Minnelide: A Novel Therapy for Pancreatic Cancer

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• Diseases of the exocrine pancreas cause considerable morbidity. In the US alone, pancreatitis causes 300,000 hospitalizations and 7,000 deaths, while pancreatic cancer claims another 38,000 deaths annually.

• Treatment is limited for both, likely reflecting an inadequate understanding of their etiological and pathophysiological mechanisms. The national economy suffers by $3.5 billion annually.
Heat Shock Proteins

• Discovered serendipitously by Ritossa in 1962.
• One of the mechanisms that have evolved to ensure the survival of living cells under stressful conditions.
• One of the most highly conserved mechanisms of cellular protection.

Thermal Stress Induces HSP70 Expression in the Pancreas

![Graph showing HSP70 levels in control (Con) and heated (Heated) conditions.](image)
Effect of Prior Thermal Stress on Parameters Associated with Caerulein-Induced Pancreatitis


Transgenic Mice Over-expressing HSP70 are Protected Against Pancreatitis

Dawra R and Saluja AK, unpublished
Effect of Caerulein Administration on the Parameters Associated with Pancreatitis in HSP70 Transgenic Mice

Pancreatic MPO Activity

Acinar Cell Necrosis

HSP70 and Pancreatitis

Bhagat et al., Gastroenterology (2002)
Frossard et al., Gut (2002)
Pancreatic Cancer

• Pancreatic cancer is the most lethal cancer known to humankind.
• **45,000** Americans will be diagnosed with pancreatic cancer this year, and most everyone will succumb to the disease.
• Pancreatic cancer causes more than **250,000** deaths worldwide per year.

Current Treatments for Pancreatic Cancer

• Current therapies for pancreatic cancer are not very effective.
• **Gemcitabine**, approved more than 14 years ago, provides a 1.5 month survival advantage.
• **Tarceva** adds 10 more days.
HSP70 in Pancreatic Cancer

HSP70 is Overexpressed in Pancreatic Cancer Cells

Aghdassi A...Saluja AK, Cancer Research (2007)
HSP70 Expression in Pancreatic Cancer Patients

* *p<0.002; n=7 patients

Aghdassi A…Saluja AK, Cancer Research (2007)

HSPs and Pancreatic Cancer Hypothesis

Blocking HSPs should increase apoptosis in cancer cells thereby slowing the growth and spreading of tumor cells.
Quercetin inhibited HSP70 and caused tumor regression

Heat Shock Protein 70 Increases Tumorigenicity and Inhibits Apoptosis in Pancreatic Adenocarcinoma

Ali Aghdassi, Phoebe Phillips, Vikas Dudeja, Dhara Dhaulakhandi, Rifat Sharif, Rajinder Dawra, Markus M. Lerch and Ashok Saluja

Cancer Research, Feb 2007
Triptolide – A Diterpenoid Triepoxide

Has been used to treat inflammatory disorders such as rheumatoid arthritis.

tripterygium wilfordii Hook. f.
(‘Thunder God Vine’*)

Triptolide Kills Pancreatic Cancer Cells In Vitro

Triptolide Reduces Pancreatic Tumor Growth *In Vivo*


Department of Surgery

Triptolide Induces Pancreatic Cancer Cell Death via Inhibition of Heat Shock Protein 70


Department of Surgery

Disadvantage of Triptolide

• Soluble only in organic solvent
• Limits clinical utility
• No IP (Patent protection)

Minnelide™: A Novel Prodrug of Triptolide

• In collaboration with Dr. Gunda Georg and Satish Patil, we have synthesized a highly water-soluble analog of triptolide.
• Minnelide is as effective as triptolide in killing tumor cells in both in vitro and in vivo models of pancreatic cancer.
• Minnelide is safe in mice at relatively high doses.
• A patent to protect intellectual property has been granted to UMN.
Disclosure

Dr. Saluja has a significant financial interest in and is the Chief Scientific Officer and a consultant for Minneamrita, a company which may commercially benefit from the results of this research. This relationship has been reviewed and managed by the University of Minnesota in accordance with its conflict of interest policies.

Effect of Minnelide™ on Pancreatic Cancer Mortality in Mice

- MiaPaCa-2 cells were orthotopically implanted into pancreas.
- 10d post-implantation, Minnelide™ was administered at doses indicated for 60d.
- Treatment was discontinued and mice sacrificed 30d after stopping treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1mg/kg</td>
<td>0.15mg/kg/bid</td>
</tr>
<tr>
<td>No of mice at beginning</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>No of mice alive at day 95</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

Chugh, Sangwan… Saluja (Sc Tran Med Oct. 2012)
MiaPaca-2 cell-induced orthotopic tumors in nude mice - Average Tumor Size On Day of Death

*# of mice found with tumors / # of mice in group

Minnelide Increases Survival in an Orthotopic Pancreatic Cancer Model

Day 1: Aspc-1 (2X 10⁵) implanted into pancreas (n=20)
Day 7: 0.42 mg/kg Minnelide or saline IP begins (n=10)
Day 100: Minnelide treatment stopped (n=5)
Minnelide Causes Tumor Regression in an Aspc-1 Induced Orthotopic Pancreatic Model

Day 1: Aspc-1 (2X 10^5) implanted
Day 28: Minnelide treatment
Day 85: Experiment terminated

Minnelide™ causes tumor regression in S2-013 cell-induced orthotopic pancreatic tumors
S2-013 is one of the most aggressive pancreatic tumor-derived cell lines.

Saline

Minnelide™
**Minnelide™** decreases tumor metastasis in mice bearing S2-013-induced orthotopic tumors

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>CONTROL</th>
<th>MINNELIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>5/8</td>
<td>0/10</td>
</tr>
<tr>
<td>Kidney</td>
<td>5/8</td>
<td>0/10</td>
</tr>
<tr>
<td>Abdominal Wall</td>
<td>8/8</td>
<td>2/10</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>8/8</td>
<td>0/10</td>
</tr>
<tr>
<td>Spleen</td>
<td>8/8</td>
<td>1/10</td>
</tr>
<tr>
<td>Ascites</td>
<td>8/8</td>
<td>0/10</td>
</tr>
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</table>

**Minnelide™** significantly decreased the tumor metastasis.

**Minnelide decreases tumor burden in a human xenograft**

![Graph showing tumor volume over days treated with Minnelide and saline]

Department of Surgery

Unversity of Minnesota

Driven to Discover

Department of Surgery
Minnelide decreases tumor burden in a human xenograft
Minnelide causes tumor regression in a human xenograft

![Graph showing tumor size over days of treatment with saline, 0.21 mg/kg Minnelide, and 0.42 mg/kg Minnelide.]
Orthotopic Pancreatic Cancer Model

<table>
<thead>
<tr>
<th>Average Tumor Weight (gms)</th>
<th>Control</th>
<th>Gemcitabine</th>
<th>Minnelide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>1.2</td>
<td>0.6</td>
</tr>
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</table>


Neuroblastoma

- One of the common pediatric tumors
- Advanced stage cases highly aggressive
  - Resistant to conventional chemotherapy
  - 5-year survival is 30-40%
Results: Tumor Growth *In Vivo*

**Tumor Volume in cm³**

- **Triptolide**
- **Control**

**Tumor Mass in gm**

- **Triptolide**
- **Control**


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**Hsp-70 in Residual Tumors**

<table>
<thead>
<tr>
<th>Negative Control</th>
<th>Hsp-70 IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Triptolide</td>
<td></td>
</tr>
</tbody>
</table>

Triptolide therapy for neuroblastoma decreases cell viability in vitro and inhibits tumor growth in vivo

Journal of Surgical Research, in press

Role of HSP70 in Triptolide-Mediated Cell Death of Neuroblastoma


Minnelide is Also Effective in Colon, Ovarian, Osteosarcoma and Several Other Cancers
Minnelide: A Prodrug of Triptolide is Soluble in Water

RESEARCH ARTICLE

PANCREATIC CANCER

A Preclinical Evaluation of Minnelide as a Therapeutic Agent Against Pancreatic Cancer


Pancreatic cancer is one of the most lethal human malignancies with an all-stage 5-year survival frequency of <5%, which highlights the urgent need for more effective therapeutic strategies. We have previously shown that triptolide, a diterpenoid, is effective against pancreatic cancer cells in vitro as well as in vivo. However, triptolide is poorly soluble in water, limiting its clinical use. We therefore synthesized a water-soluble analog of triptolide, named

Minnelide in combination with death receptor therapy

• Pancreatic cancer is highly resistant to monotherapy.

• Death receptor therapy is not very effective against pancreatic cancer
Effect of triptolide and Anti-DR5 Ab on apoptosis of pancreatic cancer cells after 24 hrs of exposure

Bar= SEM

n = 4. Run in duplicates.
Method: Annexin V

Pancreatic cancer stem cells and Minnelide
CD133+ tumors responded to therapeutic dose of Minnelide

\[ \text{in vivo} \]

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**Banerjee S……..Saluja A, unpublished**


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**Triptolide/Minnelide**

↓ **HSP 70**

↓ **Tumor Cell Death**
How does HSP70 Inhibition Results in Tumor Cell Death

Triptolide/Minnelide

↓ HSP 70

↑ LMP  ↓ Intracellular Ca$^{2+}$

Tumor Cell Death
HSP70 Stabilizes Lysosomes in Pancreatic Cancer Cells

Inhibition of HSP70 results in lysosomal permeabilization

\[ T = \text{Triptolide 0.2} \mu M \]

\[ * p \leq 0.05, n=3 \]
HSP70 Stabilizes Lysosomes in Pancreatic Cancer Cells

Inhibition of HSP70 Resulted in an Increase in Cytosolic Ca²⁺

Dudeja V...Saluja AK, Gastroenterology (2009)
HSP70 Attenuates Intracellular Calcium

Effect of Cathepsin B Inhibition and Cytosolic Ca^{2+} Chelation on Cell Death After HSP70 Downregulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Triptolide 0.2uM</th>
<th>BAPTA 10uM</th>
<th>CA074me 10uM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triptolide 0.2uM</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BAPTA 10uM</td>
<td>-</td>
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<td>CA074me 10uM</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Dudeja V…Saluja AK, Gastroenterology (2009)*
Mechanisms by Which Triptolide Downregulates Heat Shock Proteins

Triptolide Downregulated HSF1 Expression

Time after triptolide treatment

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSF-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Fold change in mRNA expression over control

- Untreated
- 100nM triptolide
Heat Shock Protein 70 Inhibits Apoptosis in Cancer Cells through Simultaneous and Independent Mechanisms
Gastroenterology, 2009, May

Pro-survival Role of Heat Shock Factor 1 In the Pathogenesis of PancreateoBiliary tumors
Amer J Physiol, 2011
Clinical Trials for Minnelide

A Phase 1, Multi-Center, Open-Label, Dose-Escalation, Safety, Pharmacokinetic, and Pharmacodynamic Study of Minnelide™ Given Daily for 21 Days Followed by 7 Days Off Schedule in Patients With Advanced GI Tumors.

As of this week fourteen patients have been enrolled

Trial Period: August 2013 - August 2015

Acknowledgements

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Bruce Blazar, MD
Gunda Georg, PhD

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National Pancreas Foundation
Robert and Katherine Goodale Foundation
University of Minnesota
Imagine we can make a difference

Steve Jobs 1955 - 2011
Ralph Steinman 1943 - 2011