Generating immunogenic animal models of pancreatic ductal adenocarcinoma

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Cancer-immunity cycle

1. Release of cancer cell antigens (cancer cell death)
2. Cancer antigen presentation (dendritic cells/APCs)
3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumors (CTLs)
5. Infiltration of T cells into tumors (CTLs, endothelial cells)
6. Recognition of cancer cells by T cells (CTLs, cancer cells)
7. Killing of cancer cells (immune and cancer cells)
Many factors limit efficacy of anti-tumor T cells

Weak tumor antigens

Limit T cell activation

Cancer antigen presentation

Limit innate activation

Priming and activation

Anti-CTLA4
Anti-CD137 (agonist)
Anti-OX40 (agonist)
Anti-CD27 (agonist)
IL-2
IL-12

Tumor antigen presentation

Vaccines
IFN-α
GM-CSF
Anti-CD40 (agonist)
TLR agonists

Release of cancer cell antigens

Chemotherapy
Radiation therapy
Targeted therapy

Limit T cell access/recognition

Limit T cell activation

Trafficking of T cells to tumors

Trafficking of T cells to tumors

Infiltration of T cells into tumors

Tumor

Recognition of cancer cells by T cells

Anti-VEGF

CARs

Killing of cancer cells

Anti-PD-L1
Anti-PD-1
IDO inhibitors

Chen DS and Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity.* July 2013
• Immunotherapy efficacy **uncertain** in PDAC

Spranger S, Gajewski T - J Immunother Cancer (2013)

Adapted from Merck graphic “Immunoregulatory Therapy in Oncology: Bending the survival curve,” Slide courtesy N. Joshi
KPC model of developing PDAC

Current Model: KPC Mouse

\[ \text{Kras}^{\text{LSL.G12D}}; p53^{\text{R172H}}; \text{PdxCre}^{\text{tg}}/+ \]

- Tumors are induced in the pancreas
- Develops full range of precancerous lesions seen in humans
- Similar morphology
- 80% of PDA metastasizes
- Resistant to chemotherapy

Can we introduce neoantigens into more translationally relevant pancreatic cancer models?
**NINJA:** iNversion INduced JOneed neoAntigen system

[Diagram showing the process of gene manipulation involving Kras WT, p53 WT, Cre recombinase, Kras G12D, p53 null, Doxycycline, Tamoxifen, and resulting cell types (Normal cell, Transformed cell, Neoantigen+ Transformed cell).]

- **Normal cell (GEMM model)**
- **Transformed cell**
- **Neoantigen+ Transformed cell**

**Locations:**
- Pancreas
- Lung
- Muscle
NINJA lung tumors infiltrated by immune cells

Control tumor
(Cold tumor)

NINJA+ tumor
(Hot tumor)

T cells
Neoantigen
B cells
Lung cells
Making an autochthonous immunogenic PDAC model

Current Model: KPC Mouse

Kras<sup>LSL.G12D/+;p53<sup>R172H/+;PdxCre<sup>tg/+</sup></sup>

- KP (no Cre)
  - No tumor
- KP pdx1-Cre
  - Focal tumors
  - Expressing neoantigens
- KP pdx1-Cre/NINJA
  - Focal tumors
  - Neoantigen throughout tissue

Brittany Fitzgerald, Gena Foster
KPC NINJA pancreatic cell lines:

16 week Kras\(^*\) p53 fl/+ NINJA/+ pdx1-cre

Early culture of cells (d2)

Current culture of cells (d36)

OFF

FlpoER

OFF

neoAntigen

OFF

neoAntigen

ON

neoAntigen

Ad FLPo

72 hours post-infection

Neoantigen off

GFP+

3%

GFP+

43.8%

Neoantigen ON

Brittany Fitzgerald

Gena Foster
Pancreatic organoids
Making organoids from normal mouse pancreas

Organoids from human PDAC – PDX tissue

Genomics/CyToF

Tumor infiltrating lymphocyte (TIL) expansion

Passage in mice

Immunocompromised Mouse

Day 10, 4-6 months

Surgical resection - Ronald Salem - Marie Robert

Co-culture?

In vitro screens

In vivo analyses?

Organoid culture

Gene Yoo
Ryan Sowell
Sue Kaech
Generating PDAC organoids from endoscopic biopsies

Only 15% of patients have resectable disease at diagnosis.

Organoids derived from biopsies can capture full spectrum of PDACs – including surgically non-resectable tumors and tumors of various stages.

Human PDAC organoids can be reliably established using endoscopic biopsies in a period of ~7 days.

Gene Yoo / James Farrell
Human PDAC Organoid Library

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- Transplantable tumor models in immunocompromised animals?
- Source of tissue for analysis / hypothesis generation
Orthotopic transplant into pancreas
Serial transplant to drive tumor progression
Mechanistic analyses of immune function / therapy

Tuveson Cell 2015 Transplant of mPanIN derived organoid
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