SMAC Mimetics as new Anticancer Agents

Alex A. Adjei,
Roswell Park Cancer Institute
Buffalo, NY
USA
Overview of Signal Transduction Pathways
Centrality of mitochondria to cancer cell function and survival

Kroemer and Pouyssegur, Cancer Cell 2008
Inhibitors of Apoptotic Proteins (IAPs)

- IAPs inhibit the apoptotic cascade by
  - Blocking caspase activation
  - Promoting proteosomal degradation of caspases
- 8 IAPs (XIAP, cIAP-1, cIAP-2, IAP-2, ML-IAP, NAIP, Survivin, Apollon) described
- IAP expression correlated to chemoresistance and tumor aggressiveness
- Rational Targets for Cancer Therapy
SMAC – Second Mitochondrial-derived Activator of Caspases
SMAC Overview

• Smac (second mitochondrial-derived activator of caspases)
  – Released from mitochondria upon receipt of cell death stimulus
  – Interacts with multiple Inhibitor of Apoptosis Proteins (IAPs):
    • Relieves their inhibitory effect on caspases
    • Inhibits NF-kB activation affecting tumor growth, survival and metastases

• Smac mimetic drugs are conceptually unique
  – Relevant to treating all types of cancer by targeting fundamental mechanisms of cancer cell survival and resistance
  – Target survival and resistance proteins that are downstream from other cancer therapy targets enabling synergy with many other therapies
SMAC Mimetics are IAP Antagonists

Inhibitors of Apoptosis

SM-164

(IC$_{50}$ < 1nM)
SMAC Mimetics

Activating Apoptosis Downstream of Most Cancer Therapies

- Rationally-designed therapeutics that target fundamental anti-apoptotic mechanisms of tumor cell survival and resistance to apoptosis.

- Apoptosis is the main mechanism through which innate responses and therapies destroy cancer cells.

- SMAC mimetics act alone or with other therapies to overcome pivotal cancer resistance mechanisms.
All Smac Mimetics are Based on the Structure of the N-terminal Tetrapeptide of Native Smac
Smac - Mechanism of Action

- **Cytotoxic Effectors (Chemo/Targeted/XRT)**
- **Extrinsic Pathway**
- **Intrinsic Pathway**

**NF-κB**
- **Canonical** Pathway
- **Non-Canonical** Pathway

**Smac**
- **cIAP1**
- **XIAP**

**BAFF**
- **TNFα**
- **TRAIL**

**Cell Survival**
- **Immune Activation**

**CASPASES 3/7**
- **Caspase 9**
- **Caspase 8, 10**
SMAC Mimetics Mimic the Activity of Smac – the Endogenous IAP Inhibitor

- TNFα
- TNFR1
- cIAP-1
- XIAP
- TRAIL
- DR4/DR5
- Complex I
- Complex II
- CASPASE 8 and 10
- proCASPASE 8 and 10
- cFLIP
- Cyto c
- Type I
- Type II
- tBID
- Canonical NF-κB Activation
- TL32711
- CASPASE-9
- CASPASE-3,7
- APOPTOSIS
- CELL SURVIVAL
- CASPASE-8 and 10
- CASPASE-3,7
Effect of SMAC Mimetics on NFκB Pathways

**Canonical Pathway**

- **Rapid inhibition of canonical pathway**

**Non-canonical Pathway**

- **Slow activation of non-canonical pathway**

### SMAC Mimetic

- **Inflammation**
  - cIAP1 ubiquitylates RIP1 to maintain activation.
  - TL32711 removes cIAP1.

- **Lymphoid Development**
  - cIAP1 ubiquitylates NIK leading to degradation.
  - TL32711 removes cIAP1 leading to NIK upregulation.
# SMAC Mimetics in Clinical Trials

<table>
<thead>
<tr>
<th>Company</th>
<th>SMC</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegera / Human Genome Sciences</td>
<td>AEG40826/ HGS1029</td>
<td>Advanced solid tumours / relapsed or refractory lymphoid malignancies (iv)</td>
</tr>
<tr>
<td>Ascenta</td>
<td>AT-406</td>
<td>Advanced solid tumours and lymphomas (po)</td>
</tr>
<tr>
<td>Genentech / Roche</td>
<td>GDC-0152/ RG7419</td>
<td>Locally advanced or metastatic malignancies (iv)</td>
</tr>
<tr>
<td>Novartis</td>
<td>LCL161</td>
<td>Solid tumours (po, weekly and daily)</td>
</tr>
<tr>
<td>Tetralogic</td>
<td>TL32711</td>
<td>Advanced or metastatic solid tumours / refractory solid tumours or lymphoma (iv)</td>
</tr>
</tbody>
</table>
TL32711 Overview

• Potent IAP selective dimer:
  – Preferential suppression of cIAP1, but also inhibits cIAP2, XIAP, and ML-IAP but no effect on Survivin
  – Marked synergy with TNFα and TRAIL in vitro
  – Broad combination synergy with chemotherapy without increased toxicity
  – Favorable ADME characteristics
  – Good pharmaceutical properties for IV dosing
TL32711 Mechanism of Action

• Selectively binds to cIAP1 vs. cIAP2 and induces rapid autodegradation of cIAP1 in all cell types
  – Following treatment of sensitive tumors
    • Autocrine TNF and exogenous TNF and TRAIL bind to death receptors
    • Cytokines activate pro-apoptotic pathway via caspase-8 activation
    • Elevated procaspase-8 levels in tumors provide tumor selectivity for killing
    • Rapidly shuts off canonical NFkB pro-survival pathway
    • cIAP2 degradation via cIAP1 ubiquitinylation not required for killing
  – Following treatment of normal cells
    • cIAP2 can replace cIAP1 function and may play protective role
      – In sensitive tumor cells, strong apoptotic signaling overwhelms any cIAP2 effect

• Binds to and antagonizes XIAP function in all cells
  – De-represses caspase-9 inhibition activity generated via intrinsic pathway
  – De-represses executioner caspase-3 and -7 activity that represents the final block in apoptosis pathway
TL32711 Key Preclinical Efficacy Data

- Rapid and potent target suppression of IAPs
  - Occurs in all cell types and tissues within 1 hr of drug exposure
  - Prolonged target suppression in tumor vs. normal tissues
  - PK/PD relationship between target suppression and anti-tumor activity

- TL32711 activity in multiple tumor models
  - Activity in both primary tumor and established tumors
  - Activity in hematologic and solid tumor models
  - Complete regressions with prolonged survival in xenograft models
  - In vivo efficacy retained in larger tumors

- Potent synergy with TNF$\alpha$ and TRAIL
- Potent synergy with multiple chemotherapies
cIAP1 Suppression Correlates to Cancer Cell Death

MDA-MB-231 Breast Tumor Cells

24 hr Treatment

TL32711

<table>
<thead>
<tr>
<th>0</th>
<th>10^{-13}</th>
<th>10^{-12}</th>
<th>10^{-11}</th>
<th>10^{-10}</th>
<th>10^{-9}</th>
<th>10^{-8}</th>
<th>10^{-7}</th>
<th>10^{-6}</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>cIAP1</td>
<td><img src="image1.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image2.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image3.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image4.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image5.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image6.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image7.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image8.png" alt="Image of cIAP1 expression" /></td>
<td><img src="image9.png" alt="Image of cIAP1 expression" /></td>
</tr>
<tr>
<td>cIAP2</td>
<td><img src="image10.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image11.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image12.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image13.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image14.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image15.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image16.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image17.png" alt="Image of cIAP2 expression" /></td>
<td><img src="image18.png" alt="Image of cIAP2 expression" /></td>
</tr>
<tr>
<td>PARP</td>
<td><img src="image19.png" alt="Image of PARP expression" /></td>
<td><img src="image20.png" alt="Image of PARP expression" /></td>
<td><img src="image21.png" alt="Image of PARP expression" /></td>
<td><img src="image22.png" alt="Image of PARP expression" /></td>
<td><img src="image23.png" alt="Image of PARP expression" /></td>
<td><img src="image24.png" alt="Image of PARP expression" /></td>
<td><img src="image25.png" alt="Image of PARP expression" /></td>
<td><img src="image26.png" alt="Image of PARP expression" /></td>
<td><img src="image27.png" alt="Image of PARP expression" /></td>
</tr>
<tr>
<td>GAPDH</td>
<td><img src="image28.png" alt="Image of GAPDH expression" /></td>
<td><img src="image29.png" alt="Image of GAPDH expression" /></td>
<td><img src="image30.png" alt="Image of GAPDH expression" /></td>
<td><img src="image31.png" alt="Image of GAPDH expression" /></td>
<td><img src="image32.png" alt="Image of GAPDH expression" /></td>
<td><img src="image33.png" alt="Image of GAPDH expression" /></td>
<td><img src="image34.png" alt="Image of GAPDH expression" /></td>
<td><img src="image35.png" alt="Image of GAPDH expression" /></td>
<td><img src="image36.png" alt="Image of GAPDH expression" /></td>
</tr>
</tbody>
</table>

% cIAP1 remaining

% Viability (Annexin/PI)

% cIAP1 degradation

% Viability (Annexin/PI)
TL32711 Causes Tumor Regressions at Well Tolerated Doses in a MDA-MB-231 Xenograft Model

Efficacy Greater than Docetaxel Administered at the MTD

**Tumor Regression Dose**
- Vehicle Control (12.5% captisol; IP; Q3Dx5)
- Docetaxel (20 mg/kg; IV; Q4Dx3)
- TL32711 (10 mg/kg; IP; Q3Dx5)
- TL32711 (5 mg/kg; IV; Q3Dx5)

**Tumor Stasis Dose**
- Vehicle
- Docetaxel 20 mg/kg (q4dx3x2)
- TL32711 1.25 mg/kg
TL32711 Plasma and Tumor Pharmacokinetics in Mice

TL32711 Plasma and Tumor Pharmacokinetics in Mice (5 mg/kg)

- Mean Plasma Conc (ng/mL)
- Mean Tumor Conc (ng/g)
- Tumor (Model Fit)
- Plasma (Model Fit)
TL32711-induced cIAP1 Reduction is Prolonged in Tumor vs. Normal Tissue

MDA-MB-231 xenograft following single 5 mg/kg dose
TL32711 Phase I Trial

ROSWELL PARK CANCER INSTITUTE

FOX CHASE CANCER CENTER

UNIVERSITY OF PENNSYLVANIA CANCER CENTER
Measures of Target Coverage & Clinical Effect

- PBMC assay of cIAP-1 as surrogate of tumor cIAP-1 suppression
- Correlation of target suppression with Phase 1 study data
  - Apoptosis activation - circulating cleaved cytokeratin-18 levels and activated caspase-3 levels in serum
  - Clinical measures
    - Radiographic assessment - CT/MRI/FDG-PET
    - Surrogate clinical markers e.g. CEA, CA-125
- Correlation of Phase 1 PBMC target suppression drug concentration levels with non-clinical drug concentrations related to target suppression and efficacy
Degradation of cIAPs by TL32711 in Volunteer PBMCs

<table>
<thead>
<tr>
<th>TL32711 (nM)</th>
<th>0</th>
<th>6</th>
<th>18</th>
<th>56</th>
<th>167</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanes</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

**cIAP1**

**cIAP2**
AQUA IHC For Predictive Biomarkers

- Study of 11 different tumor types representing 1164 patient samples
  - Quantitating TNFα & TRAIL levels in tumor and stroma – data by June
  - Identify tumor types with highest expression levels – candidates for Phase 2 study
  - Profile tumor types of interest for other markers of sensitivity including TNFα and TRAIL receptors, cIAP-1, cFLIP, Bcl family members and pro-caspase-8

- Tumor types included
  - Ovarian – 184 patients
  - Melanoma – 80 patients
  - Breast – 261 patients (Her2+/-, ER+/-, Node +/-)
  - Colorectal – 67 patients
  - Pancreatic -39 patients
  - NSCLC – 156 patients
  - Head & Neck – 181 patients
  - Esophageal – 91 patients
  - Bladder – 32 patients
  - Prostate – 24 patients
  - Kidney – 49 patients

### TNFα Staining in Ovarian Tumor

<table>
<thead>
<tr>
<th>TNFα</th>
<th>Cytokeratin</th>
<th>Dapi</th>
<th>Combination</th>
</tr>
</thead>
</table>

| Ovarian Papillary serous CA – High Expression in Epithelial component |

| Ovarian Endometrioid CA – High Expression in Stroma |
Ovarian Serous CA – High Expression in Epithelial Components

Ovarian Papillary Serous CA – Stroma Expression only
## TL32711 Single Agent Phase 1 Study Dose Escalation Status

<table>
<thead>
<tr>
<th>Cohort No.</th>
<th>Dose (mg/m²)</th>
<th>% Increase from Prior Dose</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>AUC (ng.h/mL)</th>
<th>cIAP1 Supp at 24hrs</th>
<th>cIAP1 Supp at 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.18</td>
<td></td>
<td>10</td>
<td>11</td>
<td>25-50%</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>0.36</td>
<td>100%</td>
<td>22</td>
<td>20</td>
<td>25-50%</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>0.72</td>
<td>100%</td>
<td>41</td>
<td>37</td>
<td>25-50%</td>
<td>20%</td>
</tr>
<tr>
<td>4</td>
<td>1.44</td>
<td>100%</td>
<td>86</td>
<td>114</td>
<td>&gt;75%</td>
<td>50%</td>
</tr>
<tr>
<td>5</td>
<td>2.88</td>
<td>100%</td>
<td>258</td>
<td>254</td>
<td>&gt;75%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>6</td>
<td>5.76</td>
<td>100%</td>
<td>293</td>
<td>329</td>
<td>&gt;75%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>7</td>
<td>11.5</td>
<td>100%</td>
<td>664</td>
<td>669</td>
<td>&gt;85%*</td>
<td>&gt;60%*</td>
</tr>
<tr>
<td>8</td>
<td>17.2</td>
<td>50%</td>
<td>1338</td>
<td>1657</td>
<td>&gt;85%*</td>
<td>&gt;75%*</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>50%</td>
<td>2000 (est)</td>
<td>2500 (est)</td>
<td>Pending</td>
<td>Pending</td>
</tr>
</tbody>
</table>
TL32711 Pharmacokinetics
(Cohorts 1 through 8 Total Drug Levels)

Phase Ia Plasma Concentration
TL32711 Mean +/- SD
(Days 1 and 15 Combined)

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>0.18</th>
<th>0.36</th>
<th>0.72</th>
<th>1.44</th>
<th>2.88</th>
<th>5.76</th>
<th>11.5</th>
<th>17.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>9.9</td>
<td>22.4</td>
<td>41.3</td>
<td>86.1</td>
<td>258.2</td>
<td>292.5</td>
<td>664.5</td>
<td>1338</td>
</tr>
<tr>
<td>AUC (ng.h/mL)</td>
<td>10.5</td>
<td>20.0</td>
<td>37.3</td>
<td>113.6</td>
<td>254.3</td>
<td>328.7</td>
<td>669.3</td>
<td>1657</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>1.9</td>
<td>2.5</td>
<td>3.5</td>
<td>6.7*</td>
<td>7.1*</td>
<td>6.3</td>
<td>6.1</td>
<td>34.6</td>
</tr>
</tbody>
</table>

*Excludes 3 subjects (02-402, 03-501 and 01-502) with long terminal t₁/₂ values (73-87 h)
Dose Level 9

Free AUC (ng.hr/ml)

Dose (mg/m2)

26 mg/m² (Predicted)

Max Dose Tested - Rat (weekly x 3)

Tumor Regressions - Mice
HNSTD - Rat (weekly x 4)

Tumor Stasis - Mice
HNSTD - Dog (weekly x 4)
Apoptosis Pathway Activation by TL32711
Caspase-3 dependent Cleaved Cytokeratin-18 Serum Levels
Apoptosis Pathway Activation by TL32711
Activated Caspase-3/7 Serum Levels

Cohort 1
Caspase-Glo

Cohort 2
Caspase-Glo

Cohort 3
Caspase-Glo

Cohort 4
Caspase-Glo

Cohort 5
Caspase-Glo

Cohort 6
Caspase-Glo

Cohort 7
Caspase-Glo

Cohort 8
Caspase-Glo
Degradation of cIAP1 and Apoptosis Pathway Activation in Tumor Biopsy

01-703 Biopsy Western Blot Analysis

cIAP1 degraded by ~90% after TL32711 treatment
Activated caspase-8 seen
Intensity of intact PARP lower suggesting cleavage by Western
# Phase 1 Clinical Comparison

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Bio-Effect</th>
<th>Dose Level</th>
<th>Bio-Effect</th>
<th>Dose Level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TL32711</strong></td>
<td><strong>Bio-Effect</strong></td>
<td><strong>Dose Level</strong></td>
<td><strong>Bio-Effect</strong></td>
<td><strong>Dose Level</strong></td>
</tr>
<tr>
<td>1 (0.18mg/m²)</td>
<td>• cIAP-1 suppression (50-60%)</td>
<td>1 to 5 (10/20/40/80/160 mg/pt)</td>
<td>• cIAP-1 suppression</td>
<td>1 (0.1 mg/m²)</td>
</tr>
<tr>
<td></td>
<td>• IL-6 suppression</td>
<td></td>
<td>• M30 and M65 increases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• No cytokine elevation</td>
<td></td>
<td>• no tox</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• No AEs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (0.36mg/m²)</td>
<td>• cIAP-1 suppression (70-75%)</td>
<td>6 (320 mg/pt)</td>
<td>• cIAP-1 suppression</td>
<td>2 (0.2 mg/m²)</td>
</tr>
<tr>
<td></td>
<td>• Elevation of activated caspase-3 activity in serum</td>
<td></td>
<td>• M30 and M65 increases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• No cytokine elevation</td>
<td></td>
<td>• no tox</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• No AEs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (0.72 mg/m²)</td>
<td>• cIAP-1 suppression (80-90%)</td>
<td>8 (900 mg/pt)</td>
<td>• Dose-related increased cytokines</td>
<td>4 (0.6 mg/m²)</td>
</tr>
<tr>
<td></td>
<td>• 3 subjects without AEs</td>
<td></td>
<td>• High prevalence of N/V</td>
<td>• Grade 3 INR</td>
</tr>
<tr>
<td></td>
<td>• No cytokine elevation</td>
<td></td>
<td></td>
<td>• Grade 3 Supraventricular Tach</td>
</tr>
<tr>
<td></td>
<td>• No significant inc for casp-3 or CK levels</td>
<td></td>
<td></td>
<td>• Grade 3/4 lymphocytopenia</td>
</tr>
<tr>
<td>4 (1.4 mg/m²)</td>
<td>• cIAP-1 suppression (80-90%) and prolonged</td>
<td>9 (1800 mg/pt)</td>
<td>Grade 3/4 - N/V</td>
<td>5 (0.9 mg/m²)</td>
</tr>
<tr>
<td></td>
<td>• Well tolerated</td>
<td></td>
<td></td>
<td>• cIAP-1 suppression (80-90%)</td>
</tr>
<tr>
<td>5 (2.8 mg/m²)</td>
<td>Well tolerated</td>
<td>10 (2400 mg/pt)</td>
<td>Capsule formulation ongoing</td>
<td>6, (1.4 mg/m²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• cIAP-1 suppression (80-90%)</td>
<td>• 1 DLT (AST G3, Amylase G3, Lipase G4, Fatigue G3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 (2.1 mg/m²), 8 (3.2 mg/m²)</td>
<td>• cIAP-1 suppression (80-90%)</td>
</tr>
</tbody>
</table>
1 – Carboolatin/Paclitaxel (AUC -6, 175mg/m², q3 weeks)
2 – Irinotecan (350 mg/m² q3 weeks)
3 – Docetaxel (75 mg/m² q3 weeks)
4 – Gemcitabine (1000mg/m² qweek x 3 of 4 weeks)
5 - Liposomal Doxorubicin (40 mg/m² q4 weeks)
Summary

- Alterations in IAPs are found in many cancers and are associated with chemoresistance, disease progression and poor prognosis.
- SMAC mimetics inhibit IAPs.
- Cancer cells are very sensitive to SMAC mimetics in the presence of TNF alpha.
- SMAC mimetics synergize with TRAIL and multiple chemotherapy agents.
- Single agent activity is expected in tumors with secretion of TNF alpha in microenvironment.
- Combinations with chemotherapy are expected to be efficacious.
THANKS!

alex.adjei@roswellpark.org