NYU Langone MEDICAL CENTER

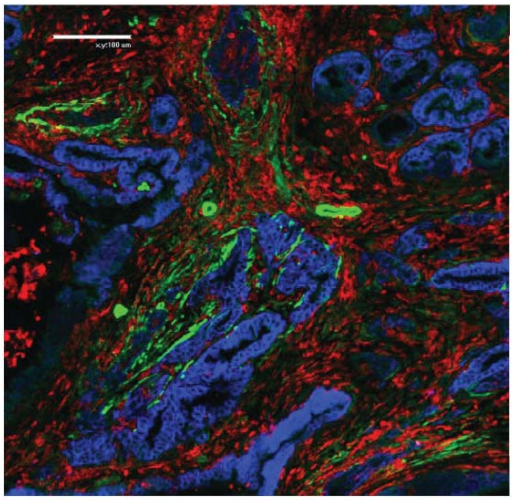
Microbial Programming of the Tumor Microenvironment

George Miller, MD
NYU School of Medicine

Disclosures

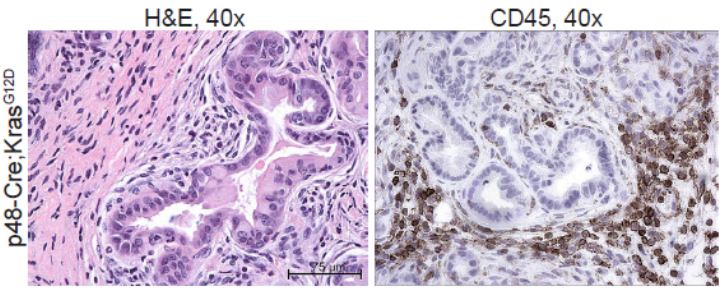
- RCA with GSK
- Co-Founder NYBO Therapeutics

Inflammatory Context of Pancreatic Cancer



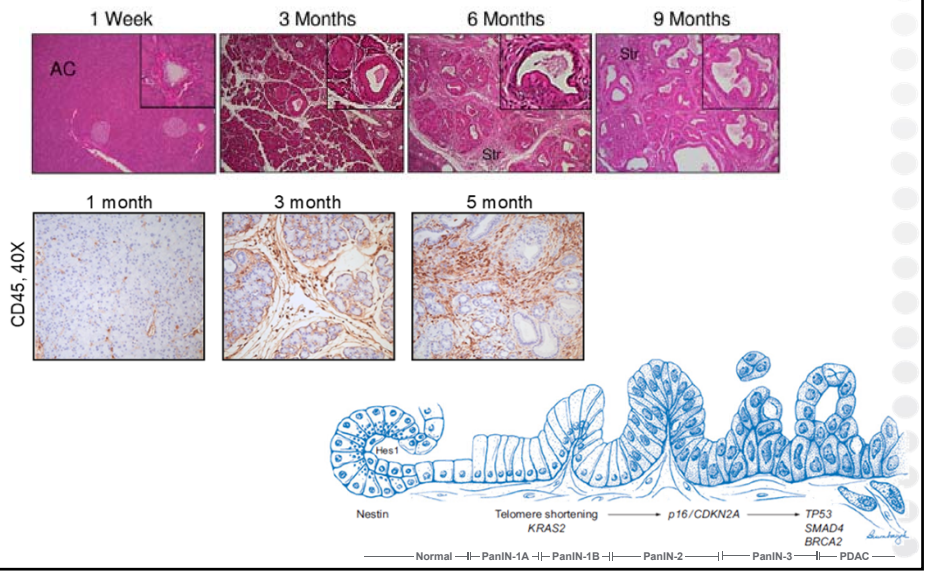
Feig et al., Clin Can Res 2012

Inflammatory Context of Pancreatic Cancer



Mallen-St.Clair et al., Genes & Development 2012

Inflammation is a Precursor to Dysplasia



Inflammation is Necessary for Pancreatic Oncogenesis

Cancer Cell
Article

Chronic Pancreatitis Is Essential for Induction of Pancreatic Ductal Adenocarcinoma by K-Ras Oncogenes in Adult Mice

Carmen Guerra,[1,*] Alberto J. Schuhmacher,[1] Marta Cañamero,[2] Paul J. Grippo,[3] Lena Verdaguer,[1] Lucía Pérez-Gallego,[2,6] Pierre Dubus,[4] Eric P. Sandgren,[5] and Mariano Barbacid[1,*]
[1]Molecular Oncology Program
[2]Biotechnology Program
Centro Nacional de Investigaciones Oncológicas, E-28029 Madrid, Spain

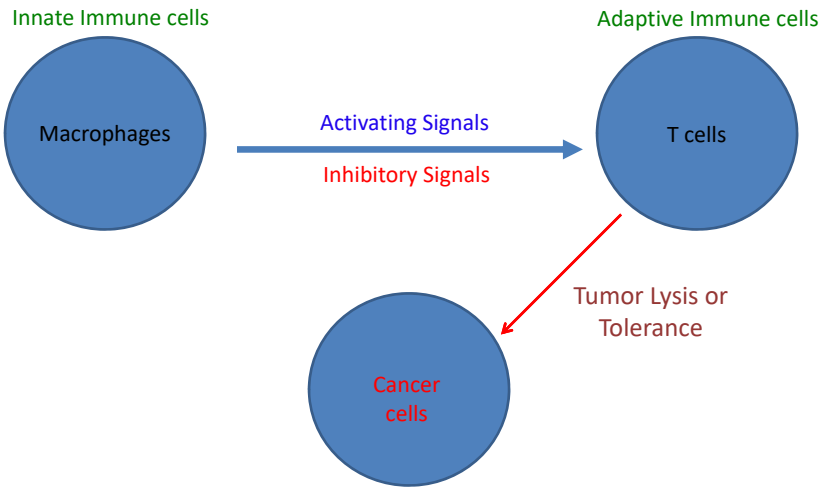
SIGNIFICANCE

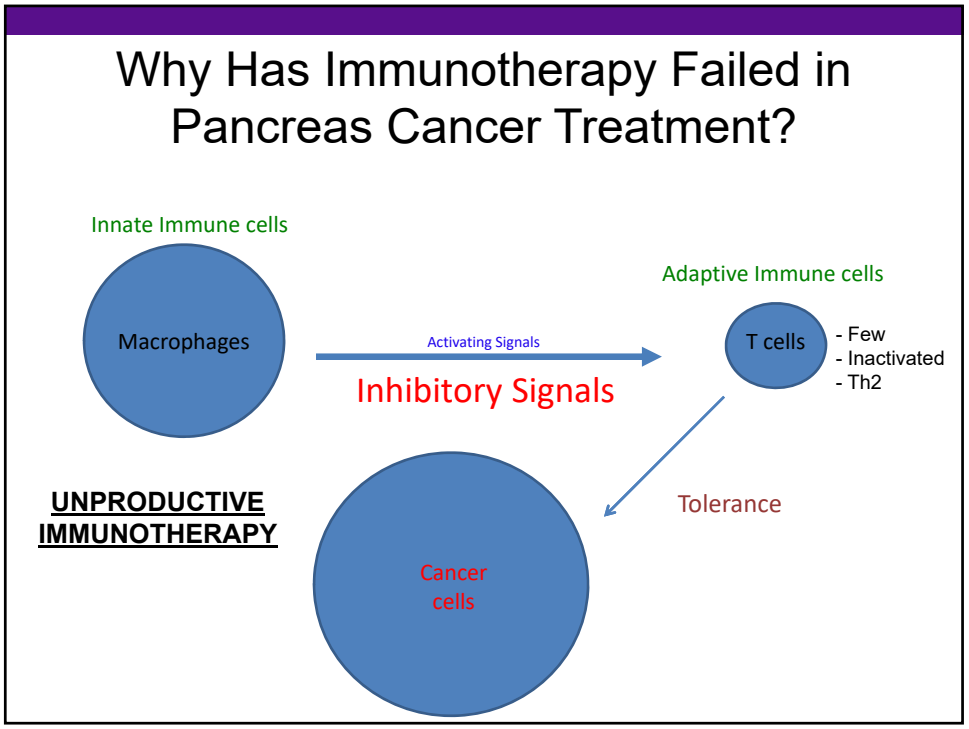
Human PanINs and PDA have been faithfully reproduced in mouse models by expressing an endogenous K-*Ras* oncogene in pancreatic lineages during embryonic development. Here, we describe a mouse model that allows controlled temporal expression of an endogenous K-Ras^{G12V} oncogene in cells of acinar and centroacinar origin. These mice develop the full spectrum of PanINs and invasive PDA when K-Ras^{G12V} expression is allowed during embryonic development. Surprisingly, K-Ras^{G12V} expression in adult mice does not result in neoplastic development unless they undergo chronic pancreatitis. Previous epidemiological studies have identified pancreatitis as a risk factor for human PDA. Thus, close monitoring of people who may have suffered pancreatic tissue damage may help to identify PDA patients in the early stages of the disease.

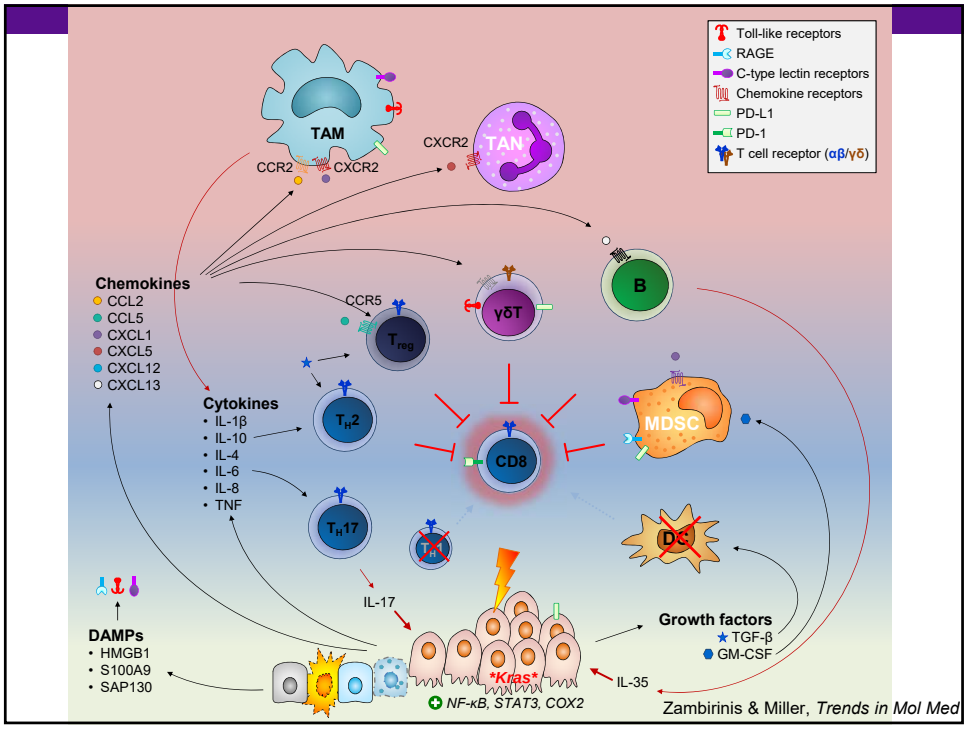
Cancer Cell *11*, 291–302, March 2007

Why has pancreatic cancer immunotherapy failed?

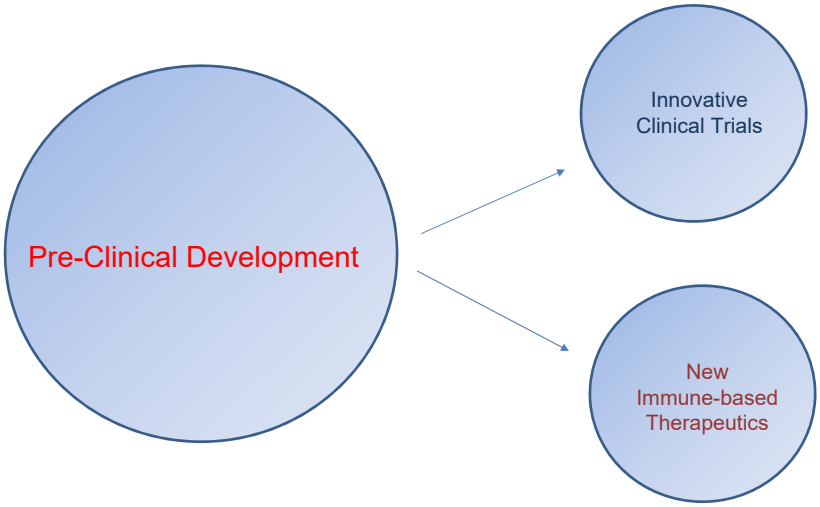
Cancer Immunology




Why Has Immunotherapy Failed in Pancreas Cancer Treatment?
Innate Immune cells
Macrophages
Activating Signals
Inhibitory Signals
Adaptive Immune cells
T cells
- Few
- Inactivated
- Th2
UNPRODUCTIVE IMMUNOTHERAPY
Tolerance
Cancer cells

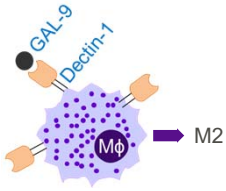

Toll-like receptors
RAGE
C-type lectin receptors
Chemokine receptors
PD-L1
PD-1
T cell receptor (αβ/γδ)
TAM
CCR2
CXCR2
TAN
CXCR2
Chemokines
CCL2
CCL5
CXCL1
CXCL5
CXCL12
CXCL13
CCR5
Treg
γδT
B
Cytokines
IL-1β
IL-10
IL-4
IL-6
IL-8
TNF
TH2
CD8
MDSC
TH17
TH1
DC
IL-17
DAMPs
HMGB1
S100A9
SAP130
Kras
NF-κB, STAT3, COX2
IL-35
Growth factors
TGF-β
GM-CSF
Zambirinis & Miller, Trends in Mol Med

Immunotherapy Pipeline



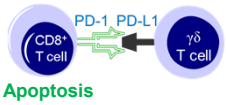
Pancreas Cancer Immunotherapy: From Basic Discovery to Development of Clinical Trials & New Therapeutics

Nature Medicine 2017



Design of αGAL-9 Ab
NYBO Therapeutics

Cell 2016

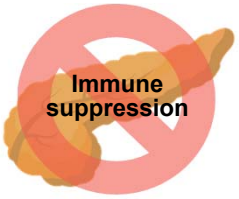


Apoptosis

Design of α-γδ T cell Ab
NYBO Therapeutics

NYU Langone Health

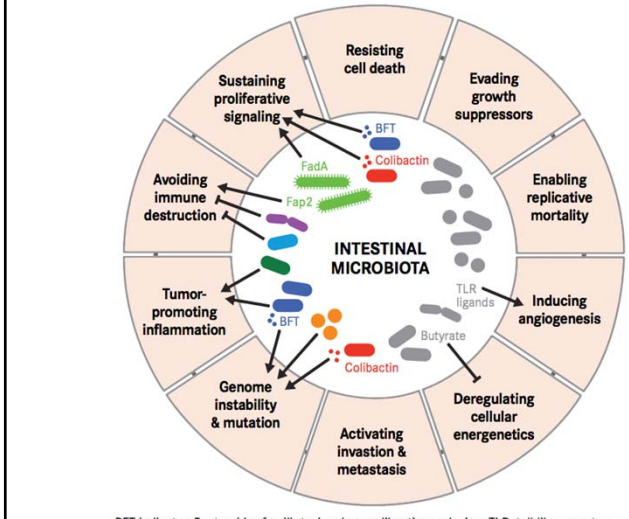
Pancreas Cancer Immunotherapy: From Basic Discovery to Development of Clinical Trials & New Therapeutics



Nature 2016
Clinical Trial (RIP1 Inhibitor)

Cancer Discovery 2018
Clinical Trial (Antbiotics + αPD-1)

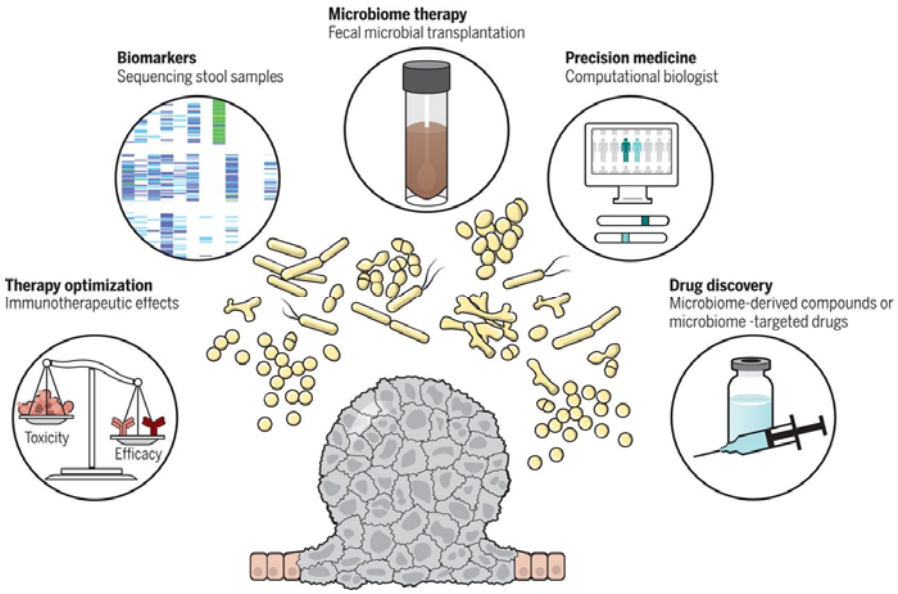
Gastroenterology 2016
Clinical Trial (RT+ αM-CSFR)



BFT indicates *Bacteroides fragilis* toxin; pks+, colibactin-producing; TLR, toll-like receptor.

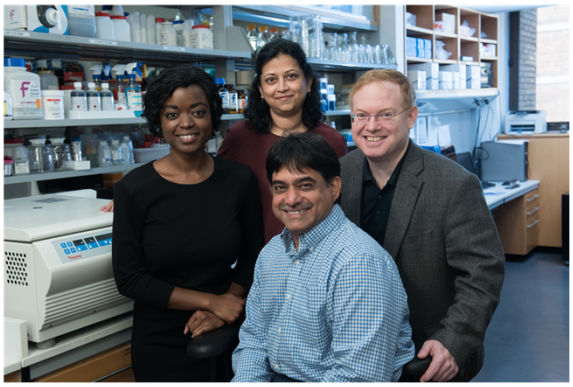
A growing body of clinical evidence has uncovered links between the microbiota and the Hallmarks of Cancer. These include butyrate, a short-chain fatty acid; colibactin, a genotoxin; and FadA and Fap2, bacterial mechanisms of *Fusobacterium nucleatum*.

Fulbright LE, Ellermann M, Arthur JC. The microbiome and the hallmarks of cancer. *PLoS Pathog.* 2017;13(9):e1006480. doi :10.1371/journal.ppat.1006480.



Smruti Pushalkar

Mautin Hundeyin



Deepak Saxena

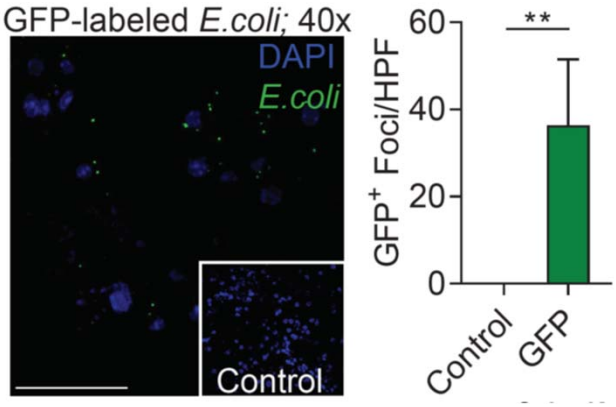
Hypothesis

In genetically susceptible hosts the microbiome alters the risk of pancreatic carcinogenesis and can promote aggressive disease biology

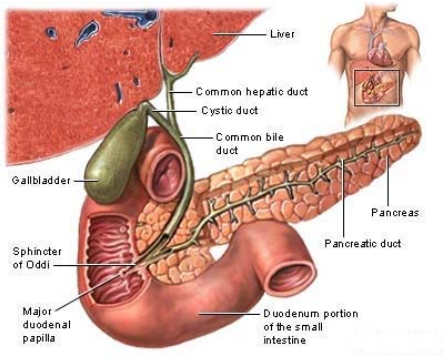
Question

Can Gut Bacteria Access the Pancreas?

Gut Bacteria can access the pancreas



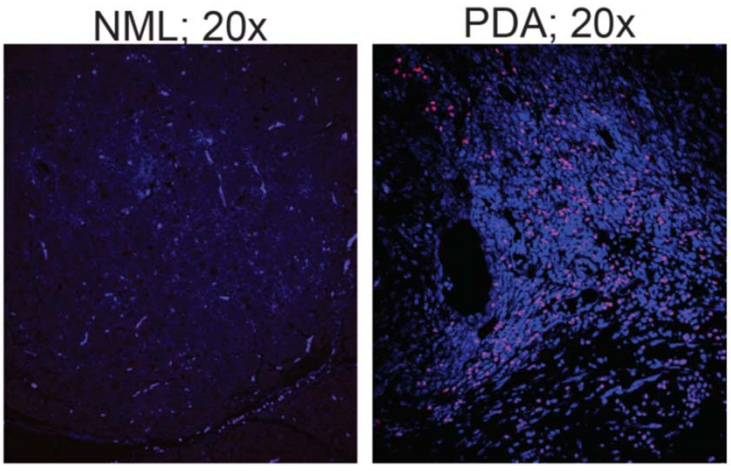
Gut bacteria can access the pancreas



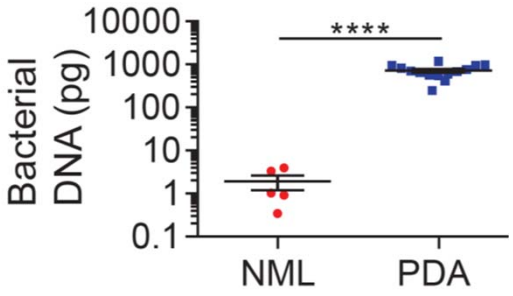
Experimental Question

- Is the pancreatic microbiome altered in PDA?

Markedly more abundant intra-pancreatic microbiome in human PDA



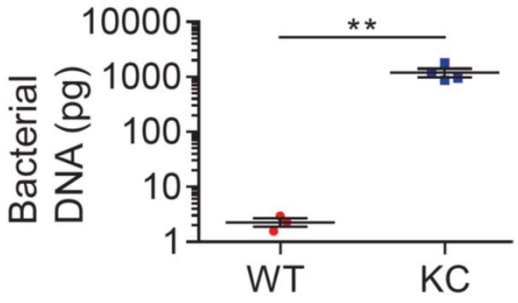
Markedly more abundant intra-pancreatic microbiome in human PDA



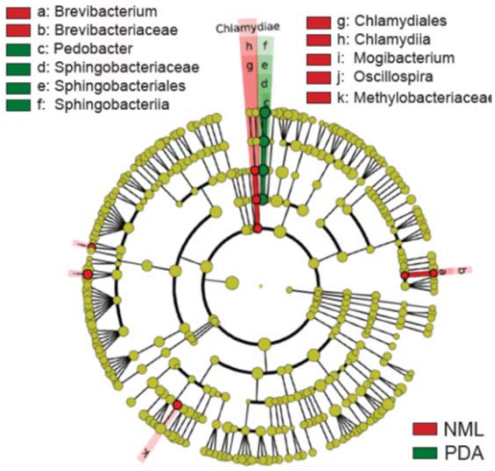
Mouse Models of PDA

- “KC” – Kras, pre-invasive
- “KPC” – Kras + p53, invasive

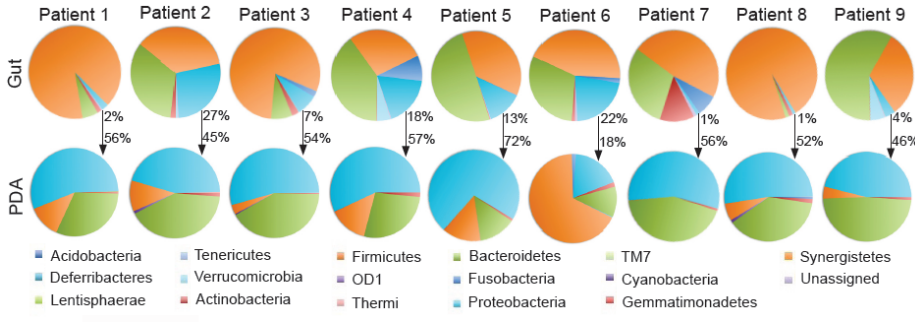
1000X Abundant Intra-Pancreatic Microbiome in Murine PDA



Comparison of Pancreatic Microbiome in Healthy vs PDA Patients



Proteobacteria is enriched in the human intra-pancreatic microbiome



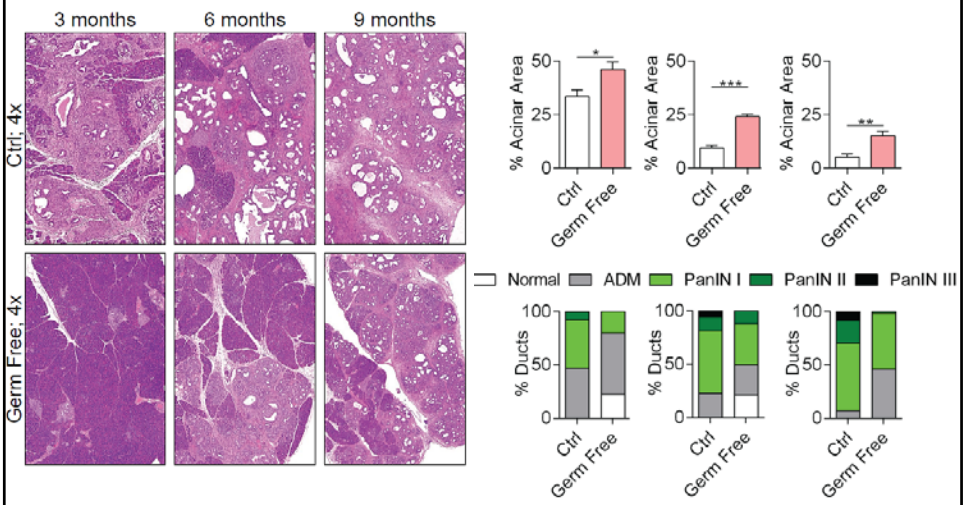
Question

How does ablation of the microbiome affect the progression of pancreatic oncogenesis?

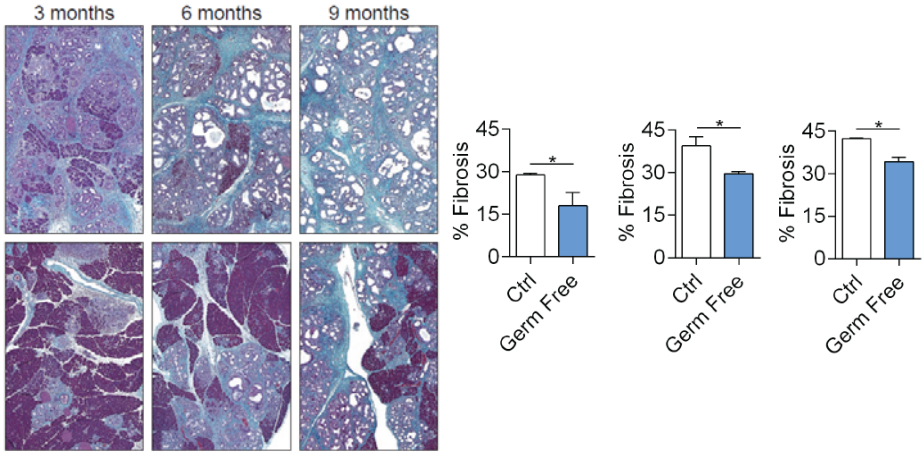
Germ Free Mice
National Gnotobiotic Rodent Research Center (UNC)

- KC mice sterilely re-derived
- Maintained in barrier tight isolators
- Mice sacrificed at 3, 6, and 9 months
- Compared PDA progression to non-sterile KC mice

Germ Free KC Mice are Protected against PDA

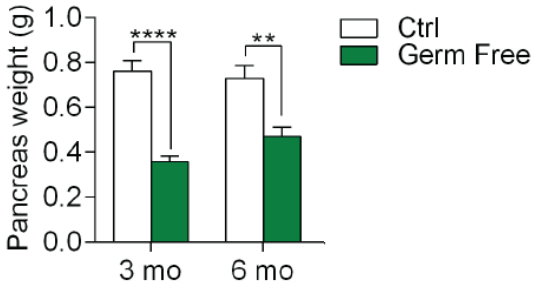


Germ Free KC Mice are Protected against PDA



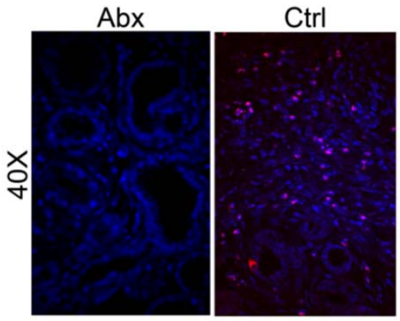
Pushalkar et al, *Cancer Discovery* 2018

Germ Free Mice are Protected against Pancreatic Oncogenesis (KC model)

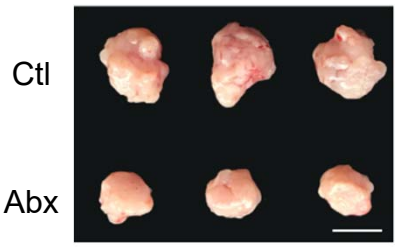


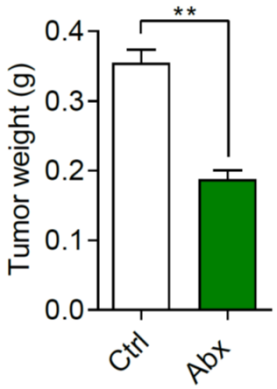
Ablative Oral Antibiotic Regimen

- Vancomycin
- Ampicillin
- Neomycin
- Flagyl



Bacterial Ablation Protects Against PDA (Orthotopic KPC Model)

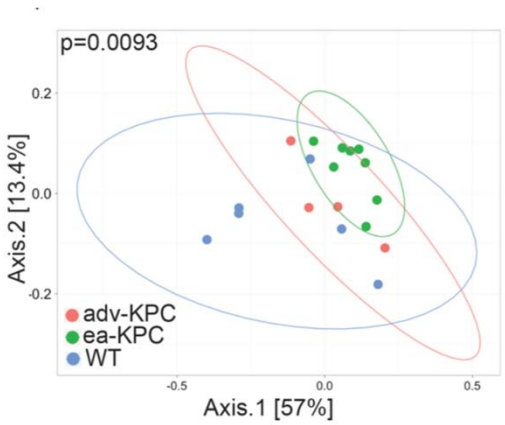


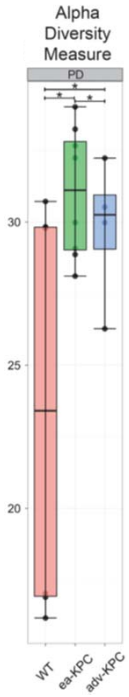


Question

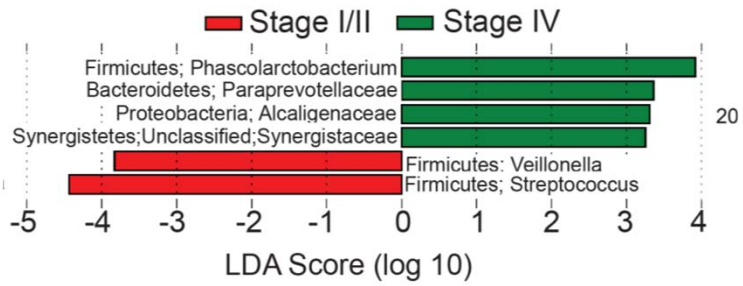
Do hosts with PDA exhibit bacterial dysbiosis in the gut?

Stage-specific microbiome in PDA



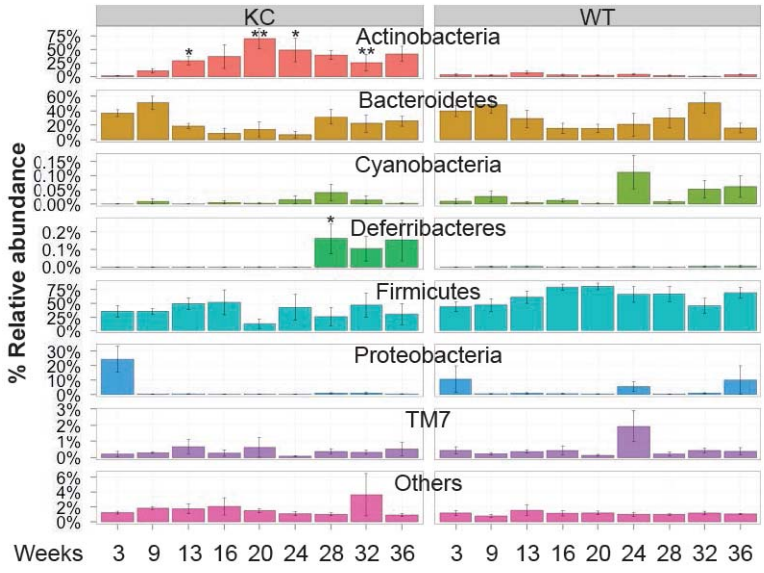


Stage-specific microbiome in human PDA

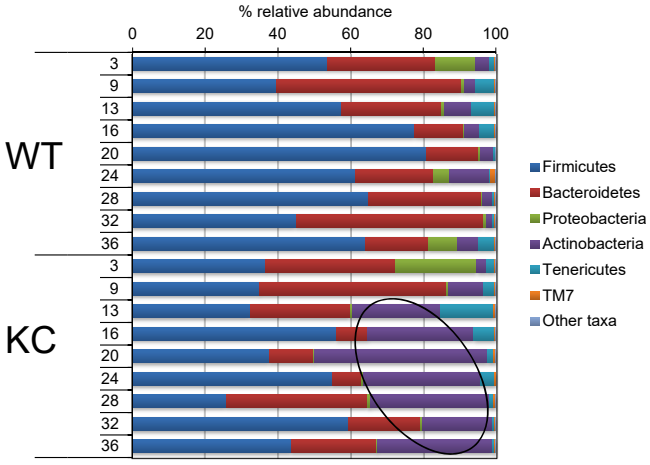


The Gut Microbiome is Distinct in Murine PDA

Phyla

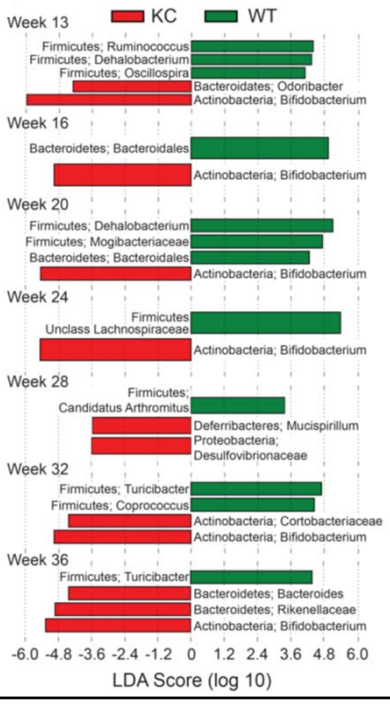


Taxonomic distribution of gut microbiome in WT and KC mice



The Gut Microbiome is Distinct in Murine PDA

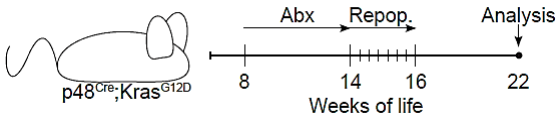
LDA Analysis

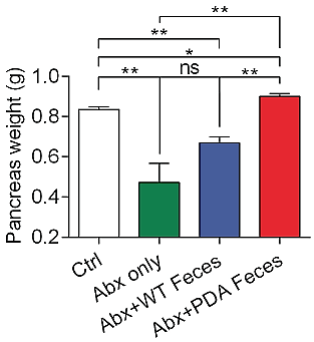


Question

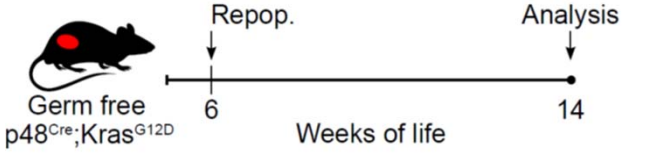
Does direct gut repopulation with "Good" or "Bad" Microbes alter PDA Progression?

The Gut Microbiome in PDA mice promotes Pancreatic Oncogenesis

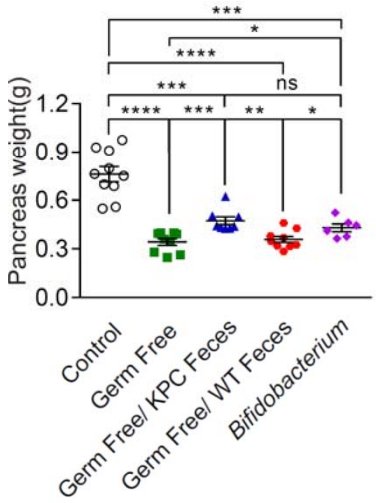




Does single bacterial transfer accelerate PDA?



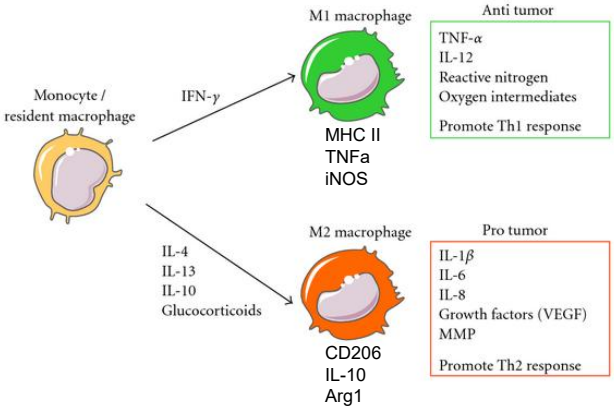
Single bacterial transfer accelerates PDA



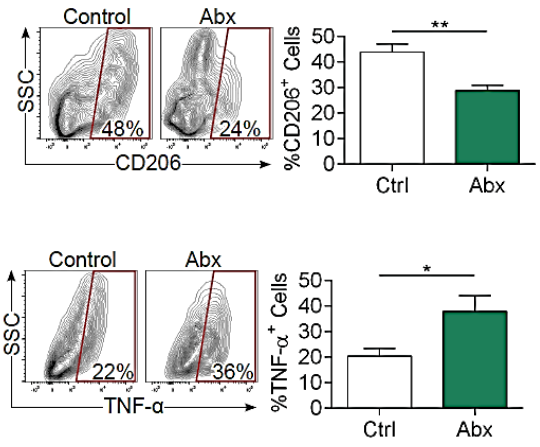
Hypothesis

Modulating the gut microbiome affects anti-tumor immunity in PDA

M1 vs M2 macrophages



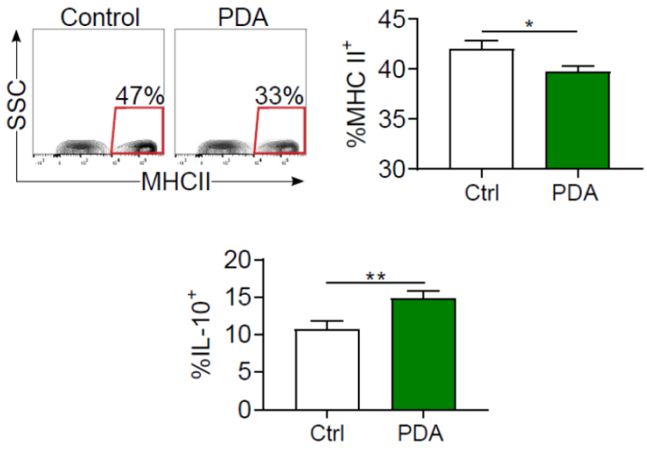
The Gut Microbiome in PDA mice promotes expansion of M2-like macrophages



Experimental Question

- Can gut bacterial extract directly affect macrophage polarization?

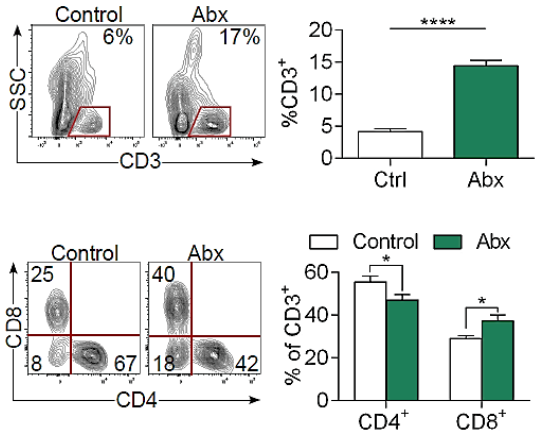
Extract from PDA gut microbiome induces suppressive macrophage phenotype



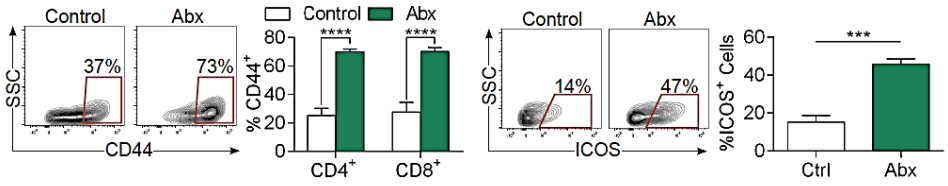
Experimental Question

- Does bacterial ablation enhance adaptive anti-tumor immunity?

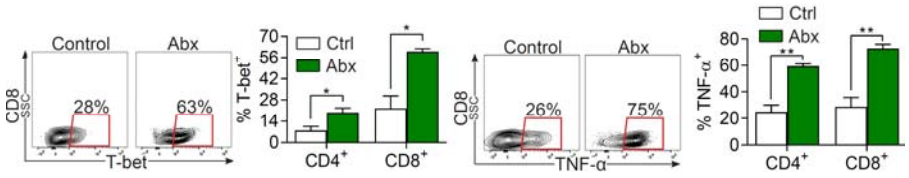
Microbial ablation expands CD8⁺ T cell Infiltration in PDA



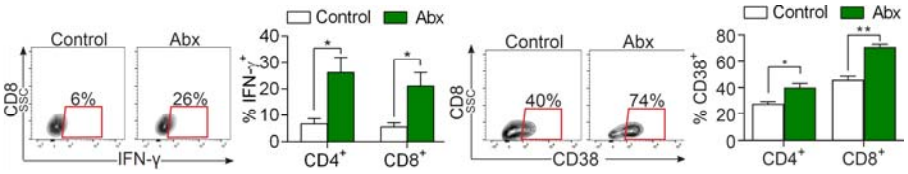
Microbial ablation results in T cell activation in PDA



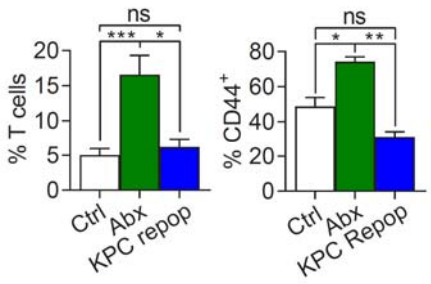
Microbial ablation induces a cytotoxic CD8⁺ T cell phenotype and Th1-polarization of CD4⁺ T cells



Microbial ablation induces a cytotoxic CD8⁺ T cell phenotype and Th1-polarization of CD4⁺ T cells



Repopulation with PDA-associated microbiome reverses the immunogenicity associated with bacterial ablation



Experimental Question

- Is tumor protection associated with bacterial ablation in PDA T cell dependent?

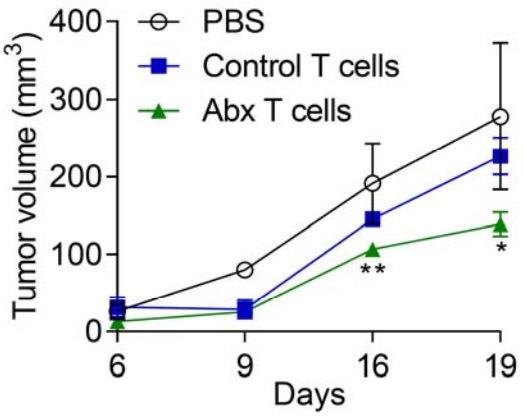
Experimental Question

- Is tumor protection associated with bacterial ablation in PDA T cell dependent?

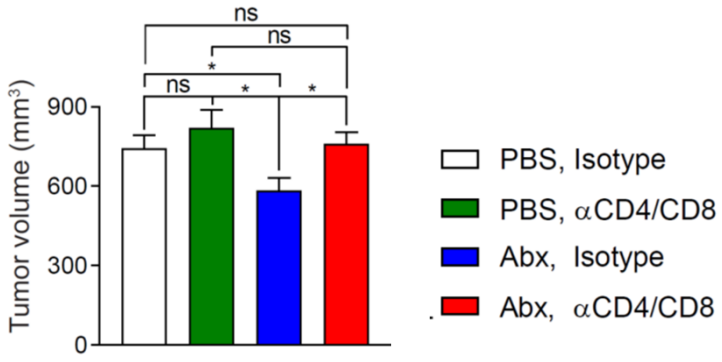
  - T cell adoptive transfer experiments

  - T cell depletion experiments

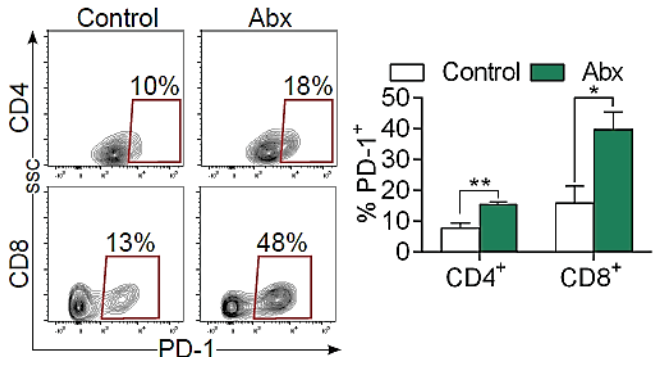
Adoptive Transfer of Tumor-Infiltrating T cells from antibiotic treated mice (but not control mice) is protective against PDA



T cell depletion reverses tumor-protection associated with bacterial ablation in PDA



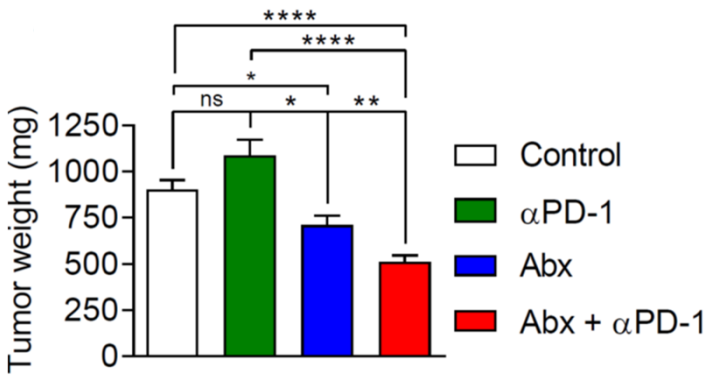
Microbial ablation induces results in higher T cell expression of PD-1



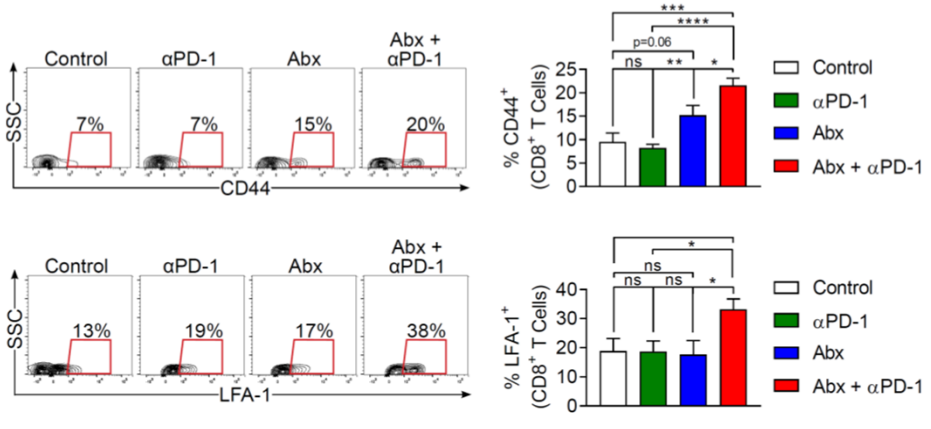
Experimental Question

- Would antibiotic ablation of gut bacteria enable PD-1 targeted immunotherapy?

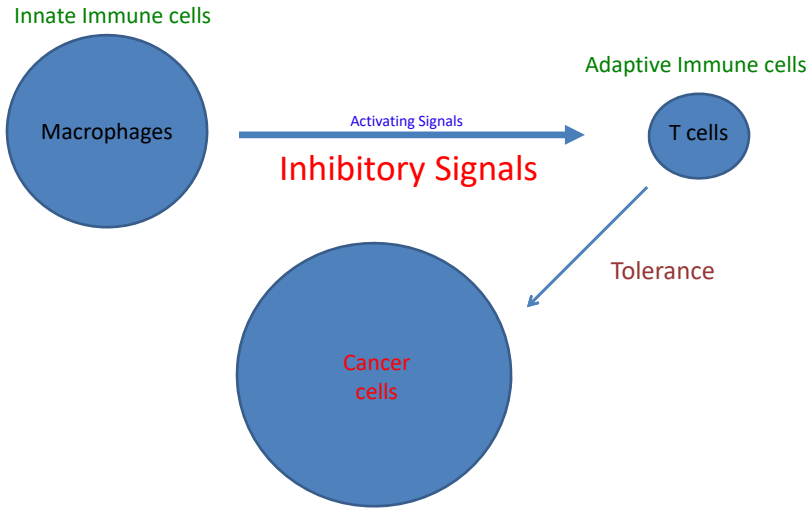
Microbial ablation enables efficacy for checkpoint-based immunotherapy in PDA



Microbial ablation enables efficacy for checkpoint-based immunotherapy in PDA



Experimental Question? Are microbiota responsible for macrophage-mediated adaptive immune collapse in PDA

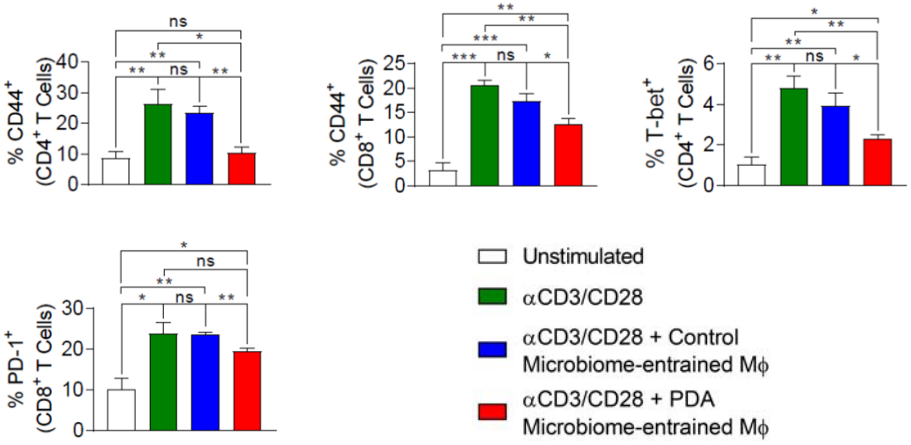


Experimental Strategy

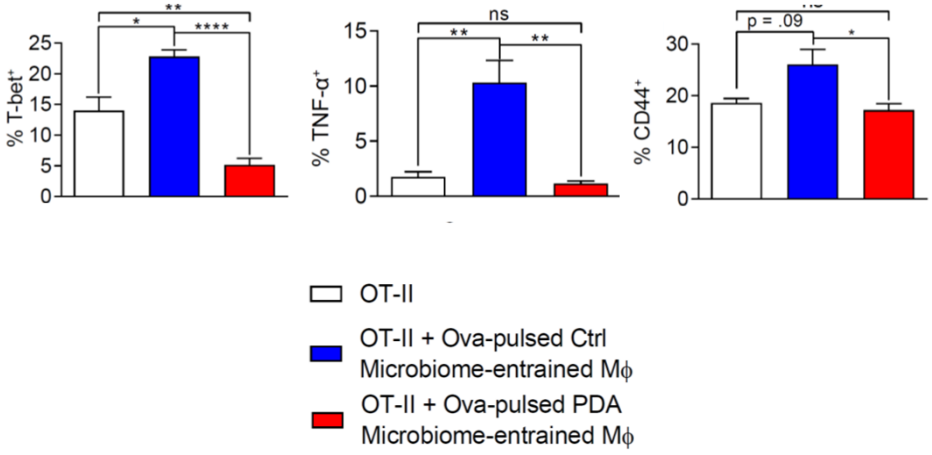
- T cell activation assays in the context of PDA-microbiome-entrained macrophages

Unstimulated
αCD3/CD28
αCD3/CD28 + PDA Microbiome-entrained Mϕ
αCD3/CD28 + Control Microbiome-entrained Mϕ

Extract from PDA gut microbiome induces a suppressive macrophage phenotype



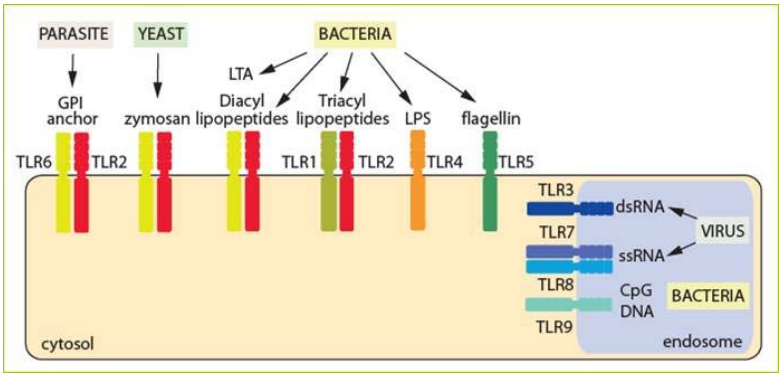
Extract from PDA gut microbiome disables the capacity of macrophage to present antigen



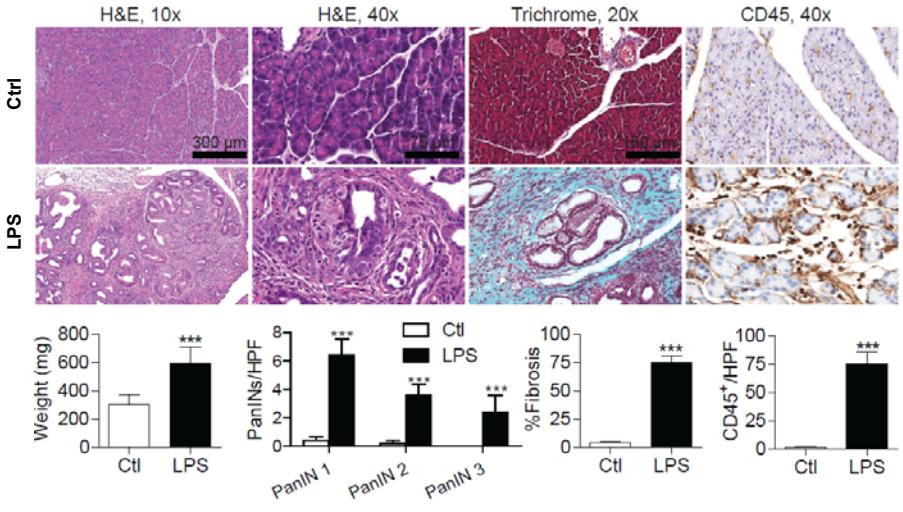
Question

- How does the gut microbiome affect macrophage programming in PDA?

Microbes can induce pattern recognition receptor signaling in macrophages

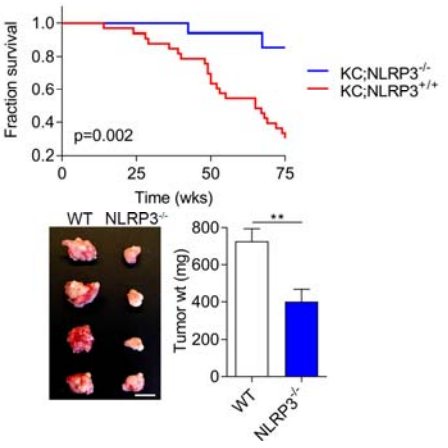


TLR4 activation accelerates pancreatic cancer

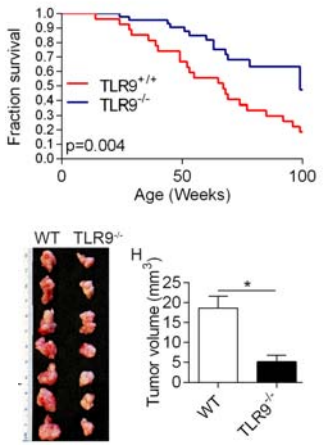


Ochi et al. J Exp Med. 2012

NLRP3 and TLR9 promote PDA

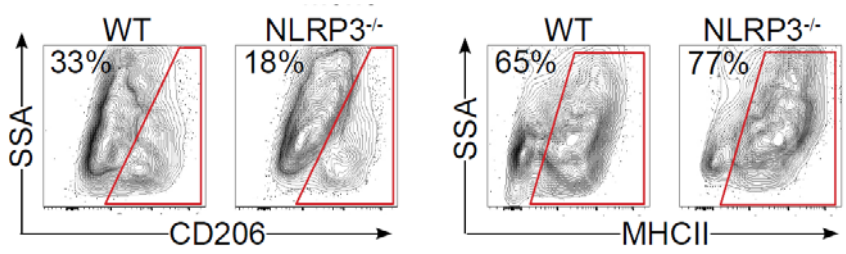


Daley et al, *JEM* 2017

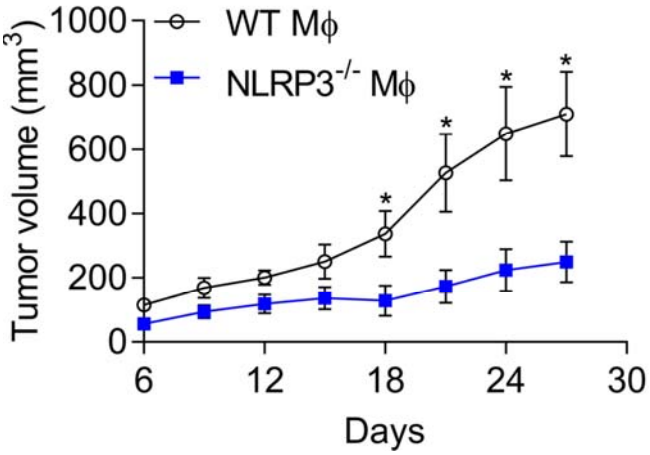


Zambirinis et al, *JEM* 2015

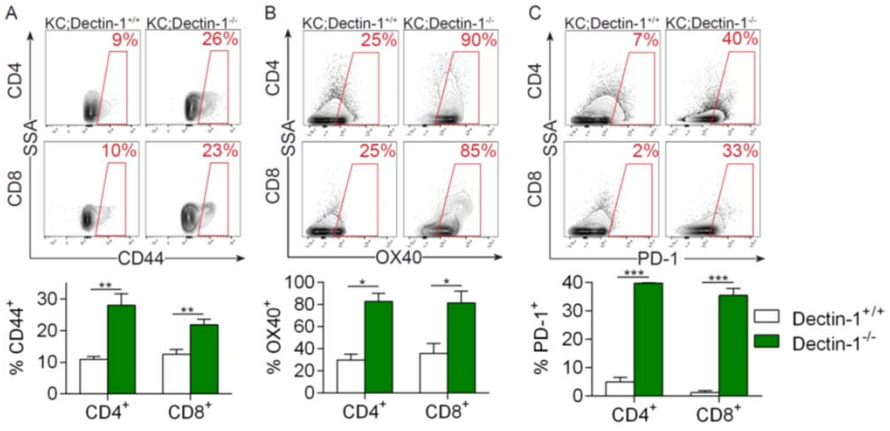
NLRP3 signaling in TAMs promotes M2 differentiation



NLRP3 signaling is necessary for TAMs to accelerate PDA



Dectin-1 deletion results in CD4+ and CD8+ T cell activation in PDA draining lymph nodes



Daley et al, *Nature Medicine* 2017

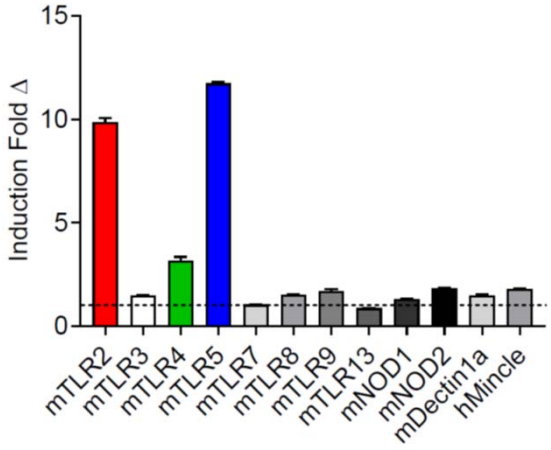
Hypothesis

- The PDA microbiome induces crippling immune-suppression in PDA via diverse pattern recognition receptor (PRR) activation

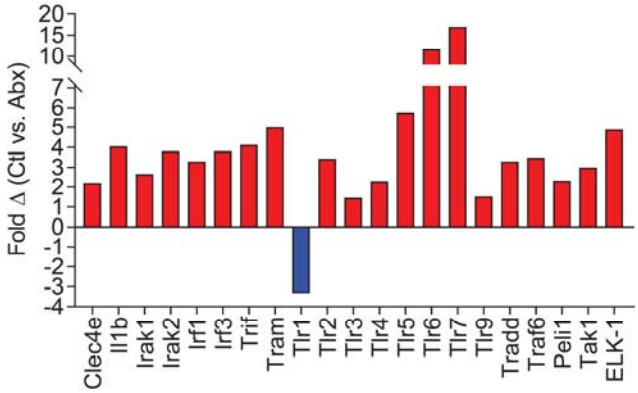
Experimental plan

- Test extract from PDA vs control microbiomes on PRR reporter cell line activation

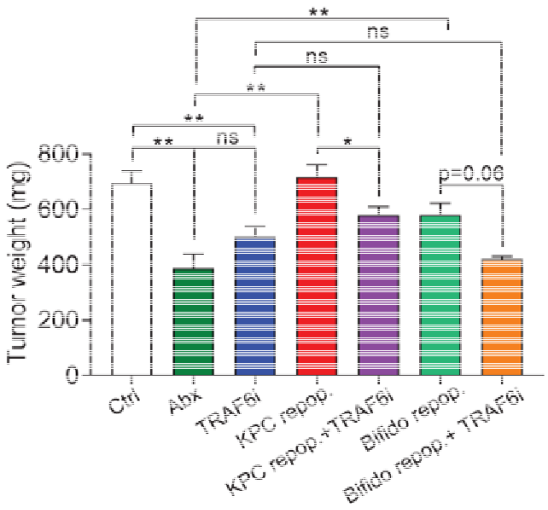
Extract from the PDA gut microbiome induces higher PRR signaling than normal gut microbiome



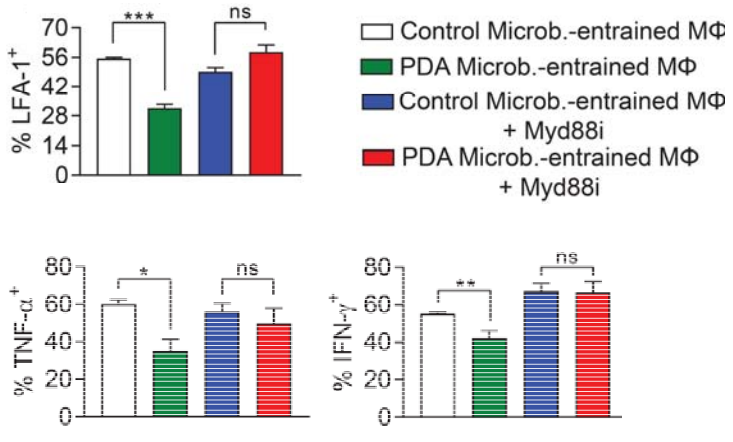
Upregulation of TLR signaling in KC vs KC germ-free pancreata



The PDA microbiome promotes oncogenesis via TLR signaling

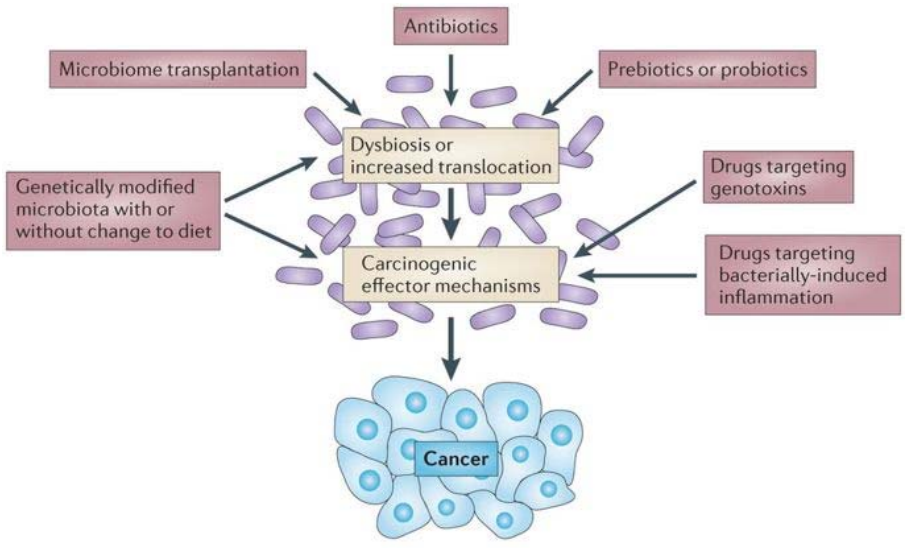


PDA microbiome-entrained macrophages are suppressive of T cell immunity in a TLR-dependent manner

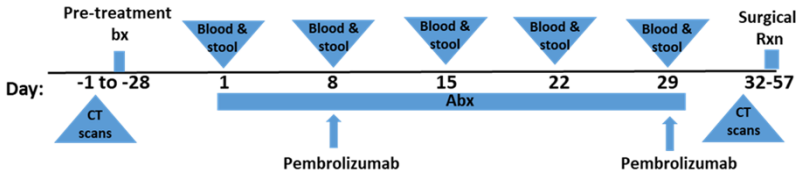


Summary

- Micorbiota are >1000X more abundant in the cancerous pancreas.
- PDA is associated with gut bacterial dysbiosis.
- The PDA-associated microbiome, including *Bifido.* and *Fuso.* species, promote PDA progression.
- The PDA microbiome induces higher TLR2, TLR4, and TLR5 signaling in innate immune cells.
- Bacteria induce suppressive macrophage programming via TLR signaling. This leads to T cell suppression.
- Targeting the microbiome is protective against PDA in a T cell dependent manner and enables efficacy for PD1 based therapy.



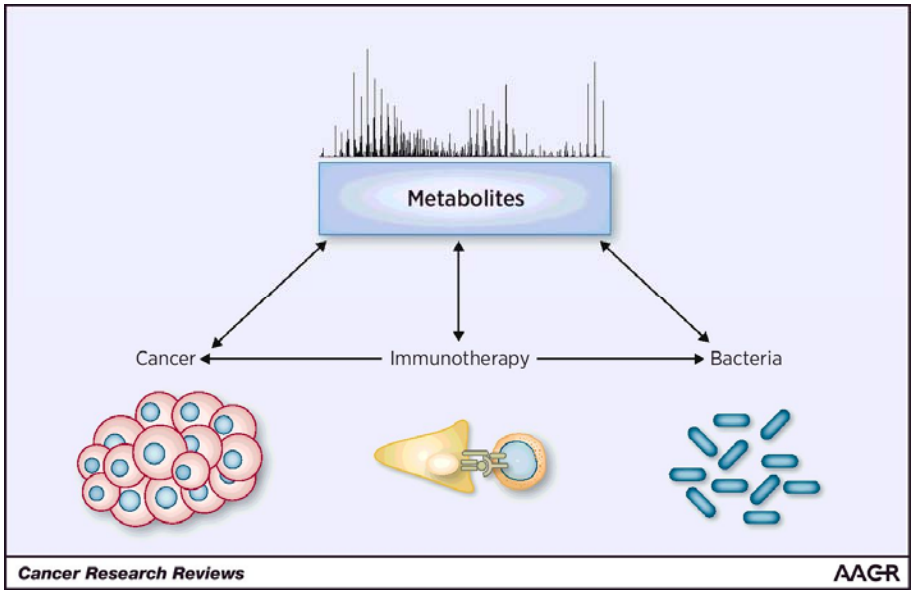
Investigator-initiated neo-adjuvant Phase 1b "window" clinical trial in early PDA



- Resectable pancreatic cancer
- Initial 7 day lead-in with abx
- Initial 6 patients will be evaluated for DLT's
- If < 2 DLT's in the 1st 6 patients, then dose expansion in 14 addt'l patients
- If >1 DLT in 1st 6 patients, then study paused and different dose/schedule considered
- Following surgical resection, R0 resection rate and tumor regression grade will be evaluated by a dedicated pancreatic surgical pathologist and scored based on both the tumor regression

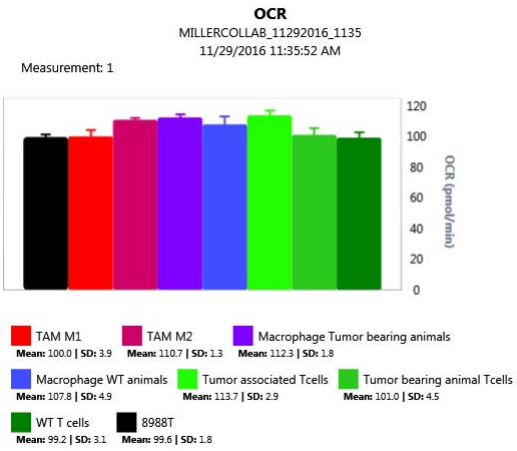
Intervention:
Ciprofloxacin 500mg PO BID days 1-29
Metronidazole 500mg PO TID days 1-29
Pembrolizumab 200mg IV days 8 and 29

MERCK

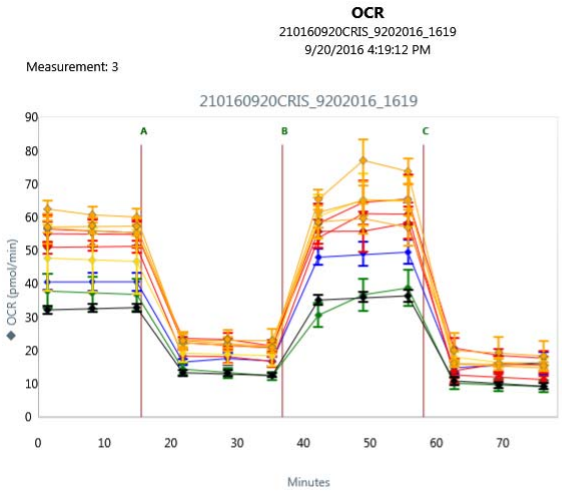


Do macrophages provide nutrient support to tumor cells?

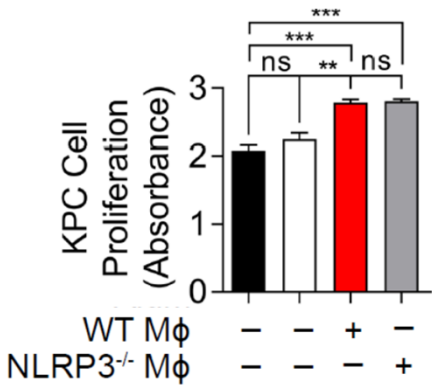
Tumor-associated macrophages increase OCR in PDA cells



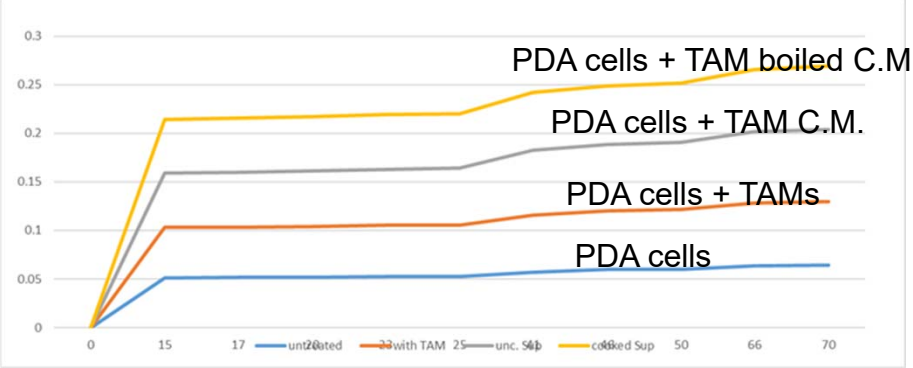
Tumor-associated macrophages increase OCR in PDA cells



Tumor-associated Macrophages Increase Tumor Cell Proliferation



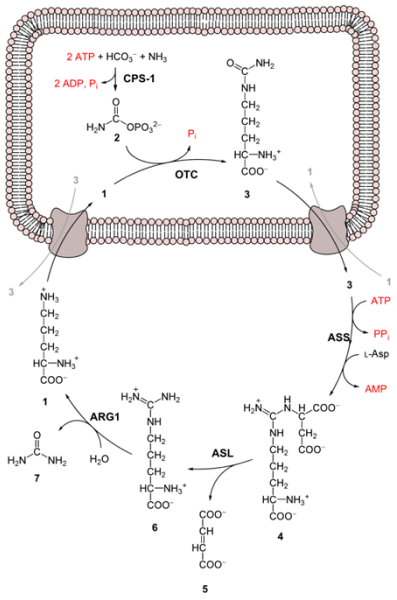
Metabolites from Tumor-Associated Macrophages Increase Tumor Cell Proliferation



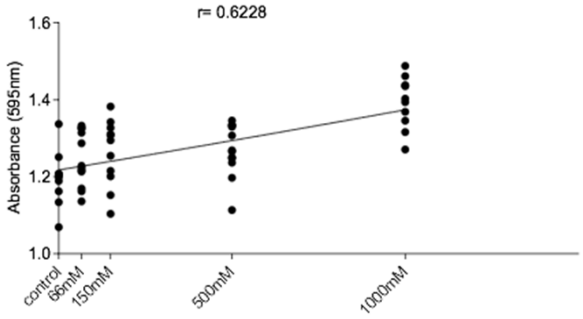
TAMs Produce High Levels of Urea Cycle Intermediates

	TAM CM vs PDAC CM		*TAM CM vs T-cell media*	
Metabolite	**Fold Change**	***p-value***	**Fold Change**	***p-value***
ADP	8.51	***0.0051***	1.43	***0.036***
dAMP	6.12	***0.0296***	7.24	***0.026***
ornithine	1.74	*0.0038*	6.62	*0.003*
sarcosine (Alanine)	1.62	***0.0010***	1.20	***0.022***
Urea	1.49	***0.0032***	1.44	***0.003***
alanine	1.44	***0.0009***	1.24	***0.001***
L-arginino-succinate	1.23	***0.0092***	1.70	***0.002***
arginine	0.86	***0.0022***	0.77	***0.0009***
creatine	0.85	***0.0128***	0.73	***0.0046***
dimethylglycine	0.53	0.0592	0.64	***0.0171***
fructose-6-phosphate	0.43	***0.0024***	0.68	0.0680
hypoxanthine	0.39	***0.0002***	0.39	***0.0198***
inosine	0.33	***0.0011***	0.40	***0.0040***
D-sedoheptulose-1-7-phosphate	0.30	***0.0015***	0.51	***0.0322***
guanine	0.16	***0.0036***	0.44	***0.0087***

Urea Cycle

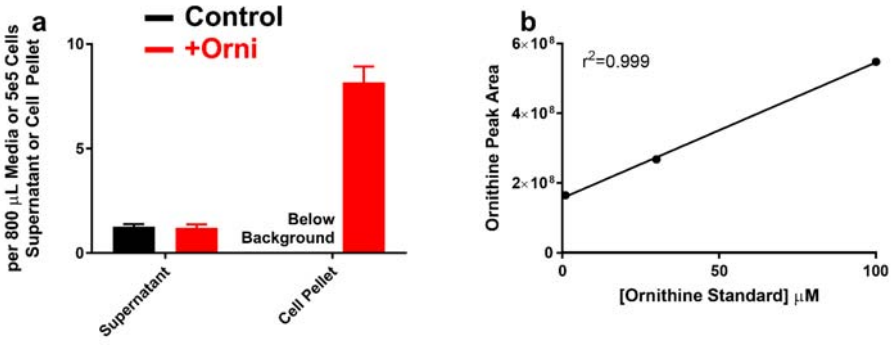


Ornithine supplementation may accelerate tumor cell growth



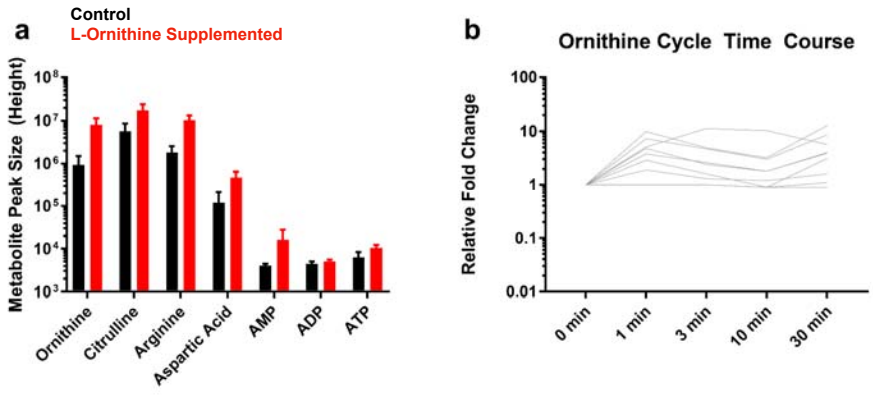
Ornithine (mM)

Tumors take up MΦ-derived Ornithine

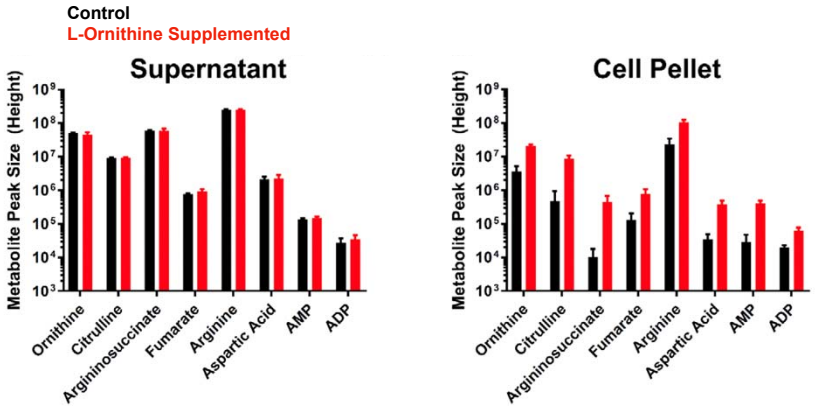


Q: What do tumor cells use this ornithine for?

Tumors use MΦ-derived Ornithine to fuel urea cycle

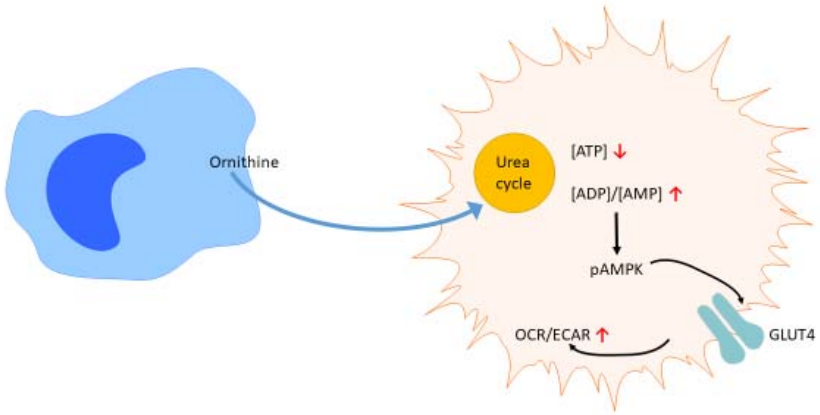


Tumors use MΦ-derived Ornithine to fuel urea cycle

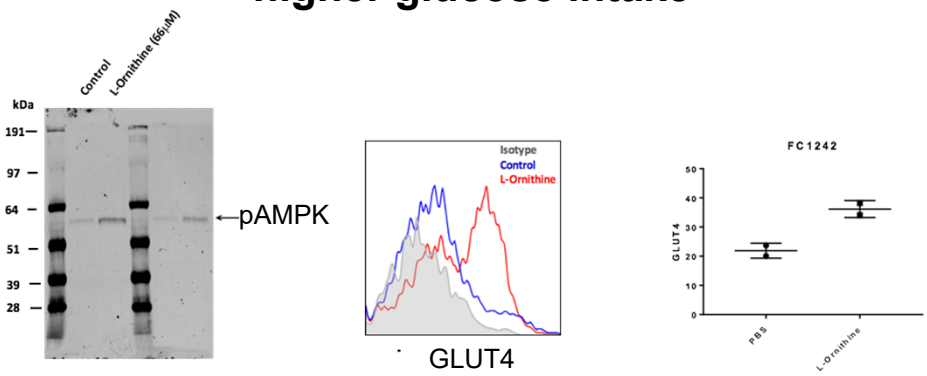


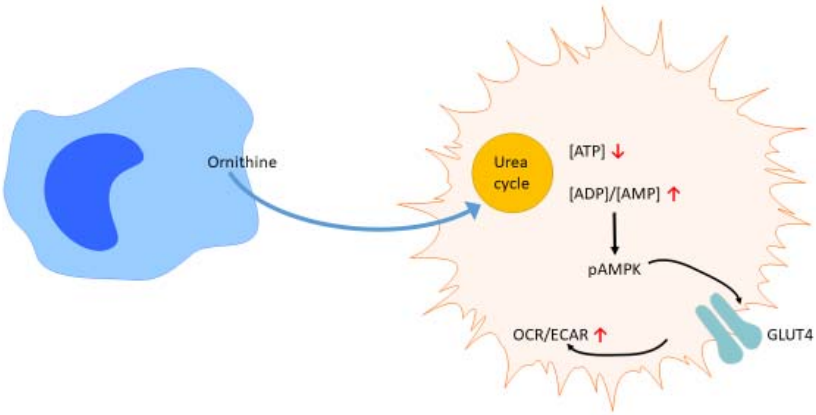
Q: Can ornithine impact other aspects of tumor metabolism?

Q: How does the increased urea cycle flux impact other aspects of tumor metabolism?



Ornithine supplemented tumors have higher AMPK signaling and possibly higher glucose intake

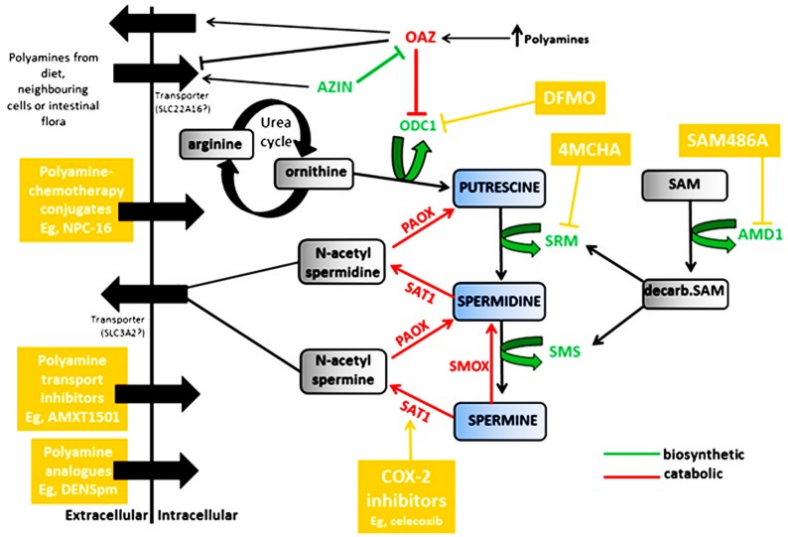




Possible Directions and Questions to Explore

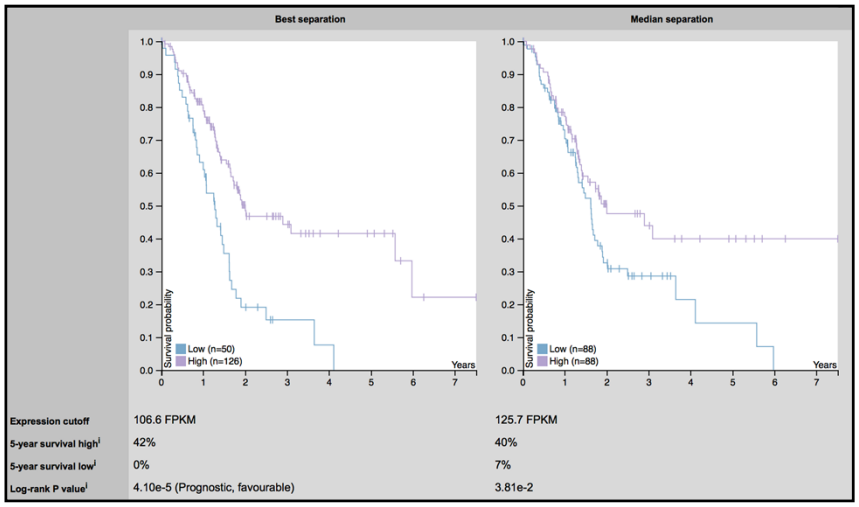
- i. What does this ornithine help the tumors do? Polyamines? Arginine-based compounds? ADP/ATP ratio to fuel glycolysis?
- ii. Where is the macrophage-derived ornithine coming from?

Arginine/Ornithine can be used to generate Polyamines



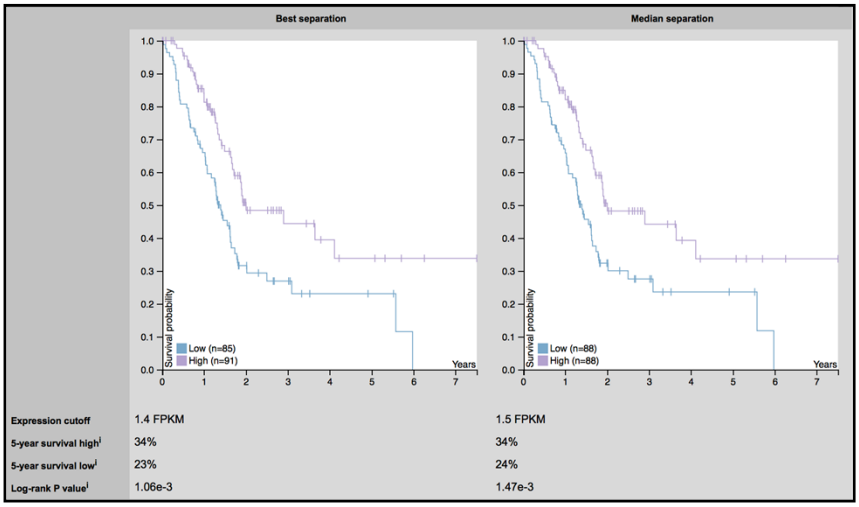
Gamble LD, Hogarty MD, Liu X, et al. Polyamine pathway inhibition as a novel therapeutic approach to treating neuroblastoma. Front Oncol. 2012;2:162.

Prognostic Value of Polyamine Shunt Enzymes
OAZ1



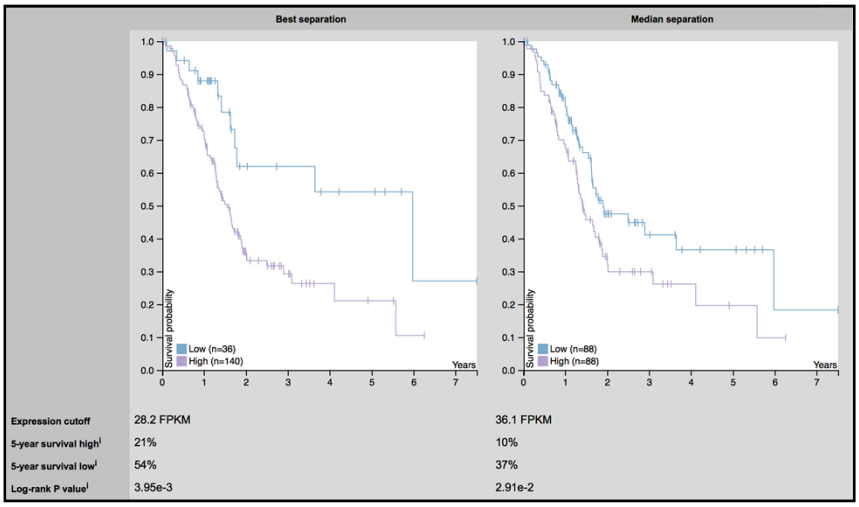
	Best separation	Median separation
Expression cutoff	106.6 FPKM	125.7 FPKM
5-year survival high	42%	40%
5-year survival low	0%	7%
Log-rank P value	4.10e-5 (Prognostic, favourable)	3.81e-2

Prognostic Value of Polyamine Shunt Enzymes
AZIN2



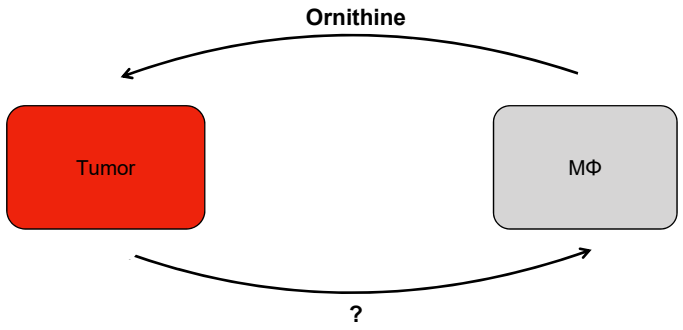
	Best separation	Median separation
Expression cutoff	1.4 FPKM	1.5 FPKM
5-year survival high	34%	34%
5-year survival low	23%	24%
Log-rank P value	1.06e-3	1.47e-3

Prognostic Value of Polyamine Shunt Enzymes
SMS



Expression cutoff	28.2 FPKM	36.1 FPKM
5-year survival high[i]	21%	10%
5-year survival low[i]	54%	37%
Log-rank P value[i]	3.95e-3	2.91e-2

Can tumor metabolism influence MΦ phenotype?



Thank you!!!

Smruti Pushalkar

Mautin Hundeyin

Deepak Saxena