

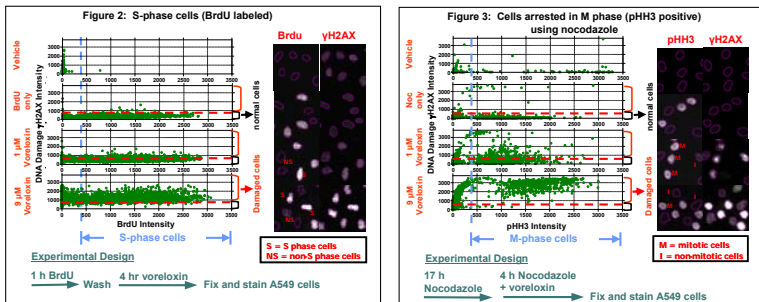
Voreloxin (formerly SNS-595) is a potent DNA intercalator and topoisomerase II poison that induces cell cycle dependent DNA damage and rapid apoptosis in cancer cell lines

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ABSTRACT - UPDATED

Background: Voreloxin (formerly SNS-595) is a replication-dependent agent that induces DNA damage, G2 arrest and apoptosis by selective intercalation of DNA and poisoning of topoisomerase II (Stockett et al., Hawtin et al., AACR 2008). Voreloxin is under clinical investigation in acute myeloid leukemia and ovarian cancer (Lancet et al., ASH 2007; McGuire et al., SGO 2008). Voreloxin is a naphthyridine analog, related to the quinolones, which have not previously been used for cancer treatment. To further define the mechanism of action, induction of DNA damage by voreloxin during different cell cycle phases was investigated. The role of DNA intercalation in the induction of DNA damage was studied using two voreloxin analogs, one with enhanced intercalative and cytotoxic activity, the other with no intercalative or cytotoxic activity (Stockett et al., 2008). The molecular events linking DNA damage with voreloxin-induced G2 arrest and apoptosis were also assessed. **Methods:** DNA damage and apoptosis in solid and hematologic cancer cell lines were monitored by γ H2AX foci formation and annexin V labeling along with PARP cleavage, respectively. DNA repair signaling was evaluated by western blot analysis. **Results:** Voreloxin induced dose-dependent DNA damage in S, G2 and M phases of the cell cycle, whereas G1 cells were markedly less sensitive to the drug. These data were consistent with the selectivity of voreloxin towards proliferating cells. No evidence of DNA damage was observed with the non-intercalative voreloxin analog, consistent with its absence of cytotoxicity. Induction of DNA damage in S-phase cells over the concentration range was biphasic: a dose-dependent increase was observed up to 10 μ M, at 20 μ M and beyond, reduced DNA damage was detected. Voreloxin-induced DNA damage activated ATM and ATR signaling, reflected by rapid and sustained phosphorylation of the checkpoint kinases CHK1 and CHK2. Phosphorylation of DNA-PKs was also observed. Activation of ATR signaling is consistent with the G2 arrest induced by voreloxin. At cytotoxic concentrations of voreloxin, apoptosis is induced as indicated by annexin V binding and PARP cleavage. **Conclusions:** The biphasic induction of DNA damage by voreloxin during replication is consistent with the well-characterized mechanism of action of the fluorquinolones towards bacterial gyrase (prokaryotic topoisomerase II) and intercalative topoisomerase II poisons. These data also establish the preferential cytotoxicity of voreloxin towards replicating cells (S, G2 and M phase) with cells in G1 being minimally affected.

VORELOXIN INDUCES DOSE-DEPENDENT DNA DAMAGE IN S PHASE AND M PHASE CELLS



VORELOXIN INDUCES A DOSE- AND TIME-DEPENDENT DNA DAMAGE RESPONSE THAT IDENTIFIES POTENTIAL RESPONSE MARKERS



Figure 7: DNA damage signaling and apoptosis in A549 lung carcinoma line

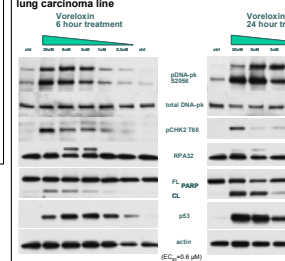


Figure 8: Voreloxin-induces pCHK1 in K562 chronic myelogenous leukemia line

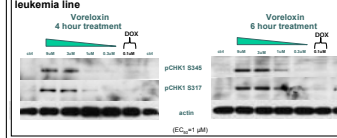
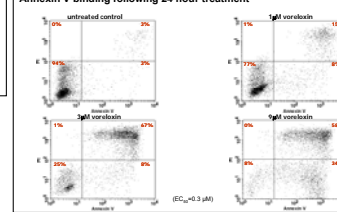
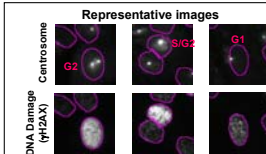
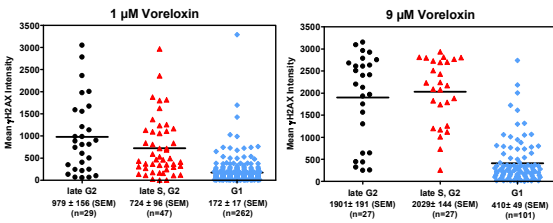


Figure 9: Voreloxin-induced apoptosis in HL-60 AML cells: Annexin V binding following 24 hour treatment



VORELOXIN INDUCES DNA DAMAGE IN S AND G2 PHASE WITH MINIMAL DAMAGE IN G1



Experimental Design
 6 h Voreloxin → Fix and stain MO59K glioma cells

At 1 μ M and 9 μ M (IC₅₀ and IC₉₀ for MO59K) voreloxin, preferential induction of DNA damage is observed in S and G2 phase cells, where minimal DNA damage is observed in non-replicating (G1) cells. Data are consistent with previous reports correlating voreloxin cytotoxicity with replication rate (Stockett et al., 2008).

Figure 4: Structure of voreloxin and analogs

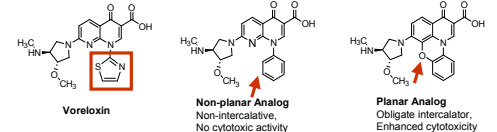


Figure 5: DNA damage in S phase cells induced by voreloxin, analogs, doxorubicin and etoposide

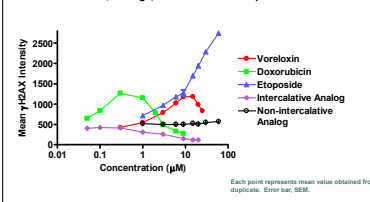
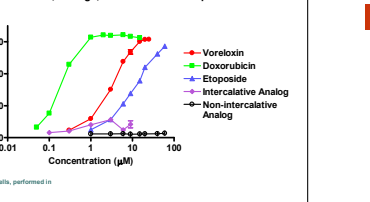


Figure 6: DNA damage in M phase cells induced by voreloxin, analogs, doxorubicin and etoposide



- Voreloxin and doxorubicin induce bi-phasic DNA damage in S phase, representative of intercalative topoisomerase II poisons. This contrasts with the non-intercalative topoisomerase II poison, etoposide.
- Voreloxin and doxorubicin reach a plateau of DNA damage in M phase.
- No evidence of DNA damage was observed with the non-intercalative voreloxin analog, consistent with its absence of cytotoxicity.
- Surprisingly, the more intercalative and cytotoxic planar fused phenyl analog of voreloxin induces minimal M phase DNA damage and low to no detectable S phase DNA damage.

SUMMARY & CONCLUSIONS

- Voreloxin preferentially induces DNA damage in replicating cells, with damage in M/G2 > S >> G1.
- The induction of DNA damage by voreloxin is consistent with its activity as a DNA intercalator and topoisomerase II poison / inhibitor, in contrast with the damage induced by the non-intercalative topoisomerase II poison, etoposide.
- The voreloxin-induced DNA-damage response indicates activation of ATR and ATM, consistent with induction of DNA damage in S and M phases of the cycle. These pathways identify potential pharmacodynamic response markers.
- Voreloxin's mechanism of action derives from its quinolone-like core, consisting of DNA-intercalation and novel inhibition of topoisomerase II that results in site-selective DNA damage and apoptosis.
- While its mechanism of action resembles doxorubicin, key features favorably differentiate voreloxin from the anthracyclines: (1) Voreloxin is not a P-gp substrate, thereby evading the most common tumor resistance mechanism; (2) Voreloxin is active in anthracycline-resistant settings; (3) Voreloxin activity is independent of p53 family proteins; (4) Voreloxin exhibits limited distribution to normal tissues relative to anthracyclines; (5) The chemically less reactive naphthyridine core produces minimal reactive oxygen species and has lower potential for cardiotoxicity than the anthracyclines.
- These data support the clinical investigation of voreloxin where the anthracyclines are broadly used, including the ongoing Phase 2 studies of voreloxin in AML and platinum-resistant ovarian cancer, and provide rationale for other tumors such as breast cancer.