Big Team Science:
The Stand Up to Cancer Pancreas Cancer
“Dream Team”

Jeffrey A. Drebin, M.D., Ph.D.
John Rhea Barton Professor and Chair
Department of Surgery
Perelman School of Medicine, University of Pennsylvania

Disclosures

• U.S. Patent #5824311, awarded 10/20/98.
  – “Treatment of tumors with monoclonal antibodies against oncogene antigens.”
  – Inventors: M.I. Greene and J.A. Drebin.
• Owned by University of Pennsylvania
• Licensed to Genentech, Inc./Roche
• Wife employed by GSK
• Studies to be discussed funded by Stand Up to Cancer, Celgene, Genentech
A Patient’s Eye View of Surgical Researchers

“The surgical investigator must be a bridge tender, channeling knowledge from biological science to the bedside and back again. He traces his origins from both ends of the bridge - he is thus a bastard, and everyone calls him this. Those at one end of the bridge say that he is not a very good scientist, and those at the other end say he does not spend enough time in the operating room.”

Francis Moore, M.D., 1958

The Surgical Investigator as Translational Researcher

• “The surgical investigator must be a bridge tender, channeling knowledge from biological science to the bedside and back again. He traces his origins from both ends of the bridge - he is thus a bastard, and everyone calls him this. Those at one end of the bridge say that he is not a very good scientist, and those at the other end say he does not spend enough time in the operating room.”

Francis Moore, M.D., 1958
Pancreatic Cancer

- 5th most common cause of cancer death in USA – 40,000/yr
- Incidence increasing
- Annual incidence approximately equals annual deaths

Pancreas Cancer is a Particularly Lethal Malignancy

- 80-90% of patients are unresectable at diagnosis
- Five year survival of resected patients traditionally 5-20%
- Chemotherapy and radiation therapy of modest benefit
- Median survival of unresectable patients at diagnosis generally < 6 months

Department of Surgery, University of Pennsylvania Health System
Is Surgical Treatment Worse than the Disease in Pancreatic Cancer?

THE ADVANTAGES OF BYPASS OPERATIONS OVER RADICAL PANCREATEO/ODENECTOMY IN THE TREATMENT OF PANCREATIC CARCINOMA

George Crile, Jr., M.D., F.A.C.S., Cleveland, Ohio

Linkage Between Pancreatectomy Case Volume and Outcome

<table>
<thead>
<tr>
<th>Hospital Cases/Year</th>
<th>&lt;1</th>
<th>1-2</th>
<th>3-5</th>
<th>6-16</th>
<th>&gt;16</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Hospitals</td>
<td>1028</td>
<td>555</td>
<td>168</td>
<td>90</td>
<td>27</td>
</tr>
<tr>
<td>% Mortality</td>
<td>18</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Surgeon Cases/Year</th>
<th>1</th>
<th>2-4</th>
<th>&gt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Surgeons</td>
<td>1057</td>
<td>1054</td>
<td>124</td>
</tr>
<tr>
<td>% Mortality</td>
<td>15</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

Survival After Pancreas Cancer Resection at Penn

Department of Surgery, University of Pennsylvania Health System
Pancreatic Cancer: Cutting Off the Fuel Supply

Co-Principal Investigators:
Jeffrey A. Drebin, MD, PhD
Craig Thompson, MD
Dan Van Hoff, MD

Initial Hypothesis

• Glutamine is a preferential fuel source for pancreas cancer
  – May facilitate novel therapeutics
  – May permit improvements in imaging
  – Understanding tumor metabolism may facilitate other therapeutic options for targeted therapeutics
SU2C Pancreas Cancer Initial Trials

- Phase II – Targeted therapy using conventional chemotherapeutics (R. Ramanathan, PI)
- Phase II, then large randomized Phase III - Gem vs Gem-nab Paclitaxel (GA) (MPACT Trial) (D. VanHoff PI)
- Phase II - GA plus Hedgehog inhibitor (D. Laheru & A. DeJesus-Acosta, PIs)
- Imaging using Glutamine-based tracers (C. Thompson, PI)
- Tissue Acquisition and Analysis (J. Drebin, PI)

Stand Up to Cancer Pancreas Dream Team

- Team meetings every 3-4 months
- Presentation to Scientific Advisory Board every 6 months
- Annual presentation by PIs to SU2C leadership
- Strongly defined timeline/deliverables, with flexibility to modify projects
Protocol to Permit the Acquisition of Samples of Tumor and Normal Tissue for Biological Endpoints in Pancreatic Cancer

Collaborators

<table>
<thead>
<tr>
<th>University of Pennsylvania</th>
<th>Princeton</th>
<th>Johns Hopkins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peter O'Dwyer</td>
<td>Jurre Kemphorst</td>
<td>Anirban Maitra</td>
</tr>
<tr>
<td>Barbara Vance</td>
<td>Josh Rabinowitz</td>
<td>Rajesh Kumar</td>
</tr>
<tr>
<td>Charles Vollmer</td>
<td></td>
<td>Victor Velaculescu</td>
</tr>
<tr>
<td>Michael Feldman</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amy Anderson</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amy Kramer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Federico Valdevieso</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jenna Gates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Tgen/VARI                  |                               |                     |
| Mike Barrett               |                               |                     |
| Matthew Chung               |                               |                     |
| Sandra Cottingham           |                               |                     |
| Michael Demeure             |                               |                     |
| Craig Webb                  |                               |                     |

| Salk Institute             |                               |                     |
| Michael Downes             |                               | Geoff Wahl          |
| Tony Hunter                |                               | Mark Wade           |
| Ron Evans                  |                               |                     |
| Harry Evans                |                               |                     |
| Michael Downes             |                               |                     |
| Tony Hunter                |                               |                     |
| Geoff Wahl                 |                               |                     |
| Mark Wade                  |                               |                     |
Specimen Flow
(informed consent obtained pre-op)

Operating Room
Blood Draw
Operative Specimen

Surgical Pathology
Frozen Section
Fresh Tumor
Snap Freezing (N₂)
tumor
normal
Processing of Blood
serum
buffy coat

O'Dwyer Lab (Penn)
Stellate Cell Isolation

Salk Institute
Stellate cell studies

Princeton
Metabolomic Profiling

Johns Hopkins
DNA Analysis

TGEN
Flow Karyotyping Nuclei

Tissue Acquisition Protocol - Results

Operating Room
Blood Draw
Operative Specimen

Surgical Pathology
Frozen Section
Fresh Tumor
Snap Freezing (N₂)
tumor
normal
Processing of Blood
serum
buffy coat

O'Dwyer Lab (Penn)
Stellate Cell Isolation

Salk Institute
Stellate cell studies

Princeton
Metabolomic Profiling

Johns Hopkins
DNA Analysis

TGEN
Flow Karyotyping Nuclei
Metabolomic Analysis of Human Pancreatic Cancer

Jurre Kamphorst and Joshua Rabinowitz
Princeton University

Sample preparation and analysis protocol

**SAMPLE PREPARATION**
- Tissue weight
- Metabolite extraction, 80% MeOH
- Lipid extraction
- Protein extraction
- Cryogenic ball mill
- 5-10 mg stored in liquid nitrogen
- 3X

**METABOLOMIC ANALYSIS**
- 3X
- Exactive
- TSQ Quantum Max
- TSQ Quantum Ultra
- Targeted Quan (-) Mode MRM
- Accurate Mass (-) Mode
- Full Scan MS
- Targeted Quan (+) Mode MRM

Surgical resection at Penn
Heatmap 1: All samples
Metabolite profiles, Tumor (T) & Normal (N)
266 metabolite-specific measurements

Fold-change in tumor vs. normal
The nutrient content of plasma proteins and lipids exceeds free glucose and amino acids

- The energy content of circulating lipids exceeds blood glucose
- The amino acid content of plasma proteins (majority albumin) exceeds free amino acids by ~200-fold

Metabolomic profile is consistent with protein degradation being the main amino acid source

F, I, L, M, P, V, Y
G, Q, S
New protein synthesis
Nucleotide synthesis

Plasma protein catabolism

Department of Surgery, University of Pennsylvania Health System
Most cultured cells cannot live off intact protein

- Albumin cannot substitute for essential amino acids in HeLa cells
- Only 3% - 6% of amino acids in HeLa cells derived from extracellular protein

Eagle and Piez, 1960
Albumin rescues pancreatic cancer cells from glutamine deprivation

Q  = Glutamine
Alb  = Albumin
EIPA  = Pinocytosis inhibitor

Pancreas Cancers Driven By Mutant K-Ras Show Enhanced Macropinocytosis
Inhibiting Macropinocytosis Inhibits K-ras Mutant Pancreas Cancer Xenografts

Tissue Acquisition Protocol – Results

Operating Room
- Blood Draw
- Operative Specimen

O’Dwyer Lab (Penn)
- Stellate Cell Isolation

Surgical Pathology
- Frozen Section
- Fresh Tumor
- Snap Freezing (N₂)
- tumor normal
- Processing of Blood serum
- buffy coat

Salk Institute
- Stellate cell studies

Princeton
- Metabolomic Profiling

Johns Hopkins
- DNA Analysis

TGEN
- Flow Cytometry Nuclei

Department of Surgery, University of Pennsylvania Health System
Flow Sorting of Aneuploid Nuclei Facilitates Cancer Specific DNA Sequencing

DNA Sequencing Identifies Patients with a Survival Advantage

- Mutation in a 3 gene chromatin regulatory gene family in about 25%
- No difference in gender, tumor location, tumor size, tumor differentiation, use of adjuvant Rx
- Nodal metastases more common in mutant tumors
- No differences in mutation frequency of other known pancreas cancer genes (k-ras, dpc4, cdkn2, p53)
## DNA Sequencing Identifies Patients with Survival Advantage

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>N</th>
<th>Chromatin Remodeling Genes</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutant</td>
<td>Wild-Type</td>
<td></td>
</tr>
<tr>
<td>Total Patients</td>
<td>52</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Age at Diagnosis (years)</td>
<td>52</td>
<td>66.8</td>
<td>67.2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Tumor Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IIA</td>
<td>14</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>IIB</td>
<td>35</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>Tumor Size (cm)</td>
<td>50</td>
<td>3.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Lymph Nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>34</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>29</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Moderate</td>
<td>21</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Well</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Neoadjuvant Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>50</td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td>Adjuvant Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>KRAS Mutant</td>
<td>46</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td>KRAS Wild-Type</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SMAD4 Mutant</td>
<td>16</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>SMAD4 Wild-Type</td>
<td>36</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>TP53 Mutant</td>
<td>41</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>TP53 Wild-Type</td>
<td>15</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>CDKN2A Mutant</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CDKN2A Wild-Type</td>
<td>20</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>
Specimen Flow
(informed consent obtained pre-op)

Operating Room
Blood Draw
Operative Specimen

Surgical Pathology
Frozen Section
Fresh Tumor
Snap Freezing ($N_2$)
tumor
normal
Processing of Blood
serum
buffy coat

O’Dwyer Lab (Penn)
Stellate Cell Isolation

Salk Institute
Stellate cell studies

Princeton
Metabolomic Profiling

Johns Hopkins
DNA Analysis

TGEN
Flow Karyotyping Nuclei

Stellate Cell Studies

Department of Surgery, University of Pennsylvania Health System
Tumors are Complex Tissues – the Role of Stroma

Current model of PSCs in carcinogenesis
Pancreas Stellate Cells Are Enriched for Vitamin D Receptors

Activation of VDR decreases cancer/activation-associated genes in primary hPSC

Implications in vivo?
Transgenic Mice

- KPC Mouse Model (Tuveson and Hingorani)
- Pancreas-specific K-Ras mutant, p53 knockout mouse model
- Reproducible development of pancreas cancer

Transgenic Pancreatic Cancer
Pre-Clinical Vitamin D Studies

- KPC mice
- Effects on tumor stroma, metastases, drug penetration and response to therapy
- Use in chemoprevention of pancreatic cancer

Other Human Pancreatic Stellate Cell Studies at Salk Institute

- Kinome profiling of pancreatic stellate cells
- Video microscopy for evaluating stellate cell – cancer cell interactions
- P53 targeting in pancreatic stellate cells
Future Directions

- Continue metabolomic analysis
- Continue sequencing analysis
  - Additional confirmatory sample sets
  - Define subsets predictive of outcome
  - Identify therapeutic targets
- Preclinical Vitamin D studies
- Clinical Vitamin D studies

Summary

- Pancreas cancer is a particularly lethal malignancy
- The Pancreas “Dream Team” successfully competed for funding from SU2C based on excellent science, clinical research experience, and the prospect of synergistic interactions among team members
- We were successful in receiving supplemental funding based on collaborative multidisciplinary success
### Collaborators

<table>
<thead>
<tr>
<th>University of Pennsylvania</th>
<th>Tgen/VARI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anirban Maitra</td>
<td>Dan VanHoff</td>
</tr>
<tr>
<td>Rajesh Kumar</td>
<td>Mike Barrett</td>
</tr>
<tr>
<td>Victor Velculescu</td>
<td>Matthew Chung</td>
</tr>
<tr>
<td>Sandra Cottingham</td>
<td>Michael Demeure</td>
</tr>
<tr>
<td>Michael Demeure</td>
<td>Craig Webb</td>
</tr>
<tr>
<td>Craig Thompson</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Princeton</th>
<th>Tgen/VARI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurre Kemphorst</td>
<td>Dan VanHoff</td>
</tr>
<tr>
<td>Josh Rabinowitz</td>
<td>Mike Barrett</td>
</tr>
<tr>
<td></td>
<td>Matthew Chung</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salk Institute</th>
<th>Tgen/VARI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ron Evans</td>
<td>Dan VanHoff</td>
</tr>
<tr>
<td>Michael Downes</td>
<td>Mike Barrett</td>
</tr>
<tr>
<td>Tony Hunter</td>
<td>Matthew Chung</td>
</tr>
<tr>
<td>Geoff Wahl</td>
<td>Michael Demeure</td>
</tr>
<tr>
<td>Mark Wade</td>
<td>Craig Webb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Johns Hopkins</th>
<th>Tgen/VARI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dan VanHoff</td>
</tr>
<tr>
<td></td>
<td>Mike Barrett</td>
</tr>
<tr>
<td></td>
<td>Matthew Chung</td>
</tr>
<tr>
<td></td>
<td>Michael Demeure</td>
</tr>
<tr>
<td></td>
<td>Craig Webb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tgen/VARI</th>
<th>Tgen/VARI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dan VanHoff</td>
<td>Dan VanHoff</td>
</tr>
<tr>
<td>Mike Barrett</td>
<td>Mike Barrett</td>
</tr>
<tr>
<td>Matthew Chung</td>
<td>Matthew Chung</td>
</tr>
<tr>
<td>Michael Demeure</td>
<td>Michael Demeure</td>
</tr>
<tr>
<td>Craig Webb</td>
<td>Craig Webb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>And Special Thanks to SU2C</th>
</tr>
</thead>
</table>

![SU2C Logo](image)