

Voreloxin is Synergistic in In Vitro Combination with Cytarabine and Additive in Combination with Azacitidine, Decitabine and Clofarabine

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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 G/C-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 (Lancet et al. ASCO 2009; Ravandi et al. ASCO 2009; Hirte et al. ASCO 2009), as well as in NSCLC and SCLC (Burris et al. ECCO 2007).

The current analysis was performed in support of a Phase 1b/2 clinical study (SPO-012) of voreloxin in combination with cytarabine in relapsed or refractory AML, and to investigate the feasibility for combining voreloxin with other agents currently in clinical use for the treatment of AML. The cytotoxicity of voreloxin in combination with cytarabine was evaluated in 3 acute leukemic cell lines (HL-60 acute promyelocytic (APL), MV4-11 Fil-3 1T3 positive AML, and CCRF-CEM acute lymphoblastic leukemia (ALL)). The cytotoxicity of voreloxin in combination with the nucleoside analog hypomethylating agents azacitidine, decitabine (pyrimidine analogs), and clofarabine (purine analog) was also studied in the HL-60 APL cell line. Cytotoxicity was assessed by proliferation inhibition.

The combined activity of voreloxin and cytarabine was evaluated using the combination index (CI) analysis method, in which each drug is serially diluted based on either 10X or 1X single agent IC₅₀ values. The combination was synergistic in the AML cell lines MV4-11 and HL-60, as demonstrated by a leftward shift in the voreloxin IC₅₀ curves and calculated CI less than 0.85. In the ALL cell line CCRF-CEM, an additive increase in cytotoxicity was observed with CI of ≤ 0.99 .

Voreloxin in combination with azacitidine, decitabine or clofarabine was evaluated after simultaneous or sequential addition of the drugs. The combinations were analyzed by: 1) serial-dilution of voreloxin combined with a fixed dose (IC₅₀) of the second compound, and 2) fixed dose (IC₅₀) of voreloxin combined with a serial dilution of the second compound. No significant change in activity was observed when compared with the single-agent activity of each compound. Sequential dosing of the agents did not alter the cytotoxicity of the reagents in combination.

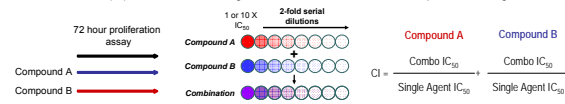
These data support the ongoing phase 1b/2 clinical study of voreloxin in combination with cytarabine in relapsed or refractory AML, and demonstrate the feasibility for combining voreloxin with other DNA damaging agents currently in clinical use for the treatment of AML.

Stockett D et al. SNS-555 is a potent anti-tumor agent that has a dual mechanism of action: DNA intercalation and site-selective topoisomerase II poisoning. Proceedings of the 99th Annual Meeting of the American Association for Cancer Research, San Diego, CA 2008 Abstract 1880.
Hawtin RE et al. Sunesis Pharmaceuticals: Activation of Double-Strand DNA Break Repair Pathways Including Homologous Recombination. Proceedings of the 99th Annual Meeting of the American Association for Cancer Research, San Diego, CA 2008 Abstract 1885.
Noble M et al. Apyrase Inhibits Topoisomerase II in Human and Mouse Cells. Proceedings, American Society of Clinical Oncology (ASCO) 2009 Annual Meeting Abstract 5582.
Borke M et al. SNS-595: Preliminary Results of Two Phase 2 Second Line Studies in Lung Cancer. ESMO 14: European Cancer Conference, Barcelona, Spain 2007.
Noble CG, Borke FM and Maxwell A. Quinolone DNA intercalation: sequence-dependent binding to single-stranded DNA affects the interaction within the gyrase-DNA complex. Anticancer Agents Chemistry. 2003 Mar 4(13):654-662.
Richter SM et al. Filo-spiro consensus of fluoropyrimidine-mediated DNA cleavage by Gram-negative and Gram-positive type II DNA topoisomerases. Nucleic Acids Res. 2007;35(18):6778-6787.
Ravandi F et al. A Phase II study of voreloxin as single agent therapy for elderly patients with newly diagnosed acute myeloid leukemia (AML). Proceedings, American Society of Clinical Oncology (ASCO) 2009 Annual Meeting Abstract 7048.
Lancet J et al. Phase IIIb 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 7055.

VORELOXIN COMBINED WITH CYTARABINE HAS ADDITIVE OR SYNERGISTIC ACTIVITY

METHOD

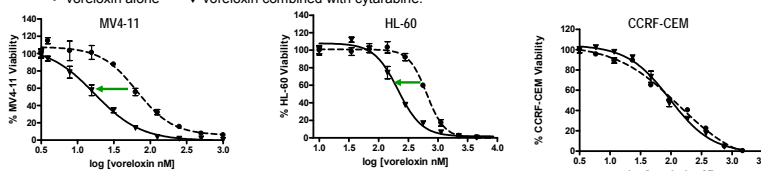
Combination index (CI) was established using combined serial dilution of both compounds, starting at either 10x (High) or 1x (Low) IC₅₀ for each agent



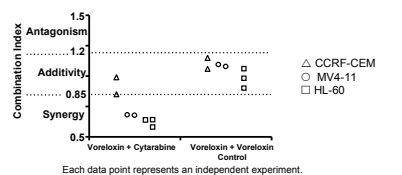
	CI
Synergy	<0.85
Additivity	0.85-1.2
Antagonism	>1.2

RESULTS

Percent viability of cells treated with:
 • voreloxin alone
 ▼ voreloxin combined with cytarabine.



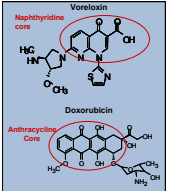
COMBINATION INDEX (CI) ANALYSIS



	Voreloxin + cytarabine activity
CCRF-CEM	Additive
MV4-11	Synergistic
HL-60	Synergistic

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 antibacterials.

- 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.

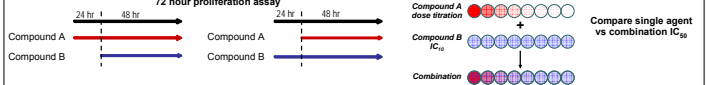
VORELOXIN COMBINED WITH AZACITIDINE, DECITABINE OR CLOFARABINE HAS ADDITIVE ACTIVITY

HL-60 cells were treated with compounds and CI indices calculated as described above. No change in activity above single agent was observed, consistent with additive activity. Voreloxin combined with itself was used as control.

Voreloxin	Azacitidine	Average CI (n=4)	Voreloxin	Decitabine	Average CI (n=4)	Voreloxin	Clofarabine	Average CI (n=4)
High	High	1.2 ± 0.3	High	High	0.8 ± 0.2	High	High	1.1 ± 0.2
High	Low	1.2 ± 0.3	High	Low	1.0 ± 0.2	High	Low	1.0 ± 0.3
Low	High	1.1 ± 0.2	Low	High	1.0 ± 0.4	Low	High	1.0 ± 0.2

ADDITIVITY RETAINED ON SEQUENTIAL COMBINATION OF VORELOXIN WITH NUCLEOSIDE ANALOGS

METHOD



RESULTS AND DATA ANALYSIS

24-hour pre-treatment with voreloxin, dose-titration or IC₁₀
 24-hour pre-treatment with azacitidine, decitabine, or clofarabine dose-titration or IC₁₀



	Combination IC ₅₀ Values (µM)			
	DMSO	Azacitidine IC ₁₀	Decitabine IC ₁₀	Clofarabine IC ₁₀
Voreloxin	0.15 ± 0.01	0.18 ± 0.04	0.16 ± 0.02	0.15 ± 0.01

	Combination IC ₁₀ Values (µM)			
	DMSO	Azacitidine IC ₁₀	Decitabine IC ₁₀	Clofarabine IC ₁₀
Voreloxin	0.51 ± 0.04	0.41 ± 0.05	0.41 ± 0.01	0.48 ± 0.08

	Combination IC ₅₀ Values (µM)	
	DMSO	IC ₁₀ Voreloxin
Azacitidine	0.53 ± 0.06	0.52 ± 0.06
Decitabine	0.81 ± 0.58	0.41 ± 0.19
Clofarabine	0.12 ± 0.01	0.14 ± 0.01

	Combination IC ₅₀ Values (µM)	
	DMSO	IC ₁₀ Voreloxin
Azacitidine	0.84 ± 0.07	0.83 ± 0.09
Decitabine	0.31 ± 0.11	0.22 ± 0.07
Clofarabine	0.1 ± 0.002	0.1 ± 0.002

SUMMARY AND CONCLUSIONS

- Voreloxin is a first-in-class anticancer quinolone derivative with Phase 2 clinical proof-of-concept in AML and ovarian cancer.
- Voreloxin combined simultaneously with cytarabine is synergistic or additive in acute leukemia cell lines.
 - These data support the ongoing clinical study of voreloxin in combination with cytarabine in relapsed/refractory AML.
- These data support the feasibility for further investigation of voreloxin in combination with other DNA damaging agents that are currently in use for the treatment of AML.
 - Voreloxin activity combined with nucleoside analogs azacitidine, decitabine or clofarabine is additive in the HL-60 acute promyelocytic cell line.
 - Sequential combination of voreloxin with nucleoside analogs retained additivity.
- 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). Abstract: Phase 1b/2 PK/PD Study of Combination Voreloxin and Cytarabine in Relapsed or Refractory AML. Poster #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.
http://www.sunesis.com/science/presentations_and_publications