Targeting Key Apoptosis Regulators for New Cancer Therapeutics

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A General Strategy
For New Anticancer Drug Design

“Removing the Road Blocks To Death’s Door”

To Promote Cancer Cells to Undergo Apoptosis
What Are The Road Blocks?

- TNF Ligand/TRAIl
- Death Receptors
- Caspase-8
- tBid
- Caspase-9
- Apoptosome
- Caspase-3
- Apoptosis
- Mitochondria
- Bax
- Cyt-c
- Apoptosis
- p53
- Radiation & chemotherapy
What Are The Road Blocks?

TNF Ligand/TRAIL → Death Receptors → Caspase-8

Bcl-2/xL/Mcl-1 → Caspase-8 → tBid → Caspase-9 → Caspase-3 → Apoptosis

Mitochondria → p53 → Bax → Cyt-c → Apoptosome

Radiation & chemotherapy
What Are The Road Blocks?

- TNF Ligand/TRAIL
- Death Receptors
- cIAP1/2
- Bcl-2/xL/Mcl-1
- Caspase-8
- tBid
- Caspase-9
- Caspase-3
- Apoptosis
- XIAP
- Apoptosome
- Mitochondria
- Radiation & chemotherapy
- p53
- Bax
- Cyt-c
What Are The Road Blocks?

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Apoptosis

Bcl-2/xL/Mcl-1

Bax

Mitochondria

p53

MDM2

Radiation & chemotherapy

XIAP

cIAP1/2

What Are The Road Blocks?
Removing the Roadblocks

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Radiation & chemotherapy

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Death Receptors

Caspase-8 → tBid → Cyt-c → Apoptosome

Caspase-9

Caspase-3 → Apoptosis

Radiation & chemotherapy

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Apoptosome
Some Critical Questions

What is the best approach to develop therapeutics to remove these roadblocks?

Do we need to remove all these roadblocks to achieve efficient apoptosis induction in tumor cells or only some of them?

Can such therapeutics have robust activity as single agents or do they have to be used in combination with other drugs?
“Good things come in small packages”

A Small-Molecule Approach
To Modulate Apoptosis
For New Therapy
Development
Bcl-2 Proteins Modulate Each Other’s Activity Through Hetero-Dimerization

- A well-defined binding pocket in Bcl-2/Bcl-xL/Bcl-w/Mcl-1
- Long but not very large interface
- Very high affinity interaction
- Primarily hydrophobic interaction

[Bcl-2, Bcl-xL, Bcl-w, Mcl-1]: [Bid, Bad, Bim, Bak, Bax]
MDM2 Protein Inhibits the Activity of p53 by Dimerization

- A well-defined and deep binding pocket in MDM2
- Small interface
- Relatively high affinity interaction
- Primarily hydrophobic interaction

p53 peptide

MDM2 protein
XIAP Inhibits Caspase-3/7/9/Smac by Hetero-Dimerization
Targeting Protein-Protein Interactions

- Largely unexplored for therapeutic development
- Traditionally very difficult
- Very few successful examples
Where Are We?

– Small-Molecule Smac Mimetics (2003-present)
  • Obtaining potent and orally active Smac mimetics
  • Clinical lead (SM-406) selected in 2007
  • Two Phase I trials started in 2010 and 2011
  • Licensed to DebioPharma for further clinical development in 2011

  • Obtaining the best-in-class orally active MDM2 inhibitors
  • Clinical candidate selected
  • Licensed to sanofi-aventis for clinical development
  • IND-enabling studies in progress
  • Phase I trial to be started in early 2012

– Small-Molecule BH3 Mimetics (2001-present)
  • First clinical compound in >15 Phase II clinical trials
  • Obtaining second generation of highly potent and optimized small-molecule inhibitors
  • IND-candidate selected
Small-molecule antagonists of IAPs

- Design of orally active Smac mimetics as IAP inhibitors
  - Through chemistry, structural biology and modeling

- Preclinical studies
  - Activity and mechanism

- Clinical trials
  - Single-agent Phase I clinical trial in solid tumors
  - Phase I clinical trial in combination with chemotherapy in leukemia
Small-molecule antagonists of IAPs

Design of orally active Smac mimetics as IAP inhibitors
  – Through chemistry, structural biology and modeling

Preclinical studies
  – Activity and mechanism

Clinical trials
  – Ongoing Phase I trial
  – Clinical trial opportunities at U Michigan
IAP Proteins Are Key Apoptosis Inhibitors

- TNFα /TRAIL
- Death receptors
- Radiation & chemotherapy
- Mitochondria
- Bak
- Bax
- Cytochrome c
- Apoptosome
- Caspase-8 → tBid → Bak Bax
- Caspase-3/7
- Caspase-9
- Apoptosis
- XIAP
Smac/DIABLO is the Endogenous Cellular Antagonist of XIAP and cIAP-1/2
XIAP Inhibits Caspase-9 and Caspase-3/-7 Through Two Different Domains

XIAP (X-linked IAP)

- CARD
- BIR1
- BIR2
- linker
- BIR3
- RING

Caspase 3/7
Caspase 9
Dimeric Smac Antagonizes XIAP by Targeting Both BIR2 and BIR3 Domains

XIAP (X-linked IAP)

- CARD
- BIR1
- BIR2
- BIR3
- RING

Protein

Caspase 3/7

Smac

Caspase 9
Dimeric Smac Antagonizes XIAP by Targeting Both BIR2 and BIR3 Domains

XIAP (X-linked IAP)

Caspase 3/7

Caspase 9

Smac Protein

Smac Protein

XIAP Bir3

Smac

Smac

XIAP Bir3
Smac/XIAP BIR3 Interaction Site Is a Drug-able Site

- A well-defined binding pocket in XIAP/cIAP1/cIAP2/ML-IAP
- Only four residues in Smac protein (AVPI)
- Relatively high affinity interaction
- An ideal drug-able site
Design of Potent and Non-peptidic Small-Molecule Smac Mimetics

Smac AVPI Peptide

Limitations
(1). Moderate affinities
(2). Not cell-permeable
(3). Not stable in vivo

Objectives
(1). High-affinity;
(2). Cell-permeable;
(3). Stable in vivo;
(4). Orally active;
Structure-Based Design of High-affinity and Orally Active Smac Mimetics

Cyclization of Valine and Proline

Smac AVPI peptide

Orally Active Smac Mimetic SM-406

XIAP BIR3
Q319
W323
Y324
E314
G307
W310
T308
D309
AVPI
K297
K299

~300 compounds
Smac Mimetic SM-406 Has High Affinities to Multiple IAP Proteins

<table>
<thead>
<tr>
<th>Compound</th>
<th>(XIAP BIR3)</th>
<th>(cIAP-1 BIR3)</th>
<th>(cIAP-2 BIR3)</th>
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<tr>
<td>Smac AVPI Peptide</td>
<td>3500 ± 300</td>
<td>188 ± 43</td>
<td>328 ± 13</td>
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<tr>
<td>SM-406</td>
<td>36 ± 6</td>
<td>1.9 ± 0.2</td>
<td>5.1 ± 0.4</td>
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SM-406 is 50-100 times more potent than Smac AVPI peptide
SM-406 is a Direct Antagonist of XIAP
Cell-Free Functional Assay

Max activation (dATP+cyt-c)  + XIAP +AT-406 (0.5 μM)
+ XIAP BIR3 (500 nM)  + XIAP +AT-406 (1 μM)
+ XIAP +AT-406 (0.1 μM)  + XIAP +SM-428 (100 μM)
Induction of cIAP-1 degradation by SM-406 in MDA-MB-231 Cancer Cells

SM-406 can effectively induce cIAP-1 degradation at 30-100 nM and within 15 min.
Small-molecule antagonists of IAPs

Design of orally active Smac mimetics as IAP inhibitors
  – Through chemistry, structural biology and modeling

Preclinical studies
  – Activity and mechanism

Clinical trials
  – Ongoing Phase I trial
  – Clinical trial opportunities at U Michigan
Mechanism of Apoptosis Induction of Smac Mimetics as Single Agent

- TNFα
  - Death receptors
  - TRAF2/FADD/RIPK
  - Smac Mimetics
    - cIAP1/2
  - TRAF2/FADD/RIPK

- Caspase-8 → tBid
- Bak
  - Bax
  - Cytochrome c
  - Mitochondria

- Caspase-9 → Apoptosome
  - XIAP

- Caspase-3/7
  - XIAP
  - Smac Mimetics

- Apoptosis
SM-406 Potently Inhibits Cancer Cell Growth in A Subset of Cancer Cell Lines
SM-406 Achieves Strong Antitumor Activity As Oral Single Agent in Mice

MDA-MB-231 Breast Cancer Xenograft Tumors in SCID Mice
SM-406 Induces cIAP-1 Degradation and PARP Cleavage in MDA-MB-231 Xenograft Tissues

<table>
<thead>
<tr>
<th>Mouse#</th>
<th>Cleaved-PARP 85kd</th>
<th>cIAP1 72kd</th>
<th>XIAP 58kd</th>
<th>Actin 42kd</th>
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Vehicle 7.5mg/kg

TXT, 24h 24h 2h 6h 16h 24h 48h

SM-406, 100mg/kg P.O

2h 6h 16h 24h 48h
PK/PD Relationship in Tumor-Bearing Mice

At 30 mg/kg, SM-406 achieves 2-4 $\mu$M of cMax in plasma and tumor tissues with T1/2 of 2-3 hrs
Strong Synergy of SM-406 In Combination with TRAIL in Breast Cancer Xenograft Model

Tumor regression was achieved with the combination of SM-406 + TRAIL, while both agents have minimal activity as monotherapy.
SM- 406 as a New Class of Anticancer Drug

- **SM-406 (AT-406)** is a good drug-like molecule with balanced hydrophobic / hydrophilic properties
  - Mol. Wt.: 562, clogP=3 and water soluble

- **Strong binding affinity to XIAP, cIAP-1 and cIAP-2 (Kᵢ = 1-25 nM to cIAP-1/2 and XIAP)**
  - Selective over other targets (Kᵢ>100uM) and antagonizing XIAP in functional assay

- **Good cellular activity as a single agent and broad synergy with TRAIL/DR mAbs, Chemo and Targeted agents etc, IC₅₀ = 150 nM in sensitive cell lines**
  - Excellent selectivity over normal cells: >100 times

- **Good PK profiles in mice, rats, dogs and NHP and excellent in vivo ADME profile and good metabolic stability**

- **Strong *in vivo* anti-tumor activity as single agent and combination at dose < MTD**
  - Active at 30 mg/kg as single, and robust synergy with chemodrugs and TRAIL

- **Well tolerated in rodents and large animals**

- **Nominated as drug development candidate in Nov 2007.**
  - **Four years of discovery research (2003-2007)**

- **IND approved in Aug 2009 and Phase I clinical trial started in Jan 2010**
Small-molecule antagonists of IAPs

- Design of orally active Smac mimetics as IAP inhibitors
  - Through chemistry, structural biology and modeling

- Preclinical studies
  - Activity and mechanism

- Clinical trials
  - Ongoing Phase I trial
  - Clinical trial opportunities at U Michigan
AT-406 is in Phase 1 Clinical Development

First-in-man study at University of Michigan, Duke University and Mayo Clinic

- Patients: Solid Tumors
- Dose-Schedule: Oral, Days 1-5 on 21-day cycle
- PD/biomarkers: tumor and surrogate tissue cIAP-1 and serum markers of apoptosis
Summary of Phase I Clinical Trial Data

• Based on the data from the first 30 patients (current dose at 600 mg)
  
• Well tolerated in patients
  • Reached 600 mg daily, oral dose
  • No grade III/IV toxicity

• Excellent pharmacokinetic parameters
  • Achieving exposure at 400 mg in plasma exceeding that by 100 mg/kg in mice
  • $c_{\text{Max}} > 10 \mu\text{M}$, $T_{1/2} = 9$ hrs at 400 mg
  • No significant accumulation

• Pharmacodynamics (biomarkers)
  • Complete and persistent cIAP degradation in tumors, skins and PBMC at 80 mg
  • Apoptosis induction by M30 at 120 mg

• Evidence of antitumor activity
  • One tumor regression observed at 400 mg
Second Clinical Trial of AT-406 in AML

AT-406 in combination with daunorubicin and cytarabine in patients with poor-risk Acute Myelogenous Leukemia (AML)
- Supported by a grant to Ascenta from the Leukemia & Lymphoma Society
- Principal Investigator: Harry Erba at U Michigan with Dale Bixby and Moshe Talpaz

Preliminary results from the first 8 AML patients
- 100 mg of AT-406 add-on standard dose-regimes of daunorubicin and cytarabine
- Well tolerated
- 6 patients achieving remission of the disease
- Response rate 75% versus ~35-40% with chemotherapy alone in relapsed, high-risk AML
Journey for Discovery and Development of AT-406

Conceived, designed, made and tested at University of Michigan
– 2003-2007

Working closely with a UM spin-off company (Ascenta) for preclinical IND-enabling and clinical trials
– 2008-2009

Tested in two Phase I clinical trials at U Michigan
• 2010-2011
• Excellent PK and tolerability in cancer patients
• Initial evidence of antitumor activity as a single agent and in combination with chemotherapy

Multiple Phase II trials planned for 2012
– Licensed to DebioPharma for clinical development and commercialization
# Acknowledgements for the IAP Project

## Wang Laboratory

- **Chemistry**
  Haiying Sun, Yuefeng Peng, Qian Cai, Rong Sheng, Dongguang Qin, Jianyong Chen,
- **Biochemistry and protein chemistry**
  Zaneta Nikolovska-Coleska, Liu Liu, Wei Gao and Han Yi
- **Cell Biology**
  Jianfeng Lu, Su Qiu, Longchuan Bai Sanjeev Kumar, Yuefeng Peng
- **Modeling and design**
  Chao-Yie Yang
- **In vivo tumor biology**
  Donna McEachern and Rebecca Miller

## Collaborators

- Jeanne Stuckey and Jennifer Meager at UM
  - **Structural biology**
- Duxin Sun Lab at UM
  - **PK/PD studies**
- Joe Gray Lab at Lawrence Berkeley National Laboratory
- Ken Pienta Lab at UM
  - **PC-3 model**

## Ascenta Therapeutics

Lance Leopold, Dajun Yang and Sanmao Kang, Ming Guo and Xiaolan Lin, Mel Sorensen

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**Funding:** NCI/NIH, BCRF, Komen, DOD, and Ascenta.
People Who Did the Work!