SNS-595 Cancer Therapeutic
Novel DNA Damage Agent

NYAS Symposium:
Pharmacologic Regulation of DNA Damage
Checkpoints to Treat Cancer

March 13th 2008

Rachael E. Hawtin, PhD
Translational Science Enables Hypothesis-Driven Drug Development

- Translational studies support interrogation throughout development continuum
  
  - **Preclinical studies** position therapeutic for early and informed Go/No Go decisions
  
  - **Correlative studies** look for evidence of pharmacodynamic activity and potential for patient stratification
  
  - **Real-time communication** between clinical and nonclinical sciences allows for iterative evolution of program strategy
  
  - **Feedback to Research** allows clinical insights to drive creative approaches to novel therapeutics
SNS-595 in Clinical Development for Acute Leukemias and Advanced Ovarian Cancer

- Clinical activity in AML, ovarian and other solid tumors
- Effective in anthracycline-resistant tumors
- Activity compares favorably to Phase 1 results of other compounds studied in relapsed/refractory AML patients
SNS-595 Mechanism Derives From Structure

- Poisons human Topo II
- Saturable, selective DNA intercalation
- Site specific DNA cleavage
- Limited to no antibacterial activity

SNS-595

- Poisons bacterial Topo II (DNA gyrase)
- Saturable, selective DNA intercalation
- Site specific DNA cleavage
- Limited to no anticancer activity
- Antibacterial drugs in use for >50 years

Ciprofloxacin

- SNS-595 targets the eukaryotic equivalent of bacterial DNA gyrase
SNS-595 Mechanism of Action

- Favorable profile over known topo II inhibitors
  - Not a P-gp substrate
  - Unaffected by p53 status
  - Active in anthracycline-resistant settings
  - Lower potential for cardiotoxicity than anthracyclines
SNS-595 Avoids Scaffold-based Toxicities Of Anthracyclines

**Naphthyridinone Core - Chemically Stable**

SNS-595

- Poisons human Topo II
- Site-specific DNA intercalation
- DNA cleavage site specific
- DNA binding saturable

**Anthracycline Core – Redox Reactive**

Doxorubicin

- Poisons human Topo II
- Non-specifically intercalates DNA
- DNA cleavage is not site specific
- DNA binding not saturable

• **SNS-595 is structurally unrelated to anthracyclines**
SNS-595 Causes Irreversible Cell Cycle Arrest and Rapid Apoptosis

Site-Selective DNA Intercalation

Topo II Poison

- ATR activation
- S phase delay

Selective DSB DNA Damage

- HRR activation
- pDNA-PK induction
- Irreversible G2 Arrest

Apoptosis
SNS-595 Intercalates DNA

- SNS-595: DNA Kd < 5 µM in an in vitro biochemical assay

Topo I intercalation assay

Neil Osheroff and Jo Ann Byl
SNS-595 Activity Requires DNA Interaction

A549 cell line, proliferation assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 μM</th>
<th>Potency Relative to SNS-595</th>
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<tbody>
<tr>
<td>SNS-595</td>
<td>0.11</td>
<td>1</td>
</tr>
<tr>
<td>SNS-595 phenyl</td>
<td>&gt;10</td>
<td>&gt;100X decrease</td>
</tr>
<tr>
<td>Non-planar – limited DNA interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNS-595 fixed ring</td>
<td>0.02</td>
<td>5.5X increase</td>
</tr>
<tr>
<td>Planar – productive DNA interaction</td>
<td></td>
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</table>
Topoisomerase II: Well Validated Oncology Target

**Therapeutic Agents “Poison” The Enzyme**

Stabilized Topo II/DNA cleavable complex

DNA → "poison" → increased DNA breaks

Topo II

- Required for correct chromosome condensation and segregation
- Alters DNA topology to relieve torsional strain during replication
  - Transient cleavable complex with DNA that causes DSB
  - DSB normally religated after breakup of cleavable complex
  - Activity peaks at G2/M phase
SNS-595 Poisons Topoisomerase II

SNS-595 treated vs. untreated cells

Isolate DNA

- DNA + or - topo II
- free topo II

Collect DNA fraction

Cellular Topo II:DNA Cleavage Complexes Stabilized

- TIIα
  - No Drug
  - 1µM SNS-595
  - 1 µM Etop
  - 10 µM Etop

- TIIβ

- Unable to religate double strand DNA breaks
- May increase forward-rate of DNA cleavage

Neil Osheroff and Jo Ann Byl
Topo IIα siRNA Knockdown Inhibits SNS-595 Mediated G2 Arrest

<table>
<thead>
<tr>
<th>Compound</th>
<th>DMSO</th>
<th>0.001uM</th>
<th>0.004uM</th>
<th>0.012uM</th>
<th>0.037uM</th>
<th>0.11uM</th>
<th>0.33uM</th>
<th>1uM</th>
<th>3uM</th>
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<td>Topo IIα KD</td>
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Appearance of G2 arrest
Topo II α siRNA Knockdown Inhibits SNS-595 Mediated G2 Arrest

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<tr>
<td>Topo II α</td>
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| SNS-595 µM     |        |         |         |         |         |        |        |     |     |
| Doxorubicin µM |        |         |         |         |         |        |        |     |     |
| Etoposide µM   |        |         |         |         |         |        |        |     |     |
| Topo II α      | 0.037  | 0.004   | 0.11    |         |         |        |        |     |     |
| competent      |        |         |         |         |         |        |        |     |     |
| Topo II α      | 0.33   | 0.037   | 3       |         |         |        |        |     |     |
| knockdown      |        |         |         |         |         |        |        |     |     |
| Fold-decrease  | 9      | 9       | 27      |         |         |        |        |     |     |
| in sensitivity |        |         |         |         |         |        |        |     |     |
SNS-595 Causes Irreversible Cell Cycle Arrest and Rapid Apoptosis

Site-Selective DNA Intercalation

Topo II Poison

- ATR activation
- S phase delay

Selective DSB DNA Damage

- HRR activation
- pDNA-PK induction
- Irreversible G2 Arrest

Apoptosis
SNS-595 Induces Cellular DNA Damage
PFGE Demonstrates Dose-Dependent dsDNA Breaks

CCRF-CEM Human Leukemic cell line
NCI GI₅₀: 0.15-0.44µM SNS-595
6hr Drug Treatment
SNS-595 Induces Site-Selective DNA Damage

- **In Vitro Site-Selective DNA Cleavage**
  - Plasmid + Topo IIα
  - [SNS-595] (µM): 0, 0.1, 0.25, 0.5, 1.0, 5, 10
  - Etoposide

- DNA

- **G/C site cleavage preference is characteristic of quinolones**

- 5' ...CCTCTTGC GGGA TATCCTCC...3'
- 3' ...GGAGAACG CCCT ATAGGAGG...5'

Neil Osheroff and Jo Ann Byl
SNS-595 Induces Dose-Dependent DNA Damage in S-phase

**Experimental Design**

- **1 h BrdU**
- **4 h 595**
- Fix and stain A549 cells
SNS-595 Causes More Extensive DNA Damage in Mitosis

Experimental Design:

0 h Nocodazole 4 h 595
Enrich mitotic cells Fix and stain A549 cells
21 h

M-Phase (pHH3) DNA damage (γH2AX)

Noc only

1 µM SNS-595

10 µM SNS-595

Damaged cells

Mitotic cells

Normal cells

Experimental Design:

0 h Nocodazole 4 h 595
Enrich mitotic cells Fix and stain A549 cells
21 h

M-Phase (pHH3) DNA damage (γH2AX)

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Noc only

1 µM SNS-595

10 µM SNS-595

Damaged cells

Mitotic cells

Normal cells

SNS-595 Causes More Extensive DNA Damage in Mitosis

DNA Damage γH2AX Intensity

Mitotic cells

Non-mitotic cells

Damaged cells

Mitotic cells

Mitotic cells

Mitotic cells

Mitotic cells
SNS-595 Induced DNA Damage Is Preferential For Replicating Cells

Correlation of DNA damage detection and cell cycle phase

M059K + SNS-595 for 6 h.
Cell Cycle phase based on centrosome number and size as determined by pericentrin staining.
SNS-595 Causes Irreversible Cell Cycle Arrest and Rapid Apoptosis

Site-Selective DNA Intercalation

Topo II Poison

- ATR activation
- S phase delay

Selective DSB DNA Damage

- HRR activation
- pDNA-PK induction
- Irreversible G2 Arrest

Apoptosis
SNS-595 Activity is Independent of p53 Status

MTT proliferation assay in HCT-116 colon cancer line

- p53 WT IC$_{50}$ 765nM
- p53 null IC$_{50}$ 877nM

Stable p53 knockdown using shRNA

HCT116-shGFP  HCT116-shp53

% activity

µM SNS-595
SNS-595 is Active In Breast Cancer Biopsies and is Independent of p63 and p73

- Activity does not require p63 or p73 (p53 family members)
- SNS-595 active against doxorubicin-resistant tumors

**Extreme Drug Resistance proliferation assay (Oncotech)**

- Blue symbols represent p63 and p73 negative samples
- Triple negative (ER-/PR-/Her2-) breast cancer biopsies
- Clinically relevant doses: 1µM SNS-595, 0.1µM doxorubicin
SNS-595 Induces DNA Damage Response Markers

- pDNA-PK is an early marker of DSB DNA repair response
- pChk1 activation indicates ATR activation
SNS-595 Induces DNA Damage Response Markers in Primary AML cells

Data inform ongoing PD analysis of PBMC from patients in Phase I study of SNS-595 in AML
Double Strand DNA Damage Repair Mechanisms Investigated

NHEJ
Includes DNA-PK

HRR

Rad51 is loaded onto DNA by BRCA2

HRR requires a multiprotein complex that includes Rad51

Tool: CHO cells mutant for HRR- Matched Rad51D +/+ vs -/-

(Daboussi et al., 2002)
Repair of SNS-595 Induced Double Strand DNA Breaks by DNA-PK is Minimal

72 hour proliferation assay

DNA-PK negative
MO59J SNS-595 $IC_{50}$ 1.6µM

DNA-PK positive
MO59K SNS-595 $IC_{50}$ 1.0µM

MO59J Are DNA-PK Null

DNA-PK

β-Actin
SNS-595 Induced DNA Damage Repair Utilizes Homologous Recombination

![Graph showing EC50 values for SNS-595 in CHO cells with/without Rad51.]

<table>
<thead>
<tr>
<th></th>
<th>HRR+ EC50 µM</th>
<th>HRR- EC50 µM</th>
<th>Fold-Change</th>
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<tbody>
<tr>
<td>SNS-595</td>
<td>0.114</td>
<td>0.005</td>
<td>23</td>
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</table>
SNS-595 Overwhelms DNA Damage Repair

1μM SNS-595
- 10X cellular IC₅₀
- Clinically relevant concentration
**SNS-595 Mechanism Suggests Potential Clinical Directions**

<table>
<thead>
<tr>
<th></th>
<th>SNS-595</th>
<th>Doxorubicin</th>
<th>Etoposide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug Class</strong></td>
<td>Naphthyridinone</td>
<td>Anthracycline</td>
<td>Epipodophyllotoxin</td>
</tr>
<tr>
<td><strong>Common Clinical App.</strong></td>
<td>-</td>
<td>Broadly used solid and hematologic cancers</td>
<td>SCLC, NSCLC, Lymphomas</td>
</tr>
<tr>
<td><strong>Primary Mechanism</strong></td>
<td>Site-Selective DNA Intercalation Topo II poison</td>
<td>Non-specific DNA Intercalation Topo II poison</td>
<td>Topo II poison</td>
</tr>
<tr>
<td><strong>DNA damage</strong></td>
<td>Selective double strand breaks</td>
<td>Single and double strand breaks</td>
<td>Single and double strand breaks</td>
</tr>
<tr>
<td><strong>P53 influence</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>P-glycoprotein substrate</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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- SNS-595 may evade common resistance mechanisms and toxicities of anthracyclines and etoposide
Preclinical Activity of SNS-595  Superior to Commonly Used  Anti-Cancer Agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Common Xenografts</th>
<th>Drug-Resistant</th>
<th>Syngeneic</th>
<th>Overall Activity*</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>SNS-595</td>
<td>8/10</td>
<td>3/3</td>
<td>3/3</td>
<td>14/16</td>
<td>87</td>
</tr>
<tr>
<td>Etoposide</td>
<td>0/10</td>
<td>0/3</td>
<td>0/2</td>
<td>0/15</td>
<td>0</td>
</tr>
<tr>
<td>CDDP</td>
<td>1/10</td>
<td>0/2</td>
<td>1/3</td>
<td>2/14</td>
<td>14</td>
</tr>
<tr>
<td>Taxol</td>
<td>7/8</td>
<td>0/3</td>
<td>0/3</td>
<td>0/6</td>
<td>50</td>
</tr>
<tr>
<td>CPT-11</td>
<td>7/10</td>
<td>0/2</td>
<td>0/3</td>
<td>0/5</td>
<td>47</td>
</tr>
<tr>
<td>Dox</td>
<td>2/10</td>
<td>0/3</td>
<td>1/3</td>
<td>1/6</td>
<td>19</td>
</tr>
</tbody>
</table>

- Resistant models include high expression of P-gp and reduced expression of Topo II

* Cut-off for activity is 70% tumor growth inhibition
Translational Science Enables Hypothesis-Driven Drug Development

- Translational studies support interrogation throughout development continuum

  - **Preclinical studies** position therapeutic for early and informed Go/No Go decisions

  - **Correlative studies** look for evidence of pharmacodynamic activity and potential for patient stratification

  - **Real-time communication** between clinical and nonclinical sciences allows for iterative evolution of program strategy

  - **Feedback to Research** allows clinical insights to drive creative approaches to novel therapeutics
SNS-595 Phase I AML: Responses Seen in Refractory Patients

Broad Therapeutic Window With Significant Biologic Activity

<table>
<thead>
<tr>
<th>Weekly</th>
<th>Dose (mg/m²)</th>
<th>18-38</th>
<th>50</th>
<th>60</th>
<th>72 MTD</th>
<th>90</th>
</tr>
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<tbody>
<tr>
<td>Patient (n)</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>CR,CRp,CRi</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Blasts &lt;5%</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
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</table>

- 13/30 (43%) patients experienced anti-leukemic activity at or above 50 mg/m²
- All 5 CR patients relapsed from or failed “7+3”
- DLT was oral mucositis
- Generally well-tolerated

Activity compares favorably to Phase 1 results of other compounds studied in relapsed/refractory patients
Activity Correlated with Time Above Threshold Concentration

Time above 1 µM SNS-595 - in vitro IC90

ASH 2007
Ongoing SNS-595 Phase 2: Emerging Evidence in Platinum Resistant Ovarian Cancer

- 1CR, 4PR (2 unconfirmed), 31/35 stable disease or better
- Activity observed in patients who have failed doxil
SNS-595 Causes Irreversible Cell Cycle Arrest and Rapid Apoptosis

Site-Selective DNA Intercalation

Topo II Poison

---

ATR activation
S phase delay

---

Selective DSB DNA Damage

---

HRR activation
pDNA-PK induction
Irreversible G2 Arrest

---

Apoptosis
SNS-595 Novel Topoisomerase II Poison: Mechanistic Summary

• SNS-595 mechanism parallels in humans that of quinolones on bacterial DNA gyrase

• SNS-595 mechanism directs clinical focus to indications where topo II poisons have demonstrated success

• SNS-595 naphthyridinone core confers advantages over other Topo II poisons

• SNS-595 evades common drug resistance caused by P-glycoprotein and p53 mutations

• Cancers with BRCA mutations (HRR impaired) may be sensitized to SNS-595
SNS-595 Novel Topoisomerase II Poison

- SNS-595 is a naphthyridinone analog that is active as a cancer therapeutic
  - Related to quinolones (ciprofloxacin)

- Objective Responses observed with SNS-595 in AML and ovarian cancers, as well as in SCLC and NSCLC
Acknowledgements

- Sunesis SNS-595 project team
  - Judy Fox
  - Bob McDowell

- Data shown in this presentation
  - Andy Conroy
  - Ute Hoch
  - Glenn Michelson
  - Jeff Silverman
  - Dave Stockett
  - Matt Suster
  - Nguyen Tan
  - Oi Kwan Wong
  - Wenjin Yang
  - Don Young

- Topo II studies at Vanderbilt University
  - Jo Ann Byl
  - Neil Osheroff

- Primary patient biopsy studies at Oncotech

- Patients on clinical trials
- Clinicians, clinical investigators and research staff
<table>
<thead>
<tr>
<th>Translational Activity</th>
<th>Impact</th>
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<tbody>
<tr>
<td>Understand role of target in disease process</td>
<td>• Sets bar for clinical activity</td>
</tr>
<tr>
<td></td>
<td>• May allow patient selection</td>
</tr>
<tr>
<td>Establish preclinical Proof of Concept</td>
<td>• Risk reduction</td>
</tr>
<tr>
<td>Understand drug’s mechanism of action</td>
<td>• <strong>Indication selection and possible patient stratification</strong></td>
</tr>
<tr>
<td></td>
<td>• Translate into preclinical PD</td>
</tr>
<tr>
<td></td>
<td>• Develop biomarkers for clinical correlative studies</td>
</tr>
<tr>
<td></td>
<td>• Rational selection of combination agents</td>
</tr>
<tr>
<td>Develop PK/PD relationship in in vivo animal models</td>
<td>• Informs clinical dose regimen</td>
</tr>
<tr>
<td>Select candidate with ADMET consistent across preclinical species</td>
<td>• PK/PD/outcome relationships more readily translates to clinic.</td>
</tr>
<tr>
<td>Indication selection based on therapeutic hypothesis and mechanistic understanding</td>
<td>• Drive earlier No Go decisions</td>
</tr>
<tr>
<td></td>
<td>• Improve PTS</td>
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