Pancreatic Cancer Research

Are we making strides to improve outcomes in the clinic?

Getting from Bench to Bedside

1. **Define** the problem(s): the **Puzzle**
2. **Create** helpful tools: what’s needed
3. **Employ** tools: pieces to the puzzle
4. **Gain** knowledge: put pieces together
5. **Design** an Approach: best ways for the job
6. **Test** new concept: parts of puzzle solved
The PUZZLE

Number and type of pieces determines the difficulty

30 pieces 120 pieces

The PUZZLE

and it can get complicated

5000 pieces, layered, 3D
The **PUZZLE**

In Pancreatic Cancer, there are

1. probably over 500 gene alterations
2. in general, 65 may be causative

So, 65 pieces? 500 pieces? More?

The **PUZZLE**

and it can get messy

stroma - fibrosis
Inflammation
fat/adipose

1000 pieces
Why?

Pancreatic Cancer is poorly detected, diagnosed, and treated

1. Location
2. Late symptoms
3. Spreads with small primary tumor
4. Dense surrounding
5. Drug resistant

Create Tools

Modeling systems that mimic human PC

1. cell culture
2. organoids
3. animals

But there are limitations

1. cell and molecular level
2. whole body effects
## Cell Lines

<table>
<thead>
<tr>
<th>HPDE</th>
<th>HPDE-Kras cells</th>
<th>Stellate cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>MiaPaCa2</td>
<td>BxPC3</td>
<td>Panc1</td>
</tr>
</tbody>
</table>

## Mouse Models

**Tools & Switches**

1. A candidate gene - usually altered in human cancer
   - Mutant Kras, mutant p53, Smad4 loss

2. A gene switch (promoter) that can target specific cell types
   - Elastase (EL), Pdx1, p48

3. Ability to genetically manipulate in order to insert the gene
   - Transgenesis, targeted manipulation, CRISPR-Cas9
Transgenesis: EL-Kras

Pancreatic Lesions from EL-Kras Mice Resemble Human Disease

human KRAS\textsuperscript{G12D} PanINs

mus Cystic Neoplasms

\textsuperscript{Cancer Res.} 2003 May 1;63(9):2016-9
**KC & KPC Mice Resemble Human Disease**

**PanINs**

**PDAC**

*human*

*mus*

Pdx1-Cre/LSL-Kras (KC)

_Cancer Cell. 2003 Dec;4(6):437-50_

Pdx1-Cre/LSL-Kras/p53<sup>R172H</sup> (KPC)

_Cancer Cell. 2005 May;7(5):469-83_

---

**Create Tools**

**Tools are more than just models**

1. certain technique/technology
2. discovery: eg. **PC subtypes**
3. combination
Genomic analyses identify molecular subtypes of pancreatic cancer

Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, etc.

*Nature*. 2016 Mar 3;531(7592):47-52

Establishing four rather unique subtypes of pancreatic cancer based on gene expression

---

Four Different **PC Subtypes**:

1. Squamous
2. Pancreatic Progenitor
3. Immunogenic
4. Aberrantly Differentiated Endocrine Exocrine (ADEX)

Defined by high expression of specific gene programs
Employ these Tools

Ask the “right” question
puzzle pieces that connect two sections
how other cell types affect cancer

Know your limitations
tools – different than in vivo human
redefining the problem
Gain Knowledge

It’s just about the data . . . but not so fast!

Things to consider:

1. Did we ask the “right” question?
2. What do our findings really mean?
3. Did we use the right tools?
4. How do we interpret the data?
5. Is this translatable?

How do we get from Bench to Bedside

1. Define the problem(s): the Puzzle
2. Create helpful tools: what’s needed
3. Employ tools: pieces to the puzzle
4. Gain knowledge: put pieces together
5. Design an Approach: best ways for the job
6. Test new concept: parts of puzzle solved
Example 1

Chemoresistance in pancreatic cancer is driven by stroma-derived insulin-like growth factors


Tying together a property of cancer cells with stromal cells secreting IGFs (related to islet cells)

---

Example 1

Define: chemoresistance caused by IGF from cells
Create tools: primary cell culture with conditioned media
Employ: cell culture, primary cells, mouse models
Gain Knowledge:
Example 2

The triterpenoid CDDO-imidazolide reduces immune cell infiltration and cytokine secretion in Kras$^{G12D}$; Pdx1-Cre (KC) mouse model of pancreatic cancer.

Leal AS, Sporn MB, Pioli PA, Liby KT. *Carcinogenesis*. 2016 Sep 22

Demonstrating that inflammation can dramatically enhance PC which can be blocked by CDDO

---

Example 2

Define: immune suppression/inflammation drives worse disease
Create tools: use existing tools
Employ: mouse models, measure blood proteins
Gain Knowledge:
Example 3

TGFβ Signaling in the Pancreatic Tumor Microenvironment Promotes Fibrosis and Immune Evasion to Facilitate Tumorigenesis


Suppression of TGFβ in immune cells can promote tumor cell killing in the pancreas

Example 3

Define: block immunosuppression to kill pancreatic tumor cells
Create tools: design new models, cells in co-culture
Employ: employ 2 mouse models, measure CTL markers
Gain Knowledge:
EL-Kras + altered TGFβ signals

H&E and Trichrome Staining of Mouse Pancreas

<table>
<thead>
<tr>
<th>EK</th>
<th>EKT2</th>
<th>EKT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td>![H&amp;E Image]</td>
<td>![H&amp;E Image]</td>
</tr>
<tr>
<td>Trichrome</td>
<td>![Trichrome Image]</td>
<td>![Trichrome Image]</td>
</tr>
</tbody>
</table>

TGFβ & Signals Increased in Co-Culture

Panc1 Cells co-cultured with hPSCs + TGFβ and/or Stromal - LY

Co-Culture
- - + + + + + + + + + + +
TGFβ
- - - + - + - + - + - + - + - + +
Stromal – LY
- - - - - - - - - - - - - - - - -

Western Blot Images:
pSMAD2, SMAD4, pERK, ERK, p21, GAPDH
TGFβ signals may support Treg cell function

CD3/Foxp3 IF colocalization and CD8 IHC Staining of Mouse Pancreas

Tgfbr1+/− may increase T-cell Cytotoxicity

Granzyme B IF and Cleaved Caspase-3 IHC of mouse pancreas
Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion

Cristovão M. Sousa, Douglas E. Biancur, Xiaoxu Wang1, Christopher J. Halbrook, Mara H. Sherman, Li Zhang, Daniel Kremer, Rosa F. Hwang, Agnes K. Witkiewicz, Haoqiang Ying, John M. Asara, Ronald M. Evans, Lewis C. Cantley, Costas A. Lyssiotis, & Alec C. Kimmelman


Stromal cells go through a quiescent program whereby they secrete alanine which becomes food for PC cells

Define: PC cells use amino acids over glucose provided by stroma
Create tools: cleverly designed metabolic chase studies
Employ: employ orthotopic model with inducible Kras expression
Gain Knowledge:
Example 4

Example 5

Metastatic Pancreatic Adenocarcinoma After Total Pancreatectomy Islet Autotransplantation for Chronic Pancreatitis
S. Muratore, X. Zeng, M. Korc, S. McElyea, J. Wilhelm, M. Bellin and G. Beilman


PC patient goes from bad to worse following islet transplant: from CP to metastatic PDAC
Example 5

Define: providing islets to CP patients to prevent diabetes
Create tools: AIT is a current technology offered to patients
Employ: surgery and AIT
Gain Knowledge:

Something that was learned:

Something for the future: miRNA profile to decipher CP from PC
MR images of mouse pancreas

wt mouse pancreas  EL-Kras mouse pancreas

MR, H&E, & 3D Images of Mouse Pancreas

MR microimage of the 7-month old Pdx1-Cre/LSL-Kras mouse pancreas Left: 2D view of a representative slice. Right: 3D volume-rendered image
How do we get from Bench to Bedside

1. **Define** the problem(s): the **Puzzle**
2. **Create** helpful tools: what’s needed
3. **Employ** tools: pieces to the puzzle
4. **Gain** knowledge: put pieces together
5. **Design** an Approach: best ways for the job
6. **Test** new concept: parts of puzzle solved

Design an Approach

An approach that **helps the patient**
working to understand both failure & success

Applying what we learn to **impact the clinic**
a few direct but most indirect

Probably **not use the same approach**
Test new concept

Before implementation in patients, test

1. if compound or approach work \textit{in vivo}, same in human PC?
2. if same, delivery parameters may be different
3. if different, why?
4. consider failed preclinical for test in human source

Time for a clinical trial: passive vs. aggressive

Example 1

Chemoresistance in pancreatic cancer is driven by stroma-derived insulin-like growth factors

PC cell chemoresistance is derived from IGF secreted from stromal cells


In lung cancer, breast cancer, and \textbf{pancreatic cancer} failed to show clinical benefit
Possible reasons for failure:
   1. complexity of the IGF-1R/insulin receptor system & downstream signals
   2. a lack of patient selection markers

Alternatives: identification of predictive markers and rational combinations

One compound used in a preclinical setting: Everolimus (IGF-1 inhibitor) – human trial?
Example 2

The triterpenoid CDDO-imidazolide reduces immune cell infiltration and cytokine secretion in Kras<sup>G12D</sup>;Pdx1-Cre (KC) mouse model of pancreatic cancer.

Demonstrating that inflammation can dramatically enhance PC which can be blocked by CDDO

Synthetic triterpenoid induces 15-PGDH expression and suppresses inflammation-driven colon carcinogenesis

*JCI* 2014; 124(6):2472-82

Other work:

Phase II clinical trials
1. pulmonary arterial hypertension;
2. reprogram the immune system and attack tumors (CTL)

Example 3

**TGFβ Signaling in the Pancreatic TME Promotes Fibrosis & Immune Evasion to Facilitate Tumorigenesis**

Suppression of TGFβ in immune cells can promote tumor cell killing in the pancreas

Galunisertib = TGFBR1 inhibitor

phase I clinical trials including combination clinical trials patients with glioblastoma, HCC, and **pancreatic cancer**.

Shows promise in HCC
Example 4

Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion

Stromal cells go through a quiescent program whereby they secrete alanine which becomes food for PC cells

How do we stop this?

Clinical trials using HCQ = inhibit the lysosome, reduce autophagy

Example 5

Metastatic PDAC After Total Pancreatectomy Islet Autotransplantation for Chronic Pancreatitis

PC patient goes from CP to metastatic PDAC
How do we get from Bench to Bedside

1. Define the problem(s): the Puzzle
2. Create helpful tools: what’s needed
3. Employ tools: pieces to the puzzle
4. Gain knowledge: put pieces together
5. Design an Approach: best ways for the job
6. Test new concept: parts of puzzle solved

HOPE
... and the rest of the UIC Gang

---

## Acknowledgements

### Main Collaborators
- Dr. Barbara Jung
- Dr. Nissim Hay
- Dr. Giamilia Fantuzzi
- Dr. HG Munshi (NU)
- Dr. David Bentrem (NU)
- Dr. Khash Khazaie (Mayo)

### Lab Investigators

#### Current:
- Ronald McKinney
- Dr. Michelle Schultz
- Dr. Carolina Perales-Torres
- Dr. Steve Waters
- Georgina Mancinelli
- Karla Castellanos
- Danny Principe

#### Animal Welfare:
- Charlie Williams
- Dr. Jeanette Purcell
- Leo Trinidad
- Tom Reynolds
- Lloyd Taylor

#### Past:
- Dr. Richard Bell
- Dr. Thomas Adrian
- Dr. Xianxong Ding
- Dr. Mark Knab
- Dr. Lifeng Cai
- Dr. Seth Krantz
- Dr. Matt Strough
- Dr. Eric Cheon
- Dr. Bhargava Mullapudi
- Dr. Yongzeng Ding
- Dr. Arash Samiei
- Dr. Laleh Melstrom

### EL-Kras and Continuing Mentorship
- Dr. Eric Sangren (UW-Madison)

### EL-Kras/TGFβ Project
- Dr. Boris Pasche (Wake-Forest)
- Dr. Qinghua Zeng
- Dr. Mike Pennison
- Dr. Laurent Bartholin (INSERM)

### Mouse MRI
- Dr. Alice Wyrwicz (NorthShore)
- Dr. PN Venkatsubramanian
- Dr. Rich Knop
- Dr. Limin Ji
- Dr. George Iordanescu

### Mouse Model Systems
- Dr. Nabeel Bardeesy (Dana-Farber/Harvard)
- Dr. Emilio Hirsch (University of Torino)
- Dr. Robert Schwabe (Columbia)

### Cell Culture Systems
- Dr. Ming-Sound Tsao (Ontario Cancer Inst.)
- Dr. Michel Ouellette (UNMC)
- Dr. Rosa Hwang (MD Anderson)
- Dr. Andrew Lowy (UCSD)

### Funding Sources:
- NIH R01 CA 161283
- NIH R21 CA 123041
- PanCAN CDA 07-20-25-GRIP
Be Bold . . . and Take the Cat by the Ears