VAL-083 is a novel N7 alkylating agent that inhibits the growth of glioma stem and non-stem cultures, including temozolomide-resistant lines

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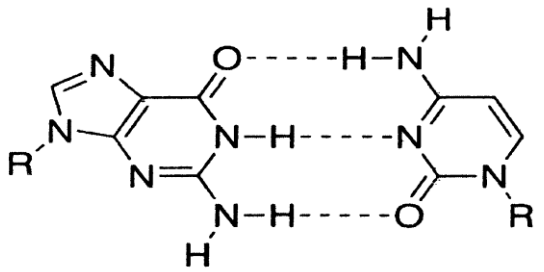
Background and Rationale

The standard of care for glioblastoma multiforme (GBM) patients is surgical resection followed by temozolomide (TMZ) and radiation (XRT). TMZ is most effective for a minority of patients that exhibit epigenetic inactivation of 0-6-methylguanine DNA methyltransferase (MGMT), a DNA repair enzyme that removes the methyl-group adducts that are caused by TMZ. Thus, adducts that are not subject to the DNA repair mechanism of MGMT might provide additional benefit to GBM patients, the majority of which express MGMT and are TMZ-resistant, or acquire resistance after TMZ administration. The N7 alkylating agent, VAL-083, is not subject to MGMT mediated repair and might therefore be a more potent chemotherapeutic. VAL-083 is a first-in-class alkylating agent that crosses the blood brain barrier and is currently in clinical trials for glioma patients with recurrent disease. We have recently shown that cancer stem cells (CSC) and their paired non-CSC cultures derived from primary GBM tissues exhibit similar responses to TMZ, with this response dependent on the presence or absence of MGMT expression (Fouse et al., 2014). We sought to investigate how our panel of stem and non-stem cultures responds to VAL-083 alone or in combination with XRT, and how the response would compare to TMZ.

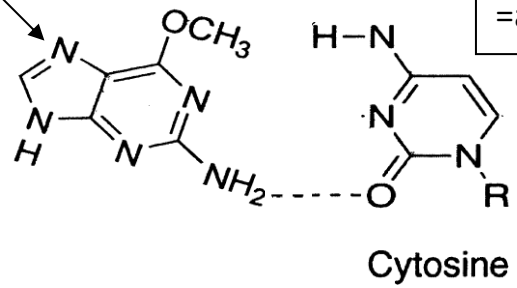
Fouse et al., Neuro Oncol. 2014 16(3): 361-71

VAL-083 Mechanism of Action



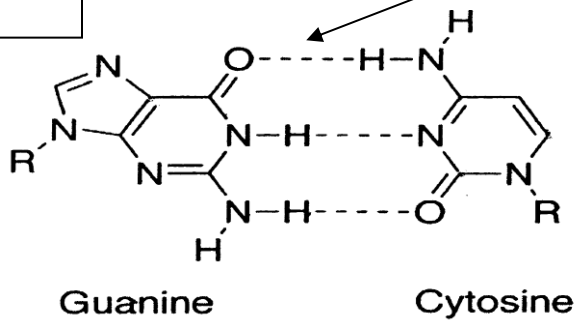
VAL-083 alkylates at N^7

TMZ methylates at O^6
Hydrogen bonds interrupted
=apoptosis



MGMT enzyme DOES NOT repair VAL alkylation

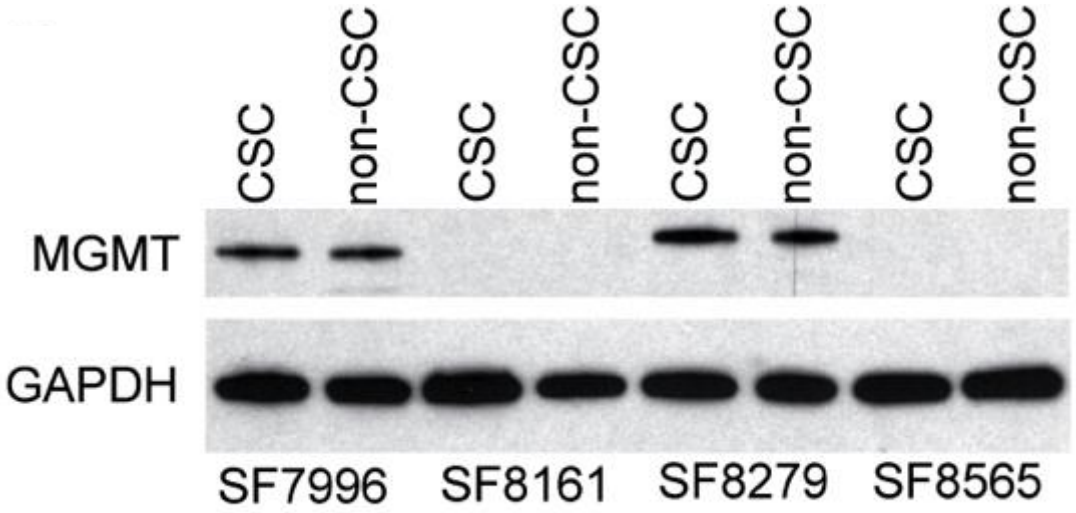
MGMT enzyme repairs TMZ methylation



Materials and Methods

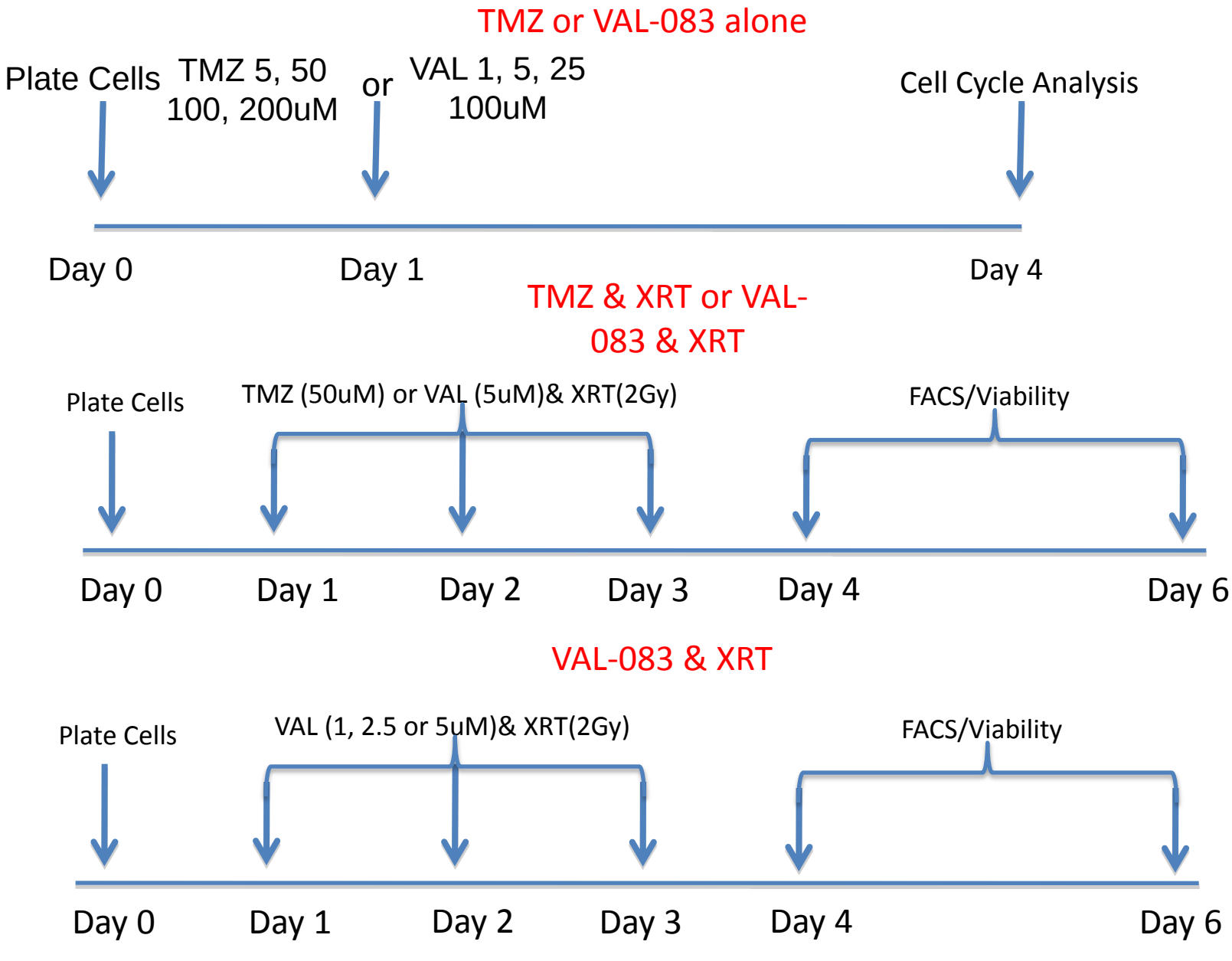
- Cell Cultures: CSCs were cultured in NSA media supplemented with B27, EGF and bFGF. Non-CSCs were grown in DMEM:F12 with 10% FBS. MGMT methylation and protein expression analysis of each culture was characterized. TMZ or VAL-083 was added to the cultures in the indicated concentrations. Depending on the experiment, cells were also irradiated with 2Gy in a Cesium irradiator

- Assays: Cell cycle analysis was performed with Propidium Iodide staining and FACs analysis. Cell viability was analyzed with CellTiter-Glo and read on a Promega GloMax

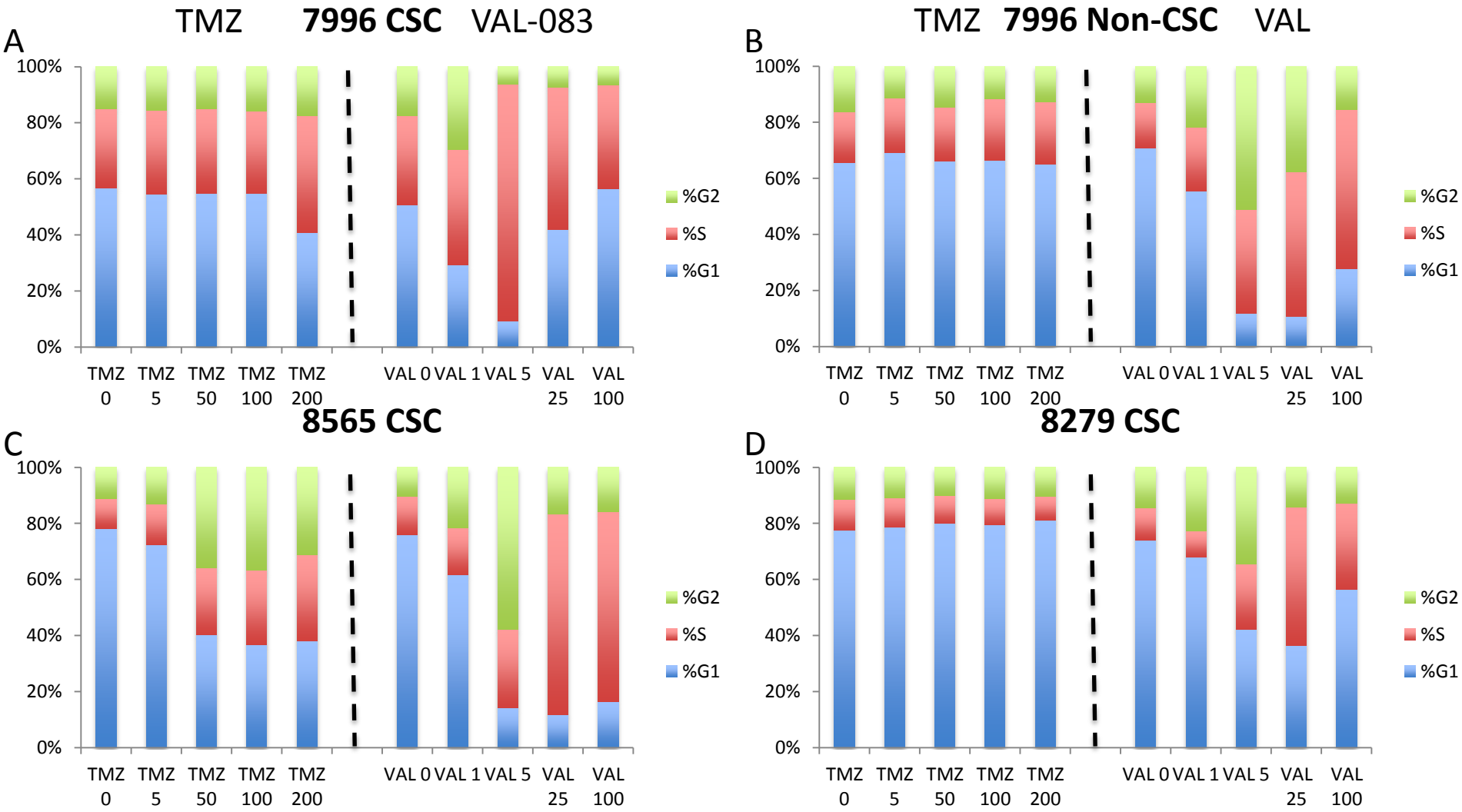


Expression of MGMT protein in primary GBM cultures. MGMT western blot analysis of protein extracts from 4 pairs of CSC and non-CSC cultures derived from primary GBM tissue

Various Treatment Regimens

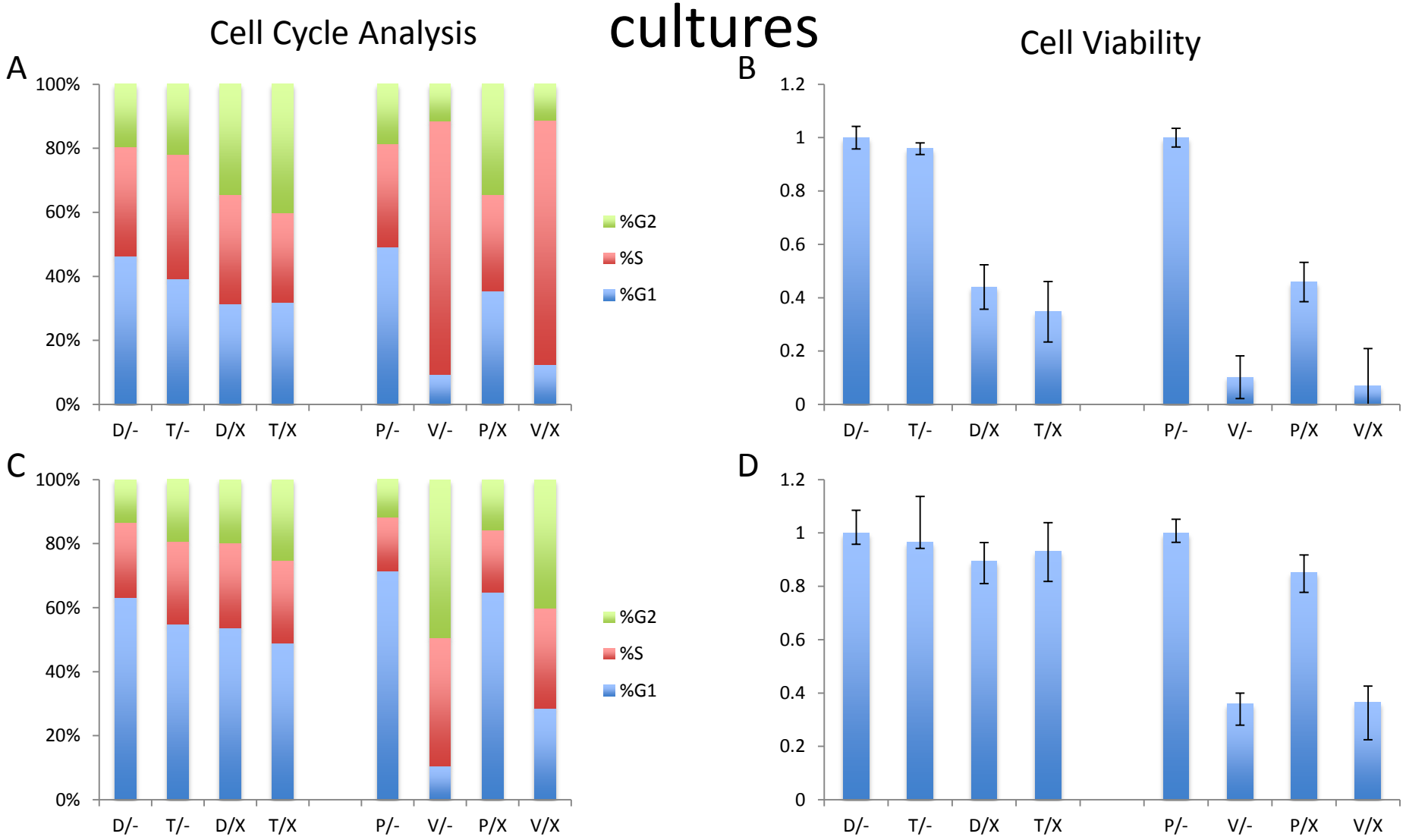


VAL-083 causes cell cycle arrest in TMZ-resistant cultures



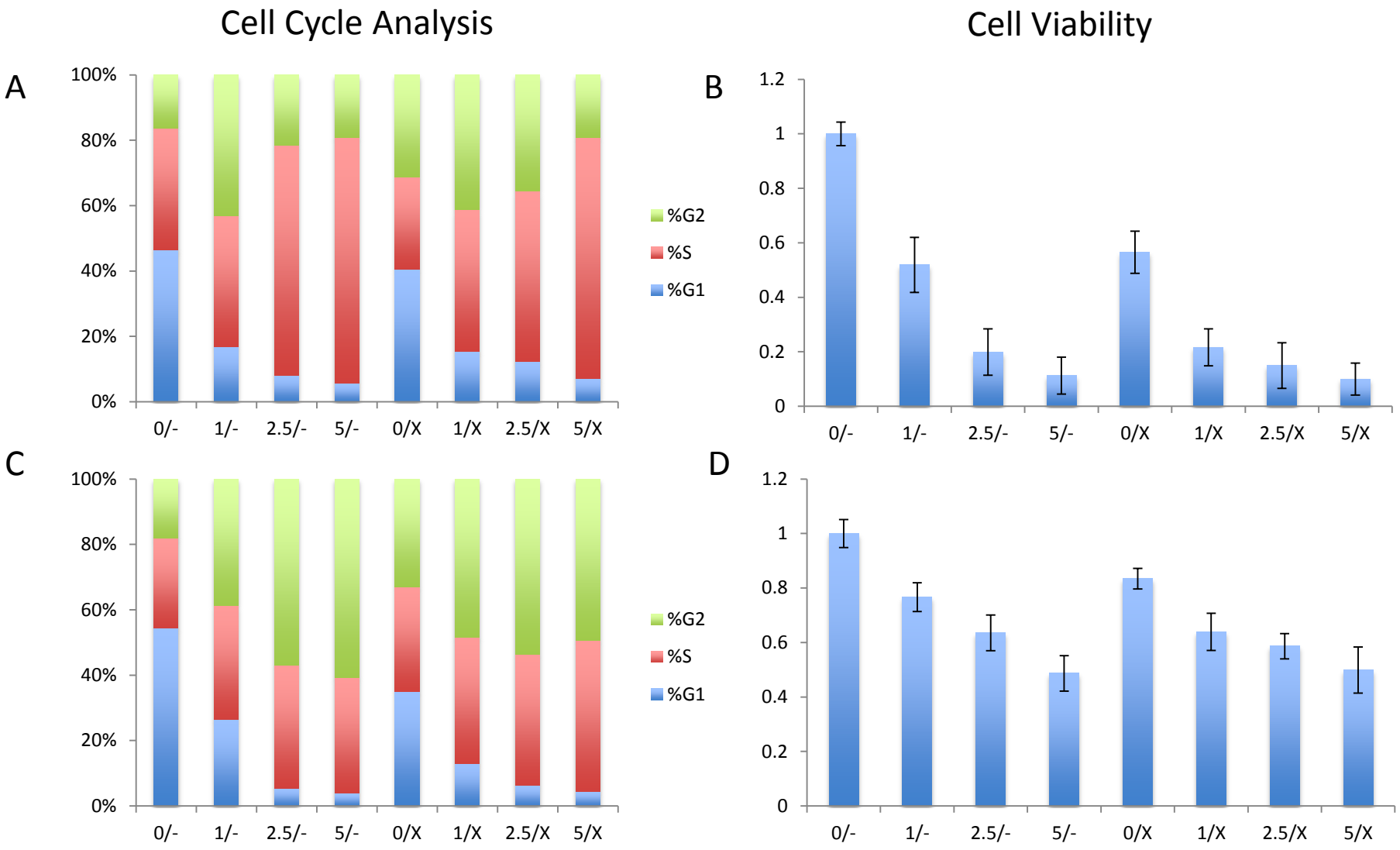
VAL-083 induces cell cycle arrest in all cultures tested. Cells were treated with either increasing doses of TMZ (5, 50 100 and 200 uM) or VAL-083 (1, 5, 25 and 100 uM) and cell cycle analysis was performed 4 days post treatment. TMZ resistant cultures (A, B, D) exhibited sensitivity to VAL-083, even at single uM doses. Furthermore, this response was not dependent on culture type as paired CSC (A) and non-CSC (B) both exhibit sensitivity to VAL-083

VAL-083 decreases cell viability in TMZ-resistant cultures



VAL-083 decreases cell viability but does not synergize with XRT at 5uM. TMZ (50uM) or VAL-083 (5uM) were added to primary CSC cultures at various doses with or without irradiation (2 Gy). Shown are cell cycle profile analysis at day 4 post treatment (A,C) and cell viability analysis at day 6 post treatment (B,D) for the paired CSC (A,B) and non-CSC (C,D) 7996 culture. Whereas these cultures are not very sensitive to TMZ, they are to VAL-083. However, the addition of XRT in both cases does not result in increase sensitivity(D = DMSO, T= TMZ, X=XRT, P=PBS)

VAL-083 functions as a radiosensitizer



VAL-083 acts as a radiosensitizer in primary CSC cultures. VAL-083 was added to primary CSC cultures at various doses (1, 2.5 and 5 uM) with or without irradiation (2 Gy). Shown are cell cycle profile analysis at day 4 post treatment (A,C) and cell viability analysis at day 6 post treatment (B,D) for two different patient-derived CSC cultures, 7996 (A,B) and 8565 (C,D)

VAL-083 Summary

- VAL-083 appears to cause cell cycle arrest and loss of cell viability at lower concentrations than TMZ
- Furthermore, VAL-083 is not affected by MGMT status or cell culture condition (Stem vs. Non-Stem) as all primary cultures tested were sensitive to VAL-083 exposure
- For all cultures tested, we see a potential additive effect of VAL-083 with XRT, particularly at low concentrations of VAL-083
- These results suggest that VAL-083 may provide a greater clinical benefit to glioma patients compared to the standard of care chemotherapy, TMZ.