Novel Therapeutics for Pancreatic Cancer

Surgical Oncology T32 Symposium
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DISCLOSURES

• PATENT and SHAREHOLDER OF MINNELIDE
• MinneAmirta
Here are a few faces you probably recognize; And a few of the thousands you probably don’t. Unfortunately, they all have one thing in common: THE #4 CANCER KILLER OF MEN AND WOMEN IN THE UNITED STATES... PANCREATIC CANCER
Actor and Supreme Court Justice
Sports Icons
U.S. Cancer Statistics

% Overall Survival

- BREAST: 74-76, 80-82, 95-00
- COLON: 74-76, 80-82
- PROST: 74-76, 80-82
- PANC: 74-76, 80-82
Pancreatic Cancer 2010: Epidemiology

- 42,680 / 37,290
- Incidence: 9 / 100,000, peak 6th and 7th decades, average age 60-65 yo
- Men > women (RR 1.35)
- AA Men 30-40% > than white males with similar smoking rates (25% and 27%, respectively)
- 1920-1978: 3-fold increase in incidence

PANC CA 9th in incidence / 4th in mortality
Stage of Diagnosis - Prostate*

*The rate of localized stage represents localized and regional stages combined.

Note: Staging according to Surveillance, Epidemiology, and End Results (SEER) historic stage categories rather than the American Joint Committee on Cancer (AJCC) staging system. For each type and race, stage categories do not total 100% because sufficient information is not available to assign a stage to all cancer cases.

Note: Staging according to Surveillance, Epidemiology, and End Results (SEER) historic stage categories rather than the American Joint Committee on Cancer (AJCC) staging system. For each type and race, stage categories do not total 100% because sufficient information is not available to assign a stage to all cancer cases.

Stage of Diagnosis - Colon and Rectum

Note: Staging according to Surveillance, Epidemiology, and End Results (SEER) historic stage categories rather than the American Joint Committee on Cancer (AJCC) staging system. For each type and race, stage categories do not total 100% because sufficient information is not available to assign a stage to all cancer cases.

Stage of Diagnosis - Pancreas

Note: Staging according to Surveillance, Epidemiology, and End Results (SEER) historic stage categories rather than the American Joint Committee on Cancer (AJCC) staging system. For each type and race, stage categories do not total 100% because sufficient information is not available to assign a stage to all cancer cases.

Bedside View

• 88% of patients non-operable
• 12% operable patients: 80% of which have systemic disease
• Actual post-operative 5-year survival: < 10%

To impact this cancer there must be a transition from a focus not only on the operation but to the disease.
NCI 2009
Funding for Major Cancers

[Bar chart showing funding for different cancers: Breast, Colon, Pancreatic. Each bar represents thousands of dollars per death.]
Cancer Workforce

**Colon Cancer**
- Basic Science: $79,000,000
- Diagnosis & Treatment: $58,000,000
- Outcomes: $14,000,000
- Models: $2,000,000
- Total: $153,000,000
- 680 Projects
- Estimated >500 NCI Investigators

**Pancreatic Cancer**
- Basic Science: $10,000,000
- Diagnosis & Treatment: $4,000,000
- Outcomes: $1,500,000
- Total: $66,500,000
- <50 Projects
- Estimated < 82 NCI Supported Investigators

<table>
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<tr>
<th>HIV / AIDS:</th>
<th>1982 ≈ 30 Million</th>
<th>3 ½ Months MS</th>
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<tbody>
<tr>
<td>2010 ≈ 2.1 Billion</td>
<td>30 Years MS</td>
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</table>
$65 million gift

11.5 million NIH award

Pancreatic Cancer SPORE '18'

Masonic Cancer Center

$65 million gift
The purpose of the UAB/UMN SPORE in Pancreatic Cancer is to make major contributions to the reduction in morbidity and mortality in this dreaded disease.

Our intent is to take major advantage of the scientific expertise, resources, and patient populations of two productive Comprehensive Cancer Centers (UAB and UMN) in pursuit of our interdisciplinary and translational pancreatic cancer research efforts.
UAB/UMN SPORE in Pancreatic Cancer

4 Core Projects

1. Biomarker discovery for early detection of pancreatic ductal adenocarcinoma – C. Klug (UAB)
2. Combined modality targeted therapy of pancreatic cancer with death receptor monoclonal antibodies – D. Buchsbaum (UAB)
3. Identifying and targeting pathways of pancreatic progression and metastasis – D. Largaespada (UMN), D. Tuveson (CRI), C. Iacobuzio-Donahue (JHU)
4. Development of oncolytic adenovirus targeting pancreatic cancer stem cells – D. Curiel (UAB) and M. Yamamoto (UMN)
CLINICAL CORE PATHWAY

All patients with a clinical diagnosis of pancreatic cancer

Informed Consent: Biopsy for tissue collection and entry into pancreatic cancer registry (both local and national familial)

EUS Biopsy Molecular analysis (98% Accuracy)

Immediate tissue FNA diagnosis

Pathology: tissue core, blood and urine collection

Multidisciplinary evaluation: CT / EUS / MRI / Surgery / Oncology / Radiological Oncology / GI / Radiology

Cytologic review

Clinical trials

Neo-Adjuvant (locally invasive)

Surgery

Primary treatment (metastatic)

Adjuvant tx
**MA-RT-Q-PCR**

<table>
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<tr>
<th>DNA Microarray</th>
<th>Amplification of RNA</th>
<th>Labeling RNA</th>
<th>Hybridization</th>
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<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td><strong>MA-RT-Q-PCR</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

**Advantages**

- Ability to analyze clinical samples where small amounts of tissue are available (2 ng total RNA / gene)
  - Frozen Tissues
  - Needle Biopsies (FNA)
- Fragmented or partially degraded RNA can be used
  - Archival Paraffin Embedded Tissues
- Rapid
  - 3 hours per microfluid card
- Quantitative
  - 5% inter- & intra-assay variation
## Micro-Fluidic Card Design

<table>
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<th>Category</th>
<th>Genes per Card</th>
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<td>Apoptosis</td>
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<tr>
<td>Pro-apoptotic</td>
<td>94</td>
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<tr>
<td>Anti-apoptotic</td>
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<tr>
<td>Drug Metabolizing Enzymes</td>
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<td>5-FU, Capecitabine, Gemcitabine, Irinotecan</td>
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<tr>
<td>DNA Repair / Methylation</td>
<td>94</td>
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<td>Transcription Factors</td>
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<td>Cell Cycle (growth &amp; maintenance)</td>
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<td>Hedgehog and Wnt Pathways</td>
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<tr>
<td>Total Number of Genes Examined</td>
<td>470</td>
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The image shows a diagram with 96 genes, divided into sections labeled 1 through 4.
Analysis of EUS-FNA from pancreatic cancer patients demonstrates a sensitivity, specificity, positive predictive value and negative predictive value for solid pancreatic masses of 94.7, 100, 100 and 85.2% respectively.

Eloubeidi et al, *Gastroenterol* 2003
• The desmoplastic stromal reaction of pancreatic adenocarcinoma greatly hampers expression studies in this tumor

• Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is used to evaluate and stage all pancreatic lesions at UAB
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<th>Category</th>
<th>Gene Name</th>
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<td>VEGF C</td>
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</table>
Development of oncolytic adenoviruses targeting pancreatic cancer stem cells

M. Yamamoto (UMN)
Adenovirus

- Un-enveloped
- Double-strand DNA virus (36kb)

Enh/φ
Problem 1: Ectopic Expression from Adenoviral Vectors

*In vivo* expression profile from CMV promoter

Liver

- Adenoviral vectors show natural hepatotropism after systemic administration.
- Effluent blood flow of the most GI organs drains into portal vein, and eventually reaches the liver.

Liver UN-targeting is necessary for practical utility.

Promoter based control (e.g. COX-2 promoter)

\[ \text{i.v. AdCMVSSTR} \rightarrow \text{Tc}^{99m} \text{-P2045} \]

From Dr. Kurt Zinn, Multimodality Imaging Lab, UAB
Conditionally Replicative Adenovirus (CRAd)
Selective promoter-based CRAd. CN706, CV739, RGD or 5/3CRAdCOX-2)

Control E1 expression with target cell specific promoter

- Normal does not express E1
  - Virus does not replicate
- Target cancer cell expresses E1
  - Viral replication and cell death
  
(i.e.)
Problem 2: Many Pancreatic Cancer Cells Are Deficient in the Primary Adenoviral Receptor CAR

Most pancreatic cancer cells lack the expression of Coxsackievirus - Adenovirus Receptor (CAR)

Establishment of CAR-independent viral entry mechanism is desired for Panc Ca CRAds
Infectivity Enhancement by Fiber Modification

**RGD modification**

RGD-4C: High affinity to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins

RGD-modified Ad5 fiber

Integrin mediated transduction

**5/3 Chimera**

Ad5 fiber

Ad3 knob

Ad3/5

Ad3 receptor

Mediated transduction


Cox-2 Promoter-Based, Infectivity Enhanced CRAds for GI cancers

\[ \Delta E1 \]

\[ \text{Intact E3} \]

CRAdcox2(F)

CRAdcox2(R)

wt fiber

RGD fiber

5/3 fiber
Cox-2 Promoter-Based CRAd

In Vivo Effect in Pancreatic Cancer Cells

Inoculate tumor cells
HS766T: cox-2 (+)
(5 x 10⁶ cells)
6-8mm

Day 0
Inject virus intratumorally
(10⁸ vp/200µl PBS)

Tumor volume (mm³)

Days after viral injection

AdCox 2 Luc
Cox 2CRAD(F)
Cox 2CRAD(R)
Wt Ad5
RGD-Cox 2CRAD(F)
RGD-Cox 2CRAD(R)
Untreated

Problem 3: The vectors need to be tested in the system closer to the clinical settings

- Subcutaneous tumors
- Intratumoral administration
- Orthotopic tumors
- Systemic administration

(Ramirez et al., in submission)
Orthotopic Pancreatic Cancer Model

- inject cells subcutaneously
- Hs766T cells $10^5$
- sacrifice
- harvest tumor
- prepare 1mm$^3$ pieces
- open abdomen
- implant the piece into the pancreatic bed atraumatically
- ready to use

Tumor Transduction After Systemic Administration in Pancreatic Cancer Orthotopic Xenograft Model

Systemic administration
CMV luciferase vector

Hs766T orthotopic implant

72 hrs

48 hrs

PBS Ad5 Luc1 AdRGD Luc1 Ad5/3 Luc1

RLU

10^1 10^2 10^3

(PBS Ad5 Luc1 AdRGD Luc1 Ad5/3 Luc1)

(Ramirez et al, in submission)
Tumor Size After Systemic CRAAd Administration in Pancreatic Cancer Orthotopic Xenograft Model

Hs766T orthotopic implant

Systemic administration

72 hrs

CRAds

28 days

Sacrifice

Tumor size measurement

Tumor Volume (mm³)

<table>
<thead>
<tr>
<th></th>
<th>Tumor Volume (mm³)</th>
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<tbody>
<tr>
<td>Ad COX2L Luc</td>
<td>120 ± 5</td>
</tr>
<tr>
<td>5/3 CRAAd COX 2F</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Ad wt</td>
<td>10 ± 1</td>
</tr>
</tbody>
</table>

(Ramirez et al, in submission)
Intratumor Viral Replication After Systemic Administration in Pancreatic Cancer Orthotopic Xenograft Model

**Methods**
- **Hs766T orthotopic implant**
- **Systemic administration**
- **CRAds**
- **28 days**
- **Sacrifice**
- **DNA extraction from tumors**
- **Determine viral copy # by real-time PCR**

**Results**
- **E4 copy number/cell**
  - Ad COX2L Luc
  - Ad wt
  - 5/3 CRAd COX 2F

(Ramirez et al, in submission)
Obstacles of existing oncolytic viral therapy:

1. Limited transduction efficiency

2. Insufficient antitumor effect

3. Lack of an effective method for monitoring adenovirus replication during clinical trials
Sodium-Iodide Symporter (NIS) as a Therapeutic and High Sensitivity Imaging Transgene

- Human protein responsible for transporting iodide in the thyroid gland
  - Its natural expression has been exploited for $^{131}$I radiotherapy of hyperthyroidism and thyroid cancer
- Radioiodine therapy has been proven to be safe and effective
  - NIS expression can be non-invasively monitored
- The imaging of hNIS-expressing tissues is widely performed in clinics with different isotopes ($^{123}$I, $^{124}$I, $^{99}$mTc$^{04-}$)
NIS-Mediated In Vivo Imaging

Ad5/3-NIS (replication-defective)

Ad5/3-ΔE3-ADP-NIS (Replication Competent)

- IP injection of 0.5 mCi of $^{99mTc\text{O}_4}$-
- SPECT/CT Imaging (1 hour after isotope injection)

NIS-uptake with Ad5/3ΔE3-ADP-NIS increased with time and peaked 5-18 days post treatment compared to 1-4 days with a non-replicative Ad5/3-NIS.

Oncolytic adenovirus-treated mice showed significantly higher $^{99mTc\text{O}_4}$-accumulation than mice injected with a replication-defective vector.
Improved Therapeutic Effect of Virotherapy in Combination with $^{131}$I Radiotherapy

- The survival rate was significantly improved when Ad5/3ΔE3-ADP-NIS was combined with $^{131}$I radiotherapy.
- These data confirms the efficient *in vivo* NIS expression from the oncolytic adenovirus and feasibility of combination of enhanced adenovirus efficacy with radiotherapy.
Novel Cox2-Controlled IFN-Expressing CRAd Showed Powerful Inhibition of Tumor Growth

Metastatic Human PDAc (S2013) 1 x 10^6 Nude Mouse

1 x 10^{10} VP

*5/3 Cox2ΔE3ADP-hu-IFN showed greater tumor reduction compared to non-replicative 5/3-hu-IFN

\( p < 0.05 \)
Cox2-CRAd with IFN Has Increased Potency in a Fully Syngeneic Immunocompetent Hamster Model

- **RGD Cox2 ΔE3ADP-ham-IFN** significantly outperformed RGDCOX2CRAd without IFN and showed tumor shrinkage throughout the experiment.
Combination of Viro-/Radiation Therapy *In Vivo* Drastically Decreases Tumors in Syrian Hamster

Cell Line: HP1
Inoculated 2 x 10^6 cells

Virotherapy:
RGD_{ΔE3}ADP_{IFN}-Hamster
Injected 2x10^{11} vp/tumor

Radiation Therapy:
Radiated 20 Gy/leg

Three Days After:
Viro-/Radiation Treated "Combi" hamster was healthy and lively until the

Combination Therapy Hamster Preliminary

- Viral/Radiation Treatment n=2
- Untreated Control n=4
- Radiation Treatment n=3
Clinical Translation

- Pre-clinical Efficacy
- Preliminary Toxicology Studies
- Development of Phase I Clinical Trial

*Pre-IND Letter to FDA for Approval of CRAd as Treatment for Pancreatic Cancer*
HSP70 in Pancreatic Cancer

Pancreatic Cancer Cells

HSP70

Cell Death
Expression of HSP70 in Normal Pancreas versus (Macro-dissected) Tumor

p = <0.0001
Triptolide

- Has been used to treat inflammatory disorders such as rheumatoid arthritis.
- Triptolide treatment decreases Heat shock protein 70 level, and inhibits pancreatic tumor growth (Phillips et.al, Cancer Res. 2007).
UAB/UMN SPORE in Pancreatic Cancer

Triptolide
Minnelide: A Novel Prodrug of Triptolide

• 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 animal models of pancreatic cancer.

• Preliminary studies using Minnelide suggest that it is non-toxic at therapeutic doses

• A patent has been filed
UAB/UMN SPORE in Pancreatic Cancer

Minneolide
Minnelide causes tumor regression in MIA PaCa-2-induced orthotopic pancreatic tumors

* = p<0.05

<table>
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<th>Dose</th>
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<td>Triptolide</td>
<td>0.2mg/kg</td>
<td>once a day × 60days</td>
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<tr>
<td>Minnelide</td>
<td>0.28mg/kg</td>
<td>once a day × 60days</td>
</tr>
</tbody>
</table>

Mean Tumor Weight (g)
Minnelide causes cell death in pancreatic cancer cell lines

Viability (% control)

MIA PaCa-2
Panc-1
S2-013
S2-VP10

Minnelide
Triptolide

(*= p<0.05)
Minnelide decreases HSP70 expression

MiaPaCa-2
Treatment 24hours
n=3
Triptolide significantly reduced pancreatic tumor growth in mice. The panel on the right above shows representative photographs of tumors dissected from the pancreas and corresponding photo of tumor in situ.
Minnelide Decreases Tumor Burden in S2-013 Induced Orthotopic Pancreatic Tumors

Day 1: S2-013 (1X 10^6) implanted into pancreas (n=20)
Day 7: 0.42 mg/kg Minnelide or saline IP begins
Day 28: Experiment terminated

*p = 0.000497
**p = 6.84221E-08

Average Tumor Weight (gms)
Average Tumor Volume (mm^3)

S2-013 (Metastatic)
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>0.1mg/kg</th>
<th>0.15mg/kg/bid</th>
<th>0.3mg/kg</th>
<th>0.6mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No of mice at</strong></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td><strong>beginning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No of mice alive</strong></td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td><strong>at day 95</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Minnelide increases survival in an orthotopic pancreatic cancer mouse model

Day 1: Aspc-1 (2X 10^5) implanted into pancreas (n=20)
Day 7: 0.42 mg/kg Minnelide or saline IP begins (n=10 for each cohort)
Day 100: Minnelide treatment stopped (n=5).
January 28, 2011 = 265 days
Minnelide decreases HSP70 expression

CONTROL  MINNELIDE

TUNEL

HSP70
Minnelide causes tumor regression in S2-013 cell-induced orthotopic pancreatic tumors. S2-013 is one of the most aggressive pancreatic tumor-derived cell line.
Minnelide Decreases Loco-regional Spread of Orthotopic Pancreatic 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>
</tbody>
</table>

S2-013 (Metastatic)

Day 1: S2-013 (1X 10^6) implanted into pancreas (n=20)
Day 7: 0.42 mg/kg Minnelide or saline IP begins
Day 28: Experiment terminated
Clinical Translation
University CTM/ITDD
Drug development Pilot Project

- Pre-clinical Efficacy
- Formal Toxicology Studies
- Development of Phase I Clinical Trial
  October 2011
- 3.5 million dollar investment by U of Minn.

Pre-IND Letter to FDA for Approval of
Triptolide as Treatment for Pancreatic Cancer
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  University of Minnesota

University of Minnesota
Just as when you see a turtle on a fence post or see program or person rise to a new level of achievement, you realize it didn't get there by itself!

FAITH   FAMILY   FRIENDS
My most pleasant dreams and worst nightmares of Minnesota