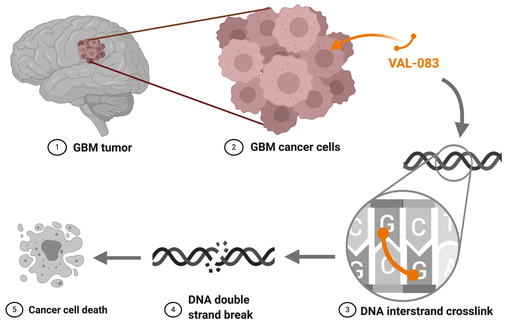


*FIGURE 1. VAL-083 induces interstrand crosslinks at $N^7$-guanines leading to DNA double-strand breaks, mediating cell cycle arrest and cell death[2-4].*

## CONCLUSIONS

In these ***MGMT-unmethylated, TMZ-resistant*** *ex vivo* and *in vivo* PDOX GBM models:

- VAL-083 showed superior antitumor efficacy against patient-derived GBM organoids compared to TMZ, independent of epigenetic background and treatment history.
- VAL-083 showed strong *in vivo* anti-tumor activity, significantly reducing tumor volume and tumor growth rate.
- The tumor growth rate reduction effect was further augmented in combination with bevacizumab.
- VAL-083-induced DNA damage was specific to the GBM tumor and minimal in normal brain tissue, demonstrating VAL-083 specificity for tumor cells.
- These results provide rationale for clinical investigation of VAL-083 in *MGMT*-unmethylated treatment-naïve and recurrent GBM.

## REFERENCES

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2. Zhai B, et al. Cell Death Dis. 2018; 9(10):1016
3. Peng et al. Acta Pharmacol Sinica 2017: 1–10
4. Fouse et al. Neuro-Oncology 16 (2014)
5. Golebiewska et al. bioRxiv 2020.04.24.057802
6. Golebiewska et al. Brain (2013)
7. Stieber&Golebiewska et al. Acta Neuropath (2014)
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9. Figures 1, 2, and 5 created with BioRender.com

## 3D GBM ORGANOIDS DERIVED FROM 18 PATIENT-DERIVED ORTHOTOPIC XENOGRAFT (PDOX) GBM MODELS OF DIFFERENT (EPI)GENETIC BACKGROUND

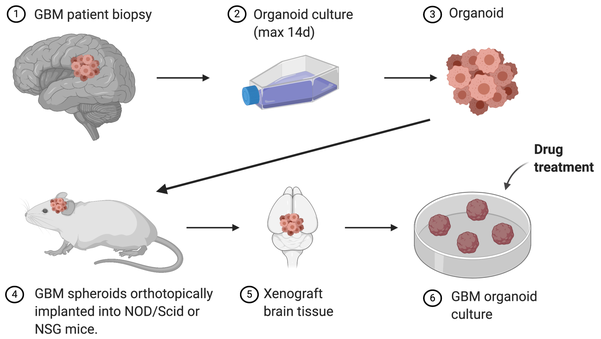



*FIGURE 2. 3D GBM organoids were derived (with proper consent) from patient GBM tissue, grown in 3D culture for 2 weeks and implanted into immunodeficient mice[5-8]. Upon sacrifice, xenograft brains were minced and organoids were grown in 3D culture for drug efficacy studies.*

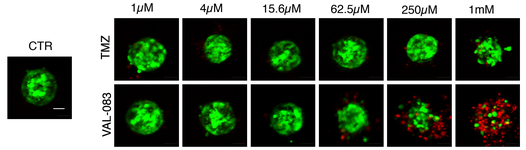



*FIGURE 3. Drug response assessment in 3D GBM organoids with standardized size (green = viable cells, red = dead cells). Representative images are shown for the T434-derived organoids. Scale bare = 50μM.*

***RESULTS:*** *VAL-083 killed T434-derived GBM organoids in a dose-dependent manner.*

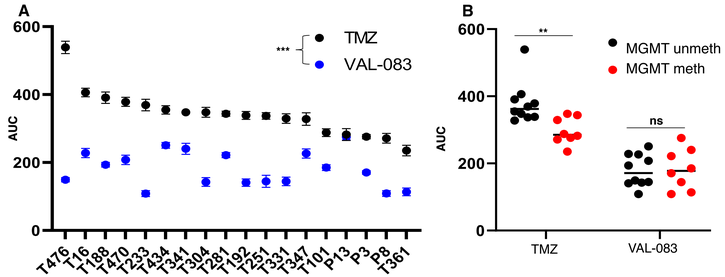
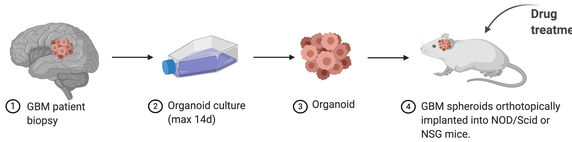



*FIGURE 4. (A) Quantification of area under the curve (AUC) upon exposure to TMZ or VAL-083 for 18 patient-derived GBM organoid models. Mean AUC +/- SEM. (B) Mean AUC upon exposure to TMZ or VAL-083 in organoids from 18 MGMT unmethylated or methylated GBM tumors.*

***RESULTS:*** *MGMT unmethylated tumors were less responsive to TMZ than methylated tumors, while VAL-083 response was independent of MGMT status. VAL-083 was generally more effective than TMZ against GBM organoid models (*** p-value < 0.0001, unpaired t-test)*

## PATIENT-DERIVED ORTHOTOPIC XENOGRAFT (PDOX) MODEL OF MGMT-UNMETHYLATED GLIOBLASTOMA




- T16 xenografts are **MGMT unmethylated**
- Xenograft T16 tumors contain **25% CD133+** cells.
- Bevacizumab treatment leads to **hypoxia** and increased glucose consumption in T16 PDOX model.

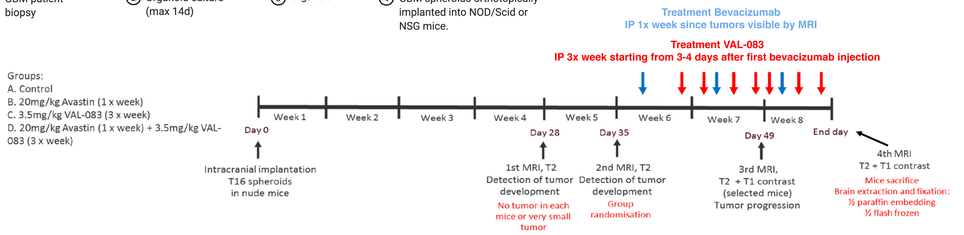



*FIGURE 5. Patient-derived GBM tissue from patient T16 was grown in 3D culture for 2 weeks and implanted intracranially into immunodeficient mice[5-8]. The mice were randomized into four groups on day 35 and treated according to the schedule (bevacizumab=blue, VAL-083=red). Tumor development was measured by MRI on days 28, 35, 49 and 56.*

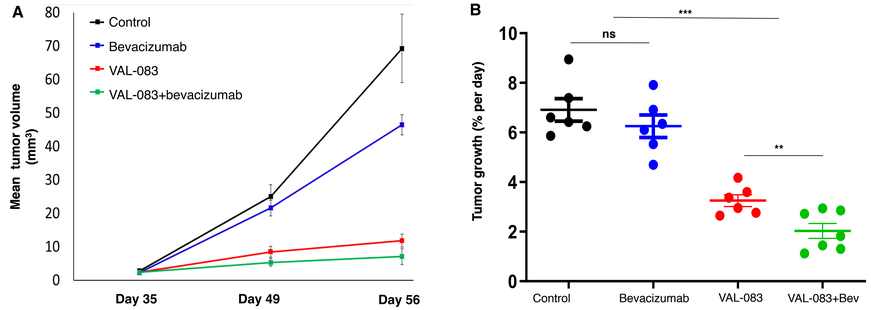



*FIGURE 6. (A) Mean tumor volume in mice from the four treatment groups as determined by MRI on days 35, 49 and 56 (n=6-7). (B) Tumor growth rate between groups was calculated during entire study (day 35 vs. 56, n=6-7, *** p-value <0.0001, unpaired t-test).*

***RESULTS:*** *VAL-083 treatment led to significant reduction of tumor growth over time in the T16-PDOX in vivo model both as single treatment and in combination with bevacizumab.*

## VAL-083 SPECIFICALLY TARGETS GBM TUMOR CELLS, *in vivo*

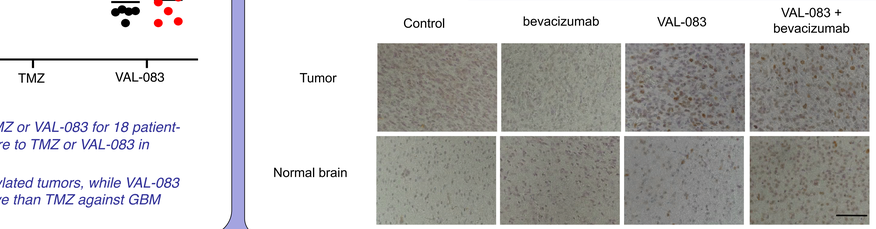



***FIGURE 7.*** *IHC for H2AX-P (a marker for DNA double-strand breaks) in PDOX sections ((IHC in paraffin tissue sections, brown=H2AX-P; Blue= hematoxylin positive nuclei).*

***RESULTS:*** *VAL-083 induced high levels of H2AX-P in GBM tumor cells but not in normal brain cells. Minor induction of H2AX-P was observed in subpopulations of normal brain cells (bottom panels). Scale bar = 100 μm.*