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Humanized mice models of cancer immunotherapy

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Farmington, CT

sitc

Society for Immunotherapy of Cancer

#SITC2016

Presenter Disclosure Information

Karolina Palucka, MD, PhD

The following relationships exist related to this presentation:

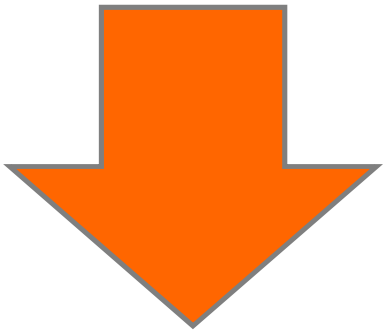
Merck : Consulting, pending grant support

Approach to construction of humanized mice

- Adoptive transfer of human cells
- Transplant of human hematopoietic cells with or without accessory tissues in conditioned immunodeficient host
- Genetic editing of immunodeficient hosts
- Genetic editing of immunocompetent mice

Major Humanized Mice Strain Platforms

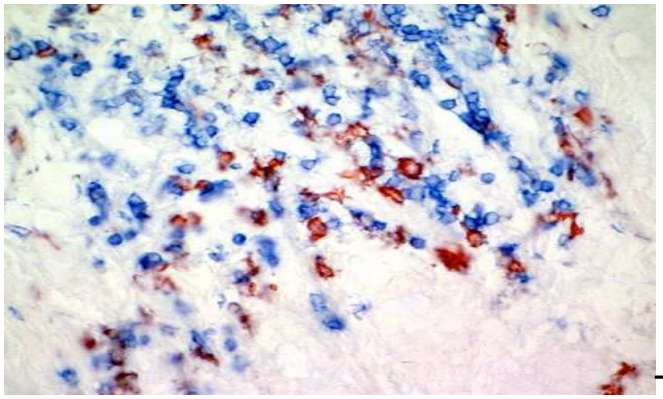
NSG	**NOD-*scid IL2rg*^{null}**	**Jackson Laboratory**
NOG	**NOD-*scid IL2rg*^{Trunc}**	**CIEA (Tokyo)**
NRG	**NOD-*Rag1*^{null} *IL2rg*^{null}**	**Jackson Laboratory**
BRG	**BALB/c-*Rag2*^{null} *IL2rg*^{null}**	**Yale/Univ. Hosp. Zurich**
	”MISTRG” Rongvaux, 2014 *Nat Biotech* 32;364	
H2^{d}RG	**Stock-H2^{d}-*Rag2*^{null} *IL2rg*^{null}**	**Pasteur Institute**
C57BL/6	***Rag2*^{null} *IL2rg*^{null} *CD47*^{null}**	**NIAID/Stanford Univ.**



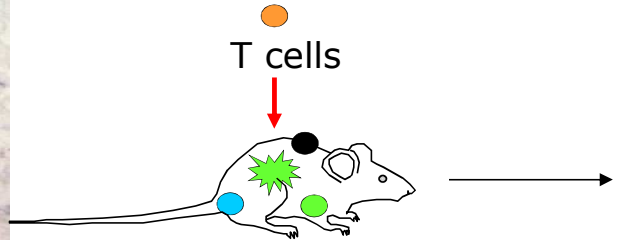
Use cases

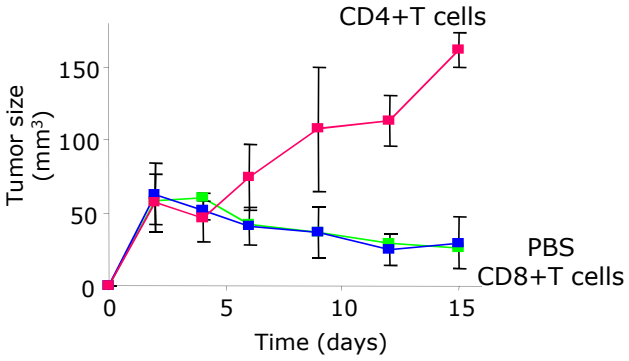
1st generation Onco-Humice

Bell et al, J Exp Med 1999

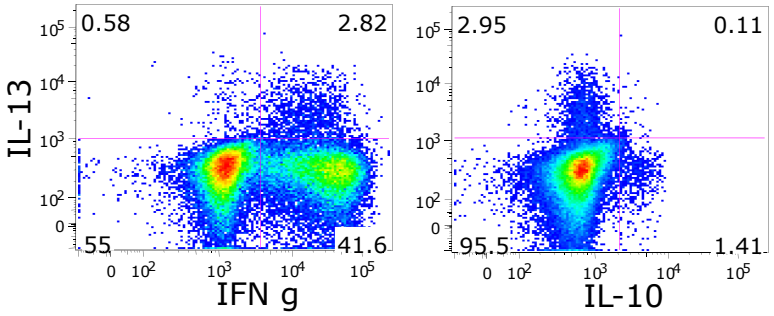


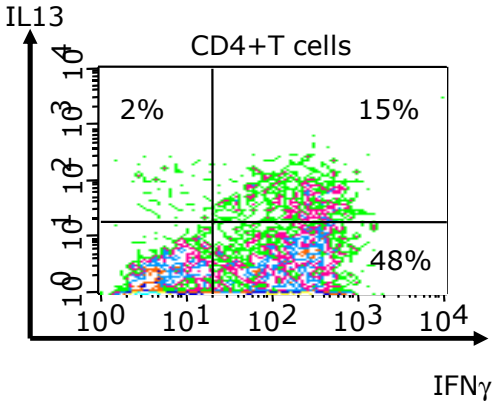
From patients to 1st generation Onco-Humice back to patients

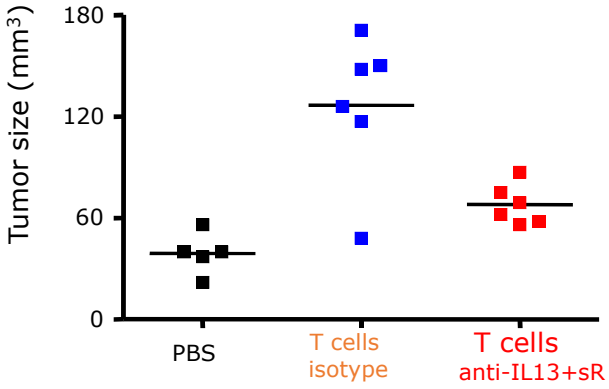




CD4+T cells | **CD83+ mature DCs**

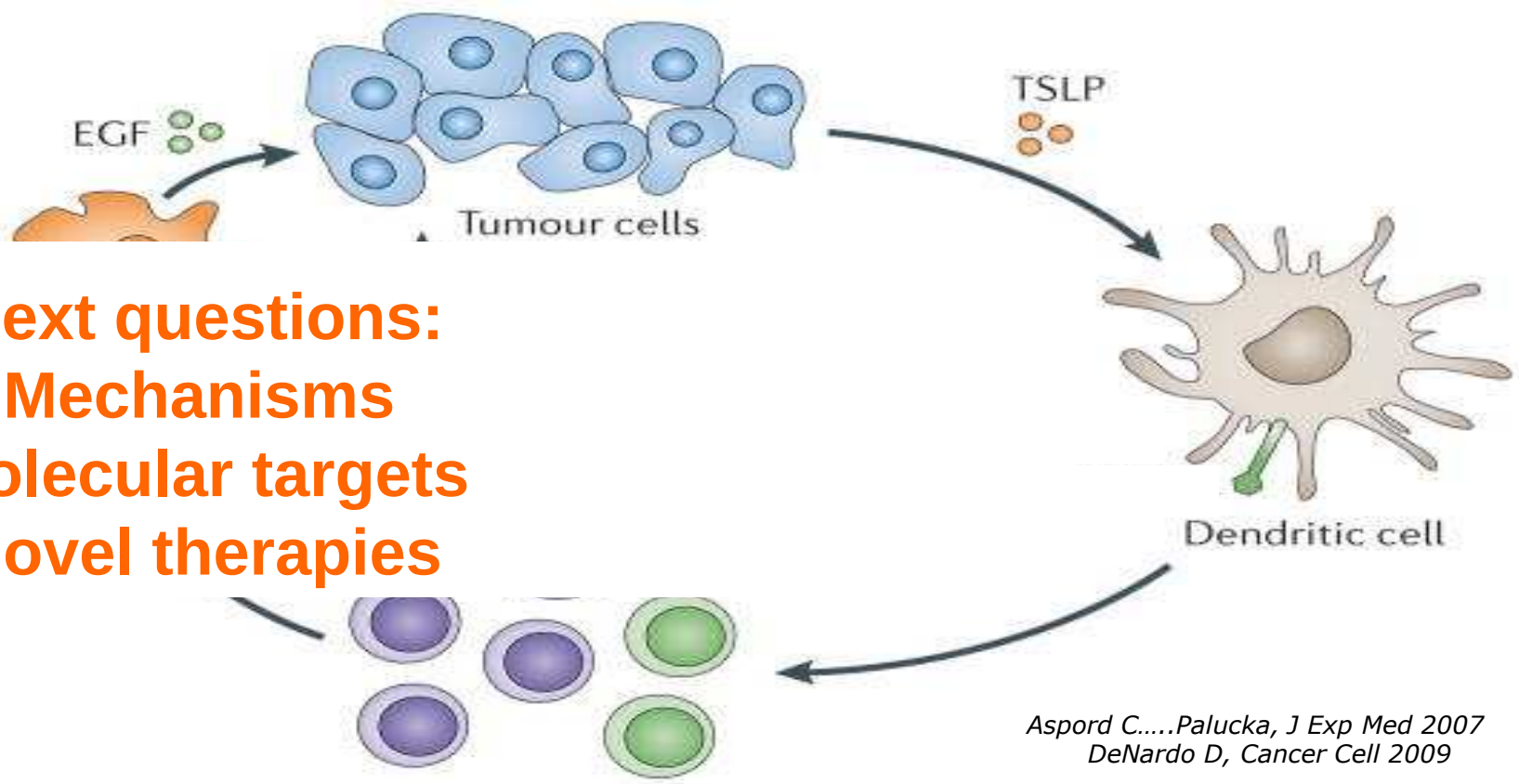






The Jackson Laboratory

Tumor promoting inflammation in breast cancer



Next questions:
Mechanisms
Molecular targets
Novel therapies

Aspord C…..Palucka, J Exp Med 2007
DeNardo D, Cancer Cell 2009

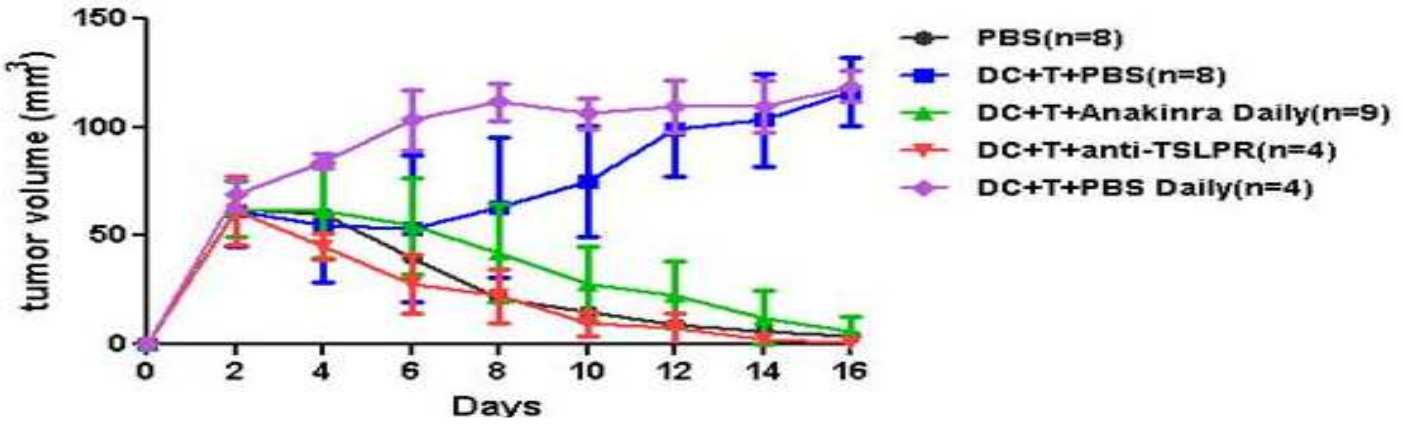
Pedroza-Gonzalez…Palucka, J Exp Med 2011
Coussens, Zitvogel, Palucka, Science 2013

The Jackson Laboratory

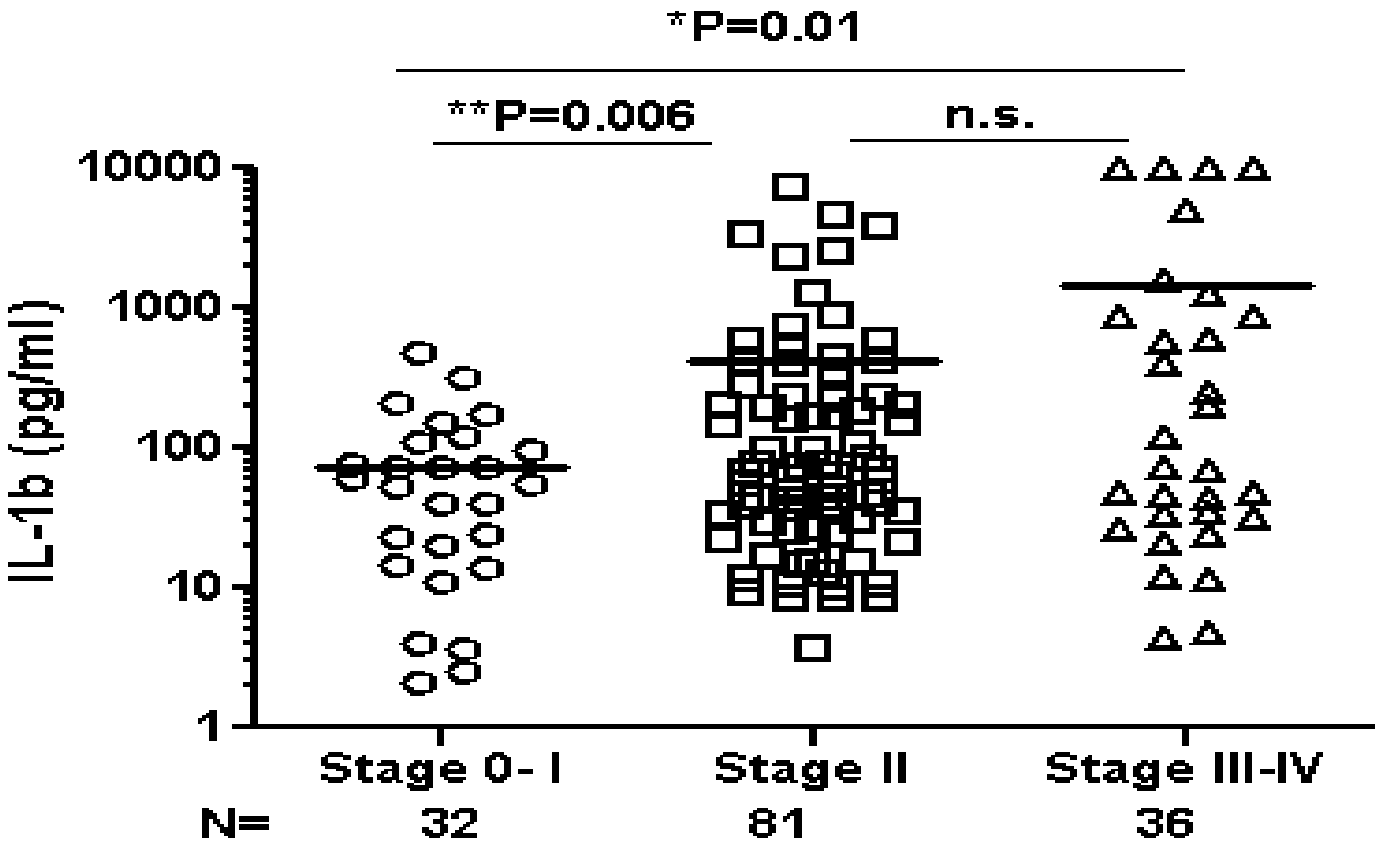
IL1 blockade prevents breast cancer progression

Inject human DCs,
T cells
Plus Anakinra
or PBS daily

Analysis

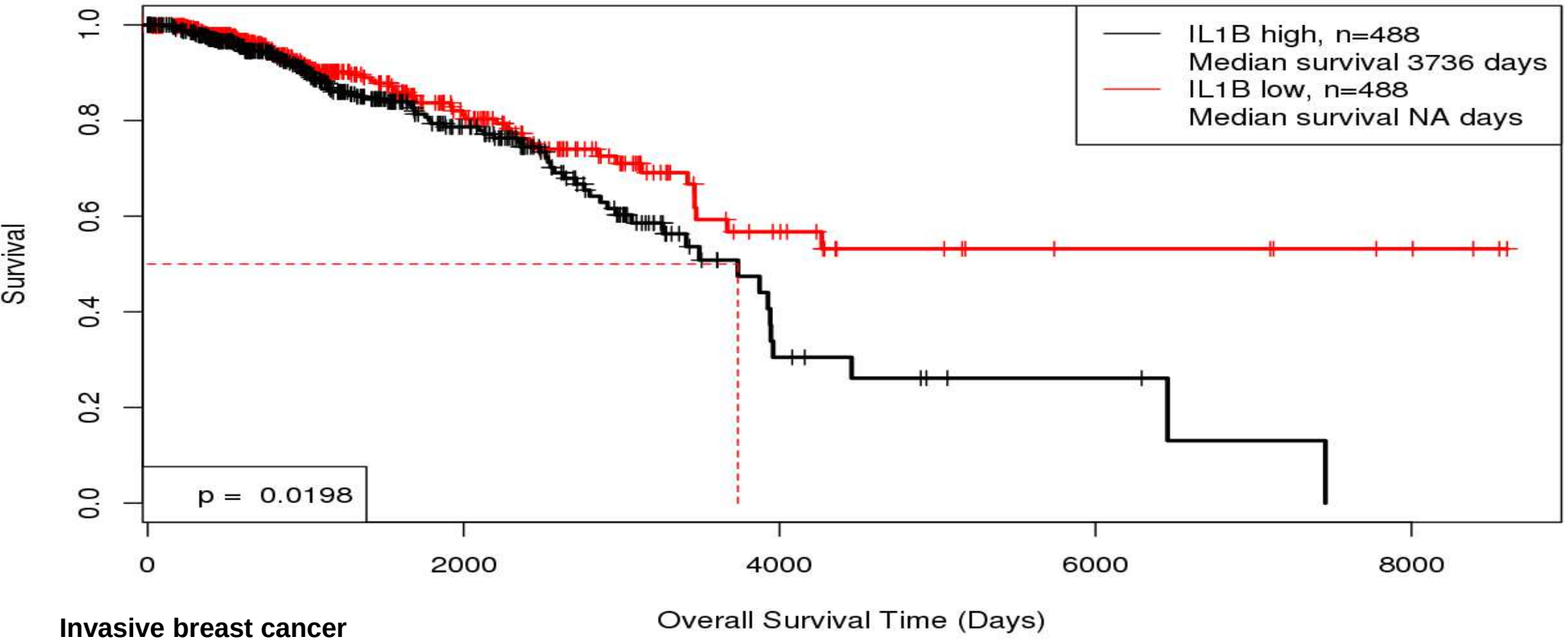


IL1β upregulation in breast cancer is clinically relevant



Connie Xu, Tina Wu

Patients with high IL1β transcription in breast cancer have decreased long-term survival

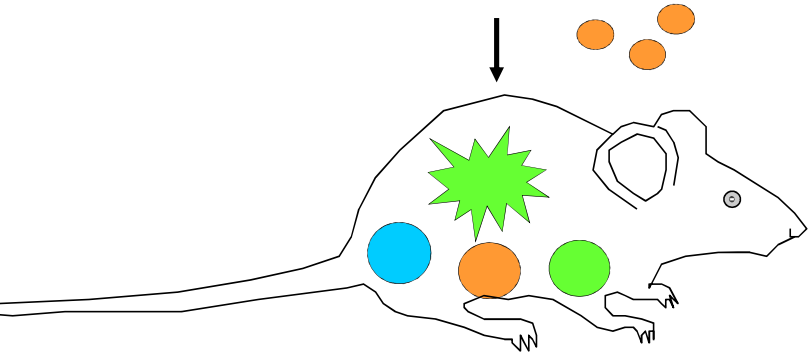


Invasive breast cancer
Diagnosis
Median expression

Tina Wu, Joshy George

Breast Invasive Carcinoma (TCGA, Cell 2015)

Adoptive T cell transfer

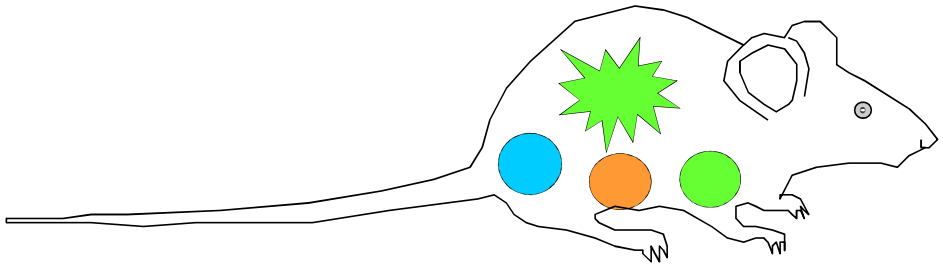


NOD-SCID β2m$^{-/-}$mouse
Adult CD34^{+} HPCs

Palucka et al., Blood 2003
Yu et al., Blood 2008
Yu et al. Immunity, 2013
Graham et al *Vaccine* 2016.

Aspord C, J Exp Med 2007
Pedroza-Gonzalez, J Exp Med 2011
Wu et al, Cancer Immunol Res 2013

Endogenous T cells



NOD-SCID γ c $^{-/-}$ or Rag2$^{-/-}$γ c $^{-/-}$ mouse
Fetal CD34^{+} HPCs &
thymus and liver

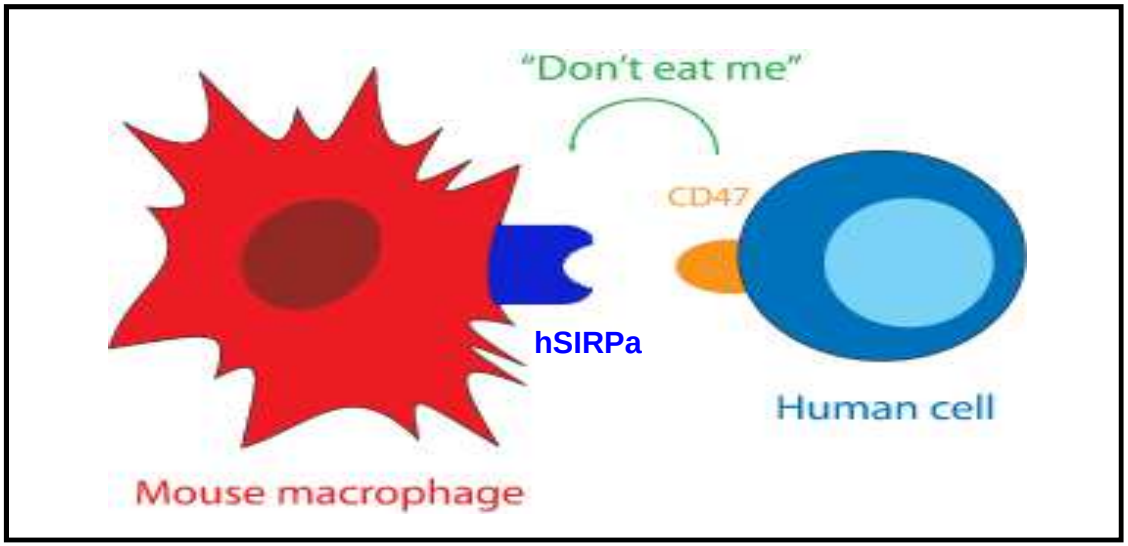
MISTRG: Human M-CSF/IL-3, GM-CSF/SCF/SIRPa/TPO
NSG-SGM3:SCF/GM-CSF, IL-3
DRAG: HLA-DR4
NSG-A2: HLA-A2.1

Traggiai et al., Science 2004
Shultz et al., JI 2005
Melkus et al., Nat Med 2006
Billerbeck et al., Blood 2011
Rongvaux et al, Nat Biotech 2014

2nd generation humanized mice: Genetic humanization of cytokine encoding genes

M-CSF$^{h/h}$
IL-3/GM-CSF$^{h/h}$
hSirpα^{tg}
TPO$^{h/h}$
RAG2$^{-/-}$
IL2RGamma$^{-/-}$

- Myeloid development
- Phagocytic tolerance
- Longterm maintenance of functional HSCs
- Immunosuppression (no mouse T, B, NK cells)

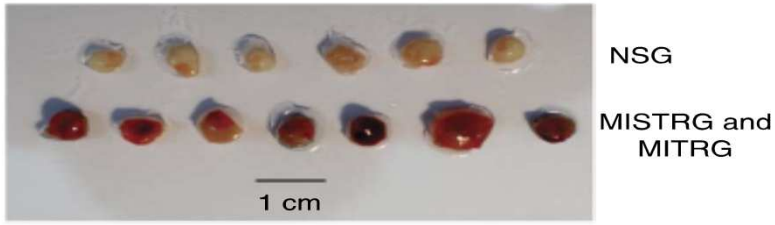


Rongvaux *et al.*, Nat. Biotech., 2014

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

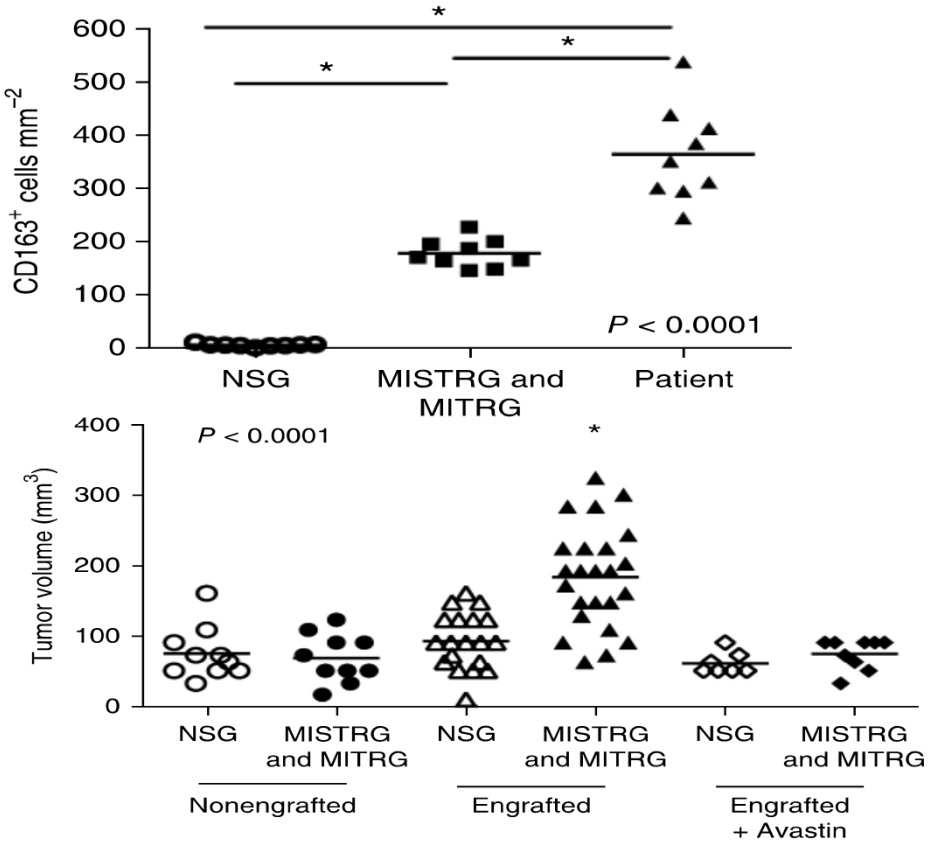
Anthony Rongvaux[1,10], Tim Willinger[1,10], Jan Martinek[2,3], Till Strowig[1,9], Sofia V Gearty[1], Lino L Teichmann[4,5], Yasuyuki Saito[6], Florentina Marches[2], Stephanie Halene[7], A Karolina Palucka[2], Markus G Manz[6] & Richard A Flavell[1,8]

Functional human monocytes and macrophages



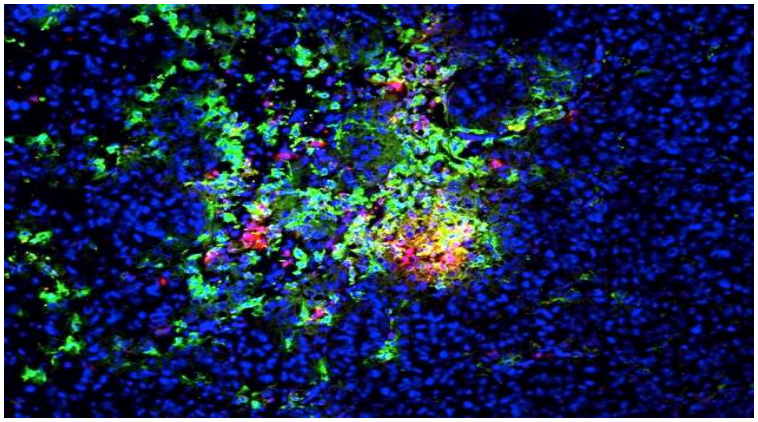
Human melanoma

NATURE BIOTECHNOLOGY ADVANCE ONLINE PUBLICATION
published online 16 March 2014; doi:10.1038/nbt.2858

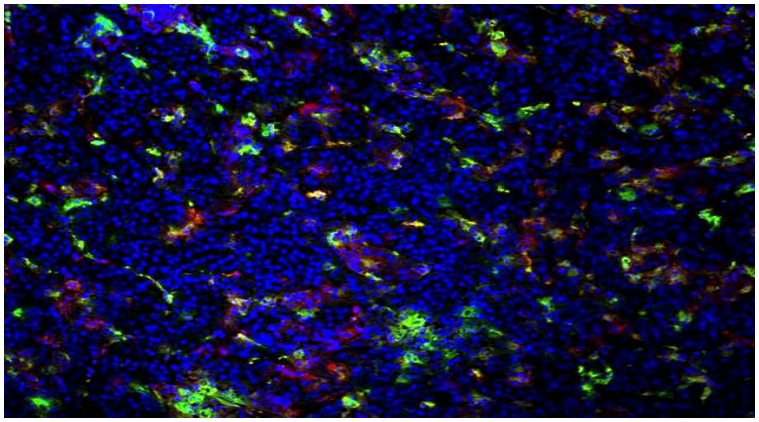


Macrophage infiltrate in experimental and patient tumor

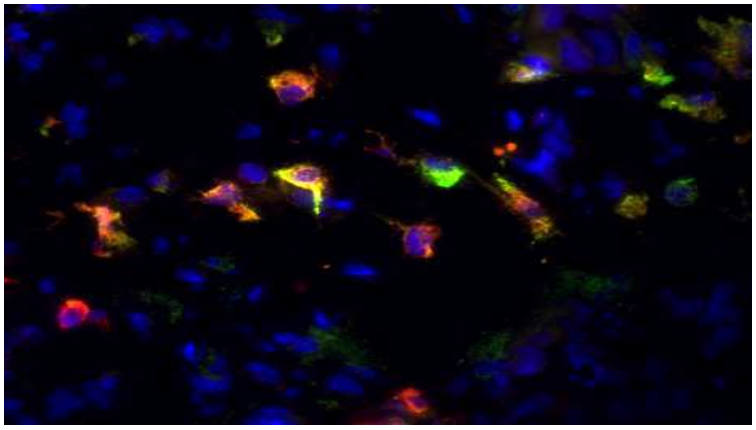
MISTRG

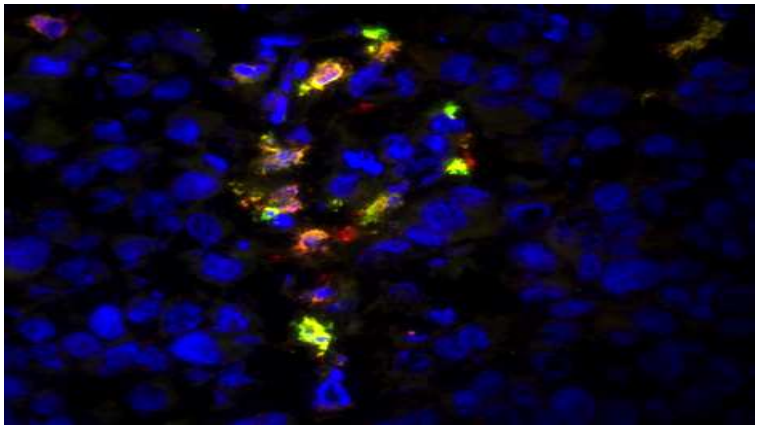


PATIENT



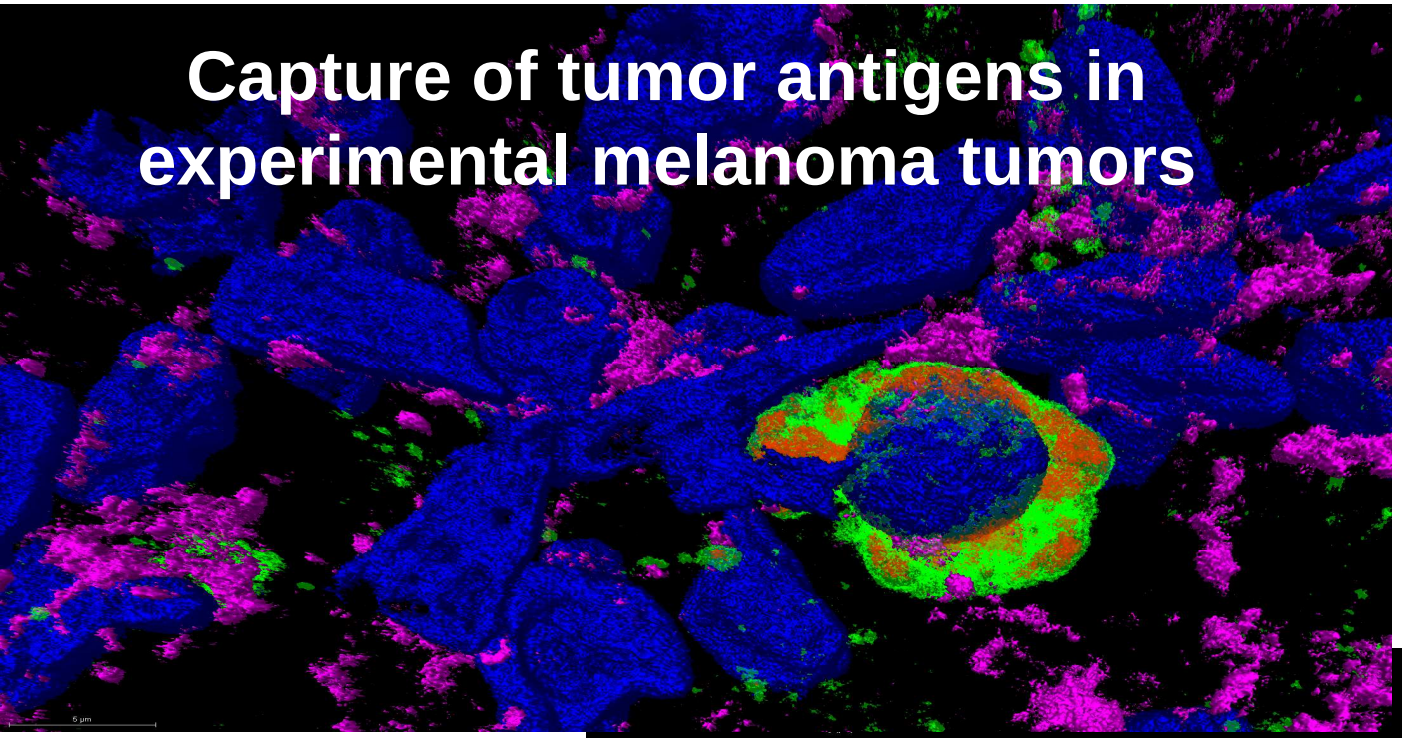
DNA
HLA-DR
CD163





DNA
CD163
CD206

Anthony Rongvaux[a*], Tim Willinger[a*], Jan Martinek et al. Nat Biotechnology 2014

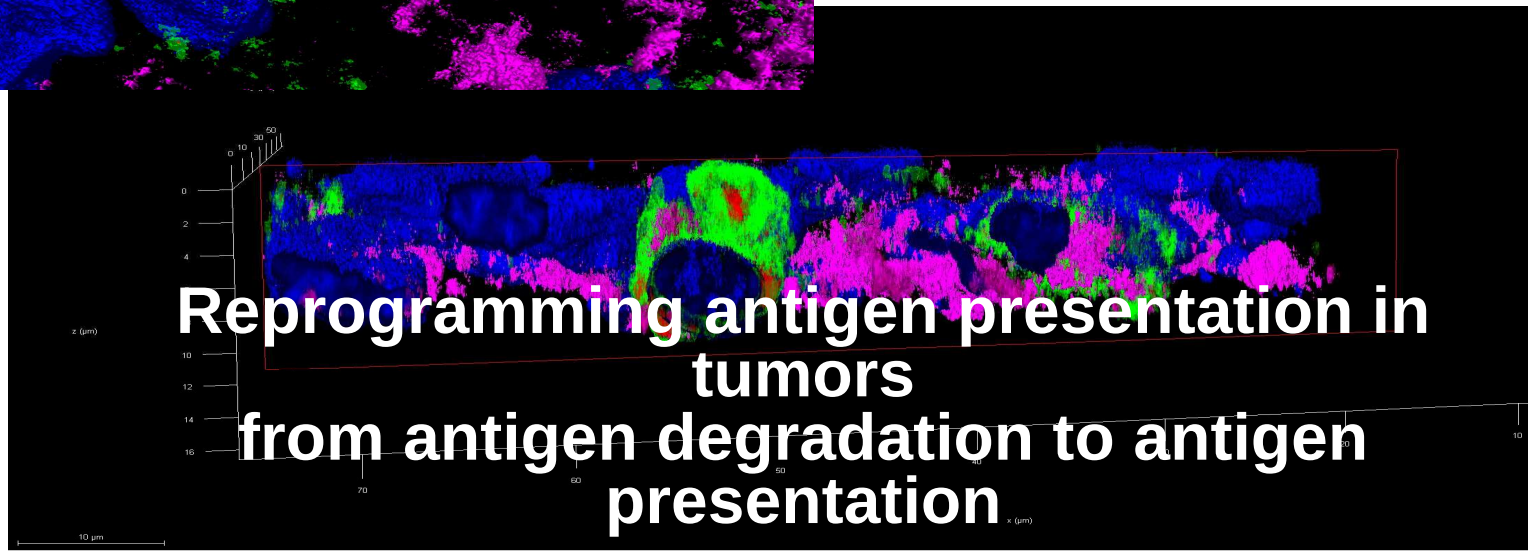


DAPI

CD206

CD163

gp100



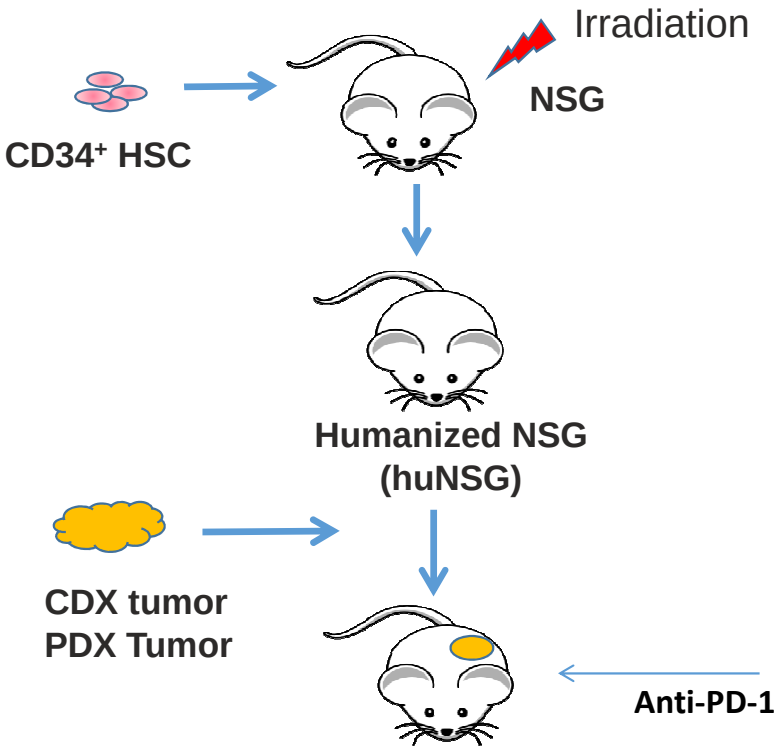
Applying humanized mice to studies on checkpoint inhibitors

- Hu-NSG & Hu-NSG-SGM3
- PDX or human cancer cell engrafted
  - Partial HLA match
  - CD34 donor variability; utilize multiple donors
  - Anti-PD-1, CTLA-4,
  - ~15% PDX tumor rejection
  - ~85% PDX/ cell line tumor growth
    - ~70% of these we have statistically significant tumor growth reduction with keytruda treatment
    - 100% statistical significant decrease in tumor PD-1 detection with keytruda treatment

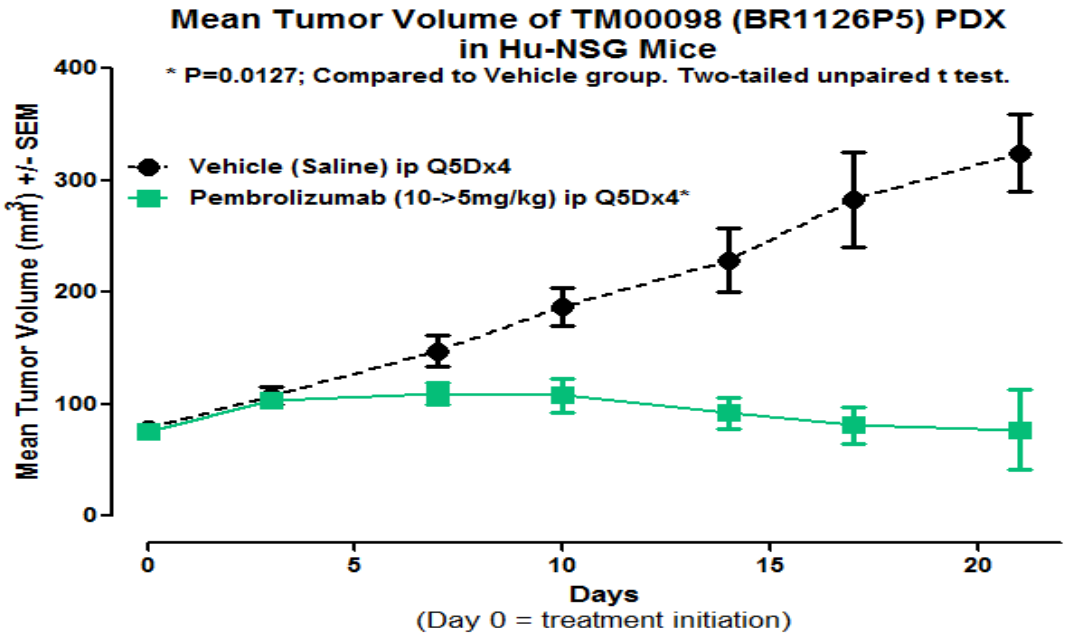
JAX – In Vivo Pharmacology Services

Jim Keck, Minan Wang, Li-Chin Yao, Mingshan Cheng and Danying Cai

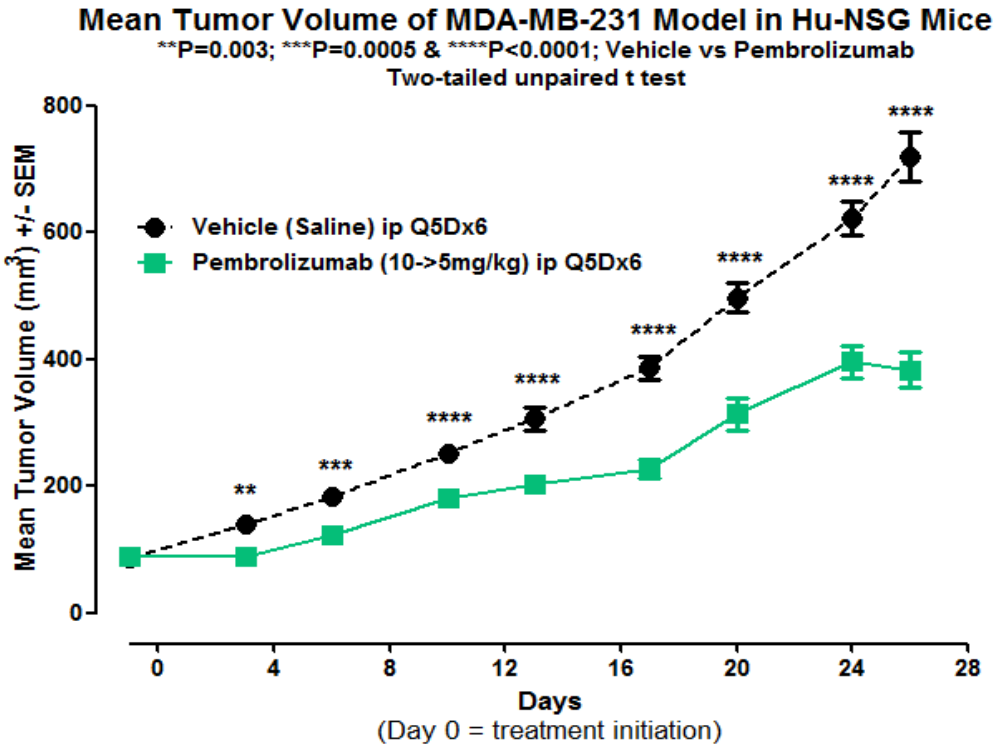
Humanized breast cancer PDX
Evaluating the effect of anti-PD-1

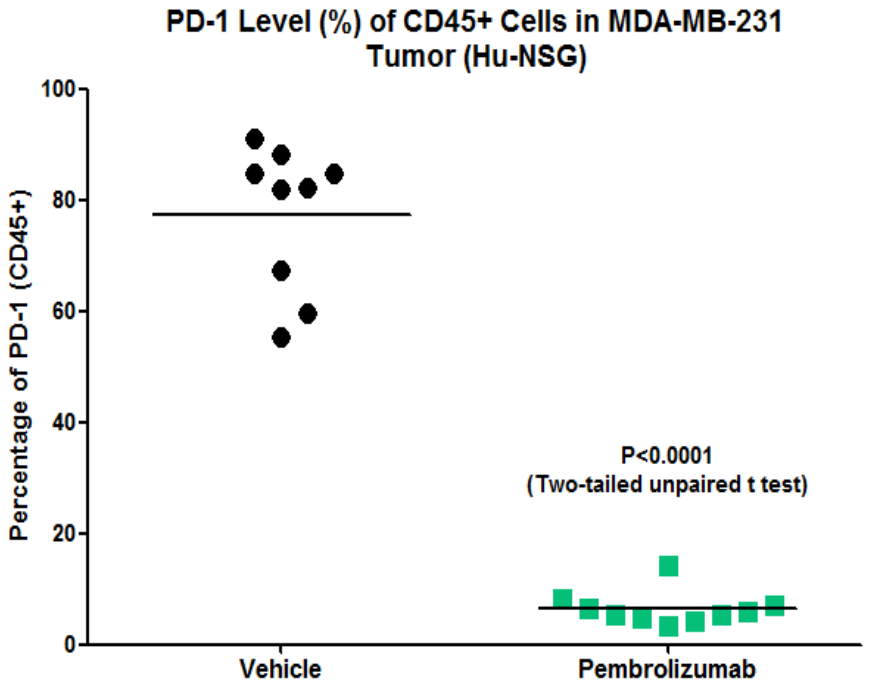


Humanized NSG (HuNSG) with Tumor (human immune system and human tumor)

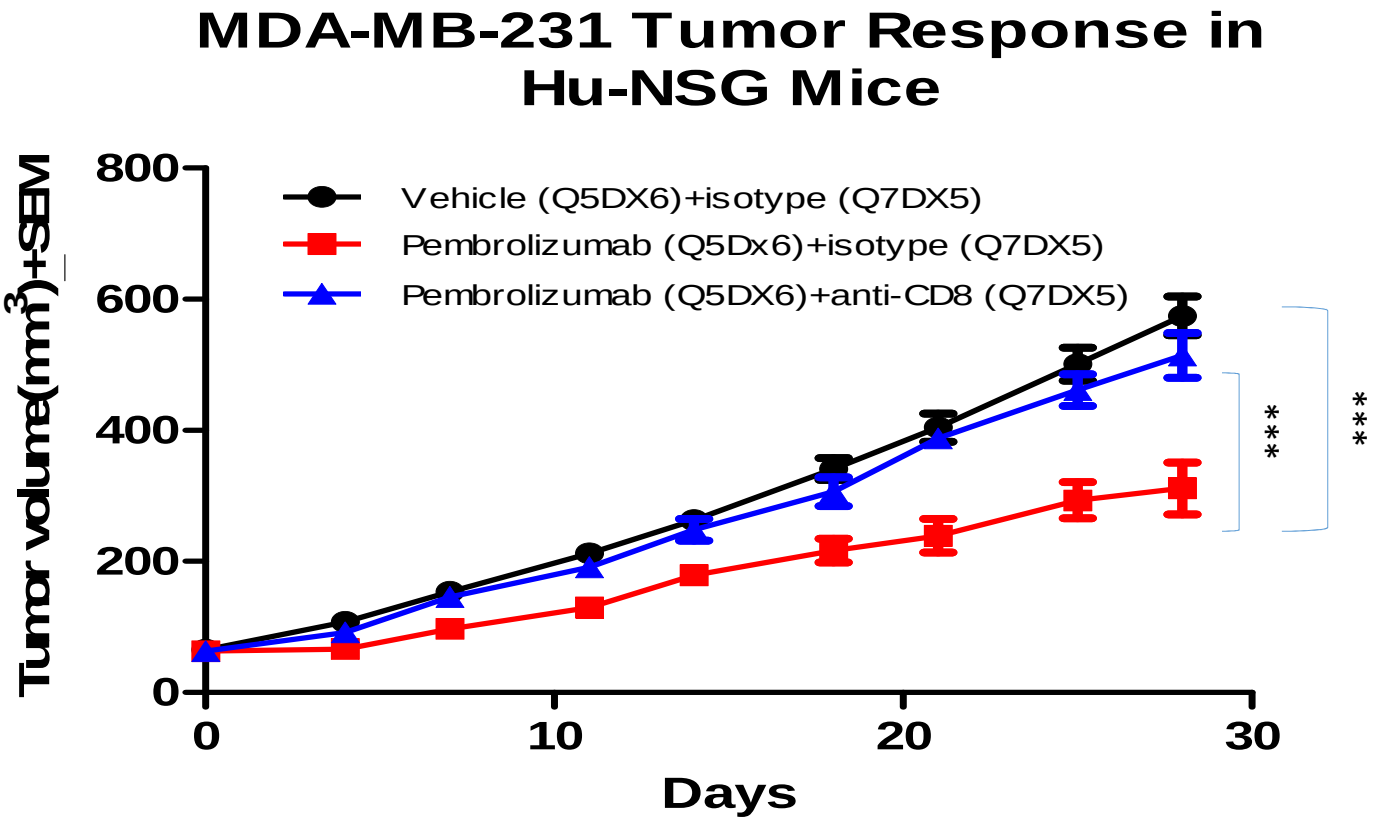


Evaluating the Effects of anti-PD-1 on breast cancer CDX in humanized NSG mice



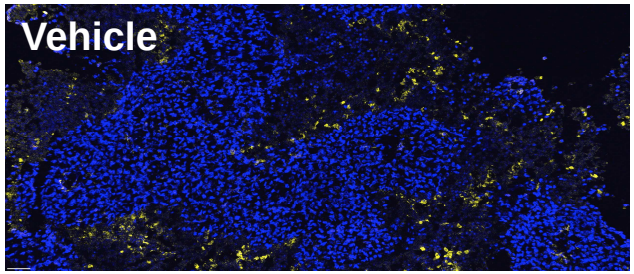


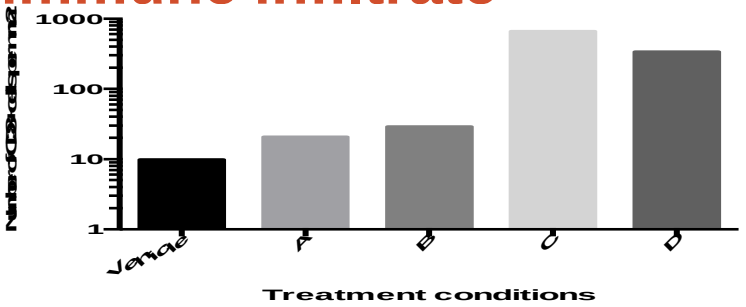
Efficacy of PD-1 blockade in humanized NSG mice bearing breast cancer CDX is $CD8^+$ T Cell Dependent

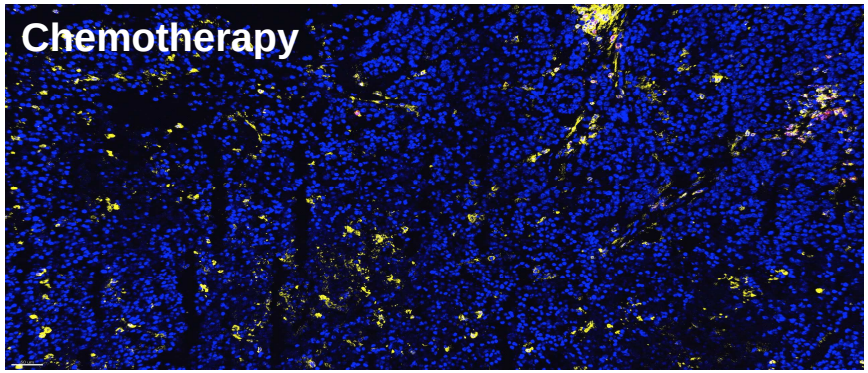


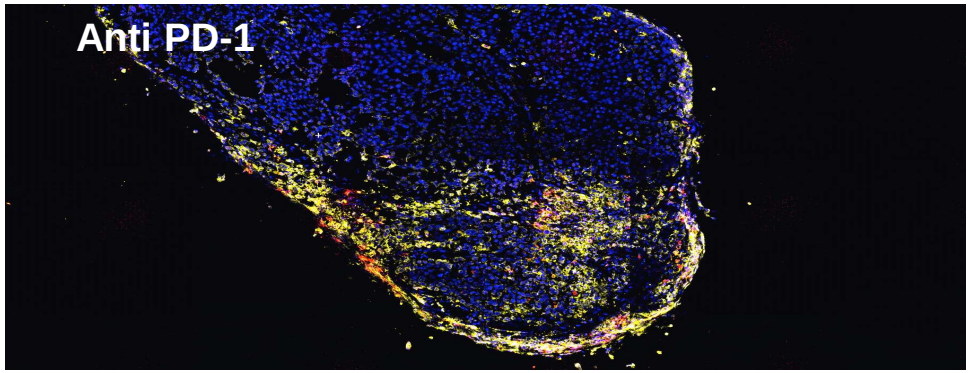
***P<0.0001; two-way ANOVA followed by Bonferroni post-tests.

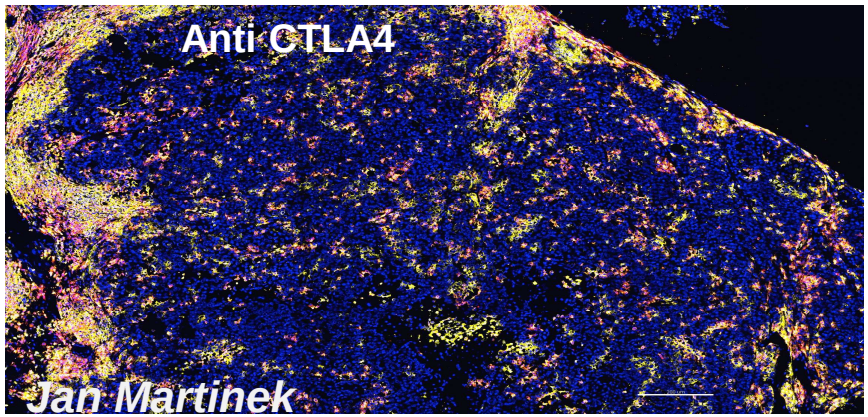
Immunotherapy changes the tumor immune infiltrate

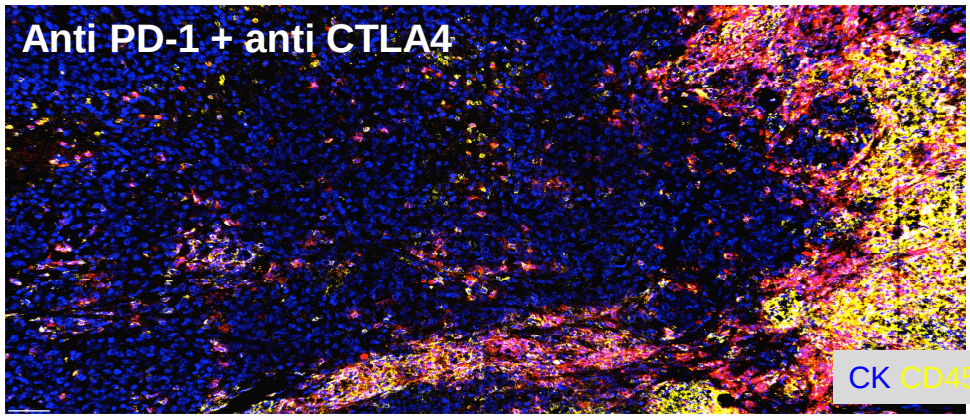




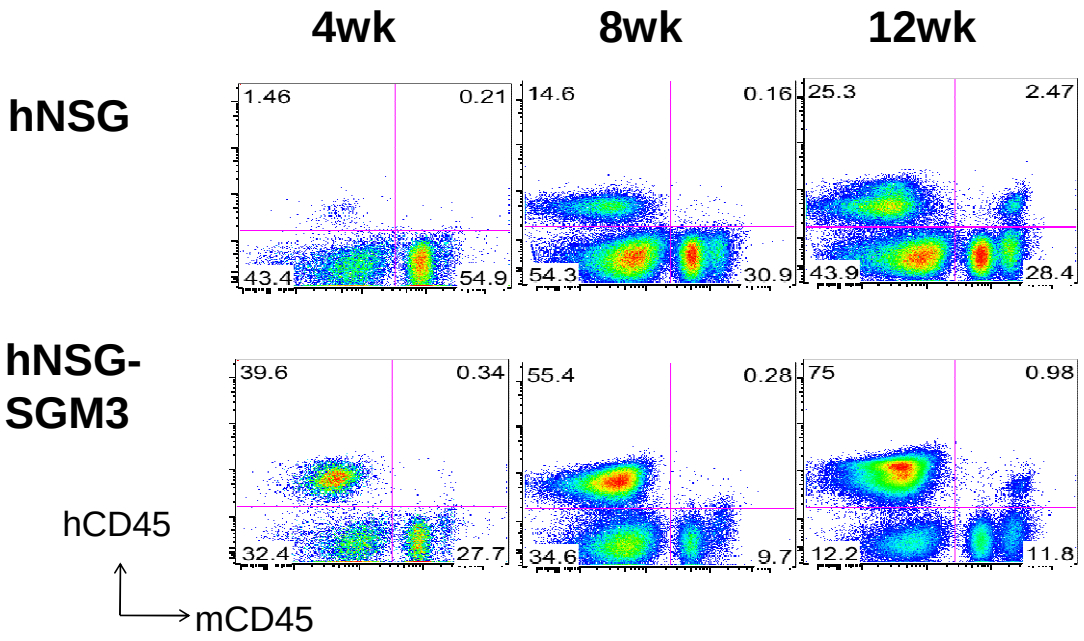


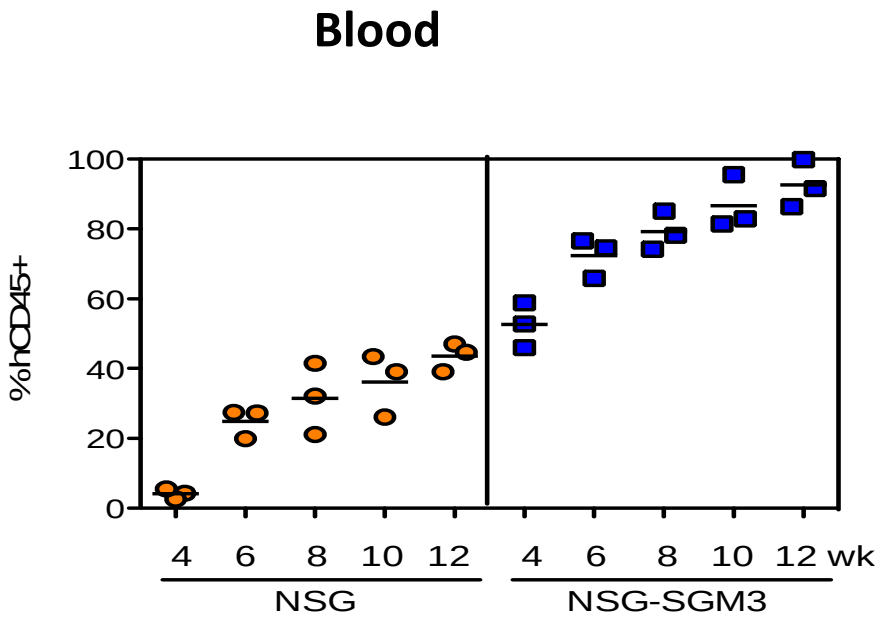






Better reconstitution of human cells in the blood of NSG-SGM3 (SCF GM-CSF IL-3) mice than NSG mice





Junni Yu

Humanized NSG-SGM3 mice favor the development of CD4+ T cells

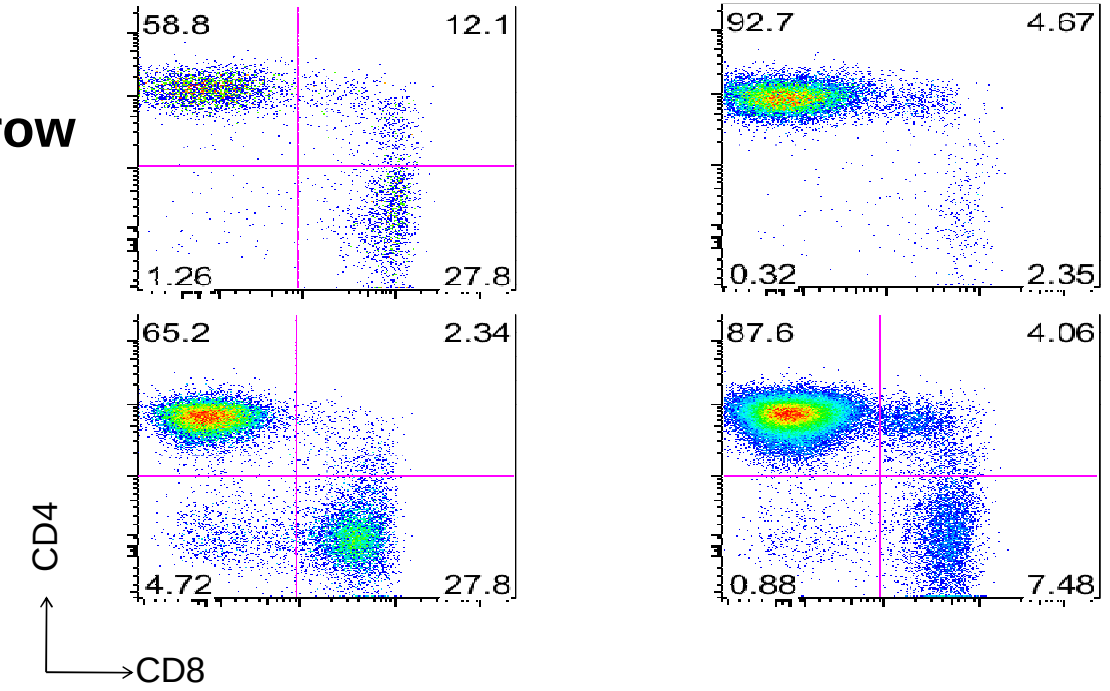
CD3+ T cells

hNSG

hNSG-SGM3

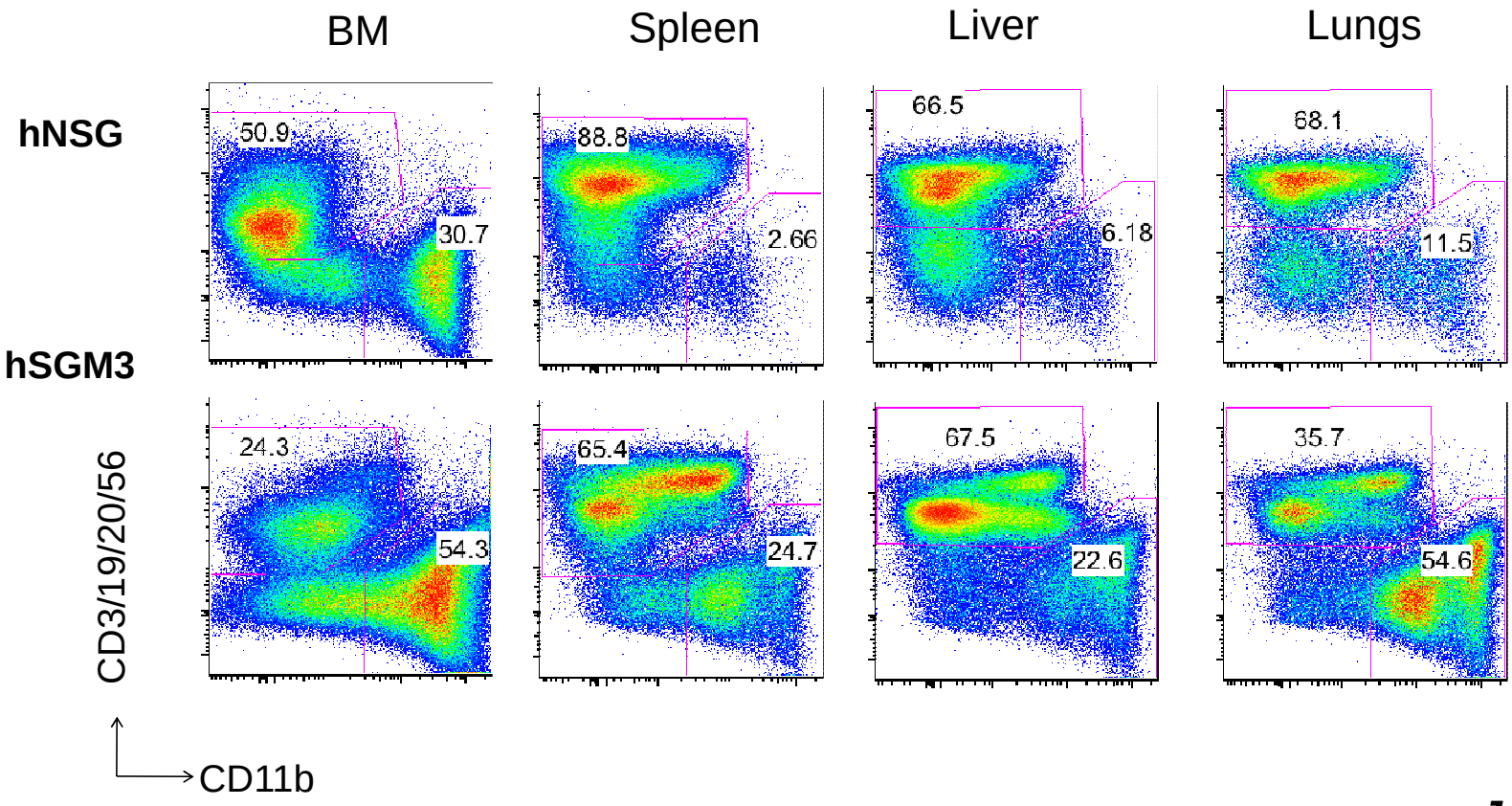
Bone Marrow

Spleen



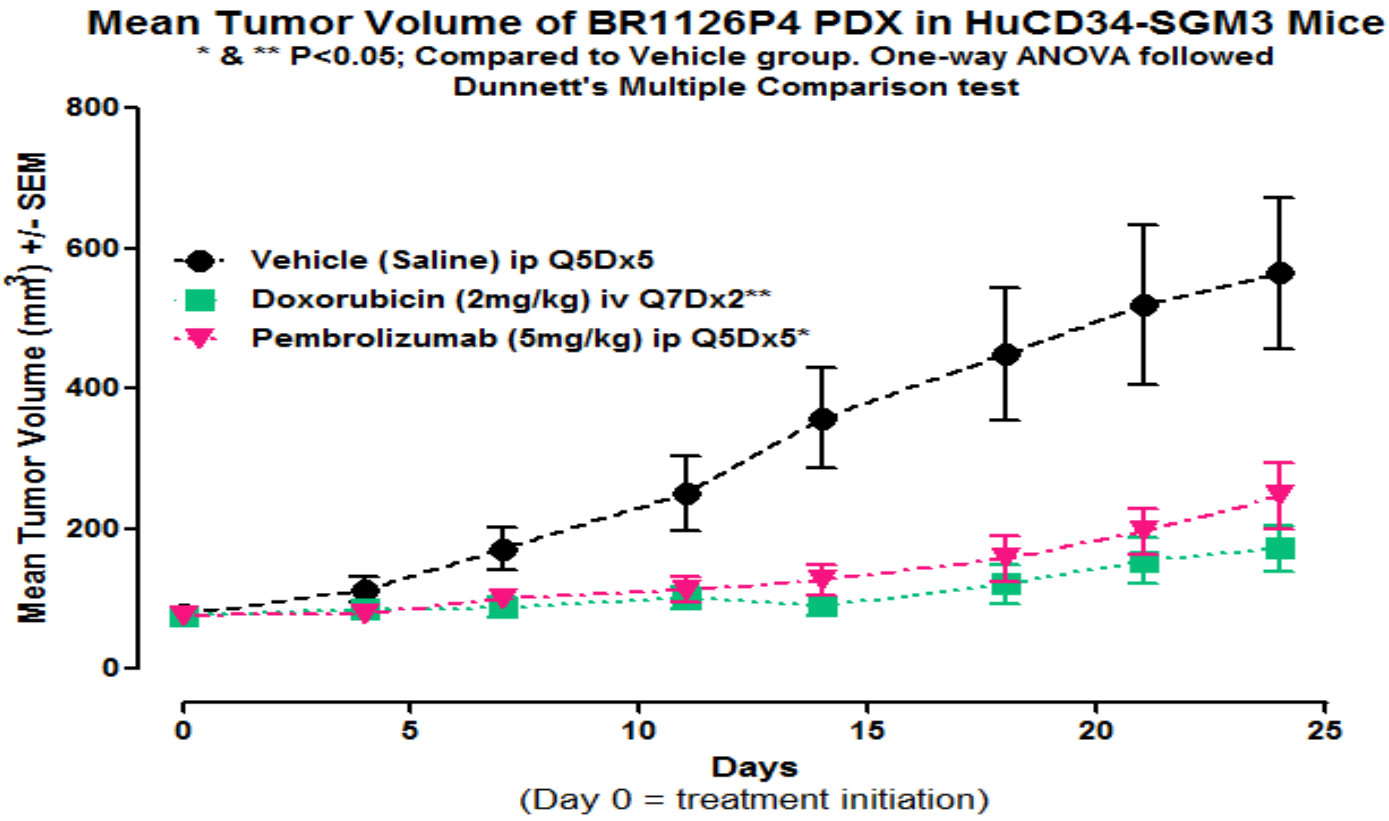
Junni Yu

CD11b^{+} myeloid compartment in periphery of hNSG-SGM3 mice



Junni Yu

Preliminary Efficacy Results of TNBC PDX in HuCD34 NSG-SGM3 Mice



Jim Keck, Li-Chin Yao

Examples of progress in the field on humanized mice based on host modification

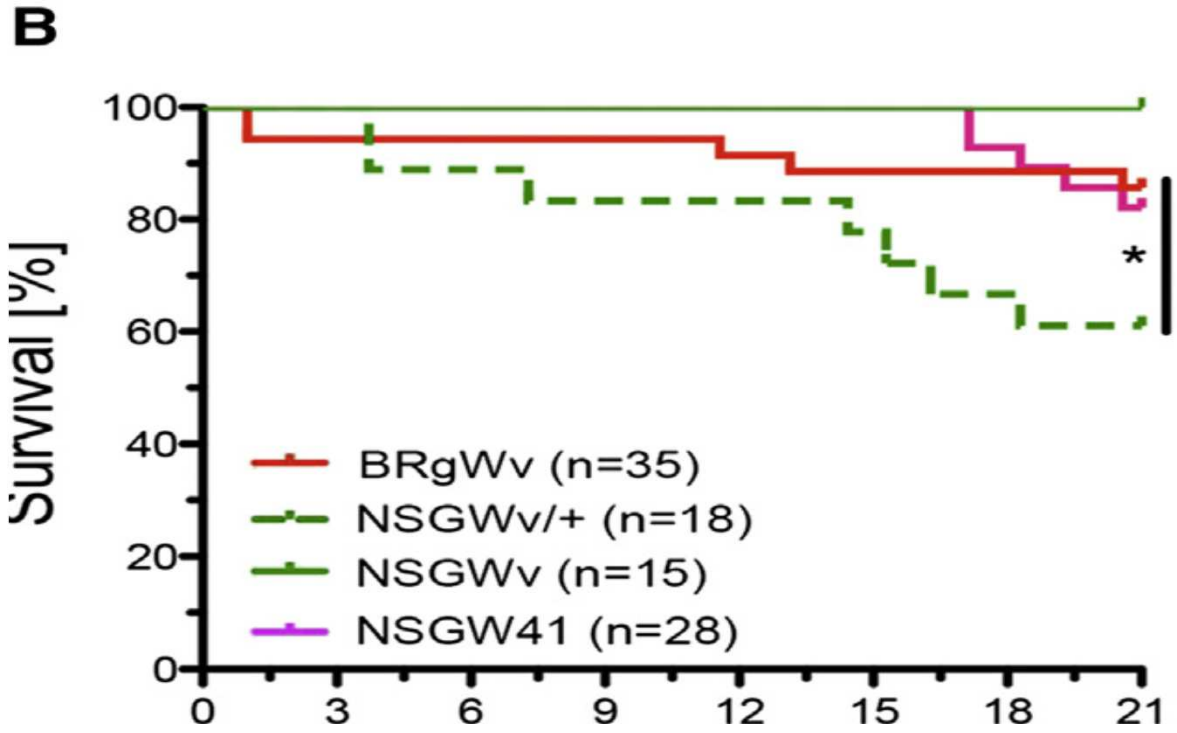
- Richard Flavell: next generation MISTRG mice with IL15&IL15Ra
- Madhav Dhodapkar: MISTRG6 for B cell malignancy

Nat Med Nov 2016

- Susann Rahmig: NSG with mouse kit mutant (Kit^{w41}) for engraftment *Cell Stem Cell 2014*
- Roberta Pelanda: BAFF for Ab
- Dale Greiner: NSG-SGM3 with CSF1-tg for macrophages and IL2-tg for NK cells
- Inessa Schwab: NSG-FcRg-ko for IVIG ***Cell Rep. 2015***

NSG with mouse kit mutant (Kit^{w41})

- Human HSCs engraft efficiently into adult immune-deficient Kit mutant mice
- *Kit* mutation enables human HSC engraftment without irradiation conditioning
- Human HSCs show robust multilineage engraftment and self-renewal in mice

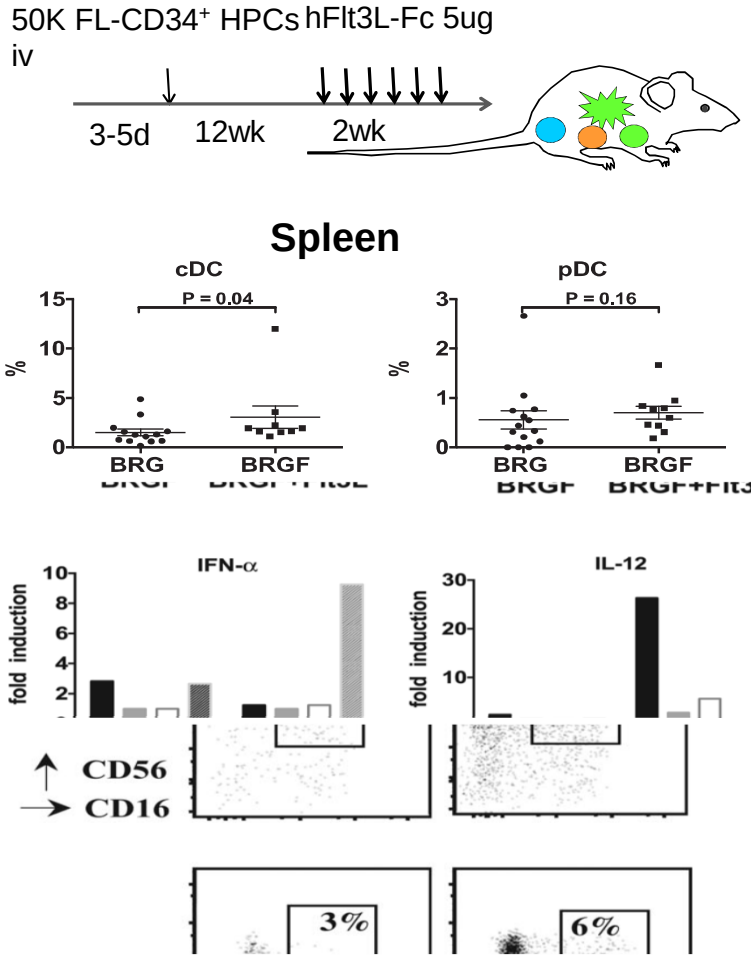


Cosgun et al., Cell Stem Cell 2014

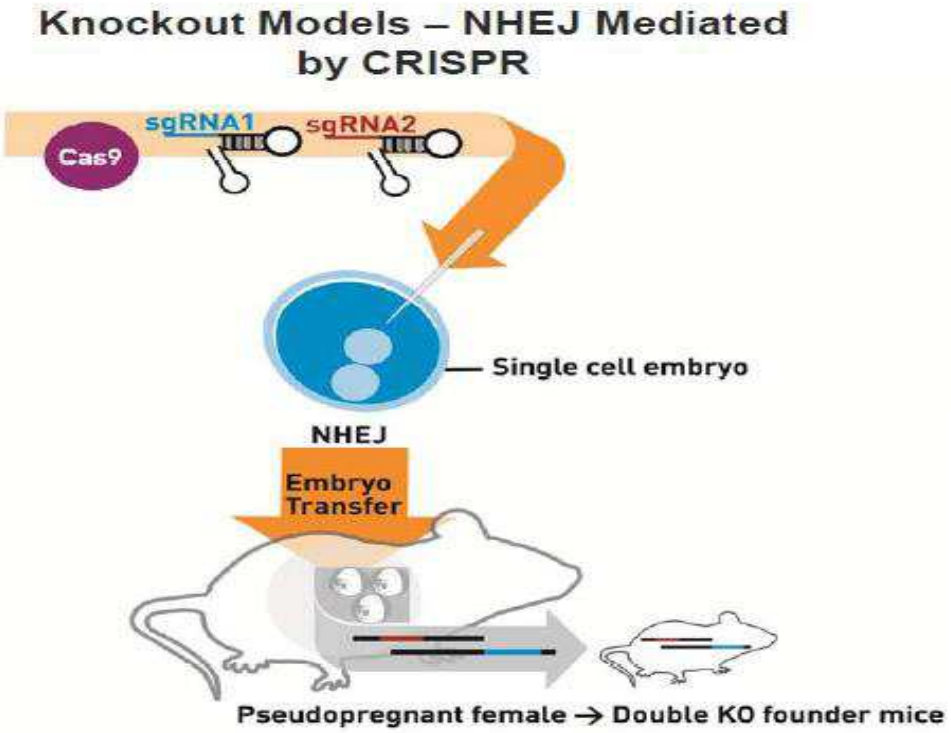
BRG with mouse Flt3 mutant (BRGF)

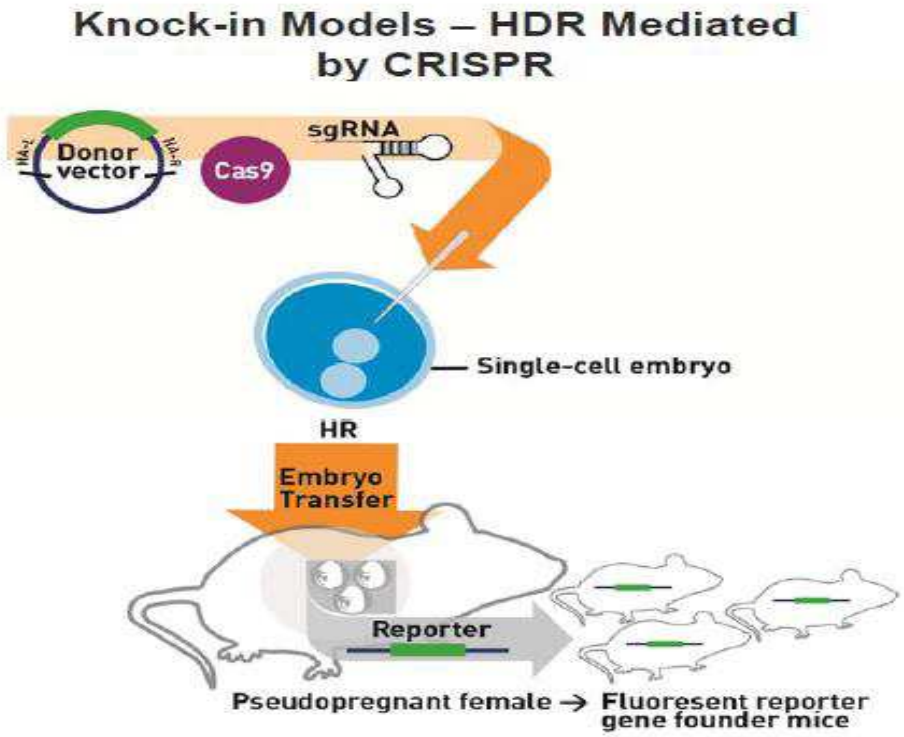
- BRGF mice have reduced cDC and pDC compartments, increased Flt3L levels and deficit to Flt3L stimulation
- Human cDCs and pDCs develop from hCD34+ precursors can be specifically boosted with exogenous Flt3L
- Increased human T and NK-cell homeostasis after boosted with exogenous Flt3L

Li et al., Eur J Immunol 2016



Modified NSG mice via CRISPR-Cas mediated genome editing





JAX Laboratories: Next Generation of Humanized Mice

CRISPR editing of the host and of human cells

Tg expression of human factors

Cytokines
HLA molecules
Microenvironmental factors (SIRPα)
Hormones (prolactin)

Reduction of mouse immunity

H2 molecules
Thymus
Macrophages
Granulocytes
Dendritic Cells
Chemokine receptors
Interferon receptors
Toll-like receptors

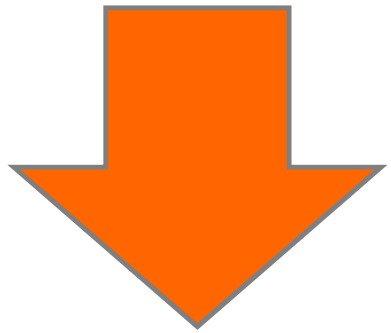
Human cancer models

Leukemias and lymphomas
Solid tumors
Role of human stroma

Shultz Nat Rev Immunol 2012

Current challenges

- **Mouse thymic environment and human T cell maturation and selection**
- **Practical constrains in making autologous humanized mice**
- **Source of HPCs: bone marrow, blood, iPS**
- **The variance in microbiome**



Design the experiment
Design the mice

Thanks to our patients
Thanks to funding organizations

Chun Yu
Jan Martinek
Florentina Marches
Te-Chia Wu
Patrick Metang
John Graham
Pierre Authie
……

Jim Keck and colleagues at JAX Sacramento
Susie Airhart

Lenny Shultz
Carol Bult
…..and many colleagues at JAX MG

Anthony Rongvaux
Richard Flavell
….and many external collaborators

Jacques Banchereau