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

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Abstract: Dianhydrogalactitol (VAL-083) is a structurally unique bi-functional alkylating agent causing methylation of N⁷-guanine and inter-strand DNA crosslinks. VAL-083 is a small water-soluble molecule that readily crosses the blood-brain-barrier. In China, VAL-083 is approved as a chemotherapeutic drug for the treatment of chronic myelogenous leukemia (CML) and lung cancer. In the United States, VAL-083 has been evaluated in more than 40 National Cancer Institute (NCI)-sponsored phase I and phase II clinical trials. Preclinical studies and clinical trial data suggested antineoplastic effects of VAL-083 in a variety of malignancies, including lung cancer, brain tumors, leukemia, cervical, and ovarian cancer. Here we report new insights into VAL-083 mechanism of action by showing that VAL-083 leads to irreversible cell cycle arrest and cell death caused by replication-dependent DNA damage. In lung (H2122, H1792, H23 and A549) and prostate (PC3 and LNCaP) cancer cell lines, VAL-083 treatment caused irreversible cell cycle arrest in late S and G₂ phase as measured by propidium iodide (PI) and immunofluorescent (IF) staining in synchronized cultures. Importantly, VAL-083 was cytotoxic to all cell lines tested (IC₅₀ range 3.1 - 25.7 μ M). Western blot and IF analyses of DNA repair markers were employed to investigate the DNA damage response induced by VAL-083 in cancer cells. VAL-083 treatment led to phosphorylation of the proximal DNA double-strand break (DSB) sensor Ataxia Telangiectasia Mutated kinase (ATM), the single-strand DNA-binding Replication Protein A (RPA32), and the histone variant H2A.X (γ H2A.X). Importantly, the DNA damage was specific to cells in S phase indicating that VAL-083-induced DNA crosslinks translates into more severe DNA lesions during replication. Furthermore, S/G₂ phase cell cycle arrest and increased phosphorylation of γ H2A.X in cancer cells persisted after pulse-treatment with VAL-083, indicating irreversible DNA lesions. Taken together, VAL-083 displayed broad anti-neoplastic activity in lung and prostate cancer cells through the induction of replication-dependent DNA damage. Elucidation of the molecular mechanisms underlying VAL-083 cytotoxicity in cancer cells will help in identifying and predicting efficacy of combination treatments.

Background:

Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent causing N⁷-guanine alkylation and inter-strand DNA crosslinks. VAL-083's cytotoxic activity is independent of MGMT-expression in various cancer cells and cancer stem cells, suggesting a mechanism that is distinct from that of other alkylating agents (e.g. temozolomide and BCNU). Preclinical and clinical trial data suggest that VAL-083 may have 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 aim to investigate the signaling events responsible for VAL-083 activity against cancer.

Figure 1: VAL-083 leads to N⁷-guanine inter-strand DNA crosslinks¹.

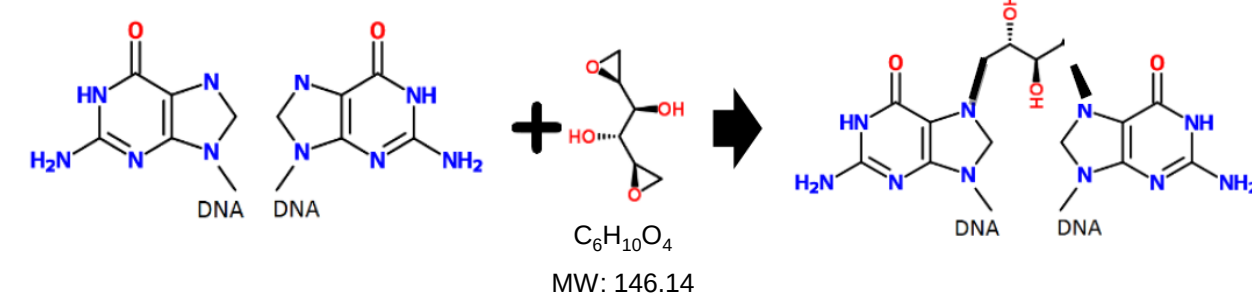
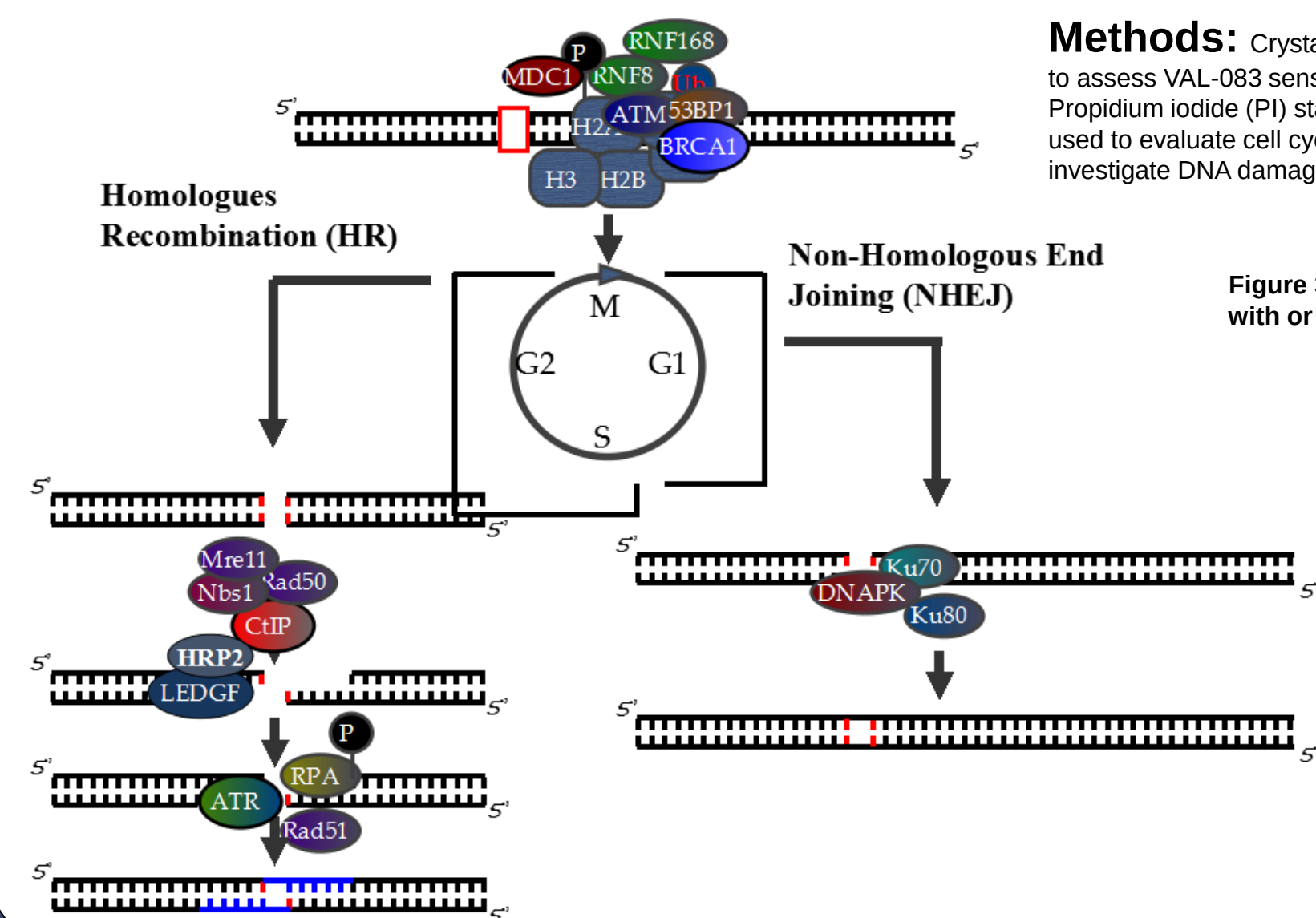


Figure 2: DNA double-strand break (DSB) repair mechanisms: HR and NHEJ



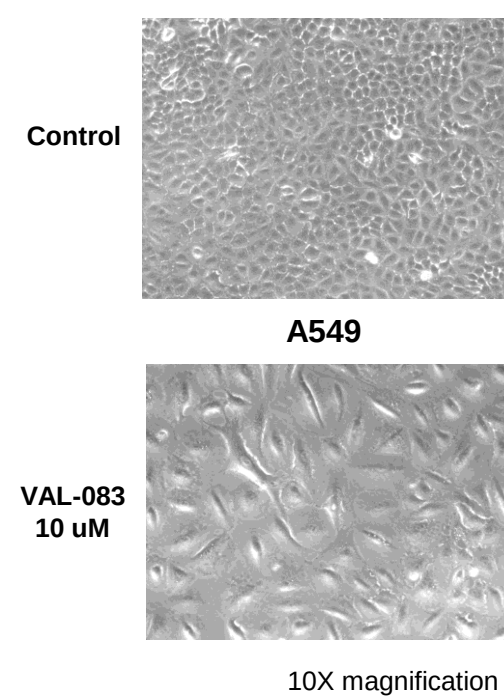
Hypothesis:

- 1) VAL-083-triggered DNA inter-strand crosslinks lead to DNA double strand breaks (DSBs) during replication;
- 2) VAL-083-induced replication-dependent DNA damage activates homologous recombination repair mechanism that is restricted to S and G₂ phases of cell cycle;
- 3) the anti-cancer effect of VAL-083 depends on the capability of tumor cells to repair VAL-083-induced DNA lesions as well as other signaling events responsible for cell cycle arrest and/or cell death;
- 4) alterations in DNA repair mechanisms, DNA damage signaling pathways, or stress-induced survival pathways may confer VAL-083 resistance in certain cancer cells.

Methods:

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

Figure 3: Representative images of A549 cells with or without 10 μ M VAL-083 treatment for 72h.



Results:

Figure 4: 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.

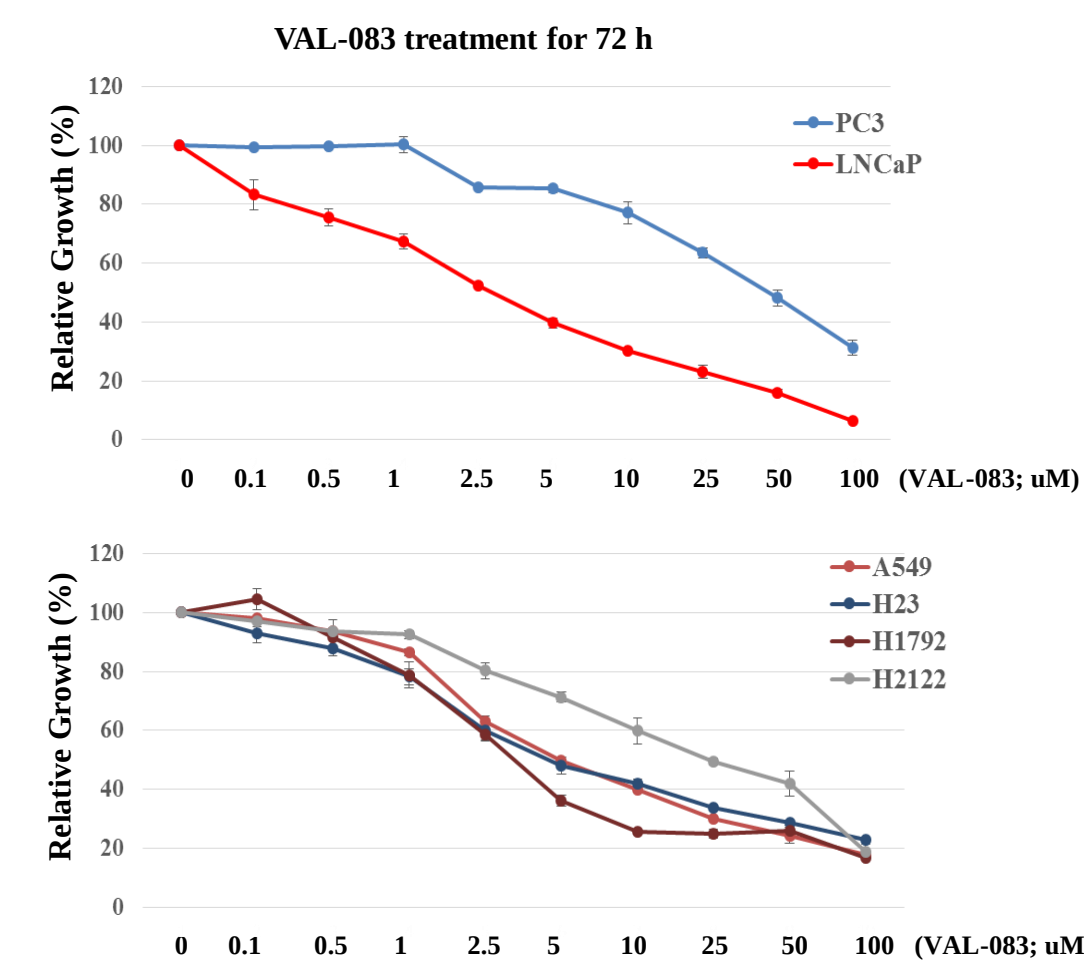


Figure 5: VAL-083-induced DSB triggers phosphorylation of H2A.X (increased γ H2AX), which could be detectable at around 24 h.

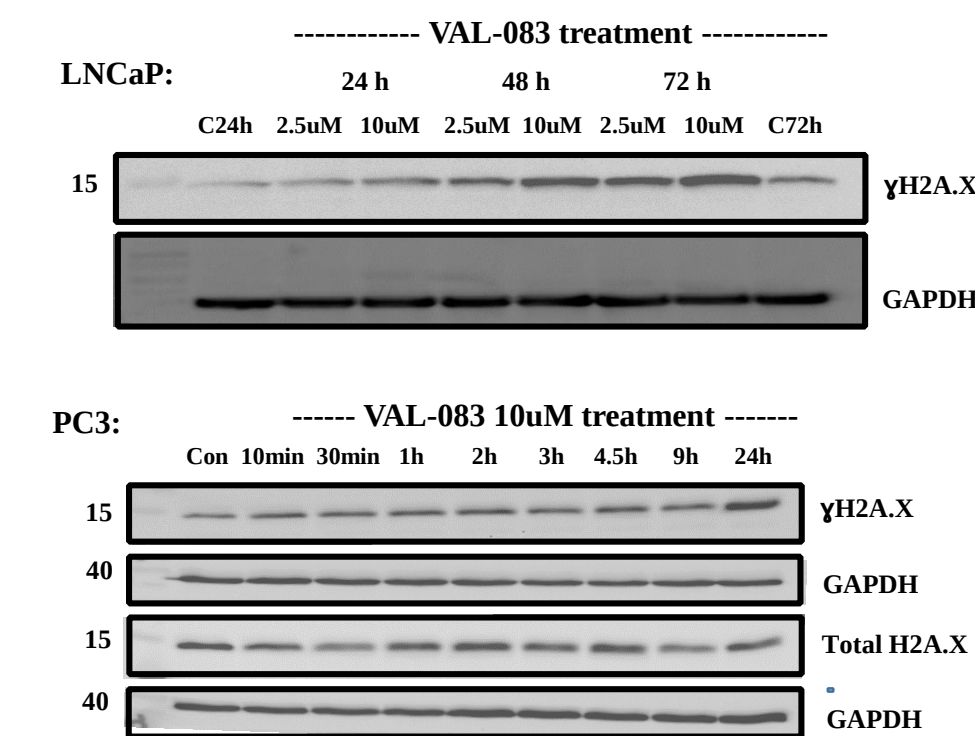


Figure 6: VAL-083 treatment led to cell cycle arrest at S/G₂ phase (serum starvation for 24 h before 5 μ M VAL-083 treatment).

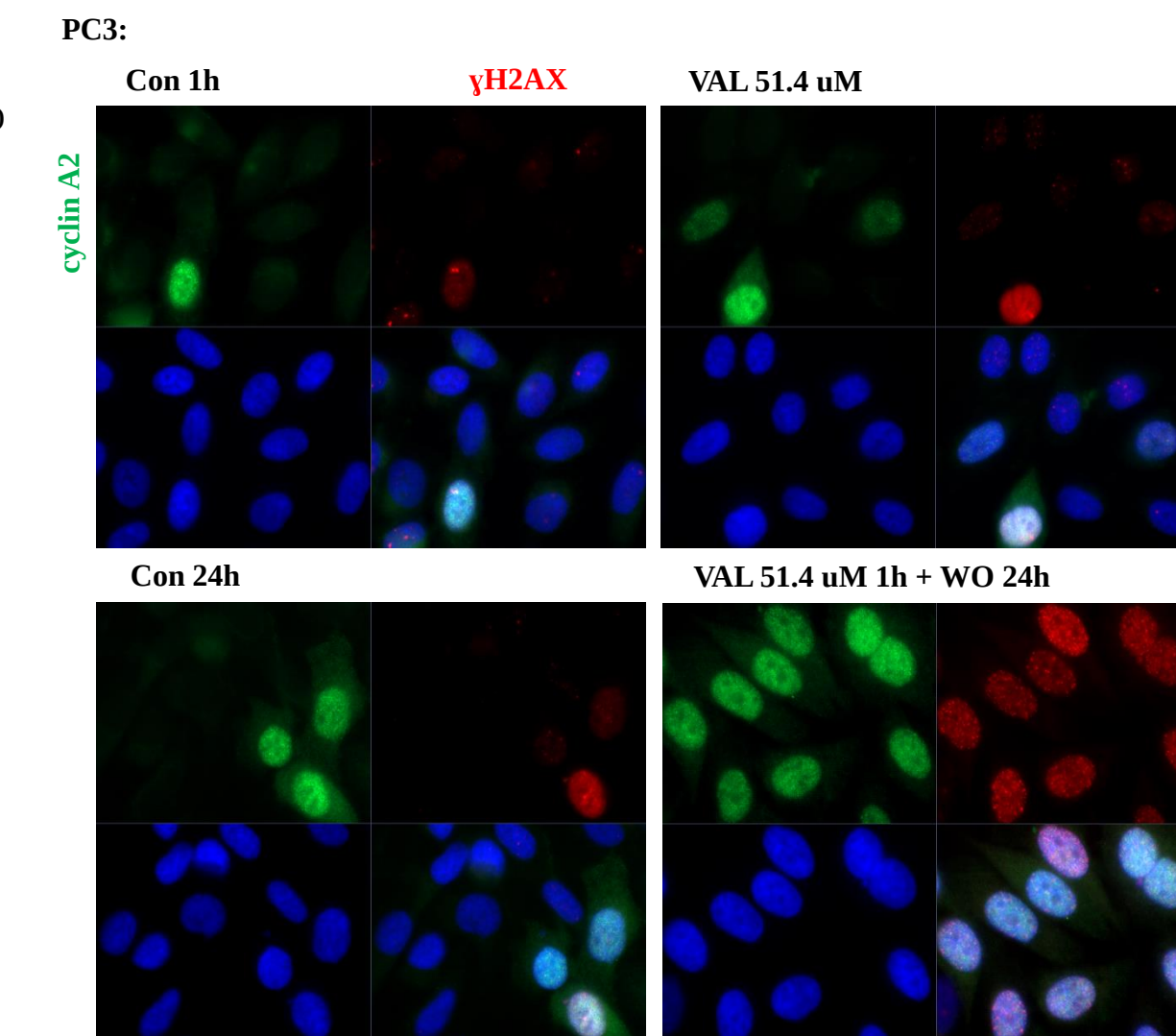
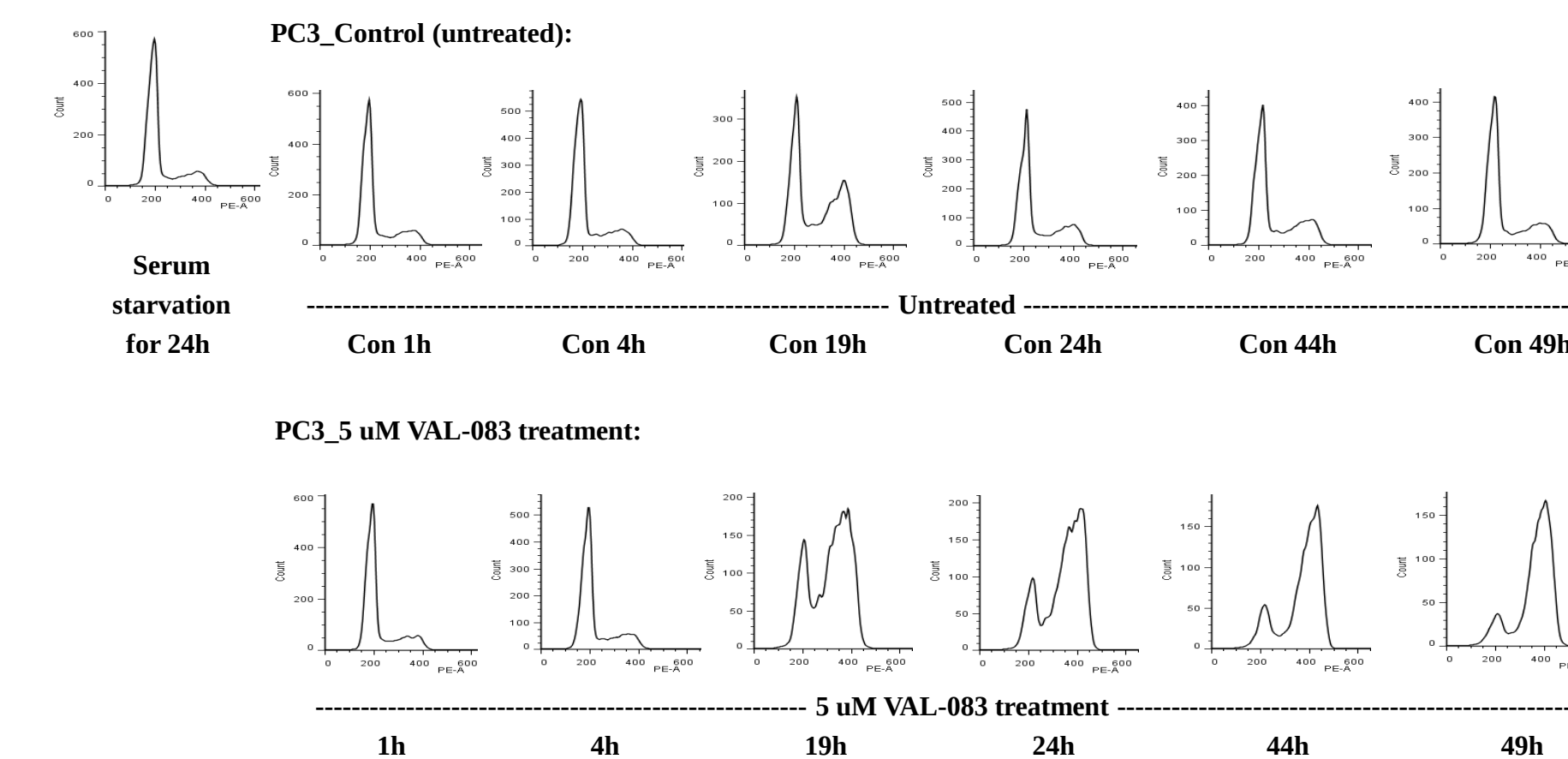
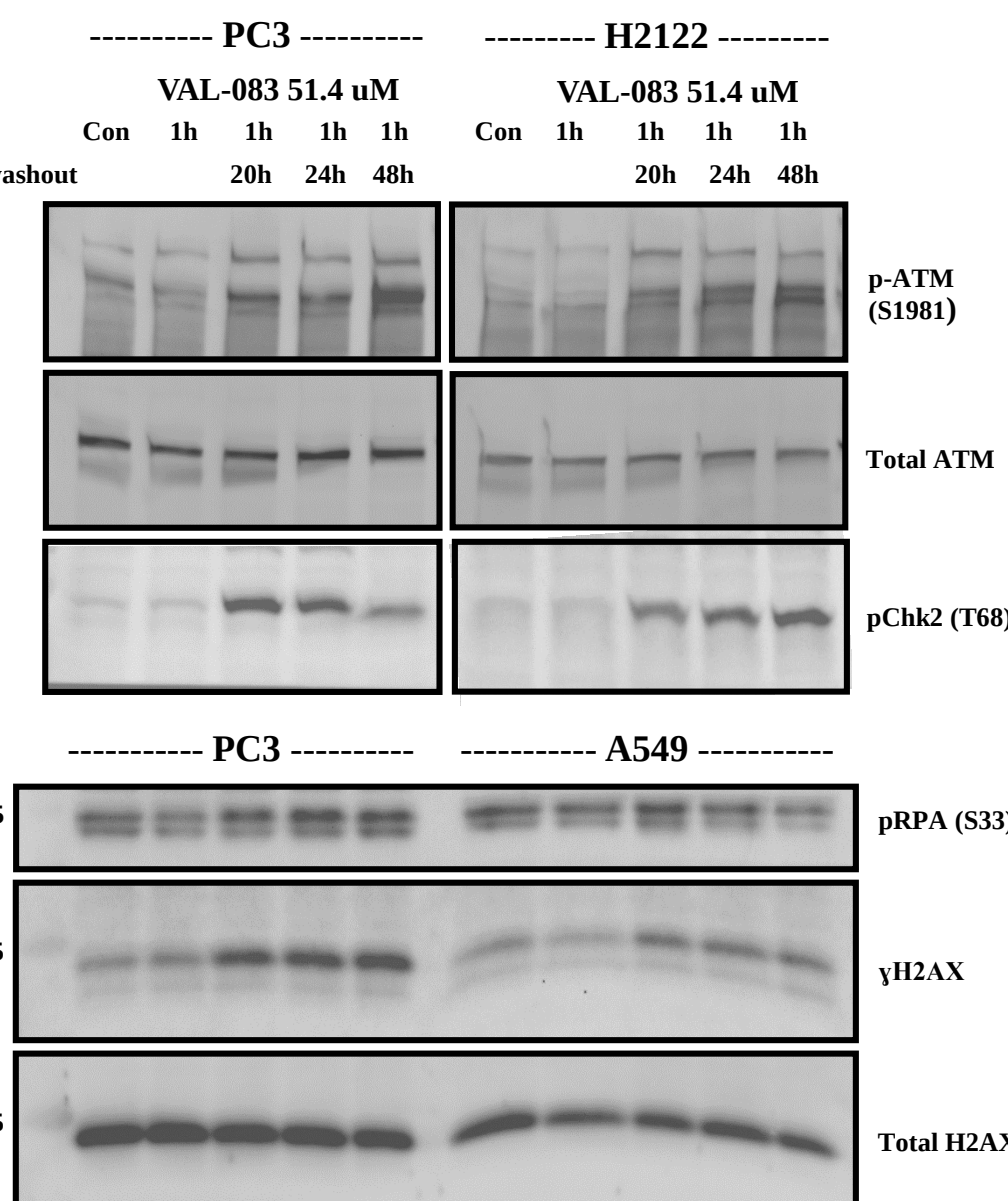
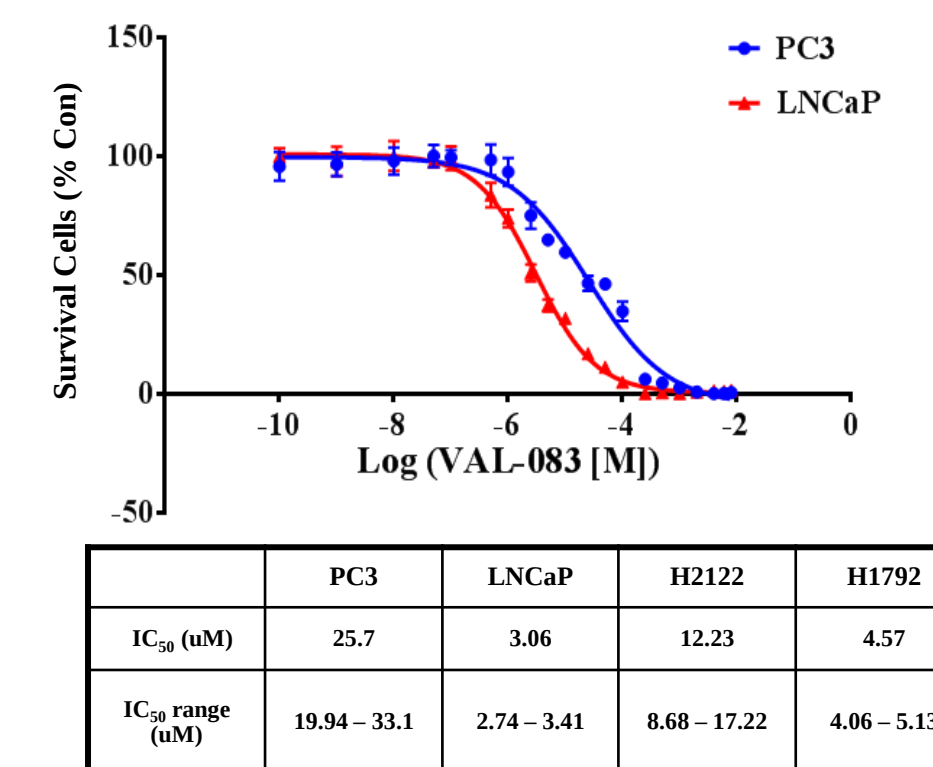


Figure 8: VAL-083 pulse treatment activated DNA damage signaling pathway as demonstrated by expression of phospho-ATM (S1981), phospho-Chk2 (T68), phospho-RPA32 (S33) and γ H2A.X which persisted for 24 - 48 h after removal of VAL-083 from the medium.

Figure 9: VAL-083 pulse treatment induced replication-dependent DNA damage. Serum starvation for 24 h before VAL-083.

PC3 cells:	γ H2A.X + cyclin A2 +
Con 1h	2.8 %
VAL 1h	2.6 %
Con 24h	16.5 %
VAL 1h + WO 24h	90.3 %

Figure 7: IC₅₀ of VAL-083 treatment in different cancer cells for 72h.



Conclusions:

1. VAL-083 displayed broad anti-tumor activity in lung and prostate cancer cells.
2. VAL-083 treatment causes rapid and durable DNA crosslinks leading to irreparable DNA double-strand breaks, S/G₂ phase cell-cycle arrest and cell death in cancer cells.
3. Elucidation of the molecular mechanisms underlying VAL-083 cytotoxicity in cancer cells will offer help for effective combination therapies.

References: 1. Institoris E, et al. Cancer Chemother Pharmacol (1989)24:311-3.

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