

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

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## ABSTRACT

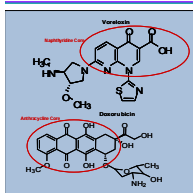
Voreloxin is a first-in-class anticancer quinolone derivative (AQD), and is currently in clinical trials in acute myeloid leukemia (AML) and platinum-resistant ovarian cancer. Clinical responses have been observed in these indications (Laroui et al., 2008; Ravandi et al., 2008; McGuire et al., 2008). Voreloxin's mechanism of action involves DNA intercalation and inhibition of topoisomerase II that induces site-selective DNA double-strand breaks (DSB), G2 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 (Hawtin et al., 2008). 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 on sensitivity to voreloxin was evaluated by proliferation assay in cells mutant and complemented for functional BRCA2 (V-C8 and V-C8-B2). Activity in these assays was compared with doxorubicin. In cells mutant for BRCA2 an approximately 5-fold increase in sensitivity was identified for voreloxin as compared to cells expressing functional BRCA2 (IC<sub>50</sub> 0.14 mM vs 0.72 mM). Doxorubicin sensitivity was increased approximately 4-fold (IC<sub>50</sub> 0.05 mM vs 0.19 mM). These studies were extended to the human sarcoma cell line U-2OS, 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 Extreme Drug Resistance (EDR) proliferation assay (Oncotech). Voreloxin was potent in samples that were resistant to the topoisomerase II inhibitors doxorubicin and / or etoposide, and was also active in cisplatin-resistant samples. Voreloxin at 1 μM (the plasma concentration sustained for approximately 24 hr 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.

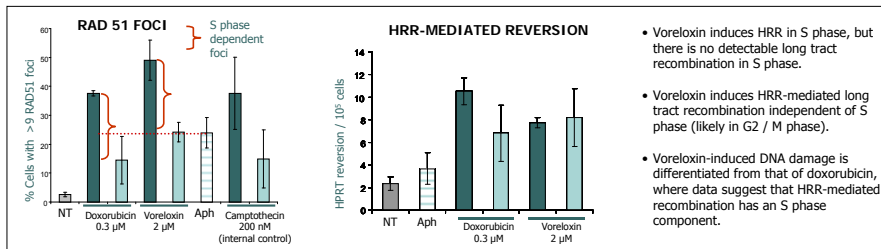
In conclusion, voreloxin induces DNA DSBs that are repaired by HRR, and BRCA2 mutations sensitize cells to the agent. Voreloxin is active in breast cancer biopsies, including those resistant to other topoisomerase II inhibitors. Combined with potent activity in triple-negative breast cancer biopsies 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.

## VORELOXIN HAS A VALIDATED MECHANISM OF ACTION WITH DISTINCT ADVANTAGES OVER ANTHRACYCLINES



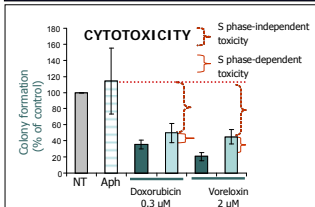
- Voreloxin: Novel topoisomerase II inhibitor and DNA intercalator
- Active in anthracycline-resistant settings
    - Not a P-glycoprotein substrate
    - Unaffected by p53, p63 or p73 status
    - Not a CYP450 inhibitor or inducer
    - Low potential for drug-drug interaction
    - Low potential for cardiotoxicity than anthracyclines
  - Anthracyclines generate substantial Reactive Oxygen Species (implicated in cardiotoxicity), unlike voreloxin

## VORELOXIN INDUCES TWO DISTINCT HOMOLOGOUS RECOMBINATION REPAIR (HRR) PROCESSES



- Voreloxin induces HRR in S phase, but there is no detectable long tract recombination in S phase.
- Voreloxin induces HRR-mediated long tract recombination independent of S phase (likely in G2 / M phase).
- Voreloxin-induced DNA damage is differentiated from that of doxorubicin, where data suggest that HRR-mediated recombination has an S phase component.

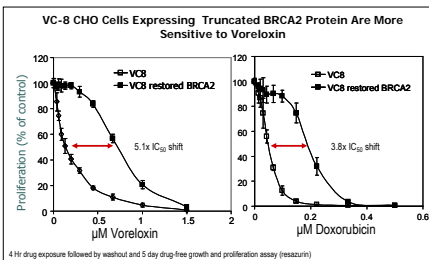
## VORELOXIN HAS BOTH S PHASE DEPENDENT AND INDEPENDENT CYTOTOXICITY



**Methodology: HRR induction and cytotoxicity were investigated in SPDB CHO cells.**

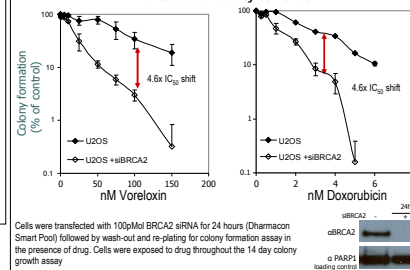
- SPDB cells contain a partial duplication of the HPRT gene that inactivates function. A functional gene is regenerated through long tract recombination mediated by HRR (Lundin et al., Mol Cell Biol (2002) 22, 3689-3693).
- Doxorubicin – a promiscuous DNA intercalator and topoisomerase II poison - was included as comparator.
- Aphidicolin was used to induce a block in early S phase.
- Equitoxic doses of voreloxin and doxorubicin were compared.
- Cytotoxicity was measured as colony growth inhibition.
- HRR was measured as (a) RAD51 focus formation (b) HPR1 reversion / recombination.

## BRCA2 DEFICIENT CELLS ARE MORE SENSITIVE TO VORELOXIN



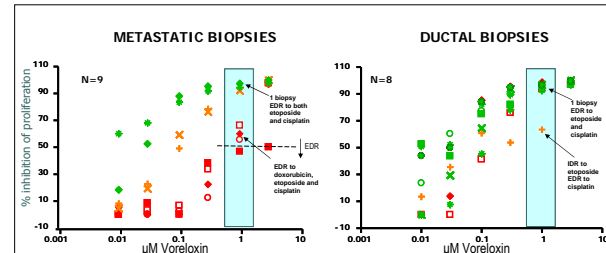
- Tumors with BRCA mutations may be particularly sensitive to voreloxin.

## BRCA2 Knockdown In U2OS Human Sarcoma Cells Increases Sensitivity To Voreloxin



Cells were transfected with 100pM Doxorubicin siRNA for 24 hours (Dharmacon Smart Pool) followed by wash-out and re-plating for colony formation assay in the presence of drug. Cells were exposed to drug throughout the 14 day colony growth assay.

## VORELOXIN IS CYTOTOXIC TOWARDS BREAST CANCER BIOPSIES



Relevant plasma concentrations for voreloxin and doxorubicin were based on clinical plasma concentration vs. time profiles.

- ≥1 μM and ≥0.1 μM plasma concentrations of voreloxin or doxorubicin, respectively, are sustained for >24 hrs in treated patients.

- 1 μM voreloxin inhibited >90%: 8/9 Ductal biopsies, 4/8 Metastatic biopsies.
- Only 1/17 biopsies was EDR (<50% inhibition) to 1 μM voreloxin.
  - This same biopsy was EDR to doxorubicin (0.1 μM) etoposide (8.5 μM) and cisplatin (1.67 μM).
- Voreloxin was cytotoxic in samples resistant to clinically relevant concentrations of doxorubicin, etoposide and cisplatin.

## SUMMARY AND CONCLUSIONS

- Homologous Recombination Repair (HRR) is required for 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 (γH2AX) in G2/M>S>>G1 (Wong et al., 2005).
- 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 (Laroui et al., 2008).
- 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.

Wong et al., 2008, EORTC/ACR Annual Meeting, Voreloxin (Dorame) (SUNES-595) is a potent DNA intercalator and topoisomerase II poison that induces cell cycle dependent DNA damage and rapid apoptosis in cancer cell lines.  
 Hawtin et al., 2008, AACR Annual Meeting, Ex Vivo Activity of SUNES-595 Against Biopsies of Acute Myeloid Leukemia, Triple Negative Breast and Ovarian Cancers Supports Ongoing and Potential Clinical Indications, #3030