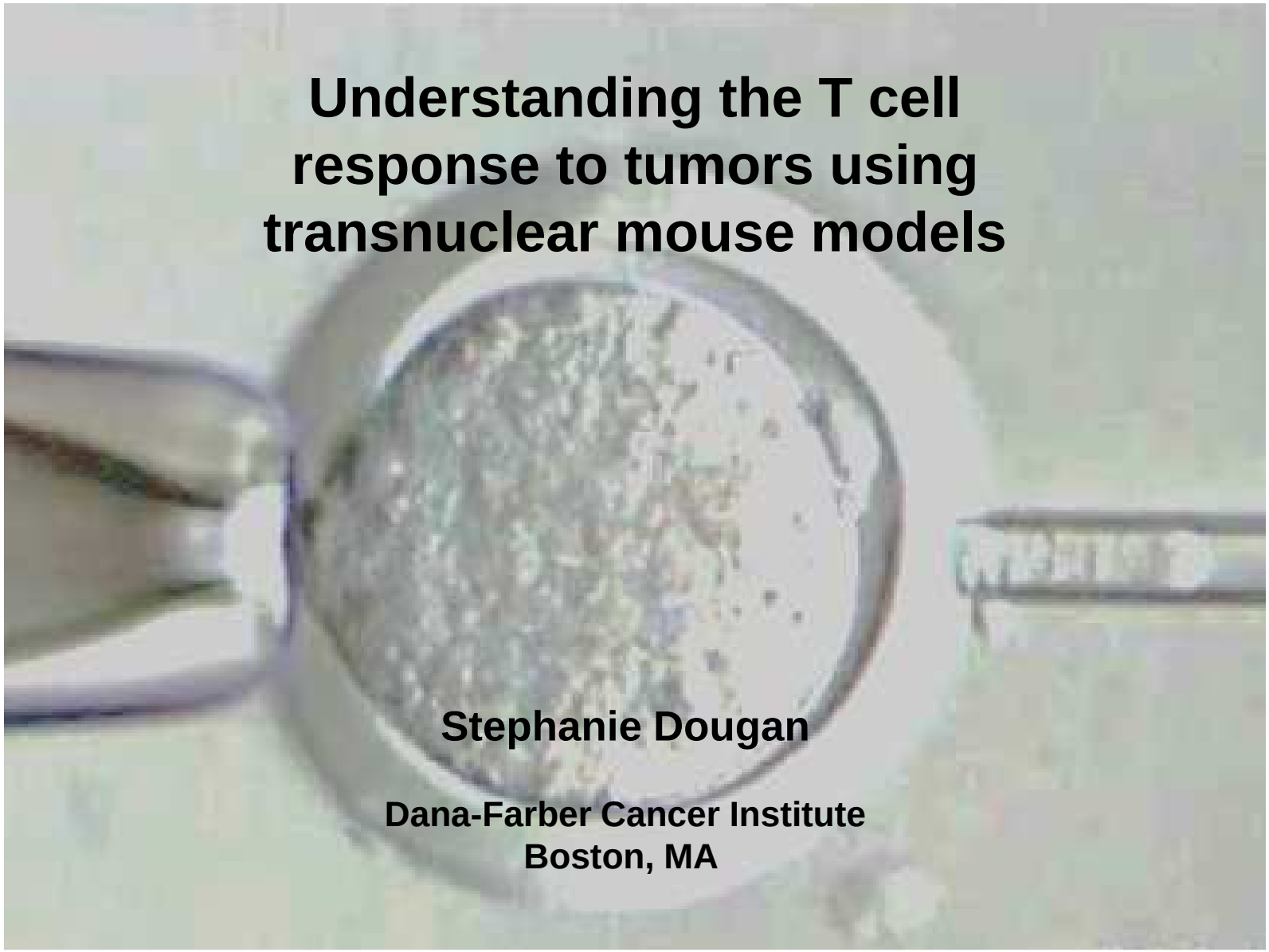
Understanding the T cell response to tumors using transnuclear mouse models



Stephanie Dougan

Dana-Farber Cancer Institute
Boston, MA

Presenter Disclosure Information

Stephanie Dougan

The following relationships exist related to this presentation:

No Relationships to Disclose

Melanoma:

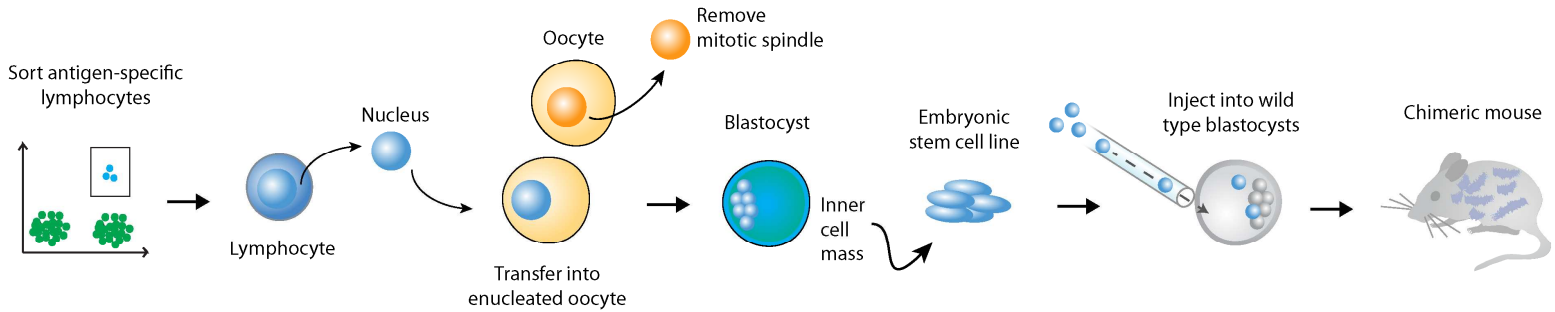
Responds well to immunotherapy (CTLA-4 and/or PD-1 blockade)

Pancreatic ductal adenocarcinoma:

Responds poorly to most therapies, notable for being one of few cancer types to fail immunotherapy

7% survival at 5 years

Transnuclear mice



Advantages of transnuclear mice:

1) T cells are isolated directly from the tumor – no time spent in tissue culture

2) TCR genes are in their endogenous loci, under physiological control – no other genetic manipulations

3) Multiple, independent lines can be made against the same antigen

4) High-throughput

5) Rapid

TRP1 TN mice: models of CD8 T cell response to melanoma

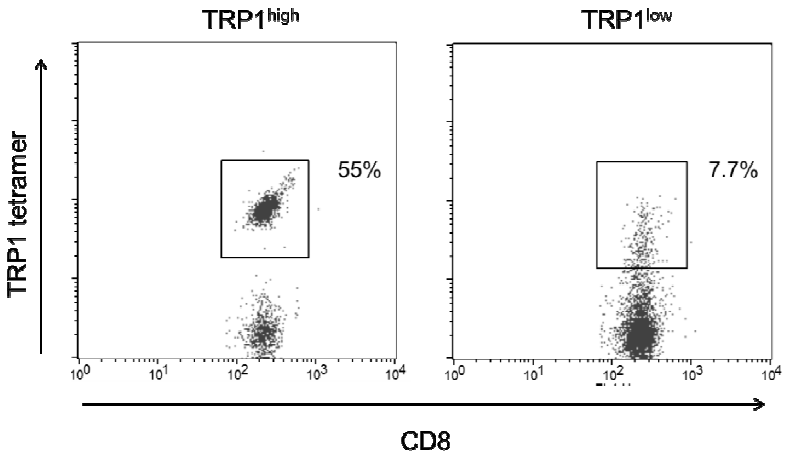
TRP1^{low}

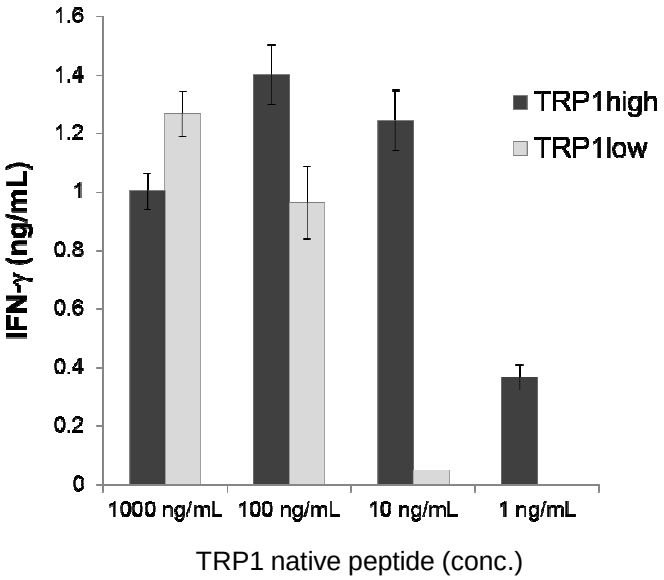
TRP1^{high}

Two lines of TN mice derived from CD8 T cells specific for tyrosinase-related protein 1 (TAPDNLGYA) presented by H-2D^{b}

Dougan et al., Cancer Immunology Research 2013

Two lines of TN mice recognize the same TRP1 epitope, but differ in affinity



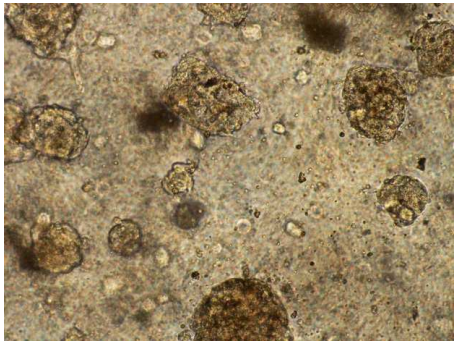


TRP1^{high} $t_{1/2}$ = 41 min

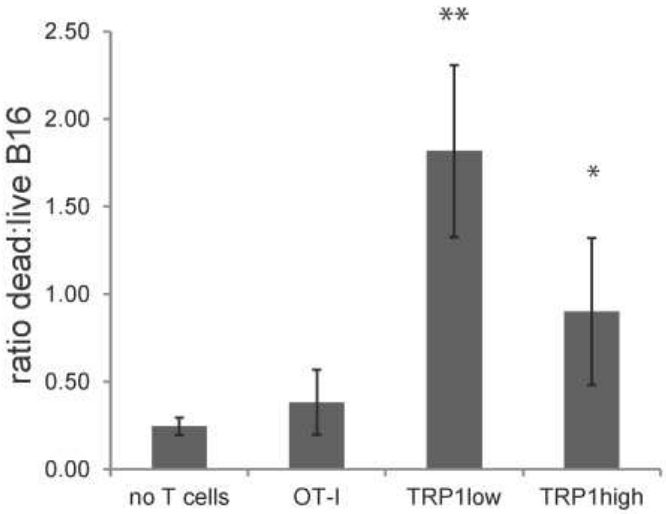
TRP1^{low} $t_{1/2}$ = 4.9 min

Dougan et al., Cancer Immunology Research 2013

Both high and low affinity TRP1 cells can be cytotoxic in 3D tissue culture



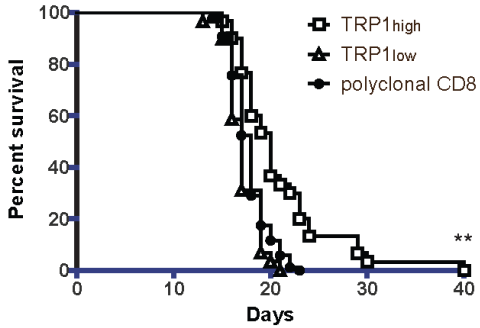
Cytotoxicity assay using matrigel co-culture of B16 cells with activated CD8 T cells



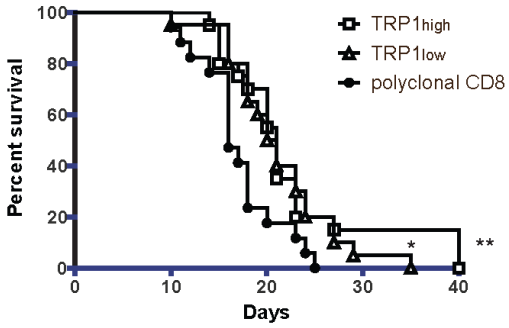
Dougan et al., Cancer Immunology Research 2013

Upon activation, both high and low affinity CD8 T cells delay tumor growth in vivo

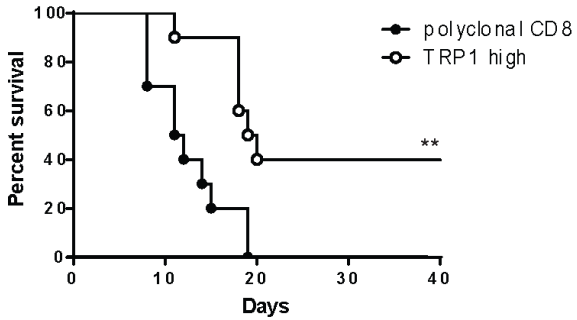
Naive



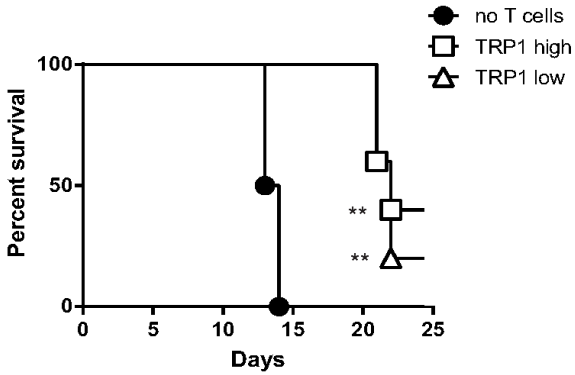
Peptide-activated



Anti-CD3/CD28 activated

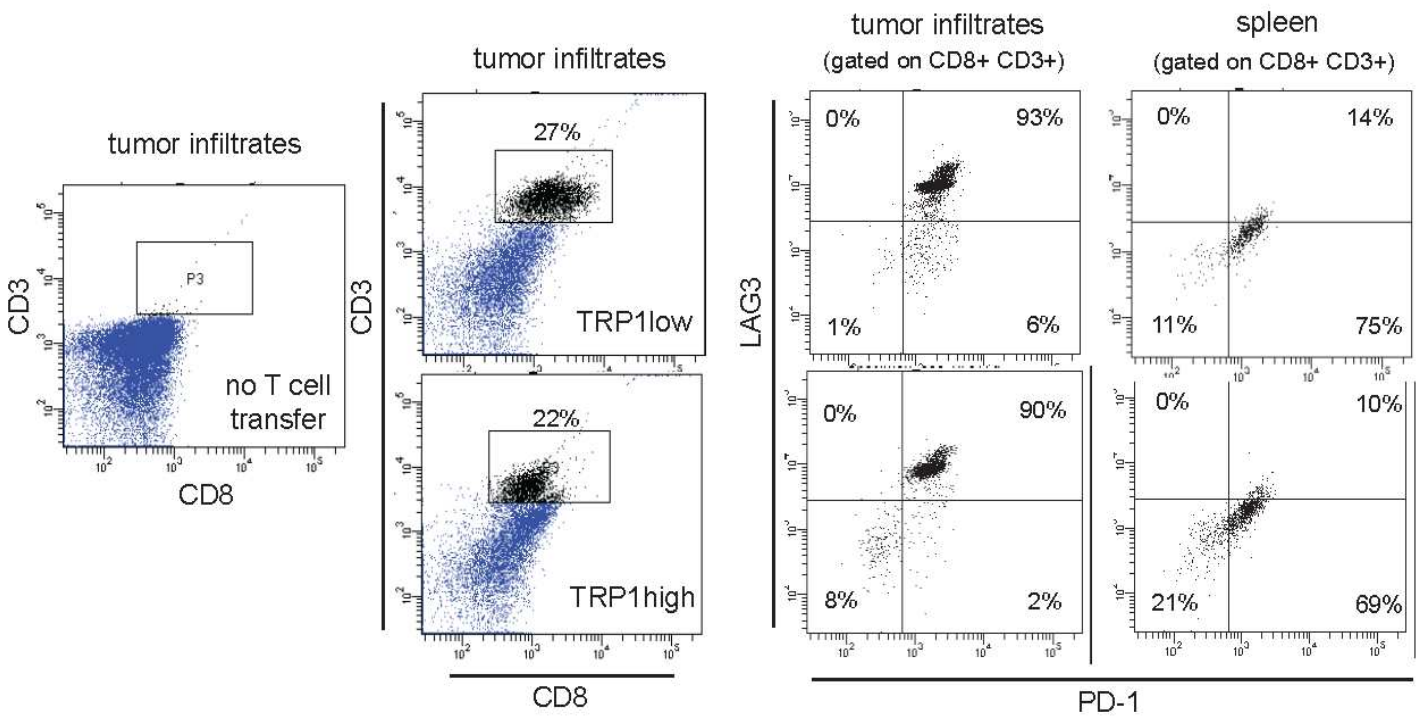


Homeostatic expansion (RAG1-/-)



Dougan et al., Cancer Immunology Research 2013

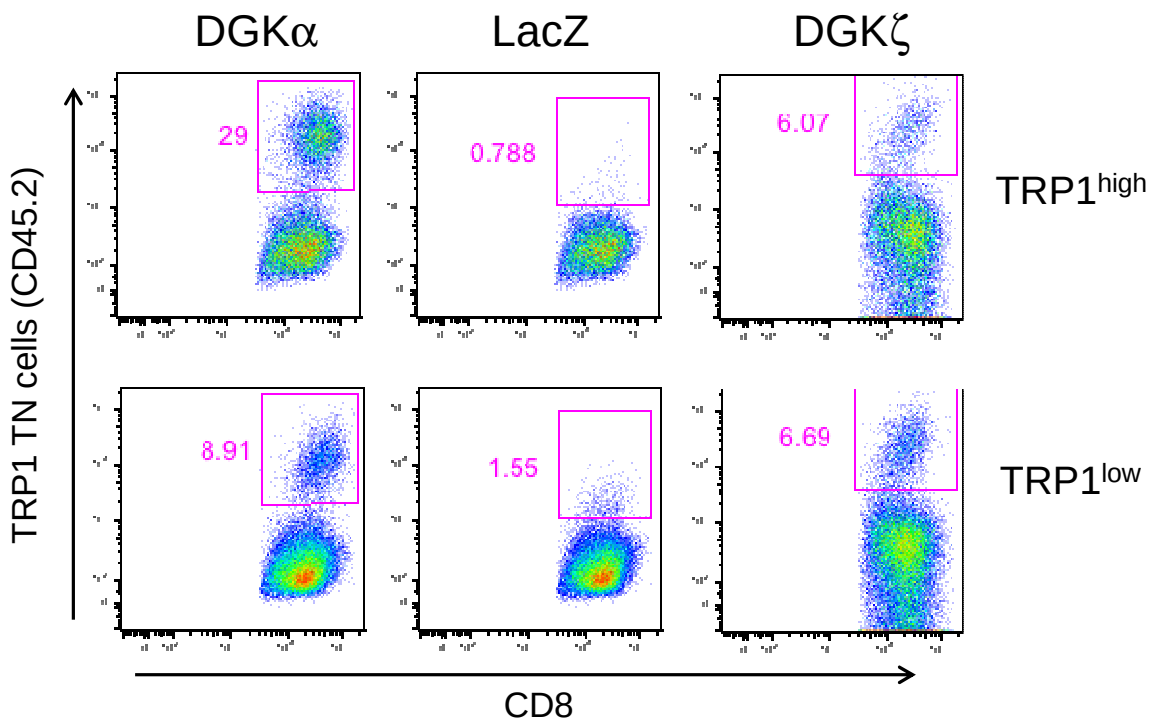
TRP1 TN cells transferred into RAG1-/- hosts and reisolated from tumors show markers of exhaustion



Increased LAG3, PD-1, Tim3 and CTLA-4 expression

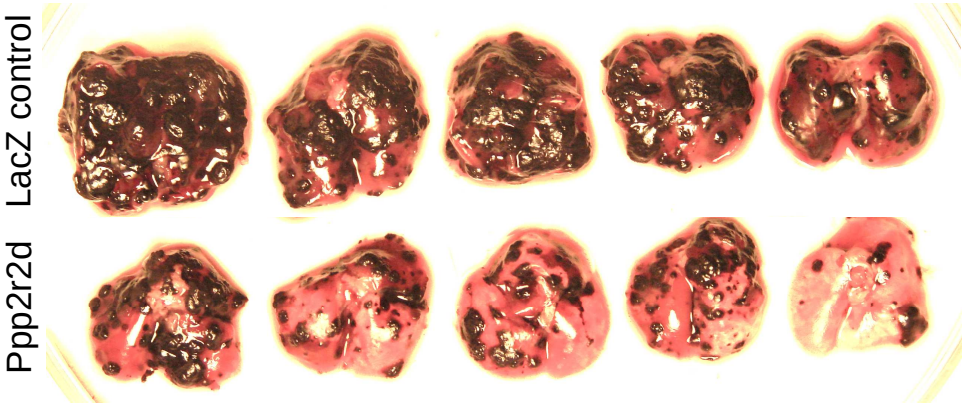
Dougan et al., Cancer Immunology Research 2013

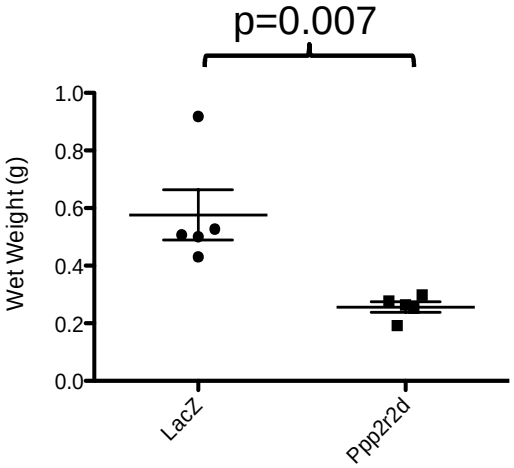
Candidate gene silencing leads to increased TRP1 accumulation in tumors



Collaboration with Kai Wucherpfennig

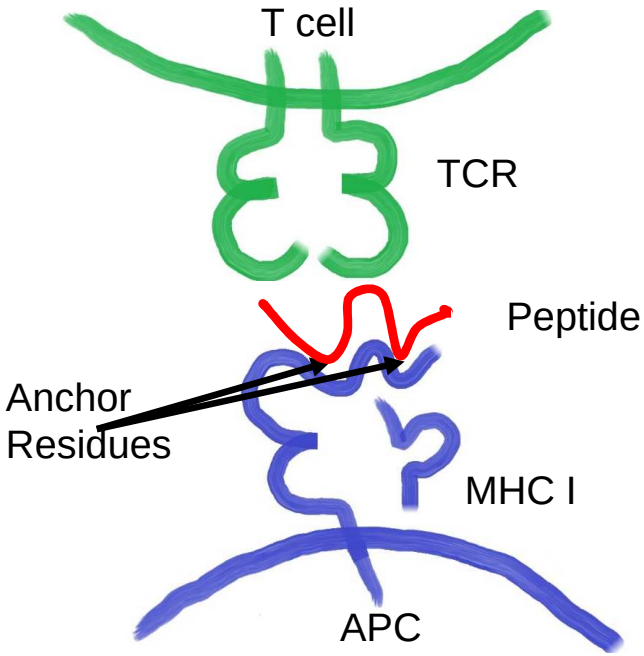
Silencing of Ppp2r2d enhances anti-tumor effect of TRP1 TN cells





Collaboration with Kai Wucherpfennig

How does TCR affinity affect activation and anti-tumor activity?

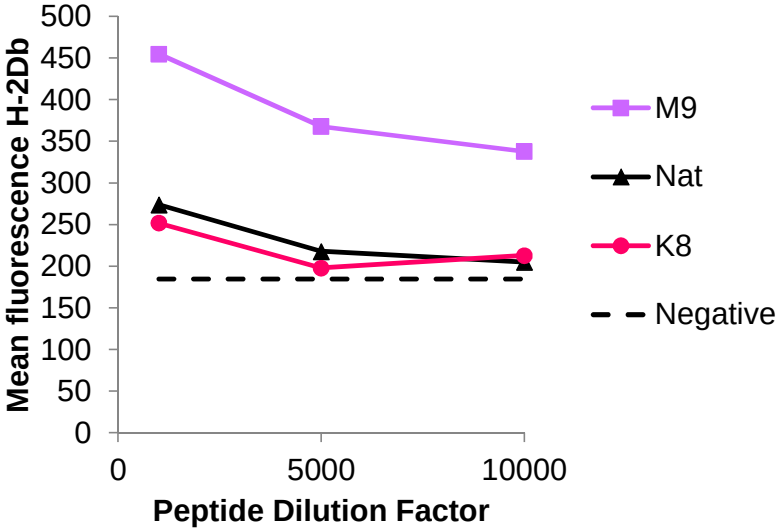


<u>Altered Peptide Ligands</u>

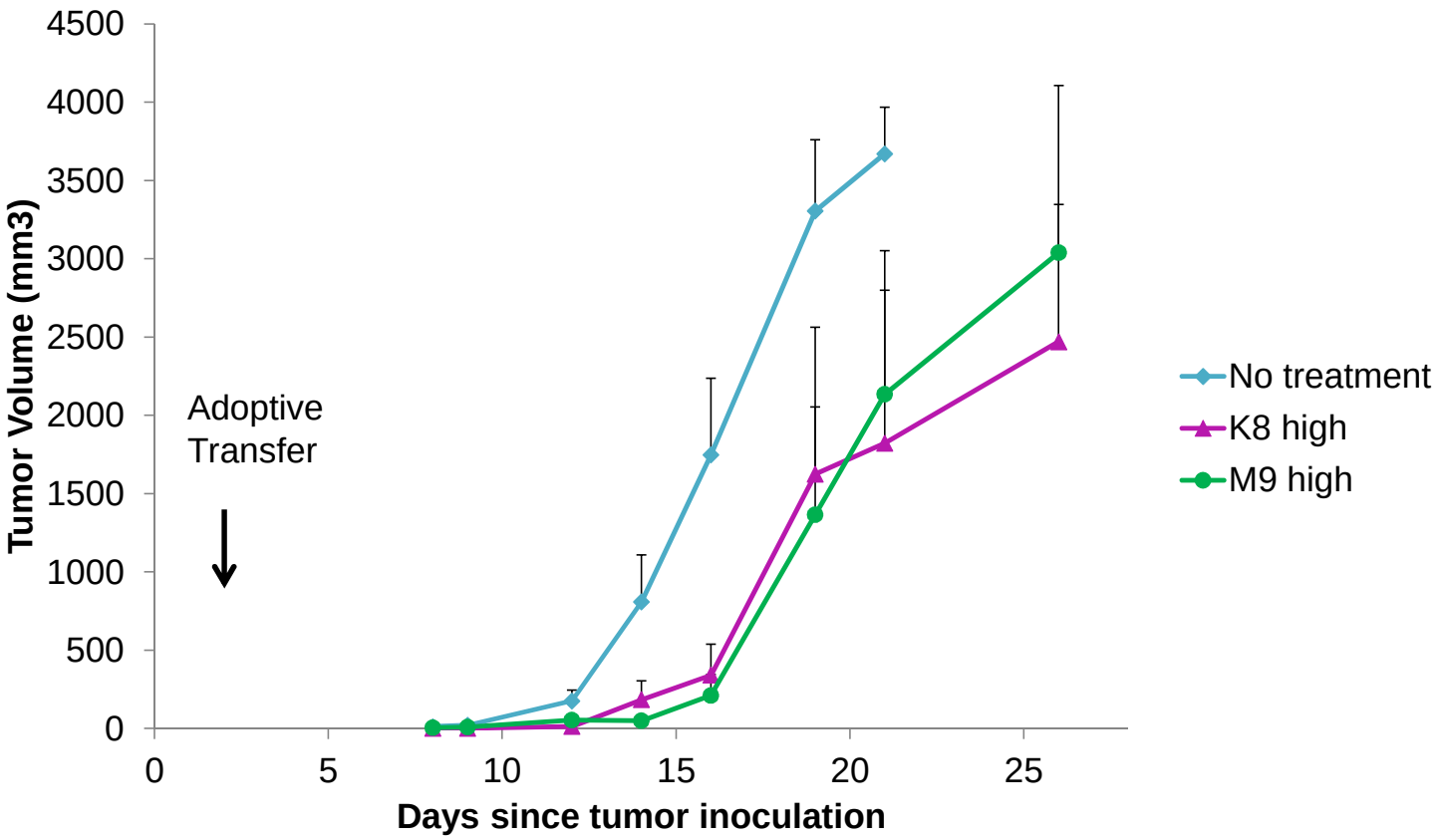
Native peptide: TAPDNLGYA

Reference (M9): TAPDNLGY**M**

K8: TAPDNLG**KM**

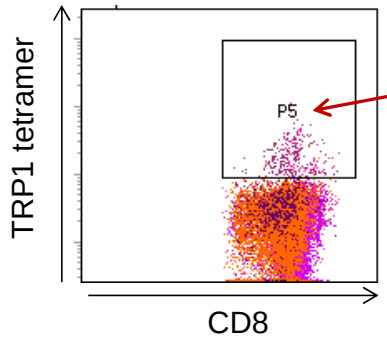


TRP1^{high} T cells activated by M9 or K8 delay growth of B16



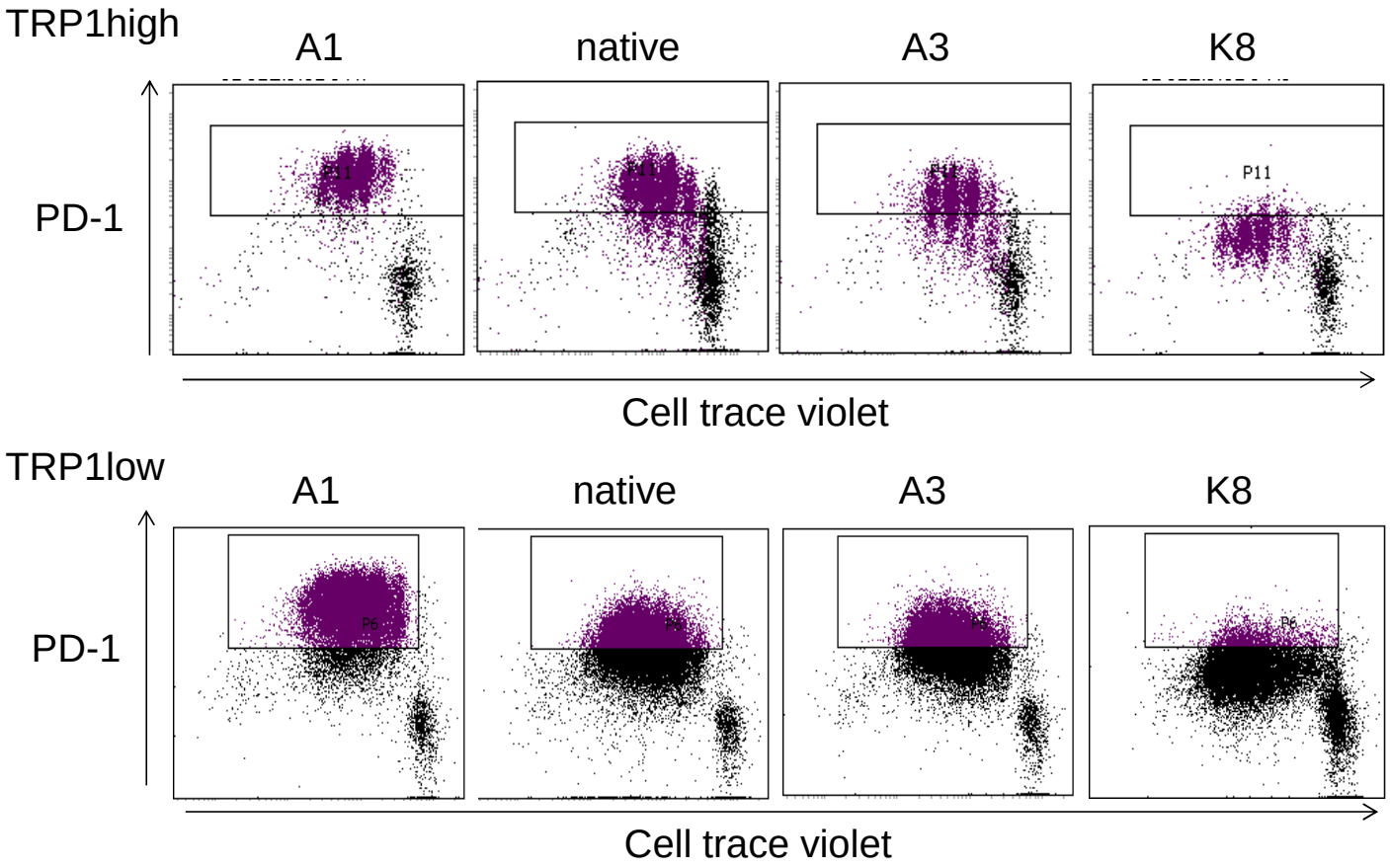
K8 activation results in more long-term survivors

	Total number mice	Long term survivors	Survived rechallenge
TRP1low K8	15	3	1/3
TRP1high K8	15	2	2/2
TRP1low M9	15	1	0/1
TRP1high M9	15	0	0



Memory T cells in spleen
3 months after transfer

K8 activated TRP1high and TRP1low cells remain PD-1 low



Summary for melanoma

Both high and low affinity CD8 T cells are capable of mounting anti-tumor responses.

TRP1 transnuclear mice are good models to study anti-tumor immunity.

K8 activation leads to good control of tumor growth and induction of long-term memory.

K8 activated CD8 cells are PD-1 low and have a unique transcriptional profile.

Can we exploit the genes differentially regulated in K8 activated cells to enhance CD8 T cell memory?

Pancreatic ductal adenocarcinoma

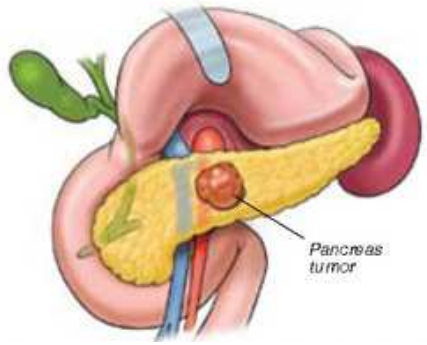
Poor prognosis

Highly metastatic

Hypovascular

Dense stroma

Poor drug delivery

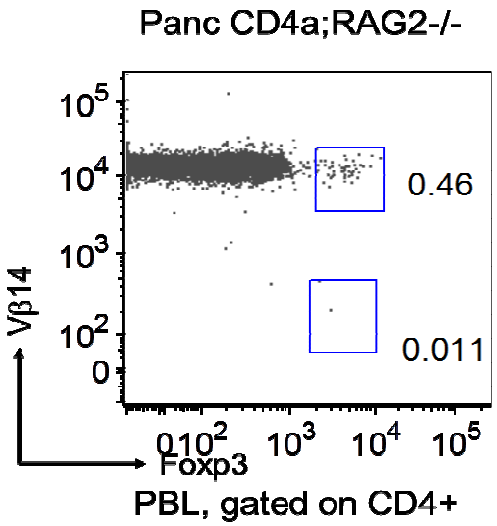


Very few T cell infiltrates (mostly Tregs)

Transnuclear mice from pancreatic tumor-infiltrating CD4 T cells

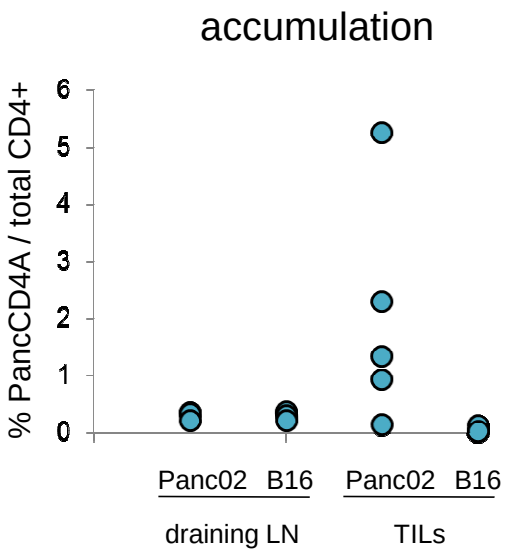
Panc CD4a: TCR Vβ14+

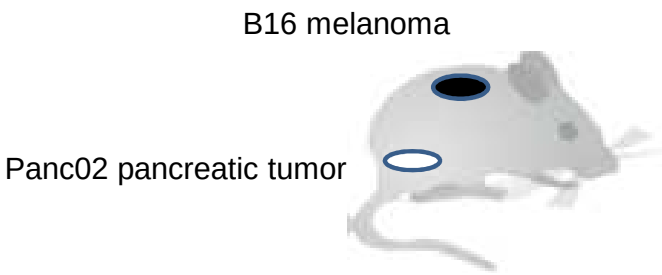
Germline-transmitting chimera



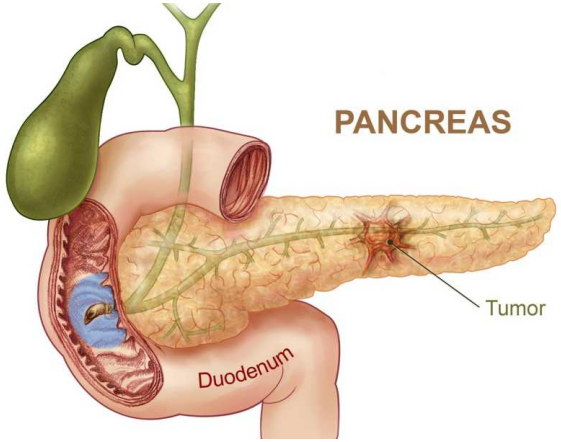
Normal frequency of Foxp3+ Tregs

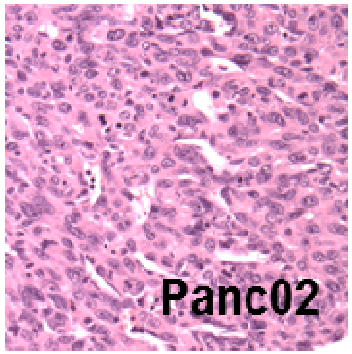
PancCD4A cells specifically infiltrate pancreatic tumors



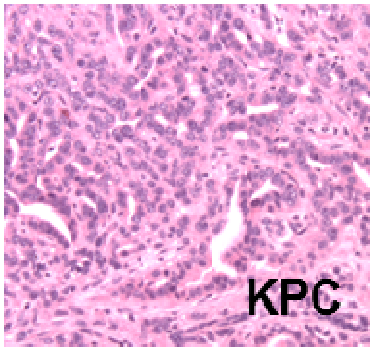


Orthotopic injections of cancer cells directly into pancreas



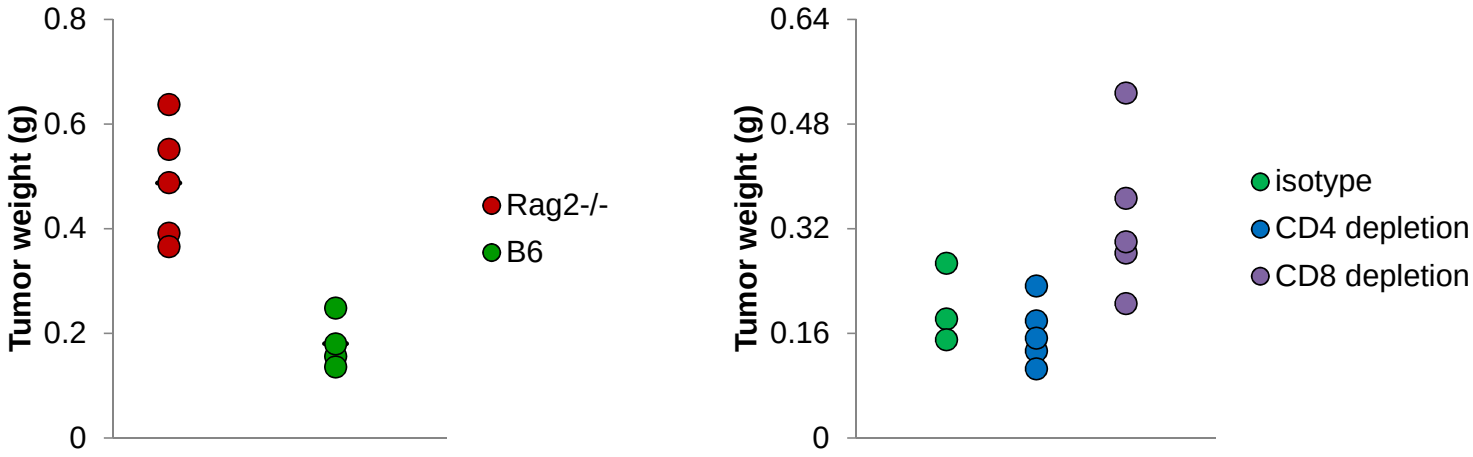


609 SNPs



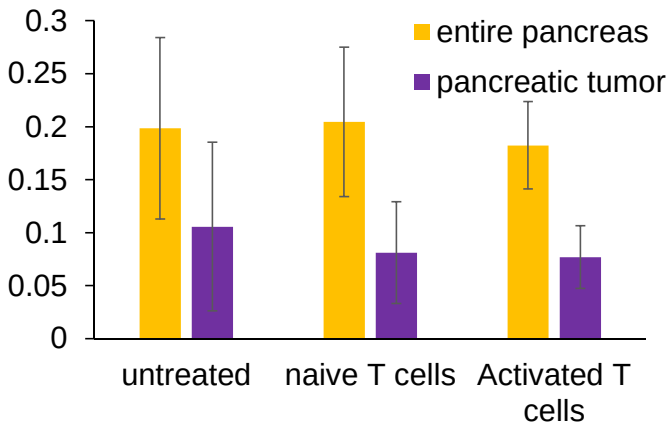
~30 SNPs

CD8 T cells are important in control of orthotopic Panc02

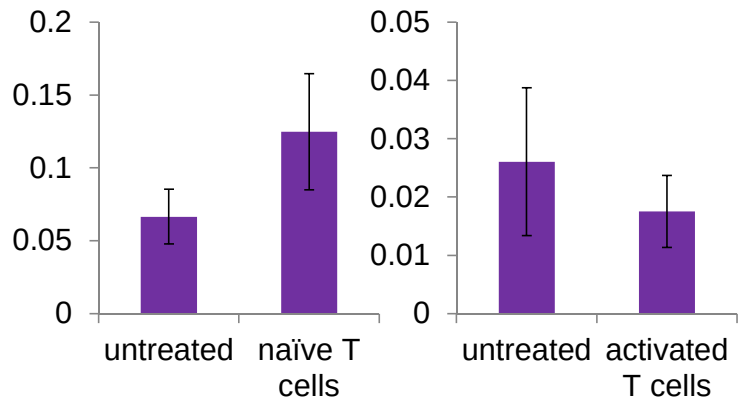


PancCD4a cells affect tumor growth only when given early

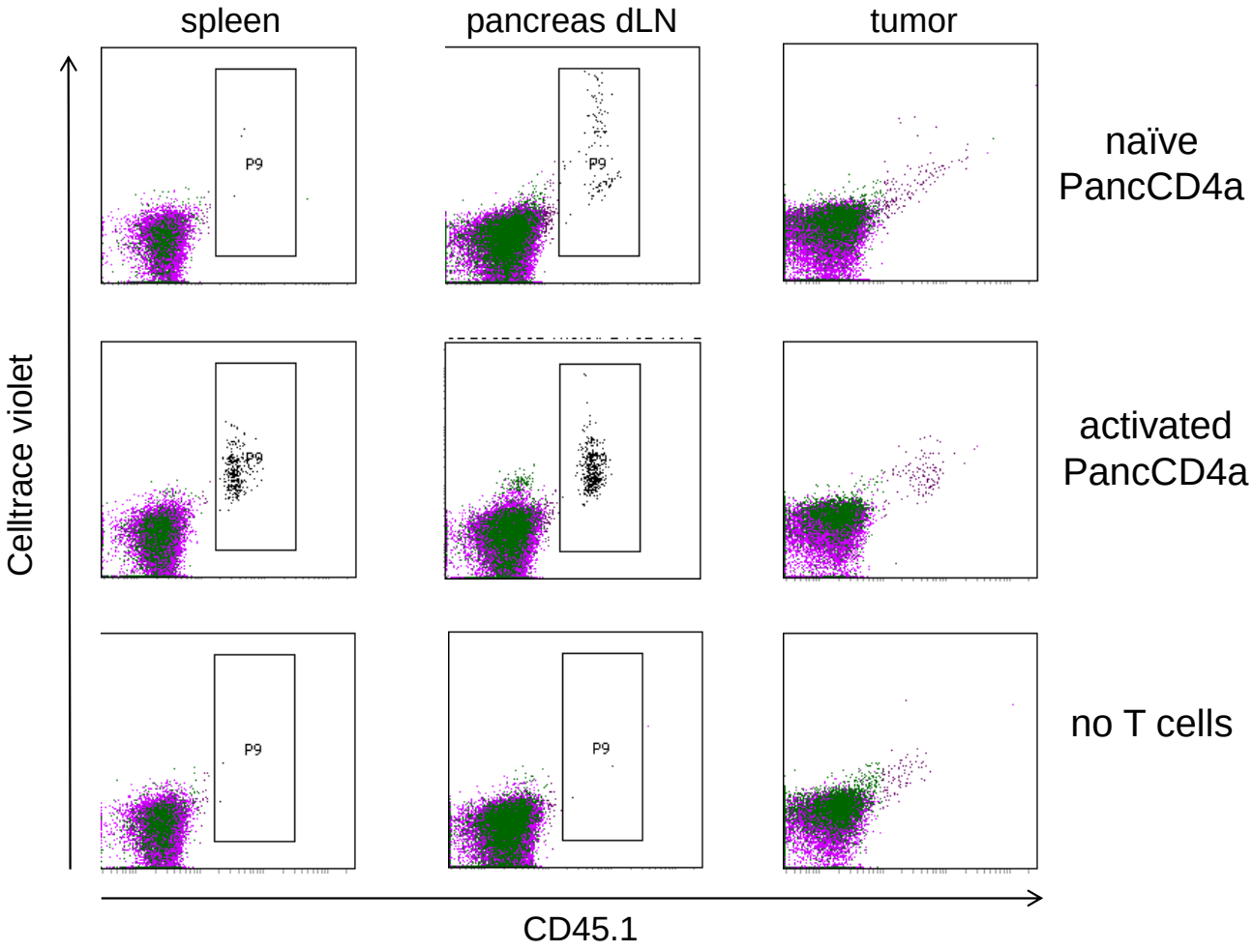
PancCD4a cells given **5** days after tumor



PancCD4a cells given **1** day after tumor



Transferred PancCD4a cells proliferate in pancreas draining LN



VHH Antibodies

Readily expressed in bacteria

Small size (15 kDa) allows deep tissue penetration

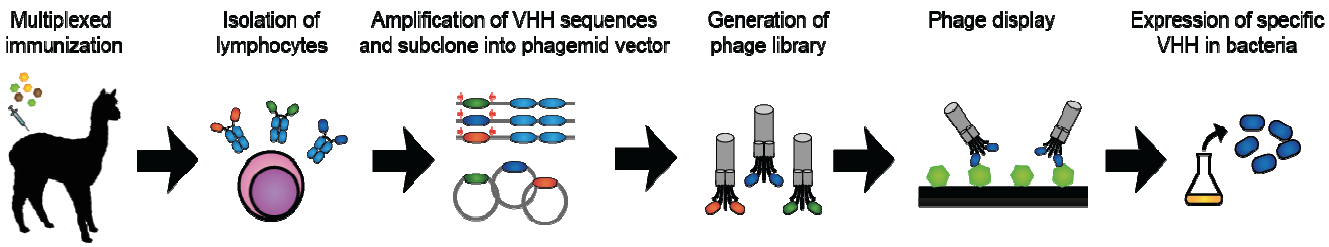
Rapidly cleared after injection

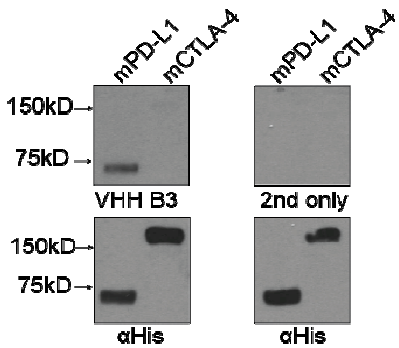
No FcR or complement engagement or cross-linking

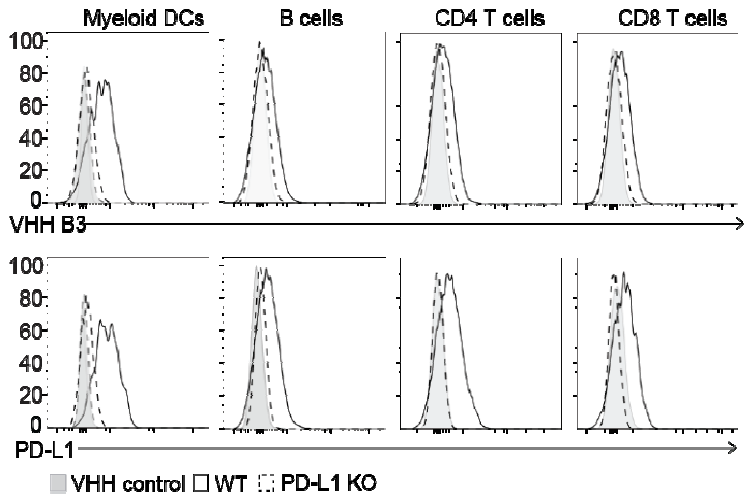
Expressed with LPETG tag for modification by sortase

In collaboration with Hidde Ploegh, Jessica Ingram and Michael Dougan

Alpaca antibody B3 is specific for PD-L1

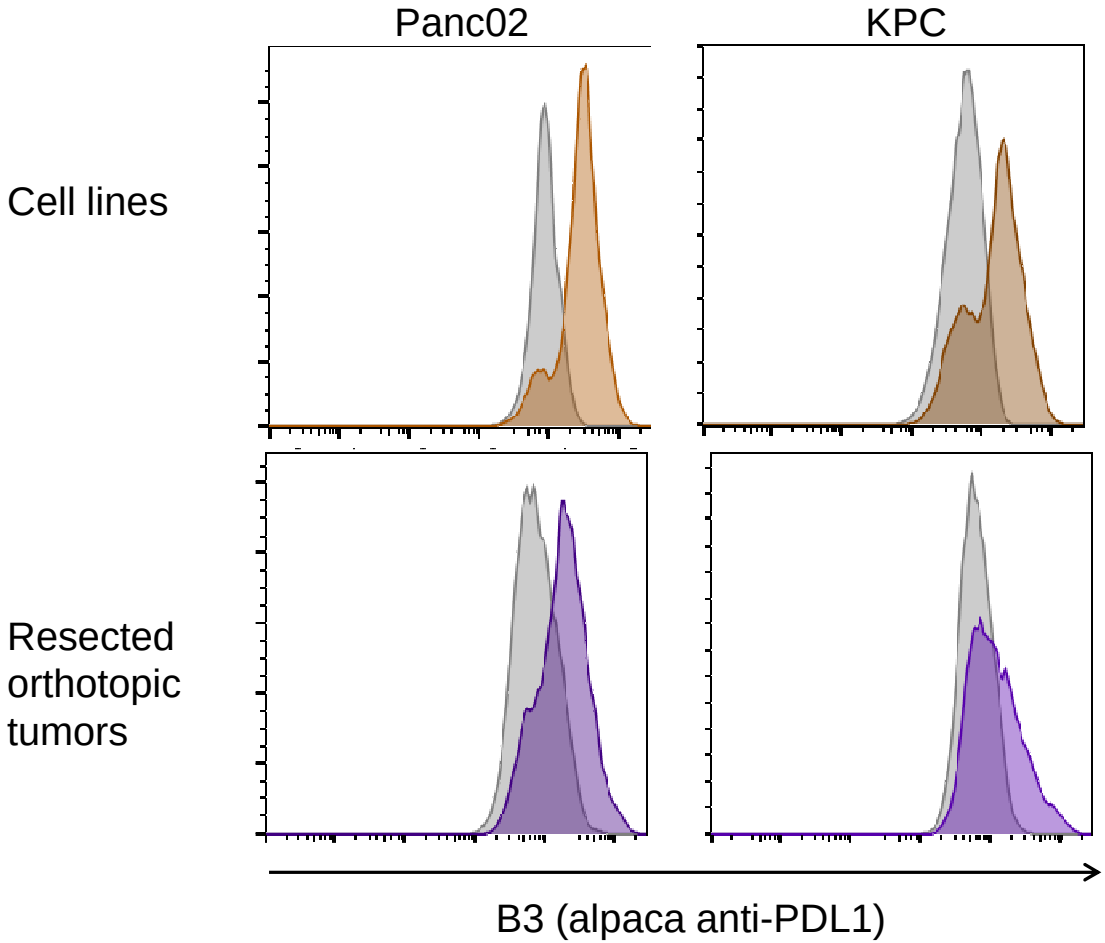




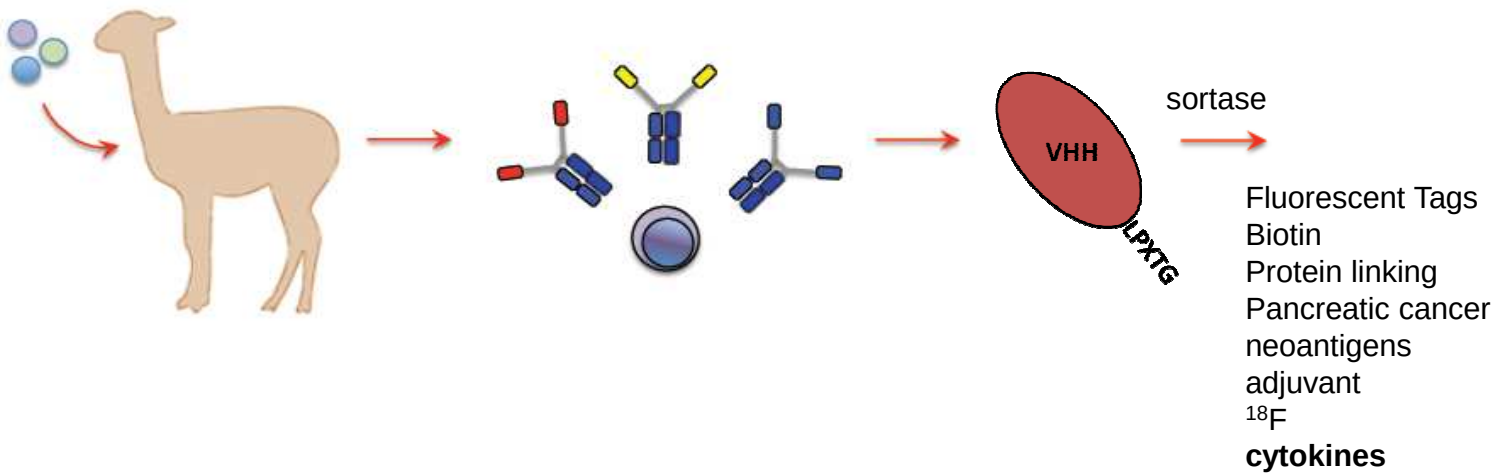


Hidde Ploegh, Jessica Ingram and Michael Dougan

Pancreatic tumor cells express PD-L1

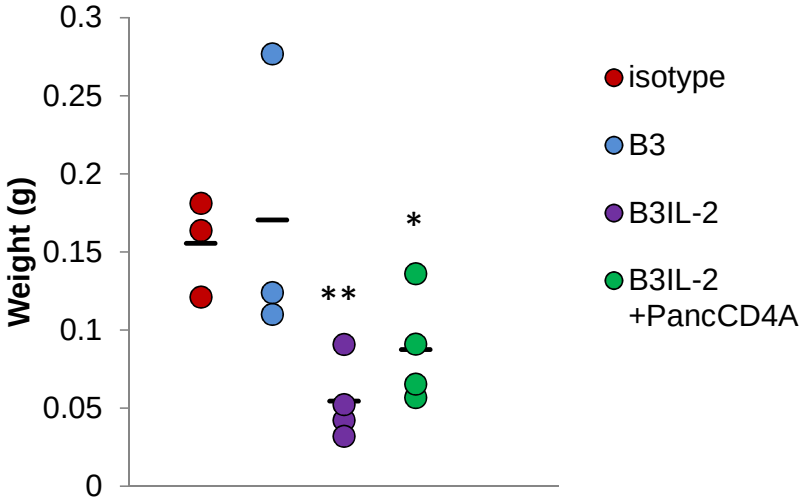


Alpaca-derived anti-PD-L1 conjugated to IL-2

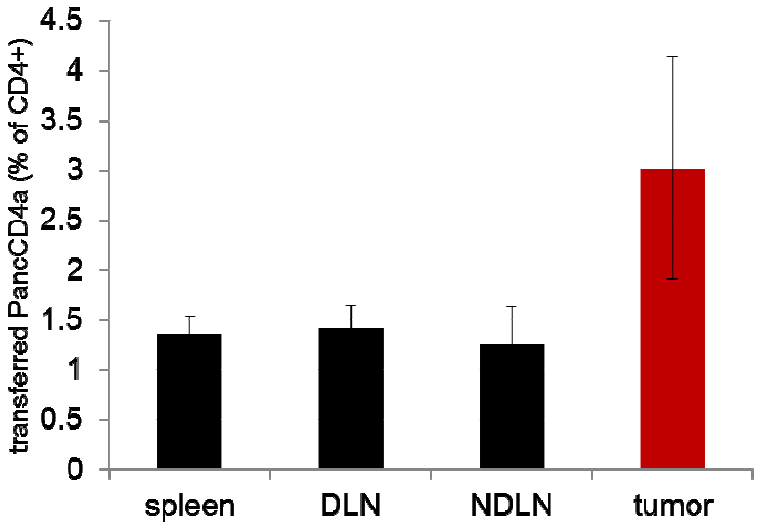


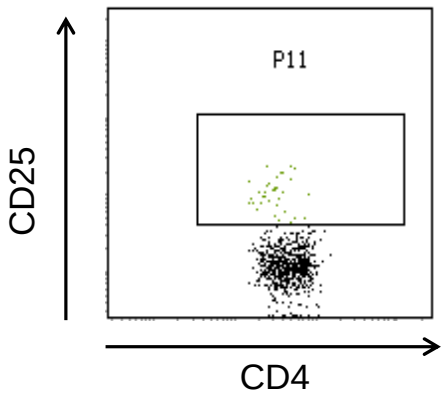
In collaboration with Hidde Ploegh, Jessica Ingram and Michael Dougan

Very low dose B3-IL2 decreases pancreatic tumor burden



Very low dose B3-IL2 allows infiltration of PancCD4a T cells





gated on CD45.1 transferred cells

Immunotherapy for pancreatic cancer

Anti-PDL1 alpaca antibodies can target immunotherapeutic agents to pancreatic tumors.

Targeted IL-2 increases CD8 T cells, increases T cell trafficking to tumors, and decreases overall tumor size.

Transnuclear PancCD4a cells can be used to monitor CD4 T cell trafficking and lineage choice.

Intratumoral anti-CD40 generates systemic immune responses against non-treated tumors and may synergize with targeted radiation therapy.

Acknowledgements

Paul Tyler
Mariah Servos
Patrick Bruck
Eleanor Clancy-Thompson
Cherry Ng
Albert Li

Hidde Ploegh (Whitehead)
Rudolf Jaenisch (Whitehead)
Kai Wucherpfennig (DFCI)
Wilfred Ngwa (DFCI/BWH)
Rajiv Kumar (DFCI)
Michael Dougan (MGH)
Jessica Ingram (Whitehead)
Anirban Maitra (MDACC)

Funding

PANCREATIC CANCER ACTION NETWORK®
ADVANCE RESEARCH. SUPPORT PATIENTS. CREATE HOPE.

Claudia Adams Barr Award for Cancer Research
Pancreatic Cancer Action Network Pathway to Leadership Award
Melanoma Research Alliance Young Investigator Award
HMS Shore Fellowship

Melanoma
Research Alliance