

Clinical Evidence of Mechanism-Based Pharmacodynamic Activity in Voreloxin-Treated AML Patients

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ABSTRACT

Voreloxin is a first-in-class anticancer quinolone derivative that intercalates DNA and poisons topoisomerase II (Stockett et al. and Hawtin et al. AACR 2008). This leads to replication-dependent, site-selective DNA double-strand breaks (DSB) targeting G2-rich sequences that are characteristic sites of quinolone-induced DNA cleavage (Noble et al.; Richter et al. 2007; Stockett et al. AACR 2008). A consequence of this DNA damage is G2 arrest and cell death by apoptosis. Voreloxin is under clinical investigation in acute myeloid leukemia (AML) and ovarian cancer. Clinical responses have been observed in these indications (Lanctot et al. ASCO 2009; Ravandi et al. ASCO 2009; Hirte et al. ASCO 2009), as well as in NSCLC and SCLC (Burriss et al. ECCO 2007).

The current analysis was performed in support of the ongoing phase 1b/2 study clinical study (SPO-0012) of voreloxin in combination with cytarabine in relapsed or refractory AML. The purposes were to: (1) characterize voreloxin-induced biomarkers of mechanism-based pharmacodynamic (PD) activity in cell lines and peripheral blood mononuclear cells (PBMC) from AML patients; (2) differentiate between the cellular PD DNA damage responses to voreloxin and cytarabine; (3) profile patient PBMC before and following the combination treatment with voreloxin and cytarabine in SPO-0012, to evaluate PD markers of cellular response; (4) investigate potential correlations between cellular PD markers of DNA damage and clinical outcome.

Based on our understanding of voreloxin's mechanism of action, pharmacodynamic (PD) markers of S phase delay, G2 arrest and DNA damage following treatment with voreloxin were profiled in cell lines and then evaluated in primary AML cells. These included markers of stalled replication forks (pRPA-32, pCHK1) and DNA DSB (DNA-PKcs, p53, and pCHK2). Cellular response to cytarabine was also characterized in these in vitro model systems. Cytarabine is a mainstay of treatment in AML, administered for 7 days per treatment cycle in combination with daunorubicin on days 1-3 (the "7+3" treatment schedule). Cytarabine is incorporated into DNA and causes inhibition of DNA polymerase resulting in decreased DNA synthesis and repair. DNA damage responses were observed with cytarabine, but these occurred later than with voreloxin treatment.

Voreloxin induction of pDNA-PKcs and pCHK2 were chosen for PD assessments in the ongoing phase 1b/2 study of voreloxin in combination with cytarabine in relapsed or refractory AML. PBMC were purified from blood samples collected from patients at time points pre- and post-dosing. Upregulation of pDNA-PKcs and pCHK2 was detected within 2 hours post-dose, providing evidence of mechanism-based PD responses to voreloxin. Inconsistent quality of PBMC preparation at individual sites precluded correlation of PD response with clinical outcome.

Stockett D et al. SNS-595 is a potent anti-tumor agent that has a dual mechanism of action: DNA intercalation and site-selective topoisomerase II poisoning. Proceedings of the 30th Annual Meeting of the American Association for Cancer Research, San Diego, CA, 2009 Abstract 1950.
Hawtin RE et al. Sensitivity to SNS-595 is Related to Activation of Double Strand DNA Break Repair Pathways Including Homologous Recombination. Proceedings of the 30th Annual Meeting of the American Association for Cancer Research, San Diego, CA, 2009 Abstract 1959.
Hirte H et al. A phase II trial of SNS-595 in women with platinum resistant ovarian cancer. Proceedings, American Society of Clinical Oncology (ASCO) 2009 Annual Meeting Abstract 5552.

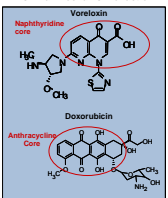
Burriss N et al. SNS-595: Preliminary Results of Two Phase 2 Second Line Studies in Lung Cancer. ECCO 14. European Cancer Conference, Barcelona, Spain 2007.
Noble CB, Baranov PM and Weaver J. Mechanism of DNA intercalation: sequence-dependent binding to single-stranded DNA reflects the interaction within the gyrated DNA complex. Anticancer Agents Chemother. 2003; 10(4):384-62.

Ravandi N et al. First cycle combination of fluoropyrimidine mediated DNA cleavage by G2-arrest and G-protein-type II DNA topoisomerases. Nucleic Acids Res. 2007; 35(18):6207-61.
Ravandi N et al. A phase II study of voreloxin as single agent therapy for elderly patients (SP) with newly diagnosed acute myeloid leukemia (AML). Proceedings, American Society of Clinical Oncology (ASCO) 2009 Annual Meeting Abstract 7548.

Lanctot J et al. Phase 1b pharmacokinetic/pharmacodynamic (PK/PD) study of combination voreloxin and cytarabine in relapsed or refractory AML patients. Proceedings, American Society of Clinical Oncology (ASCO) 2009 Annual Meeting Abstract 7050.

VORELOXIN IS AN ANTICANCER QUINOLONE DERIVATIVE (AQD)

Voreloxin is structurally unrelated to the anthracyclines, but has a similar mechanism of action



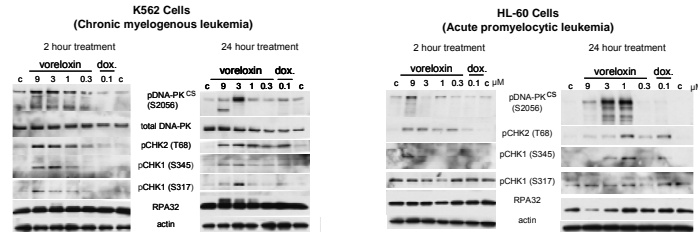
Voreloxin is a naphthyridine analog, closely related in structure to the quinolone antibiotic, but has a different mechanism of action.

- Voreloxin intercalates DNA and poisons topoisomerase II, causing site-selective DNA damage and apoptosis.
- Site selective DNA damage is analogous to the quinolones in bacterial DNA.
- Broad therapeutic index
- Evades common drug resistance pathways of P-gp mediated efflux and p53 mutation.
- Anthracyclines intercalate DNA and poison topoisomerase II, but are structurally unrelated to the quinolones.
- Voreloxin is active in anthracycline-resistant settings.
- Low potential for cardio/organ toxicity due to more chemically stable structure and minimal production of reactive oxygen species (ROS).
- Low risk of CYP450-mediated drug-drug interaction.

VORELOXIN INDUCES DOSE- AND TIME-DEPENDENT MECHANISM-BASED PHARMACODYNAMIC MARKERS OF RESPONSE

Voreloxin induces dose- and time-dependent markers of S phase delay and the DNA damage response in leukemic cell lines

Cells were treated with a dose-titration of voreloxin (0.3-9 μM), 0.1 μM doxorubicin or vehicle alone (C) and samples taken at 2 hours (left hand panels) and 24 hours (right hand panels) for analysis.

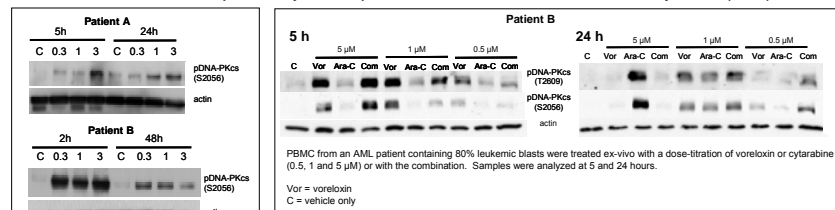


K562 are p53 mutant and HL-60 are p53 null. We have previously reported voreloxin-induced p53 expression in the A549 lung cancer cell line (Wong et al. EORTC 2008).

Protein marker	Represents	Voreloxin-induced effect?
pDNA-PK	DNA DSB / NHEJ	Early in K562 cells - detectable at 2hrs. Delayed in HL-60 cells, detectable at 24 hrs $\geq 1 \mu\text{M}$
pCHK2	DNA DSB / ATM activation	Early - detectable at 2hrs and lasting through 24hrs
pCHK1	Replication stress / ATR activation and HRR	Early in K562 cells - detectable at 2 hrs $>0.3 \mu\text{M}$ and lasting to 24 hrs. Delayed and weaker in HL-60 cells - detectable at 24 hrs
pRPA32 (reduced mobility shift)	Replication stress / Single-stranded DNA stretches at replication fork	Late in K562 cells - detectable at 24 hrs $\geq 1 \mu\text{M}$. Not detected in HL-60 cells

VORELOXIN-INDUCED PHARMACODYNAMIC MARKERS ARE DETECTABLE IN PRIMARY AML BLASTS

The mechanism-based pharmacodynamic response to voreloxin is differentiable from that to cytarabine (Ara-C)



PBMC from an AML patient containing 80% leukemic blasts were treated ex-vivo with a dose-titration of voreloxin or cytarabine (0.5, 1 and 5 μM) or with the combination. Samples were analyzed at 5 and 24 hours.

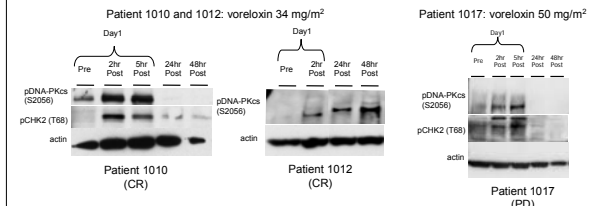
Vor = voreloxin
C = vehicle only

Detection of pDNA-PKcs

	Voreloxin	Cytarabine (Ara-C)
Time hr	2, 5, 24, 48 hours	24 hours
Concentration	$\geq 0.3 \mu\text{M}$	$\geq 1 \mu\text{M}$

VORELOXIN-INDUCED MECHANISM-BASED PHARMACODYNAMIC RESPONSE IN CLINICAL SAMPLES

Samples from patients enrolled in SPO-0012, a dose-escalation/expansion phase 1b/2 study of voreloxin plus cytarabine in relapsed/refractory AML.



Samples shown are from patients who received voreloxin d1, d4 + cytarabine CIV, d1-5, 400 mg/m²/day

- No PD response was detectable at voreloxin doses of 10 or 20 mg/m² (N=6).
- The DDR response was detected in 15 of 23 samples (65%) analyzed from patients who received voreloxin doses of $\geq 34 \text{ mg/m}^2$.
 - The PD response was observed both in Schedule A patients who received cytarabine CIV, d1-5, 400 mg/m², and in Schedule B patients who received bolus cytarabine, delivered as a 2 hr IV infusion 1g/m²/day, d1-5.
- Because of the low number of patient samples and variable PBMC sample quality, no correlations between PD and clinical response are feasible.

SUMMARY AND CONCLUSIONS

- Voreloxin is a first-in-class anticancer quinolone derivative with Phase 2 clinical proof-of-concept in AML and ovarian cancer.
- Data reported here demonstrate mechanism-based pharmacodynamic activity of voreloxin in patients from the ongoing clinical trial of voreloxin in combination with cytarabine in relapsed/refractory AML.
 - pDNA-PKcs and pCHK2 are identified as mechanism-based pharmacodynamic markers of response to voreloxin that translate from in vitro studies in cancer cell lines to voreloxin-treated patient PBMC
 - The DNA damage response to voreloxin is differentiable from that to cytarabine.
- The DNA damage response was activated in PBMC from AML patients who received voreloxin doses $\geq 34 \text{ mg/m}^2$.
 - These data establish clinical proof-of-mechanism, but based upon patient PBMC sample number and quality, correlation with clinical outcome is not feasible.
- Clinical development of voreloxin continues with ongoing studies in AML and platinum-resistant ovarian cancer.

Clinical development updates will be presented at the 2009 Annual Meeting of the American Society of Hematology (ASH):

- Phase 1b PK/PD Study of Combination Voreloxin and Cytarabine in Relapsed or Refractory AML Patients. Abstract #635, oral presentation, Monday Dec 7, 4:30PM - 6PM session time, presentation 5:30PM
- Phase 2 Dose Regimen Optimization Study of Voreloxin as Single Agent Therapy for Frontline, Elderly AML. Abstract # 1037, poster presentation, Saturday December 5, 9AM - 7:30PM viewing, 5:30-7:30PM presentation.