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# **Non-clinical Models for Safety Assessment of Immuno-oncology Products**

September 6, 2018 | Washington, DC

@FDAOncology

@AACR



Join the conversation with #OCEIONonClinModels

# Workshop Cochairs:

- John K. Leighton, PhD
- Haleh Saber, PhD
- Julie Scheider, PhD

# Welcome

## Speakers:

John K. Leighton, PhD



## **Non-Clinical Models for Safety Assessment of Immuno-Oncology Products**

**September 6th, 2018**

**Marriott Wardman Park, Washington, DC**

John K. Leighton  
Director, Division of Hematology Oncology Toxicology  
Office of Hematology and Oncology Products, CDER



# **FDA Alerts Healthcare Professionals and Oncology Clinical Investigators about Two Clinical Trials on Hold Evaluating KEYTRUDA® (pembrolizumab) in Patients with Multiple Myeloma**

**Update [September 20, 2017]:** The FDA has informed multiple investigators who have ongoing clinical trials using PD1/PD-L1 oncology drugs in combination with immunomodulatory agents or in hematologic malignancies combined with other classes of drugs whether their trials must be temporarily stopped to allow for modifications or must be permanently stopped.

**[August 31, 2017]** Based on data from two recently halted clinical trials, the U.S. Food and Drug Administration today is issuing this statement to inform the public, health care professionals, and oncology clinical investigators about the risks associated with the use of KEYTRUDA® (pembrolizumab) in combination with dexamethasone and an immunomodulatory agent (lenalidomide or pomalidomide) for the treatment of patients with multiple myeloma. KEYTRUDA® (pembrolizumab) is not approved for treatment of multiple myeloma.



Contents lists available at ScienceDirect

## Regulatory Toxicology and Pharmacology

journal homepage: [www.elsevier.com/locate/yrtph](http://www.elsevier.com/locate/yrtph)

## An FDA oncology analysis of immune activating products and first-in-human dose selection



Haleh Saber\*, Ramadevi Gudi, Michael Manning, Emily Wearne, John K. Leighton

*US Food and Drug Administration, Center for Drug Evaluation and Research, Office of Hematology and Oncology Products, 10903 New Hampshire Ave, Silver Spring, MD 20903, United States***Table 4A**Examples of antibodies with FIH doses at  $\leq 50\%$  RO.

Target or class[date of IND submission]	FIH dose	HHD (time from IND submission to this dose)	RO <sup>a</sup> at HHD	Ratio of HHD to FIH dose
Checkpoint stimulator [2014]	200 mcg	1200 mg (2 yr)	Saturated	6000
Checkpoint stimulator [2014]	36 mcg	3.6 mg (1 yr)	90%	100
Checkpoint stimulator [2015]	6 mcg	18 mg (1 yr)	Saturated	3000
Checkpoint stimulator [2014]	1.5 mcg	30 mg (1 yr)	Saturated	20,000
Checkpoint stimulator[2010]	6 mcg	120 mg (5 yr)	Saturated	20,000
Expected to activate the immune system based on experience with another product targeting the same antigen [2009]	6 mcg	1.2 g (5 yr)	Saturated	200,000

HHD: highest human dose with acceptable toxicities, at the cut-off date.

Note: doses are converted to a flat dose using 60 kg as the body weight.

<sup>a</sup> Estimated based on in vitro binding data.

# Current Status

- A 2016 FDA publication on CPI/CPS surveyed current approaches to developing these products
  - Some data gaps were identified
  - Additional work on IO products has been published by several different groups
- In Sept 2017 NCI/JAX sponsored a Think Tank on immune interventions in oncology
  - Focused on modeling opportunities in mice and human specimens
  - Uses and limitations of mouse models
  - Development of biomarkers
  - Research needs
- Regulatory science data gaps were not discussed
- FDA research on humanized mouse models is ongoing

# Nonclinical Models for Immunotherapy

- Mouse xenografts
- Syngeneic mice
- Genetically-modified mice
- Humanized mice
- Companion animals
- Organs-on-a-chip/organoids/etc.

# Review Challenges

- Definitions of pharmacologic activity and MABEL are vague
  - Generally derived from in vitro data for IO products
- Lack of transparency in translating functional assays/receptor occupancy to FIH dose
  - Not all sponsors use the same approach and details are sometimes lacking
- Challenges in integrating NHP pk/ADME and xenograft data into FIH dose selection/safety assessments
- Challenges in using other relevant factors (e.g., receptor turnover/tumor expression) in FIH dose selection

# Discussion Topics

- Current challenges in nonclinical development of IO products
  - How to fill in gaps? Existing paradigm or innovative approaches?
- Nonclinical models for IO products
  - Advancing the field with improved models?
  - Utility of these models in better predicting clinical outcome of safety? Efficacy?
  - Will one model be sufficient to study multiple targets?
  - Pros and cons of using different PD models to evaluate the activity and safety of IO products?
  - Use in FIH dose selection?



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# Morning Session

## Speakers:

Marcela V. Maus, MD, PhD

Sarah Javid, PhD

Karolina Palucka, MD, PhD

Gregory Beatty, MD, PhD

Amy K. LeBlanc, DVM

Lei Zheng, MD, PhD



# Current non-clinical models for immuno-oncology (T cell) products

*Marcela V. Maus, MD, PhD*

*FDA-AACR Non-clinical models for safety assessment of immuno-oncology products*

*Washington, DC, September 6, 2018*



HARVARD MEDICAL  
SCHOOL



MASSACHUSETTS  
GENERAL HOSPITAL

CANCER CENTER

## Statement(s) of the obvious

- There is no animal model that can fully predict the safety, either on- or off-target effects, of a particular new drug in human patients
- Immuno-oncology in particular attempts to modulate a interplay between multiple immune cell types, the tumor, and normal tissues
  - No animal has a fully human immune system, a tumor, and normal human tissues
- Immuno-oncology 'drugs' do not get cleared by the liver/kidney with defined half-lives and kinetics
- Patients are typically ill with the disease and have had prior treatments, whereas animals are asymptomatic and get only 'first-line' treatments

# Goals of animal models for immuno-therapies

- Pre-clinical: a path to IND and testing in humans
  - Human T cells are now used as drugs to treat disease; the human T cell is the investigational product!
  - Focus on efficacy (and ideally toxicity, but this is less developed)
  - Animal models not required for most T cell products on path to IND but most sponsors/investigators do use them
- Post-clinical: Modeling observed effects from clinical setting
  - Adverse effects, cytokine release syndrome, neurotoxicity, resistance mechanism
- Basic science:
  - Modeling tumor environment/interactions/basic immunology
  - Modeling a human disease (autoimmunity/transplantation/cancer)

# NSG mice: the current standard for path to IND

- NSG mice
  - Xenografted with human tumor lines/pdx
  - Investigational agent is human T cell
  - Advantages:
    - Well established, easy to buy or breed, path to IND is feasible
    - Sustains engraftment of human T cells and tumor cells
    - Can discriminate anti-tumor efficacy of different T cell constructs with dose and timing in 4-12 week models
  - Limitations:
    - Does not model on or off-target toxicity against human tissues
    - Does not model cytokine release syndrome/neurotoxicity/macrophage activation syndrome
    - Does not model long-term efficacy due to xeno-GvHD

## Other mice

- SCID/beige
  - Have some NK cells and better macrophages than NSG
  - Do not engraft as many tumors or T cells as well
- Athymic/nude
  - Have good NK and macrophages, but many human tumors don't engraft at all – not used very often
- Syngeneic mouse models
  - Few available (mouse CD19, mouse EGFRvIII)
  - Use mouse T cells, mouse CARs, mouse tumor – no longer studying the human T cell or the investigational product, but can model microenvironment
  - Do not develop clinical toxicities observed with human CAR T cells (cytokine release syndrome/MAS, neurologic toxicity)

# Reminder of how the field got to axi-cel

Published in final edited form as:

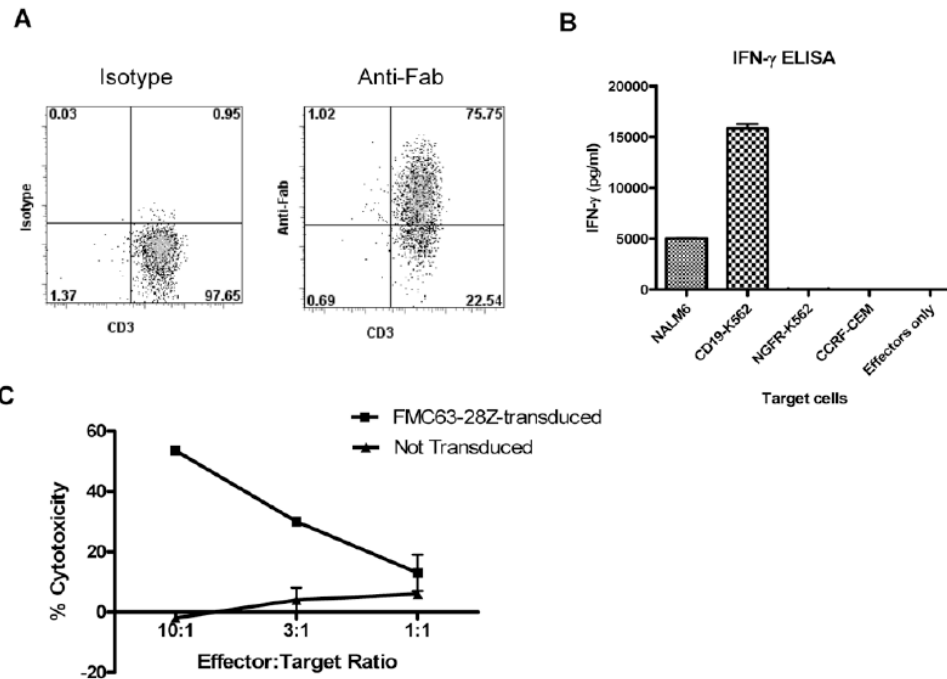
*J Immunother.* 2009 September ; 32(7): 689–702. doi:10.1097/CJI.0b013e3181ac6138.

## Construction and Pre-clinical Evaluation of an Anti-CD19 Chimeric Antigen Receptor

James N. Kochenderfer<sup>\*</sup>, Steven A. Feldman<sup>\*</sup>, Yangbing Zhao<sup>\*</sup>, Hui Xu<sup>\*</sup>, Mary A. Black<sup>\*</sup>,  
Richard A. Morgan<sup>\*</sup>, Wyndham H. Wilson<sup>Ψ</sup>, and Steven A. Rosenberg<sup>\*</sup>

<sup>\*</sup>Surgery Branch of the National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

<sup>Ψ</sup>Metabolism Branch of the National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

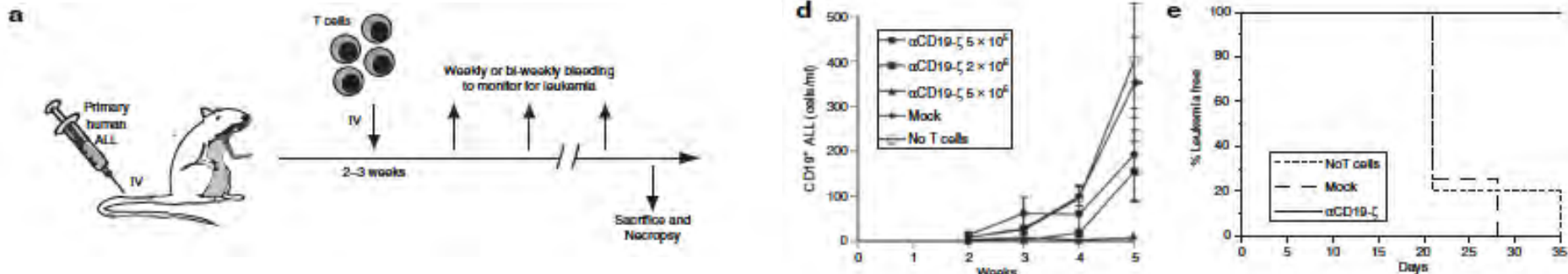


# Reminder of how we got to... tisagenlecleucel

## Chimeric Receptors Containing CD137 Signal Transduction Domains Mediate Enhanced Survival of T Cells and Increased Antileukemic Efficacy *In Vivo*

Michael C. Milone<sup>1,2</sup>, Jonathan D. Fish<sup>3,4</sup>, Carmine Carpenito<sup>1</sup>, Richard G. Carroll<sup>1</sup>, Gwendolyn K. Binder<sup>1</sup>, David Teachey<sup>3,4</sup>, Minu Samanta<sup>2</sup>, Mehdi Lakhali<sup>1</sup>, Brian Gloss<sup>1</sup>, Gwenn Danet-Desnoyers<sup>5</sup>, Dario Campana<sup>6,7</sup>, James L. Riley<sup>1,2</sup>, Stephan A. Grupp<sup>3,4</sup> and Carl H. June<sup>1,2</sup>

<sup>1</sup>Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania USA; <sup>2</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania USA; <sup>3</sup>Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania USA; <sup>4</sup>Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; <sup>5</sup>Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA; <sup>6</sup>Department of Oncology, St Jude Children's Research Hospital, Memphis, Tennessee, USA; <sup>7</sup>Department of Pathology, St Jude Children's Research Hospital, Memphis, Tennessee, USA






## Then what happened?

- CAR T cells in humans were much more potent than expected. Long term remissions seen in the first 5 patients treated at multiple centers.
- Toxicities of cytokine release syndrome (CRS) and neurologic toxicity became apparent at about patient 4...
- You know the rest
- Why wasn't any of this predicted?
  - CRS (and neurotoxicity) requires a myeloid compartment (IL-6, endothelial cell activation.... ) that interacts with activated T cells

# Humanized mice: need to be engrafted with human cord blood to get chimeras, and skew toward myeloid

MODEL	 huNOG-EXL	 Humanized NSG-SGM3 (or hu-CD34-SGM3)	 Humanized MISTRG
STRAIN	hGM-CSF/hIL3-NOG	NSG™-SGM3	MISTRG
ALSO KNOWN AS	IL34GM-Tg	NSGS	-
NOMENCLATURE	NOG.Cg-Prkdc <sup>tm1</sup> Il2rg <sup>tm1</sup> Tg(5V40)/hIL3,CSF2,IL3- T310-uraTac	NOG.Cg-Prkdc <sup>tm1</sup> Il2rg <sup>tm1</sup> Tg(CMV- IL3,CSF2,KITLG)IEav/MloY5zJ	C:129S4-Rag2 <sup>tm1.1UW</sup> Csfl <sup>tm1.1UCSF</sup> Flv-Csf2/Il3 <sup>hmlUCSF2.1.1UW</sup> Thpa <sup>tm1.1TPOJW</sup> Il2rg <sup>tm1.1UW</sup> Tg(SIRPA)1Flw/J
BACKGROUND	NOG (NOG strain background)	NSG™ (NOG strain background)	Mixed BALB/c x 129S4
CYTOKINES EXPRESSED (other modifications)	Human GM-CSF (CSF2) Human IL-3	Human GM-CSF (CSF2) Human IL-3 Human KITLG (SF)	Human GM-CSF (CSF2) Human IL-3 Human M-CSF (CSF1) Human TPO Human SIRPa
LIFESPAN AFTER CD34+ HSC ENGRAFTMENT	Up to 7 months reported. High chimeric ratio mice develop anemia after engraftment. <sup>1</sup>	Up to 4 months in ongoing studies. Mice develop sporadic anemia after engraftment. <sup>2</sup>	3 weeks after engraftment reaches 10-20% chimerism in peripheral blood if pre- conditioned with irradiation (~10-12 weeks post- engraftment); lifespan may be prolonged by using less potent stem cells, lower cell numbers or avoiding pre- conditioning. <sup>6</sup>
OTHER COMMENTS	Stable engraftment through lifespan of mouse.	Loss of human graft after 3-4 months. <sup>3</sup>	
TERMS OF USE	Label license – no signatures or license fees required. May be used for contract or sponsored studies when purchased under for-profit terms and price.	Research institutions require an MTA, companies require a license prior to shipping. <sup>7</sup>	Not available to companies or for commercial use. <sup>8</sup>
AVAILABLE FROM	Taconic Biosciences ( <a href="http://taconic.com/hunog-exl">taconic.com/hunog-exl</a> ) Naïve: <a href="http://taconic.com/13395">taconic.com/13395</a>	The Jackson Laboratory	The Jackson Laboratory (DOI: <a href="https://doi.org/10.1002/1097-4644(201601)18(1)&lt;10::aid-jmri1001&gt;3.0.co;2-1">10.1002/1097-4644(201601)18(1)&lt;10::aid-jmri1001&gt;3.0.co;2-1</a> )

# Two new mouse models that model cytokine release syndrome published in Nature Medicine, May 2018

## **Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells**

Margherita Norelli<sup>1,2</sup>, Barbara Camisa<sup>1</sup>, Giulia Barbiera<sup>3</sup>, Laura Falcone<sup>1</sup>, Ayurzana Purevdorj<sup>1</sup>, Marco Genua<sup>3</sup>, Francesca Sanvito<sup>4</sup>, Maurilio Ponzoni<sup>4</sup>, Claudio Doglioni<sup>4</sup>, Patrizia Cristofori<sup>5</sup>, Catia Traversari<sup>6</sup>, Claudio Bordignon<sup>2,6</sup>, Fabio Ciceri<sup>2,7</sup>, Renato Ostuni<sup>3</sup>, Chiara Bonini<sup>2,8</sup>, Monica Casucci<sup>1</sup> and Attilio Bondanza<sup>1,2\*</sup>

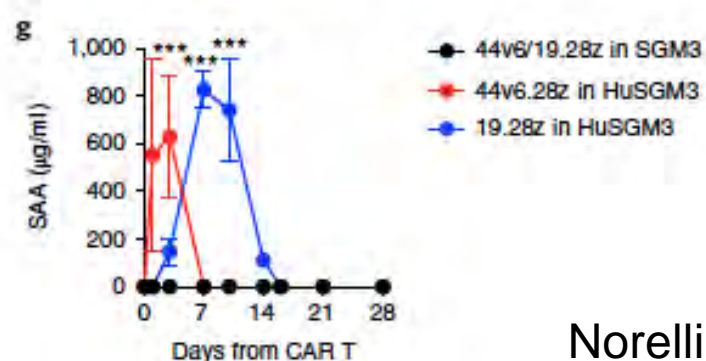
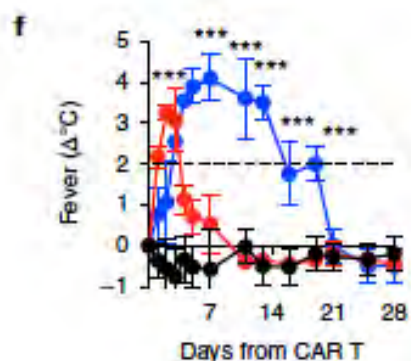
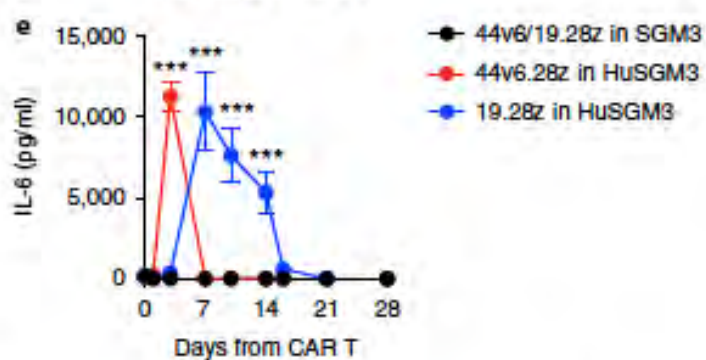
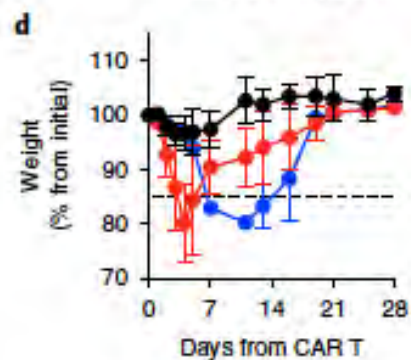
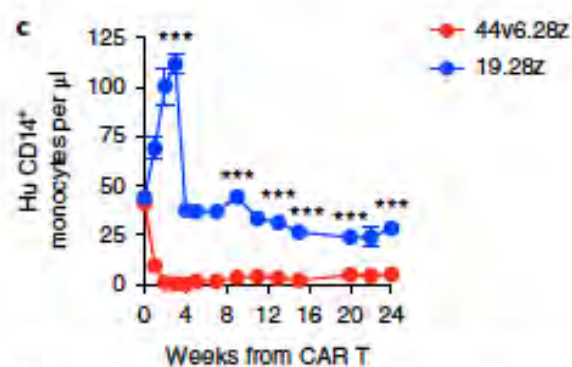
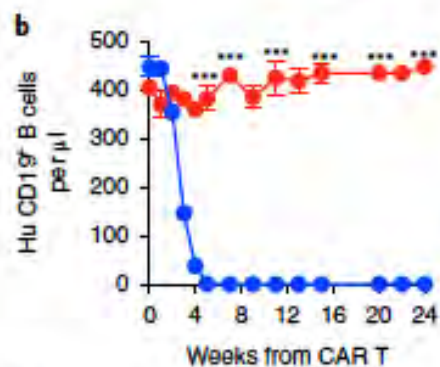
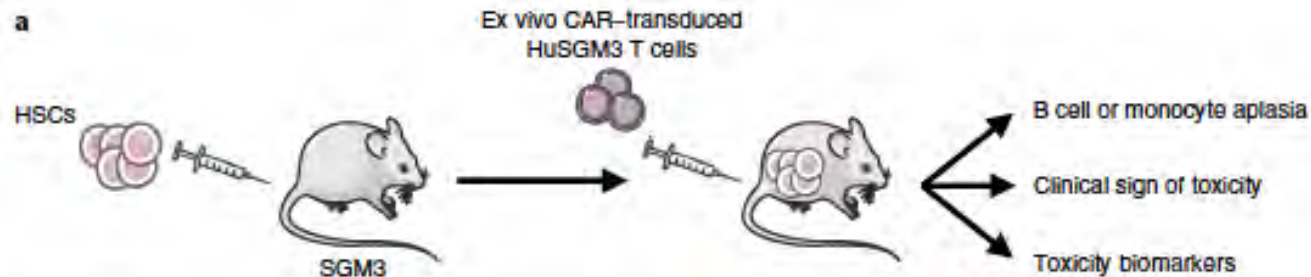
NSG mice transgenic for human IL-3, GM-CSF, and stem cell factor (SGM3)  
sublethal irradiation of newborn mice (time delay of even 2 days abrogated effect)

injected with human cord blood hematopoietic stem and progenitor cells

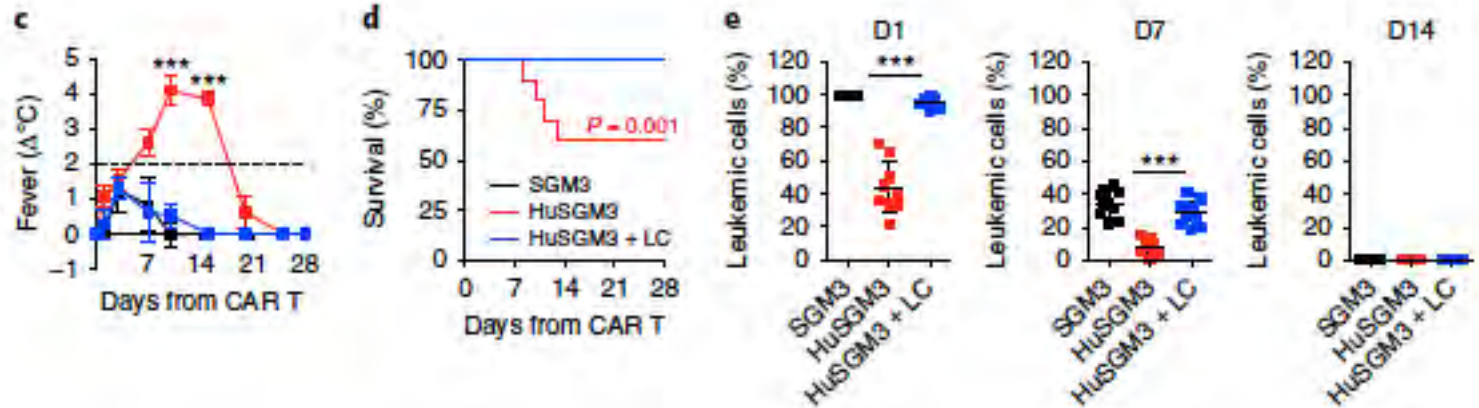
Reconstitute hematopoiesis with B cells, monocytes, T cells, myeloid cells

nHuSGM3 mouse T cells hyporesponsive to I-Ag7

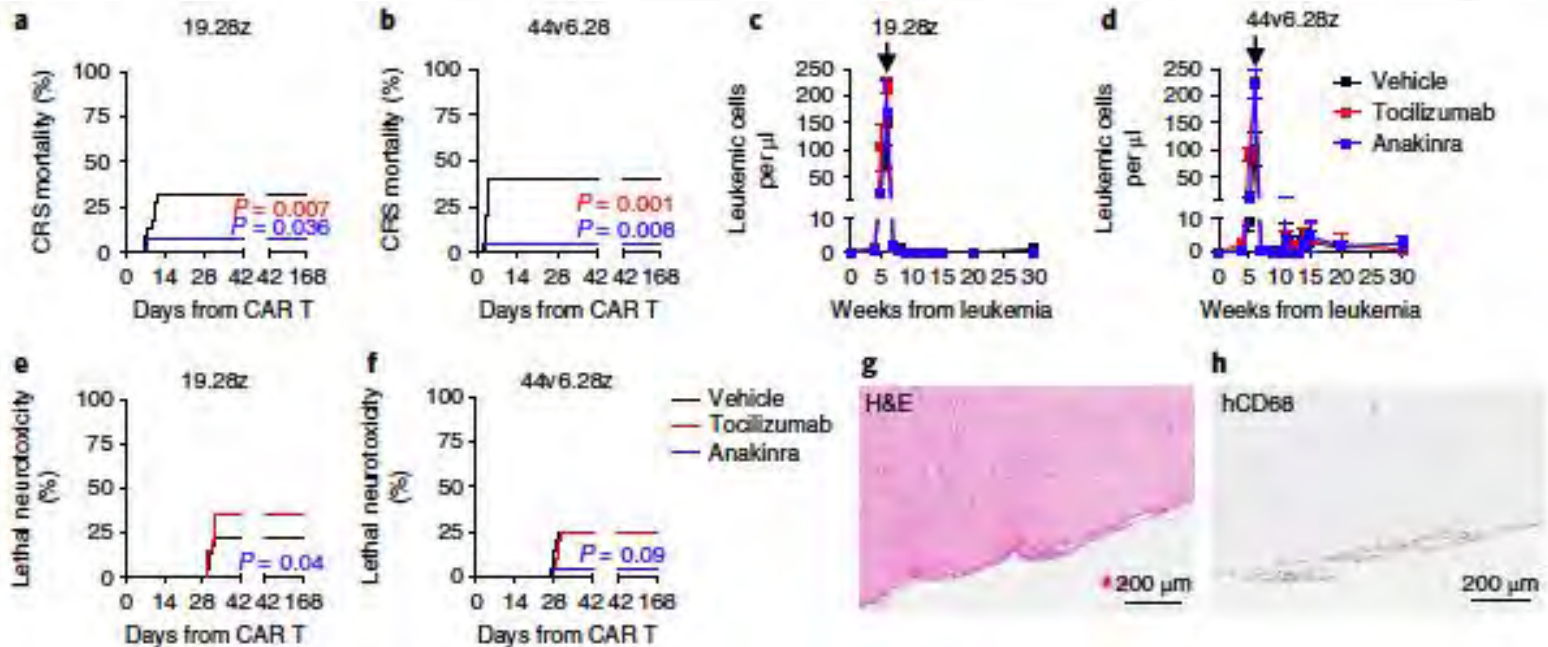
but respond to allo (I-Ad) and xeno (human) – which means you need T cells from the same cord or from another mouse injected with same donor (with lower transduction efficiency than mature peripheral blood T cells...?)



Monocyte  
depletion  
ablates  
CRS (but  
also help  
clear ALL)



Ankinra ,  
Toci  
work for  
CRS, but  
only anakinra  
for  
neurotox





## CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade

Theodoros Giavridis<sup>1</sup>, Sjoukje J. C. van der Stegen<sup>1</sup>, Justin Eyquem<sup>1</sup>, Mohamad Hamieh<sup>1</sup>,  
Alessandra Piersigilli<sup>2</sup> and Michel Sadelain<sup>1\*</sup>

### SCID-Beige mice

injected intraperitoneal tumors – allows for larger tumor burden before lethality  
large numbers required (3e6 Raji ip → 3 weeks → 30e6 CART ip)

CRS developed 2-3 days post-CART (19-28z)

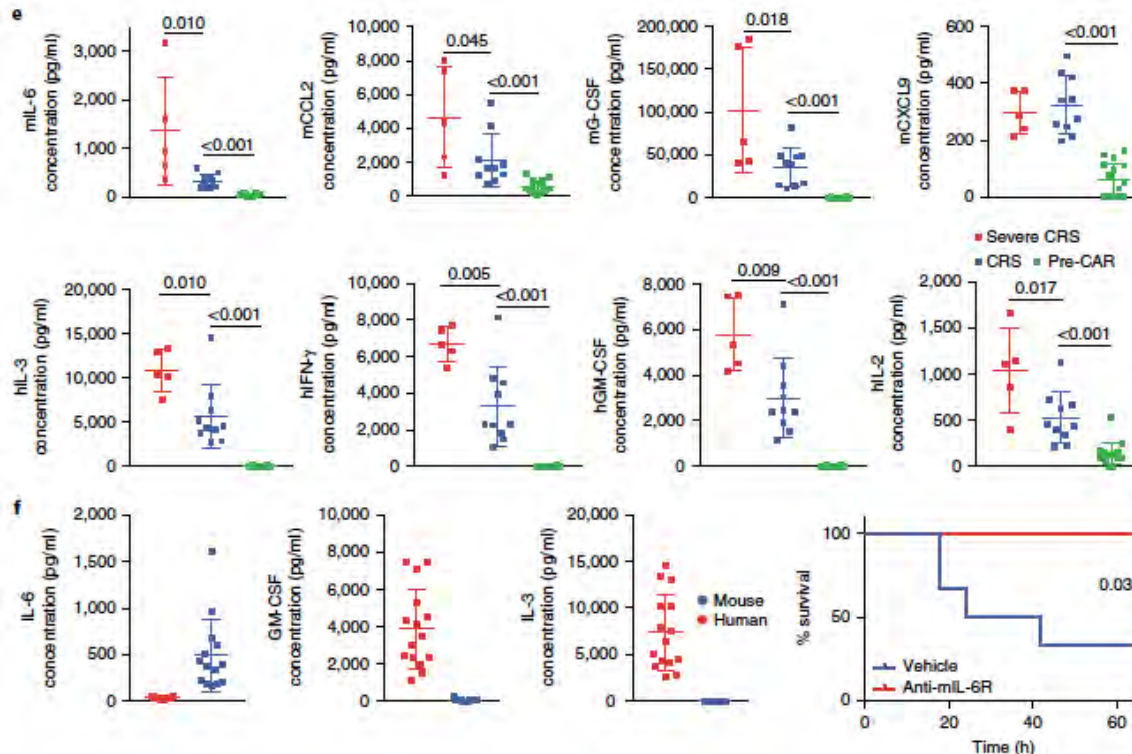
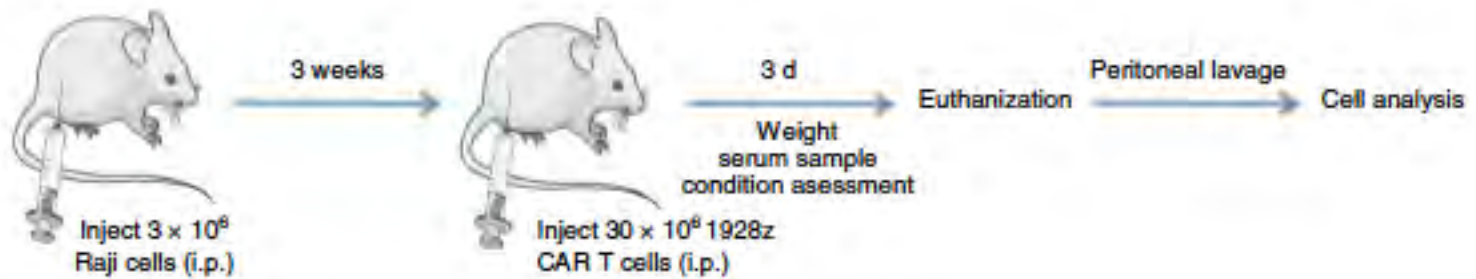
CRS = reduced activity, malaise, piloerection, weight loss... mortality  
increased serum amyloid (murine equivalent of CRP)

mixed human/murine cytokine profile: mIL-6, mG-CSF, hGM-CSF, hIFN $\gamma$ , hIL2

hIFN $\gamma$  and hGM-CSF don't bind murine receptors

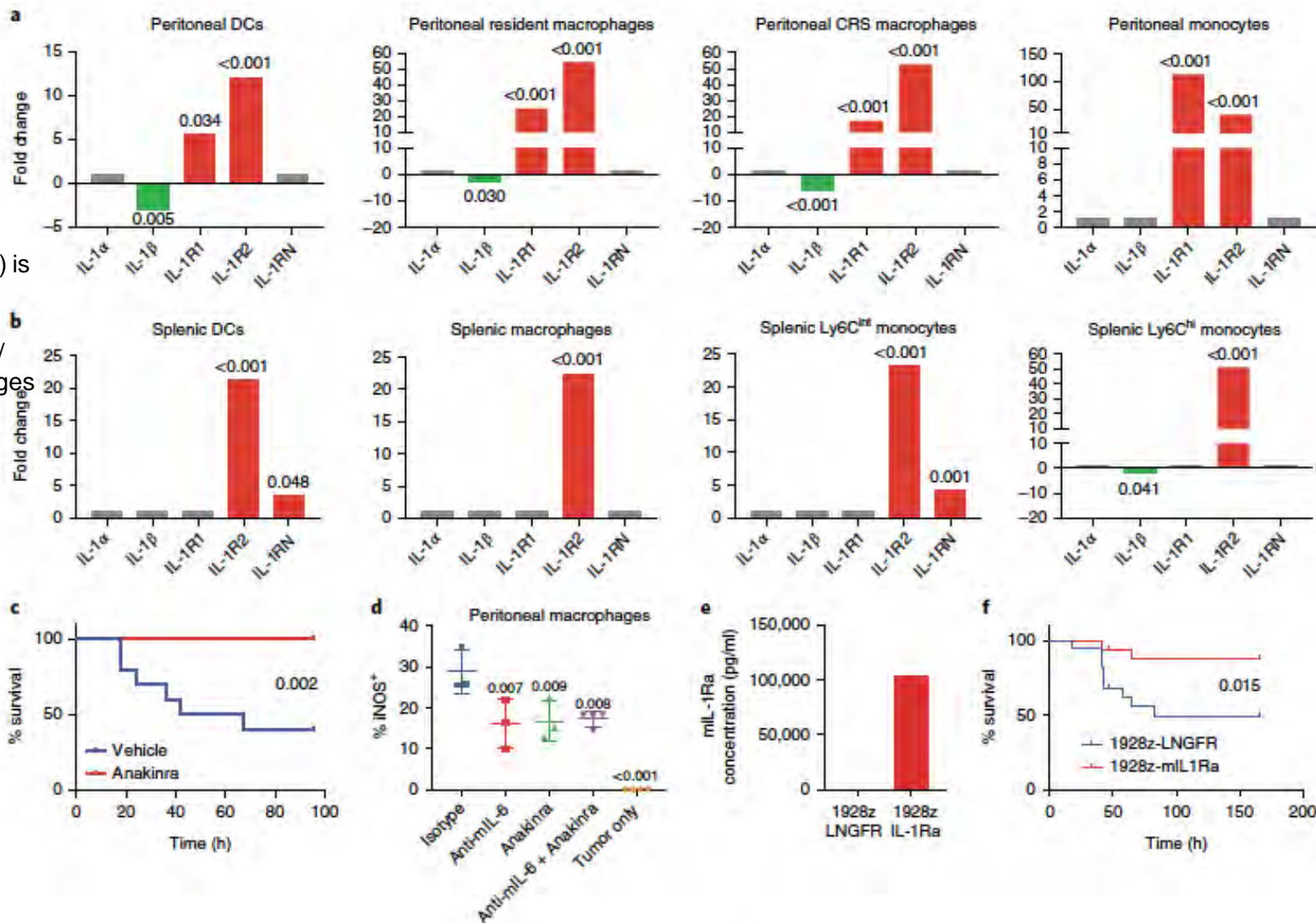
→ unclear what triggers murine innate immunity

treatment with anti-murine IL-6 receptor blocking antibody prevented mortality

**a**



IL-1R1 (functional) is up only in peritoneal monocyte/macrophages



# Non-mouse: Canines

- Limited reagents to grow/study canine T cells; outbred; useful for veterinarians and pet owners. Unknown toxicity manifestations
- Canine signaling domains? Canine viral vector envelopes?

## **Feasibility and Safety of RNA-transfected CD20-specific Chimeric Antigen Receptor T Cells in Dogs with Spontaneous B Cell Lymphoma**

M Kazim Panjwani<sup>1</sup>, Jenessa B Smith<sup>2</sup>, Keith Schutsky<sup>2</sup>, Josephine Gnanandarajah<sup>1</sup>, Colleen M O'Connor<sup>3</sup>, Daniel J Powell Jr<sup>2</sup> and Nicola J Mason<sup>1</sup>

Molecular Therapy, 2016

## **Toward Immunotherapy With Redirected T Cells in a Large Animal Model: Ex Vivo Activation, Expansion, and Genetic Modification of Canine T Cells**

Melinda Mata; Juan F. Vera; Claudia Gerken; Cliona M. Rooney; Tasha Miller; Catherine Pfent; Lisa L. Wang; Heather M. Wilson-Robles; Stephen Gottschalk

J Immunotherapy, 2014

## Non-mouse: Non-human primates

- Expensive, limited; poor tolerance of chemotherapy. Some neurologic toxicities modeled (but CD20 not CD19), and not clearly useful for human translation

### Chimeric Antigen Receptor T Cell-Mediated Neurotoxicity in Nonhuman Primates

Cancer  
Discovery,  
2018

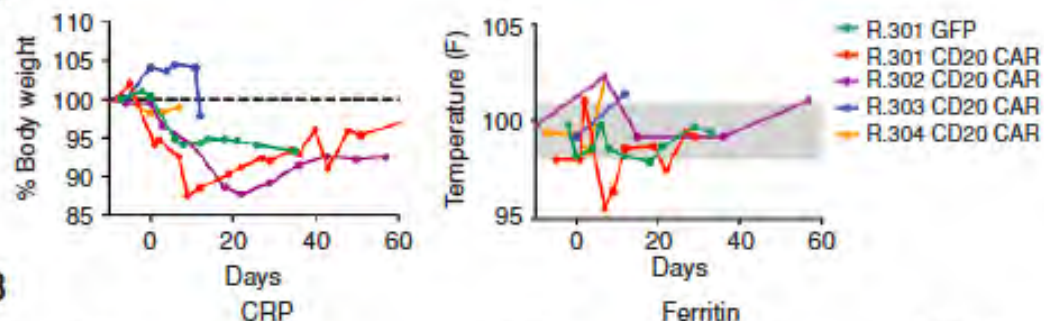
Agne Taraseviciute<sup>1,2,3</sup>, Victor Tkachev<sup>1,2,3</sup>, Rafael Ponce<sup>4</sup>, Cameron J. Turtle<sup>2</sup>, Jessica M. Snyder<sup>5</sup>, H. Denny Liggitt<sup>5</sup>, David Myerson<sup>2,6</sup>, Luis Gonzalez-Cuyar<sup>6</sup>, Audrey Baldessari<sup>7</sup>, Chris English<sup>7</sup>, Alison Yu<sup>1</sup>, Hengqi Zheng<sup>1,3</sup>, Scott N. Furlan<sup>1,2,3</sup>, Daniel J. Hunt<sup>1</sup>, Virginia Hoglund<sup>1</sup>, Olivia Finney<sup>1</sup>, Hannah Brakke<sup>1</sup>, Bruce R. Blazar<sup>8</sup>, Carolina Berger<sup>2</sup>, Stanley R. Riddell<sup>2</sup>, Rebecca Gardner<sup>1</sup>, Leslie S. Kean<sup>1,2,3</sup>, and Michael C. Jensen<sup>1,2,3</sup>

- Autologous rhesus macaque T cells transduced with anti-CD20 CAR, given back after cyclophosphamide (no tumor)

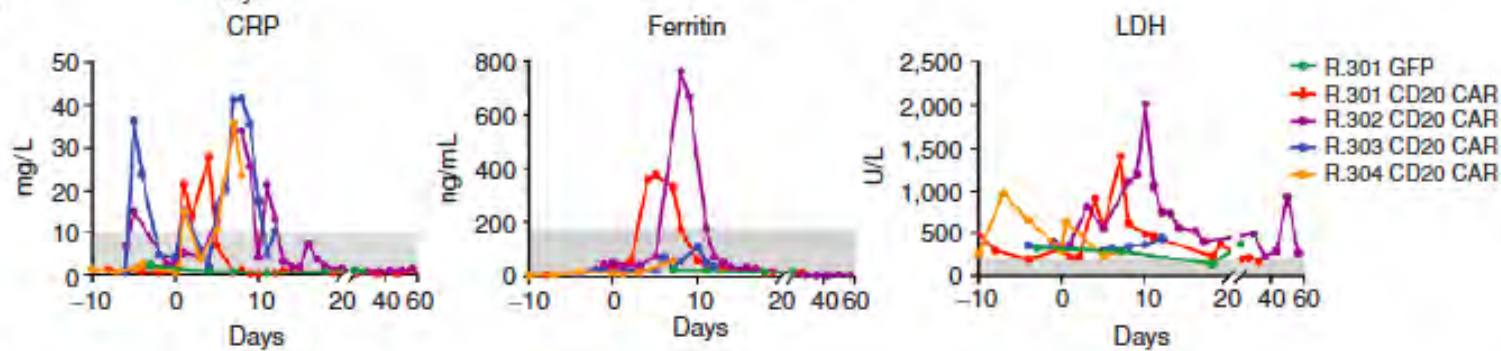


# Cytokine release syndrome in NHP (RM)

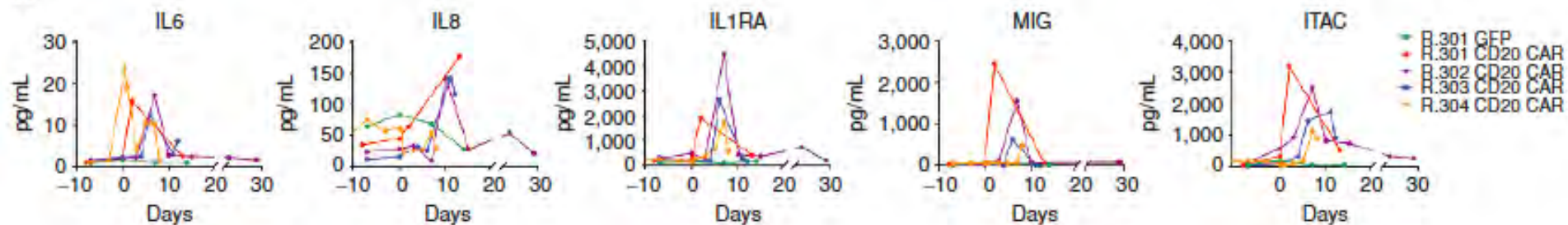
**A**



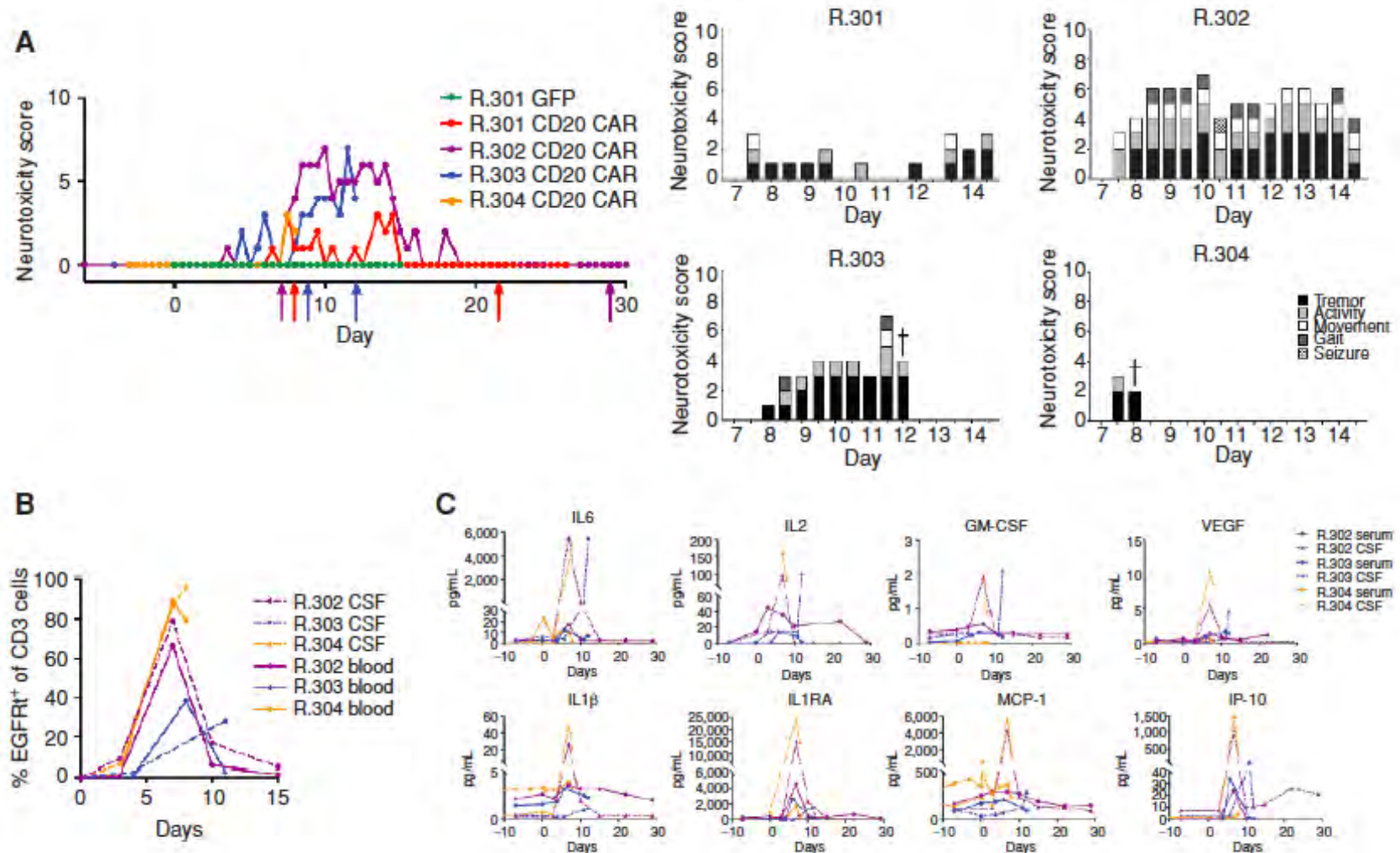
**B**



**C**



# Neurologic toxicity in RM: elevated cytokines in CSF; diffuse infiltration of CAR+ and CAR- T cells in brain parenchyma



# Blue-sky thinking

- NSG mice engrafted with a panel of viable human tissues for sale
  - We developed a skin-grafted NSG mouse to test potential skin-related toxicity
  - Imagine NSG mouse with a little graft (subcu? Orthotopic?) of human heart, lung, brain, liver, skin, HSC. Other desirable tissues could include gut or blood vessel.
  - Could be used to model on-target and off-target toxicity
- NSG mouse that develops macrophage activation syndrome / cytokine release syndrome

# Blue-sky thinking

- NSG mice engrafted with a panel of viable human tissues for sale
  - We developed a skin-grafted NSG mouse to test potential skin-related toxicity
  - Imagine NSG mouse with a little graft (subcu? Orthotopic?) of human heart, lung, brain, liver, skin, HSC. Other desirable tissues could include gut or blood vessel.
  - Could be used to model on-target and off-target toxicity
- Humanized mice that are inbred (serially transplanted and for sale) so that human T cells can be matched/syngeneic to the other immune cells (stem cells, B cells, myeloid cells)



# Who knew?

- Who knew that modeling the human immune system and immunotherapies in animals could be so complicated?

To obtain slides from Sarah Javaid, PhD, please send an email to [sarah.javaid@merck.com](mailto:sarah.javaid@merck.com) with the subject: “Slide Request: FDA-AACR Non-clinical Models Workshop”

# **Humanized mice to study cancer-immune system interface**

**Karolina Palucka, MD, PhD**

**The Jackson Laboratory for Genomic Medicine  
Farmington, CT**

**FDA-AACR Workshop**

**September 6<sup>th</sup> 2018**

# Disclosure #1

The following relationships exist related to this presentation:

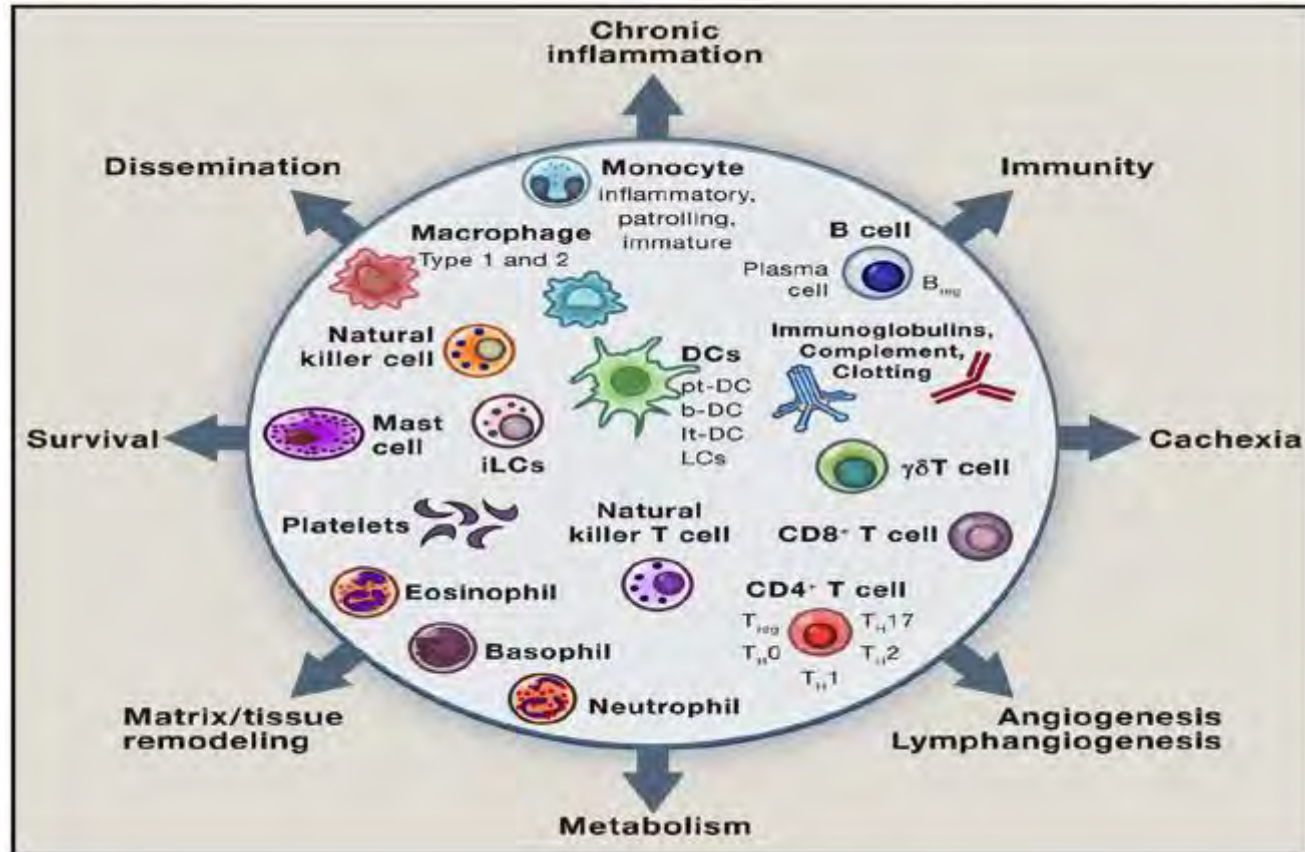
*MERCK: grant support*

# Disclosure #2

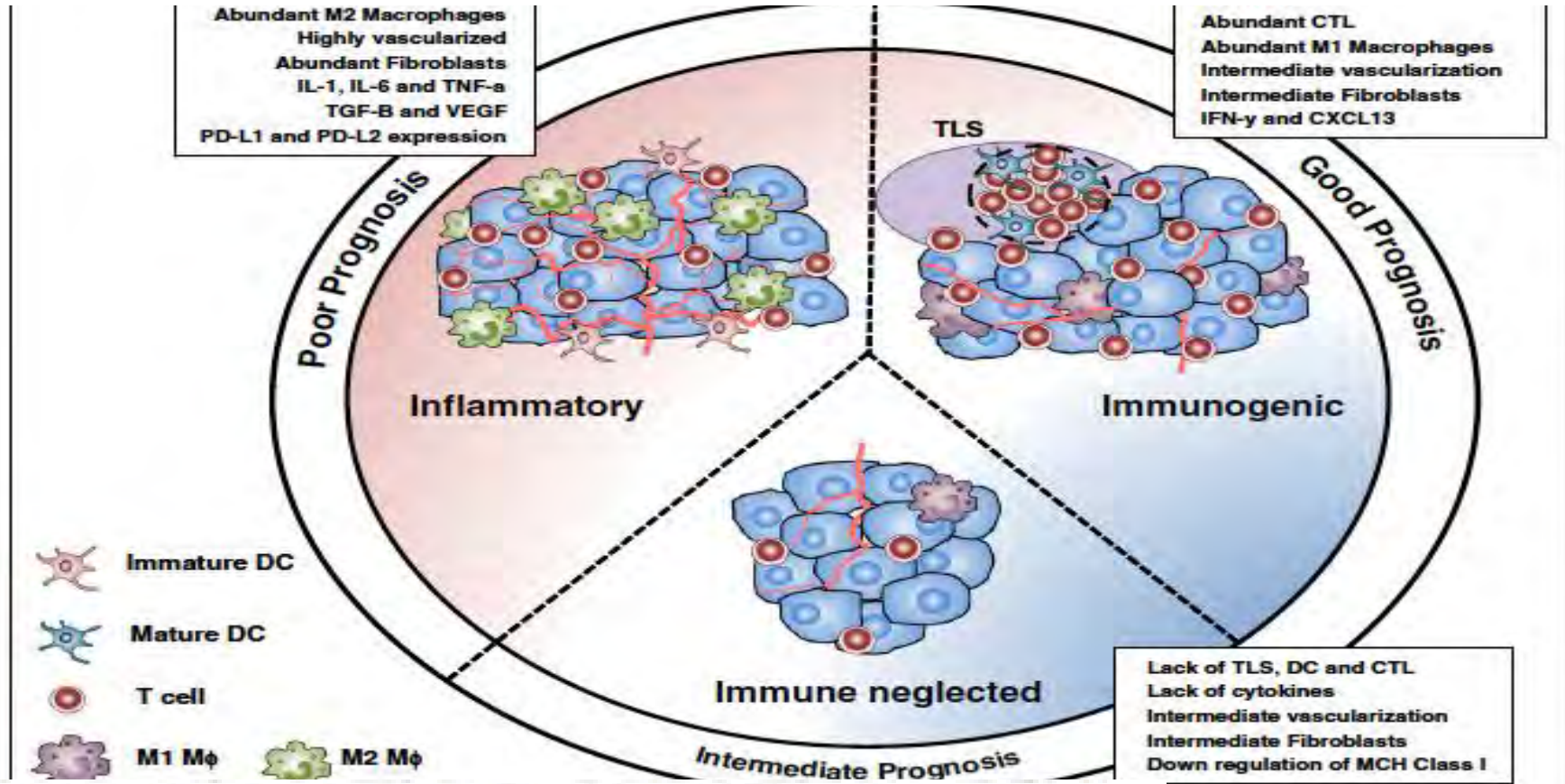
Just because the models are  
imperfect.....  
it does not mean they are wrong.....

*Bob Weinberg*

# Cancer Immune landscape is complex and heterogeneous



# Different types of tumor microenvironments differentially impact efficacy of immunotherapies





# Why humanized mice?

---

- **Adaptive immunity:** key features are well conserved between mouse and human. But....differences in affinity, antigens, longevity
- **Innate immunity:** numerous differences between mouse and human

## **Of Mice and Not Men: Differences between Mouse and Human Immunology**

*Javier Mestas and Christopher C. W. Hughes<sup>1</sup>*

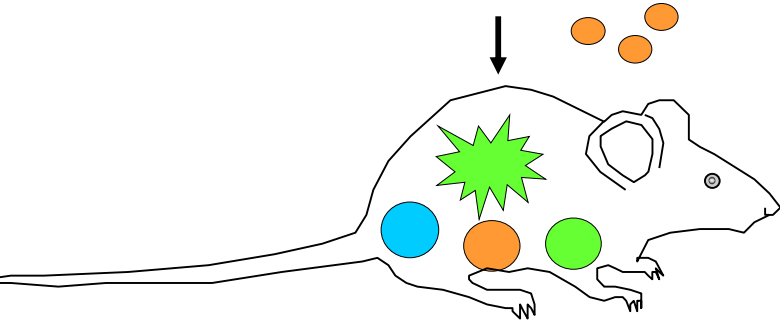
*J Immunol* 2004; 172:2731-2738; ;

doi: 10.4049/jimmunol.172.5.2731

<http://www.jimmunol.org/content/172/5/2731>

- **Cancer vaccines:** mouse studies positive,  
Many human phase III trials fail

# Adoptive T cell transfer



NOD-SCID  $\beta 2m^{-/-}$   
mouse  
Adult CD34<sup>+</sup> HPCs

*Palucka et al., Blood 2003*

*Yu et al., Blood 2008*

*Yu et al., Immunity 2013*

*Yu et al., JI 2014*

*Graham et al., Vaccine 2016*

*Aymeric, Yu et al., Science Immunology 2017*

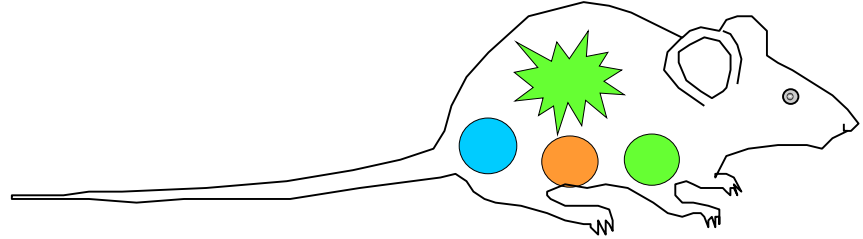
*Aspord C, J Exp Med 2007*

*Pedroza-Gonzalez, J Exp Med 2011*

*Wu et al, Cancer Immunol Res 2013*

*Wu et al, Cancer Res 2018*

# Endogenous T cells



NOD-SCID  $\gamma c^{-/-}$  or Rag2 $^{-/-}$   $\gamma c^{-/-}$   
mouse  
Fetal/cord blood CD34<sup>+</sup> HPCs

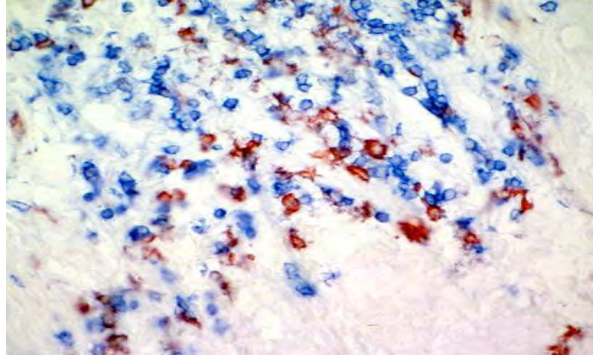
**MISTRG: Human M-CSF/IL-3,  
GM-CSF/SCF/SIRPa/TPO  
NSG and NSG-SGM3:SCF/GM-CSF, IL-3**

*Rongvaux et al, Nat Biotech 2014*

*Wang et al FASEB J 2018*

# Breast cancer subverts dendritic cell maturation to induce Th2 cells promoting cancer progression

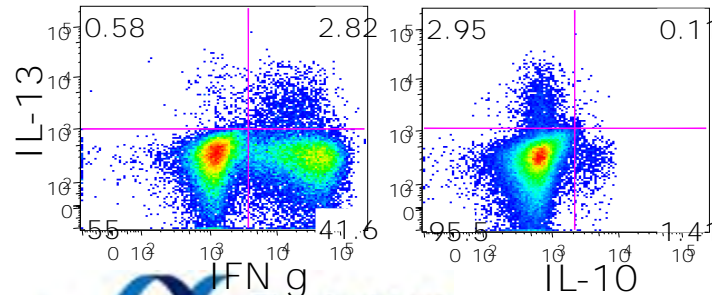
## From patients to 1<sup>st</sup> generation Onco-Humice back to patients



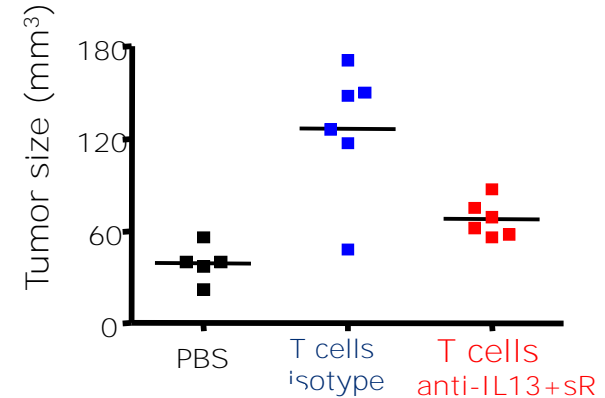
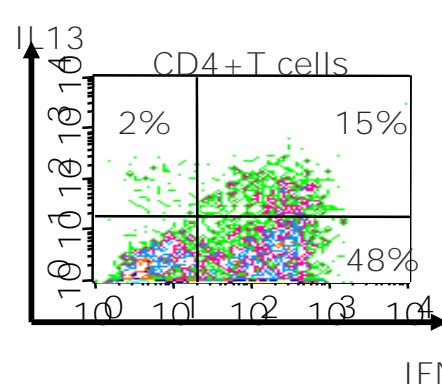
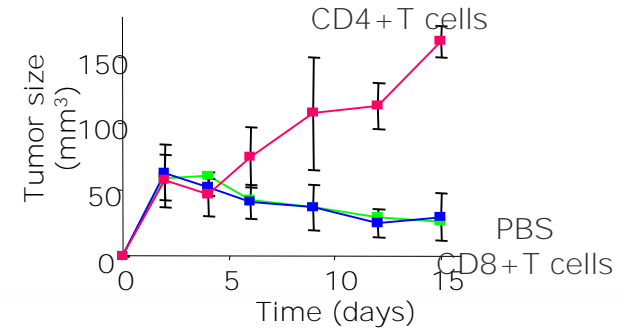
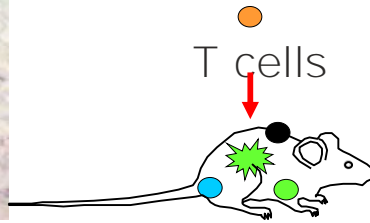
*Bell et al, J Exp Med 1999*

CD4+T  
cells

CD83+  
mature DCs



*Aspord C....Palucka, J Exp Med 2007*



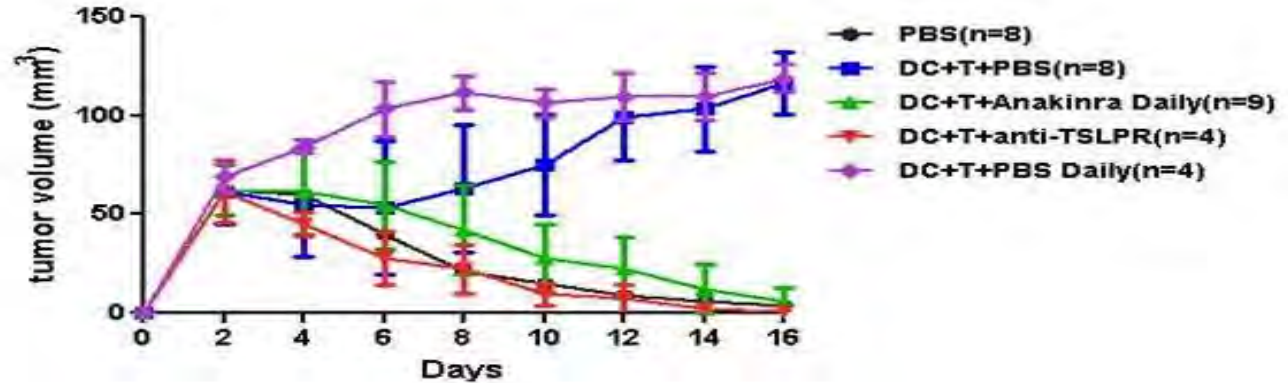
*Pedroza-Gonzalez...Palucka, J Exp Med 2011*

# IL1 receptor antagonist (Anakinra) prevents IL-13 production and breast cancer progression in experimental tumors

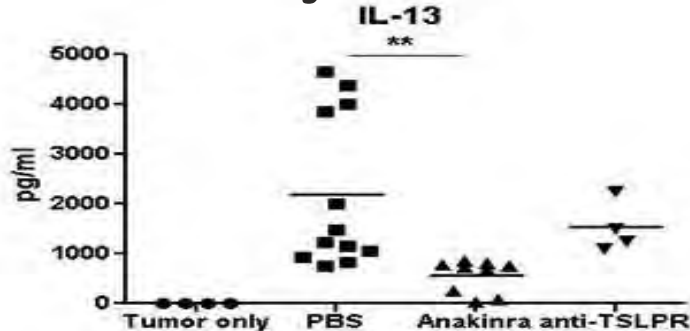


Inject human DCs,  
T cells  
Plus Anakinra  
or PBS daily

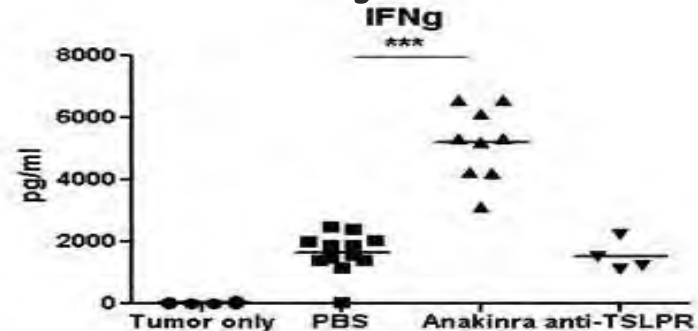
Analysis



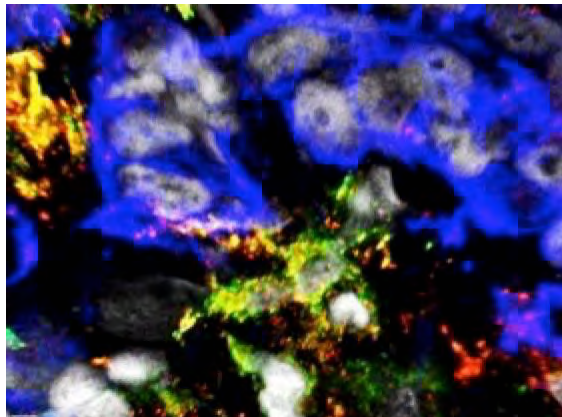
Block of pro-tumor Th2  
cytokines



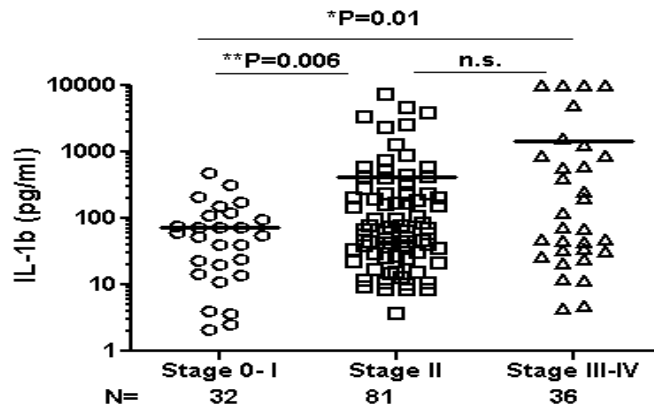
Increase of anti-tumor Th1  
cytokines



# From humanized mice to patients: Anakinra blocks signature of inflammation in patients



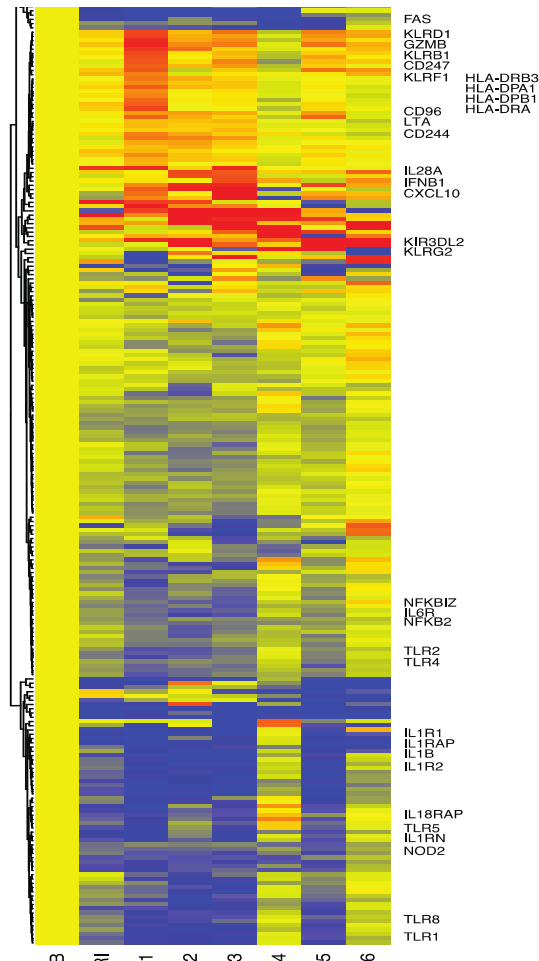
Nucleus Cytokeratin CD11c IL1b



Exploratory clinical trial  
(IRB 012-099)  
Combination of IL-1 blockade  
with chemotherapy in  
metastatic TNBC

*Joyce O'Shaughnessy*  
*Robin Young*  
*Virginia Pascual*  
*Romain Banchereau*  
*Clinical Team*  
*Patients*

## Blood transcriptome

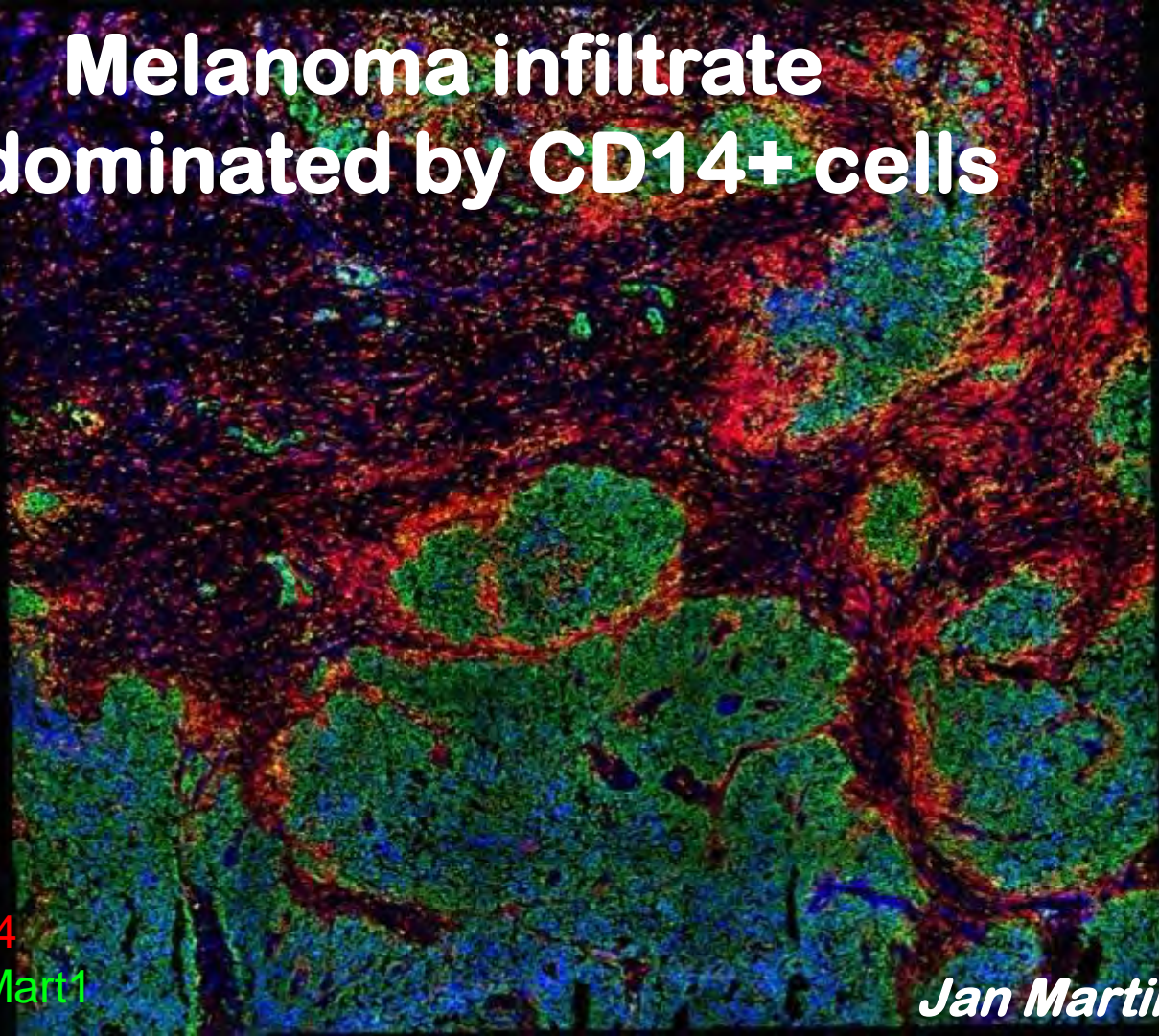


*Wu et al. Cancer Res 2018*



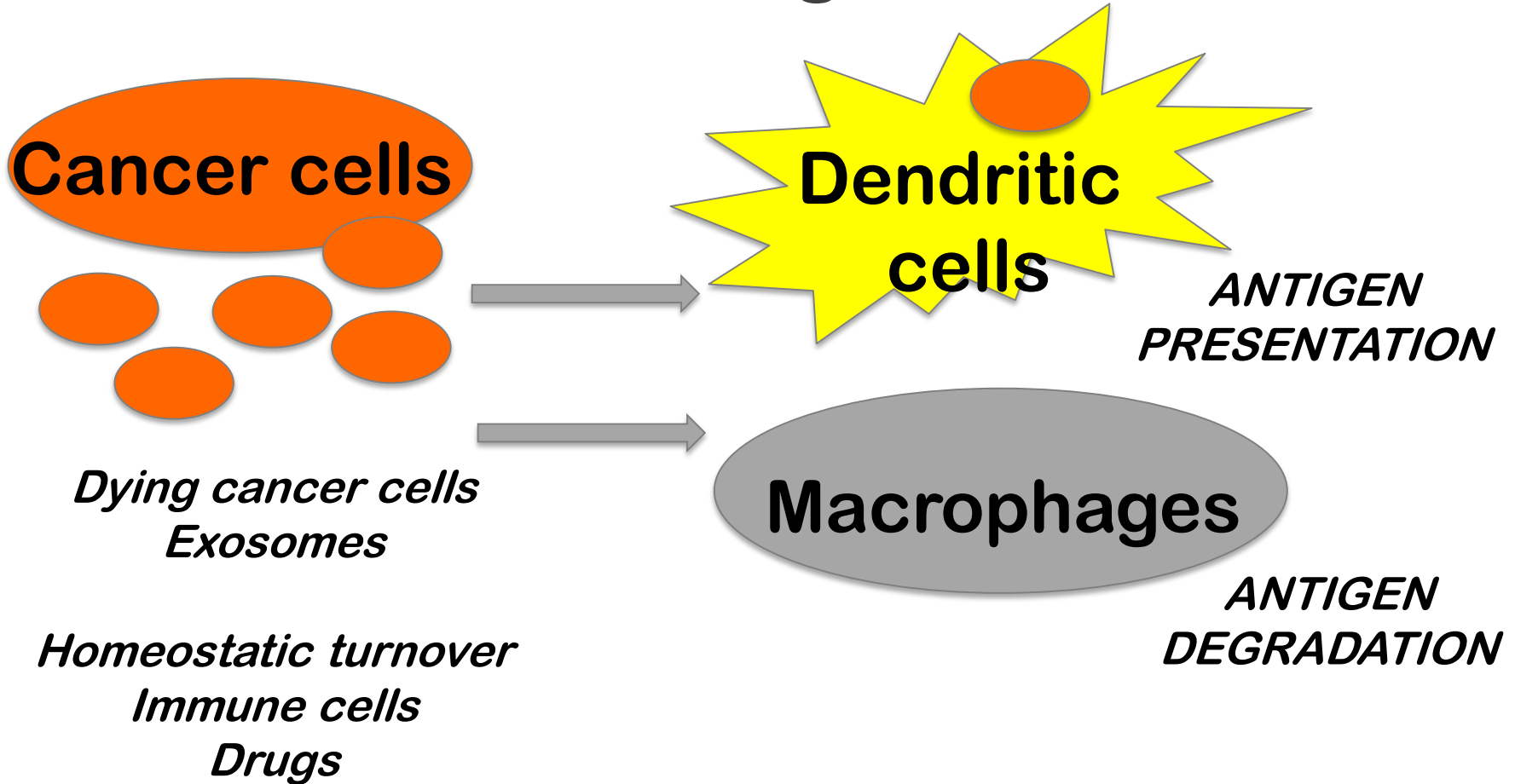
# Melanoma infiltrate is dominated by CD14+ cells

Nucleus DAPI  
T cells CD3  
Macrophages CD14  
Melanoma gp100 Mart1



*Jan Martinek*

# The fate of antigen in tissue





# In vivo models of human myeloid cells and human melanoma

---

- MISTRG for the encoded proteins M-CSF (CSF-1), IL-3/GM-CSF, SIRPα and TPO in the *Rag2-Il2rg*- background (Rongvaux, Martinek, Palucka... et al, Nat Biotech 2014).

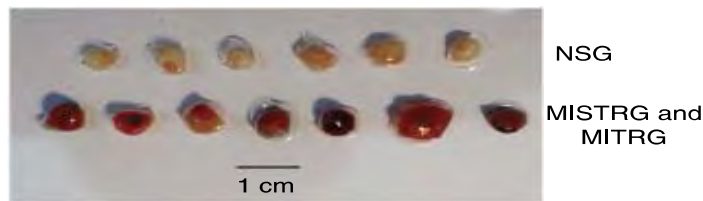
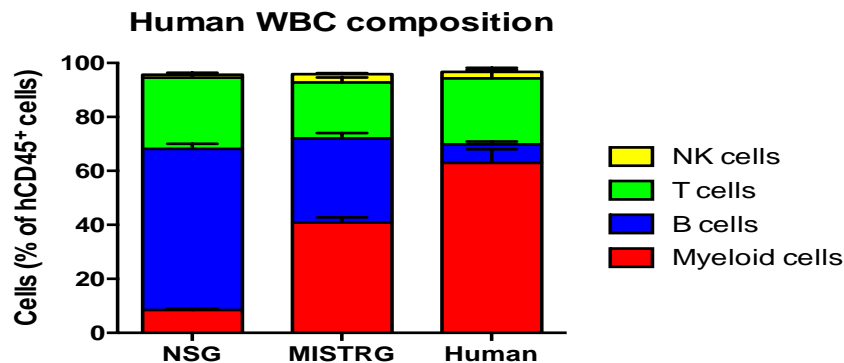
CSF-1-dependent myeloid compartment

- NSG-SGM3 strain, an immunodeficient strain that expresses transgenes for human SCF and GM-CSF/IL-3 (Billerbeck et al., Blood 2011; Coughlan et al., Stem Cells Dev 2016).

CSF-1-independent myeloid compartment

# Development and function of human innate immune cells in a humanized mouse model

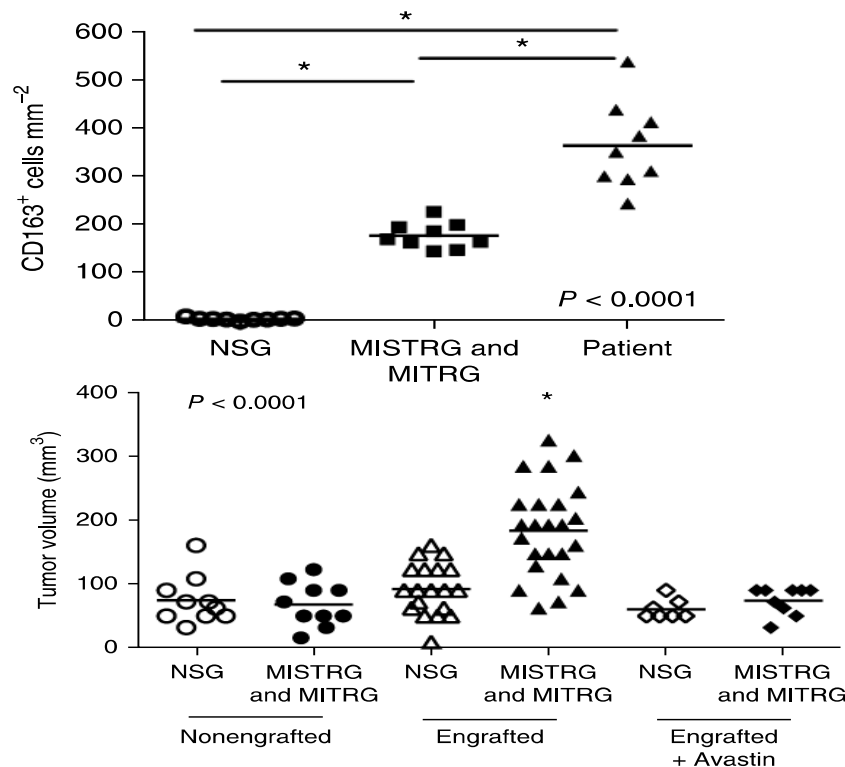
Anthony Rongvaux<sup>1,10</sup>, Tim Willinger<sup>1,10</sup>, Jan Martinek<sup>2,3</sup>, Till Strowig<sup>1,9</sup>, Sofia V Gearty<sup>1</sup>, Lino L Teichmann<sup>4,5</sup>, Yasuyuki Saito<sup>6</sup>, Florentina Marches<sup>2</sup>, Stephanie Halene<sup>7</sup>, A Karolina Palucka<sup>2</sup>, Markus G Manz<sup>6</sup> & Richard A Flavell<sup>1,8</sup>



## Human melanoma

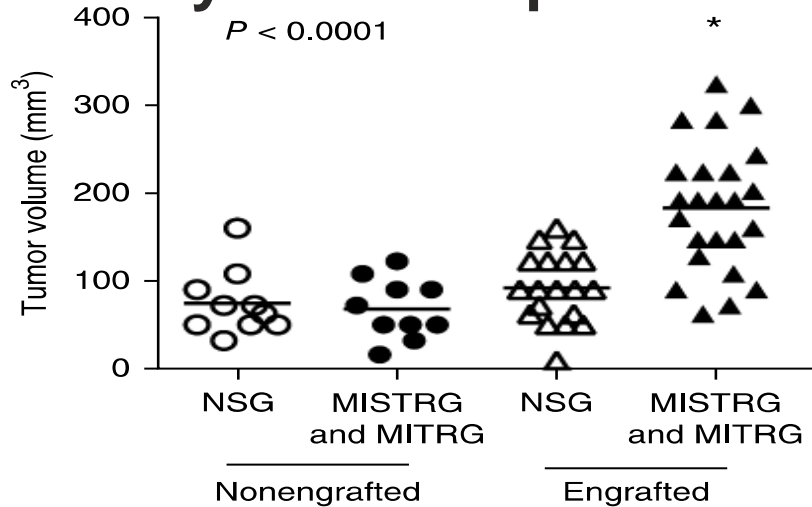
NATURE BIOTECHNOLOGY ADVANCE ONLINE PUBLICATION

published online 16 March 2014; doi:10.1038/nbt.2858

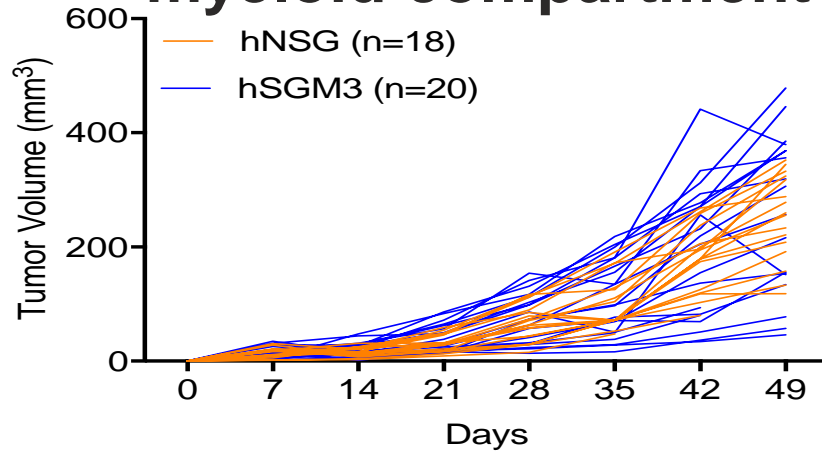


# Human myeloid cells differentially impact progression of “primary” experimental Me275 melanoma tumors in different models

## CSF-1 dependent myeloid compartment



## CSF-1 independent myeloid compartment

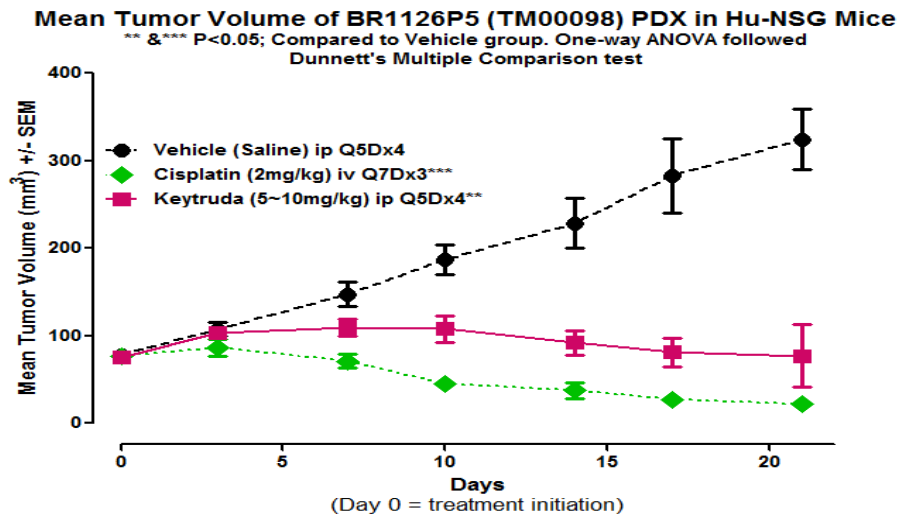


Jan Martinek

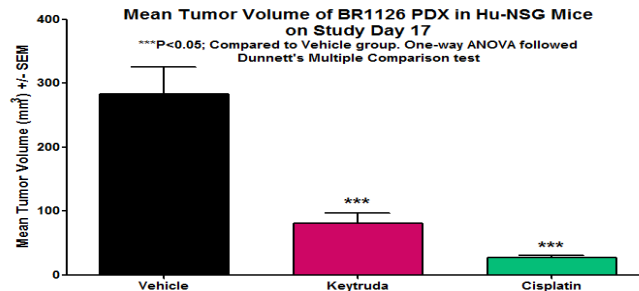
Chun I (Junnjie) Yu

# Evaluating checkpoint inhibitors:

## Pembrolizumab and Cisplatin Inhibit Growth of the breast cancer BR1126 PDX Model in Hu-CD34 NSG™ PDX Mice



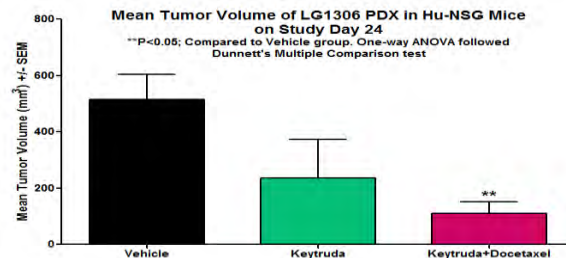
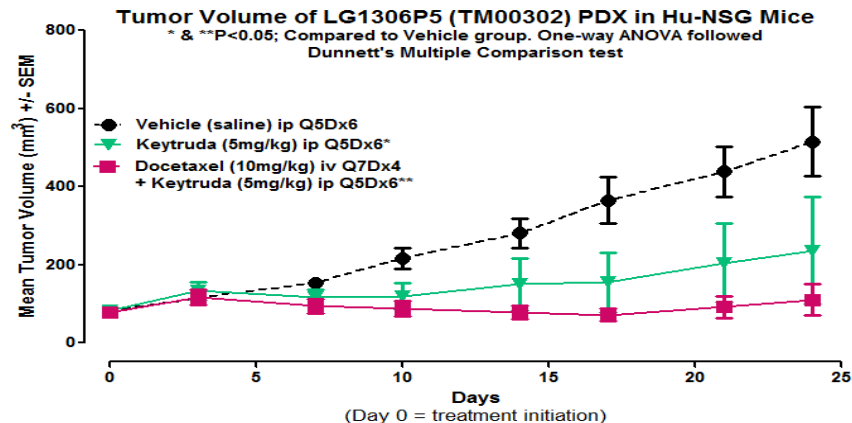
- HuCD45+ in Hu-NSG mice: >25%
- BR1126 PD-L1 surface expression: 56.9%



HLA match	CD34 <sup>+</sup> HPC donor		
Tumor	1	2	3
BR1126	HLA-C, DPA1	HLA-A,DQA1, DPB1, DPA1	HLA-C, DPA1

*Jim Keck Lab, JAX Sacramento  
Wang et al FASEB J 2018*

# Efficacy Results of Pembrolizumab +/- Docetaxel on lung cancer LG1306 PDX Tumors in Hu-NSG<sup>TM</sup> Mice

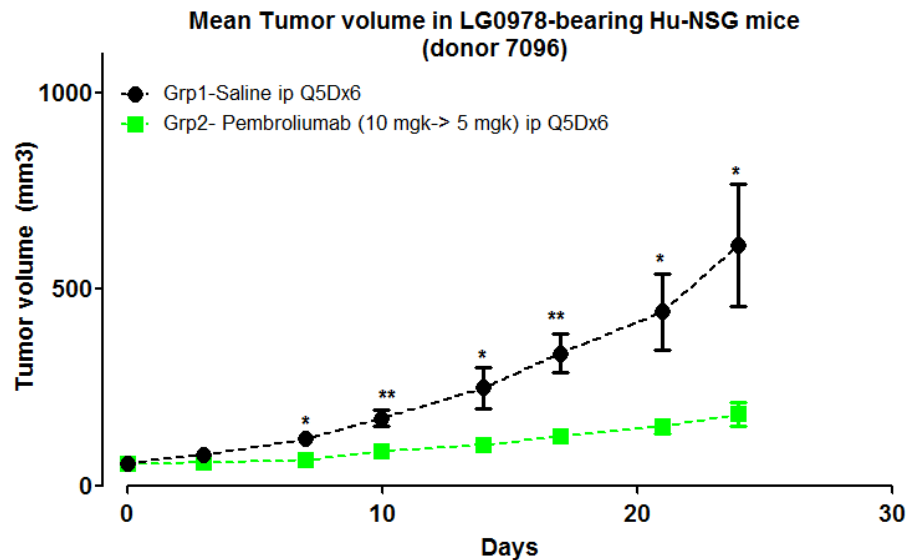
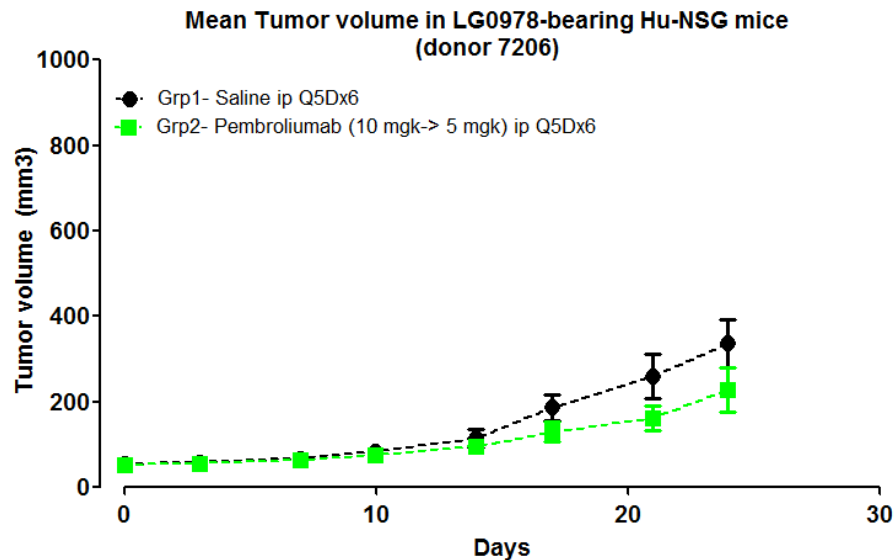


- Two or more CD34 donors
- Fresh tumor tissue engraftment
- HuCD45+ more than 20%
- LG1306 PD-L1 surface expression: 89.1%

HLA match	CD34 <sup>+</sup> HPC donor	
Tumor	1	2
LG1306	HLA-DRB4, DQA1, DQB1	No match

*Jim Keck Lab, JAX Sacramento  
Wang et al FASEB J 2018*

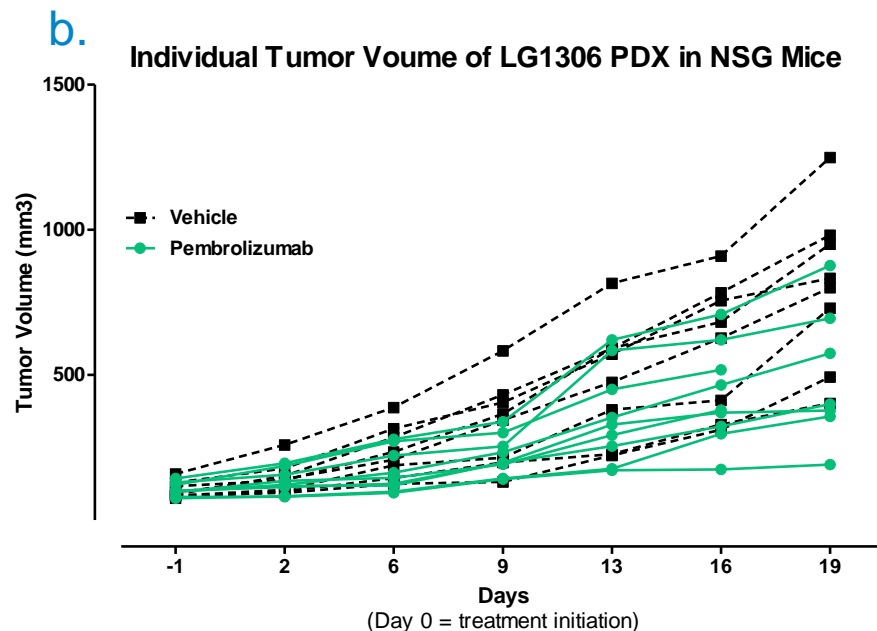
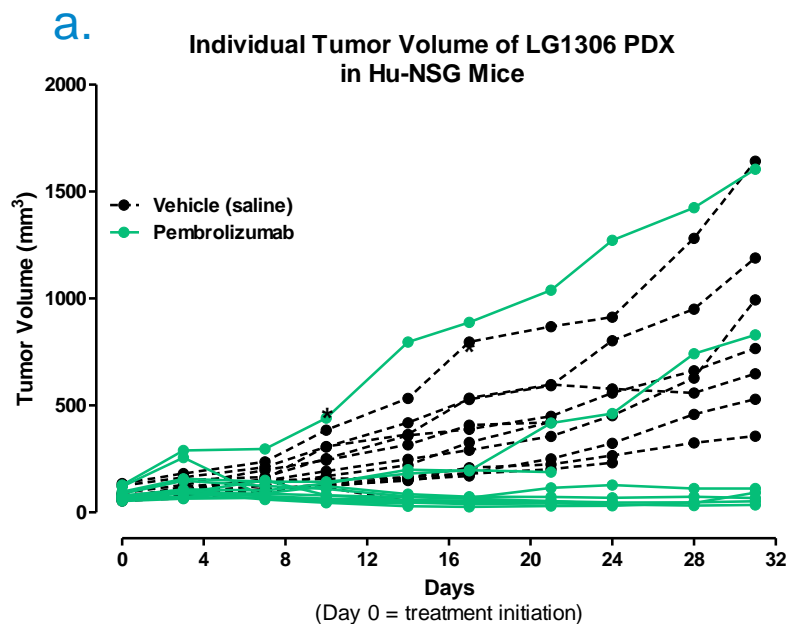
# Donor Variation in Response to Pembrolizumab Treatment in LG0978 Onco-Hu-NSG<sup>TM</sup> mice



*Jim Keck Lab, JAX Sacramento  
Wang et al FASEB J 2018*



# Mechanisms: Efficacy of Pembrolizumab Depends on Human Immune Cells

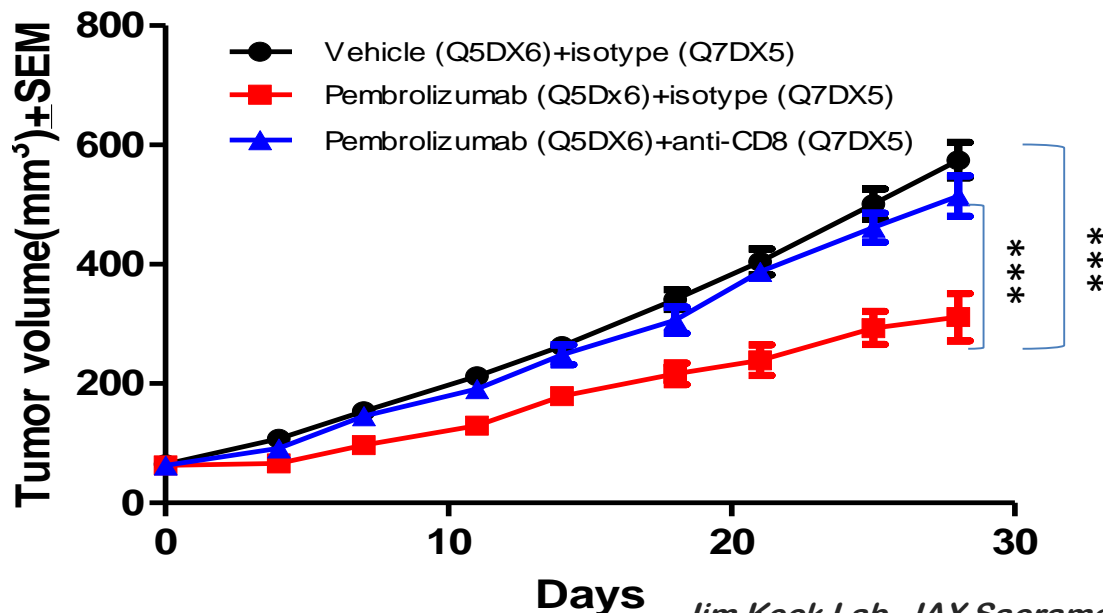
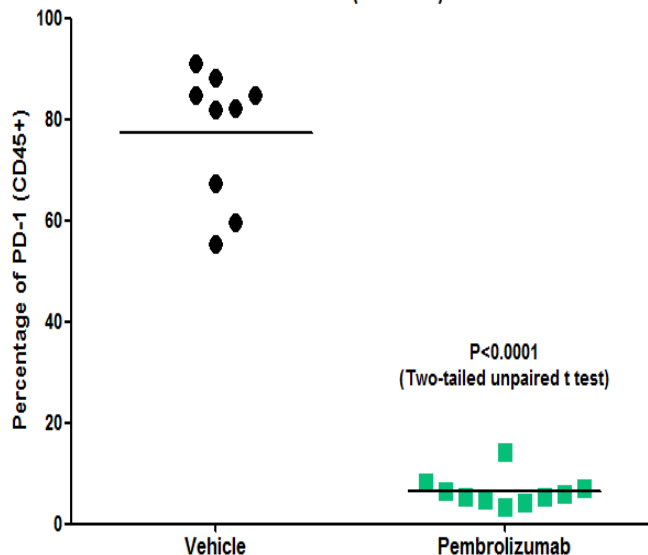


*Jim Keck Lab, JAX Sacramento  
Wang et al FASEB J 2018*

# Mechanisms: Efficacy of PD-1 blockade in humanized NSG mice bearing breast cancer CDX is CD8<sup>+</sup> T Cell Dependent

## MDA-MB-231 Tumor Response in Hu-NSG Mice

PD-1 Level (%) of CD45<sup>+</sup> Cells in MDA-MB-231 Tumor (Hu-NSG)

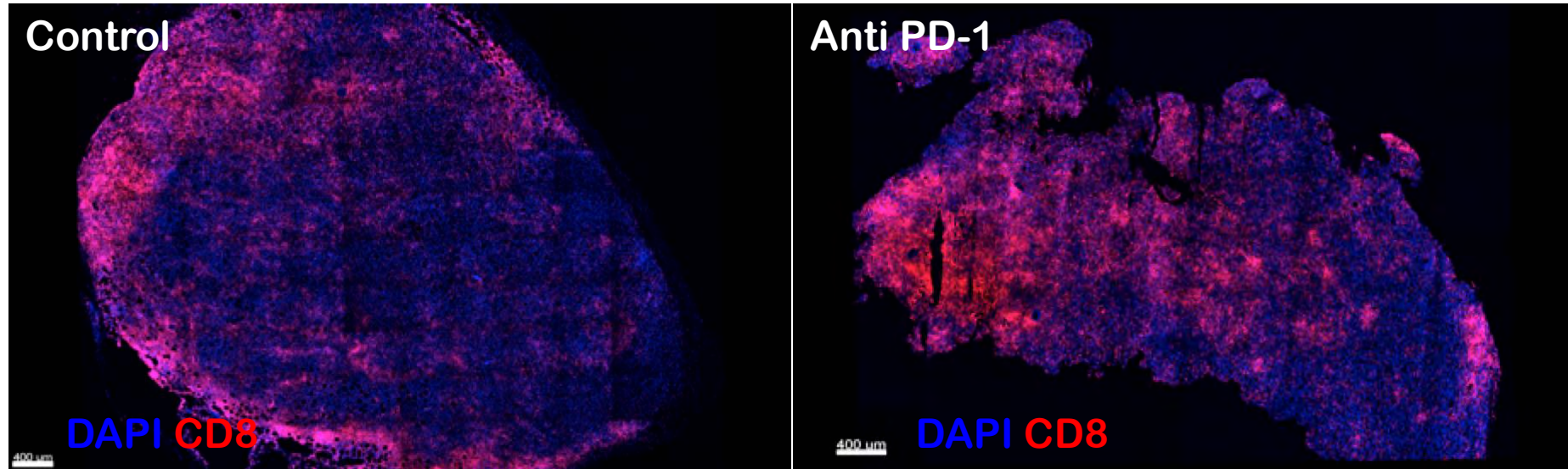


\*\*\*P < 0.0001; two-way ANOVA followed by Bonferroni post-tests.

Jim Keck Lab, JAX Sacramento  
Wang et al FASEB J 2018

# Redistribution of CD8 infiltrate after anti PD-1 treatment

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*Jim Keck Lab, JAX Sacramento  
Jan Martinek, Palucka Lab, JGM Connecticut  
Wang et al FASEB J 2018*

# Humanized mice:

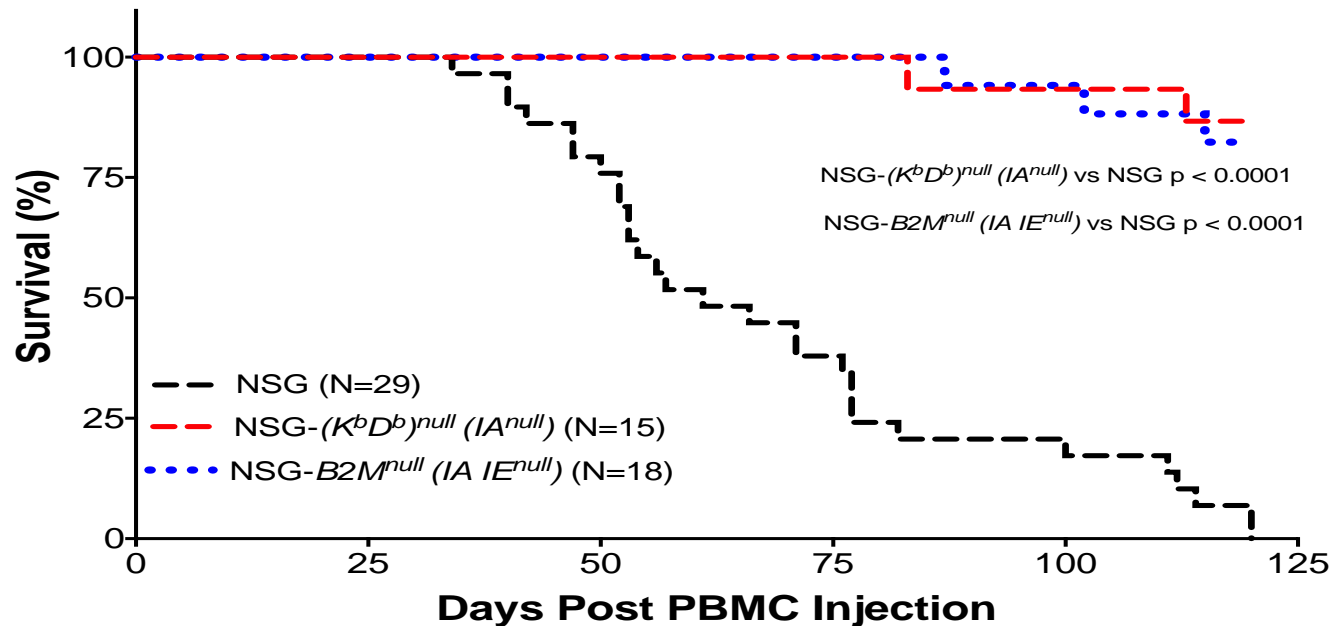
## Current challenges and opportunities

---

- **Engraftment with HPCs**  
Lack of human cytokines impairs HSC growth & differentiation  
**Source of HPCs: bone marrow, blood, iPS**
- **Mouse hosts**  
Mouse myeloid cell function  
Murine MHC
- **Suboptimal lymphoid architecture and immune function**  
T cell education in context of mouse MHC (H2) antigens  
Poor lymph node development, lack of FDCs no germinal centers  
Low levels of humoral immunity, impaired Ig class switching

# Host editing: NSG- $(K^bD^b)^{null}$ ( $IA^{null}$ ) and NSG- $B2M^{null}$ ( $IA/IE^{null}$ )

## Mice show Increased Survival Following Injection with Human PBMC



8-12 week-old mice were injected IP with  $1 \times 10^7$  human PBMC

*Courtesy of Lenny Shultz, JMG Bar Harbor*

# JAX Laboratories: Next Generation of Humanized Mice

---

## CRISPR editing of the host and of human cells

*Lenny Shultz, JMG Bar Harbor*  
*Jim Keck, JAX Sacramento*  
*Karolina Palucka, JGM Connecticut*

### Expression of human factors

Cytokines

HLA molecules

Microenvironmental factors  
(SIRP $\alpha$ )

Hormones (prolactin)

### Reduction of mouse immunity

H2 molecules

Thymus

Macrophages

Granulocytes

Dendritic Cells

Chemokine receptors

Interferon receptors

Toll-like receptors

### Role of human stroma



# Emerging Model for Human Immunotherapy

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Novel targets  
Novel drugs  
Novel biomarkers



Human in-mouse

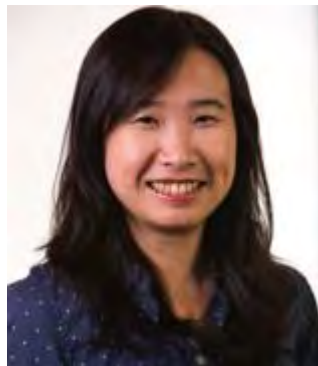
Genomic engineering  
Cell transplant  
Tissue transplant



**Jan Martinek**



**Chun I Yu**



**Tina Wu**



**Elaheh  
Ahmadzadeh**



**Florentina  
Marches**



**Kyung In Kim**



**Vanessa  
Oliveira**



**Deb  
Shurberg**

***Thanks to our  
patients; funding  
organizations  
and our collaborators***

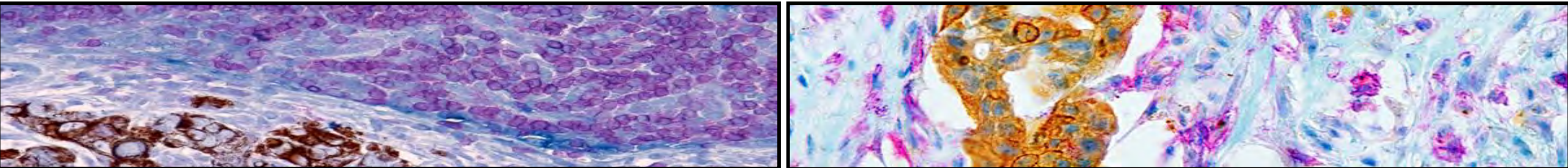
**Jim Keck  
Susie Airhart  
Lenny Shultz  
Carol Bult  
Rick Maser**

**Anthony Rongvaux  
Michael  
Richard Flavell**

**Jacques Banchereau**

# Evaluating IO toxicities in syngeneic immunocompetent mouse models

## *Lessons, Opportunities and Challenges*



**Gregory L. Beatty, MD PhD**

Assistant Professor

Director of Translational Research, Pancreatic Cancer Research Center

Department of Medicine

Division of Hematology/Oncology

University of Pennsylvania School of Medicine

# Disclosures

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## **Consulting or Advisory Role:**

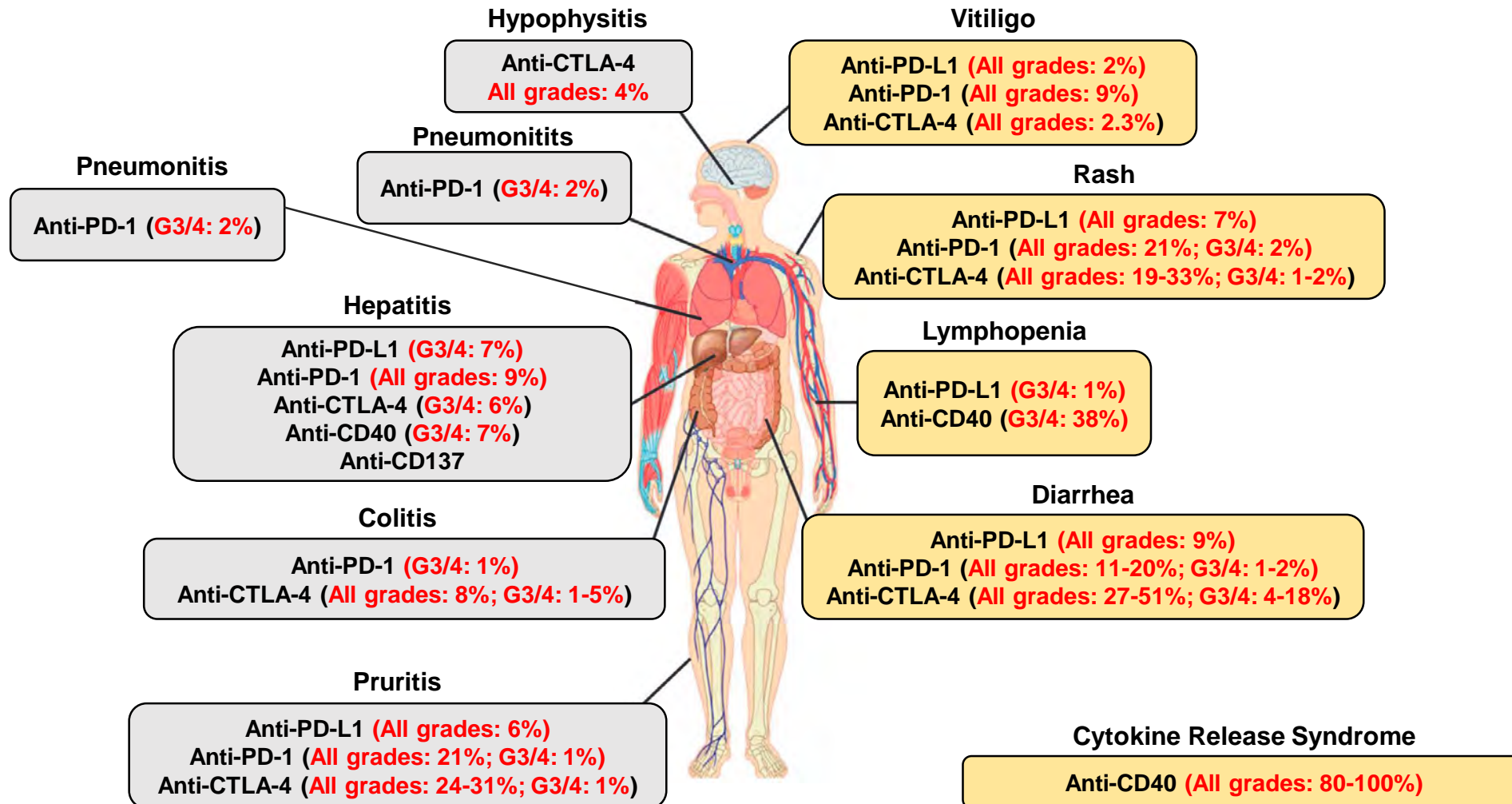
Seattle Genetics  
Aduro Biotech  
AstraZeneca  
Bristol-Meyers Squibb  
Genmab  
BiolineRx

## **Research Funding:**

Incyte  
Bristol-Meyers Squibb  
Verastem  
Halozyme  
Biothera  
NewLink  
Janssen

# Immunotherapy can provoke a wide range of immune-related toxicities

## Immune antagonists and Immune agonists



# Modeling the immune system Of **Mice** and **Humans**

## Human Blood

50-70% neutrophils  
30-50% lymphocytes

**Neutrophils:** rich in defensins

## Human HSC

*c-kit<sup>low</sup>, flt-3<sup>+</sup>*



## Mouse Blood

10-25% neutrophils  
75-90% lymphocytes

**Neutrophils:** lack defensins

## Mouse HSC

*c-kit<sup>high</sup>, flt-3<sup>neg</sup>*



# Known immunological differences between mice and humans

## Of Mice and Humans

Table I. Summary of some known immunological differences between mouse and human

	Mouse	Human	Notes	Refs.
Hematopoiesis in spleen	Active into adulthood	Ends before birth		
Presence of BALT	Significant	Largely absent in healthy tissue		9
Neutrophils in periph. blood	10–25%	50–70%		10
Lymphocytes in periph. blood	75–90%	30–50%		10
Hematopoietic stem cells	<i>c-kit<sup>high</sup>, flt-3<sup>+</sup></i>	<i>c-kit<sup>low</sup>, flt-3<sup>+</sup></i>		11
TLR2 expression on PBL	Low (induced on many cells including T cells)	Constitutive (but not on T cells)	Binds lipopeptides	88
TLR3	Expressed on DC, Mac. Induced by LPS	Expressed by DC. No LPS induction	Binds dsRNA	88, 89
TLR9	Expressed on all myeloid cells, plasmacytoid DC and B cells	Expressed only on B cells, plasmacytoid DC and N	Binds CpG	90, 91
TLR10	Expressed on granulocytes	Expressed on monocytes		
Sialic acid Neu5GC expression	Pseudogene	Widely expressed	Binds pathogens	92
CD33	Expressed on granulocytes	Expressed on monocytes	Binds sialic acids	93
Leukocyte defensins	Absent	Absent	neutrophils	14
Paneth cell defensins	Processed by MMP7. Stored pre-processed	Stored as pro-form. Processed by trypsin		94, 95
Paneth cell defensins	At least 20	Two		13
Macrophage NO	Induced by IFN- $\gamma$ and LPS	Induced by IFN- $\alpha/\beta$ , IL-4 <sup>+</sup> anti-CD23		17
CD4 on macrophages	Absent	Present		96
Predominant T cells in skin and mucosa	$\gamma\delta$ TCR (dendritic epidermal T cells—DETC)	$\alpha\beta$ TCR		40
$\gamma\delta$ T cells respond to phospho-antigens	No	Yes		97
CD1 genes	CD1d	CD1a,b,c,d		41
NK inhibitory Rs for MHC 1	Ly49 family (except Ly49D and H)	KIR		20
NKG2D ligands	H-60, Rae1 $\beta$	MIC A, MIC B, ULBP	NK activating Rs	98
BMLP receptor affinity	Low	High		99
Fc $\alpha$ RI	Absent	Present		21
Fc $\gamma$ RIIA, C	Absent	Present		22
Serum IgA	Mostly polymeric	Mostly monomeric		21
Ig classes	IgA, IgD, IgE, IgG1, IgG2a*, IgG2b, IgG3, IgM * absent in C57BL/6, /10, SJL and NOD mice, which have IgG2c	IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM		23
Ig CDR-H3 region	Shorter, less diverse	Longer, more diverse		100
BLNK deficiency	IgM <sup>high</sup> B cells in periphery	No peripheral B cells		25, 26
Btk deficiency	Normal pre-B and immature B	Blocks pro-B to pre-B transition		28
$\lambda 5$ deficiency	"leaky" block at pro-B to pre-B transition	Blocks pro-B to pre-B transition		28
CD38 expression on B cells	Low on GC B cells, off in plasma cells	High on GC B cells and plasma cells		29
B cell CD5 and CD23 expression	Mutually exclusive	Co-expression		29
IL-13 effect on B cells	None	Induces switch to IgE		24
Thy 1 expression	Thymocytes, peripheral T cells	Absent from all T cells, expressed on neurons		32
Effect of $\gamma_c$ deficiency	Loss of T, NK, and B cells	Loss of T, NK, but B cell numbers normal		33, 34
Effect of Jak3 deficiency	Phenocopies $\gamma_c$ deficiency	Phenocopies $\gamma_c$ deficiency		
Effect of IL-7R deficiency	Blocks T and B cell development	Only blocks T cell development		
ZAP70 deficiency	No CD4 <sup>+</sup> or CD8 <sup>+</sup> T cells	No CD8 <sup>+</sup> T but many non-CD4 <sup>+</sup>		
Complement 8 deficiency	Embryonic lethal	Viable		
Interleukin 10	Absent	Present		
IFN- $\alpha$ promotes Th1 differentiation	No	Yes		
Th expression of IL-4 and IFN- $\gamma$ expression by cultured Th	Th1	Sometimes both		51
CD28 expression on T cells	On 100% of CD4 <sup>+</sup> and CD8 <sup>+</sup>	On 80% of CD4 <sup>+</sup> , 50% of CD8 <sup>+</sup>		54
ICAM3	Normal B cell numbers and function, normal IgM levels	B cells immature and severely reduced in number, low IgM	Possibly age-related	55–57
B7-H3 effects on T cells	Inhibits activation	Promotes activation		101–2
ICAM3	Absent	Present	DC-SIGN ligand	103–4
P-selectin promoter	Activated by TNF and LPS	Unresponsive to inflammation		58
GlyCAM	Present	Absent		105
MHC II expression on T cells	Absent	Present		59–61
Kv1.3 K <sup>+</sup> channel on T cells	Absent	Present	Regulates Ca flux	64, 65
MUC1 on T cells	Absent	Present	Regulates migration?	106
Granulysin	Absent	Present	In CTL	43

(Table continues)

Table I. Continues

	Mouse	Human	Notes	Refs.
CXCR1	Absent	Present		66, 67
IL-8, NAP-2, ITAC, MCP-4, HCC-1, HCC-2, MPIF-1, PARC, cotaxin-2/3	Absent	Present	Chemokines	66, 67
MRP-1/2, lungkine, MCP-5	Present	Absent	Chemokines	66, 67
IFN- $\gamma$ effects in demyelinating disease	Protective in EAE	Exacerbates MS		4, 69–70
DTH lesions	Neutrophil-rich	Lymphocyte-rich		73, 74
Constitutive MHC II on EC	Absent	Present		80
EC present Ag to CD4 <sup>+</sup> T	No	Yes	Memory T only	75–77
T cell dependence for CD2-ligand interactions	Absent	Present	CD2 ligand	82
CD2-ligand interaction on CD40 on EC	Lower affinity, with CD48	Higher affinity, with CD58		82
Vascularized grafts tolerogenic?	Yes	No		83, 84
Microchimerism induces graft tolerance?	High success rate	Low success (expts. in non-human primates)		5
Passenger lymphocytes	Account for graft immunogenicity	Do not account for graft immunogenicity		7
				6

CD40 on EC

Mouse  
Absent

Human  
Present

Th expression of IL-10

Mouse  
Th2

Human  
Th1 and Th2



# Syngeneic and Transgenic Models

## Advantages and Disadvantages in their use for studying IO toxicities

Transplantable Tumor Models	Spontaneous Tumor Models	Non-tumor models
<b>Examples:</b> <ul style="list-style-type: none"><li>• MC38 subcutaneous implantation</li><li>• Pan02 orthotopic implantation</li></ul>	<b>Examples:</b> <ul style="list-style-type: none"><li>• <i>Kras</i><sup>G12D/+</sup>; <i>Trp53</i><sup>R172H/+</sup>; <i>Pdx-1</i> Cre (KPC)</li><li>• MMTV-PyMT</li></ul>	<b>Examples:</b> <ul style="list-style-type: none"><li>• Wild-type mice</li><li>• PD-1 knockout mice, etc</li></ul>

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<b>Advantages:</b> <ul style="list-style-type: none"><li>• Rapid/reproducible tumor growth</li><li>• Cell lines can be genetically-modified</li></ul>	<b>Advantages:</b> <ul style="list-style-type: none"><li>• Stochastic tumor development faithfully recapitulates tumor microenvironment</li><li>• Emergence of immune tolerance mechanisms</li></ul>	<b>Advantages:</b> <ul style="list-style-type: none"><li>• Repeated long-term administration of IO drugs is feasible</li></ul>

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<b>Examples:</b> <ul style="list-style-type: none"><li>• MC38 subcutaneous implantation</li><li>• Pan02 orthotopic implantation</li></ul>	<b>Examples:</b> <ul style="list-style-type: none"><li>• <i>Kras</i><sup>G12D/+</sup>; <i>Trp53</i><sup>R172H/+</sup>; <i>Pdx-1</i> Cre (KPC)</li><li>• MMTV-PyMT</li></ul>	<b>Examples:</b> <ul style="list-style-type: none"><li>• Wild-type mice</li><li>• PD-1 knockout mice, etc</li></ul>
<b>Advantages:</b> <ul style="list-style-type: none"><li>• Rapid/reproducible tumor growth</li><li>• Cell lines can be genetically-modified</li></ul>	<b>Advantages:</b> <ul style="list-style-type: none"><li>• Stochastic tumor development faithfully recapitulates tumor microenvironment</li><li>• Emergence of immune tolerance mechanisms</li></ul>	<b>Advantages:</b> <ul style="list-style-type: none"><li>• Repeated long-term administration of IO drugs is feasible</li></ul>
<b>Disadvantages:</b> <ul style="list-style-type: none"><li>• Implantation may produce inflammation and immune activation</li><li>• Limited window for intervention and monitoring</li><li>• Mouse surrogates for IO drugs may not reflect human biology</li></ul>	<b>Disadvantages:</b> <ul style="list-style-type: none"><li>• Tumor latency period can be long</li><li>• Costly</li><li>• Tumor heterogeneity increases complexity of interpreting responses</li><li>• Mouse surrogates for IO drugs may not reflect human biology</li></ul>	<b>Disadvantages:</b> <ul style="list-style-type: none"><li>• Effects of tumor development on IO drug toxicities cannot be examined</li><li>• Mouse surrogates for IO drugs may not reflect human biology</li></ul>

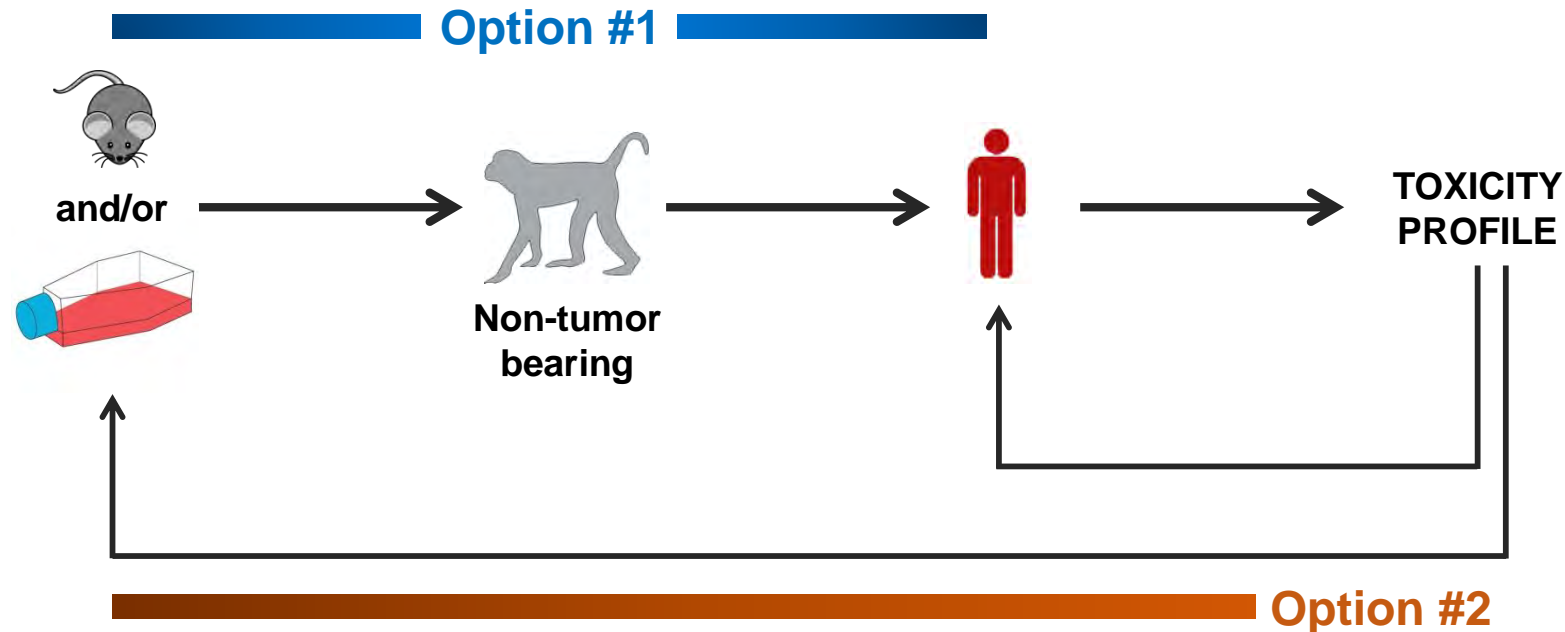
# Incorporating mouse models into the study of IO toxicities

## Predictive vs Informative

How should we incorporate preclinical models for the study of IO toxicities?

Option 1: Evaluate for potential toxicities in preclinical setting to inform translation and monitoring in patients.

Option 2: Identify toxicities that emerge after translation into patients and study them.

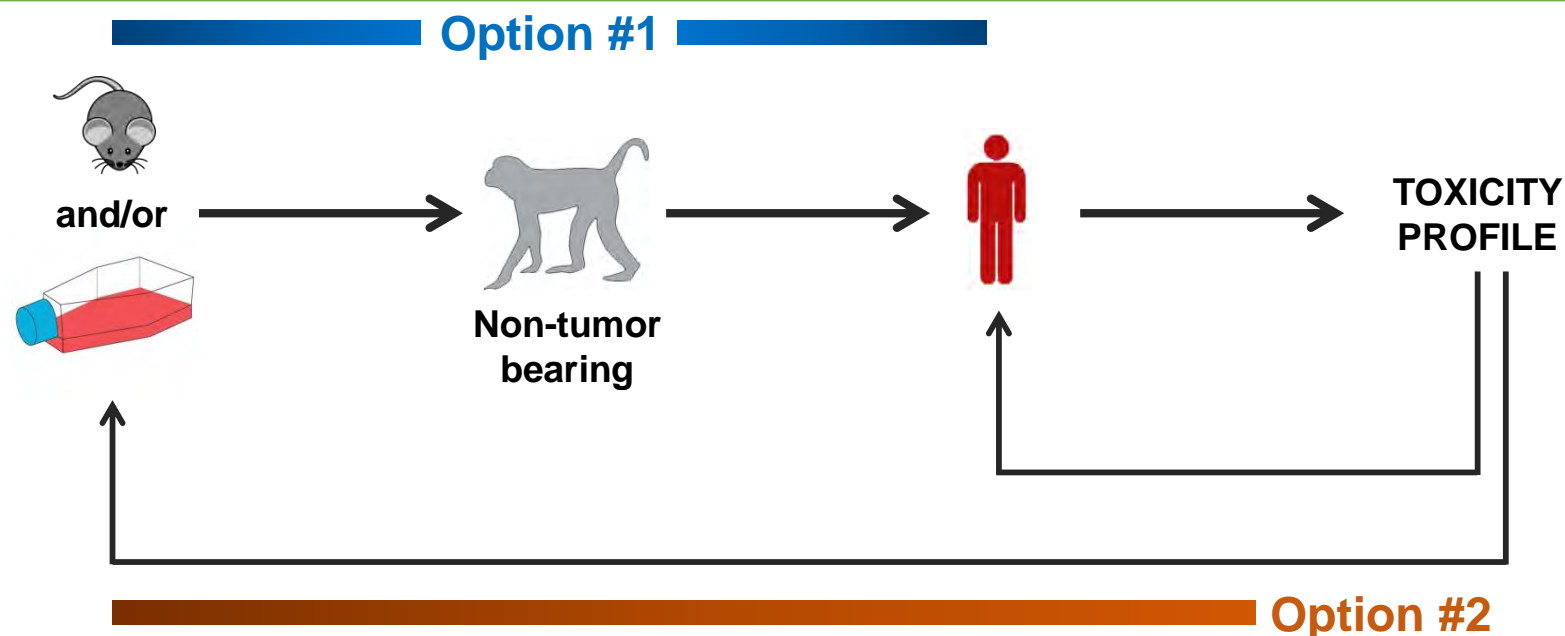




# Incorporating mouse models into the study of IO toxicities

## Predictive vs Informative

	Advantages	Disadvantages
<b>Option 1:</b> Increased monitoring for toxicities in <b>preclinical</b> models	Mechanistically important findings may emerge May inform toxicities for increased monitoring	More extensive preclinical modeling – longer time to development. Toxicities may not mirror those seen in humans How to standardize models used? Heterogeneity between models?
<b>Option 2:</b> Model <b>clinically-relevant</b> toxicities to inform biology and interventions	Preclinical studies are scientifically focused	Which models to use? Is there a non-primate drug surrogate? Mouse/human biology may be distinct



## Example 1: Modeling irAEs related to anti-CTLA-4 therapy

Ipilimumab (n = 1,498) (%)		
Toxicity	All Grades	Grade 3/4
GI (e.g. enterocolitis)	33	9.1
Pneumonitis	<1	<1
Hepatitis	1.6	1.1
Dermatologic	45	2.6
Hypophysitis	2.7	2.1
Thyroiditis	1.8	<1
Nephritis	<1	<1

### WARNING: IMMUNE-MEDIATED ADVERSE REACTIONS

*See full prescribing information for complete boxed warning.*

YERVOY can result in severe and fatal immune-mediated adverse reactions. These immune-mediated reactions **may involve any organ system**; however, the most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. The majority of these immune-mediated reactions initially manifested during treatment; however, a **minority occurred weeks to months after discontinuation of YERVOY**.

**Permanently discontinue YERVOY and initiate systemic high-dose corticosteroid therapy for severe immune-mediated reactions. (2.5)**

**Assess patients for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy and evaluate clinical chemistries including liver function tests, adrenocorticotrophic hormone (ACTH) level, and thyroid function tests at baseline and before each dose. (5.1, 5.2, 5.3, 5.4, 5.5)**

### Questions in the field:

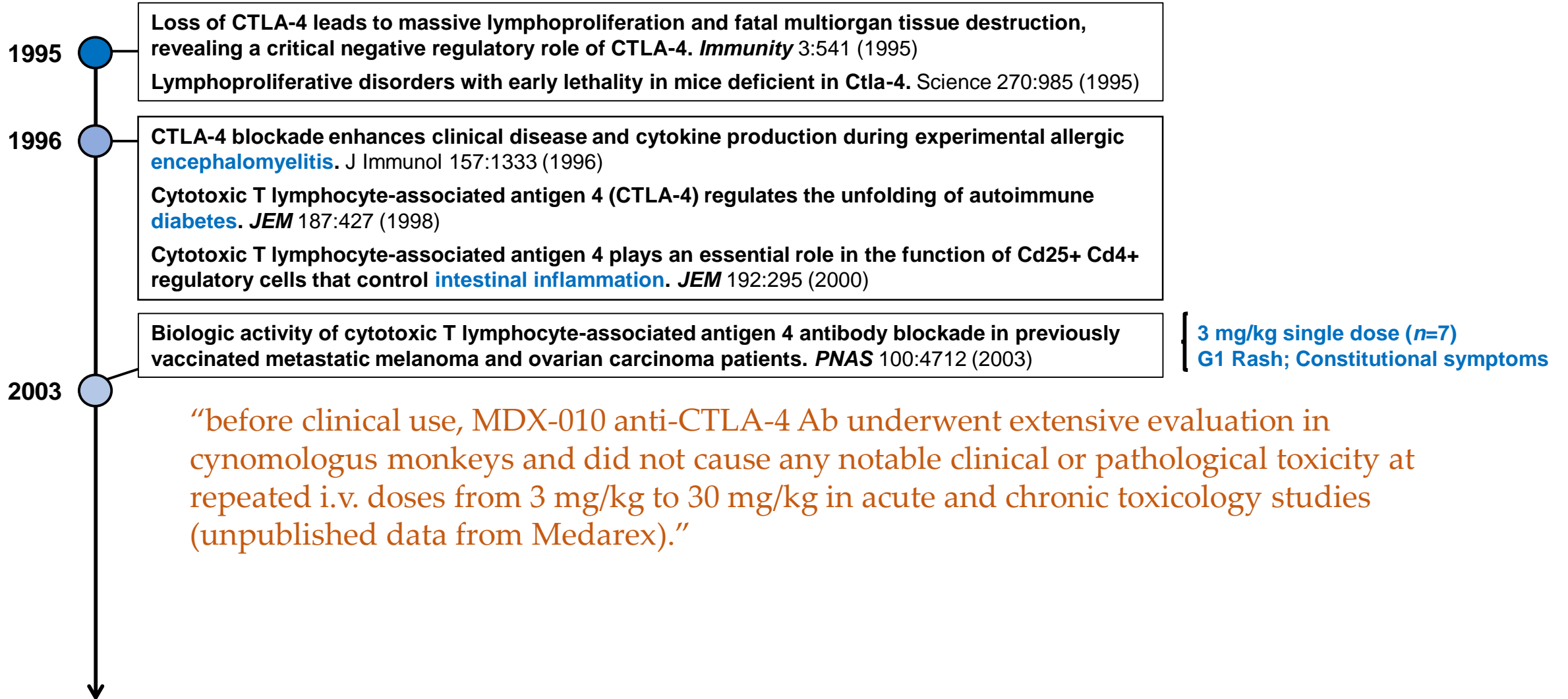
Predictability

Timing

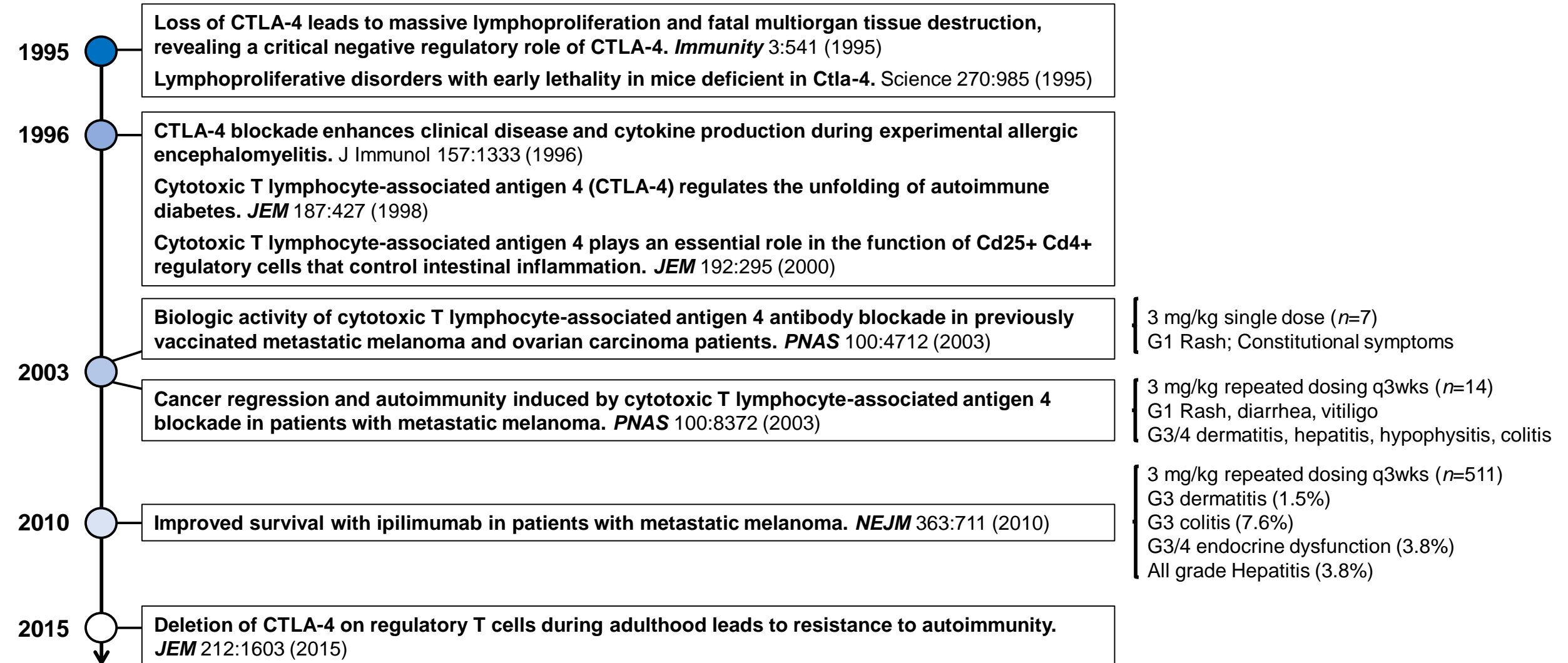
Mechanisms of Pathology

Treatment/Prevention

# Lessons from CTLA-4 antibody development



# Lessons from CTLA-4 antibody development

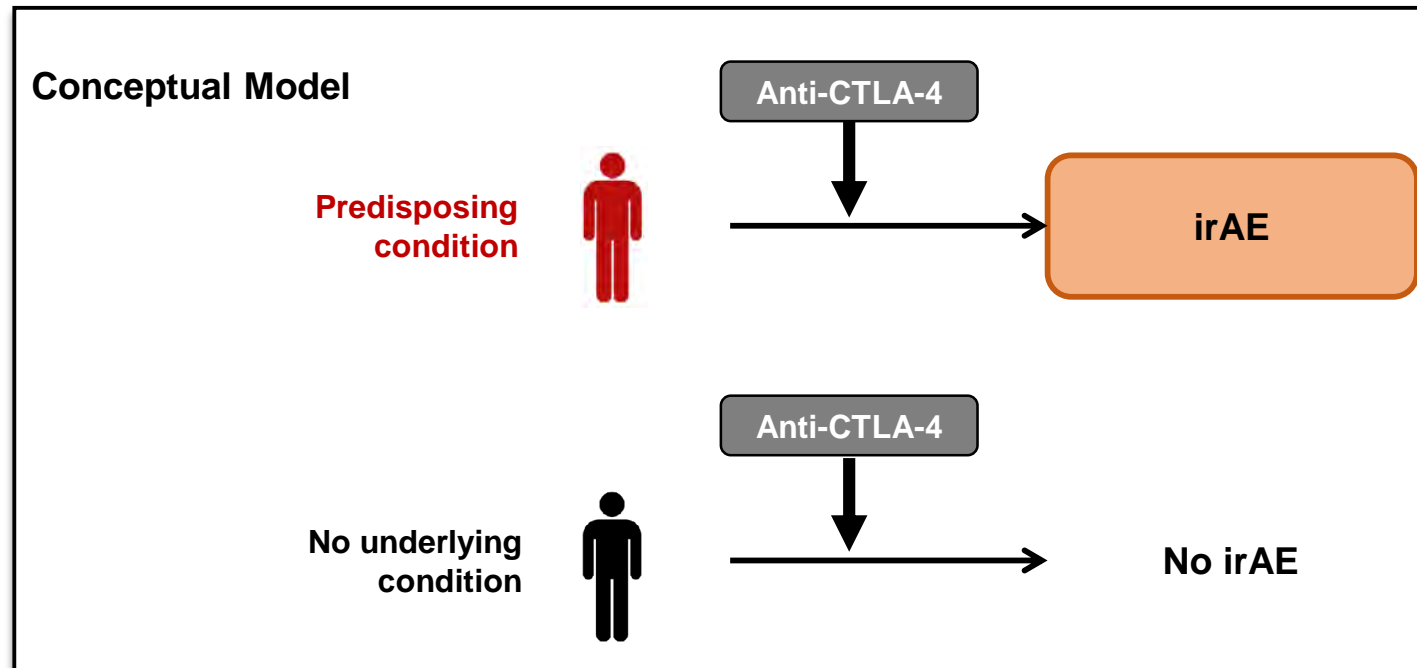


# Lessons from preclinical models evaluating CTLA-4

Transgenic models targeting the same molecule can produce widely divergent pathological outcomes

- Conditional deletion during adulthood vs genetic knockout from birth

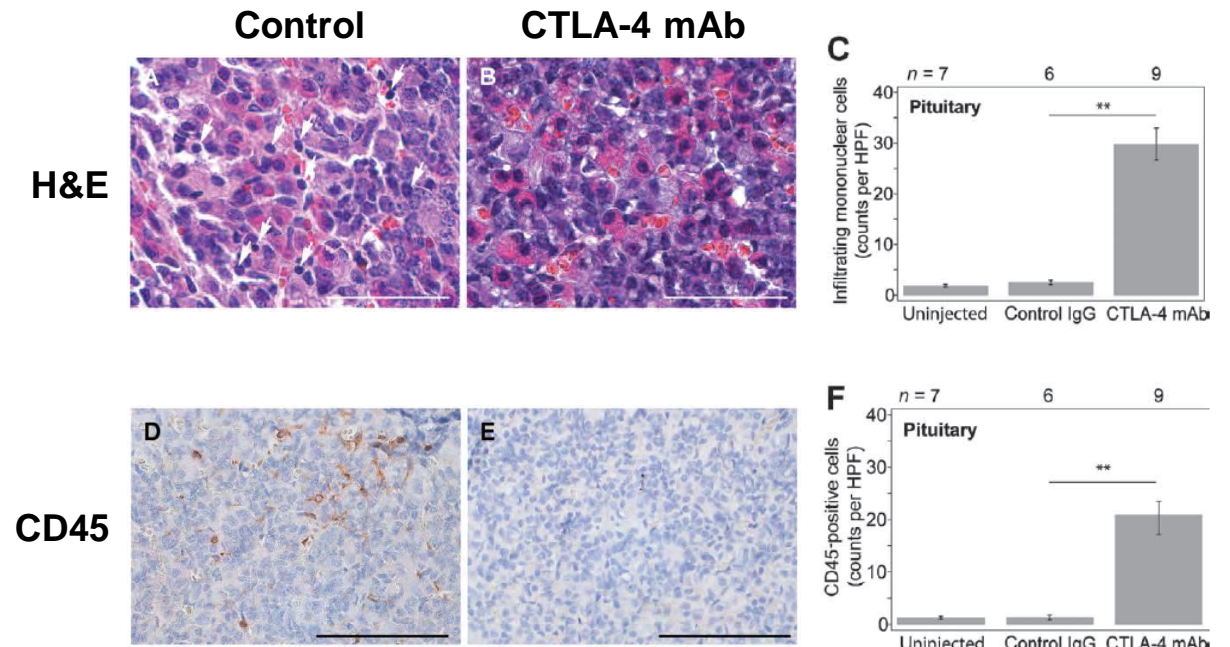
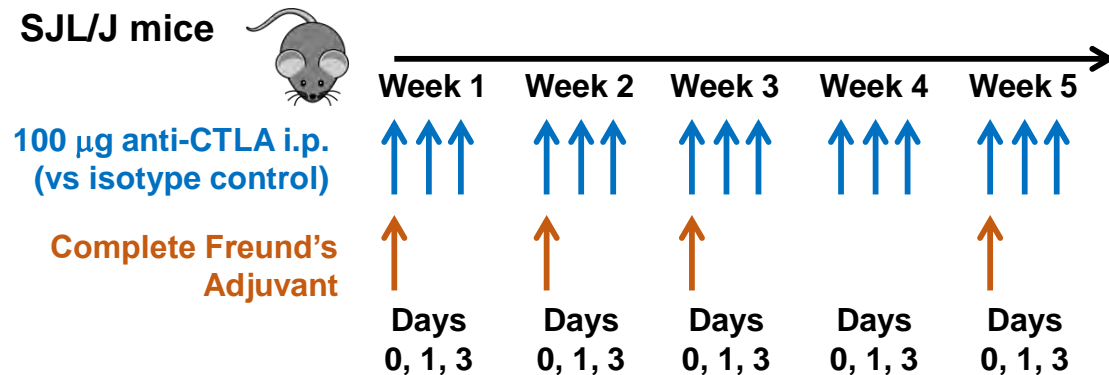
CTLA-4 blockade can impact the biology of non-malignant inflammatory conditions



# A mouse model of CTLA-4 induced **hypophysitis**

Repeated dosing in non-tumor bearing mice

**Pituitary Expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody.** Sci Transl Med 6:230ra45 (2014)



**CTLA-4 blockade induces hematopoietic cell infiltration of pituitary gland**

(No infiltration was seen in thyroid, liver, colon or skin)

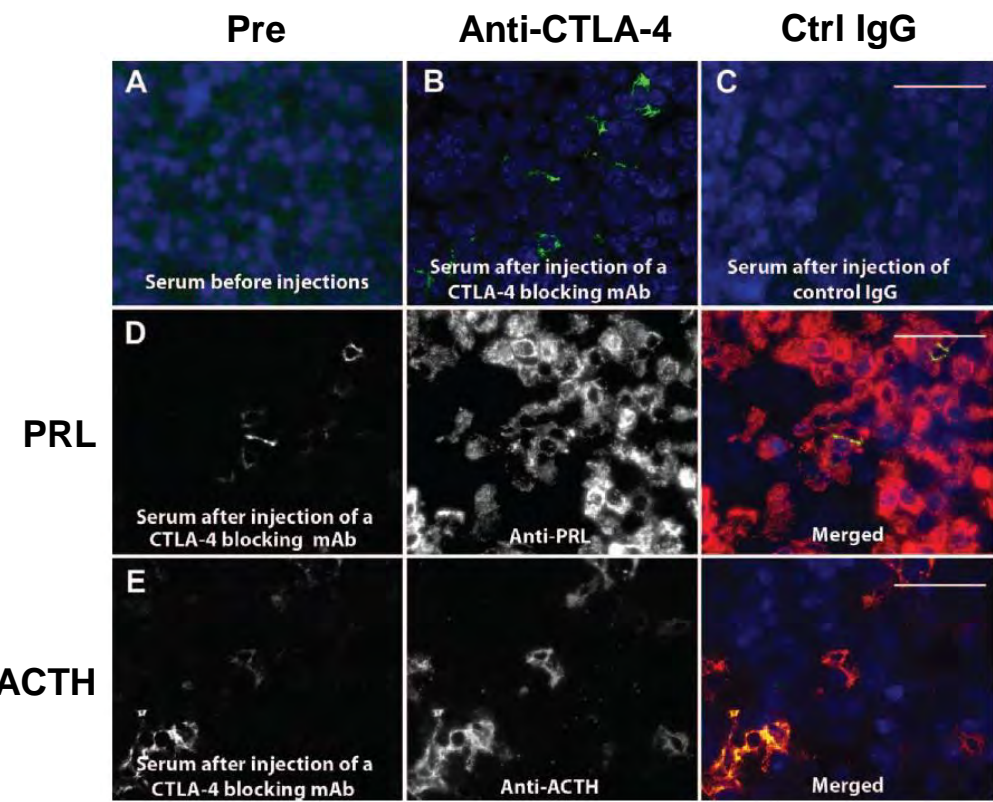


# A mouse model of CTLA-4 induced hypophysitis

## Repeated dosing in non-tumor bearing mice

Pituitary Expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. Sci Transl Med 6:230ra45 (2014)

CTLA-4 blocking induces serum antibodies with specificity for prolactin (PRL)- and ACTH-secreting cells in pituitary



ID	Sex	Age (years)	Cancer type	Clinical hypophysitis	Overall pituitary antibodies		Cell-specific pituitary antibodies				
					Before Ipi	After Ipi	TSH-secreting cells	FSH-secreting cells	ACTH-secreting cells	GH-secreting cells	PRL-secreting cells
1	M	53	Melanoma	Yes	Absent	Present	Positive	Positive	Negative	Negative	Negative
2	M	68	Melanoma	Yes	Absent	Present	Positive	Positive	Negative	Negative	Negative
3	M	59	Melanoma	Yes	Absent	Present	Positive	Negative	Positive	Negative	Negative
4	F	34	Melanoma	Yes	Absent	Present	Positive	Positive	Positive	Negative	Negative
5	F	58	Melanoma	Yes	Absent	Present	Positive	Negative	Negative	Negative	Negative
6	M	72	Melanoma	Yes	Absent	Present	Positive	Positive	Positive	Negative	Negative
7	M	65	Prostate	Yes	Absent	Present	Positive	Positive	Negative	Negative	Negative

7 of 7 patients with CTLA-4 induced hypophysitis show pituitary antibodies after beginning ipilimumab, whereas 13 of 13 patients without hypophysitis lacked pituitary antibodies

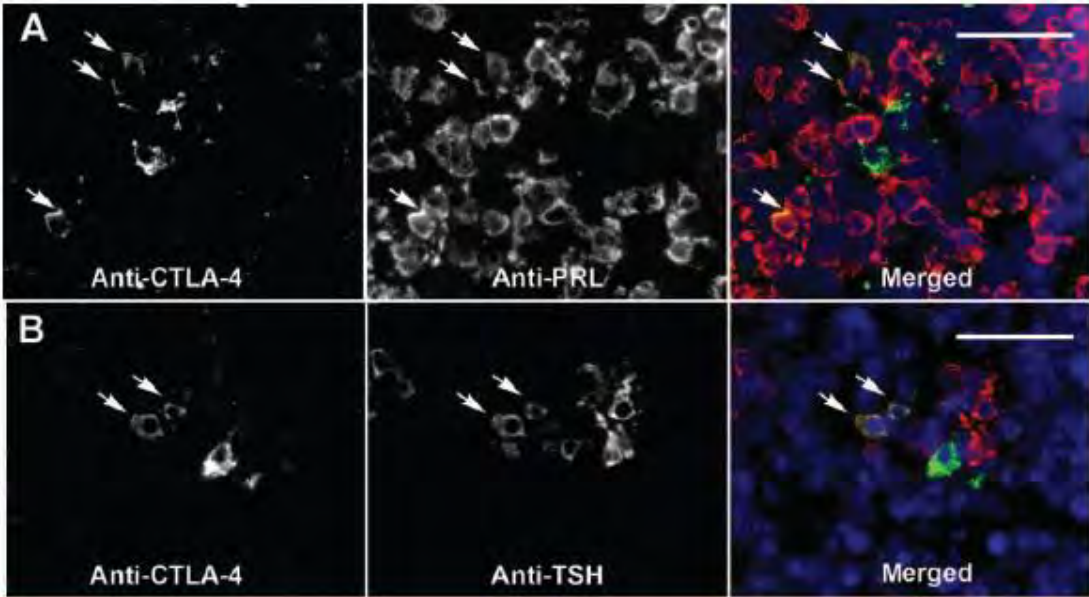
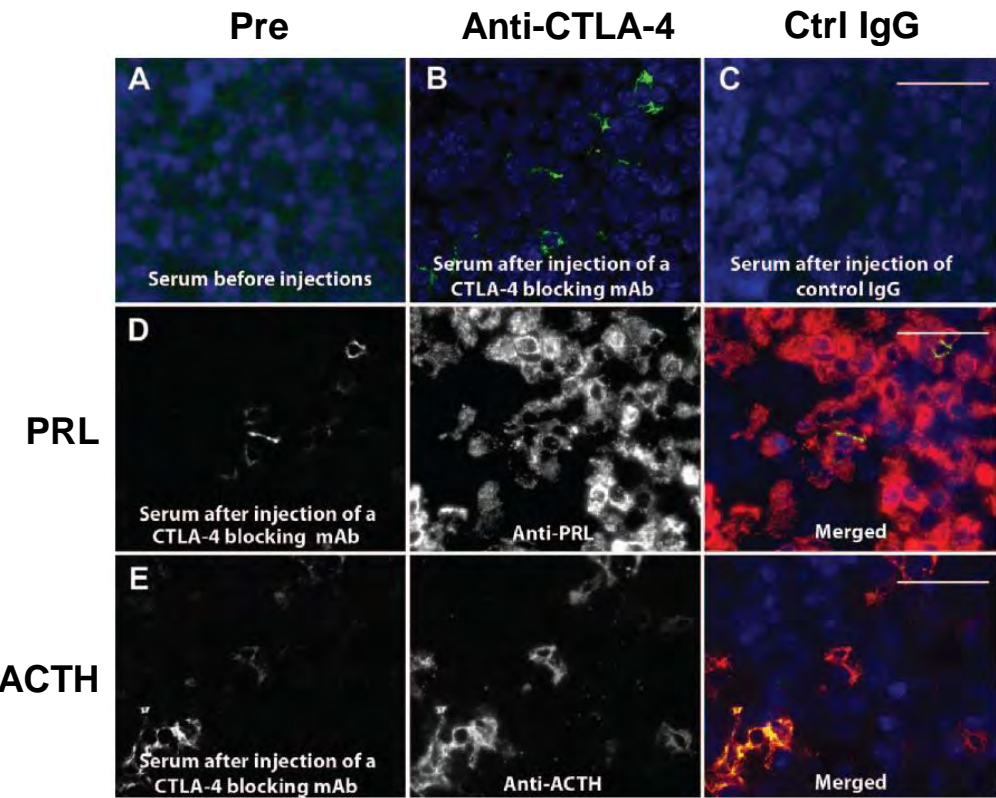


# A mouse model of CTLA-4 induced hypophysitis

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CTLA-4 blocking induces serum antibodies with specificity for prolactin (PRL)- and ACTH-secreting cells in pituitary



A subset of pituitary gland cells expressing prolactin (PRL) and TSH express CTLA-4.

Authors proposed that CTLA-4 antibodies activates complement in pituitary leading to tissue destruction.

# A mouse model of CTLA-4 induced hypophysitis

Repeated dosing in non-tumor bearing mice

Pituitary Expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. Sci Transl Med 6:230ra45 (2014)

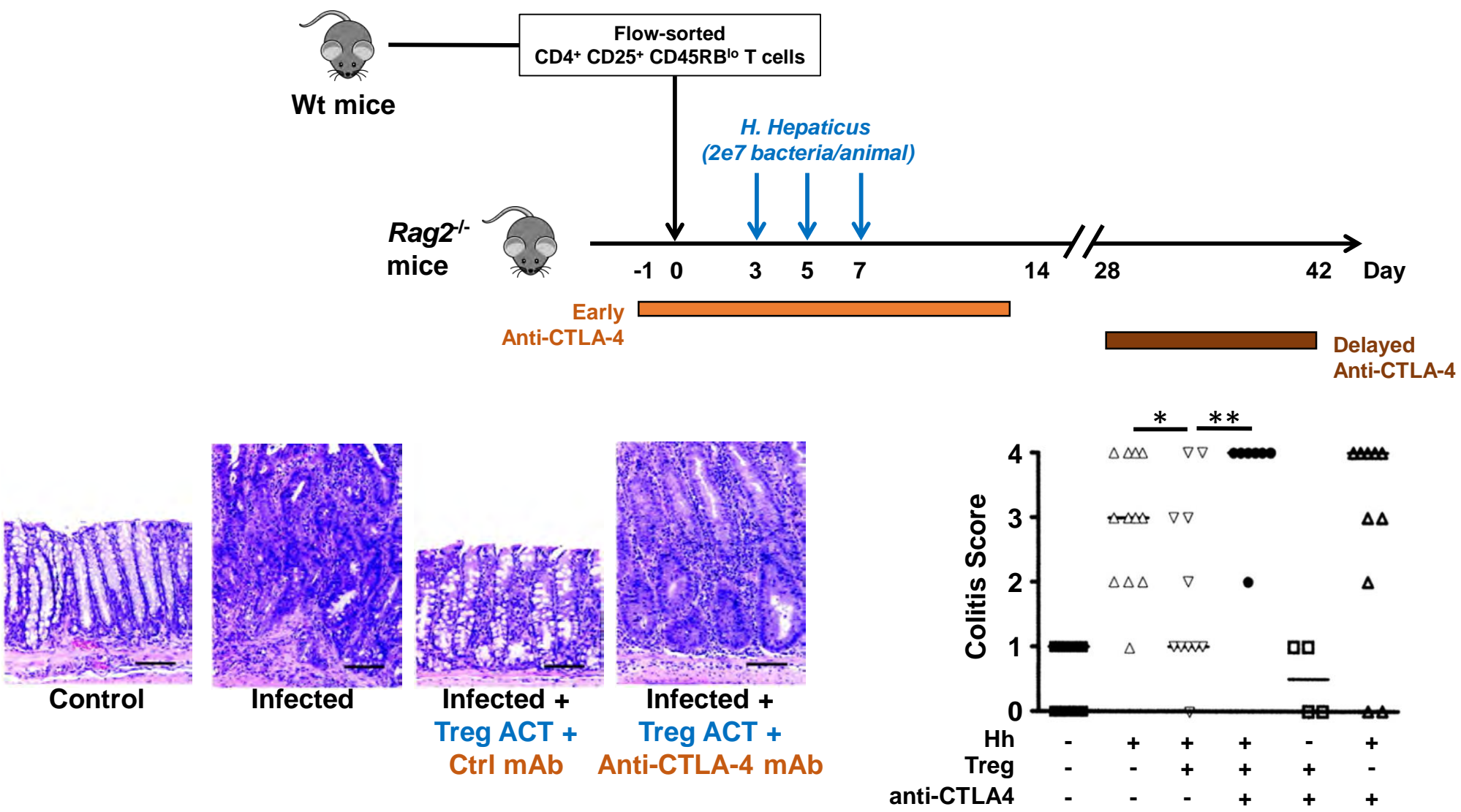
## **Model Limitations:**

1. Lack of direct evidence for CTLA-4 antibodies binding cognate antigen in pituitary
2. Unclear if model of secondary hypophysitis in mice mimics the human counterpart

# A mouse model of CTLA-4 induced colitis

Unmasking subclinical pathology with CTLA-4 blockade?

[Infect Immun.](#) 2008 Dec;76(12):5834-42



- CTLA-4 blockade led to inflammation in colon despite accumulation of Foxp3<sup>+</sup> cells.
- Delayed anti-CTLA-4 also induced inflammation in setting of infection + Treg ACT

**Notes:** Rag2<sup>-/-</sup> and wt mice were on a 129/SvEv background; hamster anti-mouse CTLA-4 mAb (UC10-4F10-11, 100 µg/animal/day i.p.); ACT, adoptive cell transfer

# A mouse model of CTLA-4 induced colitis

Unmasking subclinical pathology with CTLA-4 blockade?

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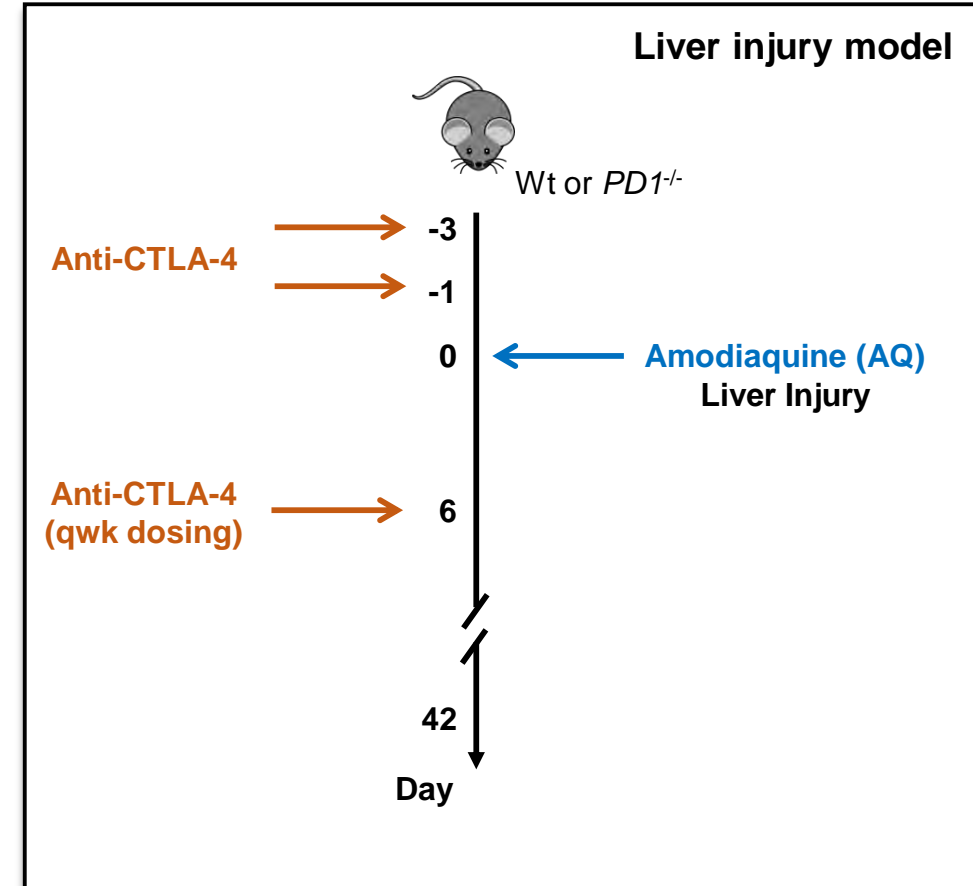
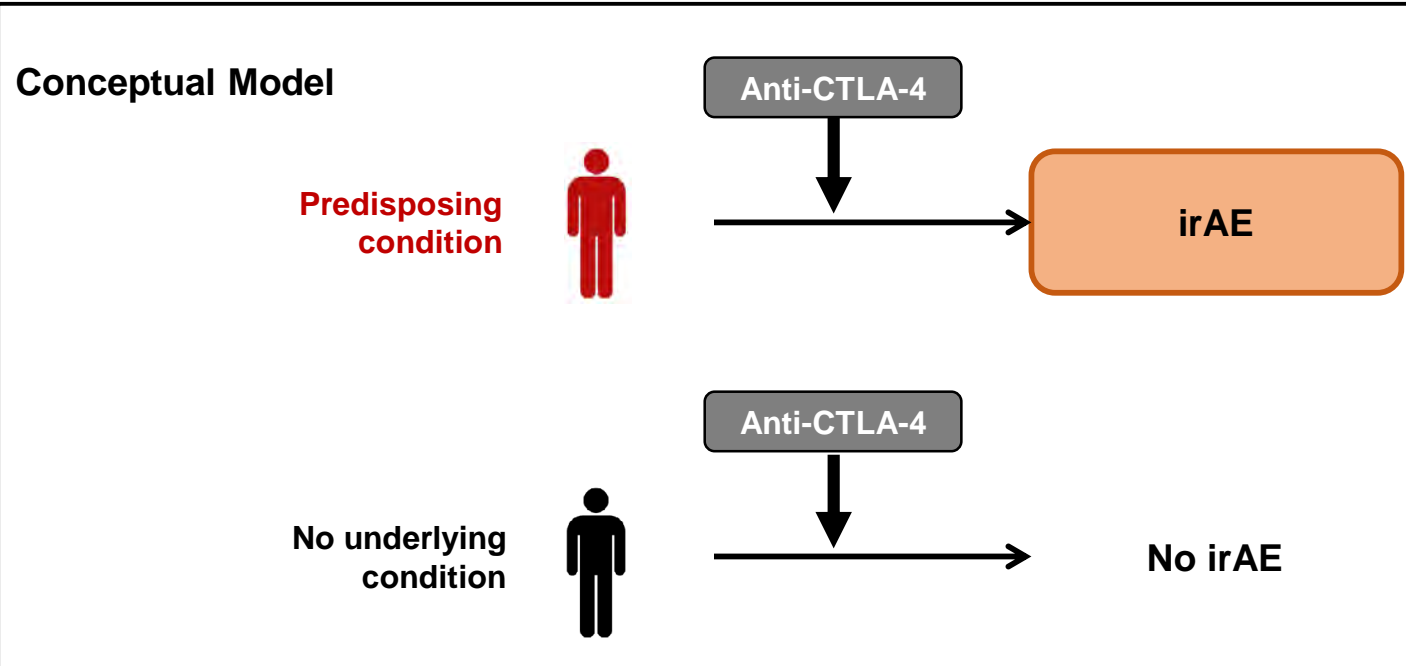
Cytotoxic T-lymphocyte-associated antigen 4 blockade abrogates protection by regulatory T cells in a mouse model of microbially induced innate immune-driven colitis. Infect Immun 76:5834 (2008)

## Model Limitations:

1. Immunocompromised model (*Rag2*<sup>-/-</sup>)
2. *H. Hepaticus* is a murine enterohepatic pathogen

# Conceptual model

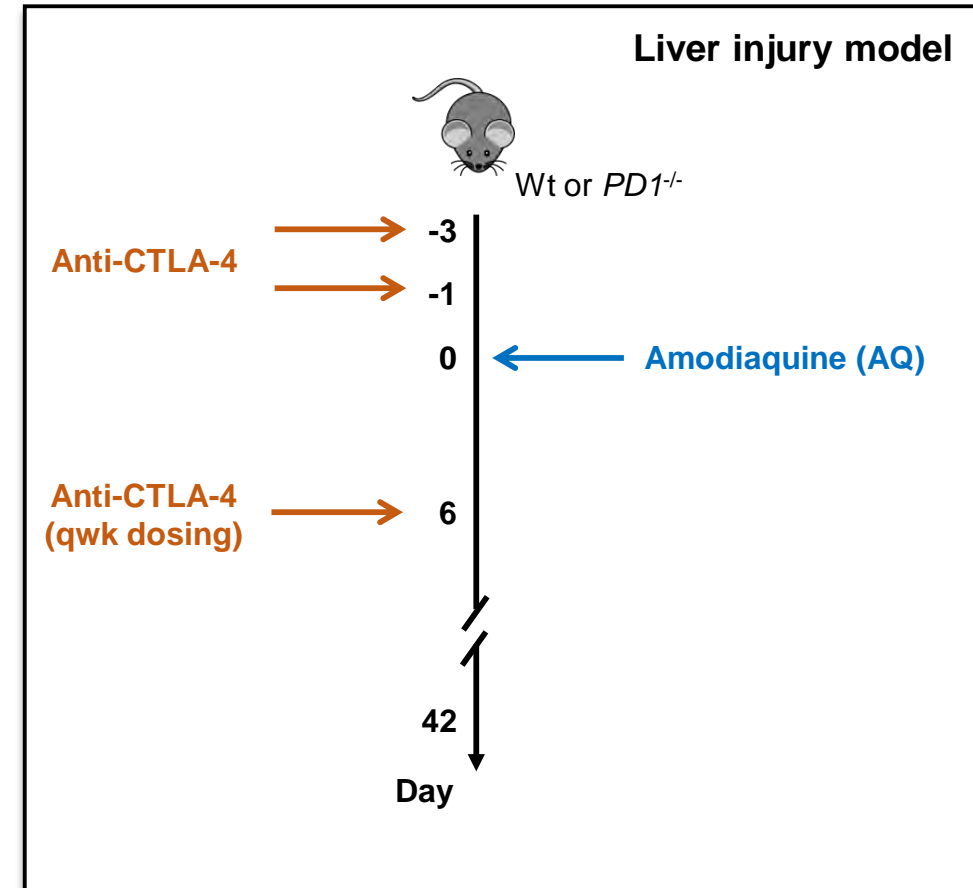
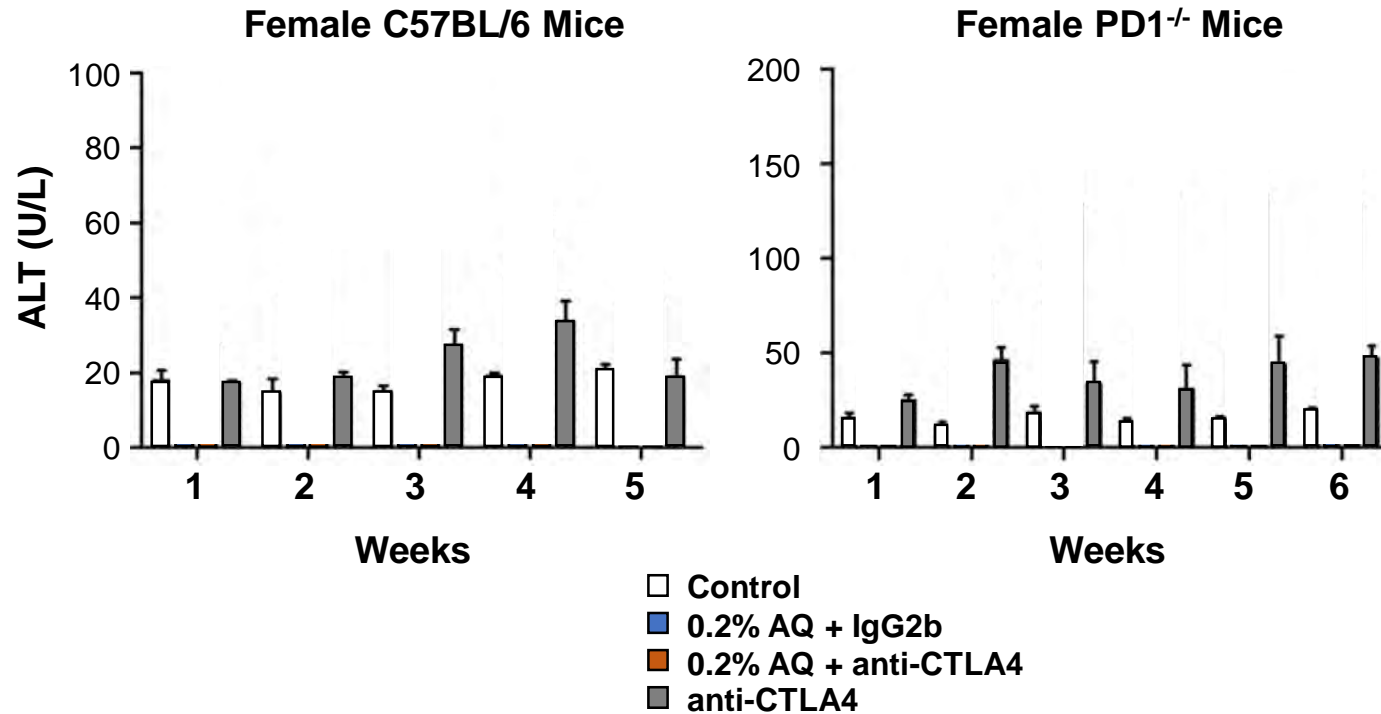
## Predisposition as a prerequisite for irAE?



Treatment of PD-1(-/-) mice with amodiaquine and anti-CTLA4 leads to liver injury similar to idiosyncratic liver injury in patients. Hepatology 61:1332 (2015)

# Liver injury predisposes to chronic hepatitis

## Impact of dual CTLA-4 and PD-1 disruption

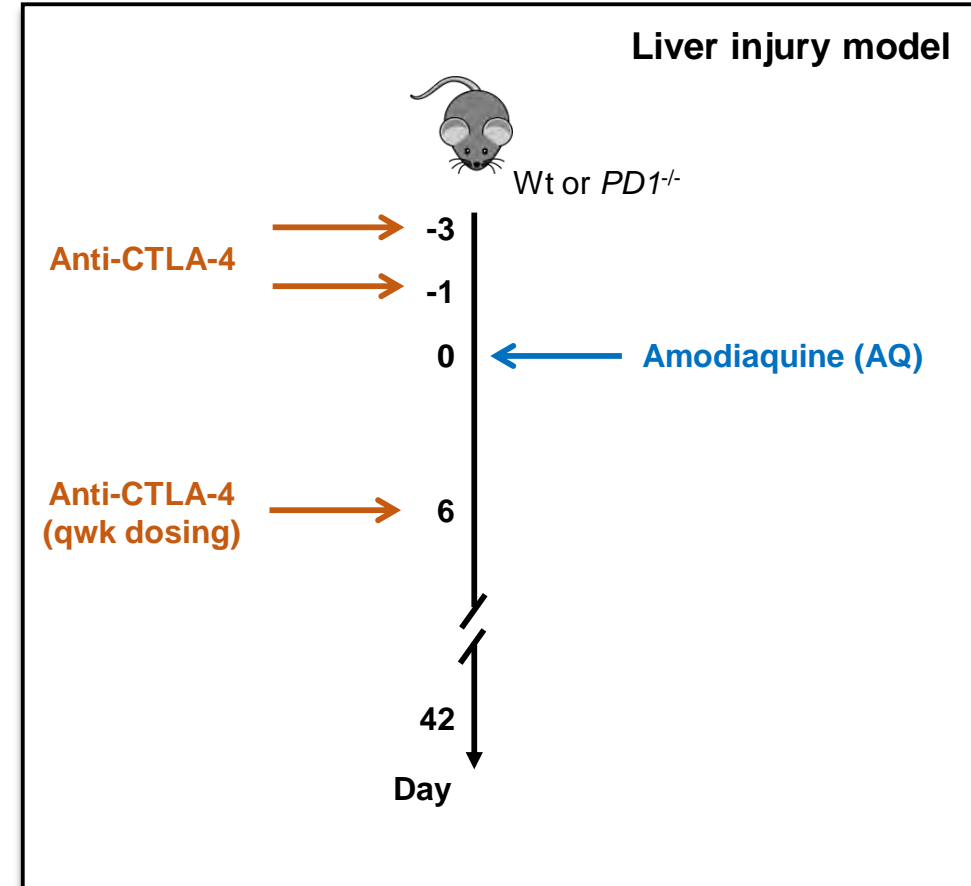
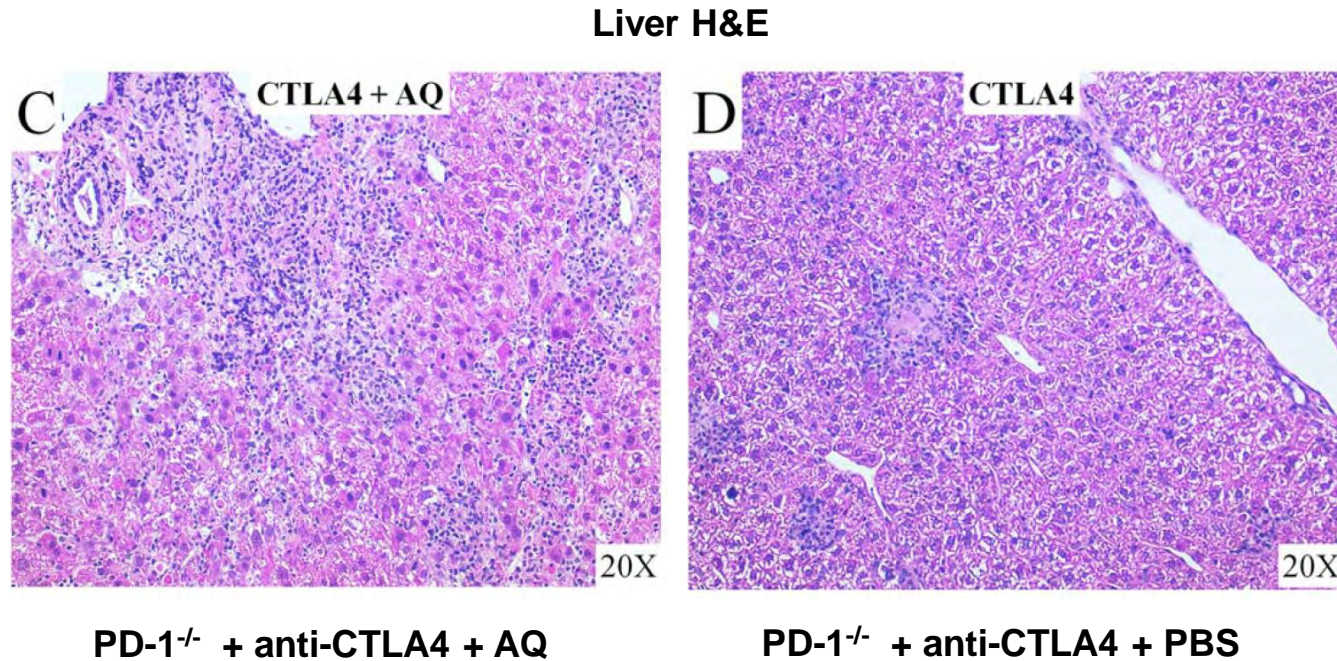


Treatment of PD-1(-/-) mice with amodiaquine and anti-CTLA4 leads to liver injury similar to idiosyncratic liver injury in patients. Hepatology 61:1332 (2015)



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# Liver injury predisposes to chronic hepatitis

## Impact of dual CTLA-4 and PD-1 disruption

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Treatment of PD-1(-/-) mice with amodiaquine and anti-CTLA4 leads to liver injury similar to idiosyncratic liver injury in patients. Hepatology 61:1332 (2015)

### **Model Limitations:**

1. Does PD-1 blockade reproduce PD-1 genetic deletion?
2. Amodiaquine is not a common drug used in cancer patients

## Example 2: Modeling irAEs related to anti-PD1 therapy

Anti-PD1 (%)		
Toxicity	All Grades	Grade 3/4
Pruritis	14-19	0
Rash	13-26	0-1
Diarrhea	1-3	1-2
Colitis	1-3	0.5-2
Elevated ALT	1-4	0.4-1
Elevated AST	2-4	0.4-4
Hypothyroidism	7-9	0
Hypophysitis	0-1	0-0.4
Pneumonitis	1-2	0.3-0.4



Oncologist. 2016 Oct; 21(10): 1230–1240.

## Management of Adverse Events Following Treatment With Anti-Programmed Death-1 Agents

# Lessons from PD-1 preclinical studies

1999

**Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor.** *Immunity* 11:141 (1999)

“Aged C57BL/6(B6)-*PD-1*<sup>-/-</sup> congenic mice spontaneously developed characteristic lupus-like proliferative [arthritis](#) and [glomerulonephritis](#) with predominant [IgG3](#) deposition...Identified a role for PD-1 in regulating GVHD. **Concluded** that PD-1 is involved in the maintenance of peripheral [self-tolerance](#) by serving as a negative regulator of immune responses.”

1996

**Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice.** *Science* 291:319 (2001)

“BALB/c mice *PD-1*<sup>-/-</sup> develop [dilated cardiomyopathy](#) with severely impaired contraction and sudden death by [congestive heart failure](#). Affected hearts showed diffuse deposition of immunoglobulin G (IgG) on the surface of cardiomyocytes. **Concluded** PD-1 contributes to [prevention of autoimmune diseases](#).”

2003

**The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice.** *JEM* 198:63 (2003)

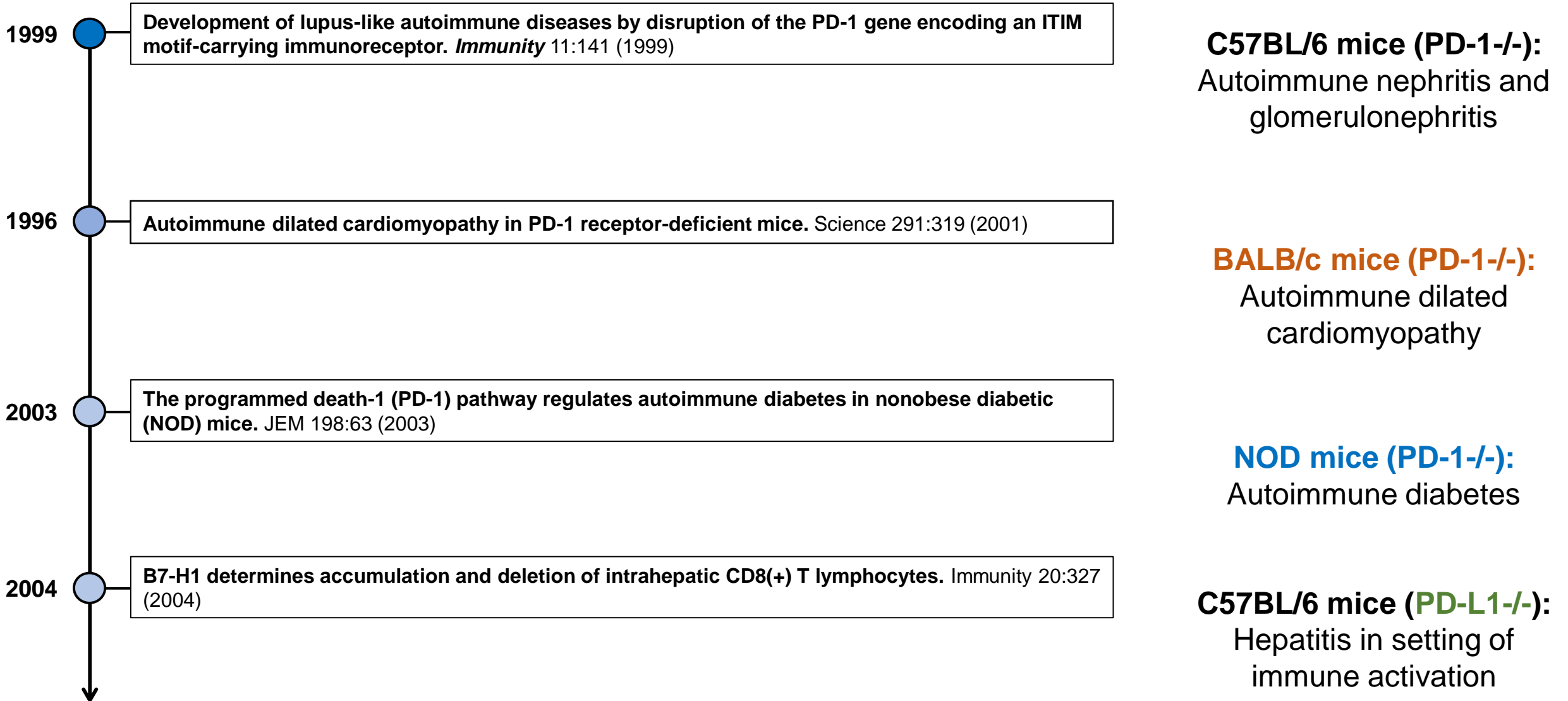
“PD-1 or PD-L1 but not PD-L2 blockade rapidly precipitated [diabetes](#) in prediabetic female nonobese diabetic (NOD) mice regardless of age (from 1 to 10-wk-old), although it was [most pronounced in the older mice](#). **Concluded** PD-1-PD-L1 pathway has a central role in regulation of induction and progression of autoimmune diabetes in NOD mouse”

2004

**B7-H1 determines accumulation and deletion of intrahepatic CD8(+) T lymphocytes.** *Immunity* 20:327 (2004)

“PD-L1<sup>-/-</sup> C57BL/6 mice show [accumulation of CD8<sup>+</sup> T lymphocytes in the liver](#). No liver pathology seen in 14 mo old PD-L1 KO mice. PD-L1 KO mice compare to wt mice demonstrate [increased liver damage in model of experimental autoimmune hepatitis](#) involving systemic ConA injection. **Concluded** PD-L1 regulates intrahepatic CD8 T cell accumulation and may contribute to inflammation, autoimmune diseases and tolerance in the liver”

# Lessons from PD-1 preclinical studies



# Lessons from PD-1 preclinical studies

1999

Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 11:141 (1999)

**C57BL/6 mice (PD-1<sup>-/-</sup>):**  
Autoimmune nephritis and  
glomerulonephritis

A role for genetics and predisposing insults (inflammation) in driving autoimmune phenomena  
in setting of PD-1/PD-L1 blockade

Autoimmune diabetes

2004

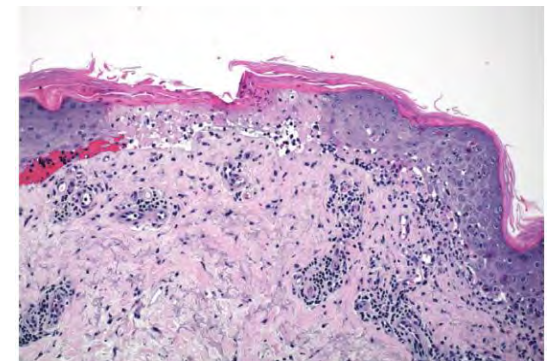
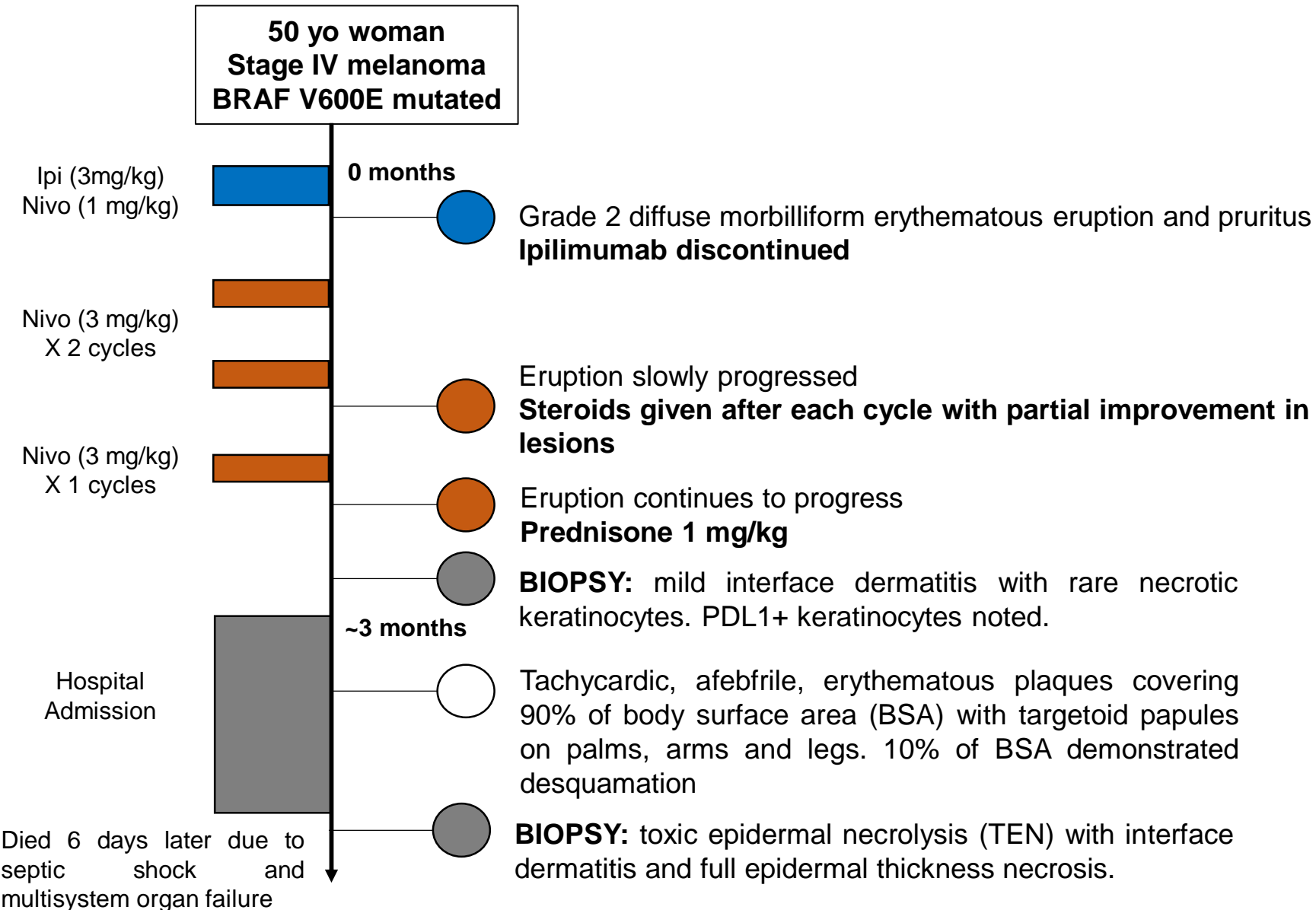
B7-H1 determines accumulation and deletion of intrahepatic CD8(+) T lymphocytes. *Immunity* 20:327 (2004)

**C57BL/6 mice (PD-L1<sup>-/-</sup>):**  
Hepatitis in setting of  
immune activation

# Rash from anti-PD1 therapy

## Unpredictable and potentially lethal

*J Cutan Pathol.* 2017;44:381–384.

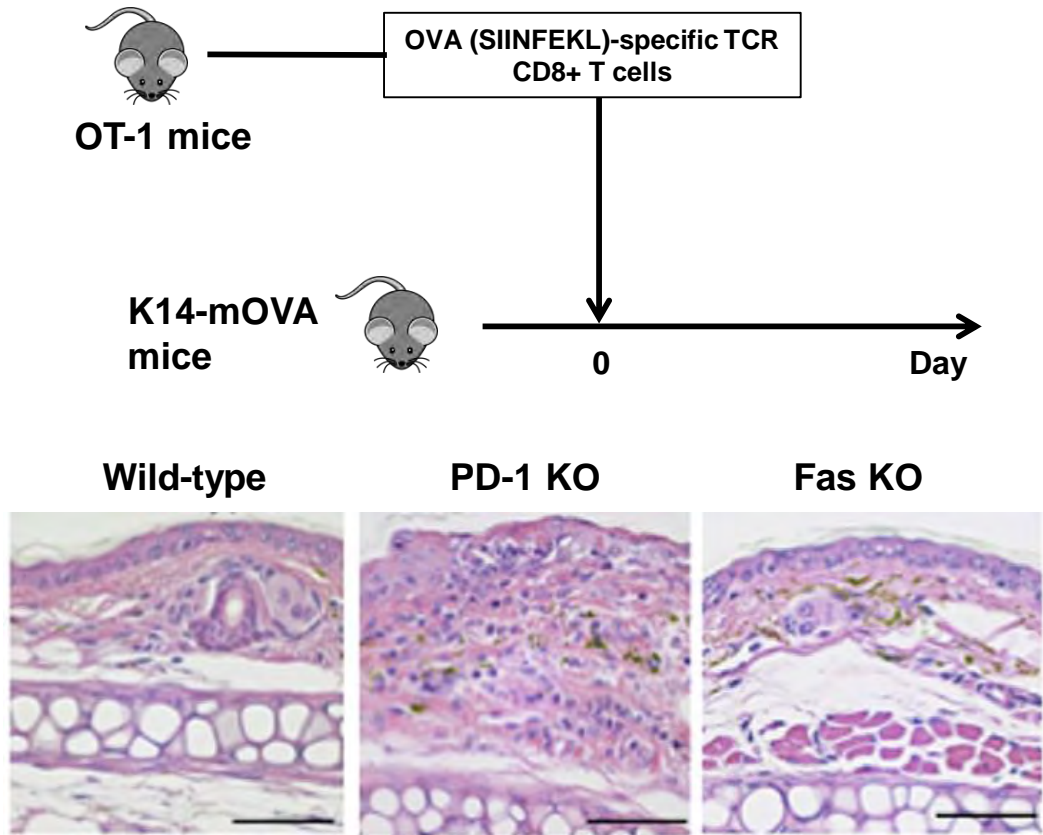




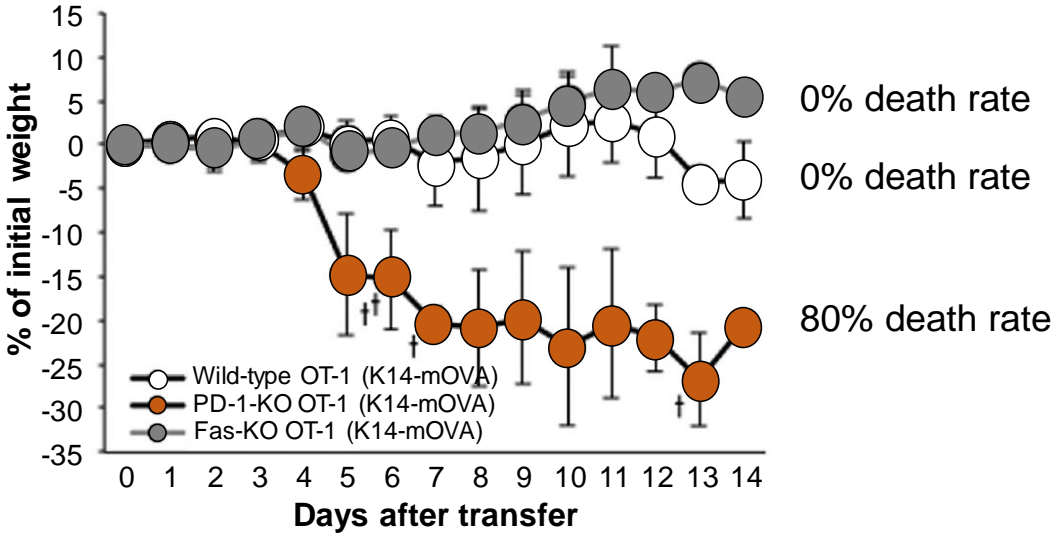
# A model to study rash from anti-PD1 therapy

## Disrupting peripheral tolerance against keratinocytes?

### Experimental Model



**Note:** Keratinocytes express PD-L1



Recipient	OT-I cell	IFN $\gamma$
K14-mOVA	Wild-type	574.0 $\pm$ 229.4
K14-mOVA	PD-1-KO	1527.9 $\pm$ 599.6*
K14-mOVA	Fas-KO	176.5 $\pm$ 17.4**
B6	Wild-type	21.2 $\pm$ 0.3**

**Note:** IL-6 and TNF also increased in PD-1 KO condition. Pathology blocked in setting of IFN, IL-6, and TNF deficiency

**K14-mOVA:** Tg mouse that expressed chicken ovalbumin (OVA) in skin and mucosal epithelia under control of the keratin 14 promoter

**Programmed cell death 1 (PD-1) regulates the effector function of CD8 T cells via PD-L1 expressed on target keratinocytes.** J of Autoimmunity 53:1-9 (2014)



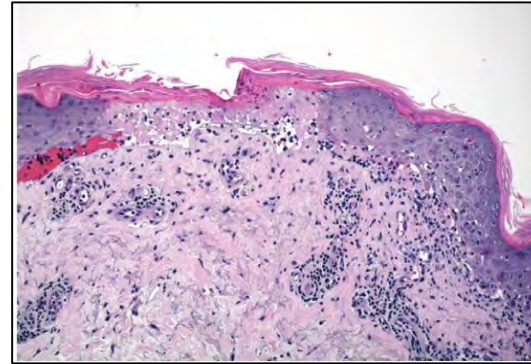
# Rash from anti-PD1 therapy

Disruption of peripheral tolerance to keratinocytes

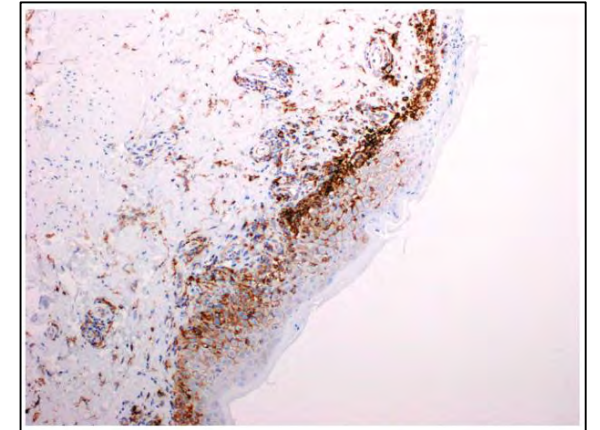
50 yo woman  
Stage IV melanoma  
BRAF V600E mutated



H&E



PD-L1



# A model to study rash from anti-PD1 therapy

## Disrupting peripheral tolerance against keratinocytes?

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Programmed cell death 1 (PD-1) regulates the effector function of CD8 T cells via PD-L1 expressed on target keratinocytes. J of Autoimmunity 53:1-9 (2014)

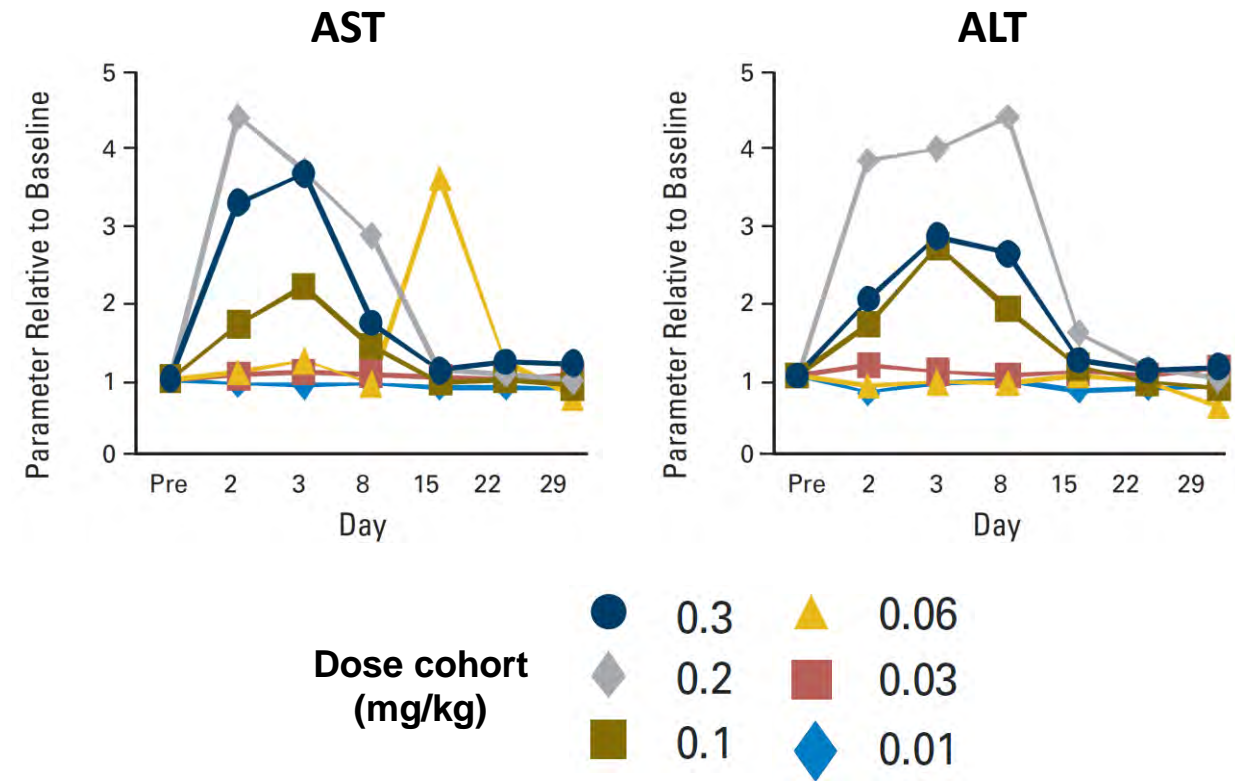
### **Model Limitations:**

1. Strong model antigen (OVA)
2. Will anti-PD1 reproduce same biology seen with genetic deletion used for ACT?

## Example 3: Immune agonists targeting CD40 can induce hepatitis

### Monitoring for acute toxicities and dependence on age

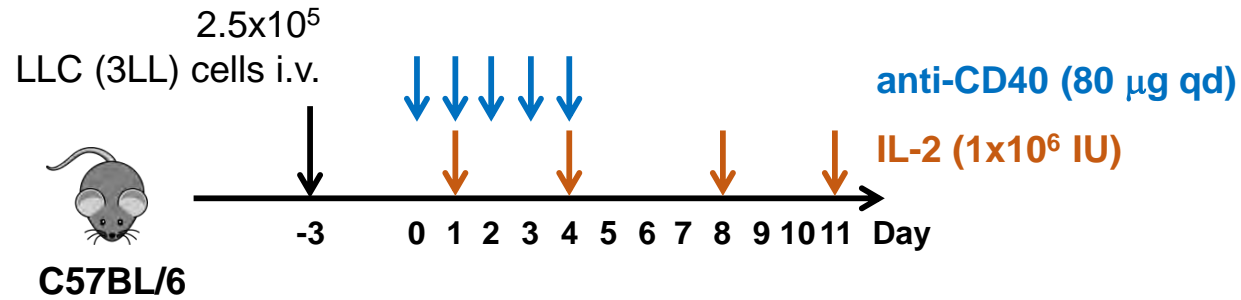
- CD40 is a member of the tumor necrosis factor (TNF) receptor superfamily
- CD40 regulates immune activation and can mediate tumor apoptosis
- CD40 is expressed by dendritic cells, B cells, monocytes and other non-hematopoietic cells (e.g. endothelial cells, platelets)
- CD40 signaling activates antigen presenting cells
- Agonistic CD40 antibodies induce a cytokine release syndrome in patients, hepatitis, and thrombocytopenia



# Mechanisms underlying toxicity with CD40 agonists

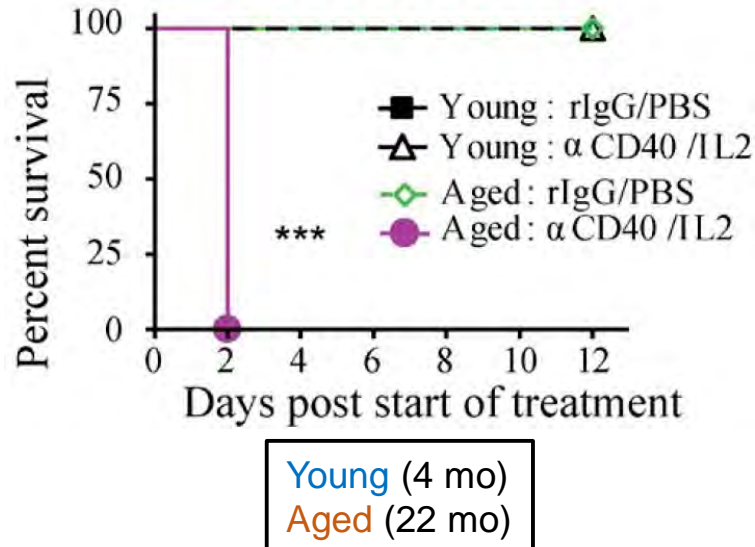
## Impact of age

### Experimental Model

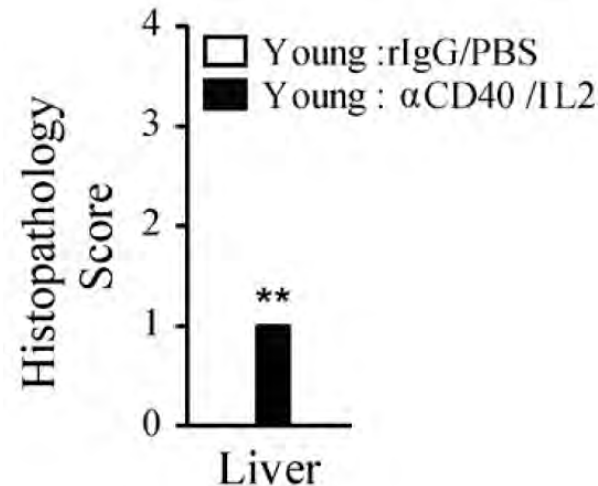


**Goal of study:** Define the impact of age on the efficacy of anti-CD40/IL-2 therapy.

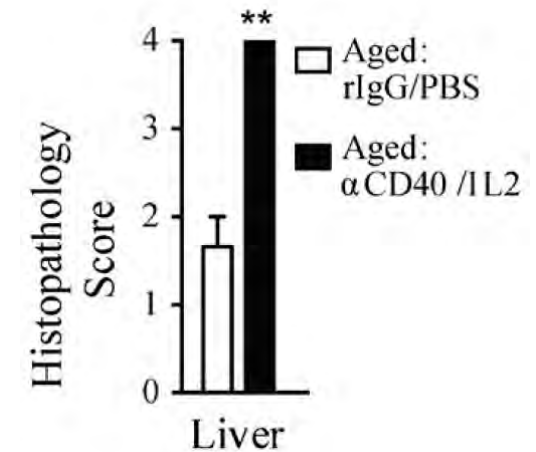
CD40/IL-2 induces lethal toxicity  
in **aged** but not **young** mice



CD40/IL-2 induces minimal hepatotoxicity  
in **young** mice



CD40/IL-2 induces severe hepatotoxicity  
in **aged** mice

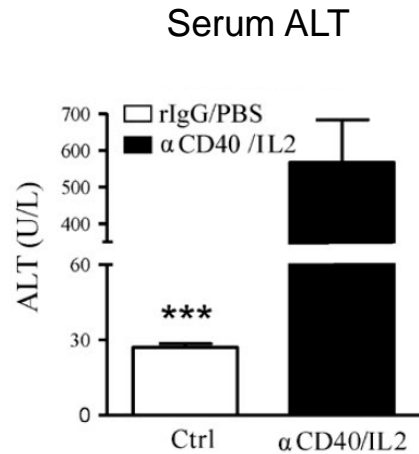




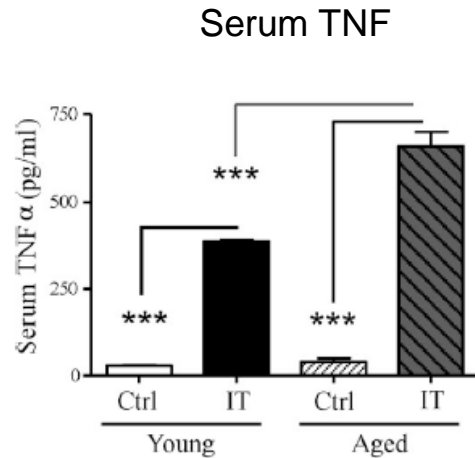
# Mechanisms underlying toxicity with CD40 agonists

## Dependence on age

### CD40/IL-2 induces transaminitis



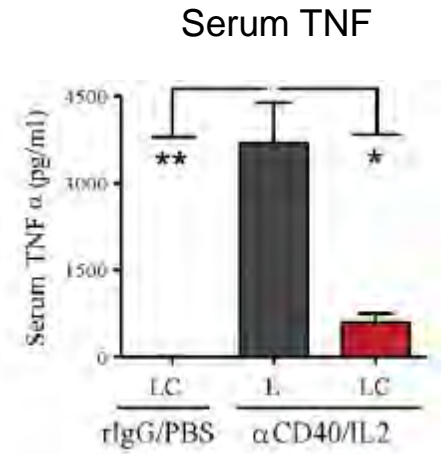
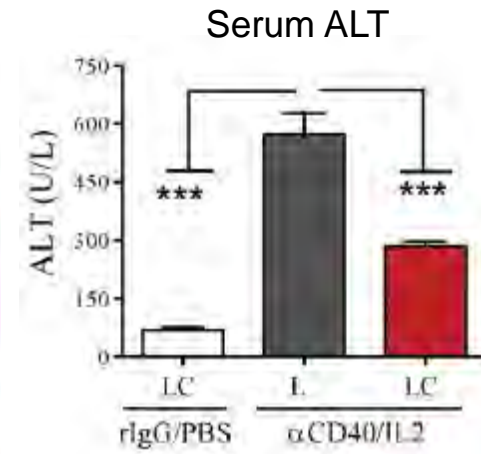
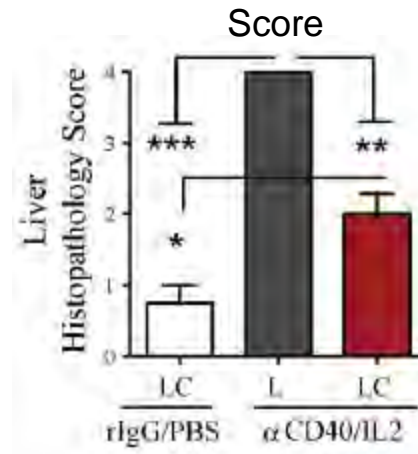
### CD40/IL-2 (IT) induces Increased serum TNF in **aged** mice



**Note:** IT also induces increases in IL-6 and IFN $\gamma$

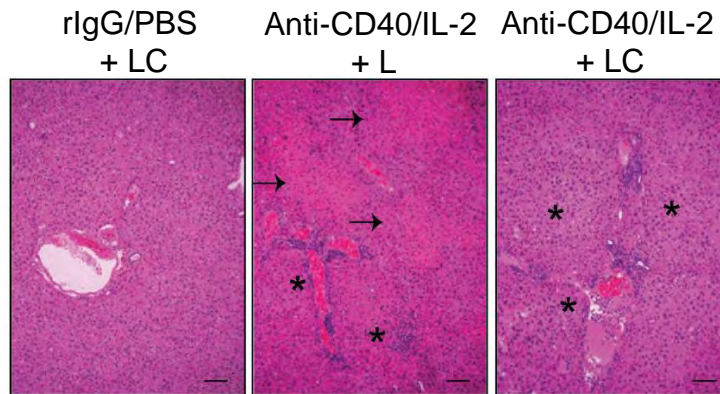
### CD40/IL-2 induced hepatotoxicity, transaminitis, and cytokine release inhibited by macrophage depletion (LC)

#### Liver histopathology

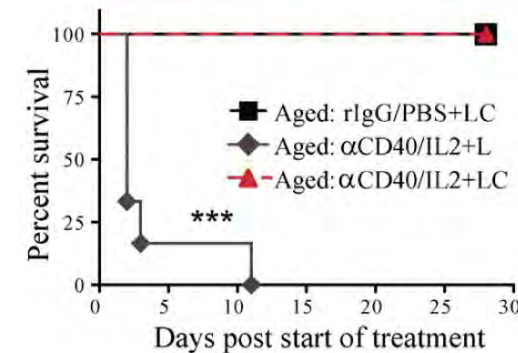


**Note:** CD4 and CD8 T cells and NK cells are not required for hepatotoxicity

### Macrophage depletion (LC) reverses CD40/IL-2 induced hepatotoxicity and lethality



\*, Lymphocytic infiltrates  
→, necrosis

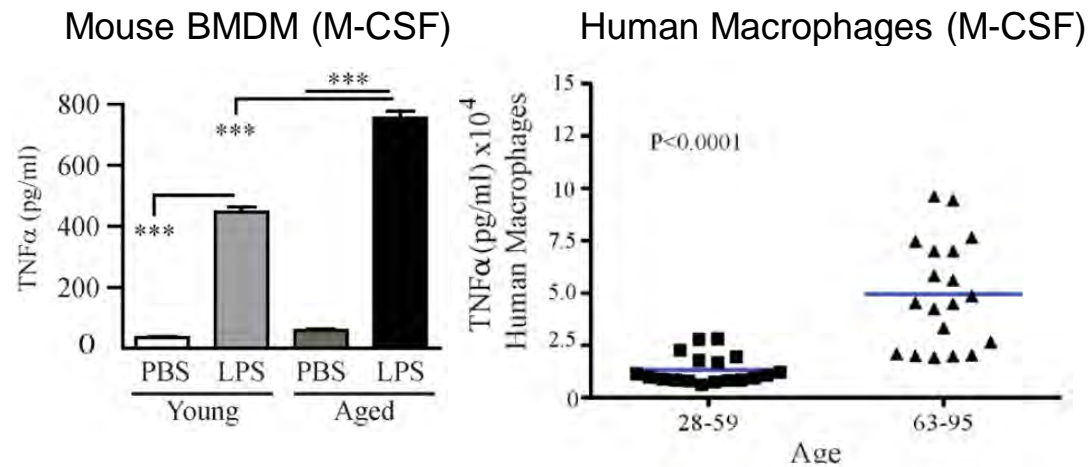


LC, clodronate liposomes

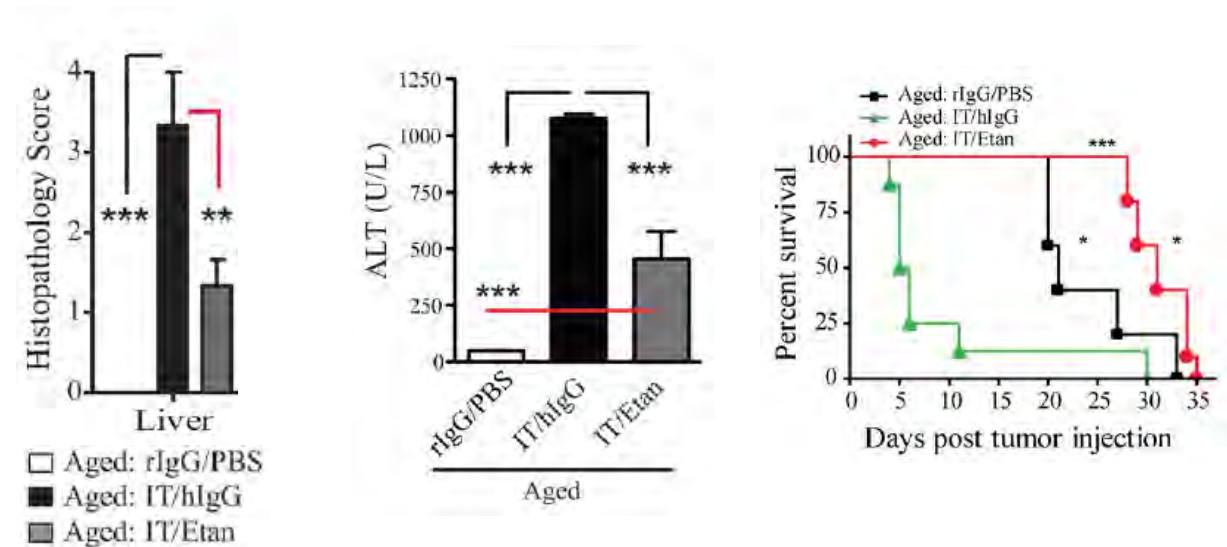
# Mechanisms underlying toxicity with CD40 agonists

## Dependence on age

Macrophages from aged vs young hosts secrete increased cytokines (TNF, IL-6)



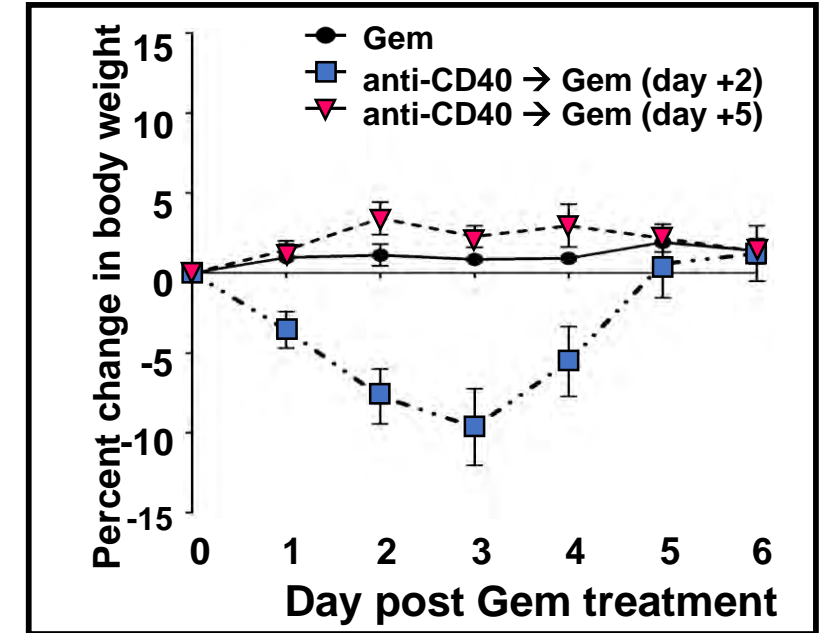
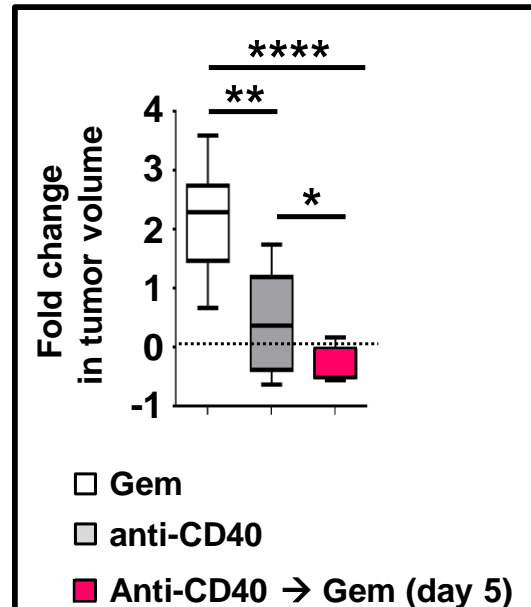
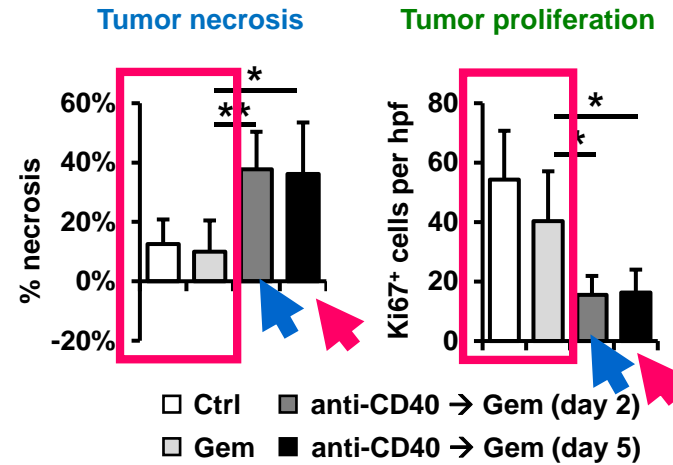
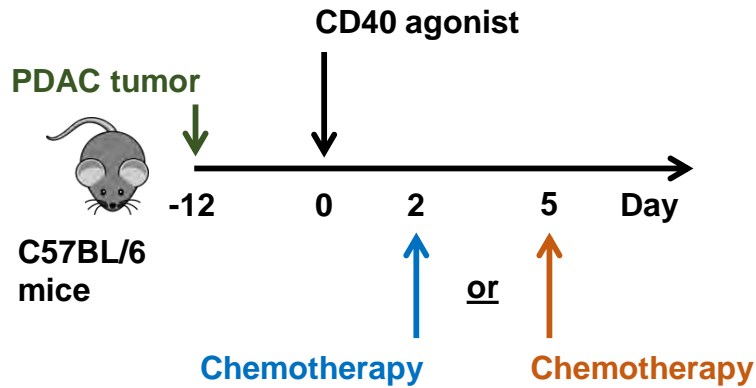
Etanercept (Anti-TNF) reverses CD40/IL-2 **toxicity** without impacting anti-tumor **efficacy**



# CD40 agonists condition for enhanced hepatotoxicity from chemotherapy

Timing of chemotherapy administration is critical

## Experimental Model

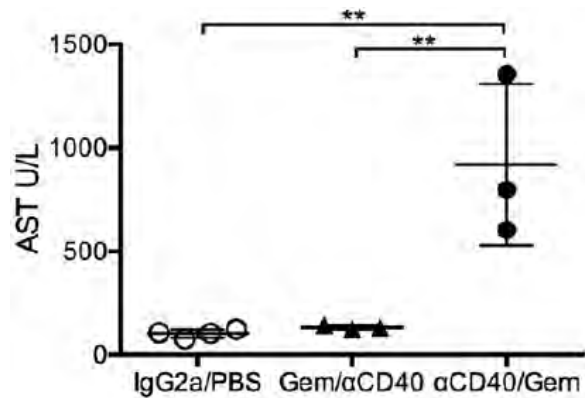
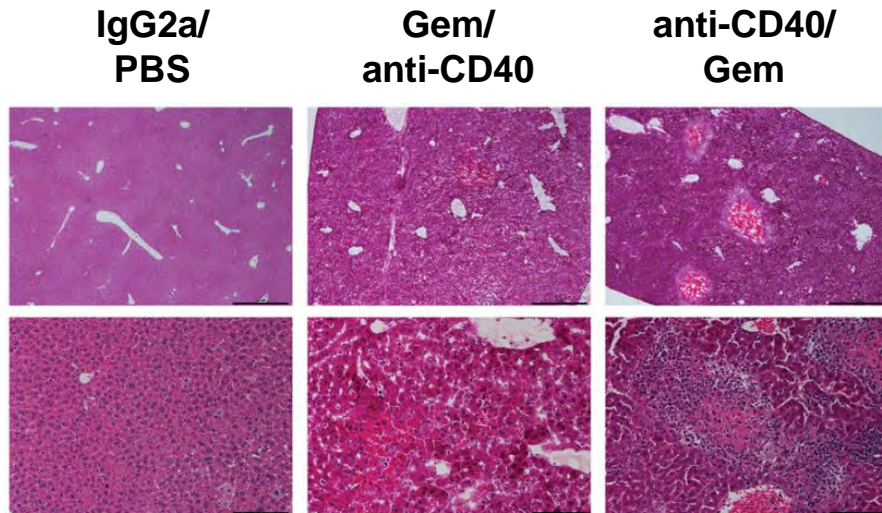




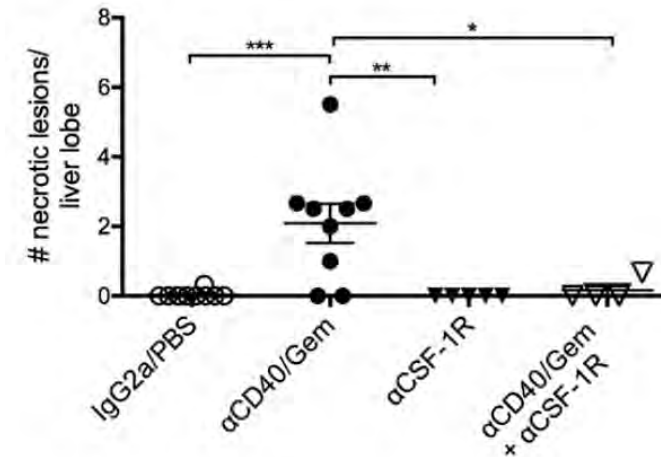
# CD40 agonists condition for enhanced hepatotoxicity from chemotherapy

## A role for macrophages

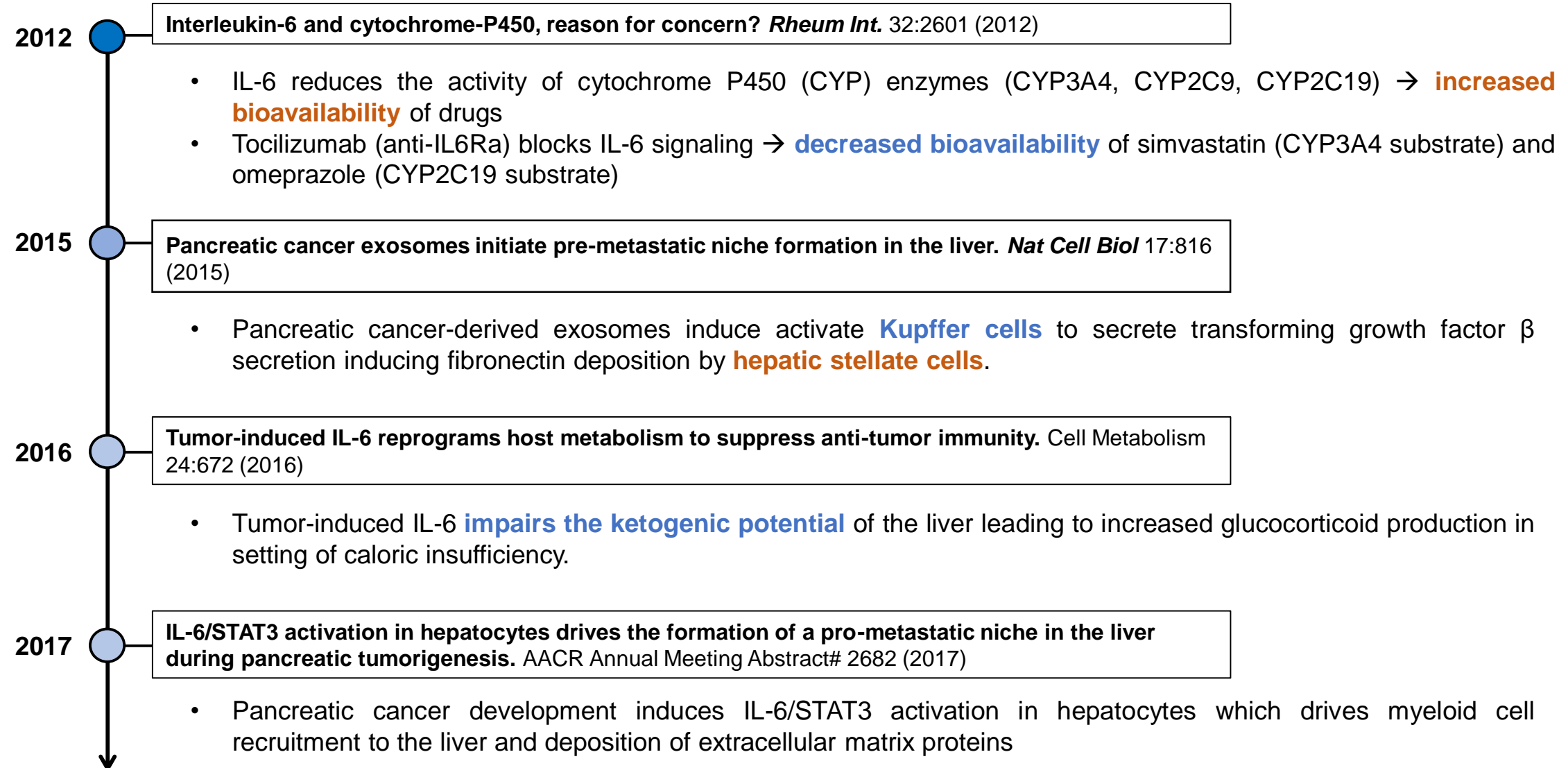
Chemotherapy administered 2 days after a CD40 agonist induces lethal hepatotoxicity with associated transaminitis<sup>1</sup>



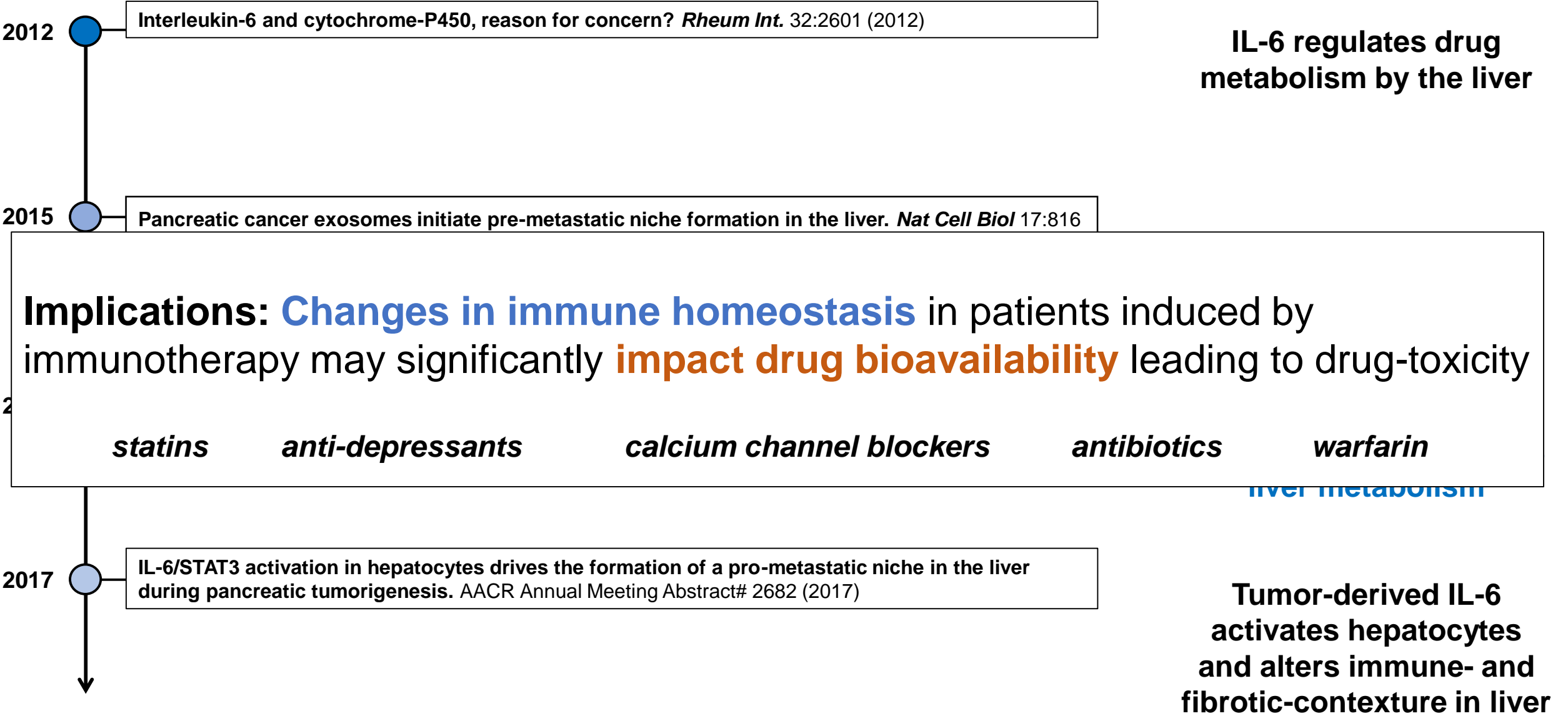
Hepatotoxicity induced by chemotherapy after a CD40 agonist is blocked by CSF1R antibodies in **tumor-bearing mice**<sup>1</sup>



# Tumor development alters liver biology



# Tumor development alters liver biology



# Challenges with modeling irAEs in mice

---

- Lack of comorbidities (non-alcoholic fatty liver disease – may affect up to 30-40% of adults, obesity, heart disease, diabetes)
- Genetics
- Diet/Microbiome
- Medications
- Exposure to community-acquired infections

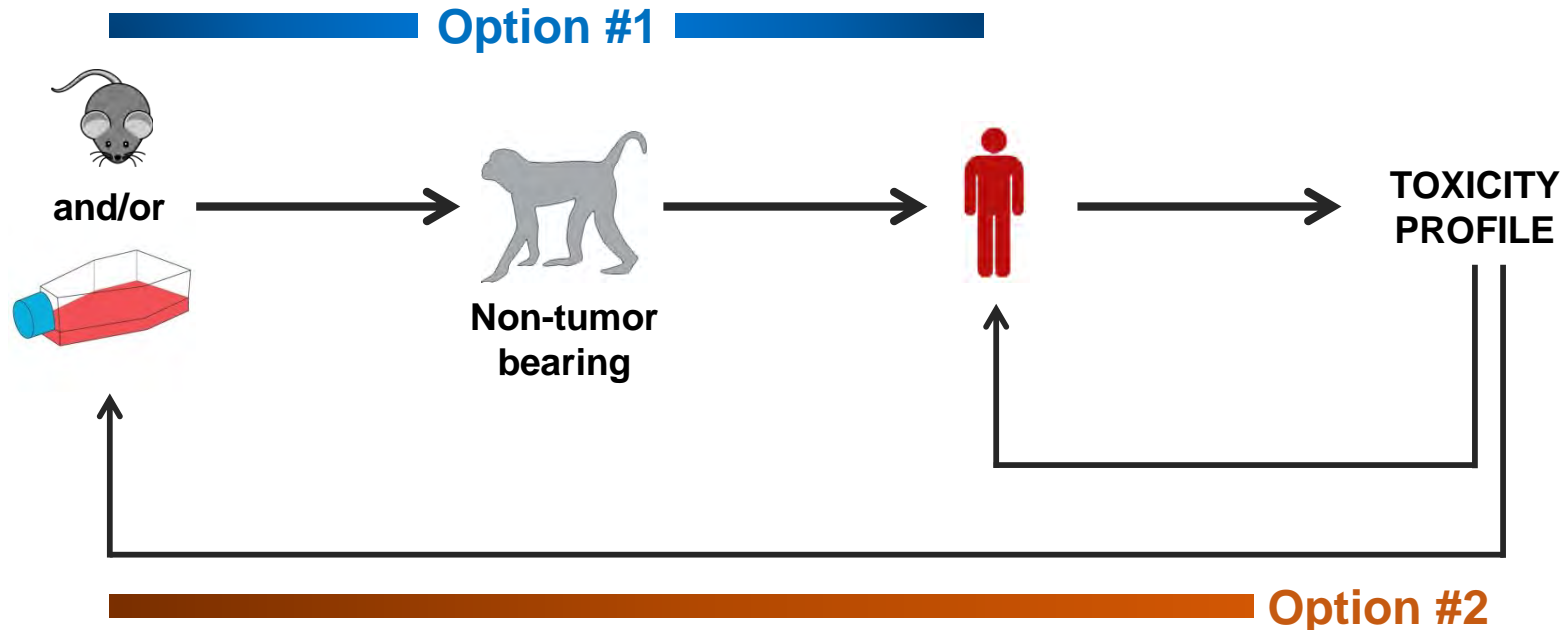
# Incorporating mouse models into the study of IO toxicities

## Predictive vs Informative

How should we incorporate preclinical models for the study of IO toxicities?

**Option 1:** Evaluate for potential toxicities in preclinical setting to inform translation and monitoring in patients.

**Option 2:** Identify toxicities that emerge after translation into patients and study them.



**Option #1:** Toxicity findings justify investigation, but because findings are not absolute predictors, detailed investigations may not be warranted at this stage of development

**Option #2:** Careful use of preclinical models may inform human pathology

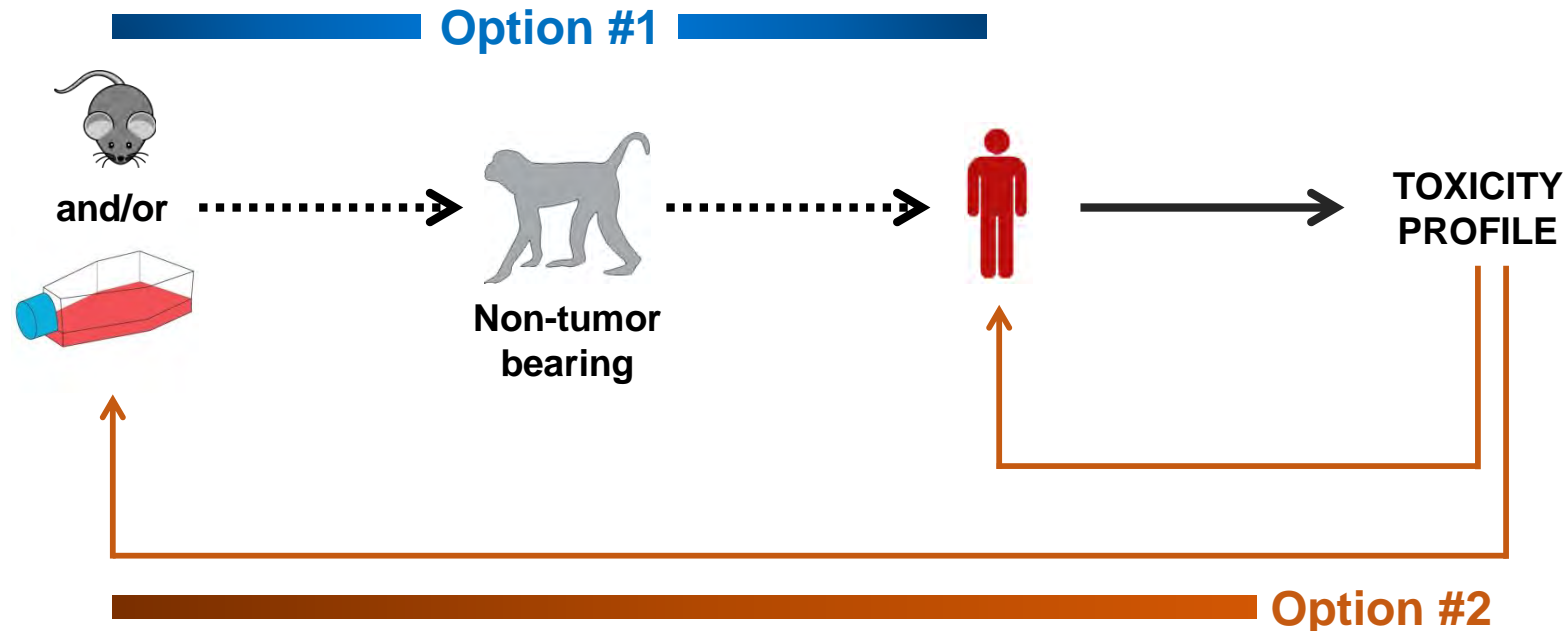
# Incorporating mouse models into the study of IO toxicities

## Predictive vs Informative

How should we incorporate preclinical models for the study of IO toxicities?

**Option 1:** Evaluate for potential toxicities in preclinical setting to inform translation and monitoring in patients.

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**Option #2:** Careful use of preclinical models may inform human pathology





## Summary Points

Beatty Laboratory  
University of Pennsylvania

- **Preclinical immunocompetent mouse models** can be used to **inform mechanisms of irAE** seen in humans treated with immunotherapy.
- There is **no perfect model** for studying irAEs. Models need to be matched to the question to be addressed.
- **Subclinical pathology** from genetic predisposition or environmental insults **may predispose to irAEs**.
- **Measuring irAEs in preclinical models can be done** via monitoring hepatic enzymes, weight loss, organ pathology, serum cytokines even in the absence of overt symptoms.
- **Immunotherapy** may alter the **tolerability to chemotherapy**.
- Tumor development and immunotherapy impact **liver biology** – implications for altered **pharmacokinetic profiles for concomitant medications**.
- **Most current models do not incorporate subclinical pathology** settings (for ex. Studying tumor biology in NOD mice predisposed to development of diabetes) - a possible **advantage for spontaneous models**.
- **Mechanisms** regulating irAE may differ between **tumor-free** and **tumor-bearing** mice?
- **Age** can impact the immune system and perhaps irAE development.
- Ameliorating **toxicity** does not necessarily mean loss of **efficacy**.
- Toxicity findings in **preclinical models are not absolute predictors** of pathology in humans but **toxicity does justify investigation**.





# Acknowledgements

**Beatty Laboratory**

University of Pennsylvania

## Beatty Laboratory

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Thomas Buckingham  
Evan Tooker  
Ishir Seth  
Fatima Chaundhry  
Colton Tom

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Janos Tanyi	Mark O'Hara
Robert Vonderheide	Ursina Teitelbaum
Kim Reiss-Binder	Ben Stanger
Michael Soulen	Andrea Troxel
Anne Chew	Gabriela Plesa
Lester Lledo	Maureen
McGarvey	
Anne Marie Nelson	Paige Porrett
Erica Carpenter	Steve Albelda
Drew Torigian	

## Center for Cellular Immunotherapies

Carl June

## TCSL

Simon Lacey  
Jos Melenhorst  
Joseph Fraietta



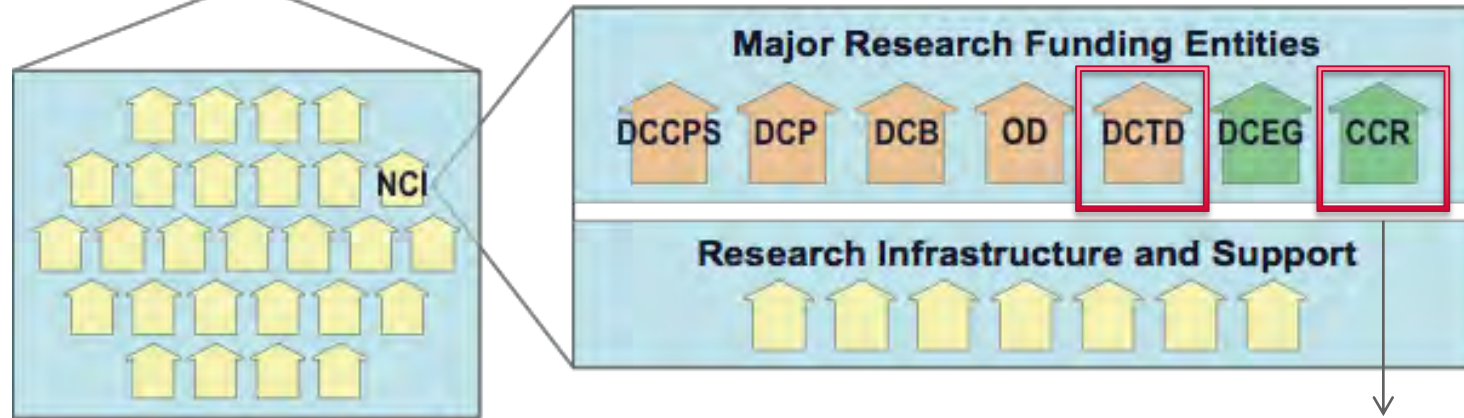
# A comparative approach to immunology drug development: Integration of canine models

*Amy LeBlanc, DVM DACVIM (Oncology)  
Director, Comparative Oncology Program  
NIH/NCI/CCR*

# The NCI's Center for Cancer Research (CCR) is part of the Intramural Research Program (IRP) of NIH



The NCI supports its mission through a combination of extramural funding (grants) and intramural (on-site) research



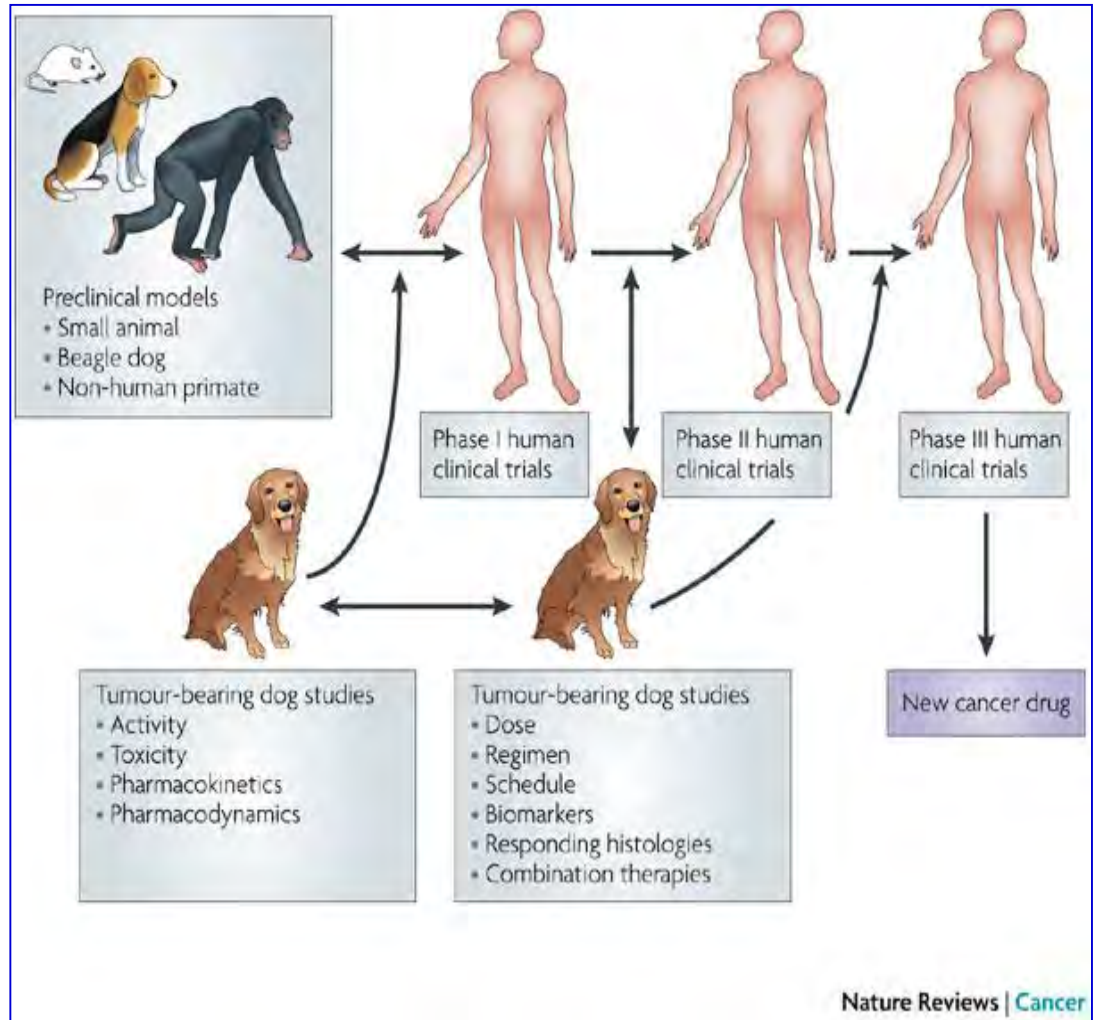
Molecular Imaging Program

Comparative Oncology Program

 Extramural research  
 Intramural research

# A Comparative and Integrated Approach to Cancer Drug Development

- ✓ Naturally-occurring, spontaneous cancers – solid tumors, hematologic cancers
- ✓ Immune-competent host
- ✓ Compressed disease progression, short survival
- ✓ Tumor/stroma heterogeneity
- ✓ Application of chemotherapy, surgery, radiotherapy; single-agent and combinations
- ✓ Resistance to therapy
- ✓ Metastasis
- ✓ No 'standard of care'





# The NCI Comparative Oncology Trials Consortium (COTC)

**Auburn University**  
Auburn, AL

**Colorado State University**  
Ft. Collins, CO

**Cornell University**  
Ithaca, NY

**Kansas State University**  
Manhattan, KS

**Iowa State University**  
Ames, Iowa

**North Carolina State University**  
Raleigh, NC

**Oregon State University**  
Corvallis, OR

**Purdue University**  
West Lafayette, IN

**Texas A&M University**  
College Station, TX

**The Ohio State University**  
Columbus, OH

**Tufts University**  
North Grafton, MA

**University of California**  
Davis, CA

**University of Florida**  
Gainesville, FL

**University of Georgia**  
Athens, GA

**University of Guelph**  
Guelph, ON Canada

**University of Illinois**  
Urbana, IL

**University of Minnesota**  
St. Paul, MN

**University of Missouri**  
Columbia, MO

**University of Pennsylvania**  
Philadelphia, PA

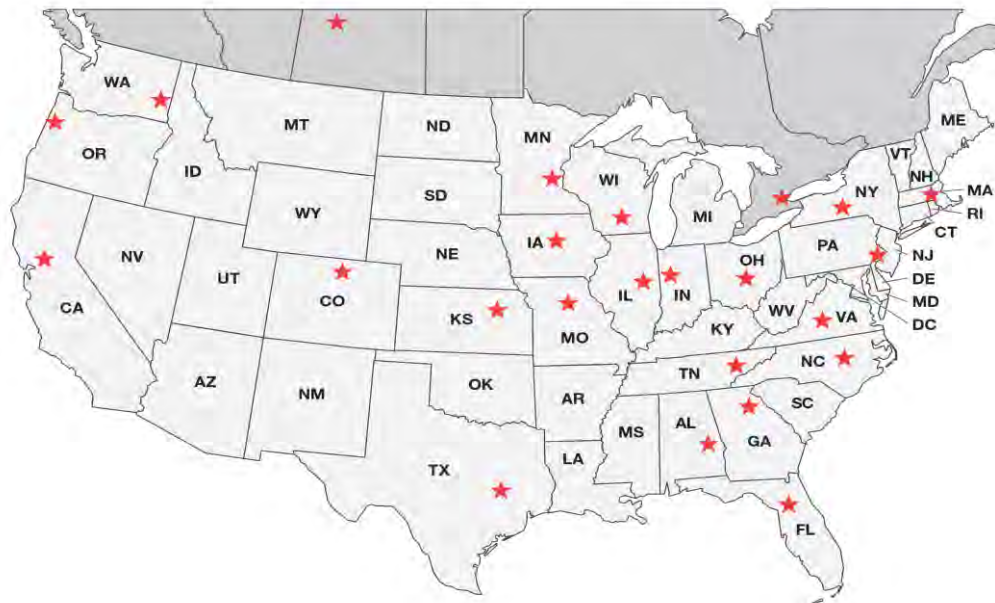
**University of Saskatchewan**  
Saskatoon, Saskatchewan

**University of Tennessee**  
Knoxville, TN

**University of Wisconsin**  
Madison, WI

**Virginia Tech**  
Blacksburg, VA

**Washington State University**  
Pullman, WA





**Reagent/Resources  
to conduct studies in  
Comparative Oncology**

**Genomics  
Proteomics  
Antibodies  
PD Core - CSU  
Contract Core  
TMAs/Cell Lines**

**Canine Comparative  
Oncology and Genomics  
Consortium**

**Advocacy for the  
Appropriate Integration of  
Comparative Oncology  
Trials**

**Academia  
Pharma  
NCI  
Regulatory Bodies**

COTC Trial	Summary of Initiated trial	No. COTC Sites	Publication Status
COTC001	Tumor specific delivery of RGD-TNF-a phage	6	PLoS One 3/2009
COTC003	PK/PD (tumor) of rapamycin in OS	4	PLoS One 6/2010
COTC005	Tolerability and biological of tumor targeted IL-2/IL-12	4	Pending
COTC006	Optimizing PD endpoints sampling by cryobiopsy technique	2	Pending
COTC007a	Trial design validation: parent Topoisomerase I inhibitor	6	Pending
COTC007b	Evaluation of three novel Indenoisoquinolines	6	Clinical Cancer Research 8/2018
COTC008	Tolerability and feasibility of long term parenteral rapamycin in OS	14	PLoS One 6/2010
 COTC010	Safety and biological activity of two immunocytokines in melanoma	6	PLoS One 6/2015
COTC013	Bioavailability of orally administered rapamycin	2	Complete/under analysis
COTC016	Feasibility of Tissue Collections and Molecular Profiling for Personalized Medicine Studies	11	PLoS One 4/2014
COTC018	Clinical evaluation of iniparib	9	PLoS One 2/2016
COTC020	PK of oral rapamycin in OS (walk-in)	5	Complete/under analysis
COTC021/022	Adjuvant rapamycin in OS when added to SOC	18	Complete/under analysis
 COTC024	Oncolytic virotherapy in canine cancer	4	Open trial
COTC026	<i>Listeria</i> -based immunotherapy in OS	11	Open trial

**One patient,  
many opportunities**

serum

whole  
blood

plasma

urine sample

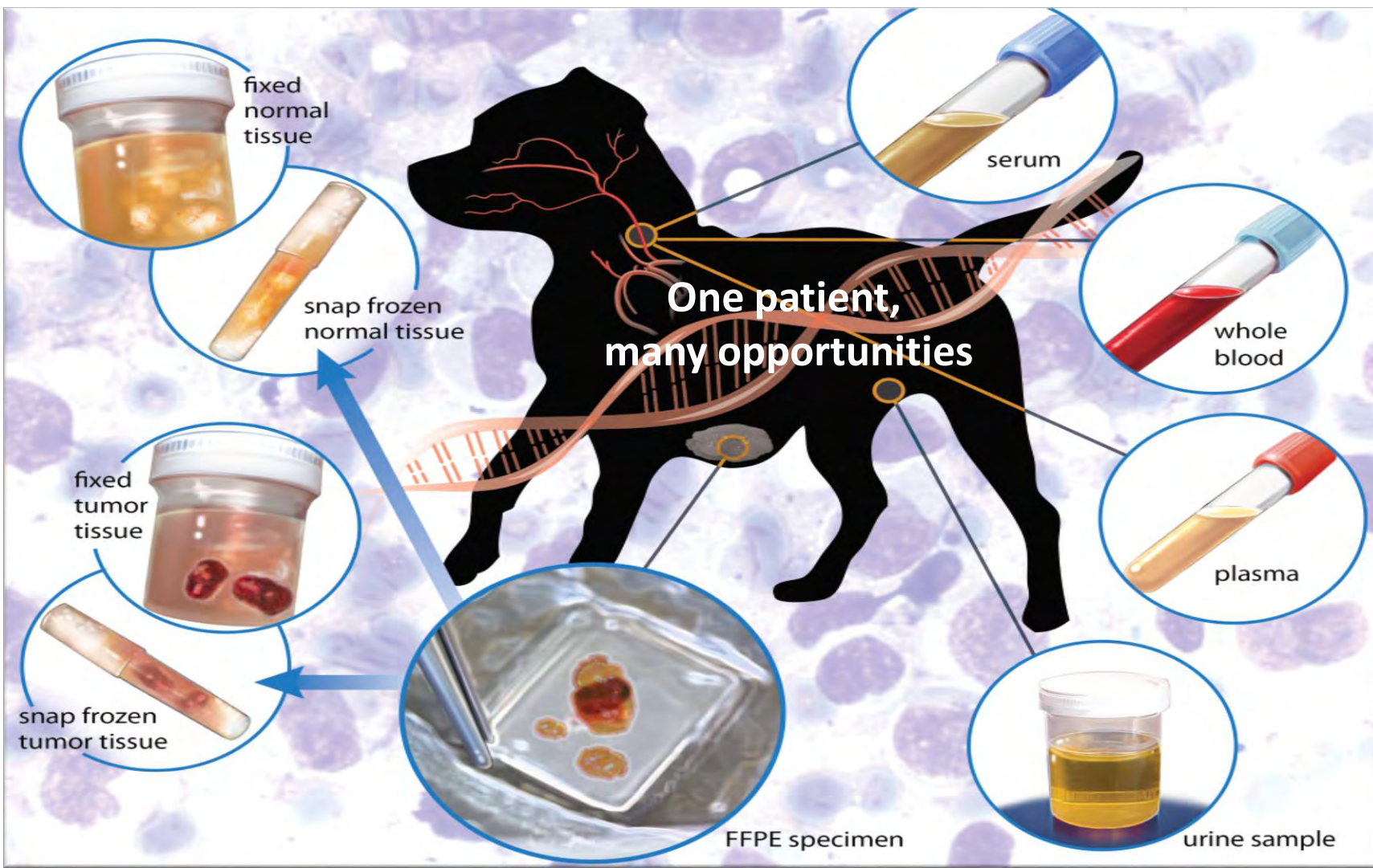
FFPE specimen

fixed  
normal  
tissue

snap frozen  
normal tissue

fixed  
tumor  
tissue

snap frozen  
tumor tissue



# Specific COTC trial examples: Advancing development of immune- oncology agents

1. *Canine melanoma and IL12-based immunocytokine therapy*
2. *Canine T-cell lymphoma and oncolytic virotherapy optimization*
3. *Canine bladder cancer and EGFR-targeted photoimmunotherapy*

# Canine melanoma and IL12-based immunocytokine therapy



# Canine and human melanoma may exhibit key differences in activating mutations, but demonstrate similar malignant potential, downstream pathway activation and biologic behavior *in vivo*



BRAF/NRAS mutations rare

? Role of c-kit

? etiology



AKT and MAPK pathway activation + loss of p16 and p53

Aggressive biologic behavior and metastasis

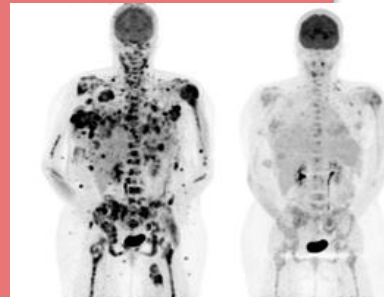
Poorly responsive to cytotoxic chemotherapy

Responsive to immunotherapies



BRAF/NRAS/c-kit mutations common but variable

UV-induced





## RESEARCH ARTICLE

# Defining the Pharmacodynamic Profile and Therapeutic Index of NHS-IL12 Immunocytokine in Dogs with Malignant Melanoma

Melissa Paoloni<sup>1</sup>, Christina Mazcko<sup>1</sup>, Kimberly Selting<sup>2</sup>, Susan Lana<sup>3</sup>, Lisa Barber<sup>4</sup>, Jeffrey Phillips<sup>5</sup>, Katherine Skorupski<sup>6</sup>, David Valli<sup>7</sup>, Heather Wilson<sup>8</sup>, Barbara Biller<sup>3</sup>, Anne Avery<sup>9</sup>, Matti Kiupel<sup>9</sup>, Amy LeBlanc<sup>1</sup>, Anna Bernhardt<sup>10</sup>, Beatrice Brunkhorst<sup>10</sup>, Robert Tighe<sup>10</sup>, Chand Khanna<sup>1\*</sup>

**1** Comparative Oncology Program, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, United States of America, **2** College of Veterinary Medicine, University of Missouri-Columbia, Columbia, Missouri, United States of America, **3** College of Veterinary Medicine and Biological Sciences, Colorado State University, Fort Collins, Colorado, United States of America, **4** School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts, United States of America, **5** College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, United States of America, **6** School of Veterinary Medicine, University of California Davis, Davis, California, United States of America, **7** School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **8** College of Veterinary Medicine, Texas A&M University, College Station, Texas, United States of America, **9** College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, United States of America, **10** EMD-Serono Research and Development Institute, Billerica, Massachusetts, United States of America

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## OPEN ACCESS

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**Academic Editor:** Nupur Gangopadhyay, University of Pittsburgh, UNITED STATES

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## Abstract

### Background

Interleukin (IL)-12 is a pro-inflammatory cytokine that mediates T-helper type 1 responses and cytotoxic T-cell activation, contributing to its utility as anti-cancer agent. Systemic administration of IL-12 often results in unacceptable toxicity; therefore, strategies to direct delivery of IL-12 to tumors are under investigation. The objective of this study was to assist the preclinical development of NHS-IL12, an immunocytokine consisting of an antibody, which targets necrotic tumor regions, linked to IL-12. Specifically this study sought to evaluate the safety, serum pharmacokinetics, anti-tumor activity, and immune modulation of NHS-IL12 in dogs with naturally occurring cancers.

### Methodology/Principal Findings

A rapid dose-escalation study of NHS-IL12 administered subcutaneously to dogs with melanoma was conducted through the Comparative Oncology Trials Consortium (COTC). Eleven dogs were enrolled in four dose-escalation cohorts; thereafter, an additional seven dogs were treated at the defined tolerable dose of 0.8 mg/m<sup>2</sup>. The expanded cohort at this fixed dose (ten dogs in total) was accrued for further pharmacokinetics and pharmacodynamics assessment. NHS-IL12 levels, serum cytokine concentrations, and peripheral blood



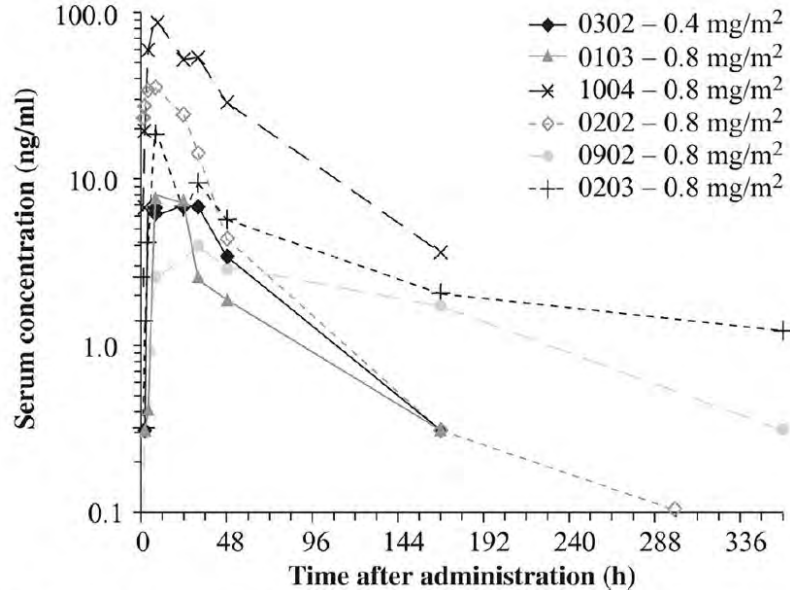
**Table 1. Study schedule for dose escalation and cohort expansion of NHS-IL12 administered subcutaneously.**

Action	Pre-Tx	Day 1	Day 2	Day 3	Day 8	Day 15	Day 22	Day 29	q. Day 8	q. 28 days
Patient eligibility	X									
Tumor measurements	X				X	X	X	X	X	X
Tumor biopsy	X				X			X		X
Draining lymph node fine-needle aspirates	X				X			X		X
Serum (IFN- $\gamma$ , cytokines, drug levels, immunogenicity)		X	X	X	X	X		X	X	X
PBMC immune cell characterization		X	X	X	X			X		X
CBC/chemistry and coagulation profiles/UA		X			X	X		X	X	X
Abdominal ultrasound/thoracic radiographs	X							X		X
NHS-IL12 subcutaneous		X						X		X
Weight measurement	X		X		X	X	X	X	X	X
Fever monitoring		X	X				X	X		
Digital photo	X				X			X	X	X

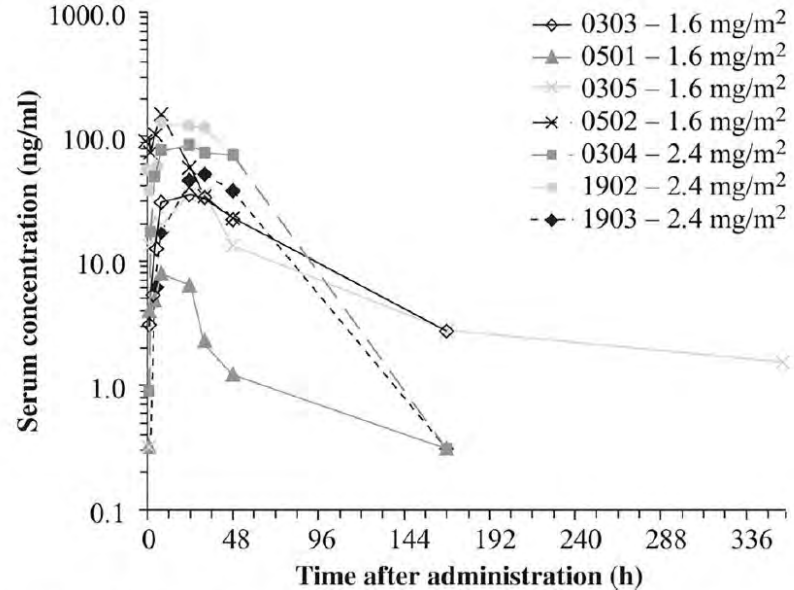
CBC: complete blood count; IFN, interferon; PBMC, peripheral blood mononuclear cell; Tx: treatment; UA: urinalysis.

# Dogs receiving NHS-IL12 subcutaneously achieve measureable drug exposures across multiple dosing cohorts

A



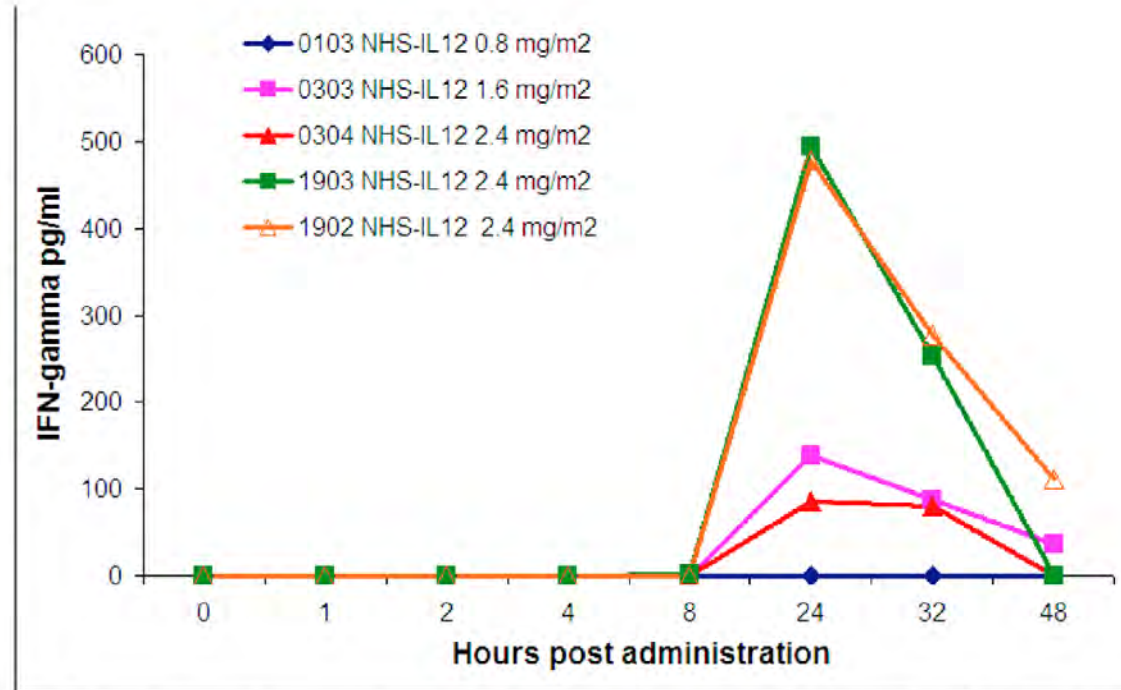
B



**Fig 1. Subcutaneous administration of NHS-IL12 resulted in measurable serum drug levels.** Serum samples were collected from dogs to define systemic exposures of NHS-IL12 after subcutaneous administration. NHS-IL12 levels were measured pre-treatment and at 1, 2, 4, 8, 24, 36, and 48 hours following administration (8-point collection) and on days 8, 15, and 29. NHS-IL12  $C_{max}$  was dose-dependent: 0.4 mg/m<sup>2</sup> and 0.8 mg/m<sup>2</sup> (A) and 1.6 mg/m<sup>2</sup> and 2.4 mg/m<sup>2</sup> (B). Clearance was prolonged in some dogs as NHS-IL12 was still measurable in five animals 14 days following treatment.

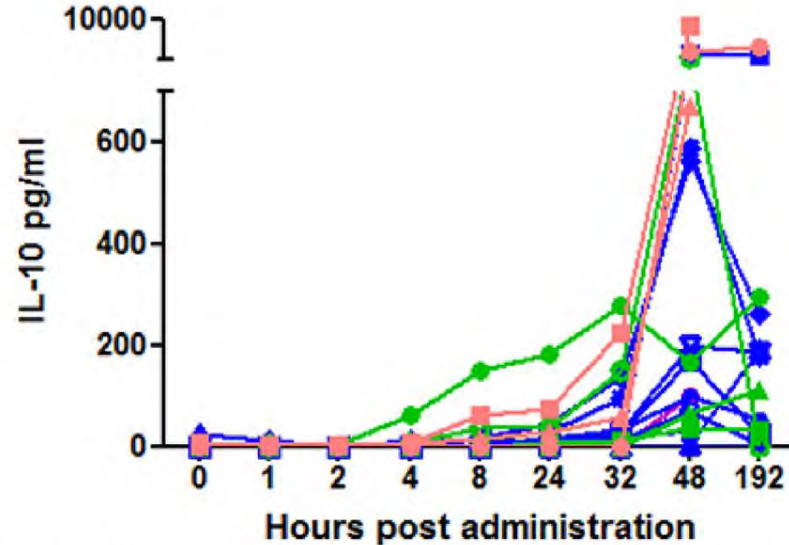


## Dogs receiving NHS-IL12 subcutaneously demonstrate pharmacodynamic response via post-treatment IFN- $\gamma$ and IL-10 release



**Fig 3. Serum IFN- $\gamma$  induction was associated with NHS-IL12 dose and the observation of adverse events.** IFN- $\gamma$  levels were measured using ELISA techniques. Induction of IFN- $\gamma$  was detectable in dogs treated with NHS-IL12 at a dose of 1.6 mg/m<sup>2</sup> or higher. IFN- $\gamma$  levels spiked sharply at 24 hours post-treatment and returned to undetectable levels by 48 hours. Elevated serum IFN- $\gamma$  (> 100 pg/ml) was associated with increased risk for toxicity. However the highest level of IFN- $\gamma$  induction was not directly linked to the most severe (Grade 4 or 5) adverse events.

# Dogs receiving NHS-IL12 subcutaneously demonstrate pharmacodynamic response via post-treatment IFN-g and IL-10 release



**Fig 2. Serum IL-10 levels increased following treatment with NHS-IL12.** Each line represents a different dog, with individual colors representing different treatment groups. Serum IL-10 levels at 48 and 192 hours were significantly different from time points 0–8 hours (Kruskal-Wallis test followed by Dunn's multiple comparison test) when data from all dogs was pooled. There was no difference in IL-10 levels at 48 hours between any of the treatment groups (Kruskal-Wallis test,  $p = .06$ , the 0.4 mg/m<sup>2</sup> group was not included because it was a single dog).

# COTC010: Deliverables

- NHS-IL12, a fully-human necrosis-targeted IgG1 antibody/IL-12 conjugate, can be safely and repeatedly administered to tumor-bearing dogs
  - PD biomarkers: Induction of IFN $\gamma$  and IL-10, CD8+ TILs all linked to exposure at the MTD
- An efficacy signal was identified in 2/7 canine oral malignant melanoma patients
- Adverse events relatable to cytokine release
  - Self-limiting lymphopenia, fever, hepatic enzymopathy
  - > 1.6 mg/m<sup>2</sup> associated with Grade 4 or 5 events (vascular leakage syndrome, thrombocytopenia, DIC)
- Cohort expansion at MTD (25.8 ug/kg or 0.8 mg/m<sup>2</sup> at Dose Level 2) allowed confirmation of tolerability, PK/PD, efficacy signal



# COTC010: Deliverables

- Re-prioritization of this agent occurred after receipt of canine data in 2010
- IND opened in 2011 for human Phase I work at NIH-CC -- recently published in *Clinical Cancer Research*
  - N = 59 patients; MTD defined as 16.8 ug/kg (Dose level 8)
  - Adverse event profile similar to dogs: fever, leukopenia, hepatic enzymopathy
  - PD data similar to dogs: IFN $\gamma$  and IL-10 induction; added findings from PBMC subsets, TCR sequencing
- Canine data comparable to human data: same agent, similar AE profile, similar MTD
- Generated in 18 months for less than \$300,000

**Date:** August 7, 2018

**For:** *Clin Cancer Res*

**First-In-Human Phase I Trial of a Tumor-Targeted Cytokine (NHS-IL12) in Subjects with Metastatic Solid Tumors**

Julius Strauss<sup>1\*</sup>, Christopher R. Heery<sup>2\*</sup>, Joseph W. Kim<sup>3</sup>, Caroline Jochems<sup>1</sup>, Renee N. Donahue<sup>1</sup>, Agnes S. Montgomery<sup>4</sup>, Sheri McMahon<sup>5</sup>, Elizabeth Lamping<sup>5</sup>, Jennifer L. Marté<sup>6</sup>, Ravi A. Madan<sup>6</sup>, Marijo Bilusic<sup>6</sup>, Matthew R. Silver<sup>7</sup>, Elisa Bertotti<sup>7</sup>, Jeffrey Schlom<sup>1</sup>, James L. Gulley<sup>6</sup>

<sup>1</sup>Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. <sup>2</sup>Bavarian Nordic, Morrisville, North Carolina. <sup>3</sup>Yale University, New Haven, Connecticut. <sup>4</sup>Uniformed Services University of the Health Sciences, Bethesda, Maryland. <sup>5</sup>Office of Research Nursing, National Cancer Institute, National Institutes of Health. <sup>6</sup>Genitourinary Malignancies Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. <sup>7</sup>EMD Serono, Darmstadt, Germany.

\* Authors contributed equally

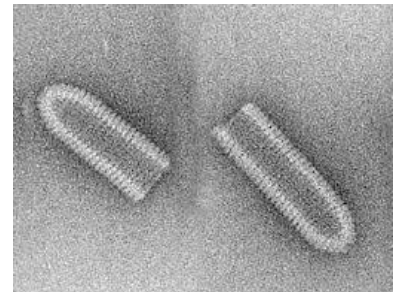
**Corresponding Author:** James L. Gulley, MD, PhD, 10 Center Drive, Room 13N240C, Bethesda, MD 20892, USA (e-mail: [gulleyj@mail.nih.gov](mailto:gulleyj@mail.nih.gov); tel: 301-480-7164)

# Canine T-cell lymphoma and oncolytic virotherapy optimization

# Oncolytic virotherapy: VSV-IFN $\beta$ -NIS\*

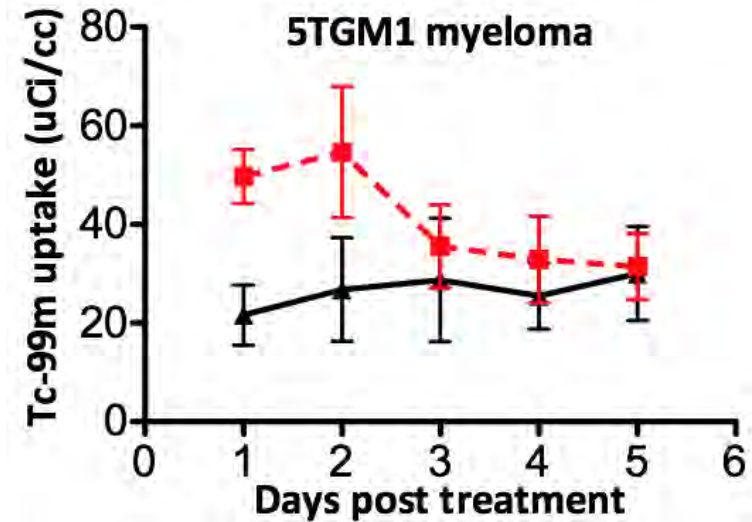
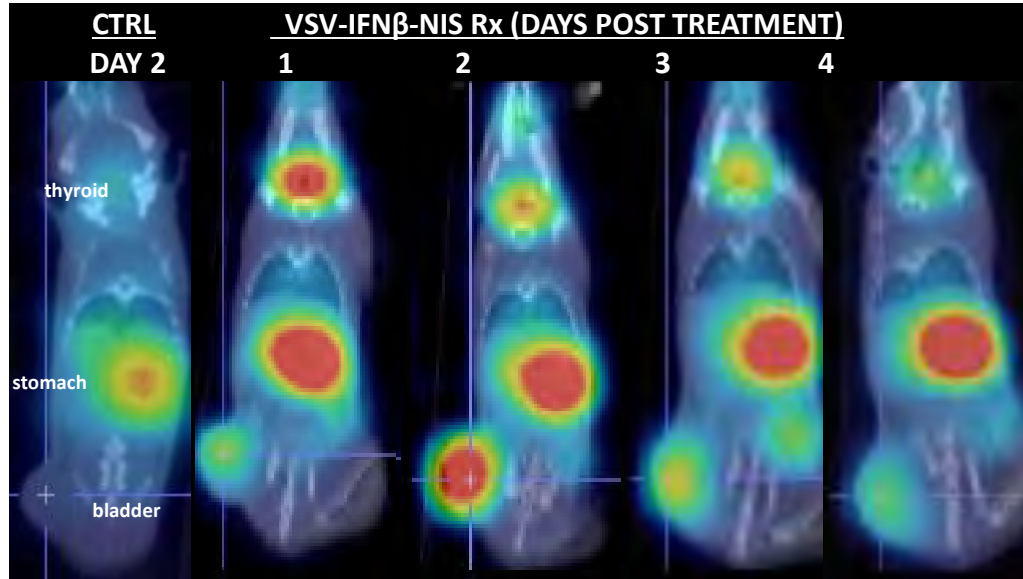
Replication-competent, lab-attenuated strain of VSV-Indiana

- IFN $\beta$  : species-specific constructs
  - Protective of normal tissues
  - Direct anti-tumor effect
  - Cross-priming of T cells – ‘vaccine’ effect
- NIS (sodium-iodide symporter)
  - Allows imaging of expressed protein with nuclear medicine techniques ( $^{99m}\text{Tc}$ ,  $^{123}\text{I}$ ,  $^{18}\text{F}$ -tetrafluoroborate)



Currently under study in human patients with solid tumors, myeloma, lymphoma, leukemia (IV and IT dosing)

# Preclinical development : VSV-hIFN $\beta$ -NIS



Naik S, et al. *Cancer Gene Therapy* 2012; 19(7): 443-450.

## Safety Studies on Intravenous Administration of Oncolytic Recombinant Vesicular Stomatitis Virus in Purpose-Bred Beagle Dogs

Amy K. LeBlanc,<sup>1,2,\*</sup> Shruthi Naik,<sup>3,\*</sup> Gina D. Galyon,<sup>1</sup> Nathan Jenks,<sup>4</sup> Mike Steele,<sup>4</sup> Kah-Whye Peng,<sup>3,4</sup> Mark J. Federspiel,<sup>3,5</sup> Robert Donnell,<sup>6</sup> and Stephen J. Russell<sup>3</sup>

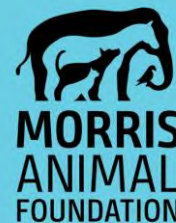
- ✓ Rapid single-dose escalation study in Beagle dogs
- ✓ Systemic administration of VSV-hIFNb-NIH at  $10^8$  to  $10^{11}$  TCID<sub>50</sub> IV
- ✓ Characterization of toxicity, immune response, shedding
- ✓ Enabled a pet dog clinical trial

### Models and Technologies

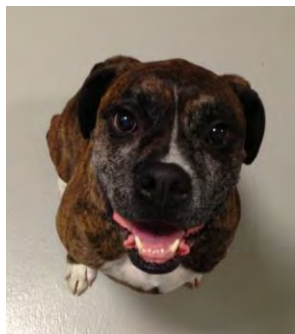
## Comparative Oncology Evaluation of Intravenous Recombinant Oncolytic Vesicular Stomatitis Virus Therapy in Spontaneous Canine Cancer

Shruthi Naik<sup>1,2</sup>, Gina D. Galyon<sup>3</sup>, Nathan J. Jenks<sup>4</sup>, Michael B. Steele<sup>4</sup>, Amber C. Miller<sup>1</sup>, Sara D. Allstadt<sup>3</sup>, Lukkana Suksanpaisan<sup>5</sup>, Kah Whye Peng<sup>4</sup>, Mark J. Federspiel<sup>6</sup>, Stephen J. Russell<sup>1,2</sup>, and Amy K. LeBlanc<sup>3</sup>

**Molecular  
Cancer  
Therapeutics**



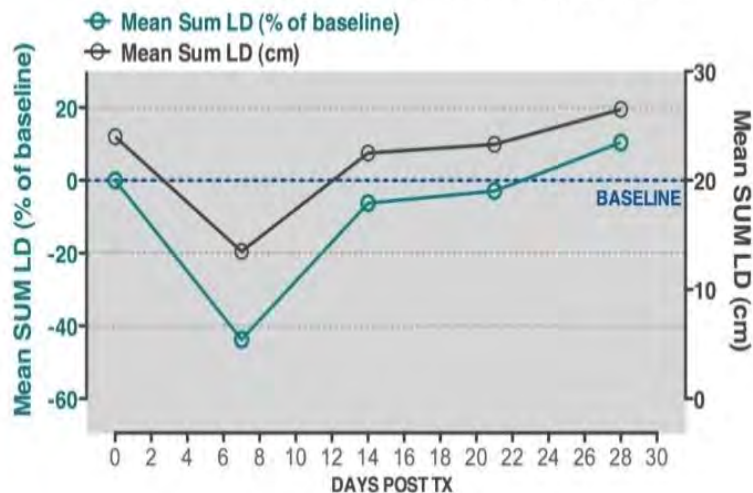




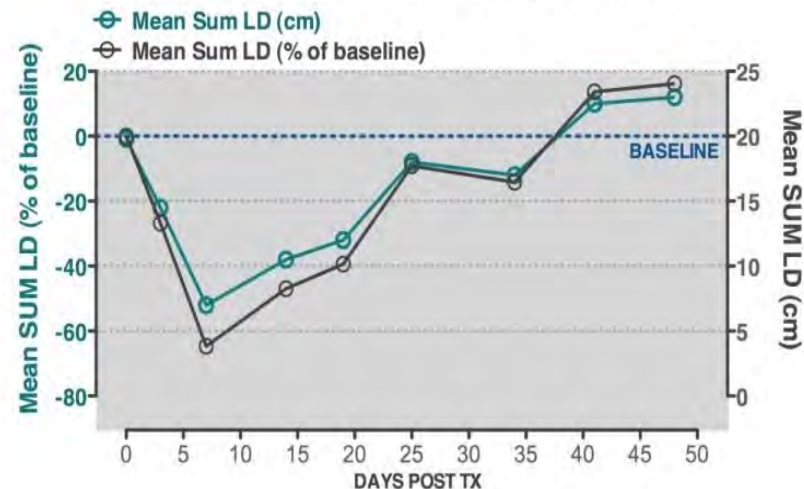
High-grade peripheral T cell LSA  
Aggressive clinical course  
MHC Class II – low expression  
CD4+/CD45+



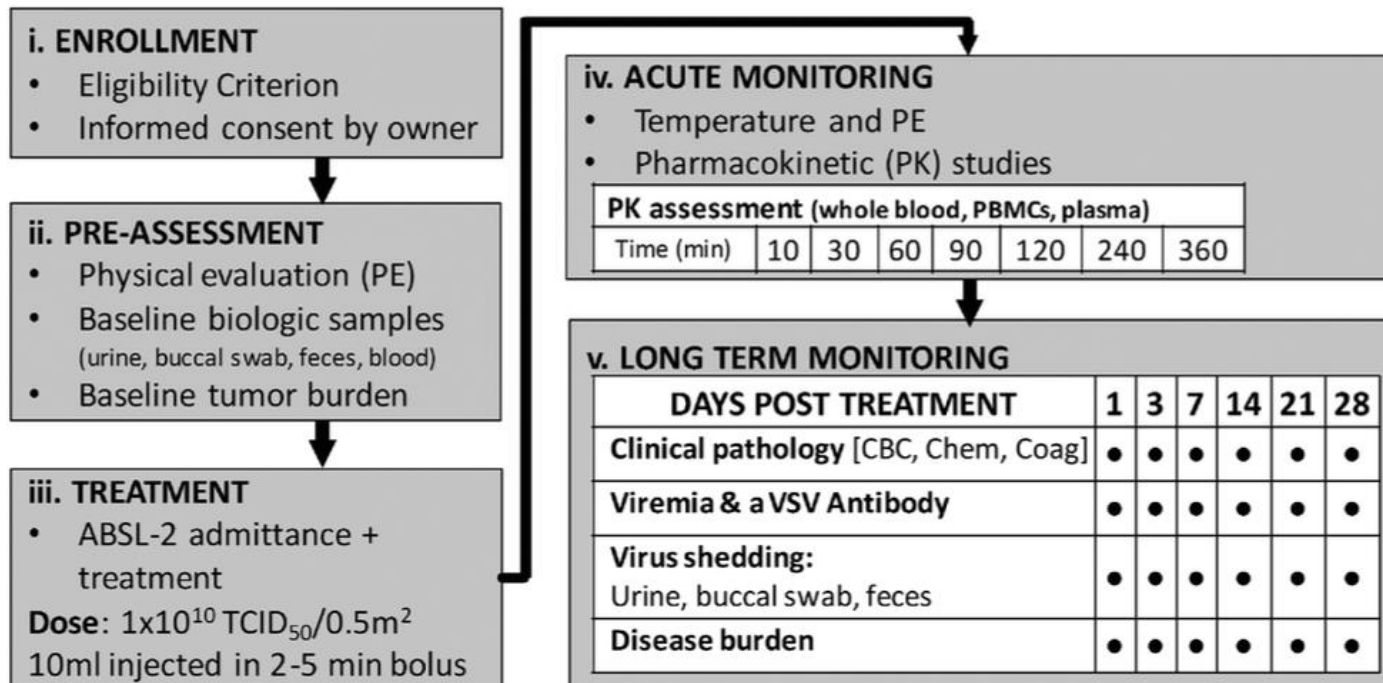
Peripheral Lymphoma disease burden (VCOG)  
Beasley: T-cell lymphoma, single IV dose VSV-hIFN-NIS



Peripheral Lymphoma disease burden (VCOG)  
Roxie: T-cell lymphoma, single IV dose VSV-hIFN-NIS



# Schema for COTC-024: Dose-escalation study of systemically-administered VSV-hINFb-NIS in dogs with cancer



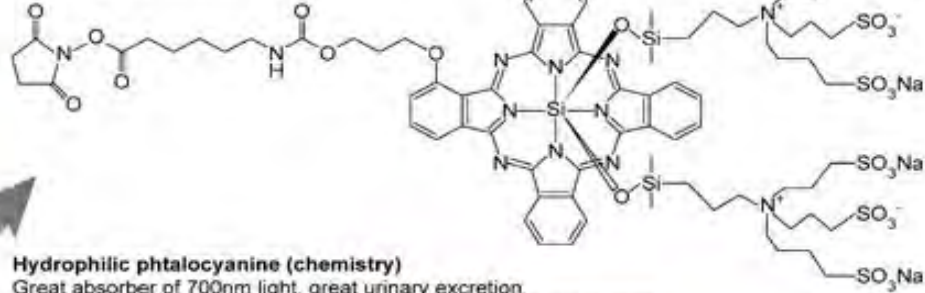
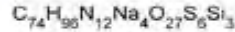
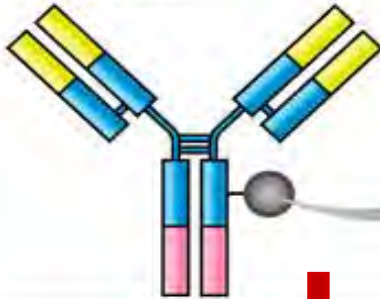
# COTC024: Deliverables

- VSV-IFNb-NIS can be safely administered systemically and intratumorally to dogs with cancer
  - Exploration of human and canine-specific IFNb constructs
  - NIS facilitates  $^{99m}\text{Tc}$ -based molecular imaging of viral trafficking *in vivo*
- Adverse events are relatable to hepatic DLT (reversible enzymopathy) and cytokine release – fully evaluable and attributable in pet dogs
- Efficacy signal in T cell lymphoma warrants further study
  - Comparative canine study linked to open IND for same agent
  - Can explore PK-PD relationships in multiple tumor types and various dosing strategies simultaneously in dogs

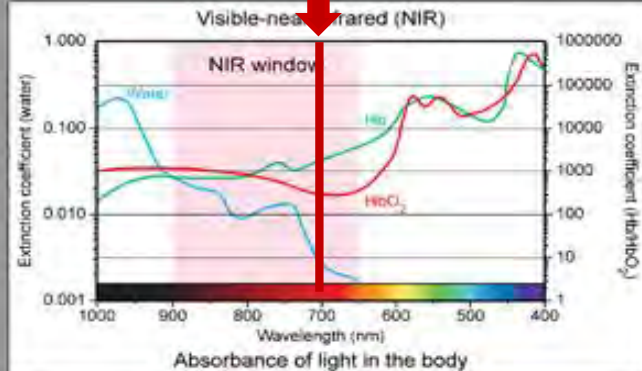
# Canine invasive urothelial carcinoma (iUCa) and EGFR-targeted photoimmunotherapy

# Near infrared photo-immunotherapy (NIR-PIT)

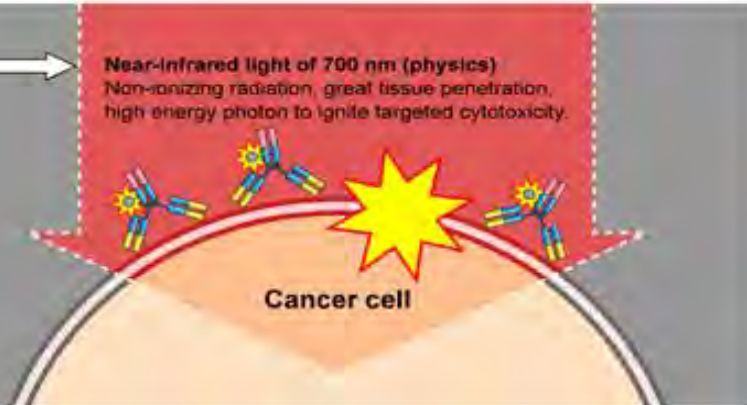
**Humanized monoclonal antibody (biology/medicine)**  
Highest binding specificity, greatest *in vivo* target delivery, applicable to the clinical practice.



**Hydrophilic phthalocyanine (chemistry)**  
Great absorber of 700nm light, great urinary excretion.  
Works as a "nano-dynamite" to damage only binding cell membrane.



**Near-Infrared light of 700 nm (physics)**  
Non-ionizing radiation, great tissue penetration,  
high energy photon to ignite targeted cytotoxicity.



# Phase 1: Multicenter results

No severe adverse side effect

9 patients (7 males, 2 females), aged 52–86 years, enrolled in to RM-1929/101 Part I study.

	Patient	Tumor site	Previous Treatment	Tumor size, CT (cm)	HPV/p16 Status	RECIST Survival	
160 mg/m <sup>2</sup>	03-101	Oropharynx	Surgery, radiation×2, cisplatinium	2.6 × 1.2	+/+	PR	1.5
	03-102	Posterior oro-and hypopharynx	Surgery, radiation	3.0 × 7.0 clinical	-/-	CR	>20m
	03-103	Right anterior tongue	Surgery, radiation ×2, carboplatin, 5-FU, cetuximab	2.8 × 0.7 × 1.3	-/-	PR	6
320 mg/m <sup>2</sup>	03-201	Right neck	Surgery, radiation, taxol, carboplatin, cetuximab, nivolumab	8.0 × 6.0 × 4.0	-/-	SD	2
	03-201	Right submandibular, submental	Surgery, radiation, cisplatinium, paclitaxel	6.0 × 4.5 submandibular, 2.3 × 1.7 submental	-/-	CR	>15m
	02-212	Left tongue base	Surgery, radiation, cisplatinium, docetaxel, cetuximab	2.0 × 1.1 × 0.9	-/-	PR	5.5
640 mg/m <sup>2</sup>	02-311	Occipital mass	Surgery, radiation, PD-1 inhibitor, cetuximab, PI3K inhibitor	2.7 × 3.3	+ /NA	PR	2.5
	05-341	Pharynx and buccal mass	Surgery, radiation, 5-FU, cisplatinium, docitaxel	6.0 × 4.0 left cheek 4.0 × 4.0 left oropharynx 5.0 × 3.0 left nasopharynx	NA/NA	----	1
	03-301	Dermal metastases, neck nodes	Surgery, radiation, cisplatinium, cetuximab, nivolumab	4.0 × 1.0, 2.0 × 1.0 right neck metastases; 2.0 × 1.0 right neck midline metastasis	-/+	CR	>16m

Abbreviations: CT = computed tomography; HPV = human papilloma virus; NA = not applicable; PD-1 = programmed cell death protein 1; PI3K = phosphoinositide 3 kinase; p16 = p16 protein; 5-FU = 5-fluorouracil



## Review

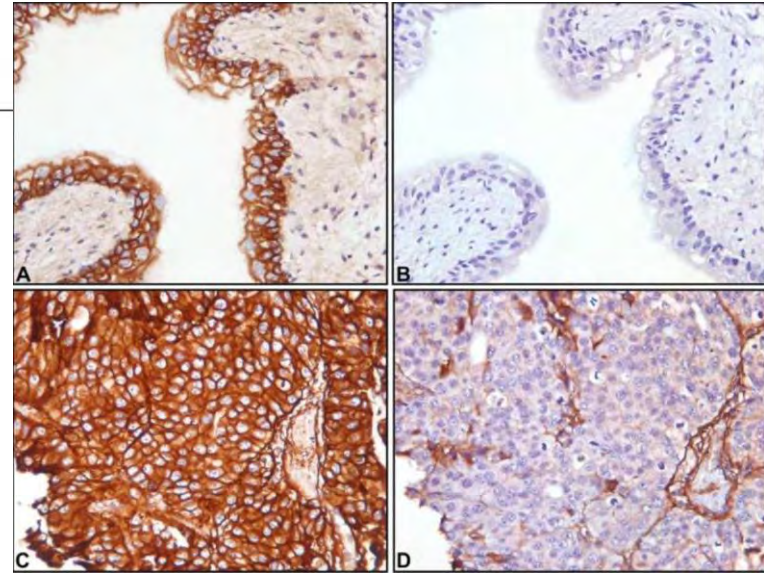
# Naturally-Occurring Canine Invasive Urothelial Carcinoma: A Model for Emerging Therapies

Breann C. Sommer<sup>a</sup>, Deepika Dhawan<sup>a</sup>, Timothy L. Ratliff<sup>b,c</sup> and Deborah W. Knapp<sup>a,c,\*</sup>

<sup>a</sup>Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA

<sup>b</sup>Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA

<sup>c</sup>Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN, USA



## Similarities between naturally-occurring canine invasive urothelial carcinoma and human invasive urothelial carcinoma

### Similarities in muscle-invasive bladder cancer between dogs and humans

Physiological age of onset

Clinical signs/symptoms

Cellular and pathological features including high grade, tumor heterogeneity, and local invasion

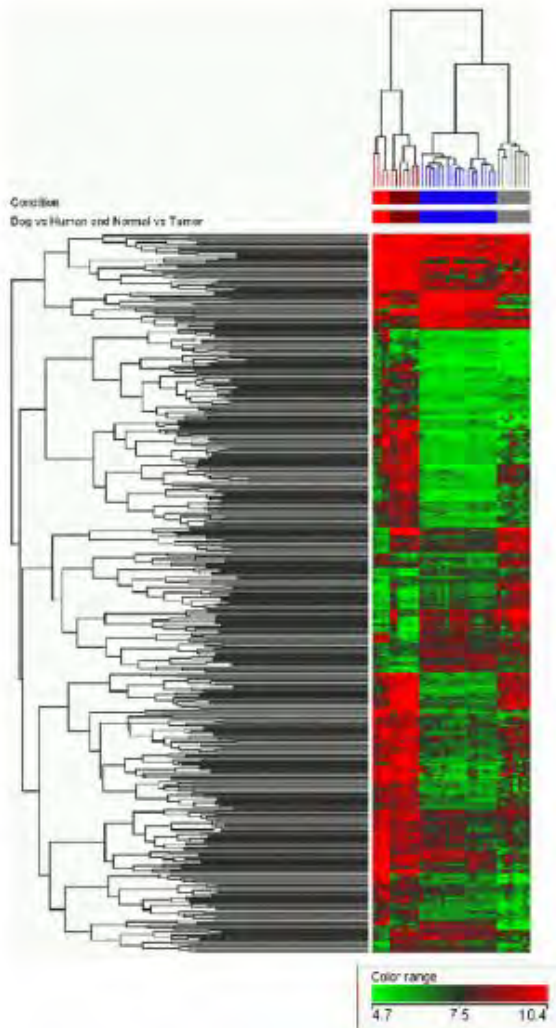
Molecular subtypes (e.g. luminal, basal)

Biological behavior (sites and frequency of metastasis)

Response to chemotherapy (e.g. cisplatin, carboplatin, vinblastine)

Shared molecular targets (e.g. *EGFR*, *CDKN2B*, *PIK3CA*, *BRCA2*, *NFκB*, *ARHGEF4*, *XPA*, *RB1CC1*, *RPS6*, *MITE* and *WT1*)

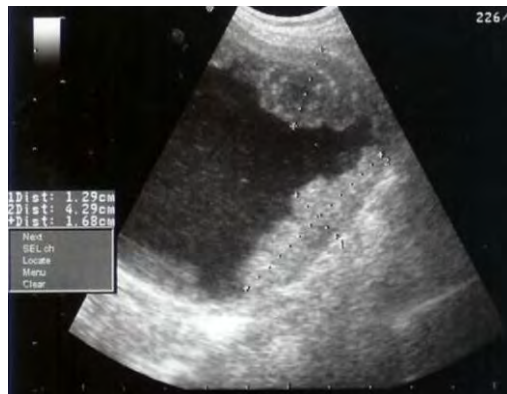
# Similar gene expression patterns between dog and human in invasive bladder cancer and dog genes cluster into luminal and basal subgroups—as in human disease



**Fig 2. Canine and human iUC samples cluster together.** A list of genes that are commonly annotated and significantly expressed (between normal and iUC,  $p < 0.05$ , FC2) in dogs and humans, was generated. Hierarchical clustering was performed on these genes ( $n = 436$ ) using Euclidean distance matrix. Figure illustrates that canine and human normal controls cluster together and these cluster separately from canine and human iUC samples. The iUC samples from dogs and humans clustered together. The color codes are: (1) red bar denoting canine normal bladder, (2) brown bar denoting normal human bladder, (3) blue bar denoting canine iUC samples, and (4) grey bar denoting human iUC samples.

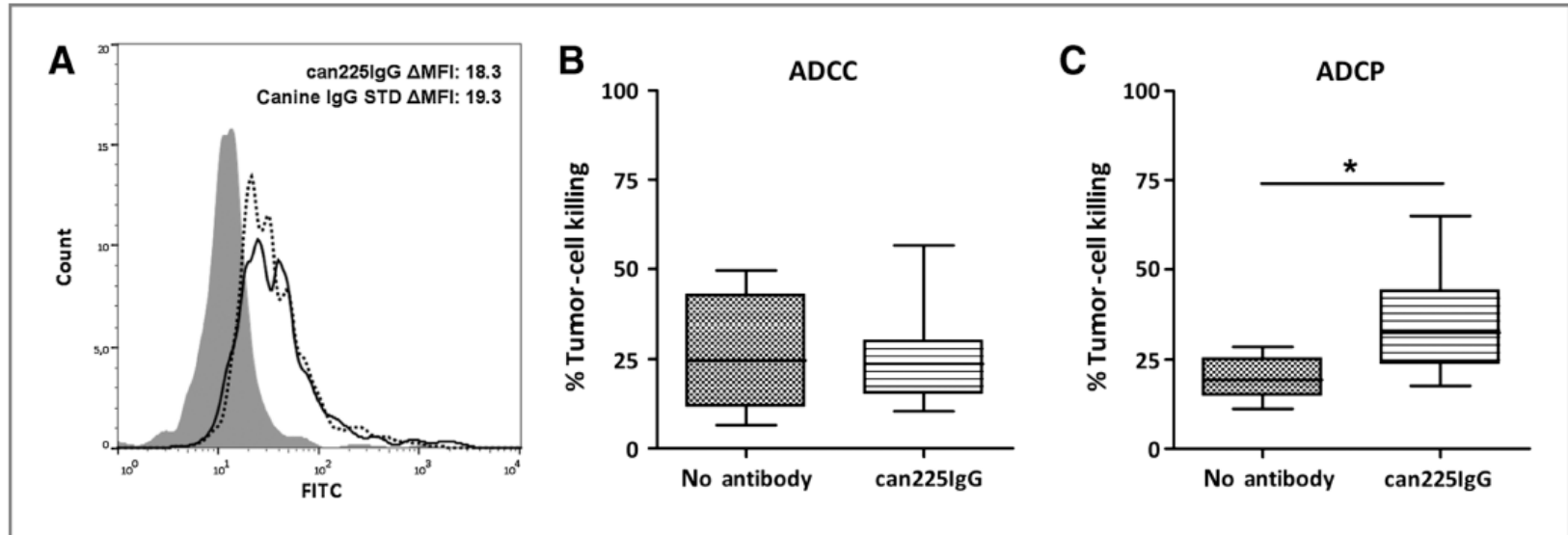
doi:10.1371/journal.pone.0136688.g002

# Dogs with naturally-occurring iUCa are ideal participants in clinical trials designed to advance new therapeutic concepts



- ✓ Known breed disposition (Scottie, Sheltie, Beagle, other small Terriers)
- ✓ Muscle-invasive at diagnosis
- ✓ Herbicide exposure increases risk
- ✓ BRAF V595E mutation present in ~ 85% of cases
- ✓ Current medical and surgical therapies largely unrewarding and toxic
  - Median PFI ~ 100-200 days
  - Local progression + metastasis to distant sites (lymph node, lung, liver, bone)

# A canine anti-EGFR antibody (can225) can reduce viability and proliferation of EGFR-overexpressing cell lines as well as induce antibody-dependent phagocytosis



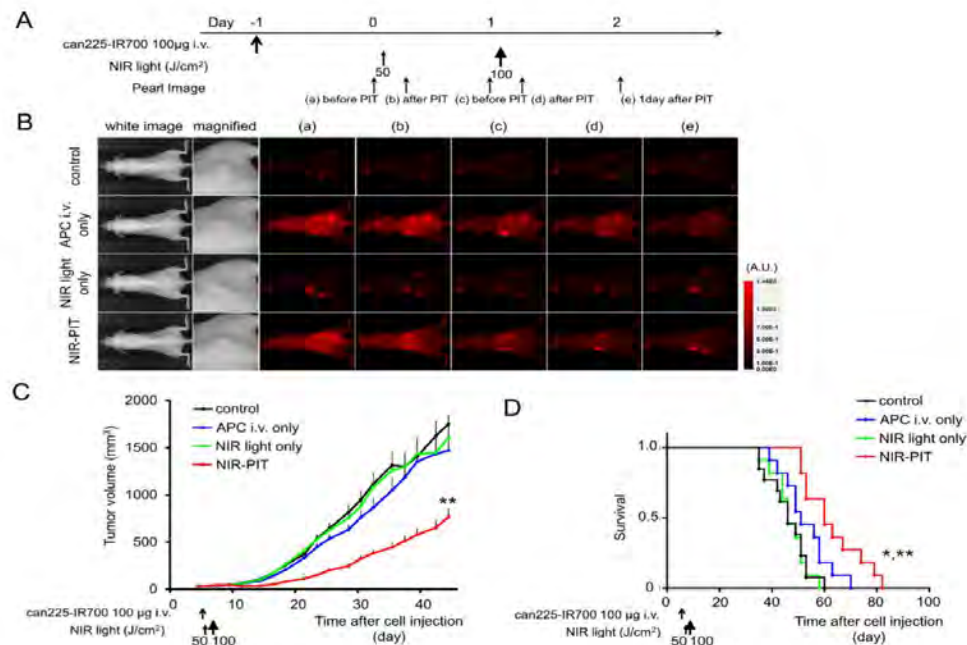


# Near infrared photoimmunotherapy targeting bladder cancer with a canine anti-epidermal growth factor receptor (EGFR) antibody

Tadanobu Nagaya<sup>1</sup>, Shuhei Okuyama<sup>1</sup>, Fusa Ogata<sup>1</sup>, Yasuhiro Maruoka<sup>1</sup>, Deborah W. Knapp<sup>2</sup>, Sophia N. Karagiannis<sup>3,4</sup>, Judit Fazekas-Singer<sup>5,6</sup>, Peter L. Choyke<sup>1</sup>, Amy K. LeBlanc<sup>7</sup>, Erika Jensen-Jarolim<sup>5,6</sup> and Hisataka Kobayashi<sup>1</sup>

Next steps:

- Translate canine EGFR-PIT to the canine bladder cancer patient in support of human translation past EGFR+ head/neck cancers
- Ultimate goal: combine with checkpoint blockade to enhance anti-tumor effect



## COTC029: Deliverables

- **Main goal: assess safety of single-dose EGFR-PIT in dogs with iUCa**
  - Proof of concept n = 8 to establish single dose safety, tolerability, efficacy
    - Serial cystoscopy, biopsy/histopathology of tumor and normal bladder tissue
  - Consider expanded cohort to explore multiple dosing options at MTD
    - Fully canine Ab-dye conjugate allows repeated dosing



# Recent efforts in canine immuno-oncology: Checkpoint molecules and correlative assays

# What do we know about canine immune checkpoints and checkpoint inhibitors?

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- Canine PD-1 and PD-L1 genes are conserved 100% among dog breeds.
- Recombinant canine PD-1 and PD-L1 proteins have been constructed and shown to bind to one another; anti-PD1 antibody blocks the binding of soluble PD-1 with canine PD-L1 expressing cells in a dose-dependent manner.
- Fresh canine tumor biopsy explant cultures mixed with activated canine PBMCs + anti-PD1 showed an increase in IFN-gamma production in the presence of anti-PD1.
- Most canine tumors express PD-L1 and increased expression is associated with the density of T cell infiltration. Immune stimuli (e.g., IFN-gamma) can further upregulate PD-L1 expression.
- Anti-PD-L1 treatment enhances IFN-gamma production from cultured tumor-infiltrating lymphocytes (TILs) from clinical specimens.
- CD8+ TIL cells from canine lymphomas have a higher PD-1 expression than CD8+ cells from normal canine lymph nodes.
- A clinical trial studying the effect of anti-PD1 in dogs with cancer has begun (sponsored by Merck Animal Health).

## RESEARCH ARTICLE

# Immunohistochemical Analysis of PD-L1 Expression in Canine Malignant Cancers and PD-1 Expression on Lymphocytes in Canine Oral Melanoma

**Naoya Maekawa<sup>1</sup>, Satoru Konnai<sup>1</sup>, Tomohiro Okagawa<sup>1</sup>, Asami Nishimori<sup>1</sup>, Ryoyo Ikebuchi<sup>1</sup>, Yusuke Izumi<sup>2</sup>, Satoshi Takagi<sup>2</sup>, Yumiko Kagawa<sup>3,4</sup>, Chie Nakajima<sup>5</sup>, Yasuhiko Suzuki<sup>5</sup>, Yukinari Kato<sup>6</sup>, Shiro Murata<sup>1</sup>, Kazuhiko Ohashi<sup>1\*</sup>**

**1** Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **2** Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **3** North Lab, Sapporo, Japan, **4** Department of Diagnostic Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **5** Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan, **6** Department of Regional Innovation, Graduate School of Medicine, Tohoku University, Sendai, Japan



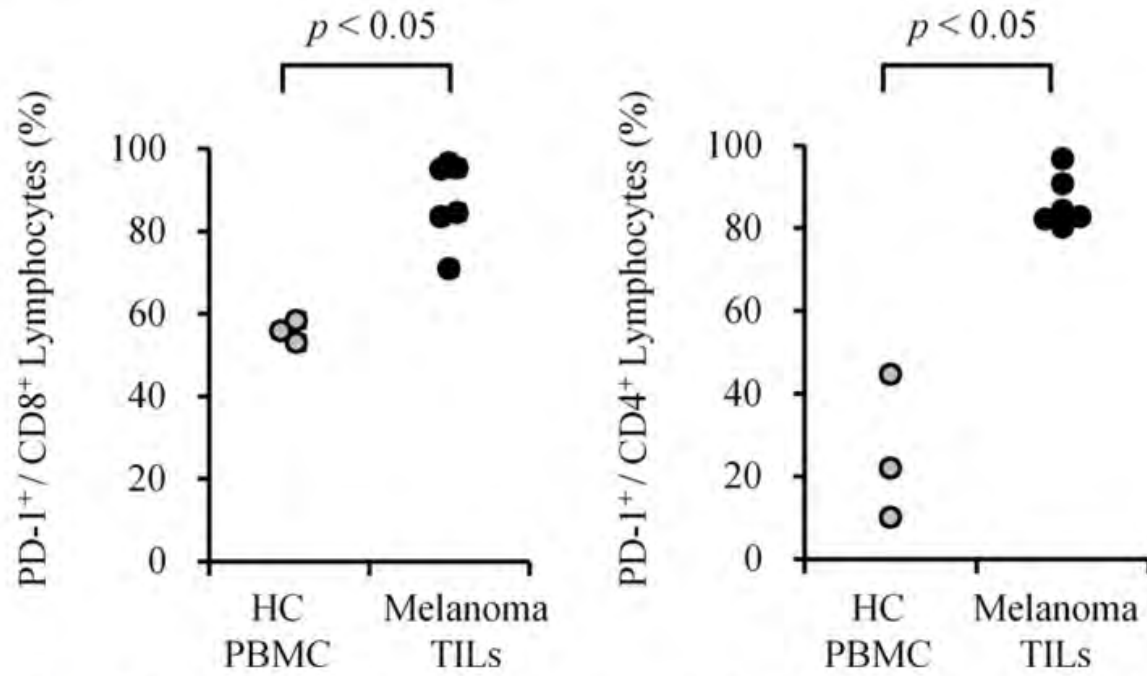
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Pathology	Positive cases/Tested samples
Melanoma (oral)	36/40
Osteosarcoma	7/10
Hemangiosarcoma	6/10
Mast cell tumor (grade III)*	3/5
Mammary adenocarcinoma**	4/5
Prostate adenocarcinoma	3/5
Squamous cell carcinoma (skin)	0/5
Diffuse large B-cell lymphoma	0/5
Nasal adenocarcinoma	0/5
Soft tissue sarcoma	0/5
Histiocytic sarcoma	0/5
Transitional cell carcinoma	0/5
Anal sac gland carcinoma	0/5

The results of immunohistochemical analysis were summarized.

\*Grading of mast cell tumor was performed in accordance with the Patnaik grading method [19].

\*\*No inflammatory mammary carcinoma was included in this study.



**Fig 4. PD-1 expression on tumor-infiltrating lymphocytes (TILs) obtained from oral melanoma.** TILs were collected from surgically excised oral melanoma tissues and the expression level of PD-1 was evaluated by flow cytometry. Left panel, PD-1 expression on CD8<sup>+</sup> lymphocytes. Right panel, PD-1 expression on CD4<sup>+</sup> lymphocytes. Peripheral blood mononuclear cells (PBMC) obtained from healthy dogs were used as control (healthy control, HC).  $p < 0.05$  was considered statistically significant (Mann–Whitney U test).

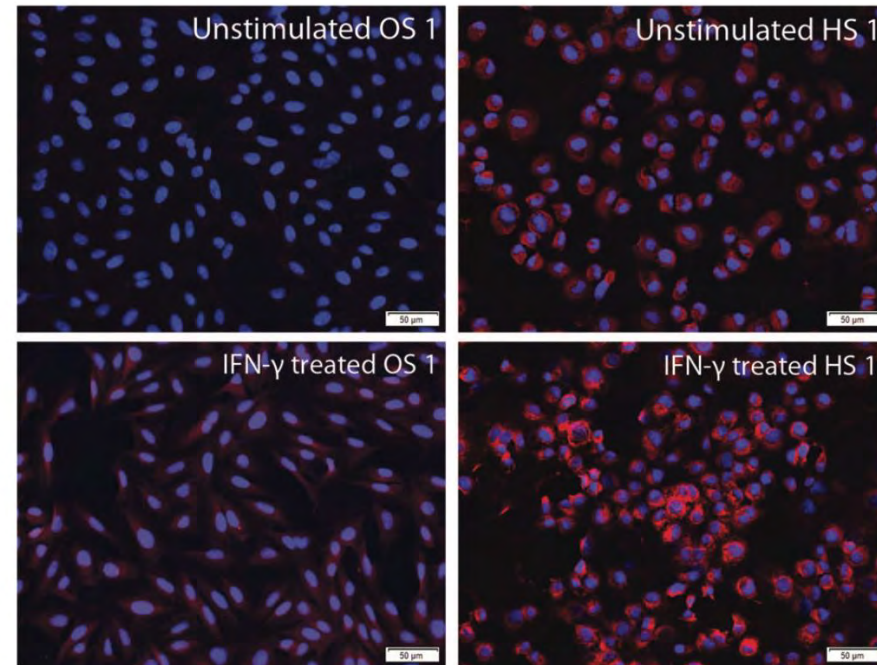


# Immune regulation of canine tumour and macrophage PD-L1 expression

G. Hartley, E. Faulhaber, A. Caldwell, J. Coy, J. Kurihara, A. Guth, D. Regan and S. Dow

Department of Clinical Sciences, Flint Animal Cancer Center, Colorado State University, Ft. Collins, CO

- Constitutive PDL-1 expression on all n = 14 canine cancer cell lines
- Significant upregulation after IFN $\gamma$  and TLR3 stimulation
- PDL-1 expression can be induced by IFN $\gamma$  exposure in canine monocytes/macrophage cultures



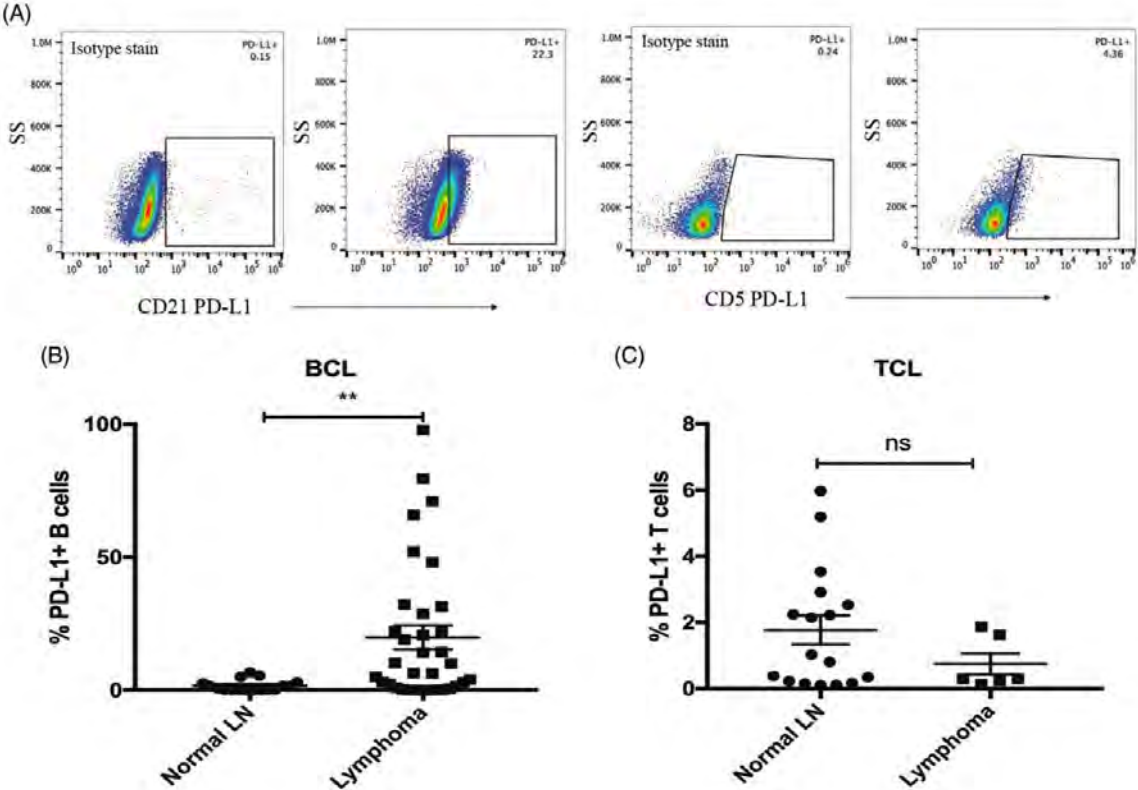


ORIGINAL ARTICLE

# Checkpoint molecule expression by B and T cell lymphomas in dogs

G. Hartley<sup>1</sup> | R. Elmslie<sup>2</sup> | S. Dow<sup>1</sup> | A. Guth<sup>1</sup>

- B cell malignancies have higher PDL-1 expression than normal B cells
- Both normal and malignant T cells have low to negative PD-1 and PDL-1 expression
- TILs from both BCL and TCL patients have increased expression of PD-1 and PDL-1 compared to normal B and T cells from healthy animals



OPEN

## A canine chimeric monoclonal antibody targeting PD-L1 and its clinical efficacy in canine oral malignant melanoma or undifferentiated sarcoma

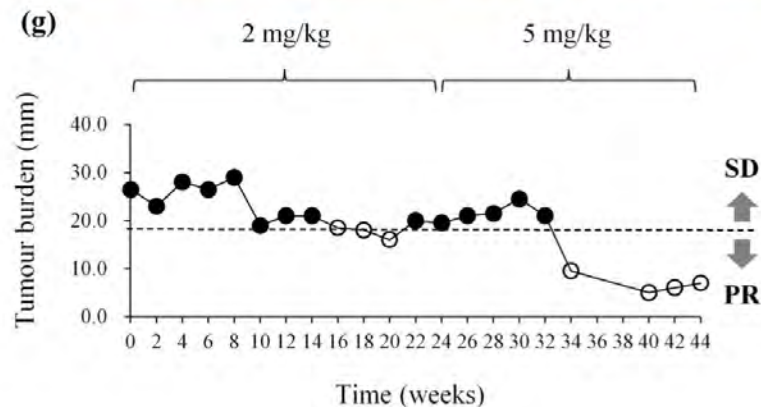
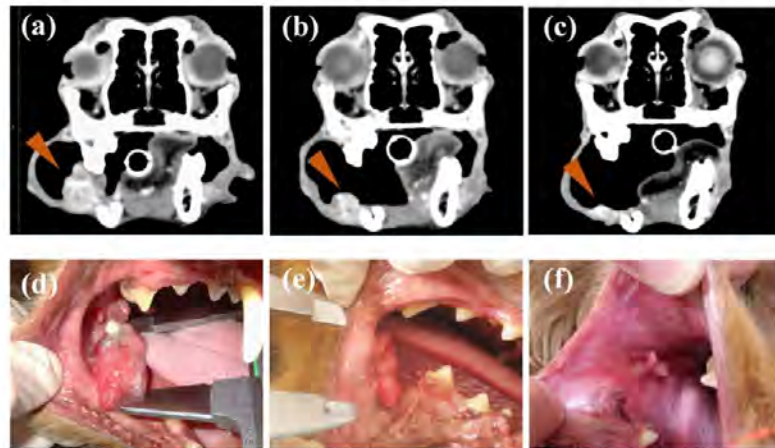
Naoya Maekawa<sup>1</sup>, Satoru Konnai<sup>1</sup>, Satoshi Takagi<sup>2</sup>, Yumiko Kagawa<sup>3,4</sup>, Tomohiro Okagawa<sup>1</sup>, Asami Nishimori<sup>1</sup>, Ryoyo Ikebuchi<sup>1</sup>, Yusuke Izumi<sup>2</sup>, Tatsuya Deguchi<sup>2</sup>, Chie Nakajima<sup>5,6</sup>, Yukinari Kato<sup>7,8</sup>, Keiichi Yamamoto<sup>9</sup>, Hidetoshi Uemura<sup>9</sup>, Yasuhiko Suzuki<sup>5,6</sup>, Shiro Murata<sup>1</sup>, Kazuhiko Ohashi<sup>1</sup>

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- Clinical trial evaluated single and multi-dose treatment of canine solid tumors
- An efficacy signal noted in 2 dogs (sarcoma and melanoma)



# Other NCI-sponsored activities: Comparative Immuno-Oncology in the Precision Medicine and Cancer Moonshot Era

# NCI Supplements to Cancer Center Grants (P30s)

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- As part of the **Precision Medicine Initiative in Oncology**, in 2016 the NCI competed and issued eight 1-year supplements to Support Research in Canine Immunotherapy via Collaboration of NCI-Designated Cancer Centers and Veterinary Medical Colleges.
- The goals of the supplement:
  - Sequence (by whole exome sequencing and RNAseq) at least 25 canine tumors (and their normal controls) in one of more of the following tumors: **B-cell lymphoma, glioma, osteosarcoma, melanoma, bladder cancer, and mammary cancer**
  - Determine the mutational load in the cancers chosen for study
  - Using appropriate computational tools, characterize neoantigens that can strongly bind canine MHC antigens
  - Describe and characterize the T lymphocyte numbers and subsets, as well as other relevant aspects of the tumor microenvironment, within the canine tumors

Institution(s)	Project Leader	Canine Cancer(s)	Title or Aims
Baylor College of Medicine/U. Florida Vet Med College/ Texas A&M/Tech U. Denmark	Jonathan Levitt, PhD/ Alan Herron, DVM	Bladder, Mammary, Melanoma	Mutational load and predicted neoantigens in canine tumors and characterization of immune infiltrate and the tumor microenvironment
U. Colorado/Colorado State U. Vet School	Jill Slansky, PhD/ Steven Dow, DVM, PhD	B-Cell Lymphoma	Immune profiling and neoantigen discovery in canine B cell lymphoma
DFCI-HCC/Tufts University Vet Med School	Katherine Janeway, MD/ Cheryl London, DVM	Osteosarcoma	A multi-institutional approach to interrogate and improve immunotherapy outcomes in osteosarcoma
Purdue University/Duke University	Deborah Knapp, DVM/ H. Kim Lyerly, MD	Bladder	Advancing immunology in dogs with naturally-occurring invasive bladder cancer: a relevant model to improve immunotherapy across molecular cancer subtypes in humans
Roswell Park Cancer Inst. /Cornell U. Vet Med	Richard Koya, MD, PhD/ Kristy Richards, PhD, MD	B-Cell Lymphoma	Immunogenic mutational load analysis for adoptive T cell therapy in canine B cell lymphoma
UC Davis/UC Davis School of Vet Med	Arta Monjazebl, MD, PhD	Glioma, Melanoma, Osteosarcoma	Evaluation of the tumor mutational landscape/neoantigens and immunophenotyping the tumor microenvironment in canine cancers
Ohio State U/OSU Vet Med School/TGEN	Peter Shields, MD/ Jeffrey Trent, PhD	Melanoma, Osteosarcoma	Immunogenomic profiling of canine melanoma and osteosarcoma
MD Anderson CC/Texas A&M	Amy Heimberger, MD/ Jonathan Levine, DVM	Glioma	Genomic and immunological canine glioma characterization

# Enter: Beau Biden Cancer Moonshot Initiative

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- In 2016 the NCI issued two RFAs (set-aside funding) for awards in 2017:
  - **Canine Immunotherapy Trials and Correlative Studies (U01)** to support:
    - Canine **clinical trials** using immunotherapeutic agents and novel combinations (of immune modulators, molecularly targeted agents, chemotherapy, and/or radiation)
    - Correlative studies that seek to describe, characterize, and understand the cellular and molecular mechanisms that determine the anti-tumor response (or non-response) in dogs with spontaneous tumors.
    - Up to 5 awards to a network of academic laboratories, veterinary medicine clinical trial sites, and veterinary pharmaceutical companies (producing canine immunotherapy agents) working together
  - **Coordinating Center (U24) assisted by the NCI's Comparative Oncology Program (COP) and an NCI Program Official that will:**
    - Help develop/implement the clinical studies in immunotherapy and combinations
    - Establish a Steering Committee and an External Advisory Board
    - Assist in the standardization of clinical and laboratory protocols
    - Manage clinical and correlative data from all sites
    - Provide statistical support
    - Facilitate sharing of agents, specimens, and data via teleconferences and a website
    - Report progress in an annual report



# 5 U01s Selected for Funding

Grant Number	PIs	Lead Institution	Title
1U01CA224182-01	DOW, STEVEN W (contact); LONDON, CHERYL A	COLORADO STATE UNIVERSITY	Optimizing novel immunotherapy combinations targeting the tumor microenvironment in canine spontaneous <b>osteosarcoma</b>
1U01CA224151-01	CHAMBERS, M R (RENEE)	UNIVERSITY OF ALABAMA AT BIRMINGHAM	Canine immuno-neurotherapeutics ( <b>glioma</b> )
1U01CA224160-01	PLUHAR, GRACE ELIZABETH (LIZ)	UNIVERSITY OF MINNESOTA	Novel combined immunotherapeutic strategies for <b>glioma</b> : using pet dogs as a large animal spontaneous model
1U01CA224166-01	CANTER, ROBERT (BOB) J (contact); REBHUN, ROBERT (ROB) B	UNIVERSITY OF CALIFORNIA AT DAVIS	Enhancing natural killer immunotherapy with first-in-dog trials of inhaled recombinant IL-15 and super-agonist IL-15 in naturally occurring canine cancers ( <b>melanoma and osteosarcoma</b> )
1U01CA224153-01	LONDON, CHERYL A (contact); RICHARDS, KRISTY L	TUFTS UNIVERSITY BOSTON	Enhancing the efficacy of immunotherapy in <b>DLBCL</b> using rational combination approaches

# U24 Selected for Funding

<b>1U24CA224122-01</b>	<b>MASON, NICOLA (NICKY) J (contact); PROPERT, KATHLEEN (KATE)</b>	<b>UNIVERSITY OF PENNSYLVANIA</b>	<b>Coordinating Center for Canine Immunotherapy Trials and Correlative Studies</b>
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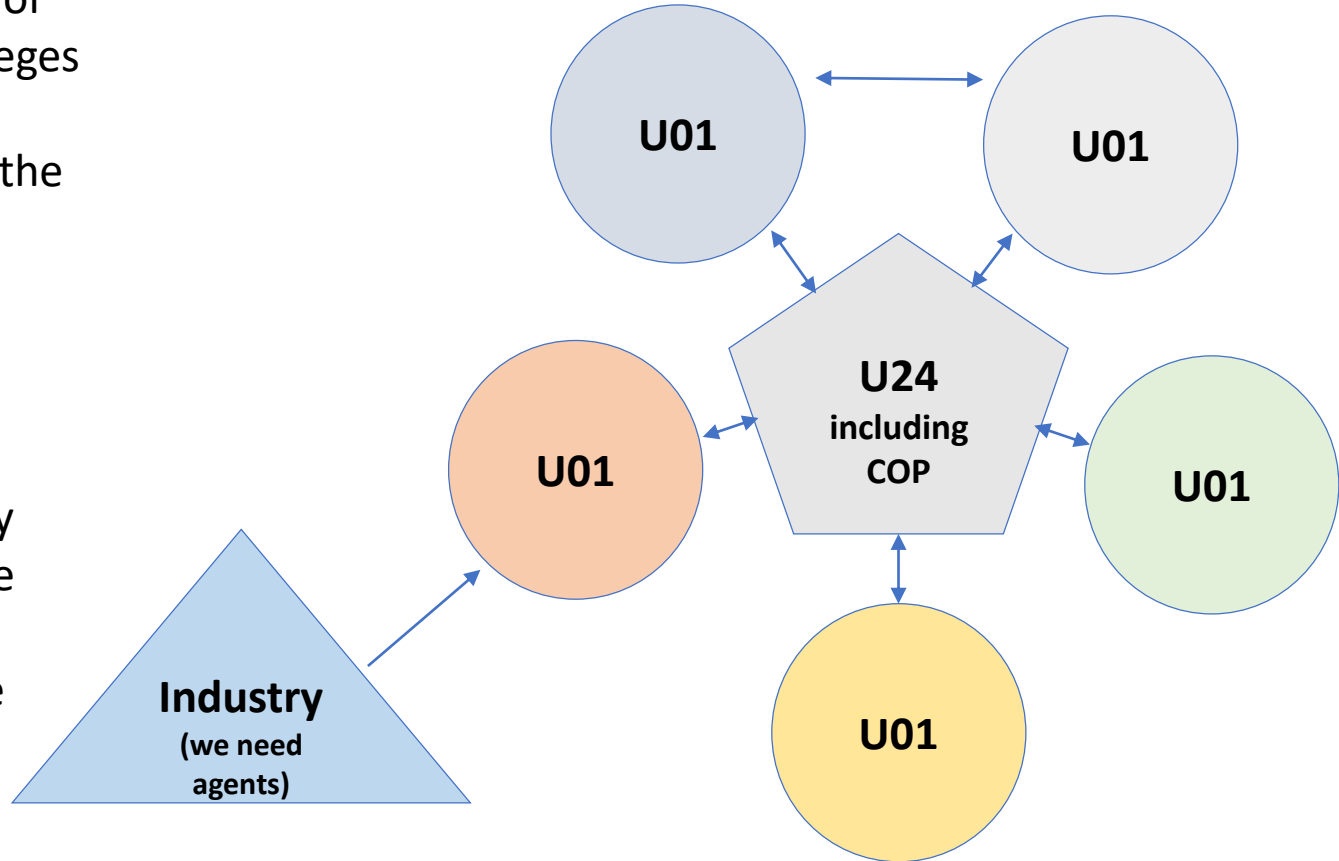
## NCI Participants:

<b>Amy LeBlanc</b>	<b>NCI Center for Cancer Research</b>	<b>Comparative Oncology Program: Heads the Comparative Oncology Trials Consortium (COTC)</b>	<b>Member of the Steering Committee</b>
<b>Connie Sommers</b>	<b>NCI Developmental Therapeutics Program</b>	<b>ImmunoOncology Branch</b>	<b>NCI Program Officer and member of the Steering Committee</b>
<b>Toby Hecht</b>	<b>NCI Division of Cancer Treatment and Diagnosis</b>	<b>Office of the Director</b>	<b>Deputy Division Director</b>

# How does this RFA work?

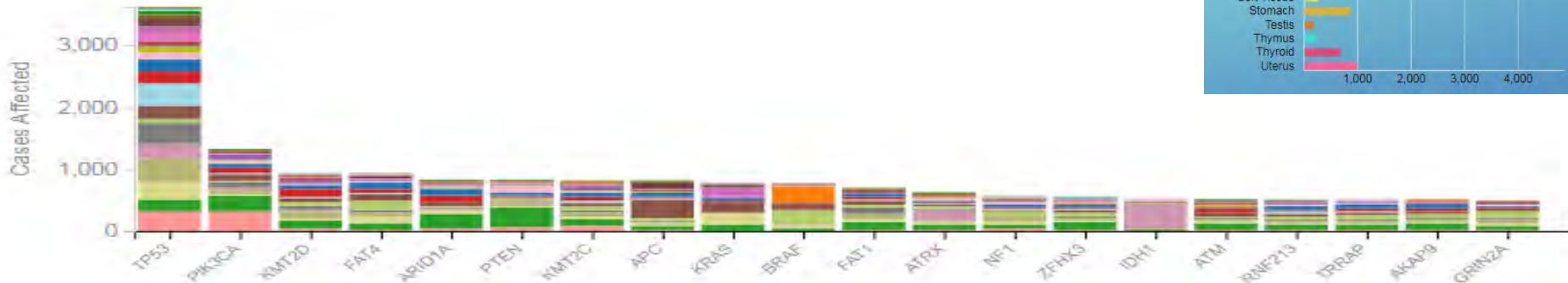
Each **U01** consists of a PI's lab(s) and one or more vet med colleges (members of the COTC—or not) for the proposed clinical studies

- Oncolytic virotherapy
- NK cell therapy
- Small molecule repurposing
- Gene/cytokine therapy



# Coming Soon: A Canine Genomics Data Commons (C-GDC)

- Patterned on the NCI Human Genomics Data Commons (GDC): <https://portal.gdc.cancer.gov/>
- Provides the cancer research community with a publicly accessible unified data repository that enables data sharing across cancer genomic studies in support of precision medicine. GDC data analysis tools allow users to interact intuitively with the GDC data and promote the development of a true cancer genomics knowledge base. >30,000 cases; >3M mutations in 22K genes



## From the NCI-COP perspective:

What lies ahead for comparative oncology's role in immunology research and development?

- Emphasis on continuing the scientific dialogue as it pertains to applicability and validity of the dog model of cancer for IO strategic advancement
- Maintain the highest standards for clinical trial design, execution and reporting
- Continue to develop of canine-specific reagents and correlative assays to support trial efforts and enhance human translation



# Acknowledgements

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NIH-NCI Center for Cancer Research

- Office of the Director (Dr. Tom Misteli, Mel Bronez)
- Molecular Imaging Program (Dr. Peter Choyke, Elaine Jagoda, Dagane Daar, Tieu Hoa, Alicia Forest, Phil Eclarinal, Karen Wong, Mirna Martinez)
- Pediatric Oncology Branch (Drs. Kathy Warren, Rosandra Kaplan, Fernanda Arnaldez)
- Radiation Oncology and Biology Branches (Drs. Deb Citrin, Murali Cherukuri, Kazu Yamamoto)
- NeuroOncology Branch (Dr. Mark Gilbert, Dr. Mioara Larion, Victor Ruiz)

NIH-NCI Comparative Oncology Program

- Christina Mazcko; Dr. Ling Ren; Dr. Shan Huang
- Dr. Hongsheng Wang
- Christine Tran Hoang
- Anusha Kambala

Vyriad/Mayo Clinic (Drs. Russell, Naik, Peng, Federspiel)

NCI Division for Cancer Treatment & Diagnosis (DCTD)

NIH Divisions of Veterinary Resources (DVR) and Radiation Safety (DRS)

NIH Investigational Probe Development Center (IPDC)

Dr. Chand Khanna

COTC member institutions, investigators, and support staff: past, present and future







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# Challenges and Opportunities in Developing Non-clinical Models for Immuno-oncology

Lei Zheng, M.D., Ph.D.

Associate Professor of Oncology and Surgery

Tumor Immunology Program, Department of Oncology

Co-Director, The Precision Medicine Center of Excellence for Pancreatic Cancer



JOHNS HOPKINS  
M E D I C I N E

# FDA-Approved Immune-Oncology Agents

Class	Mechanism of action	Agent's name	Approval (FDA)	Cancer indication
Cancer vaccines (patient's dendritic cell)	Autologous CD54+ cells activated with recombinant PAP-GM-CSF (prostatic acid phosphatase linked to granulocyte-macrophage colony-stimulating factor)	Sipuleucel-T (Provenge®)	2010	Prostate
Immunomodulatory monoclonal antibodies (blockade of checkpoint inhibitors)	Anti-CTLA-4 (IgG1)	Ipilimumab (Yervoy®)	2010	Melanoma
	Anti-PD-1 (IgG4)	Pembrolizumab (Keytruda®)	2014–2017	Melanoma; nonsmall cell lung cancer; head and neck squamous cell carcinoma; classical Hodgkin lymphoma; urothelial carcinoma; microsatellite instability-high cancer; colorectal cancer; gastric or gastroesophageal junction adenocarcinoma
	Anti-PD-1 (IgG4)	Nivolumab (Opdivo®)	2014–2017	Melanoma; nonsmall cell lung cancer; classical Hodgkin lymphoma; renal cell carcinoma; head and neck squamous cell carcinoma; urothelial carcinoma; microsatellite instability-high colon cancer; hepatocellular carcinoma
	Anti-PD-L1 (IgG1 with N298A mutation)	Atezolizumab (Tecentriq®)	2016	Urothelial carcinoma
	Anti-PD-L1 (IgG1)	Durvalumab (Imfinzi™)	2017	Urothelial carcinoma
	Anti-PD-L1 (IgG1)	Avelumab (Bavencio®)	2017	Merkel cell carcinoma
	Anti-CD3/CD19 (binding to T cell receptor and CD19 on cancer cells)	Blinatumomab (Blincyto®)	2014	Acute lymphoid leukemia
Oncolytic viruses (talimogene laherparepvec)	Enhanced activity towards tumor cells by genetically modified oncolytic herpes simplex virus type-1 (oHSV-1)	T-Vec (Imlygic®, OncoVEX <sup>GM-CSF</sup> )	2015	Melanoma
Chimeric antigen receptor T cell (CAR-T) cell therapy	Genetically modified autologous T cells to target and kill tumor cells that express CD19	Tisagenlecleucel (Kymriah®)	2017	Acute lymphoblastic leukemia
	Genetically modified autologous T cells to kill B lymphocytic tumor cells	Axicabtagene ciloleucel (Yescarta®)	2017	Diffuse large B cell lymphoma

# Nonclinical model used for Approved Immune-Oncology Agents

	Nonclinical animal model for safety assessment	Preclinical model for anti-tumor assessment
Cancer vaccines (e.g. Sipuleucel-T)	Not available	Syngeneic model
Immunomodulatory monoclonal antibodies (checkpoint inhibitors)	Non-human Primate	Syngeneic model, Knock-in model
Bi-specific antibodies (e.g. BiTE)	Non-human Primate	PDX or CDX model
Oncolytic viruses (e.g. T-VEC)	Wild-type mice and tumor bearing syngeneic mice	PDX, CDX, or Synergic model
Cell Therapy (e.g. CAR-T, TCR-T)	Not available	PDX or CDX
Cytokine (e.g. IL-2)	Non-human Primate	Syngeneic model

# **Challenges and Lessons Learned**

# Immune modulatory antibodies

- Species relevance has been an issue
- Affinity of binding to human and cynomolgus monkey PD-1 has been similar for most products
- EC50 for blocking interactions between the PD-1 receptor and its ligands is similar between species
- At exposures well above those seen clinically, there was no clear autoimmunity
- However, autoimmune toxicities were observed in the combination of anti-PD-1 and anti-CTLA-4 antibodies.



# Cynomolgus Toxicology Signals with Ipilimumab and Nivolumab Combination

Group	M/F	Treatment	Dose	Diarrhea <sup>a</sup>	Mean Spleen Weight <sup>b</sup> (g)		Spleen Pathology <sup>c</sup>	Gastrointestinal Pathology <sup>d</sup>
			mg/kg	n/N	Day 30 M/F	Day 59 M/F	n/N	n/N
1	5/5	saline control	—	0/10	3.9/2.8	3.5/3.7	0/6	0/6
2	5/5	nivolumab + ipilimumab	10/3	2/10	4.0/3.6	4.3/2.4	2/6	2/6
3	5/5	nivolumab + ipilimumab	50/10	4/10	6.1/4.47	7.5/3.2	4/5	3/5

<sup>a</sup> Incidence of repeated diarrhea (number of animals with finding/number of animal examined).

<sup>b</sup> Mean spleen weight on days 30 and 59; at day 30, 3 monkeys per sex per group with the exception of 2 males in Group 3; at day 59, 2 monkeys per sex per group.

<sup>c</sup> Incidence of lymphoid follicle hypertrophy or marginal zone expansion: number of animals with finding (n) / number of animals examined (N).

<sup>d</sup> Minimal, diffuse lymphoplasmacytic inflammation in the lamina propria with concurrent enlargement of the colonic or pelvic lymph nodes: number of animals with finding (n) / number of animal examined (N).

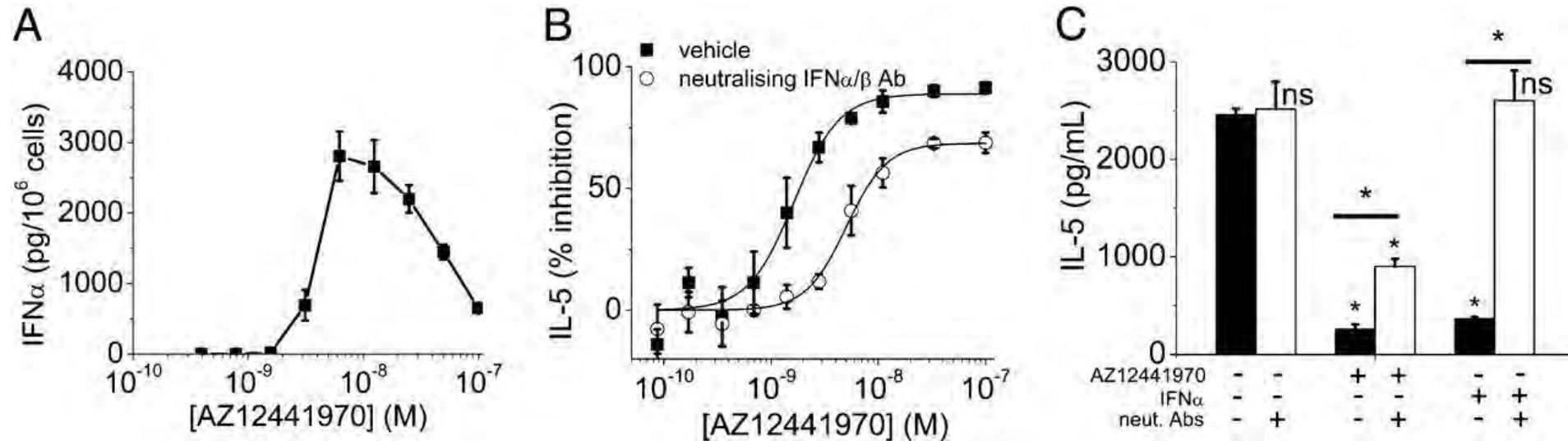
doi:10.1371/journal.pone.0161779.t002

Selby MJ, Engelhardt JJ, Johnston RJ, Lu LS, Han M, et al. (2016) Preclinical Development of Ipilimumab and Nivolumab Combination Immunotherapy: Mouse Tumor Models, In Vitro Functional Studies, and Cynomolgus Macaque Toxicology. PLOS ONE 11(9): e0161779. <https://doi.org/10.1371/journal.pone.0161779>  
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0161779>

# Antagonist vs. Agonist

- An agonistic immune stimulation is considered of higher risk compared to the immune modulation via antagonistic binding to target receptors.
- Antagonists typically exhibit a linear dose response (e.g., anti-CTLA-4 and anti-PD-1 mAbs).
- Agonists are often associated with a bell-shape dose response.

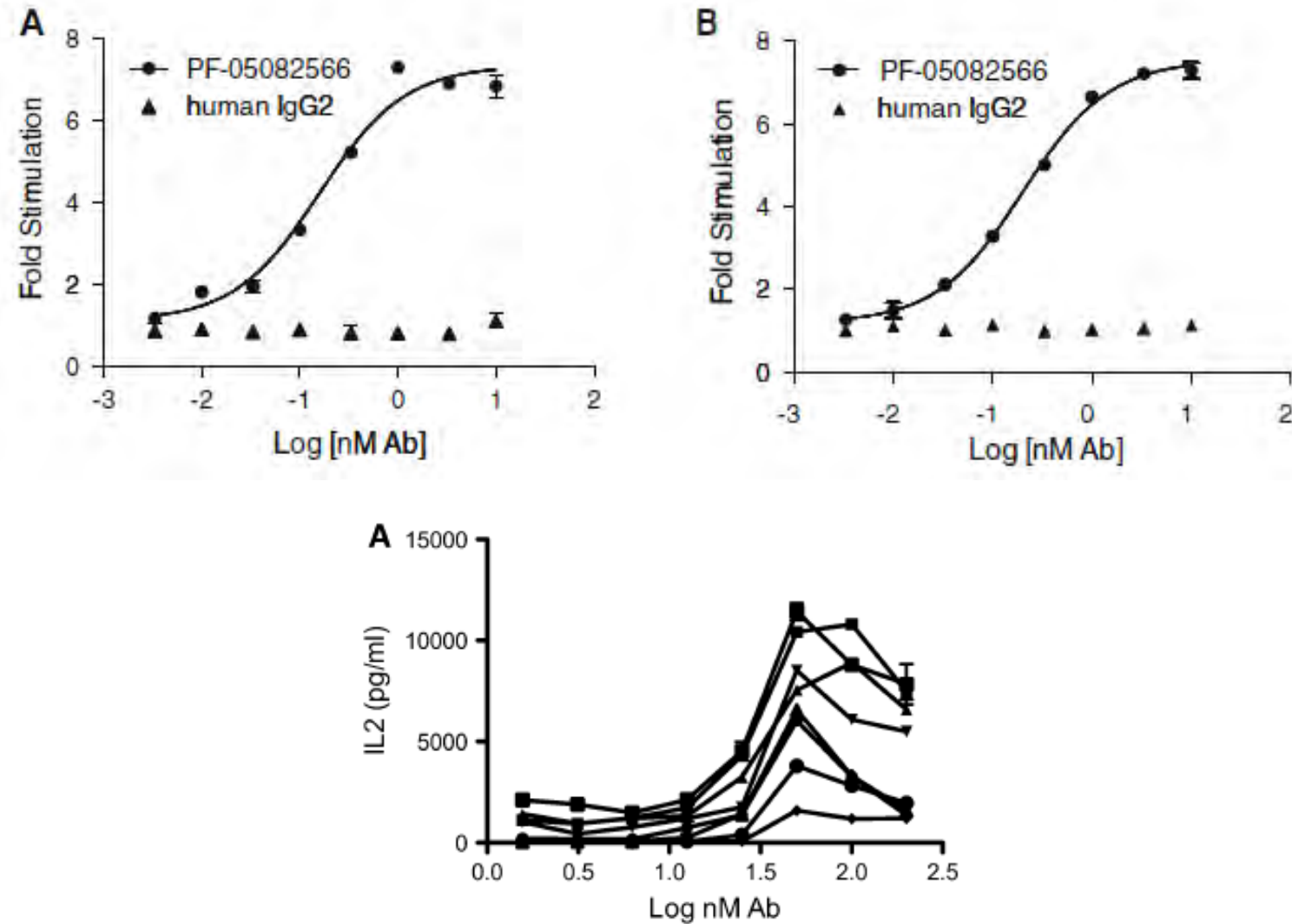
# TLR7 Agonist Induced $\text{INF}\alpha$ in a Bell-Shaped Dose Response



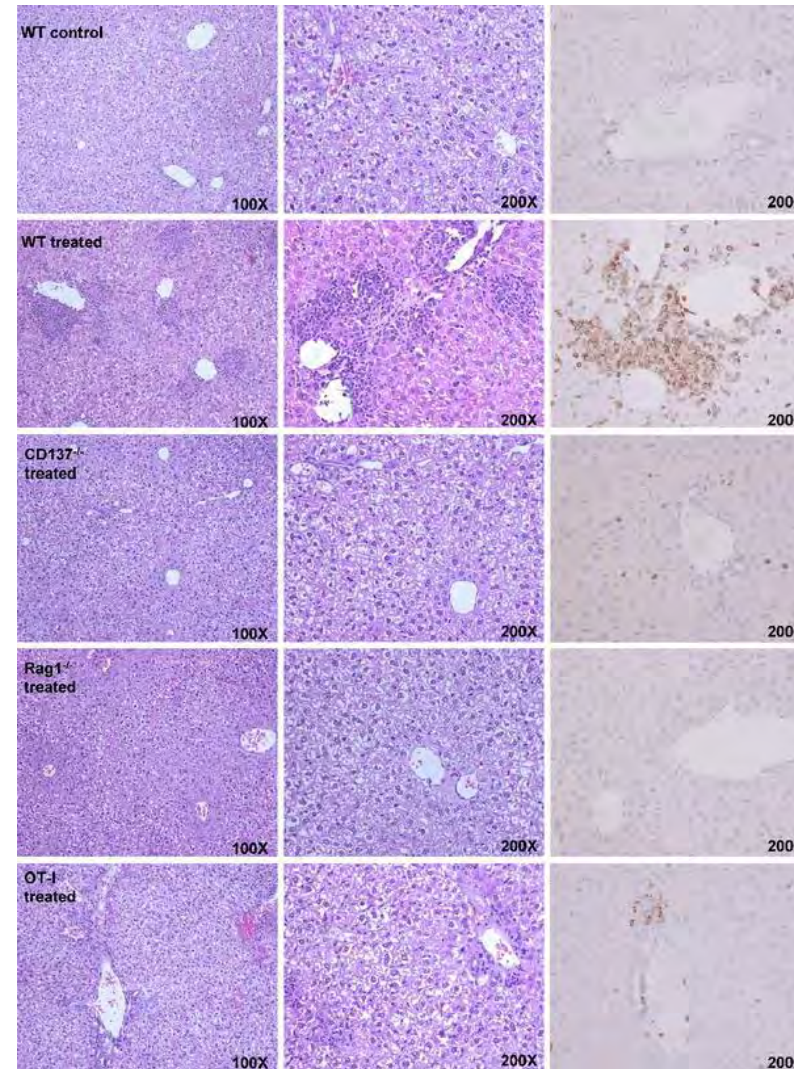
**IFN- $\alpha$  dependence of PHA-induced IL-5 production from PBMC. (A)** Human PBMC were stimulated with AZ12441970 for 20 h, and IFN- $\alpha$  levels in the medium were determined.

Susan Edwards et al. J Immunol 2013;190:2585-2592

# In vitro, Anti-CD137 agonist PF-05082566 induced the NF- $\kappa$ B reporter in linear dose response, but enhanced IL2 in a bell-shaped dose response



# Anti-CD137 agonist antibody induced mononuclear inflammation in the portal spaces of the liver and a marked increase in the CD8+ T cell infiltration in the mouse models



CD3

Dubrot et al. Cancer Immunology, Immunotherapy 2010

# **In vitro dose response is often different from in vivo dose response**

- In vitro studies of PF-05082566 anti-CD137 agonist antibody demonstrated a bell-shaped IL-2 response curve. However, this could be an artifact of in vitro culture system.
- A bell shaped response curve was not noted in vivo in the huPBL-SCID-Bg xenograft model and in cynomolgus monkeys at doses up to 10 mg/kg.



# Neither in vitro nor in vivo cytokine release assay is predictive

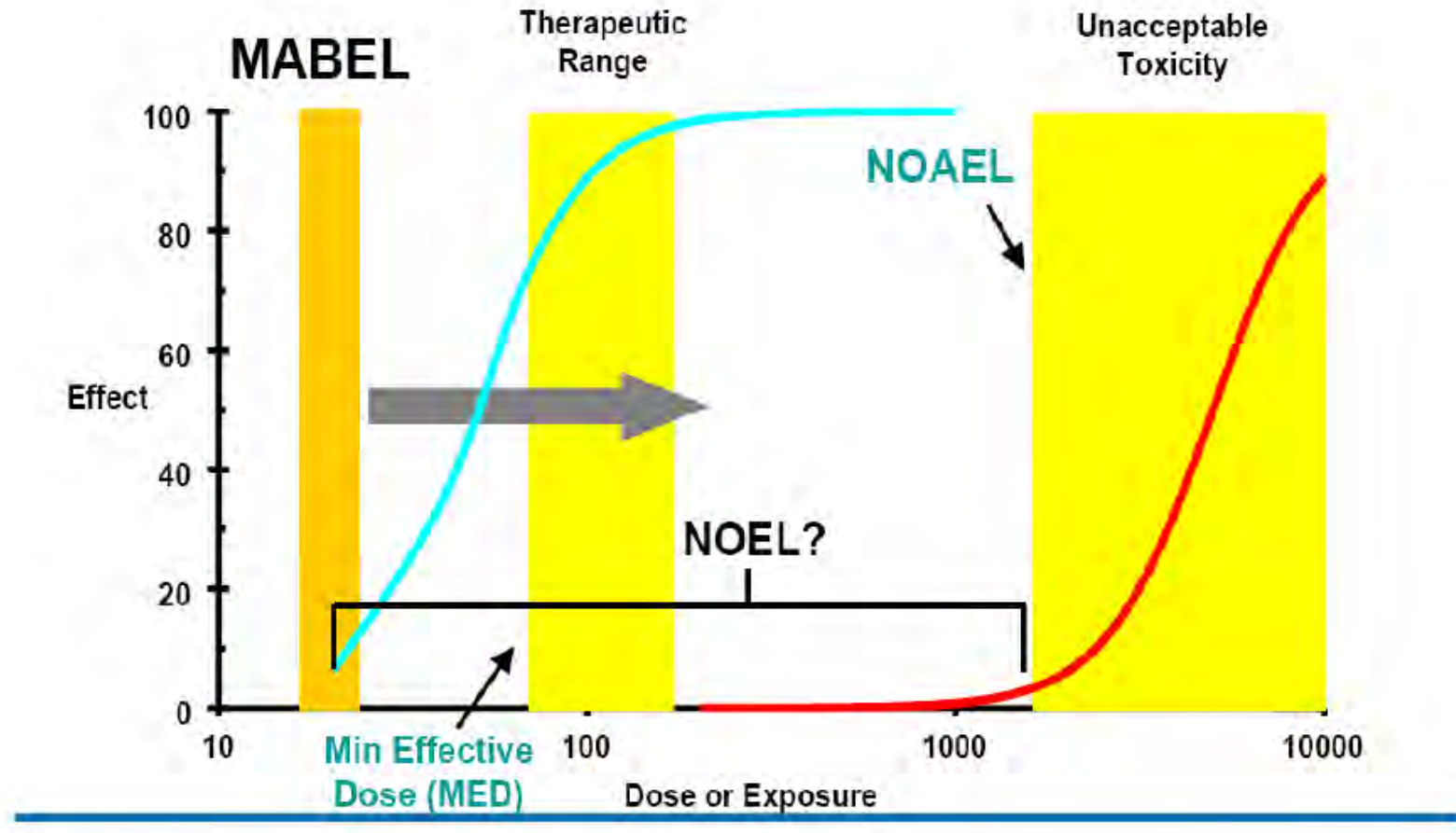
- A negative in vitro cytokine release assay result may not be reliably used to make assumptions about risk for patients.
  - In an in vitro system using soluble TGN1412 anti-CD28 agonist antibodies added to isolated human PBMCs or 1:5 diluted whole blood, no cytokine release was observed.
  - In subsequent experiments TGN1412 did stimulate pro-inflammatory cytokine release in either PBMC or 1:5 diluted whole blood test systems if the antibody was immobilized by air drying to plastic or anti-Fc antibody capture, or if the antibody was added in aqueous phase in the presence of endothelial cells.
- Only low level cytokine release was observed in the primate studies of TGN1412.
- Due to the above results, the First-in-human (FIH) dose may be calculated inappropriately; no proper interval was left between dosing the first and next patients; neither were the investigators prepared for managing the cytokine storms.

*Stebbing et al. "Cytokine storm" in the phase I trial of monoclonal antibody TGN1412: better understanding the causes to improve preclinical testing of immunotherapeutics. J Immunol 2006; 179:3325-31.*

# Special Recognition of Immune-Targeting Agonists

- ICH S9 specifically mentions concerns about using standard methods based on toxicology studies alone to set the starting dose of immune agonists:
  - For biopharmaceuticals with immune agonistic properties, selection of the start dose using a minimally anticipated biologic effect level (MABEL) should be considered.
  - Determining a MABEL relies heavily on a variety of pharmacology studies
- However, it is challenging to translating in vitro data to in vivo with immuno-oncology products and would be more challenging for IO combination

# Special Recognition of Immune-Targeting Agonists

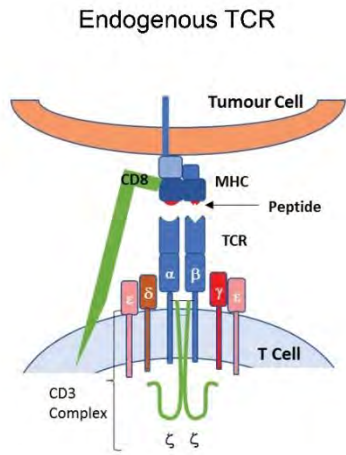


**NOAEL:** No Observed Adverse Effect Level

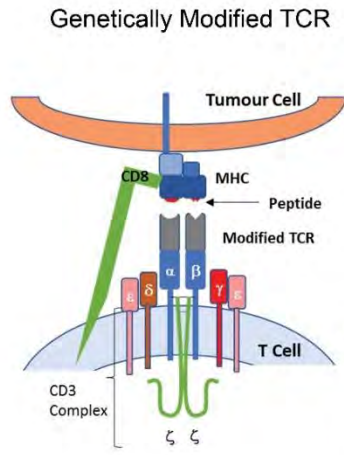
**NOEL:** No Observed Effect Level

# WHAT CAN WE LEARN FROM T CELL THERAPY?

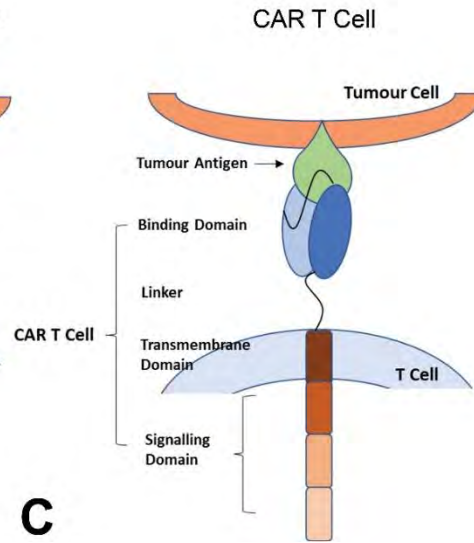
# Therapies engaging or mimicking T cells (CAR-T, TCR-T, BiTE, TCRm(imitic) antibody)



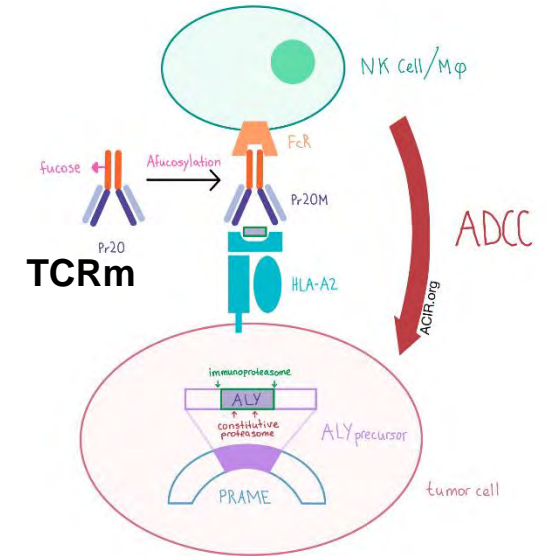
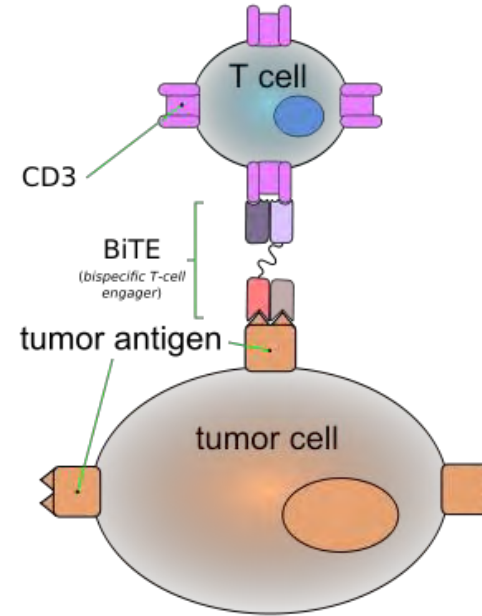
A



# B



C



# On-target Off-tumor Toxicity

**Table 2.** On-target, Off-tumor Toxicities Associated with CAR T-cell Therapies.

Disease	Target	Toxicity	Reference
B-cell malignancies	CD19	B-cell aplasia, which can be maintained long term with reported cases up to 4 years	Grupp et al. (2013); Maude, Frey, et al. (2014); Porter et al. (2015)
Colon cancer	HER2/ERBB2	Lethal pulmonary failure Suspected cytokine release following the recognition by the CAR T cells of low levels of ERBB2 on lung epithelial cells	Morgan et al. (2010)
Renal cancer	Carbonic anhydrase-IX	Liver enzyme disturbances in subjects, reaching National Cancer Institute Common Toxicity Criteria grades 2 to 4 The development of cholestasis due to expression of carboxy anhydrase-IX on bile duct epithelium. Liver biopsies showed T-cell infiltration around the bile ducts	Lamers et al. (2006); Lamers et al. (2013)
Non-Hodgkin's lymphoma/ multiple myeloma	$\kappa$ light chain	Elimination of $\kappa$ -expressing B and plasma cells However, spares the normal B cells expressing the nontargeted $\lambda$ light chain, thus potentially minimizing humoral immunity impairment	Ramos et al. (2016)

Note: CAR = chimeric antigen receptor; CD = cluster of differentiation.



# On-target Off-tumor Toxicity

**Table 4.** On-target, Off-tumor Toxicities Associated with Genetically Modified TCR T-cell Therapies.

Disease	Target	Toxicity	Reference
Melanoma	MAGE-A3 peptide (KVAELVHFL)	On-target, off-tumor toxicity due to previously undetected MAGE-A expression in the human brain. TCR also recognizes peptides: MAGE-A12 (KMAELVHFL) MAGE-A2 (KMVELVHFL) MAGE-A6 (KVAKLVHFL). Three subjects developed neurological toxicity. Two subjects died and 1 subject made a full neurological recovery	Morgan et al. (2013)
Melanoma	TCR T-cell therapy TCR recognizing melanoma antigen MART-1 (amino acids 27–35 epitope) TCR recognizing the HLA-A*02-restricted melanoma antigen gp100 (amino acids 154–162 epitope)	On-target, off-tumor reactivity, destruction of normal melanocytes in the skin, eye, and ear	Johnson et al. (2009)
Metastatic colorectal cancer	TCR recognizing the carcinoembryonic antigen (CEA) peptide: (IMIGVLVGV)	On-target, off-tumor reactivity resulting in severe transient inflammatory colitis caused by T-cell reactivity to CEA expression on normal colonic mucosa	Parkhurst et al. (2011)

Note: HLA = human leukocyte antigen; TCR = T-cell receptor.

# Off-target Off-tumor Toxicity

**Table 5.** Off-target Toxicities Associated with Genetically Modified TCR T-cell Therapies.

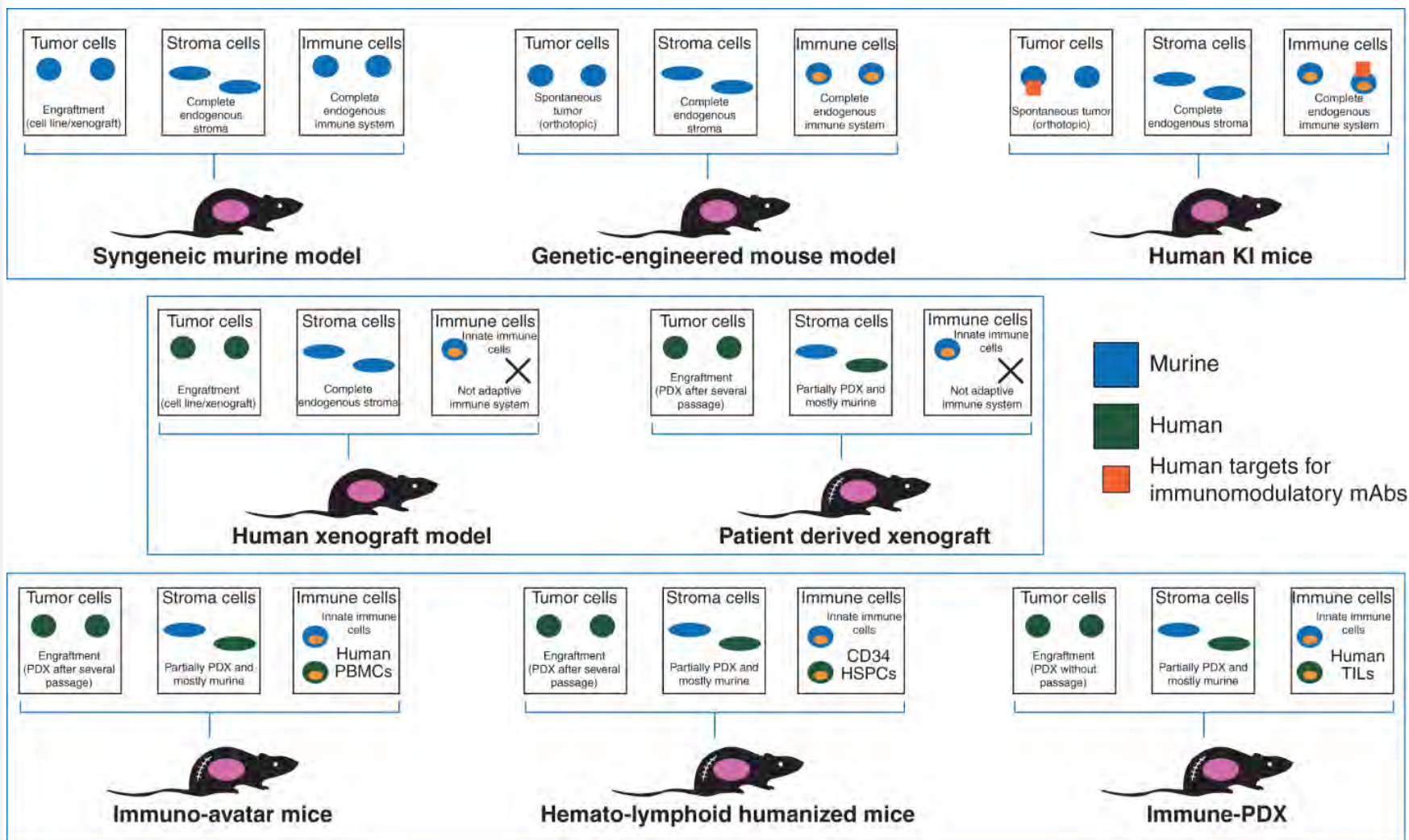
Disease	Target	Tumor Toxicity	Reference
Myeloma and melanoma	An affinity-enhanced TCR recognizing MAGE-A3 (EVDPIGHLY)	Off-target reactivity. Lethal cardiac toxicity. Two subjects died approximately 5 days' postdosing Following adverse events, <i>in vitro</i> investigations revealed cross-recognition of an off-target peptide	Linette et al., (2013); Cameron et al. (2013)

Note: TCR = T-cell receptor.

# What factors have impacts on the toxicity from cytokine release?

- On Target, Off Tumor toxicity: Target expression in non-tumor tissues
- Off Target toxicity: Cross-recognition of an off-target epitope
- **Tumor Burden: The burden of targets on tumors**

# Opportunities



From: Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies

Ann Oncol. 2016;27(7):1190-1198. doi:10.1093/annonc/mdw041

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# Nonclinical model used for Approved Immuno-Oncology Agents

	Nonclinical animal model for safety assessment	Preclinical model for anti-tumor assessment	Safety assessments in tumor-bearing preclinical models
Cancer vaccines (e.g. Sipuleucel-T)	Not available	Syngeneic model	Toxicity of adjuvant; Treatment schedules
Immunomodulatory monoclonal antibodies (checkpoint inhibitors)	Non-human Primate	Syngeneic model, Knockin model	Autoimmune toxicity, Cytokine release
Bi-specific antibodies (e.g. BiTE)	Non-human Primate	PDX or CDX model	Cytokine release, off-tumor targeting
Oncolytic viruses (e.g. T-VEC)	Wild-type mice and tumor bearing syngeneic mice	PDX, CDX, or Synergic model	Biodistribution, viral shedding and toxicology
Cell Therapy (e.g. CAR-T, TCR-T)	Not available	PDX or CDX	Cytokine release, off-tumor targeting
Cytokine (e.g. IL-2)	Non-human Primate	Syngeneic model	Biodistribution and toxicology



# What can we do?

- Low-level cytokine release in nonclinical animal models should prompt more caution.
- Mild symptoms or non-specific symptoms such as weight loss should prompt pathologic examination of toxicities including autoimmune toxicities.
- Humanized mouse models provide an opportunity of assessing treatment-related toxicities in tumor-bearing mice under clinically relevant conditions
- Toxicities should be studied in both wild-type animals and tumor-bearing mice being evaluated for anti-tumor efficacies.
- Evaluate the biology and expression of the target in the intended clinical population and models: vigorously examine off-tumor target expressions and off target effects in the preclinical model.

# Commonly Modulated Blood Cytokines Associated with Pathological Responses

Pathological response	Cytokines
Acute-phase response	IL1b, IL6, TNF- $\alpha$
Cytokine storm/release	IL2, IL6, IL8, IL10, IFN $\gamma$ , TNF- $\alpha$
Fibrosis	TGF $\beta$
Hemophagocytic syndrome	IFN $\gamma$ , IL1b, IL6, TNF- $\alpha$
Neutrophilic inflammation	IL8, MIP-1, TNF- $\alpha$
Systemic inflammatory response syndrome	IL6, MCP-1, TNF- $\alpha$
Th1 immune response	IFN $\gamma$ , IL2, IL12
Th2 immune response	IL4, IL5, IL6, IL10, IL13

# What can we do?

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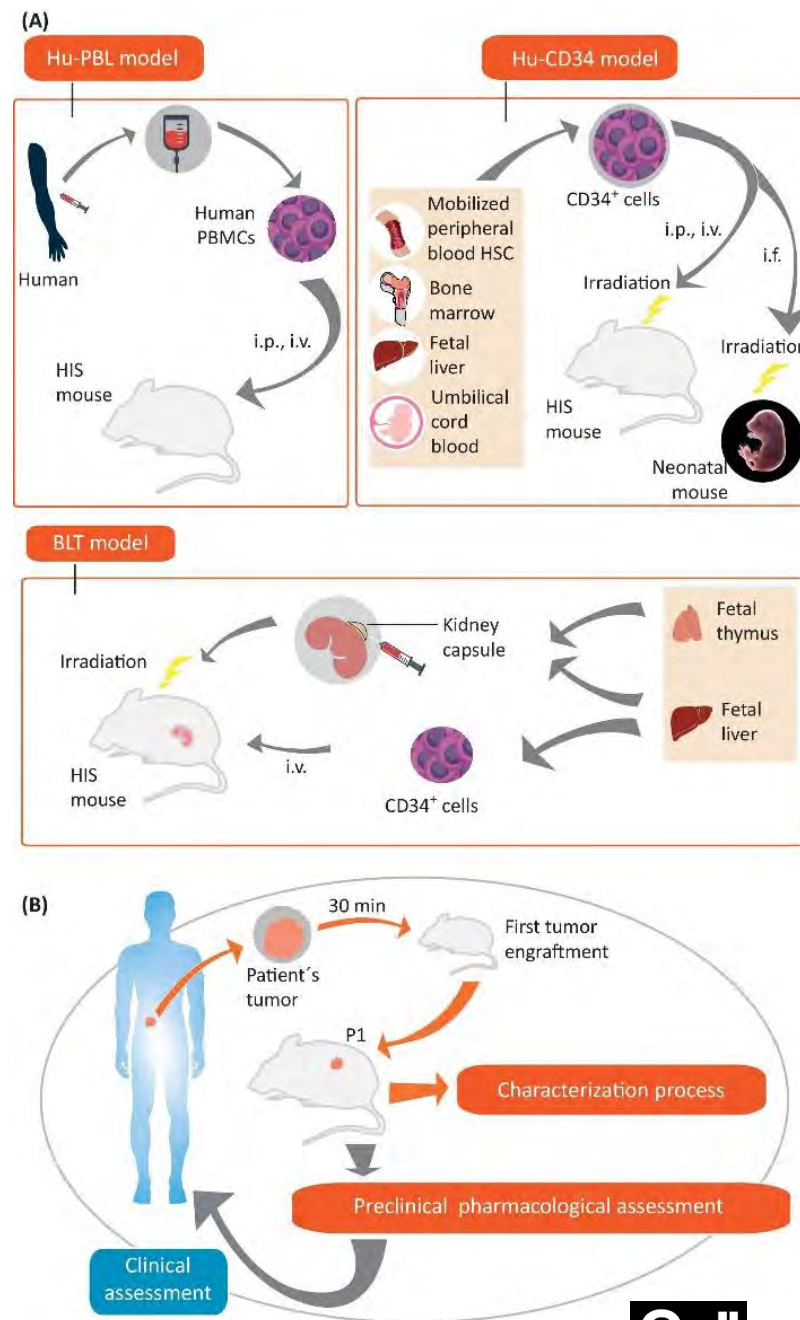
# Assess autoimmunity

- Use tumor models where the kinetics of tumor regression is slower and/or spontaneous tumor models to allow for more long term administration of immune-modulating agents
- Increase experimental sampling to detect for the presence of biochemical autoimmunity
  - Serum testing for liver damage (ALT, AST)
  - Immunohistochemistry for kidney damage (autoantibodies)
  - Changes in inflammatory cytokine profile (IL-6, TNF)
- Assess combination immunomodulatory agents in strains of mice that are more susceptible to autoimmunity
- Assess the autoimmune adverse reactions by histologic examination

# What can we do?

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- Mild symptoms or non-specific symptoms such as weight loss should prompt pathologic examination of toxicities including autoimmune toxicities.
- Humanized mouse models provide an opportunity of assessing treatment-related toxicities in tumor-free mice and in tumor-bearing mice under clinically relevant conditions
- Toxicities should be studied in both wild-type animals and tumor-bearing mice being evaluated for anti-tumor efficacies.
- Evaluate the biology and expression of the target in the intended clinical population and models: vigorously examine off-tumor target expressions and off target effects in the preclinical model.

# Humanized mice provide an opportunity of examining treatment-induced autoimmunity





# Histologic examination of autoimmune toxicities in humanized mice treated with Nivolumab

Adverse Reactions	Observed in BLT/NOG mice in the Nivolumab Pilot Experiment
Pneumonitis	Low dose: 3/4 Medium dose: 2/4 High dose: 2/4
Hepatitis	Low dose: 3/4 Medium dose: 3/4 High dose: 4/4
Nephritis	Low dose: 1/4 Medium dose: 1/4 High dose: 1/4
Rash/Dermatitis	Low dose: 1/4 Medium dose: 3/4 High dose: 2/4
Adrenalitis	Low dose: 1/4 High dose: 1/4

Reported by Dr. Kristina E. Howard from CDER  
at an FDA-AACR I-O Drug Development Workshop in October 2016

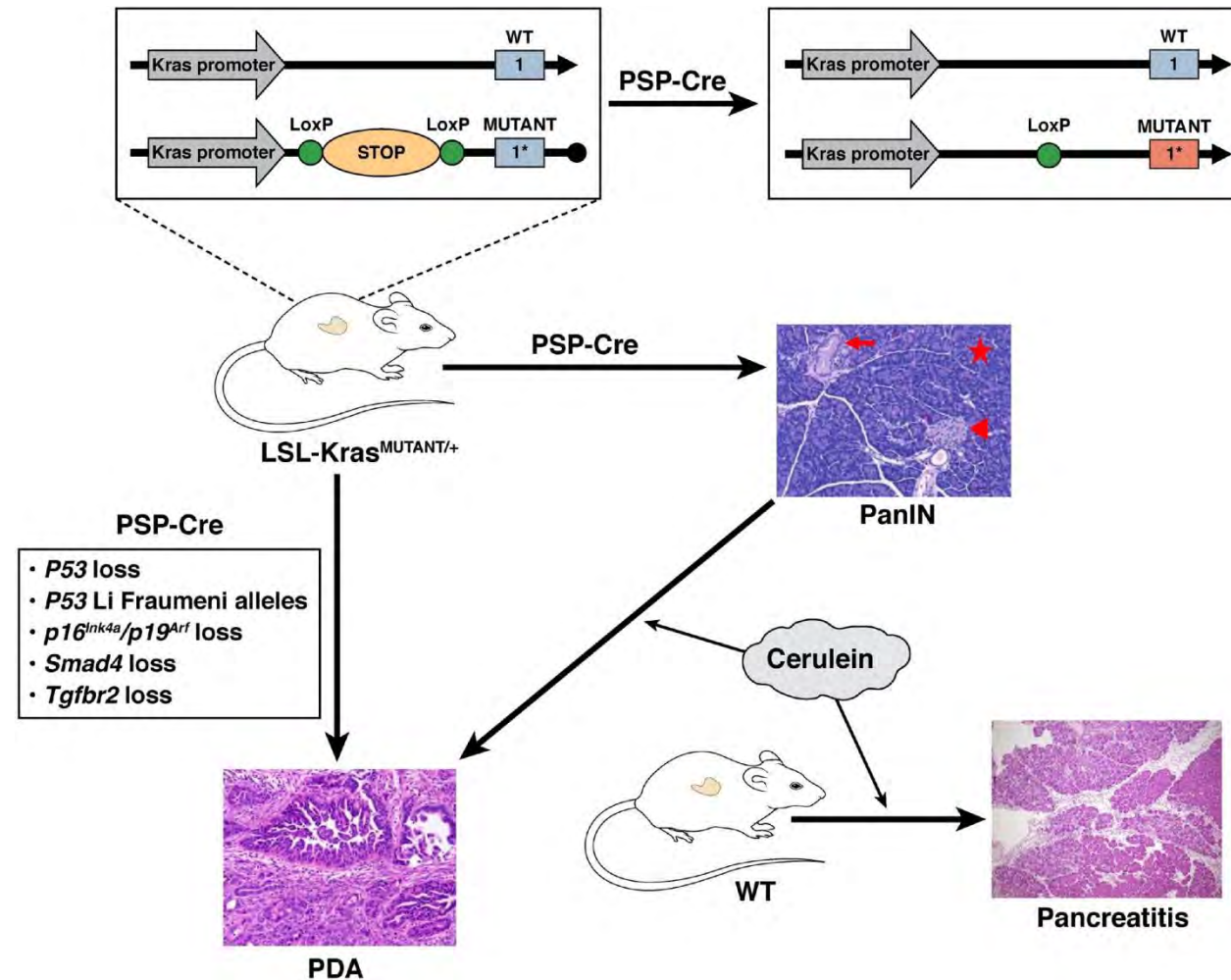
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# These would be applied to cytokines and immune agonist ligands

- **In Vivo Anti-tumor Studies.** The antitumor activity of T-VEC was studied in the xenograft model or immunocompetent syngeneic mouse model. The antitumoral effects of T-VEC after local intratumor injection or occurring systemically after injection in the contralateral, tumor-free animal flank were studied.
- **Nonclinical pharmacokinetics evaluation included single-dose and repeated-dose studies addressing biodistribution, viral shedding, and replication of T-VEC.**
  - In vivo biodistribution evaluated in **naïve or tumor-bearing** BALB/c mice following single or multiple subcutaneous, intravenous, and intratumoral dosing.
  - Viral shedding in BALB/c mice.
- **Toxicology**
  - Repeated-dose studies through the **intratumoral route** administration under clinically relevant conditions, e.g., **in tumor-bearing animals that allow viral replication as anticipated in patients**
  - Repeated-dose studies **in tumor-free mice**, after **s.c. and intravenous (i.v.) routes** of administration, to inform the safety of T-VEC under conditions that are similar to the planned clinical dosing route **in a study unconfounded by the presence of a tumor.**
  - In two of the pivotal repeated-dose studies, a group of high dose animals was used to assess biodistribution.

# Genetically Engineered (KPC) Mouse Models Resemble Spontaneous Human Pancreatic Adenocarcinoma Pathogenesis



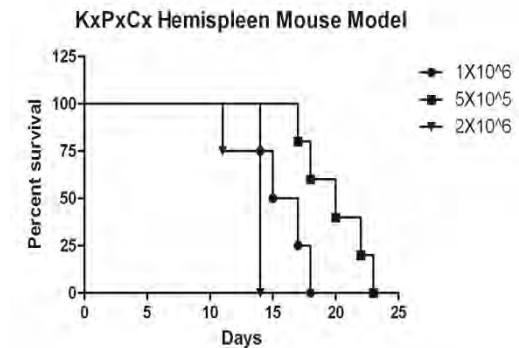
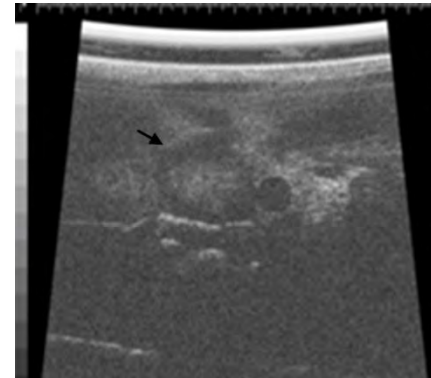
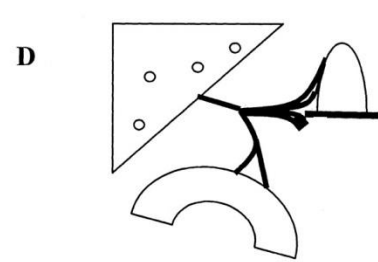
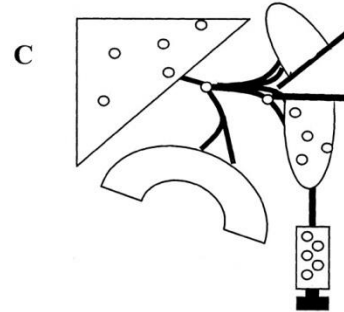
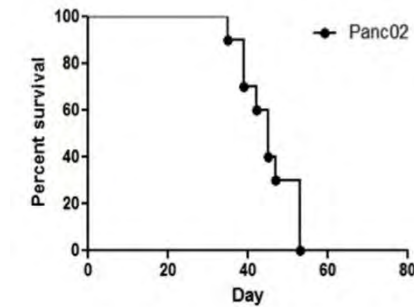
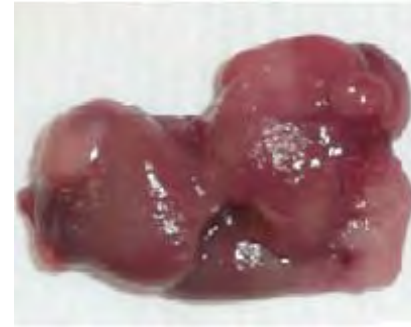
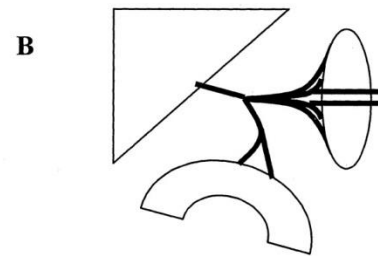
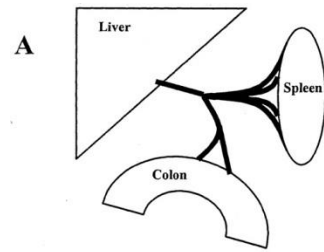
# Orthotopic pancreatic tumor transplant model



- Study uniformly implanted PDA in a heterogeneous pancreatic microenvironment
- Spontaneous metastases to liver, peritoneum and lung

Zheng et al. Plos One 2011; Foley et al. Science Signaling 2015

# Syngeneic Hepatic Metastasis Model by Hemispleen Injection



Jain, Schulick, et al. Annals Surg. Onc. 2003

Soares, Foley, Edil, Zheng, et al. JoVE. 2013

- Spontaneous formation of metastases in the liver microenvironment
- Narrow time window for metastasis formation; suitable for using survival as the endpoint
- Intratumoral injection of therapeutic agents



# Intratumoral injection of an immune agonist under the ultrasound guidance for anti-tumor efficacy, abscopal effect, and toxicity assessment in comparison to other routes of drug administration



Ultrasound measurement of liver metastases



Intratumoral injection

# What can we do?

- Low-level cytokine release in nonclinical animal models should prompt more caution.
- Mild symptoms or non-specific symptoms such as weight loss should prompt pathologic examination of toxicities including autoimmune toxicities.
- Humanized mouse models provide an opportunity of assessing treatment-related toxicities in tumor-bearing mice under clinically relevant conditions
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- Evaluate the biology and expression of the target in the intended clinical population and models: vigorously examine off-tumor target expressions and off target effects in the preclinical model.

# Determine the FIH dose

- Does response in cytokine release
  - Receptors of immune agonists are often only transiently expressed on activated T cells
- In vivo toxicology study
  - Healthy non-human primates have negligible activated T cells due to lack of relevant antigens.
- Receptor occupancy (RO)
  - Relationships between peripheral and tissue RO, between RO and efficacy/toxicity, between tumor-free model and tumor-bearing model are not well established.
- Minimal Pharmacological active dose (MPAD)
  - Difference in MPAD between mouse models and human patients. MPAD in mouse models usually projects to a FIH dose level far from the effective dose in human patients .

# Summary

- Species relevance is an issue, but not the main issue. Neither in vitro nor in vivo cytokine release assay is predictive; however, low-level cytokine release in nonclinical animal models should prompt more caution
- Increase experimental sampling to detect for the presence of biochemical autoimmunity and assess the autoimmune adverse reactions by histologic examination
- Academic tumor models, particularly the spontaneous tumor models and humanized mice provide the opportunity of assessing the toxicities with clinically relevant conditions and routes of drug administration.
- Evaluate the biology and expression of the target in the intended clinical population and tumor-bearing preclinical models
- Combine multiple measurements to determine the FIH dose. No one size fits all.



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## **Panel Discussion:**

### **Moderator:**

Haleh Saber, PhD

### **Panelists:**

Marcela V. Maus, MD, PhD

Sarah Javaid, PhD

Gregory L. Beatty, MD, PhD

Lei Zheng, MD, PhD



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## **Speakers:**

Danuta Herzyk, PhD

Helen Haggerty, PhD

Robert Li, PhD

Mariam Eljanne, PhD



# ANIMAL MODELS IN IMMUNO-ONCOLOGY DRUG DEVELOPMENT

Danuta Herzyk, PhD  
Merck Research Laboratories

FDA-AACR WORKSHOP  
SEPTEMBER 6TH, 2018



**MERCK**

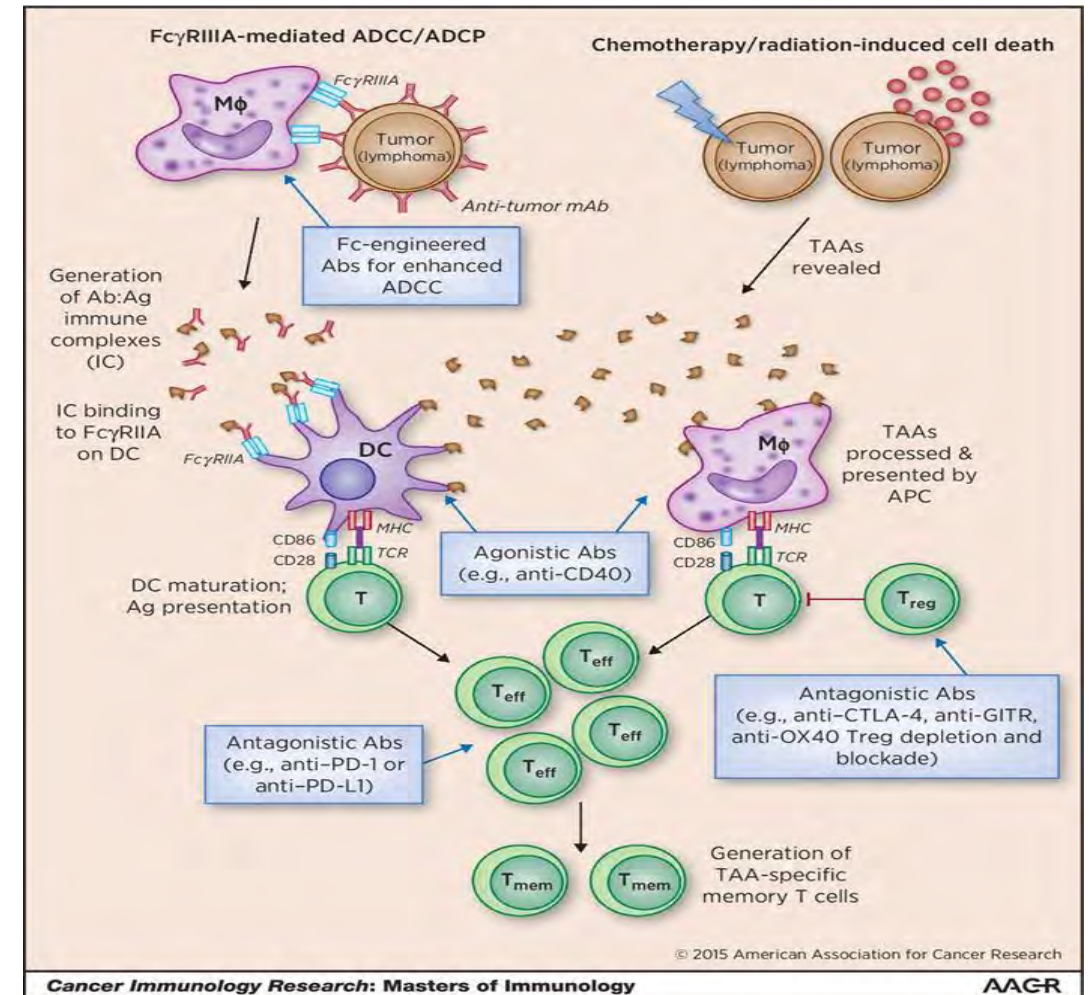
INVENTING FOR LIFE

# Immunotherapy in cancer:

## Mechanisms of Action for efficacy

### vs. immune related Adverse Effects (irAEs)

- **Similar mechanisms contribute to efficacy and toxicity**
- Stimulation of a diffuse T cell repertoire expansion counteracts tumor growth but at the same time reduces self tolerance and can lead to damage of healthy organs



David J. DiLillo, and Jeffrey V. Ravetch 2015

# Clinical safety data for Checkpoint Inhibitors (CPIs)

- **A retrospective examination of data for CTLA4 and PD-1/PD-L1 blockers**  
(Puzanov et al. J Immunotherapy of Cancer, 2017)
- **Main target organs for irAEs:**
  - Skin, gut, endocrine, lung and musculoskeletal (e.g., dermatitis, colitis, hepatitis, hypophysitis, thyroiditis, pneumonitis, inflammatory arthritis)
- The majority of irAEs are **mild to moderate** in severity
- irAEs typically have a **delayed onset and prolonged duration**
- **Greater severity with anti-CTLA4 than anti-PD-1/PD-L1 therapy**
- **Toxicity varies between the adjuvant and metastatic disease settings**
- **Cancer type may be a factor for triggering certain irAEs**, e.g., for pembrolizumab pneumonitis is reported chiefly in NSCLC, while uveitis is reported chiefly in melanoma

# Perspectives from FDA on non-clinical data to support Immuno-Oncology (IO) Biologics

- A retrospective examination of toxicology studies submitted to the INDs in support of FIH studies indicated that **toxicities in monkeys were minimal and did not predict adverse effects in patients** (Saber, Gudi, et al. 2016)
- However, not all IO agents are “equal”
  - Targeting **co-stimulatory / co-inhibitory receptors** requires concurrent antigen-specific T cell receptor (TCR) signaling for T cell activation (e.g., *checkpoint inhibitors*)
    - Associated with low incidence toxicity (in animals and patients)
  - Targeting **T cells recruitment to tumor cells** by simultaneous binding of tumor-associated antigens and a T cell specific antigen (e.g., CD3) induces T cell activation independent of antigen-specific TCR signaling, and results in strong acute cytokine-mediated responses (e.g., *BITEs, CAR-T*)
    - Associated with potent toxicity (in animals and patients)

# Framing the safety question

- Focus on biologic **CPIs**: “immune stimulation” by restoring active T-cell immune surveillance
- In general, safety risks for immune activating agents are predictable based on MOA associated with pro-inflammatory responses, therefore, immune-related “-itis” in the clinic could be expected, regardless of animal toxicology data
- Still, it is unclear which adverse effects in patients would be of greatest concern [as not predicted by animal studies]
  - MOA-mediated “-itis” in target tissues or some unexpected/unknown toxicities?
- Are the concerns about predicting risks associated with a single IO agent or with IO combination therapies?
  - If the main concern is about combinations
    - Is it for combination of multiple biologic CPIs OR CPIs with small molecule drugs?

## A closer look at animal data with CPIs

Differential toxicity profiles for different CPIs were demonstrated in knockout mice

CTLA4 blockade (single target)	PD-1/PD-L1 blockade (single target)	LAG-3 blockade (single target)
KO mice <ul style="list-style-type: none"><li>• <b>Early onset, lethal autoimmune phenotype</b></li></ul>	KO mice <ul style="list-style-type: none"><li>• <b>Late onset</b>, low incidence of minimal to mild focal lymphocytic cellular infiltrates in multiple tissues (salivary, pancreas, thyroid, lung, heart, kidney, liver, skin and/or adrenal)</li></ul>	KO mice <ul style="list-style-type: none"><li>• <b>Late onset</b>, low incidence of minimal to mild focal lymphocytic cellular infiltrates in multiple tissues (salivary, pancreas, thyroid, lung, heart, kidney, liver, skin and/or adrenal)</li></ul>



# A closer look at animal data with CPIs

Differential toxicity profiles for different CPIs were demonstrated in NHP studies

CTLA4 blockade (single target)	PD-1/PD-L1 blockade (single target)	LAG-3 blockade (single target)
<p>Cynomolgus monkeys</p> <ul style="list-style-type: none"><li>• <b>Toxic at &gt; 10 mg/kg/week</b></li><li>• Pharmacodynamics (PD) “delayed” relative to a dose administration</li><li>• Dose- and time-dependent incidence and severity of diarrhea (leading to moribund conditions) and large intestine inflammation, skin changes</li></ul>	<p>Cynomolgus monkeys</p> <ul style="list-style-type: none"><li>• <b>Well tolerated up to 200 mg/kg/week</b></li><li>• Target Engagement (TE) evident for months after stopping dosing</li><li>• Very low incidence and dose-independent (one or two out of 24-30 exposed animals) findings of mononuclear cellular infiltrates (primarily lymphocytes and/or histiocytes) in scattered organs/tissues similar to the spontaneously occurring changes (background) commonly seen in monkeys</li><li>• Effects (v. low incidence) seen in animals following treatment-free period</li></ul>	<p>Cynomolgus monkeys</p> <ul style="list-style-type: none"><li>• <b>Well tolerated up to 125 mg/kg/week</b></li><li>• TE evident during dosing period</li><li>• Findings minimal but dose-dependent</li><li>• Red skin discoloration (without histologic correlates and reversible): head, axillary regions, thoracic region, abdomen, inguinal region and/or the ventral and/or dorsal surfaces of the hind limbs</li><li>• Very slight and transient decreased albumin concentration and albumin to globulin ratio, increased fibrinogen and increased white blood cell and neutrophil counts</li><li>• Mononuclear cellular infiltrates around occasional vessels of the meninges and/or choroid plexus of the brain that represented a slight exacerbation of background changes commonly seen in monkeys</li></ul>

**Greater toxicity with anti-CTLA4 than anti-PD-1**

*More findings with anti-LAG-3 than anti-PD-1*

# Composite analysis of animal data with CPI combos

Differential toxicity between single and dual CPI blockade was observed in KO mice

CTLA4 – PD-1 dual blockade	LAG-3 – PD-1 dual blockade
<p>cKO Mice (C57BL\6; 8-10 weeks old)</p> <ul style="list-style-type: none"><li>• <b>Increased incidence and severity of lymphocytic infiltrates</b> in multiple tissues</li><li>• Mild to marked <b>lymphoid proliferation in multiple lymphoid tissues</b> (lymph nodes, Peyer's patches) compared to age-matched single KO</li></ul>	<p>KO Mice (C57BL\6; 8-10 weeks old)</p> <ul style="list-style-type: none"><li>• <b>Significantly increased incidence and severity</b> (mild-marked) <b>of lymphocytic infiltrates with changes to parenchymal tissue</b> in thyroid, pancreas, salivary (acinar atrophy); heart (fibrosis, myocardial degeneration) compared to age-matched single KO</li></ul>

# Composite analysis of animal data with CPI combos

## Differential toxicity between single and dual CPI blockade was observed in NHP studies

CTLA4 – PD-1 dual blockade	LAG-3 – PD-1 dual blockade
<p>Cynomolgus monkeys</p> <ul style="list-style-type: none"><li>• <b>Increased incidence and severity of GI and skin toxicities (“colitis and dermatitis”)</b></li></ul>	<p>Cynomolgus or Rhesus monkeys (mAbs or other constructs)</p> <ul style="list-style-type: none"><li>• Similar findings (skin, inflammatory infiltrates in meninges and/or choroid plexus of the brain) to anti-LAG-3 alone but with <b>increased incidence and/or severity</b>, and at lower doses compared to the single agent</li><li>• Findings (consistent with PD-1 blockade) not seen with anti-LAG-3 alone<ul style="list-style-type: none"><li>• One animal (out of 32 exposed) had <b>diarrhea</b> (leading to moribund conditions) ~7 weeks after the last dose received</li><li>• One animal (out of 30 exposed) had <b>inflammatory infiltrates in thyroid</b> (very slight with no signs of any clinical or biochemical alterations) ~8 weeks after the last dose received</li></ul></li></ul>

# What do animal data tell us?

- KO mice data are generally consistent with MOA-related findings
- NHP toxicology data are generally consistent with expected safety profiles
  - Findings related to immune activation driven by MOA
  - Wide range of tissues affected but low incidence and severity of findings (i.e. minimal exacerbation of background inflammation)
    - Similar (?) to clinically observed toxicity profile for single IO agent



# Most Frequently Reported ( $\geq 0.2\%$ ) Serious Adverse Events Considered Drug-Related in Patients Treated with Pembrolizumab (melanoma and lung cancer)

Preferred Term	Reference Safety Dataset for Pembrolizumab	
	n	(%)
Participants in population	2799	
Pneumonitis	44	(1.6)
Colitis	25	(0.9)
Diarrhoea	17	(0.6)
Pyrexia	10	(0.4)
Autoimmune hepatitis	8	(0.3)
Pneumonia	8	(0.3)
Adrenal insufficiency	7	(0.3)
Hyponatraemia	7	(0.3)
Dyspnoea	6	(0.2)
Hyperthyroidism	6	(0.2)
Nausea	6	(0.2)

# What do animal data tell us?

- KO mice data are generally consistent with MOA-related findings
- NHP toxicology studies (without immune stimulation by vaccination) are generally consistent with clinical toxicity profiles
  - Findings related to immune activation driven by MOA
  - Wide range of tissues affected but low incidence and severity of findings (i.e. minimal exacerbation of background inflammation)
  - Differentiation of toxicity between molecules
    - anti-CTLA4 > anti-LAG-3 > anti-PD-1
  - Combined treatments indicate [anticipated] increased toxicity compared to treatments with single agents



## Why are toxicology studies with CPIs viewed as non-predictive for AEs observed in the clinic ?

- Using **conventional risk assessment** established in toxicology, many findings in NHP studies are non-adverse
  - The effects often represent PD response and are not detrimental in the context of animal health status (physiology and organ/tissue structure)
  - It may be difficult to determine a threshold between PD and toxicity
  - Need to pay attention to both adverse and non-adverse findings
- **The immune system is the most heterogeneous organ in both humans and animals**
  - Highly variable individual sensitivity to the modulation of the immune system
  - Additional caveats: different immune status in cancer disease vs. healthy host
    - Vaccination of animals during the treatment with IO agents is postulated as more relevant paradigm
      - Does vaccination decrease or increase variability in response to IO agents?

# Are toxicology studies with IO agents helpful?

- **Animal data**, including the lack of toxicity / NOAEL at the highest dose tested **in relevant species** (when Receptor Occupancy / Target Engagement / Pharmacodynamics is demonstrated), **inform safety** profile of IO agents
  - A wide range of exposure (e.g., 2 logs) in the context of projected efficacious dose/exposure in patients
  - Insights into expected vs. unexpected changes
- FIH starting dose is based on integrative analysis of pharmacology data rather than NOAEL
  - In vitro human functional data (e.g., EC50)
  - Pharmacologically Active Dose in animal models
  - RO / TE / PD data in NHP and/or mouse

# Efficacy studies in tumor-bearing mouse models

- Established syngeneic mouse tumor models are selected based on feasibility and relevance to cancer type in humans
- Tumor growth is rapid with survival rate of ~3 weeks after tumor implantation
  - Mouse models may be useful to evaluate potential antagonistic effects of combined therapies
- In the presence of effective anti-tumor response in mouse models, safety signals (tolerability, body weight) are hardly detectable

# Combination studies of IO biologics

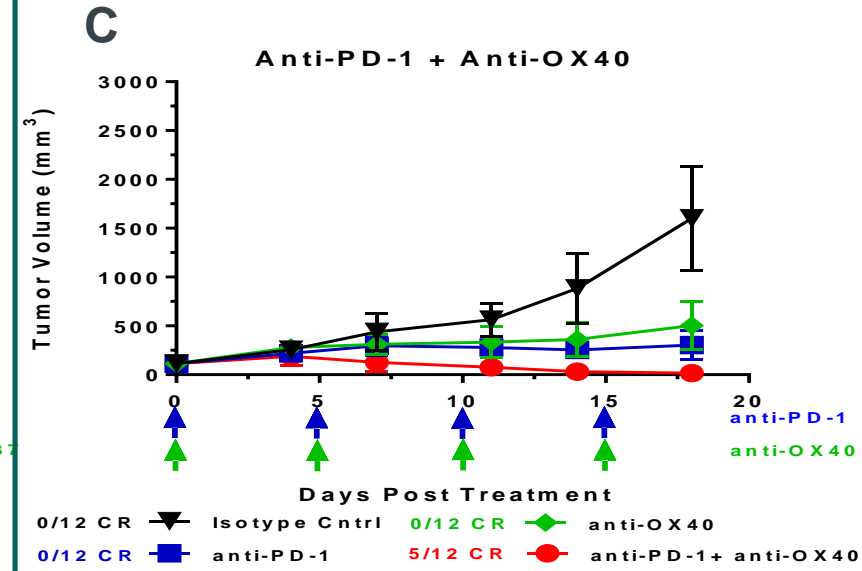
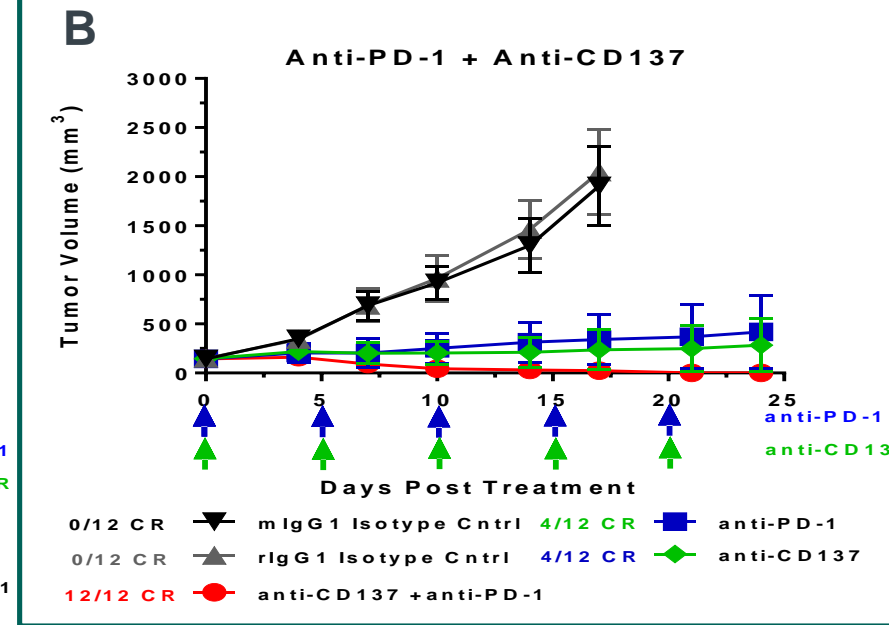
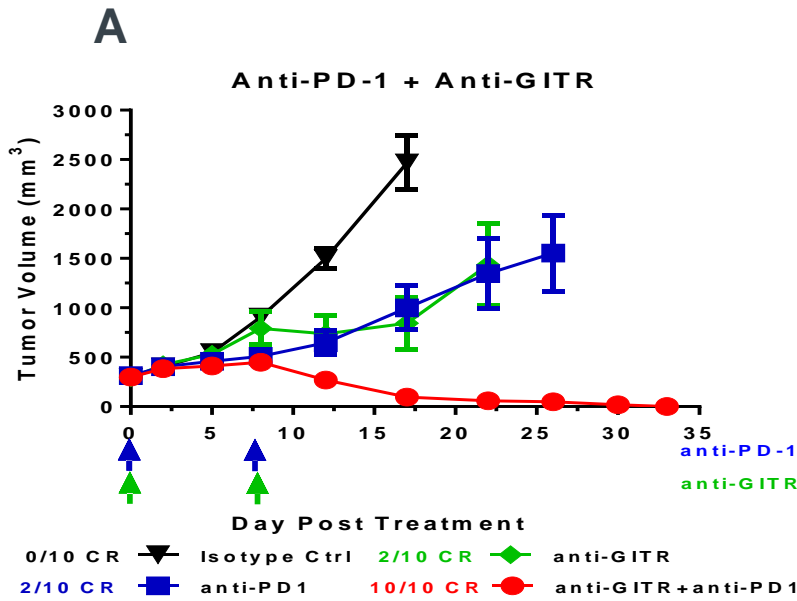


Table 2. Treatment-emergent adverse events in ≥15% of patients

Adverse event <sup>a</sup>	Pembrolizumab (2 mg/kg) + utomilumab (N = 23)			
	Treatment emergent		Treatment related	
	All grades	Grade 3-4 <sup>b</sup>	All grades	Grade 3-4
Fatigue	10 (43.5)	1 (4.3)	8 (34.8)	0
Rash	10 (43.5)	0	8 (34.8)	0
Cough	8 (34.8)	0	1 (4.3)	0
Decreased appetite	7 (30.4)	0	3 (13.0)	0
Nausea	7 (30.4)	0	3 (13.0)	0
Constipation	6 (26.1)	0	1 (4.3)	0
Pruritus	6 (26.1)	0	5 (21.7)	0
Pyrexia	5 (21.7)	0	3 (13.0)	0
Vomiting	5 (21.7)	0	1 (4.3)	0
Anemia	4 (17.4)	3 (13)	0	0
Dyspepsia	4 (17.4)	0	2 (8.7)	0
Upper respiratory tract infection	4 (17.4)	0	0	0

<sup>a</sup>None of the patients discontinued due to treatment-related adverse events.

<sup>b</sup>Treatment-related grade 3 adverse events reported in this study included adrenal insufficiency and hypokalemia (n = 1 each).

- No increased toxicity for anti-PD-1 combos with anti-GITR, anti-CD137, or anti-OX40
  - ✓ Consistent with clinical data in patients in early clinical studies
  - ❖ In contrast to data for anti-CTLA4/PD-1 combo

# Combinations of IO biologics with small molecules

- Clinical reports of increased severity and incidence of irAEs compared to monotherapy
  - Grade 1/2 reactions managed with anti-inflammatory agents
  - Grade 3/4 reactions required cessation of treatment
- Examples
  - Ipilimumab + Vemurafenib – Liver (Ribas, 2013)
  - Ipilimumab + Dacarbazine – Liver, GI, Skin (Bondarenko et al., 2011)
  - Pembrolizumab + Dabrafenib or Trametinib - Liver (Internal Communication)
  - Pembrolizumab + Preladenant - Liver (Internal Communication)
- Difficult to recapitulate in animal studies

# Animal studies with IO biologics and small molecules

- In contrast to cancer patients treated with ipilimumab and vemurafenib, **no hepatotoxicity** in **WT C57BL6 Mice** with short-term (2 week)  $R_x$  combination of **murine anti-CTLA4 + vemurafenib**

Study	Group	Vemu $AUC_{0-24}$ $\mu M \cdot hr$	Anti-CTLA4 $AUC_{0-24}$ $\mu M \cdot hr$	Results
#1	Vemu alone	4290	-	No treatment related liver injury or exacerbation associated with anti-CTLA4
	Vemu + anti-CTLA4 IgG1	4899	3650	
#2	Vemu alone	5049	-	No treatment related liver injury or exacerbation associated with anti-CTLA4
	Vemu + anti-CTLA4 IgG2a	4562	2020	
#3	Vemu alone	4711	-	No treatment related liver injury or exacerbation associated with anti-CTLA4
	Vemu + anti-CTLA4 IgG2b	4637	6340	

- In contrast to cancer patients treated with pembrolizumab and preladenant, **no exacerbation of hepatotoxicity** in **Dogs** with long-term (3 month)  $R_x$  combination of **canine anti-PD-1 + preladenant**

	Liver Enzymes (SW 1-13)	Liver Histology (SW 13)
Preladenant alone	AST (2-9X), ALT (4-27X), ALP (5-8X), GLDH (6-78X)	Hepatocellular degeneration and inflammatory changes in 2 out of 3 animals
Preladenant + anti-PD-1	AST and ALT (2X), GLDH (4X)	Hepatocellular degeneration and inflammatory changes in 1 out of 3 animals

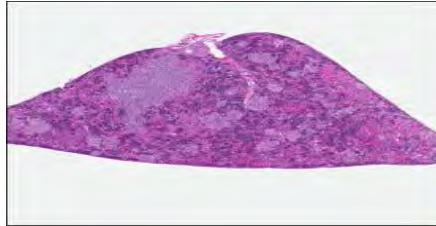


# Do humanized mouse models help?

**Ipilimumab 10 mg/kg (single dose IP) and Vemurafenib 600 mg/kg/day (14 days PO)  
Combination Study in Humanized Immune System Mice**

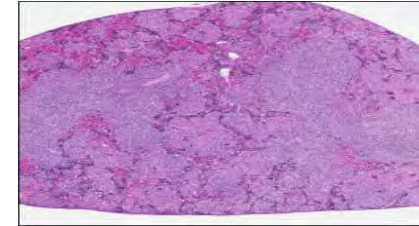
**Ipilimumab**

**Spleen  
(2X)**



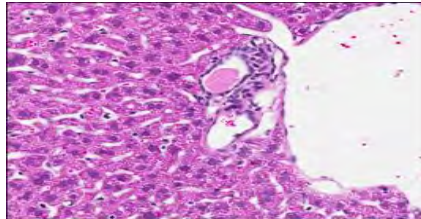
Slight mononuclear cell infiltrate

**Ipilimumab + Vemurafenib**

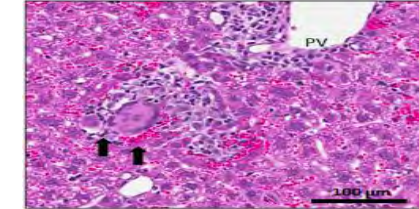


Increased size, marked infiltrate composed of lymphocytes and macrophages with multinucleated giant cells

**Liver  
(20X)**



Minimal, focal infiltrate of lymphocytes in portal region



Moderate infiltrate of lymphocytes and macrophages with multinucleated giant cells

- ✓ Mice engrafted with cord blood derived CD34<sup>+</sup> hematopoietic stem cells
- ✓ No observations in control humanized mice receiving vehicle (14 days PO)
- ✓ No hepatocellular injury but moderate to marked tissue infiltration by immune cells in liver and spleen
- ✓ Lack of injury to lung or GI (indication the absence of GVHD related change)

# Challenges with nonclinical combination studies

## Small molecule (SM) drugs

- Often associated with “traditional” off-target toxicities (liver, cardiac, renal, etc.)
- Metabolized, transported and distributed very differently from biologics
- **Most are characterized in rats and dogs**

## Biologic drugs

- Associated with on-target MOA-related / exaggerated pharmacology findings
- **Characterized in mice and monkeys**

- 
- We thought the problem was that pharmacology combination studies were conducted in tumor-bearing mice or genetically-modified mice (in which toxicity/metabolism profiles of SM drugs are largely unknown, and background histology not understood)
    - *However, anti-PD-1/preladenant study in dogs (the “right” tox species for this molecule) did not increase predictivity...*

## Summary of nonclinical data for CPIs

- **While animal data cannot predict which specific “-itis” cancer patients will develop, they provide insight to signals for potential risk.**
- In patients, individual genetics, underlying disease, co-therapies and previous therapy history (e.g., radiotherapy) likely contribute to the incidence and/or type of irAEs that manifest, or accelerate the emergence of preexisting [asymptomatic] conditions.

# High Level View

- Safety evaluation of IO agents in toxicology studies provide “directional” risk assessment rather than prediction of adverse effects in patients.
- There are many examples of correctly identified risk based on the directional evaluation as well as a few examples of unpredicted toxicity in the clinic.
- The main gap in data is for IO Biologic - SM combinations, for which severe/acute toxicities in the clinic appear to be more likely than for IO Biologic combinations.

# THANK YOU!

FDA – BioSafe - AACR Organizers

AND

*My Colleagues:*

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- *Rene De Waal Malefyt*

*Nonclinical Safety Assessment*

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- *James Monroe*
- *Michael Oropallo*
- *Claudette Fuller*
- *Paul Ciaccio*
- *Valerie Hamilton*
- *Judith Prescott*
- *Frank Sistare*

**MERCK**



# Use of Antigen Challenge in Non-Human Primate to Assess Pharmacodynamic Activity and Translation to Clinic

Helen G. Haggerty, PhD  
Bristol-Myers Squibb



# Immune system of healthy monkeys is not that of tumor bearing mice nor cancer patients

- ❑ Many mAbs to checkpoint inhibitors and costimulatory agonists  
NHP only relevant pharmacologic model
- ❑ NHP model most used to assess safety for human dosing, but has under predicted toxicities observed in patients
  - ▣ Healthy animals more quiescent immune system
  - ▣ Many targets transiently expressed upon immune activation
  - ▣ Differences in FcR binding affinities and/or activities between species
- ❑ Toxicities generally on-target, immune-mediated due to pharmacology
- ❑ Therefore, to assess safety, aid in setting safe starting dose and designing clinical trial, it is important to understand the biology of target and predicted pharmacologic dose response in humans



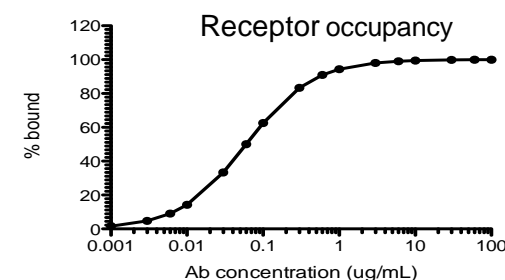
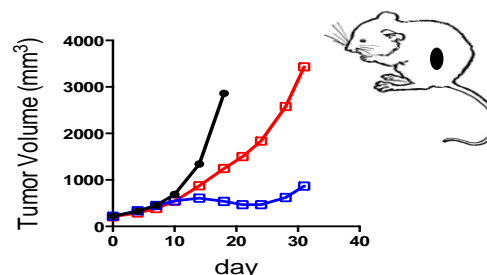
# Determining FIH Starting Dose

- ❑ NOAEL/HNSTD approach often not appropriate
- ❑ Rather established via MABEL or PAD which integrates totality of nonclinical data

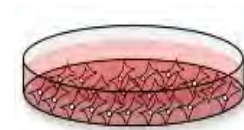
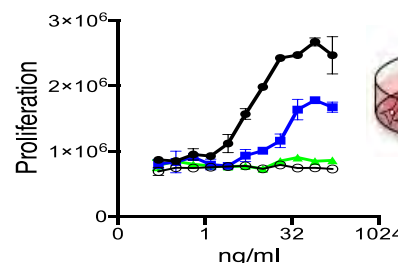
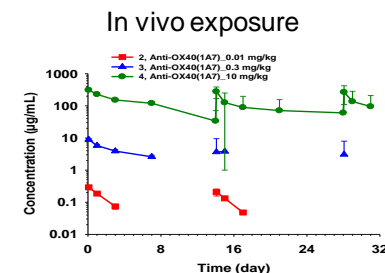
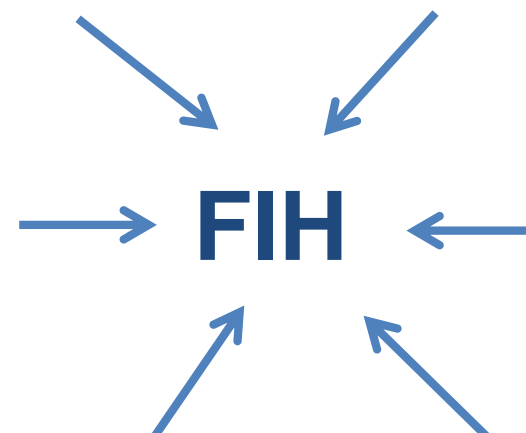
- MOA/pharmacology
- Animals in vitro/ in vivo
- PK/PD modeling
- Receptor occupancy (RO)
- Human In vitro assays

- ❑ Biomarkers of immune activation in NHP

- Improve translatability by correlating exposure, RO, PD activity, and ADA in one test system
- Help establish pharmacologically active dose and dose response
- Support relevance of nonclinical model
- Help identify clinical biomarkers



In vitro cytokine release



Safety



# Assessing Immunopharmacology in Nonclinical Studies

4

- ❑ Efficacy demonstrated in mouse tumor models with surrogate mAbs
- ❑ Tumor bearing monkey models not feasible and immune system in healthy monkey often does not provide the appropriate context to fully characterize IO agents biological activity
- ❑ Immunizing NHP with antigen, monitor alterations in immune response due to treatment and correlate with exposure, receptor occupancy and expression, and toxicity findings



# Antigen Challenge

## T-cell-dependent antibody response (TDAR)

- Immunize with antigen, collect serum, measure antigen-specific antibodies
- Many different immunogens/vaccines can be used to elicit antibody response
  - ▣ Keyhole limpet hemocyanin (KLH)
    - Multimeric immunogenic protein
    - Primary and secondary responses
    - Commonly used to assess immune suppression
    - Suboptimal dose used to assess for immune enhancement
  - ▣ Tetanus toxoid – recall responses
- Responses are CD4-mediated

## Recombinant adenovirus serotype 5 (Ad-5) vectors

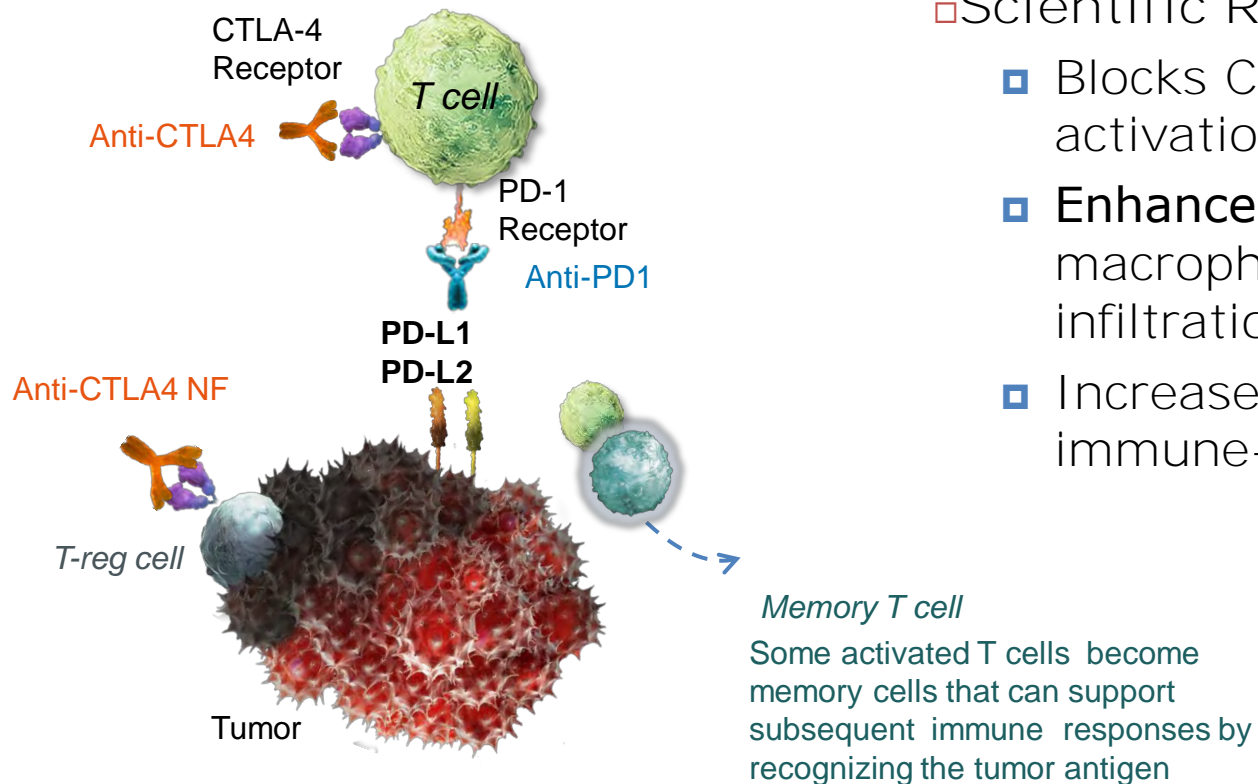
- Cytotoxic (CD8) T cells play a critical role in cancer immunity
- Viral immune responses are CD8-mediated
- Express viral proteins in replication incompetent vector
  - ▣ Adenovirus 5 encoding gag and nef SIV peptides
- MHC haplotyping is required to select for monkeys with specific alleles for recognition of immunodominant epitopes
  - ▣ >80% of Mauritian Cynos express Mafa-A1\*063
- Immunize with viral particles/vector
- Ability to measure antigen-specific T cells using viral peptide tetramer analysis

# Case Study: Anti-CTLA-4 mAb (Ipilimumab) with a non-fucosylated Fc

## CTLA4NF – ipilimumab with enhanced Fc effector function

### Scientific Rationale for Anti-CTLA4-NF:

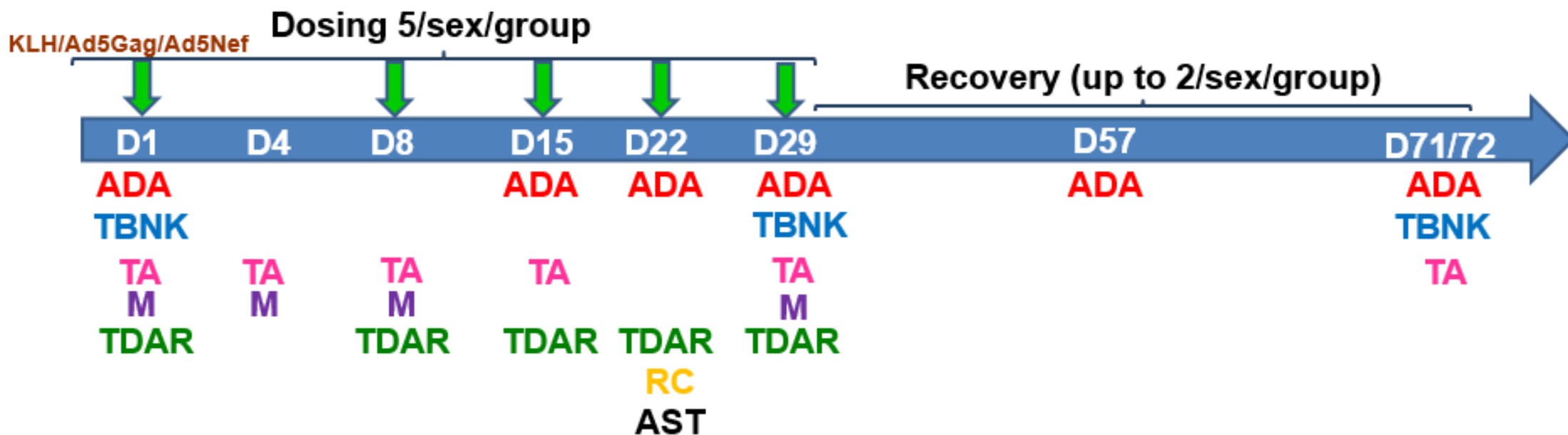
- Blocks CTLA4 similar to IPI to help stimulate T-cell activation/ proliferation
- **Enhanced FcR $\gamma$  (CD16) binding to NK/T cells, macrophages/DC, PMNs to increase immune cell infiltration**
- Increases intratumoral T-reg depletion to reverse immune-suppression



# Monkey Toxicity and PD Study Design

CTLA4-NF mAb: 3, 15, or 75 mg/kg

- ❑ Doses administered weekly to 3-5 monkeys/sex/group
- ❑ 3/sex/group necropsied on Day 30; up to 2/sex/group necropsied after 6-week recovery



ADA = anti-drug antibody, aka immunogenicity

TBNK = immunophenotyping for T cells, B cells, NK cells

M = flow cytometric immunophenotyping for activated/Treg/memory T cell populations

TA = T cell activation (Ki67, CD69)

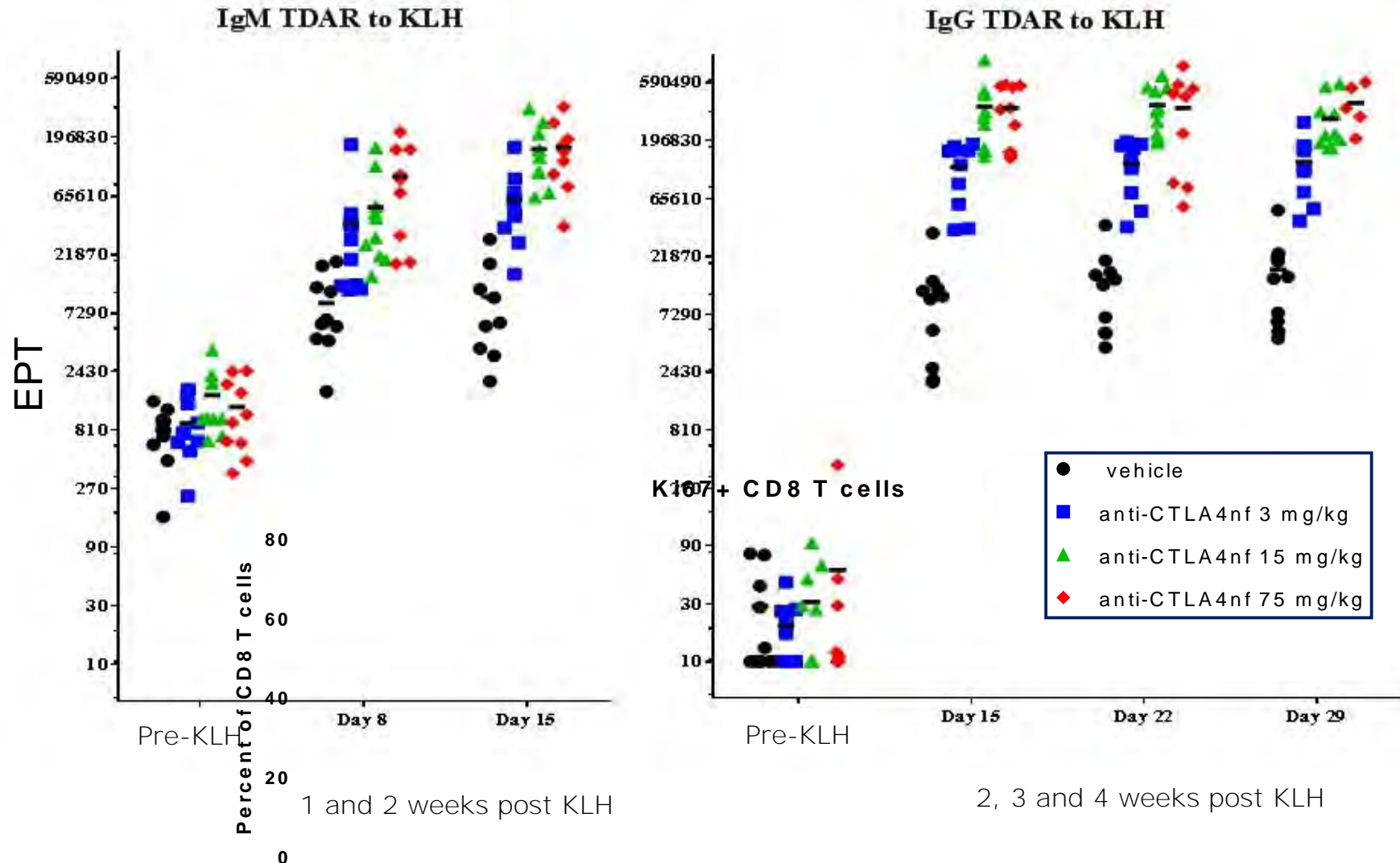
TDAR = T cell dependent antibody response to KLH

AST = Antigen specific T cells (tetramer staining)

RC = Ex vivo recall to KLH, Gag, Nef



# T-cell-dependent Antibody Response to KLH



# Antigen-Specific T-cell Phenotyping

**Ad5 peptide-specific T cells identified using fluorochrome-conjugated MHC tetramers loaded with peptides**

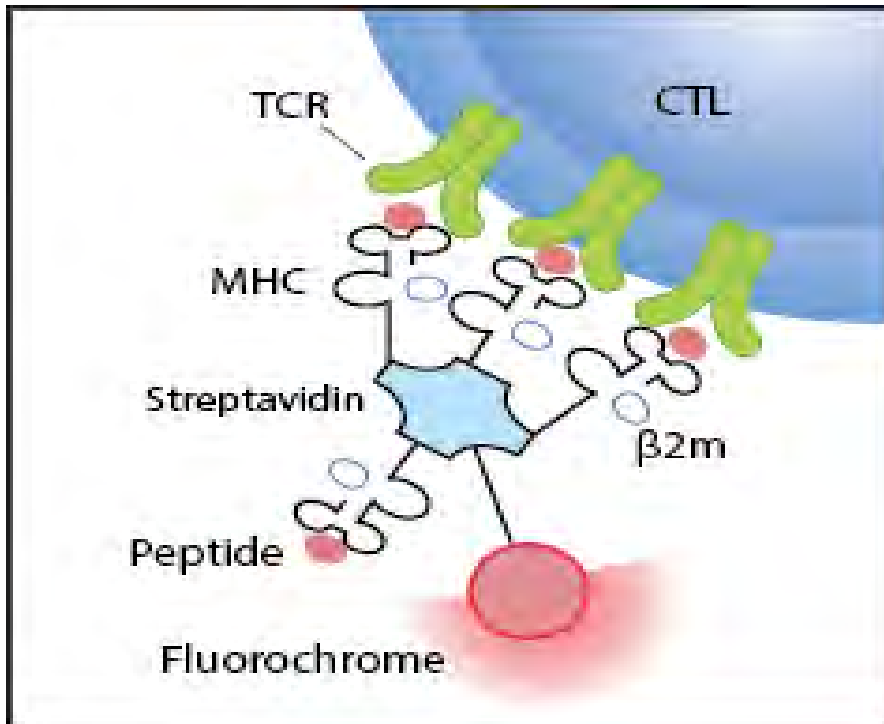


Image from [www.mblintl.com](http://www.mblintl.com)

Immunize with  
Ad5-gag and Ad5-nef



Draw blood in EDTA



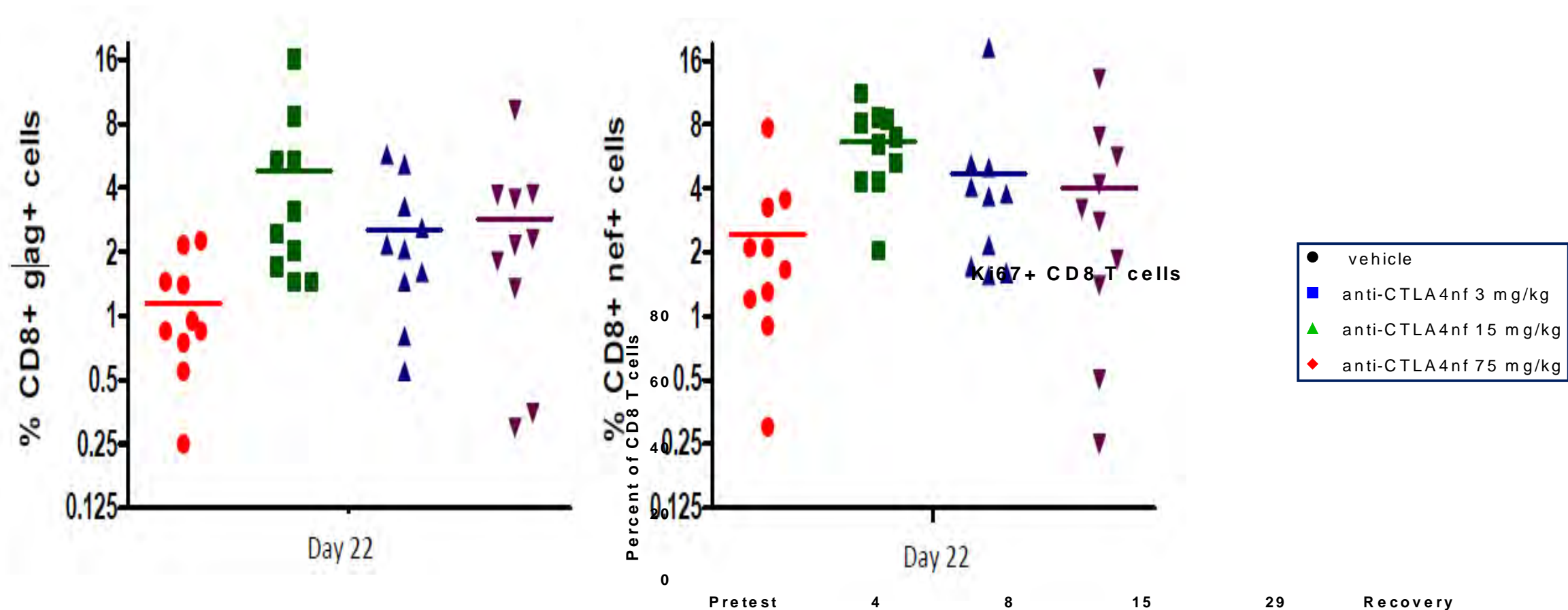
Immuno-stain with T-cell  
specific markers and  
fluorochrome conjugated  
MHC1 tetramers loaded  
with Gag and Nef peptides



Analyze on Flow Cytometer

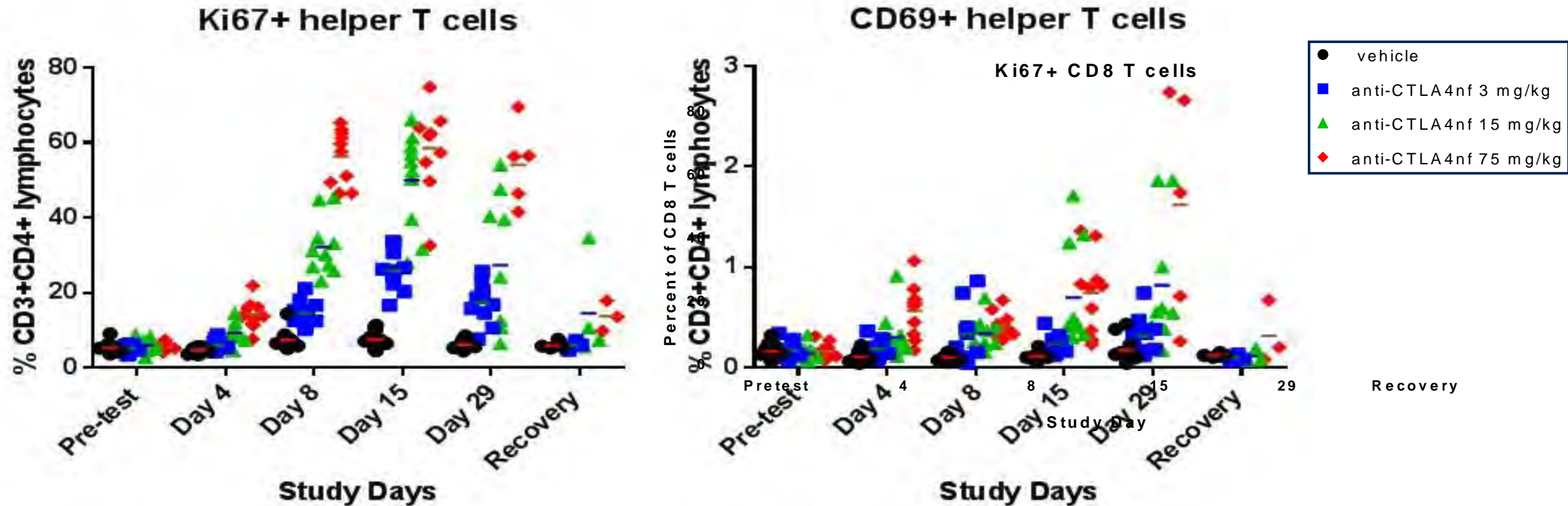
# Ad5-gag and Ad5-nef Antigen-Specific CD8+ T-cell Phenotyping (Tetramers)

Increases in percentage of gag- or nef-specific CD8+ T cells



# CD4 T-cell Activation/Proliferation

Dose-dependent increases in Ki67<sup>+</sup> and CD69<sup>+</sup> helper T cells

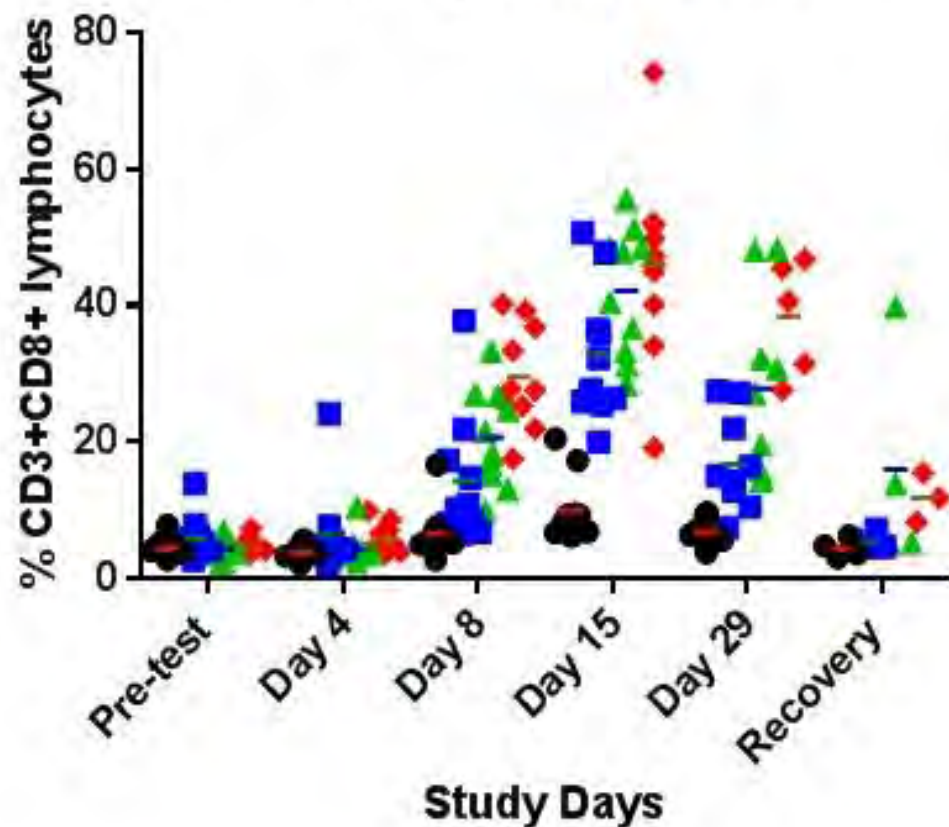




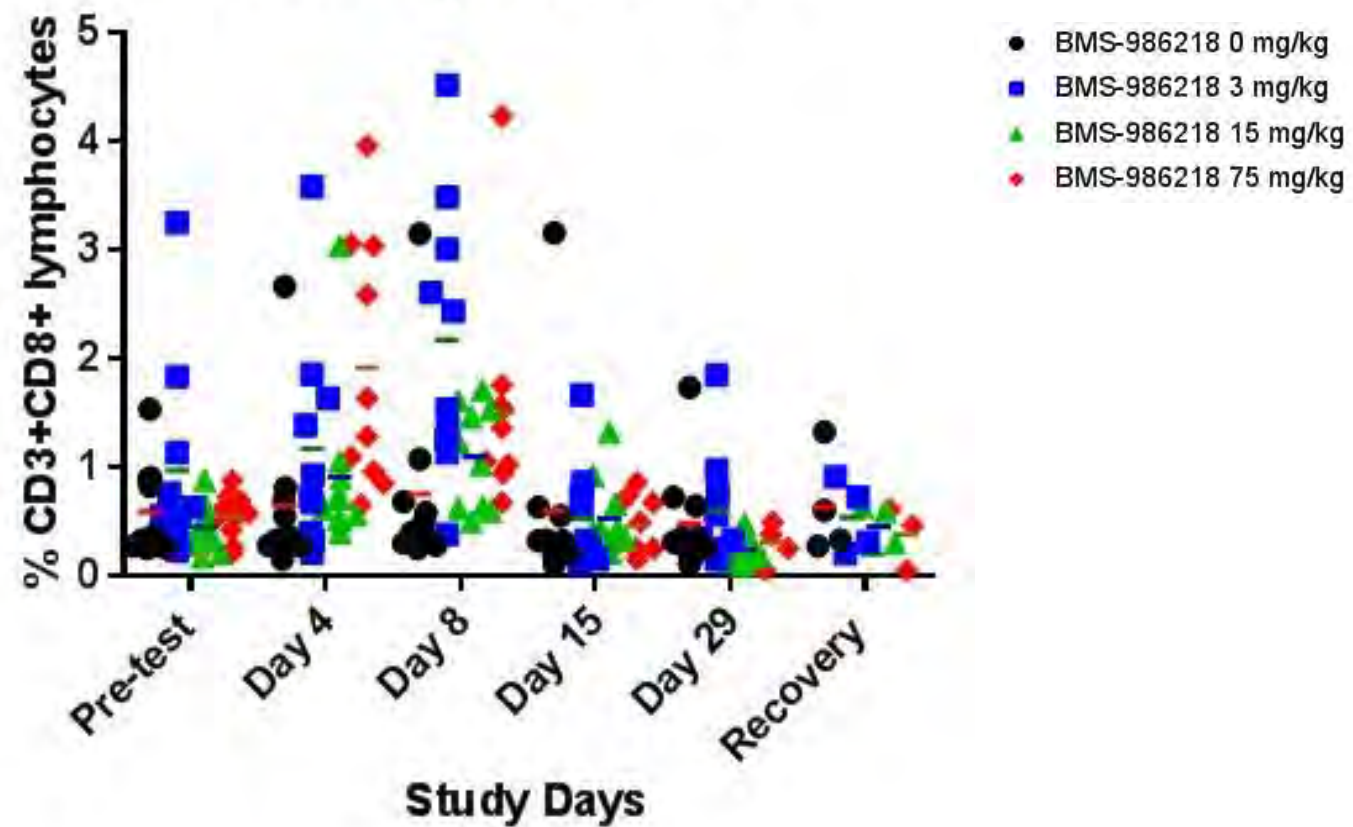
# CD8 T-cell Activation/Proliferation

Increases in Ki67<sup>+</sup> and CD69<sup>+</sup> cytotoxic T cells

## Ki67<sup>+</sup> cytotoxic T cells

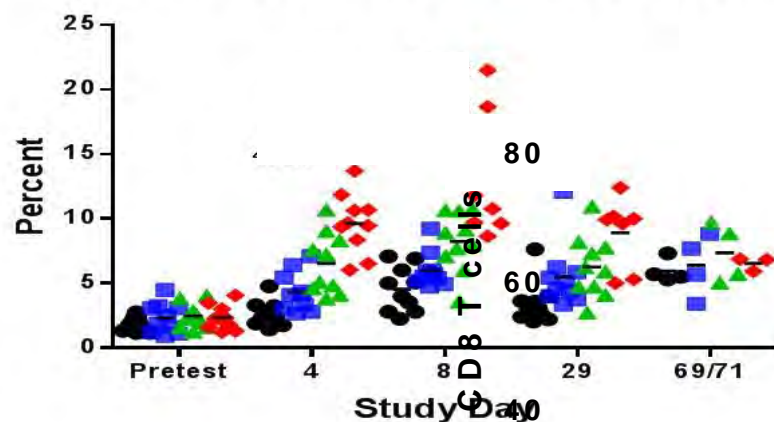


## CD69<sup>+</sup> cytotoxic T cells

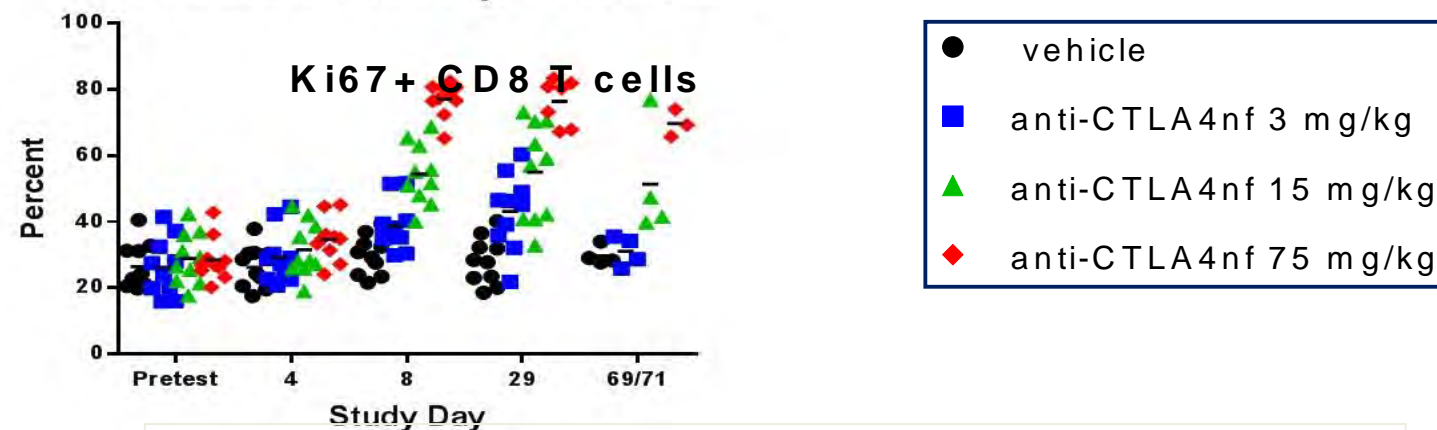


# CD4+ and CD8+ Immunophenotyping

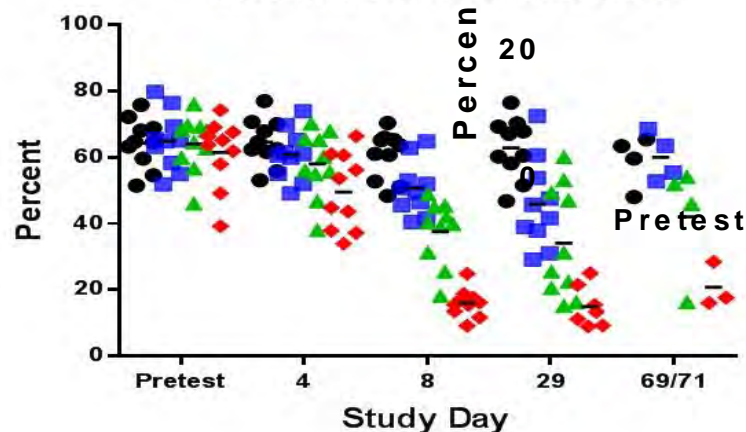
% Activated CD25+ CD4 cells



% Central Memory CD4 cells



% Naïve CD4 cells



Dose-dependent increases in other cell populations assessed including:

□ Tregs and activated CD25+ and central memory CD4+ cells

□ Corresponding decrease in Naïve CD4 cells

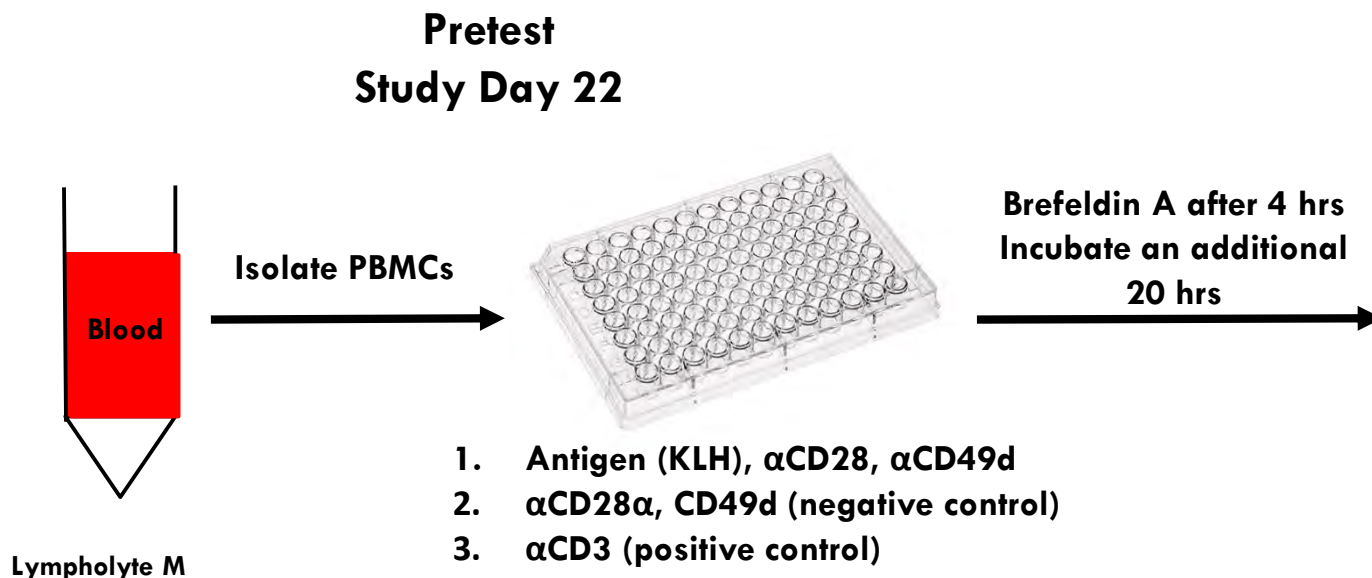
□ Similar changes in CD8+ cell types (not shown)



# Ex Vivo Recall Responses to KLH or Viral Peptides

Activation of antigen-specific cells following ex vivo stimulation with neo-antigen, measured by activation marker (CD69) and cytokine (IFN $\gamma$  and TNF $\alpha$ ) expression

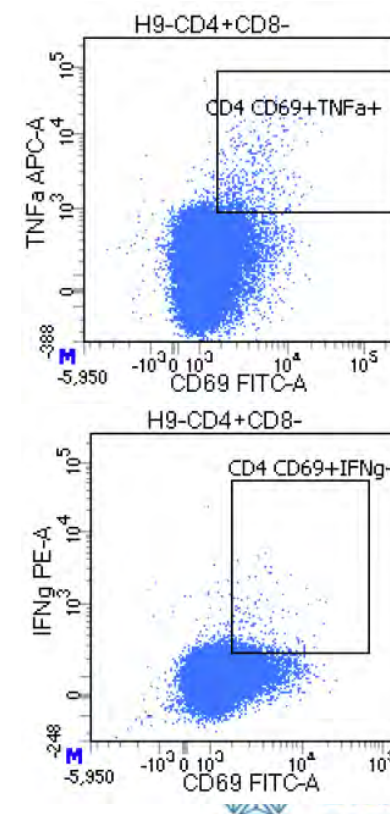
- KLH response CD4-mediated
- Gag/Nef responses CD8-mediated



## Flow cytometric analysis

**CD69, IFN $\gamma$ , TNF $\alpha$**

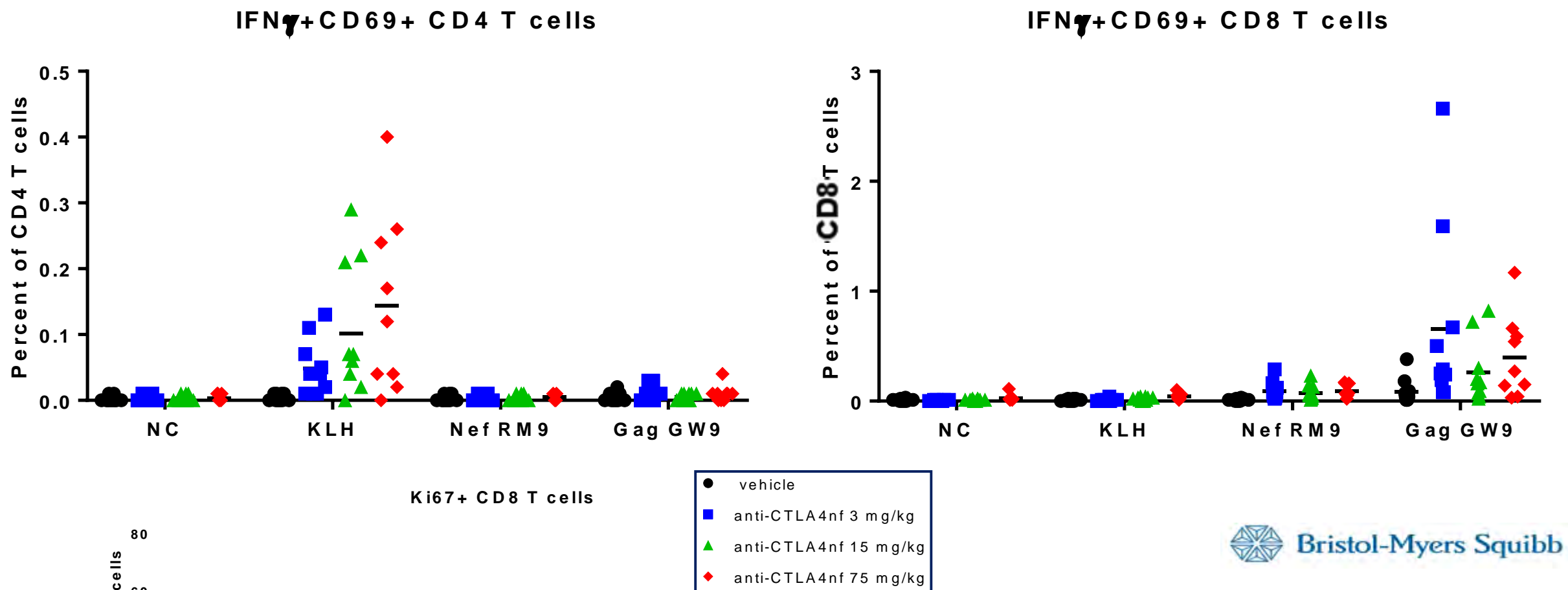
**CD4 T cells**



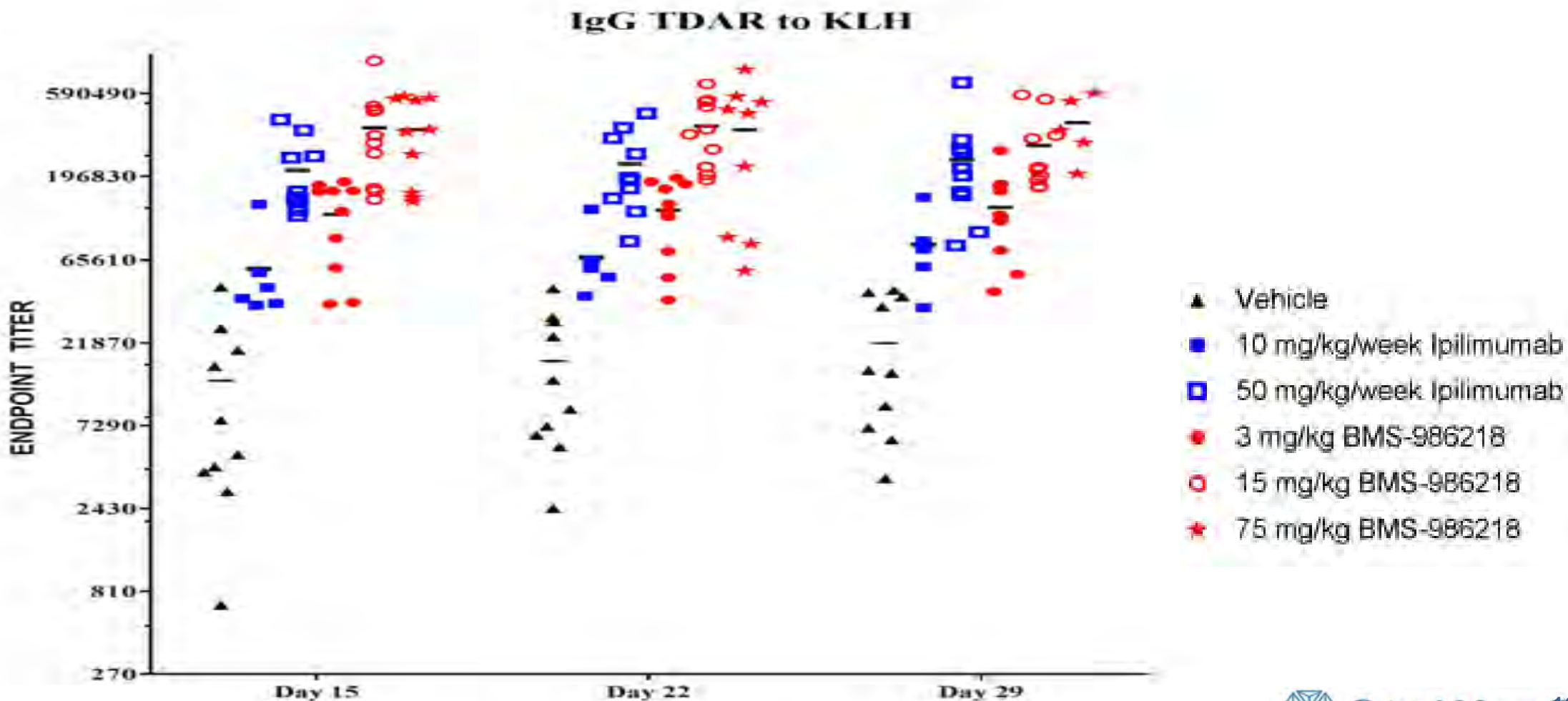
# Ex Vivo Recall Response to KLH and Gag/Nef

20

## Increases in percentages of activated CD4 (KLH) and CD8 (gag/nef) T cells



# Comparison of Ipilimumab with CTLA4NF





# Summary of CTLA4NF Case Study

- ❑ CTLA4NF caused dose-dependent increases in both CD4- and CD8-mediated responses to KLH and gag or nef, respectively, in multiple endpoints
- ❑ Toxicity was observed at all doses
  - ▣ Moderate to severe clinical observations and dose-dependent increase in incidence and severity of lymphohistiocytic infiltration into numerous tissues
- ❑ CTLA4NF enhanced PD and caused adverse findings at lower doses as compared to ipilimumab
- ❑ Impact of findings
  - ▣ PD endpoints enhanced the understanding of Fc on mechanism
  - ▣ FIH dose setting was done based on comparison to ipilimumab, with PD data used as benchmark compared to ipi to gauge differences in potency



# Costimulatory Agonist mAb Examples

mAb	Antigen	PD Response	Impact
1	KLH	Increase in TDAR to KLH Increase in ex vivo recall response in CD4+ Increase in Ki67 CD4+ T-cells	Ki67 T-cell proliferation used as PD marker in clinical trial
2	KLH	Decrease in TDAR to KLH No ADAs, consistent with suppressed TDAR Suppressed ex vivo recall response to KLH	Hypothesis: excess mAb abrogates Fc-mediated cross-linking, which is essential for activation and mAb blocks endogenous ligand binding Greater understanding of biology and impact on dose escalation
3	KLH gag/nef Tetanus toxoid	Loss of receptor expression Decrease in TDAR to KLH Reduction in gag and nef-specific CD8 T cells Decrease or no enhancement in proliferating CD4 or CD8 T-cells, resp. No effect on TDAR to Tetanus toxoid (recall)	Greater understanding of sustained receptor internalization and its impact on biologic activity Impacted clinical trial design with regard to dose escalation

# Conclusion

- ❑ Antigen challenge elicits the immune system allowing for an assessment of immune function
  - ▣ CD4- and CD8-mediated
- ❑ PD assessments when correlated with exposure, RO/RE, and toxicity data have:
  - ▣ furthered our knowledge of biological activity of IO mAb
  - ▣ help to define pharmacologic active dose and dose-response relationship to translate to humans to aid in clinical trial design
  - ▣ helped to demonstrate relevance of model in absence of toxicity
  - ▣ helped to identify clinical biomarkers





# Acknowledgements

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Pharmacokinetics

Toxicology

Veterinary Sciences

## *BMS Discovery, PCO, and Translational Medicine*

Alan Korman

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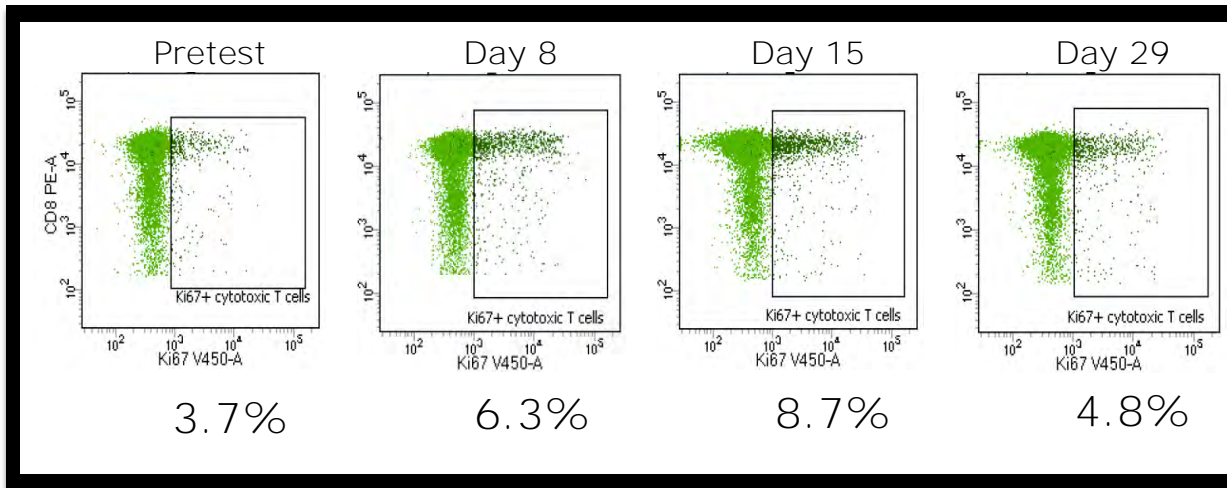
Tox Operations

Immunology Group

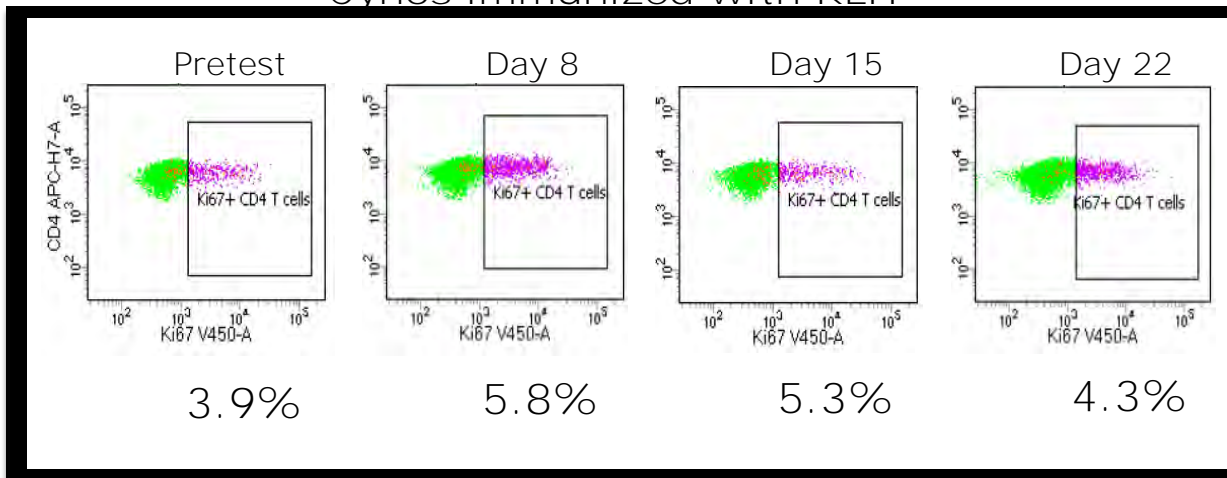
WORKING  
TOGETHER  
FOR *Patients*

# T-cell activation/proliferation

Cynos immunized with Ad5-Gag and Ad5-Nef



Cynos immunized with KLH



- Non-antigen-specific CD4+ or CD8+ T cells
- Nuclear proliferation marker
  - ▣ Ki67
- Cell-surface activation markers
  - ▣ CD69 and/or CD107a
- Multiple timepoints throughout study to assess kinetics of response
- Flow cytometry



# NCI Funding Programs That Support Development of Cancer Models

**Mariam Eljanne, PhD**

**Program Director/Division of Cancer Biology/NCI/NIH**

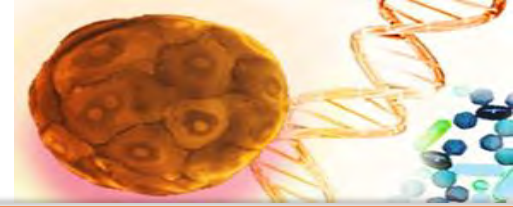
**FDA-AACR Non-clinical Models for Safety Assessment of  
Immuno-oncology Products Workshop  
September 6, 2018**



# **I. Human Cancer Models Initiative (HCMI)**

# **II. Enhancing Applicability of Mammalian Models for Translational Research**

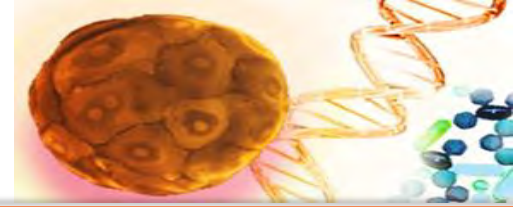
# Modeling the Diversity of Human Cancer: An Unmet Need



- Molecular characterization of cancer has identified cancer-relevant mutations that range from <1% to >50% in population frequency
- Of the ~ 1,000 cell lines commonly used in research
  - ❖ Most cannot be compared to the primary tumor
  - ❖ Clinical and outcome data is not available
  - ❖ Do not represent, or underrepresent certain molecular subtypes (e.g. TMPRSS2/ERG prostate cancer)
  - ❖ Do not represent most pediatric cancer subtypes
  - ❖ Do not represent and underrepresent common combinations of lesions
  - ❖ Certain rare cancers subtypes are either missing or are underrepresented
  - ❖ Do not reflect the ethnic and racial sub-populations
  - ❖ Do not recapitulate the relationships of a tumor and its microenvironment (e.g. stroma, immune cells, endothelium, tumor subclones)



# Human Cancer Models Initiative (HCMI) Consortium



<https://ocg.cancer.gov/programs/HCMI>

**NIH NATIONAL CANCER INSTITUTE**  
Office of Cancer Genomics

PROGRAMS / RESOURCES / NEWS & PUBLICATIONS / ABOUT OCG / DATA ACCESS

Home » Programs » HCMI

**HCMI: Human Cancer Models Initiative**

The Human Cancer Models Initiative (HCMI) is a collaborative effort between the National Cancer Institute (NCI), Cancer Research UK, and the Wellcome Trust Sanger Institute to develop new cancer models that will accelerate research and improve patient outcomes.

**Collaborations**

**Human Cancer Models Initiative**

The Human Cancer Models Initiative (HCMI) brings together world experts to build on scientific advances from the past decade to make approximately 1,000 new cancer models available as a transformative resource for researchers.

**About the Partner:**

The Human Cancer Models Initiative is a collaborative effort between the National Cancer Institute (NCI), Cancer Research UK, and the Wellcome Trust Sanger Institute. Our laboratory collaboration with the Cancer Research UK foundation Hubrecht Organoid Technology (HOT) will genetically characterise hundreds of new cancer models as a transformative resource for researchers.

**Project objectives**

The cancer models will closely mirror the cellular complexity and architecture of human tumours, being developed using new cell culture techniques, such as those that enable efficient production of conditionally reprogrammed cells and organoids. They will be generated using tumour tissue from a variety of cancer types, including rare and paediatric cancers which are often underrepresented in existing cell line collections.

The originating tumour and the derived models will be sequenced, and this information, along with pertinent clinical data, will be available to researchers.

The HCMI will serve as a transformative resource that will empower researchers to study cancer, including tumour heterogeneity, disease progression, mechanisms of drug resistance, and development of precision medicine treatments.

The HCMI is a partnership between CRUK and:

- the Wellcome Trust Sanger Institute
- the US National Cancer Institute (NCI)
- the foundation Hubrecht Organoid Technology

**Testing cancer drugs on mini tumours**

**A NEW ERA OF CANCER CELLS**

Approximately 1,000 human cancer models will be developed over the next decade. These are a useful resource for researchers and will accelerate the development of precision medicine treatments.

**Our role**

We will work with the Wellcome Trust Sanger Institute, and across a number of clinical sites, to develop and validate new cancer models, including organoids and cell lines, with an initial focus on colorectal, pancreatic, and oesophageal models. Patients will benefit from the development of precision medicine treatments.

**INTERNATIONAL COLLABORATION TO CREATE NEW CANCER MODELS TO ACCELERATE RESEARCH**

An international project to develop a large, globally accessible, bank of new cancer cell culture models for the research community launched today (Monday).

The National Cancer Institute (NCI), Cancer Research UK, the Wellcome Trust Sanger Institute and the foundation Hubrecht Organoid Technology are joining forces to develop the Human Cancer Models Initiative (HCMI) which will bring together expertise from around the world to make around 1,000 cancer cell models.

Using new techniques to grow cells, scientists can make models which will better resemble the tissue architecture and complexity of human tumours than the cell lines used today.

Dr Louis Staudt, director of NCI's Center for Cancer Genomics, said: "As part of NCI's Precision Medicine Initiative in Oncology, this new project is timed perfectly to take advantage of the latest cell culture and genomic sequencing techniques to create models that are representative of patient tumours and are annotated with genomic and clinical information. This effort is a first step towards learning how to use these tools to design individualised treatments."

Scientists will make the models using tissue from patients with different types of cancer, potentially including rare and children's cancers, which are often under-represented or not available at all in existing cell line collections.

Dr Ian Walder, Cancer Research UK's director of clinical research, said: "This exciting new project means that we can expand our resources for researchers around the world. We want scientists to have the best resources to be able to easily study all types of cancer. And these new cell lines could transform how we study cancer and could help to develop better treatments for patients."

The new models have the potential to reflect the biology of tumours more accurately and better represent the patient population.

The tumour and the derived models will be genetically sequenced. Researchers will have access to this information, as well as the anonymised clinical data about the patients and their tumour.

The HCMI collaborators aim to speed up development of new models and to make research more efficient by avoiding unnecessary duplication of scientific efforts.

Dr Matthew Garnett, group leader at the Wellcome Trust Sanger Institute, said: "New cancer model derivation technologies are allowing us to generate even more and improved cancer models for research. A concerted and coordinated effort to make new models will accelerate this process, while also allowing for rapid learning, protocol sharing, and standardised culturing methods."

Dr Hans Clevers of the foundation Hubrecht Organoid Technology, said: "We are delighted to take part in this global partnership to make new resources for researchers."

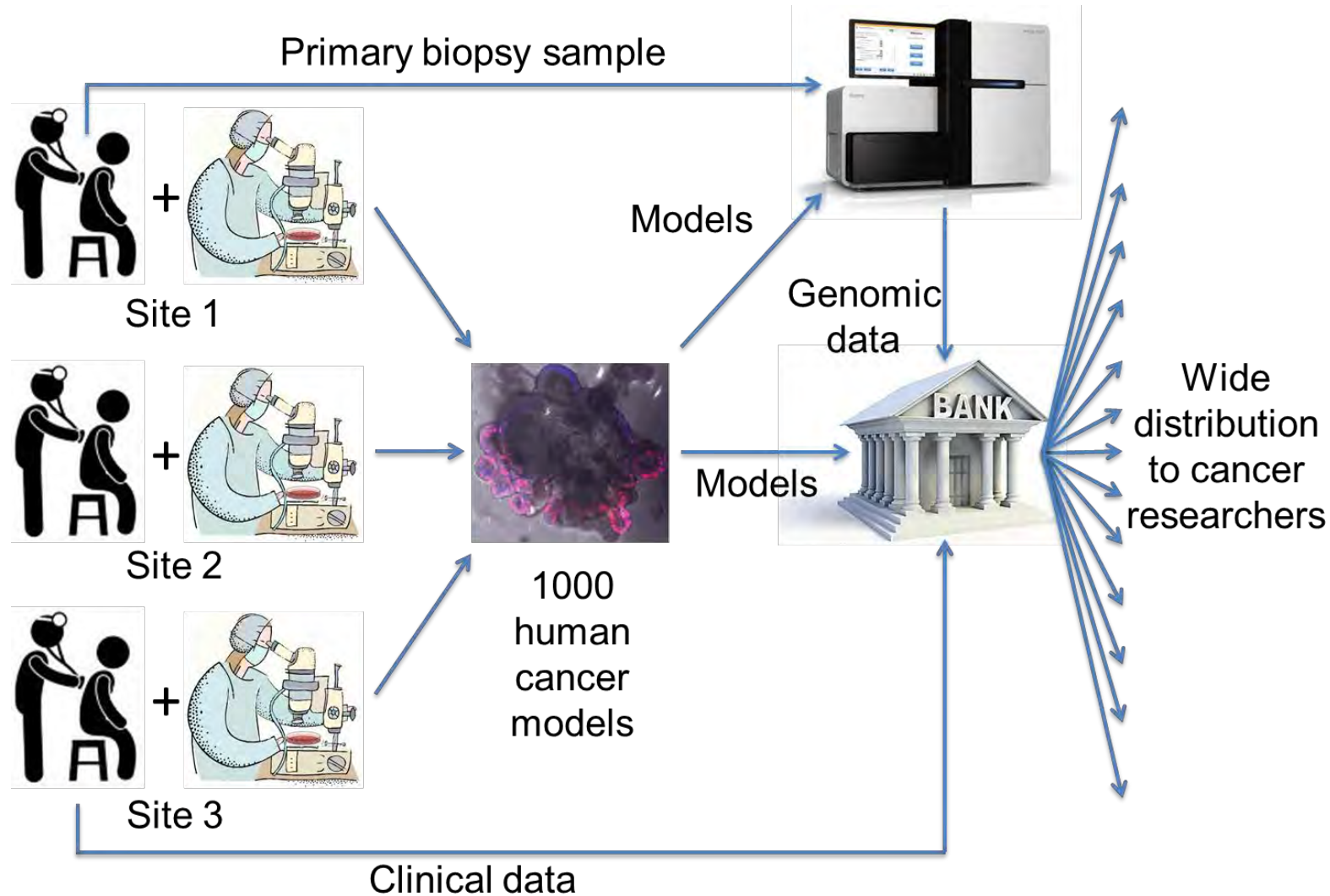
HCMI could transform research and will allow scientists to study many aspects of cellular biology and cancer, including how the disease progresses, drug resistance, and the development of precision medicine treatments.

**Read more**

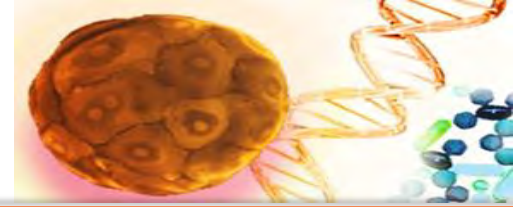
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# HCMI Pilot Design



# HCMI Core Principles



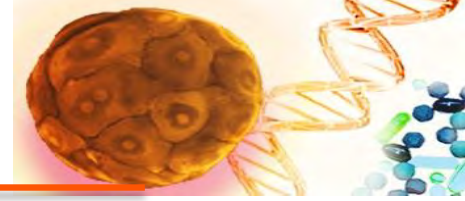
- Open distribution “community resource”
- IP issues identified and addressed for model development and distribution for academia and industry
  - ❖ Informed consent language allows
    - Model development
    - Collection of clinical data
    - Molecular characterization
    - De-linking
    - Use by academia, industry, others
    - All information collected available through Genomic Data Commons (GDC), Office of Cancer Genomics (OCG) web site and European Bioinformatics Institute
  - ❖ Distribution through ATCC
    - Reasonable Material Deposition requirements
    - Reasonable Material Transfer Agreements
- All protocols, when they are developed, will be shared through the OCG web site  
<https://ocg.cancer.gov/programs/hcmi/resources>

# HCMI Core Principles

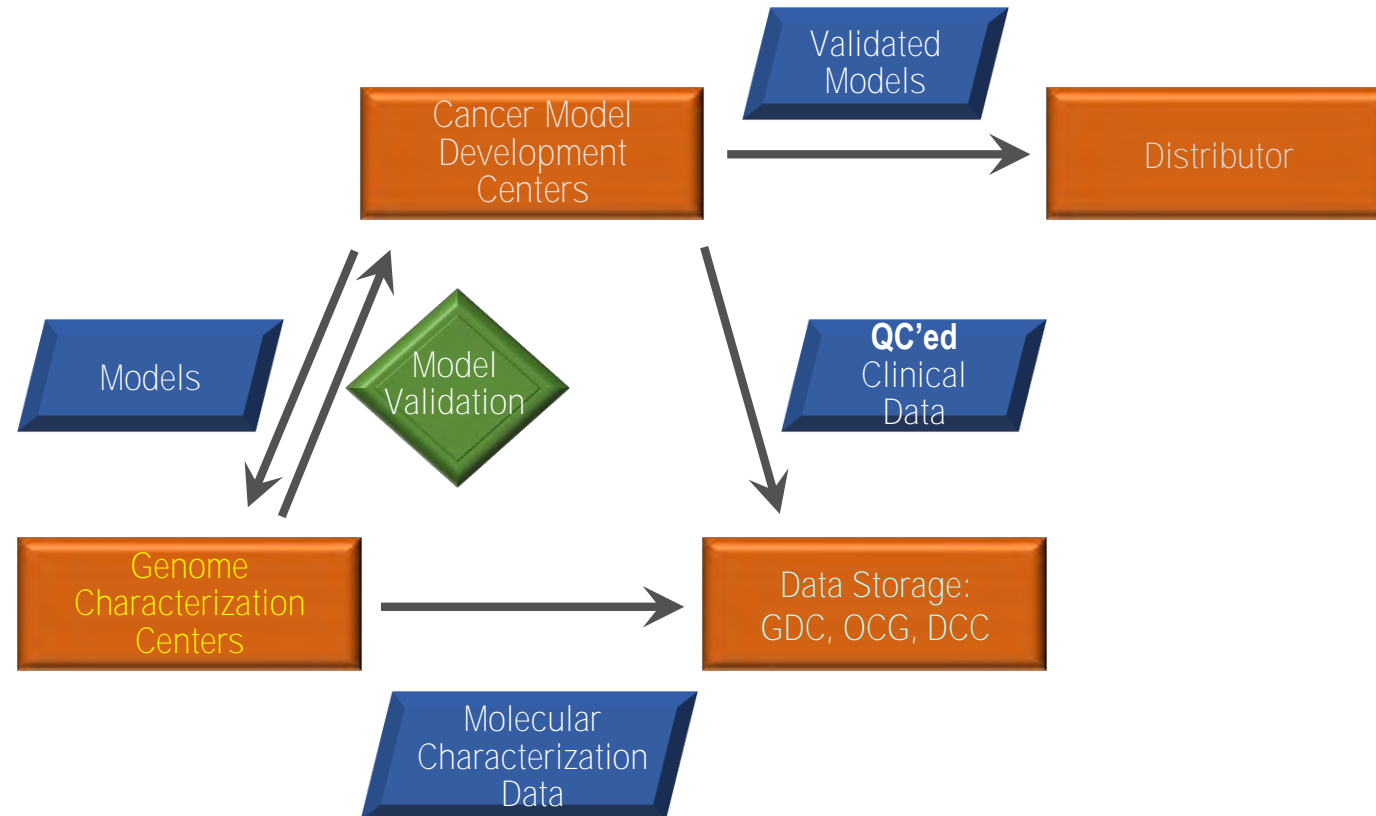


- Molecular characterization [NCI-supported models]
  - ❖ 15X WGS of model, tumor and normal DNA
  - ❖ 150X WXS of model, tumor and normal DNA
  - ❖ RNA-seq of model and tumor RNA
- WSI and HUB will also sequence DNA and RNA, details vary
- Sharing
  - ❖ Molecular characterization and clinical data will be available from the GDC or EBI
  - ❖ Protocols on the NCI HCMI web site and ATCC
- Steering Committee selected 27 data elements which will be part of the **HCMI Searchable Catalog** (NCI subcontract, work in progress)

# Cancer Models Development Process



- Patients are enrolled and clinical data is collected
- Successfully established models are sequenced
  - ❖ Important for model confirmation
  - ❖ These models are submitted for distribution
  - ❖ Outcome data is collected and the case de-linked from primary clinical files
- Molecular characterization, clinical and other data for confirmed cancer models is deposited into the GDC



Rectangle = Process  
Trapezoid = Input/Output  
Diamond = Decision  
Line with arrow = Flow line

# HCMI Pilot: Cancers



- Breast Cancer
- Colorectal Cancer
- Esophageal Cancer
- Gastroesophageal Cancer
- Glioblastoma
- Lung Cancer
- Pancreatic Cancer
- Pediatric tumors
- Rare Cancers
- Upper GI, other
- List of cancers and populations represented is expanding

# Research Projects to Enhance Applicability of Mammalian Models for Translational Research

*PARs 17-244 and 17-245*



# Rationale for PARs 17-244 and 17-245

- Lessons learned from NCI's Mouse Consortium (1999-2014).
  - ❖ Partnerships within and across research communities help to ensure model validity
  - ❖ Multiple viewpoints foster development of better models for specific purposes
  - ❖ Models to explore basic disease mechanisms may not be sufficiently complex to support some translational research requirements
  - ❖ Regular influx of new ideas into oncology modeling accelerates development of a broader spectrum of translational models
  
- Why do we need Oncology Models Forum
  - ❖ Use the Forum as a locus to nucleate cross-disciplinary groups around specific technical, scientific, or informatics challenges to improve or expand translational use of oncology models;
  - ❖ Promote effective communications and collaborations among diverse communities of research, especially those who generate or identify models and those who need to use them for patient benefit
  - ❖ Dissemination of well-validated translational models and their associated data
  - ❖ Data sharing to improve translational model selection
  - ❖ Access to detailed protocols so that model use is robust and reliable

# Purpose of PARs 17-244 and 17-245

Invite applications for projects to:

- Expand, improve, or transform the utility of mammalian cancer and tumor models for translational research.
- Show that translational mammalian models are suitable for use in pre-clinical and co-clinical settings
- Develop and characterize mammals with cancer as representative models of human disease.
- Demonstrate that mammalian models or their derivatives used for translational research are:
  - ❖ robust representations of human biology
  - ❖ Appropriate to test questions of clinical importance
  - ❖ Provide reliable information for patients' benefit
- Demonstrate how to overcome translational deficiencies of mammalian oncology models
- Define new uses of mammalian models or their genetics for unexplored translational challenges
- Advance standard practices for use of translational models
- Test approaches to validate and credential models
- Challenge current practices for how models are used translationally

### ➤ PAR-17-244: Collaborative R01s Projects

- ❖ Up to 5 years
- ❖ Up to \$450,000 direct costs per year
- ❖ Address the technical and experimental parameters that ensure effective translational use of mammalian models
- ❖ Identify and propose the means to tackle unmet translational requirements;
- ❖ Extend the range of insights and approaches that address translational oncology modeling needs
- ❖ Use the Oncology Models Forum for their collaborations, and be active members of the Forum.

### ➤ PAR-17-245: R01 Projects

- ❖ Up to 5 years
- ❖ Up to \$450,000 direct costs per year
- ❖ Narrower scope than a Collaborative R01 team
- ❖ Address one or more of the technical and experimental parameters that ensure effective translational use of mammalian models
- ❖ Identify and propose one way to address an unmet translational requirement
- ❖ If possible, take advantage of the Oncology Models Forum to facilitate collaborations, and participate in the Forum

**Projects that are suitable for this FOA include but are not limited to the examples that follow:**

- Develop and test innovative validation and credentialing strategies for different types of in vivo and in vitro translational models
- Cross-compare various closely related human or mammalian models (e.g., PDXs, and derivative organoids, cell lines, or cell line xenografts, etc.) for what each model type contributes to translationally reliable information for design, testing, or outcome evaluation of chemo- or immuno-therapy or radiation
- Utilize experimental population mammalian genetics to functionalize a cancer GWAS or population study
- Define human genetic determinants of response or resistance to immunotherapy, immuno-prevention, or chemotherapy, as well as risk of adverse events, late effects, and second malignancies
- Create novel models to fill one or more of the critical gaps in translational requirements;
- Derive and test a widely applicable tool strain for oncology modeling or imaging
- Validate and/or credential an existing model or models to enhance the range of translational uses
- Develop and test novel "humanizing" approaches for mammalian models as recipients of human transplants from tumors, metastatic deposits, or early lesions; and/or
- Develop new, reliable standard reagents to advance the existing translational uses of mammalian models, enable new uses, and enable comparisons across species.



oncologymodels.org

OMF Annotated Data Sets Analysis Tools Discussion Publications Funding Links

## Links & Resources

Web Portals	Tool Links	Image Portals
<a href="#">PDXfinder</a>	<a href="#">GenePattern (GP)</a>	<a href="#">The Cancer Image Archive Collections</a>
<a href="#">Alliance of Genome Resources</a>	<a href="#">cBioPortal</a>	<a href="#">European mutant mouse pathology database</a>
<a href="#">Collaborative Cross</a>	<a href="#">GenomeSpace</a>	
<a href="#">ARCHS</a>	<a href="#">OncoScape</a>	
<a href="#">10k-immunomes</a>	<a href="#">ImmGen</a>	
<a href="#">ICGC</a>	<a href="#">Bioconductor</a>	
<a href="#">Repositio.io</a>	<a href="#">NCI CTD2 dashboard</a>	
<a href="#">Immport</a>	<a href="#">Integrative Genomics Viewer (IGV)</a>	
<a href="#">MGI (Jackson Labs)</a>	<a href="#">MMAPPR</a>	
<a href="#">PDQ® Cancer Information</a>	<a href="#">SEER*STAT</a>	
<a href="#">TCGA Encyclopedia</a>	<a href="#">Visual Annotation Display (VLAD)</a>	
<a href="#">NCI CTD2 dashboard</a>		
<a href="#">NCI Dictionary of Cancer Terms</a>		
<a href="#">NCI Biospecimen Collection SOP</a>		
<a href="#">Clinical Data Elements</a>		
<a href="#">COSMIC</a>		

Nancy Boudreau



Questions?



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ADMINISTRATION



American Association  
for Cancer Research

FINDING CURES TOGETHER<sup>SM</sup>

## **Panel Discussion:**

### **Moderators:**

John K. Leighton, PhD, and Julie Schneider, PhD

### **Panelists:**

Alan Korman, PhD

Danuta Herzyk, PhD

Helen Haggerty, PhD

Robert Li, PhD

Mariam Eljanne, PhD