VAL-083, a novel N7 alkylating agent, surpasses temozolomide activity and inhibits cancer stem cells providing a new potential treatment option for glioblastoma multiforme

Abbas Fotovati [1], Kaiji Hu [1]Hiro Wakimoto[2], Joanna Triscott [1], Jeffery Bacha [3], Dennis Brown [3], and Sandra E Dunn [1]*

[1]*University of British Columbia, Vancouver, British Columbia, Canada;*

[2]*Massacheusetts General Hospital, Department of Neurosurgery, Boston MA,*

[3]*Del Mar Pharmaceuticals (BC) Ltd., British Columbia, Canada.*

Key words: glioblastoma multiforme, Temozolomide, drug resistance, MGMT, cancer stem cells, VAL-083

*Corresponding author: Sandra E. Dunn, Ph.D., Departments of Pediatrics, Experimental Medicine, and Medical Genetics, Child and Family Research Institute, University of British Columbia, 950 W. 28th Ave, Vancouver, BC, V5Z 4H4, 604-875-2000 ext 6015, Fax: 604-875-3120. E-mail: sedunn@mail.ubc.ca.

Abstract

Glioblastoma (GBM) remains one of the most difficult tumors to treat in part because many new agents fail to cross the blood brain barrier (BBB) and secondly due to intrinsic drug resistance. Temozolomide (TMZ) is a front-line therapy for the treatment of GBM, however, it is often ineffective due to drug inactivation by O^6-methylguanine-DNA methyltransferase (MGMT). Cancer stem cells (CSC) are a subpopulation of the tumor that resist therapy and give rise to relapse. Here we described VAL-083 a novel alkylating agent that creates N^7 methylation on DNA, which was initially intriguing because it crosses the BBB. We addressed how it compared to TMZ, whether it could be used to overcome MGMT-driven drug resistance and if it has activity against CSCs. Addressing these questions provides further preclinical support for VAL-083, which is currently undergoing human clinical trials in the USA against refractory GBM. VAL-083 inhibited U251 and SF188 cell growth in monolayer and as neurospheres better then TMZ and caused apoptosis after 72 hrs. In a 10-day colony formation assay, VAL-083 (5uM) suppressed SF188 growth by ~95%. T98G cells are classically TMZ resistant and express MGMT yet VAL-083 inhibited their growth in monolayer after 72 hrs in a dose-dependent manner (IC50<5 uM). VAL-083 also inhibited the growth of CSCs (BT74, GBM4 and GBM8) by 80-100% in neurosphere self-renewal assays. Conversely, there was

minimal effect on normal human neural stem cells. In summary, VAL-083 has better *in vitro* efficacy than TMZ against brain tumor cells, can overcome resistance associated with MGMT, and targets brain tumor CSCs demonstrating that it has the potential to surpass the standard-of-care.