Exploring the Molecular Mechanisms of Dianhydrogalactitol (VAL-083) in Cancer Treatment

Beibei Zhai[1, 2], Anne Steino[3], Jeffrey Bacha[3], Dennis Brown[3], and Mads Daugaard[1, 2]

[1]Vancouver Prostate Centre, Vancouver, Canada; [2]University of British Columbia, Vancouver, Canada;
[3]DelMar Pharmaceuticals, Inc., Vancouver, Canada and California, USA

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

VANCOUVER PROSTATE CENTRE
A UBC & VGH Centre of Excellence

DelMar PHARMACEUTICALS

Background

Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent causing N^7-guanine methylation and inter-strand DNA crosslinks. In China, VAL-083 is approved as a chemotherapeutic drug for the treatment of chronic myelogenous leukemia and lung cancer. Preclinical studies and clinical trial data suggest that VAL-083 may have potential effects in treating various cancers including lung, brain, cervical, ovarian tumors, and leukemia. However, the detailed molecular mechanisms mediating VAL-083 sensitivity or resistance in cancer is still unclear. We aimed to investigate the signaling events responsible for VAL-083 activity against cancer.

Figure 1: VAL-083 leads to N^7-guanine inter-strand DNA crosslink[1].

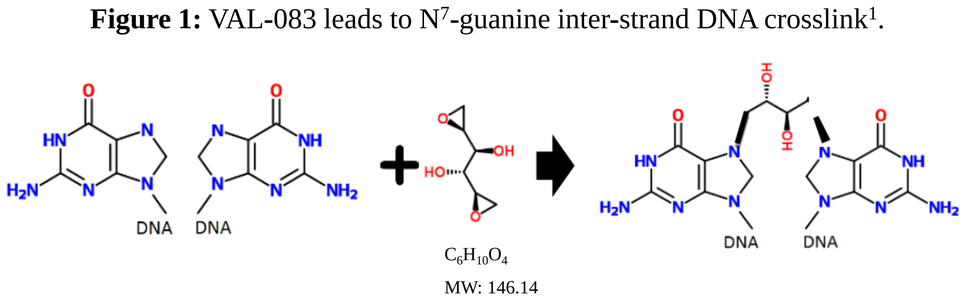
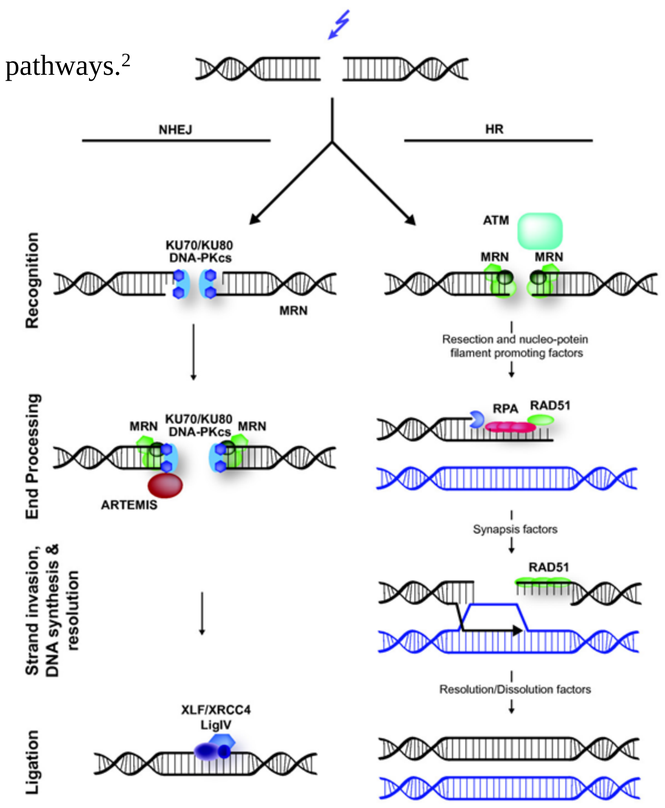


Figure 2: DNA damage repair signaling pathways.[2]



Hypothesize:

1. VAL-083 induces DNA double-strand breaks (DSBs).
2. VAL-083 cytotoxicity is due to activation of DNA damage response.
3. The antineoplastic effect of VAL-083 is dependent on cancer cells' ability to repair the VAL-083-induced DNA damage.
4. Alterations in DNA damage repair signaling pathway lead to VAL-083 sensitivity or resistance in tumor cells.

Methods

Crystal violet proliferation assays were performed to assess VAL-083 sensitivity in a variety of cancer cell lines. PI staining and immunofluorescent analyses were used to evaluate cell cycle. Western blots were used to investigate DNA damage response induced by VAL-083 treatment.

Results

Figure 3: Crystal violet proliferation assays to evaluate VAL-083 sensitivity in six cancer cell lines. Cells were treated with increasing concentrations of VAL-083 for 72 h.

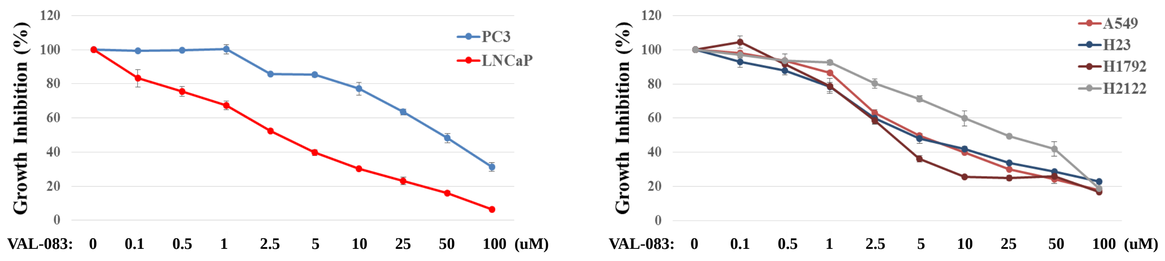
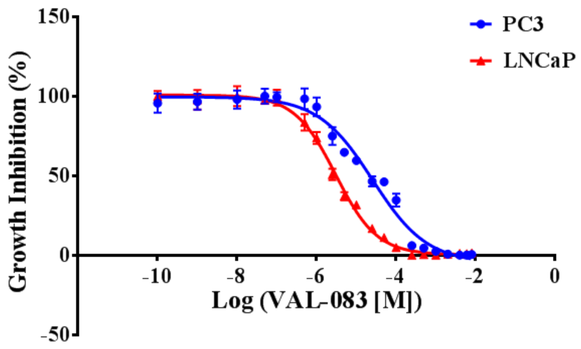


Figure 4: IC_{50} of VAL-083 treatment for 72 h.



	PC3	LNCaP	H2122	H1792
IC_{50} (uM)	25.7	3.06	12.23	4.57
IC_{50} range (uM)	19.94 – 33.1	2.74 – 3.41	8.68 – 17.22	4.06 – 5.13

Figure 5: VAL-083-induced DSB triggers phosphorylation of H2A.X (increased γH2AX), which could be detectable at around 24 h.

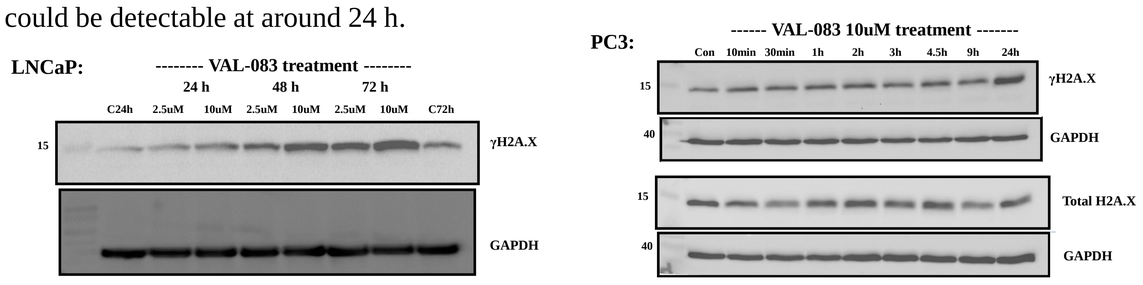


Figure 6: VAL-083-induced γH2A.X lasted for 48-72 h after removing from the medium.

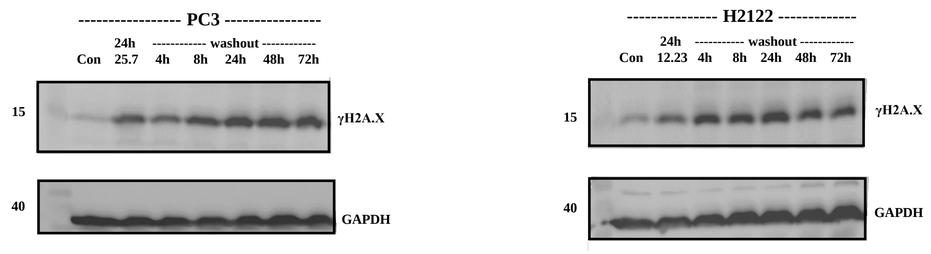


Figure 7: VAL-083 treatment led to cell cycle arrest at late S/G_2 phase (LNCaP cells were shown below as the representative images), which is similar to cisplatin treatment.

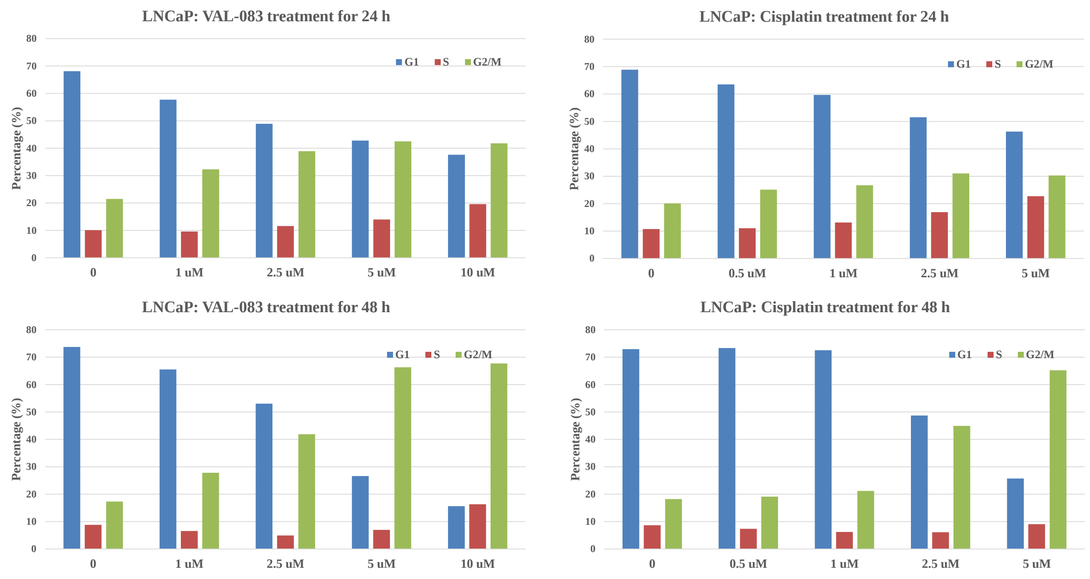


Figure 8: VAL-083 treatment activated DNA damage signaling pathways as demonstrated by expression of phospho-ATM (S1981) and phospho-RPA32 (S33), especially in PC3 and H2122 cells.

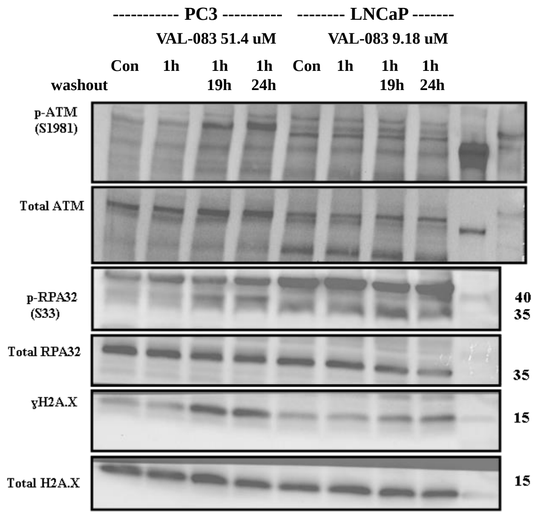
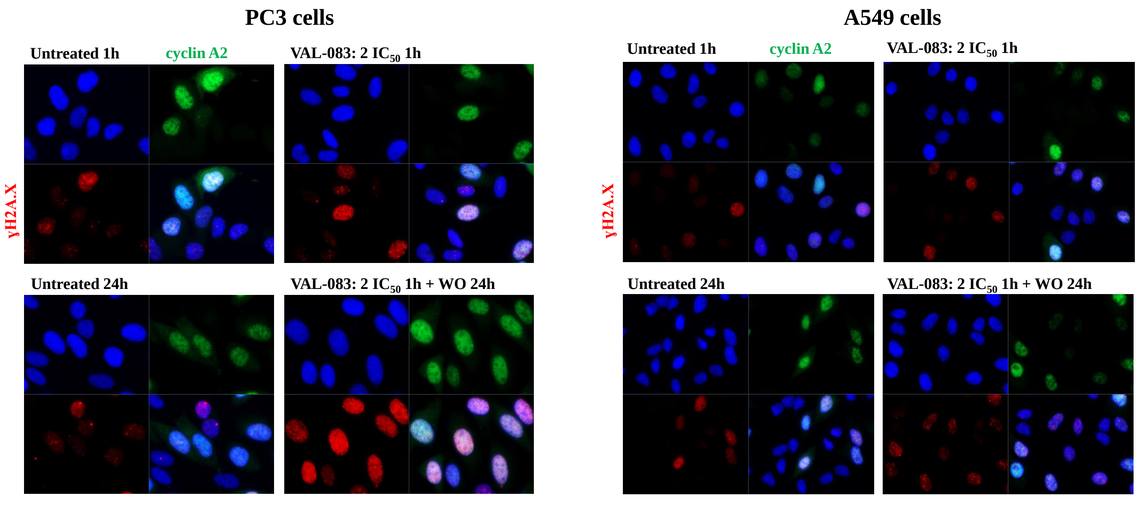


Figure 9: Immunofluorescent staining showed increased γH2A.X and late S/G_2 phase cell cycle arrest after VAL-083 treatment in PC3 cells.



Perspective Research

- Continue to explore DNA damage signaling pathways involved in VAL-083 treatment in cancer.
- in vivo studies: xenograft or syngeneic mouse model to investigate tumor response following VAL-083 treatment.
- The elucidation of the molecular mechanisms of VAL-083 will help to identify patients who would be most beneficial from VAL-083 treatment.

References

1. In vitro activity of dianhydrogalactitol alone or with platinum drugs in the treatment of non-small cell lung cancer. April 19, 2015 AACR meeting.
2. ATP-dependent chromatin remodeling in the DNA-damage response. Lans et al. *Epigenetics & Chromatin* 2012 5:4.

Acknowledgements

This project is supported by DelMar pharmaceuticals Inc and MITACS.

DelMar PHARMACEUTICALS
Mitacs
VANCOUVER PROSTATE CENTRE
A UBC & VGH Centre of Excellence

LLafave 2010