Voreloxin is active in breast cancer biopsies and potency is enhanced in a BRCA2 mutant background

Voreloxin is a first-in-class anticancer quinoline derivate (AQD), and is currently in clinical trials in acute myeloid leukemia (AML) and platinum-resistant ovarian cancer. Clinical responses have been observed in these indications (Lavender et al., 2018; Lavender et al., 2016; McLaughlin et al., 2016). Voreloxin’s mechanism of action involves DNA intercalation and inhibition of topoisomerase II that induces site-selective DNA double-strand breaks (DSBs), S2 arrest and apoptosis. Potency was reported in primary patient biopsies from triple negative breast cancer, ovarian cancer and AML, including samples that are resistant to the topoisomerase II inhibitor doxorubicin, with activity independent of p53 family members (Lavender et al., 2018). Here we report that toxic DNA damage is generated at the replication fork, triggering homologous recombination repair (HRR) and that BRCA2 mutation increases sensitivity to voreloxin. We also show that the agent is active in breast cancer biopsies from patients with ductal or metastatic disease.

The influence of BRCA2 to sensitivity to voreloxin was evaluated by proliferation assays in cells mutant and complemented for Functional BRCA2 (V-C8 and V-B8). Activity in these assays was compared with doxorubicin. In cells mutant for BRCA2 as an approximately 5-fold increase in sensitivity was identified for voreloxin as compared to cells expressing functional BRCA2 (EC50 0.14 nM vs 0.72 nM). Doxorubicin sensitivity was increased approximately 4-fold (EC50 0.15 nM vs 0.19 nM). These studies were extended to the human sarcoma cell line U-2 OS, comparing wild-type cells to those depleted for BRCA2 using siRNA. The BRCA2 influence on sensitivity to voreloxin was evaluated by clonogenic survival and compared with doxorubicin. For both drugs a 4.6-fold increase in sensitivity was identified in the BRCA2-depleted line.

Voreloxin cytotoxicity toward 9 ductal and 8 metastatic primary breast cancer biopsies was determined using the English Drug Resistance (EDR) proliferation assay (OncoTher). Voreloxin was potent in samples that were resistant to the topoisomerase II inhibitors doxorubicin and/or doxorubicin, and was also active in cisplatin-resistant samples. Voreloxin at 1 µM (the plasma concentration sustained for approximately 24 h in Phase 2 clinical studies) inhibited proliferation >90% in 8/9 ductal biopsies, with none being resistant to the agent. Proliferation was also inhibited >90% in 4/8 metastatic samples with only one being resistant to 1 µM voreloxin.

In conclusion, voreloxin induces DNA DSBs that are repaired by HRR, and BRCA2 mutations sensitize cells to the agent. Voreloxin is active in breast cancer biopsies, including those resistant to other topoisomerase II inhibitors. Combined with potent activity in triple-negative breast cancer biopsies and the known mechanism of action of voreloxin, these data support expansion of the clinical evaluation of voreloxin to include breast cancer, in which other topoisomerase II inhibitors are active.

**SUMMARY AND CONCLUSIONS**

- Homologous Recombination Repair (HRR) plays a key role in repair of voreloxin-induced DNA damage.
- The HRR processes induced by voreloxin differ with cell cycle phase.
- Voreloxin-induced HRR-mediated long tract recombination events are independent of S phase.
- Doxorubicin-induced HRR-mediated long tract recombination appears to have an S phase component.
- Both voreloxin and doxorubicin induce replication stress during S phase (visualized as RAD51 foci).
- Voreloxin-induced cytotoxicity occurs both in S phase and independent of S phase.
- These data are consistent with previously reported data showing voreloxin-induced DNA damage (vorDNA) in G2/M (Lavender et al., 2018).
- Tumors with BRCA mutations may be particularly sensitive to voreloxin.
- BRCA2 functional loss-sensitizes cells to voreloxin.
- Voreloxin is active in ductal and metastatic breast cancer biopsies, as well as in triple negative breast cancer biopsies, a population reported to be up to 80% BRCA mutant.
- Breast cancers are identified as potential indications for clinical investigation.
- Voreloxin is currently being investigated for the treatment of acute myeloid leukemia and platinum-resistant ovarian cancer.

**REFERENCES**


**http://www.sunesis.com/science/presentations_and_publications.php**