Pre-clinical mouse models of cancer immunotherapy

Anthony Rongvaux, PhD

@RongvauxLab

Fred Hutchinson Cancer Research Center

Seattle, WA

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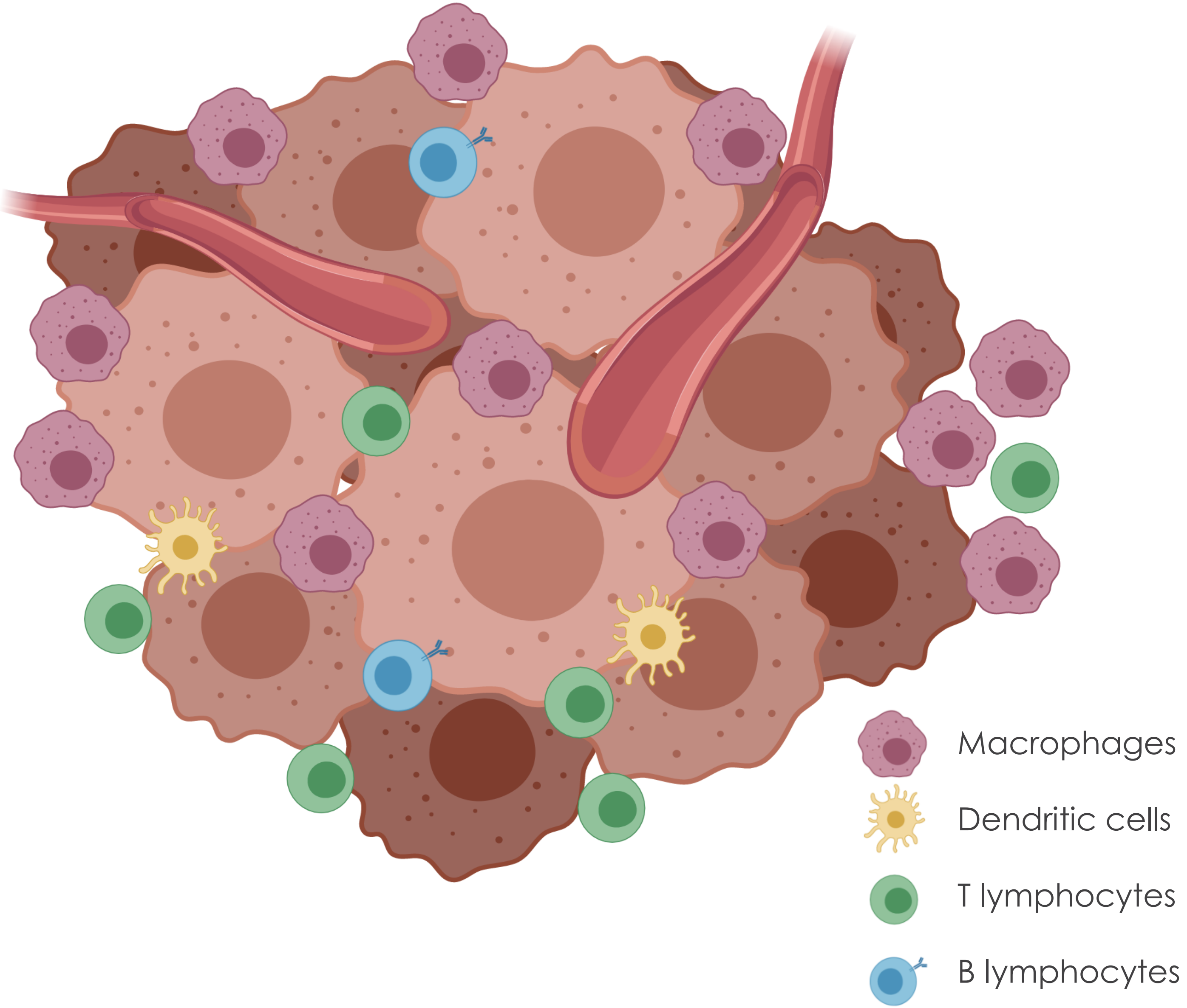
CURES START HERE

The tumor microenvironment is complex



Cell

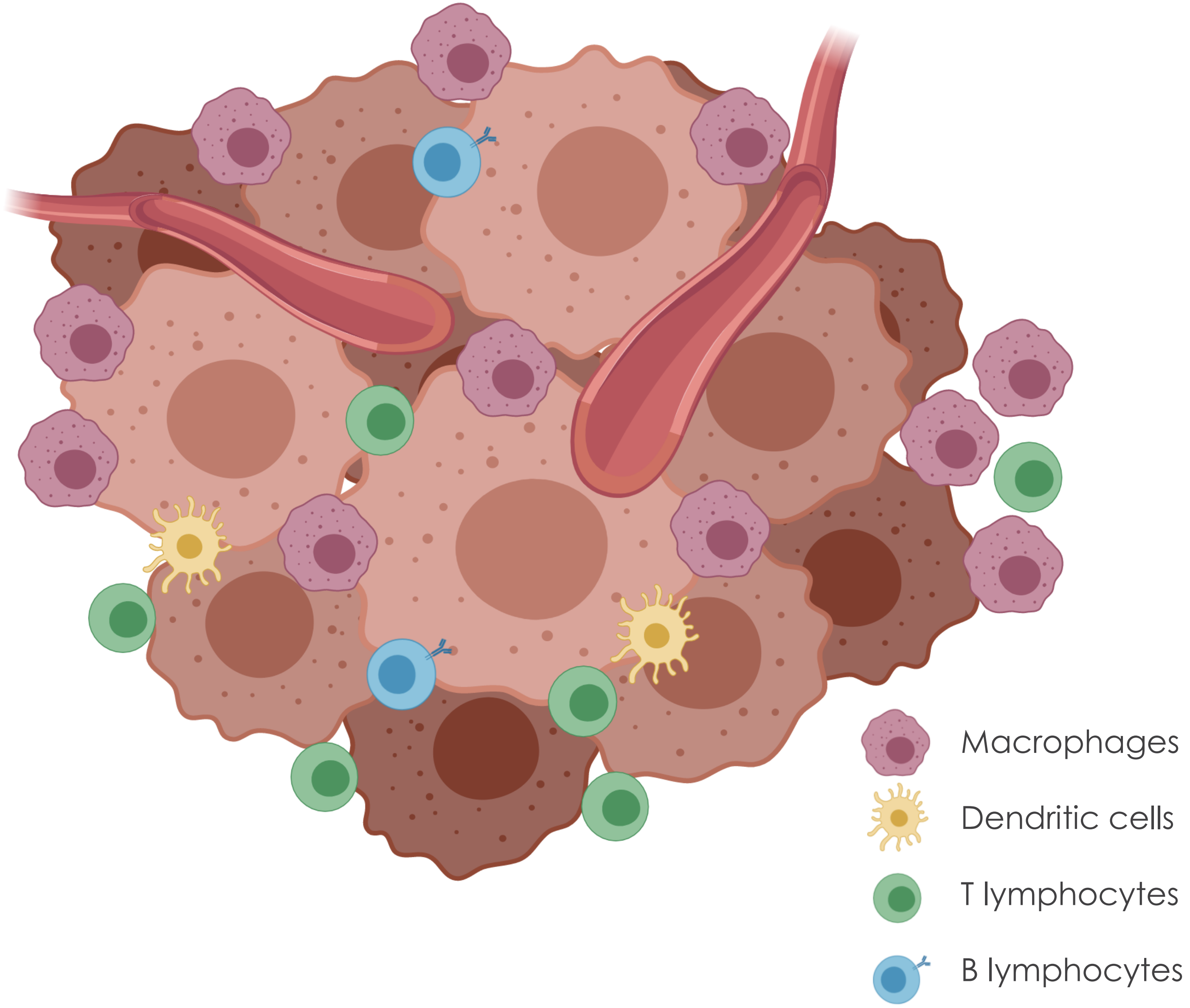
Leading Edge
Review

Hallmarks of Cancer: The Next Generation

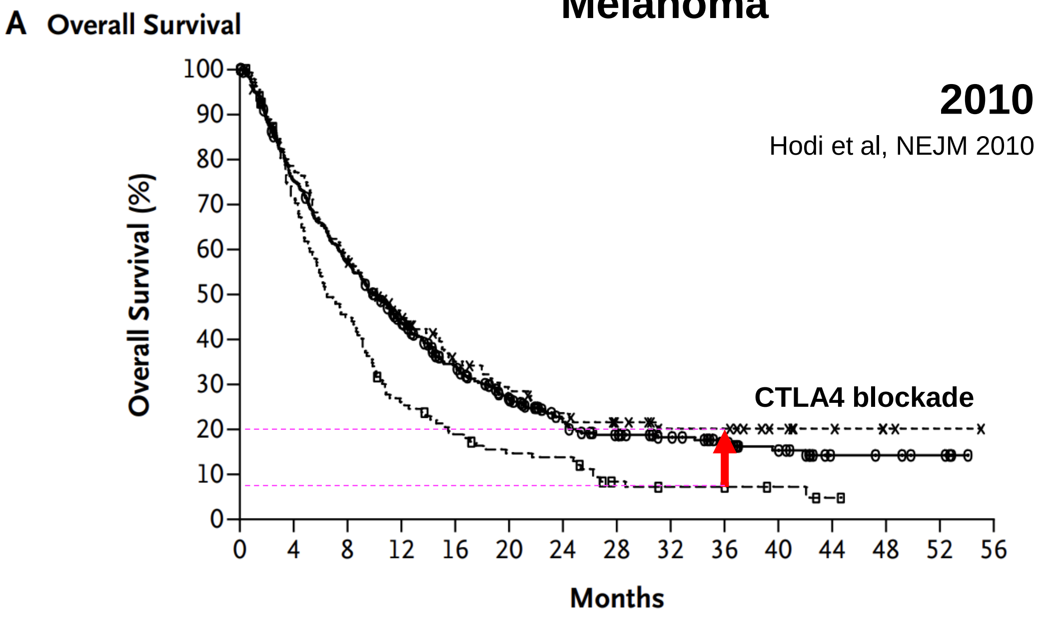
Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]

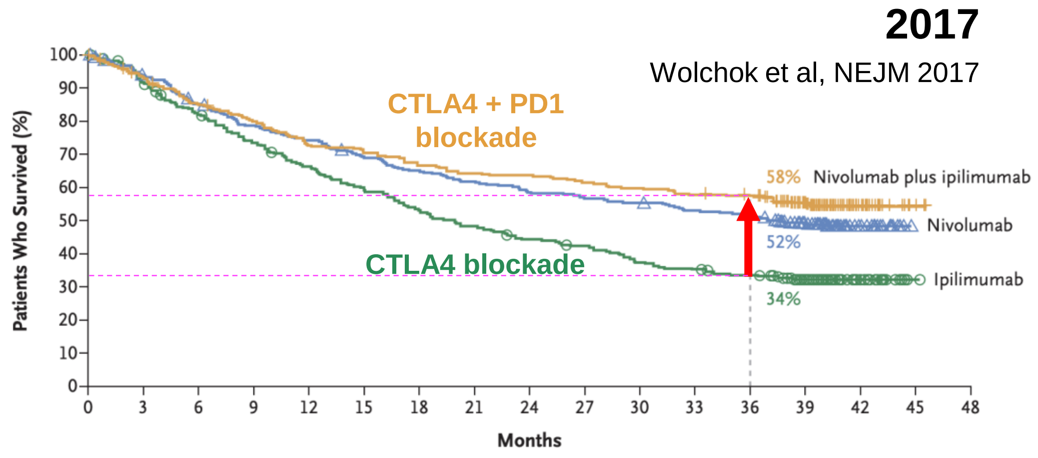
An Emerging Hallmark: Evading Immune Destruction

Evading T cell-mediated antitumoral immunity

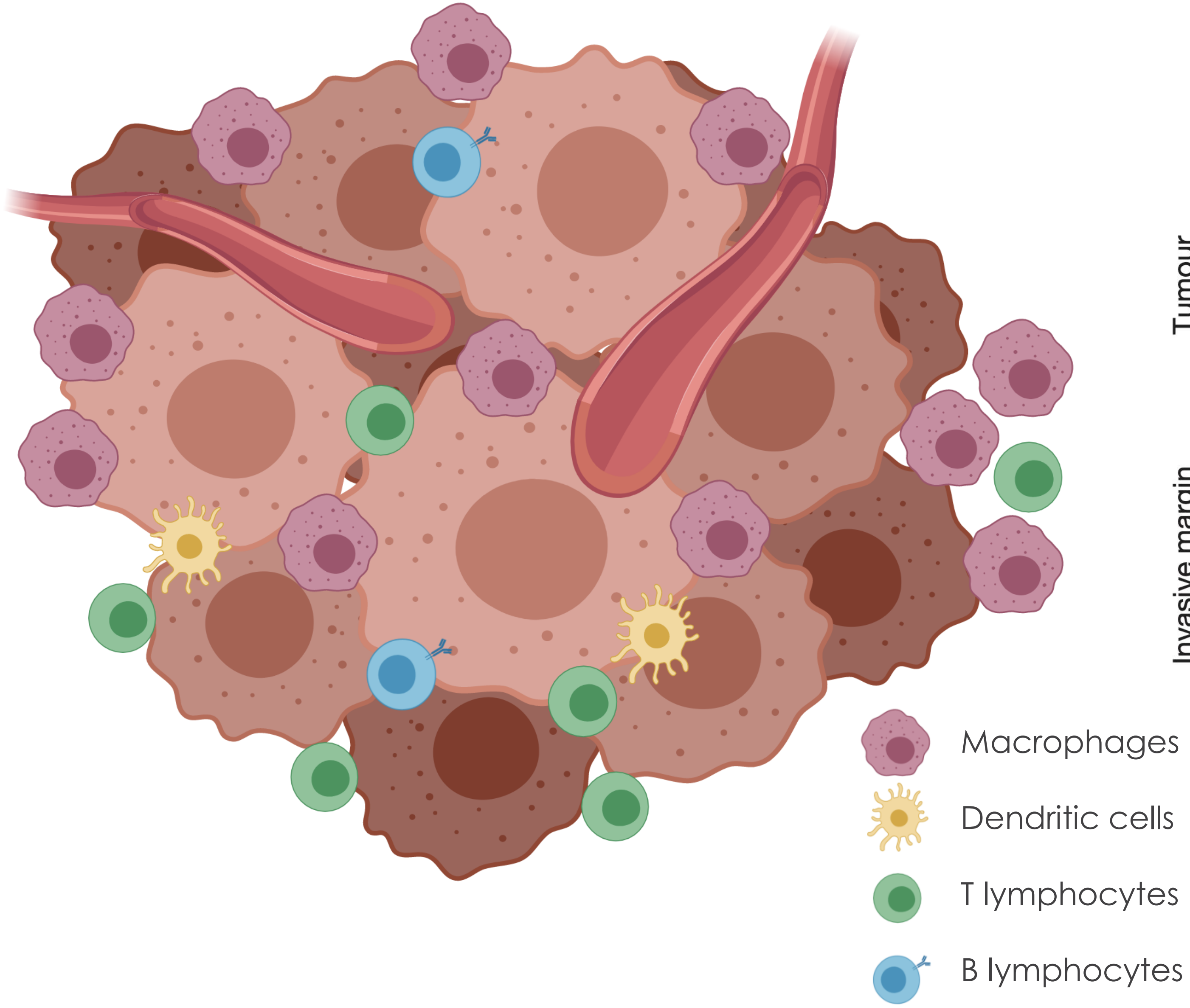


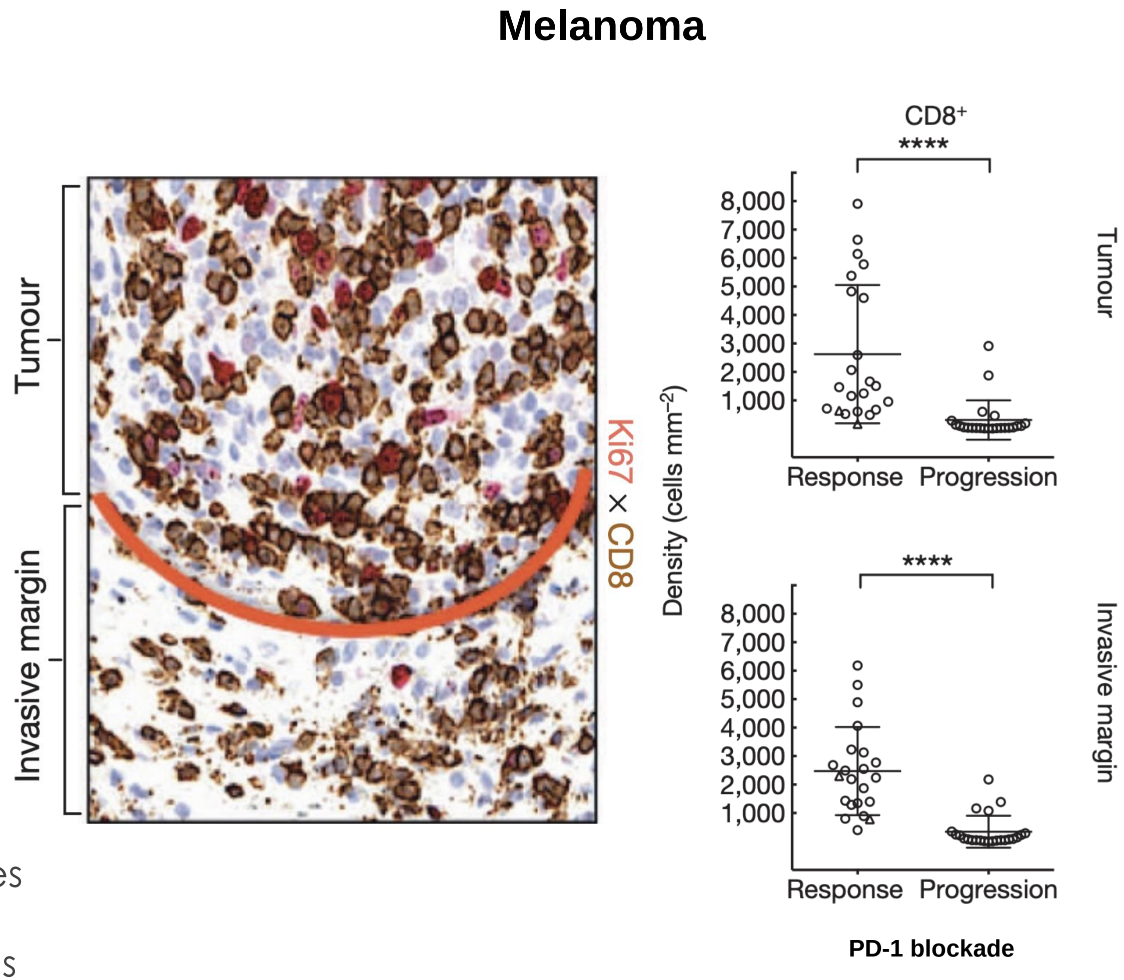
Melanoma





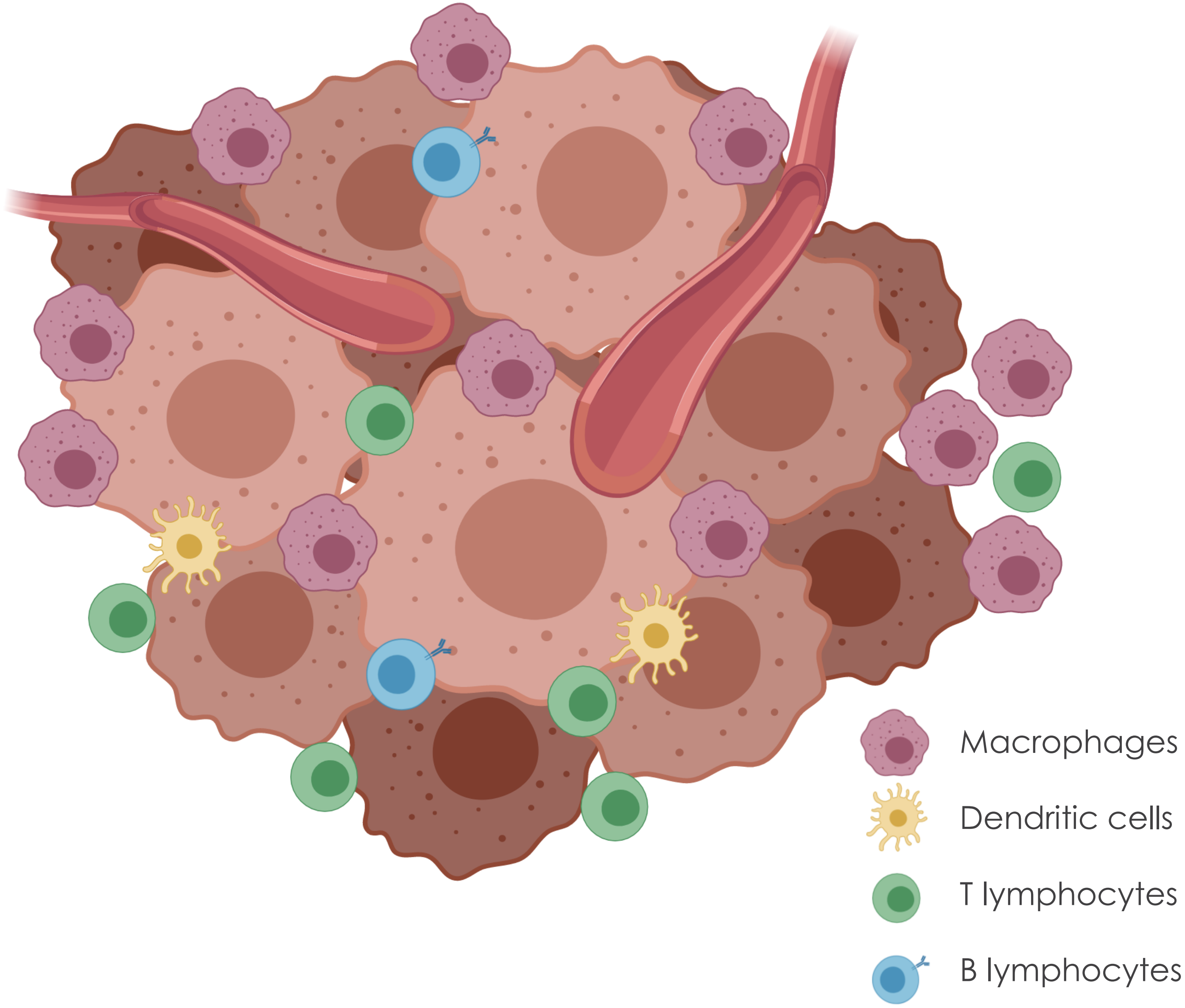
Evading T cell-mediated antitumoral immunity

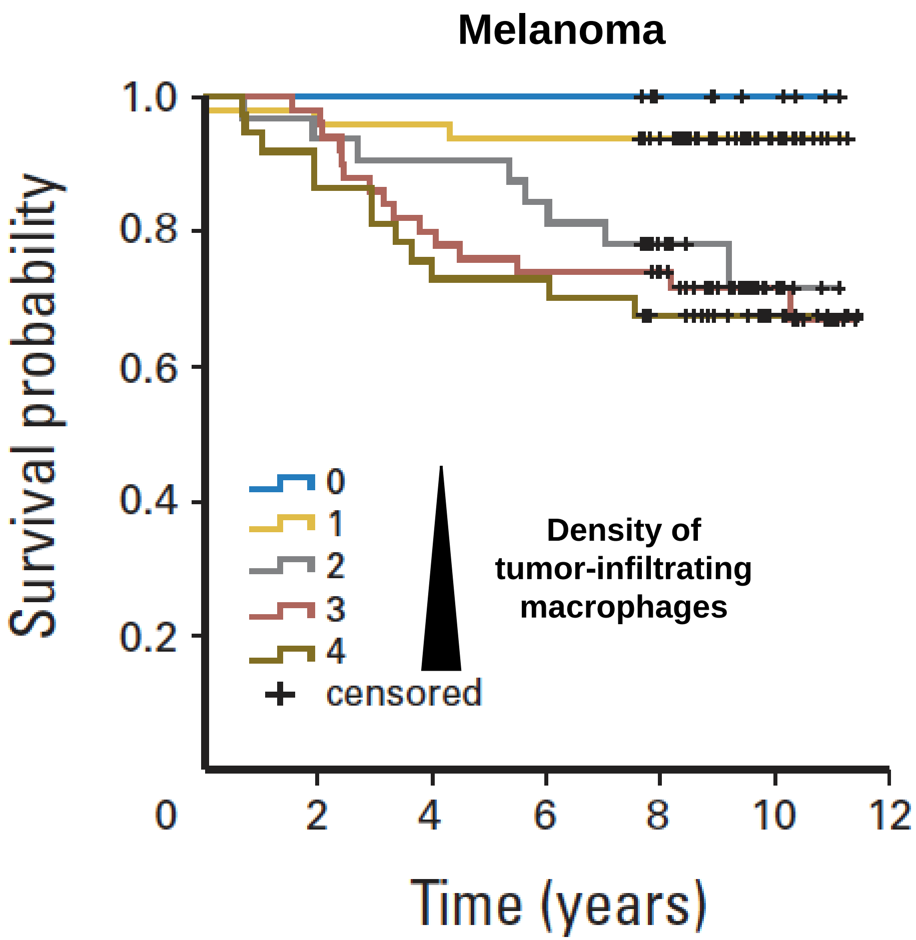




Tumeh et al, Nature 2014

Exploiting macrophage-mediated tumor support





Jensen et al, J Clin Oncol 2009

- Complex interactions between tumor and immune cells
- Decisive impact on cancer progression
- Determine responsiveness vs. resistance to (immuno)therapy
- Each patient is unique

→ The cancer/immune system interface cannot be modeled in vitro

➔ **Need for in vivo mouse models**

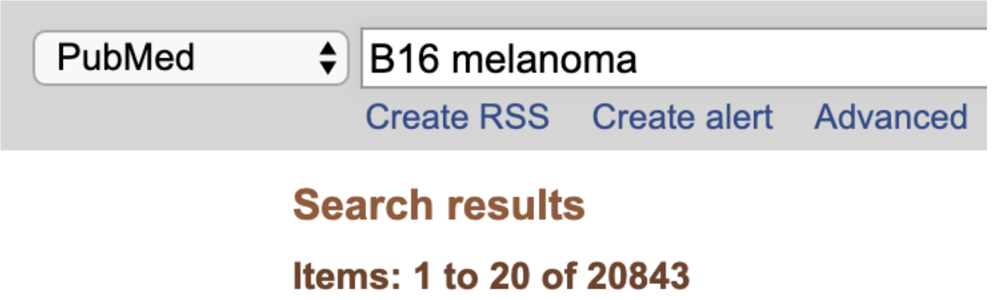
Mouse models of cancer

1) Tumor cell line implantation in immunocompetent mice
2) Genetically-engineered mouse (GEM) model of cancer
3) Patient-derived xenografts (PDX)
4) PDX in mice with a humanized immune system (immuno-PDX)

Mouse models of cancer

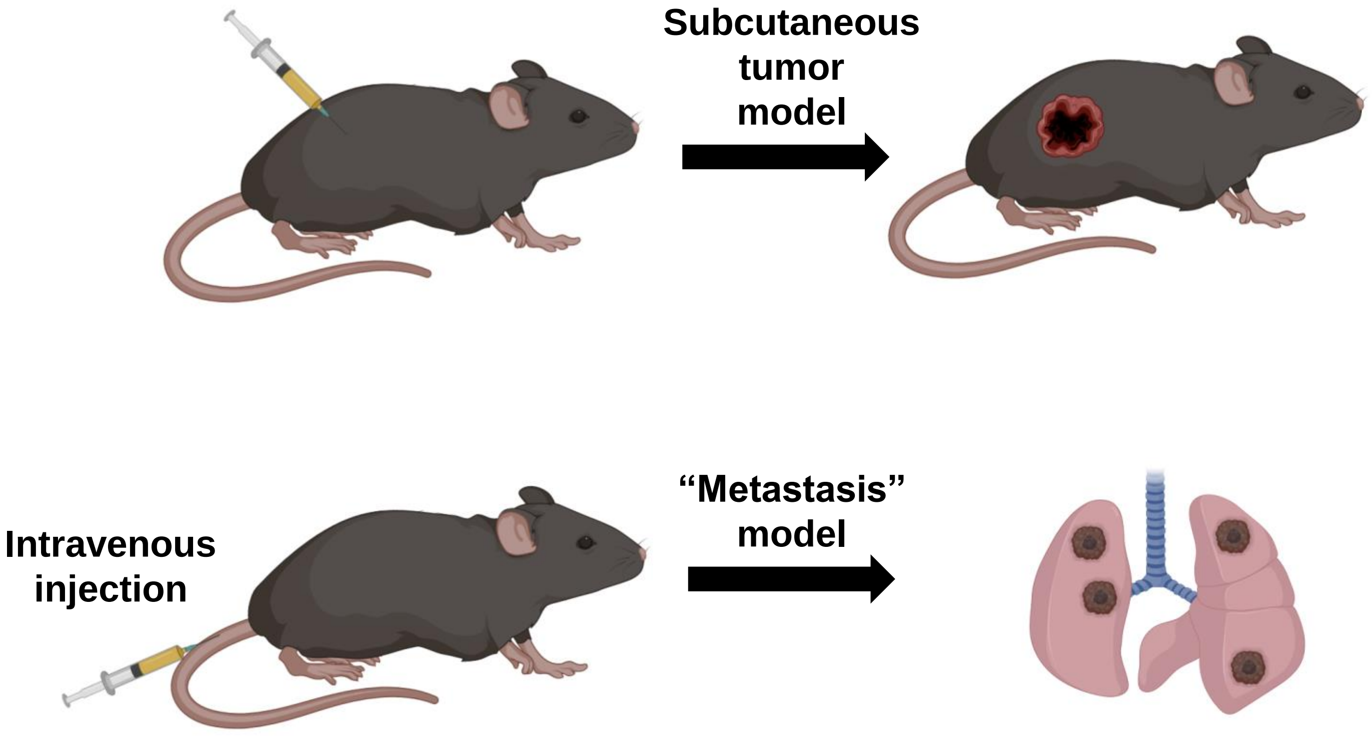
1) Mouse tumor cell line implantation in immunocompetent mice

The B16 melanoma cell line



B16 melanoma

- Spontaneous tumor isolated in 1954 from a C57BL/6 mouse
- Origin: melanin-producing epithelium cell
- Transplanted in syngeneic C57BL/6 mice

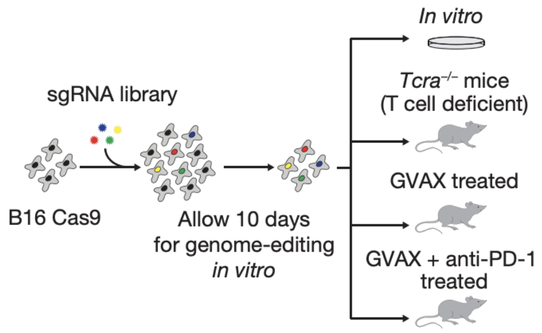


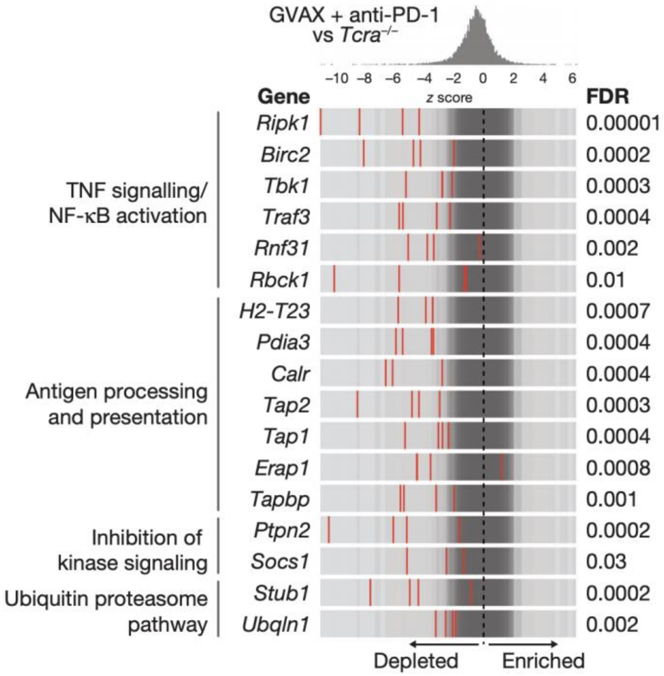
	Applications
SQ injection	Primary solid tumor
IV injection	Lung metastases
B16-luciferase	In vivo imaging
B16-OVA	Defined antigen
B16-Cas9	In vivo gRNA screen
GVAX*	Vaccination

* GM-CSF-secreting irradiated B16 cells

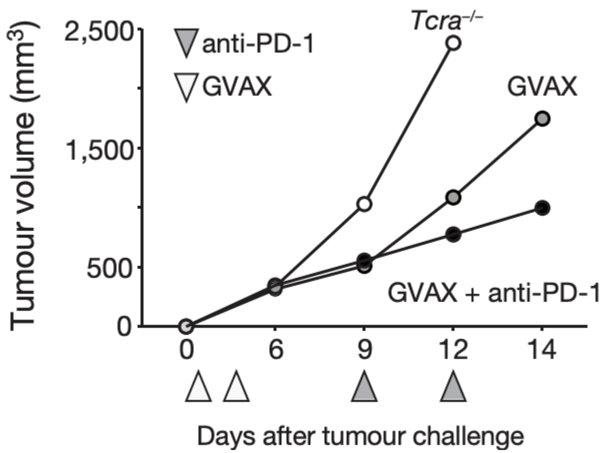
Application of the B16 model – gRNA screen

In vivo sgRNA screen

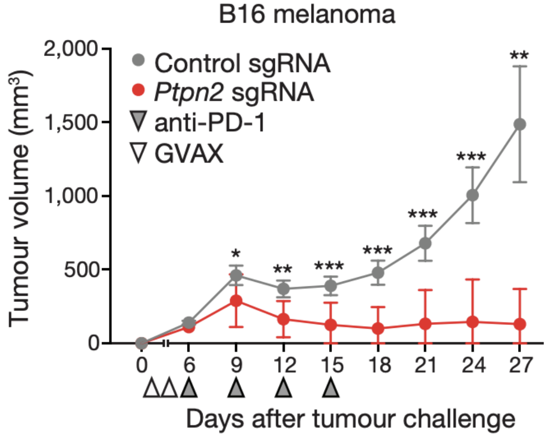




B16 responds to PD-1 blockade



Validation of one of the targets (*Ptpn2*)



PTPN2 inhibitors may enhance the efficacy of PD-1 checkpoint blockade

Manguso et al, Nature 2017

Pros and cons

	Pros	Cons	Cost
Mouse tumor implants	- Simple and reproducible - Genetic engineering	- Genetically homogenous - Artificial implantation - Mouse, not human	

Mouse models of cancer

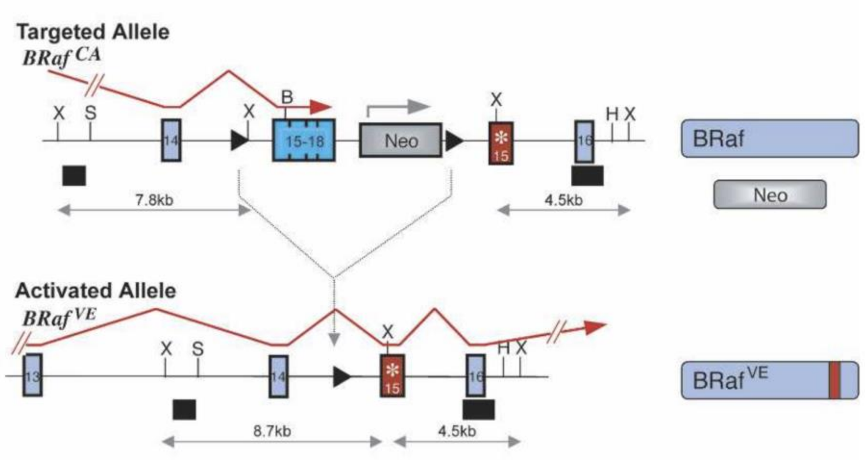
2) Genetically-engineered mouse (GEM) model of cancer

- Designed to represent cancer patients
- (Inducible) mutation of oncogene(s) and/or tumor suppressor(s)

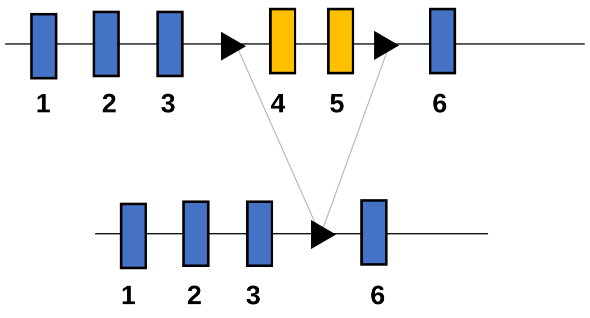
➔ Example: the BRAF/PTEN melanoma model

The BRAF/PTEN melanoma model

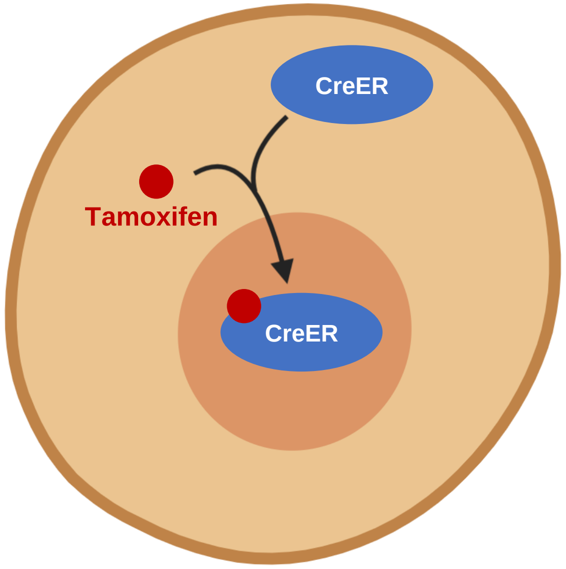
BRAF^{CA}
(Cre-activated BRAF^{V600E})



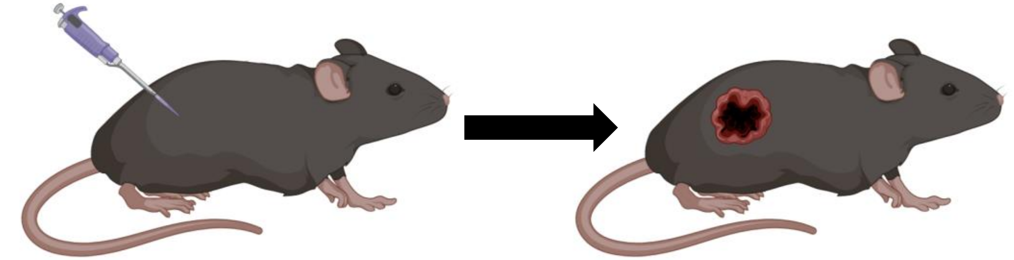
PTEN$^{fl/fl}$
(Cre-activated PTEN deletion)

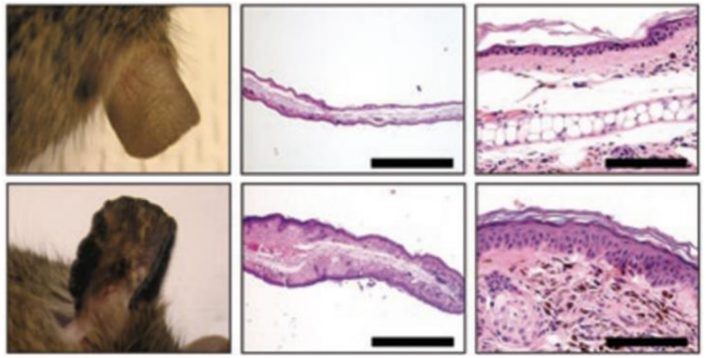


Tyr::CreER
(Melanocyte-specific
tamoxifen inducible
Cre recombinase)



Tamoxifen





Dankort et al, Genes Dev 2007
Dankort et al, Nat Genet 2009

Other examples of GEMs

Table 2. Representative Clinically Relevant Mouse Trials

Trial Design	Cancer Type	Model Type	Engineered Drivers	Drugs/ Treatment	Significance	Relevant Publications
Preclinical	Hematopoietic (APL)	GEM	PML-RARα fusion PLZF-RARα fusion	Retinoic acid	Demonstrated the efficacy of retinoic acid plus As_2O_3 in specific APL subtypes, validated in clinic	(Ablain and de Thé, 2014; Pandolfi, 2001)
Preclinical	Pancreas (Neuro-endocrine)	GEM	RIP1-Tag2	Sunitinib	Demonstrated the efficacy of Sunitinib plus Imatinib, validated in clinic. FDA approved for pancreatic cancer treatment in 2011.	(Pietras and Hanahan, 2005; Raymond et al., 2011)
Preclinical	Medulla-blastoma	GEM	$Ptc1^{+/-}$ $P53^{-/-}$	GDC-0449 (SMO inhibitor)	Demonstrated the efficacy of an Shh pathway small molecule inhibitor, validated in clinic	(Romer et al., 2004; Rudin et al., 2009)
Preclinical	Pancreas (Neuro-endocrine)	GEM	RIP1-Tag2	Erlotinib Rapamycin	Demonstrated efficacy of combining drugs targeting EGFR and mTOR	(Chiu et al., 2010)
Co-clinical	Pancreas (PDA)	GEM	LSL-$Kras^{G12D}$ LSL-$Trp53^{R172H}$ Pdx-1-Cre	Gemcitabine Nab-Paclitaxel	Provided mechanistic insight into clinical cooperation between Gemcitabine and Nab-Paclitaxel	(Frese et al., 2012; Goldstein et al., 2015)
Co-clinical	Pancreas (PDA)	GEM	LSL-$Kras^{G12D}$ LSL-$Trp53^{R172H}$ Pdx-1-Cre	CD40 monoclonal antibody Gemcitabine	Demonstrated that targeting stroma was effective in treatment of metastatic PDA	(Beatty et al., 2013)
Co-clinical	Lung (NSCLC)	GEM	$KRAS^{G12D}$ $p53^{fl/fl}$ $Lkb1^{fl/fl}$	Selumetinib Docetaxel	Validation of improved response of adding Selumetinib to Docetaxel treatment	(Chen et al., 2012; Jänne et al., 2013)
Co-clinical	Lung (NSCLC)	GEM	EML4-ALK fusion	Crizotinib Docetaxel Pemetrexed	GEM model predicted clinical outcome of drug combinations	(Chen et al., 2014; Lunardi and Pandolfi, 2015)

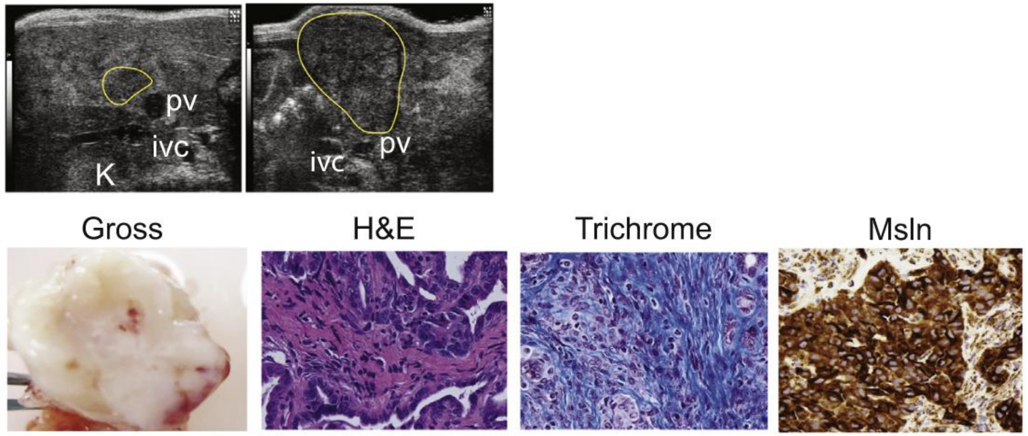
Day et al, Cell 2015

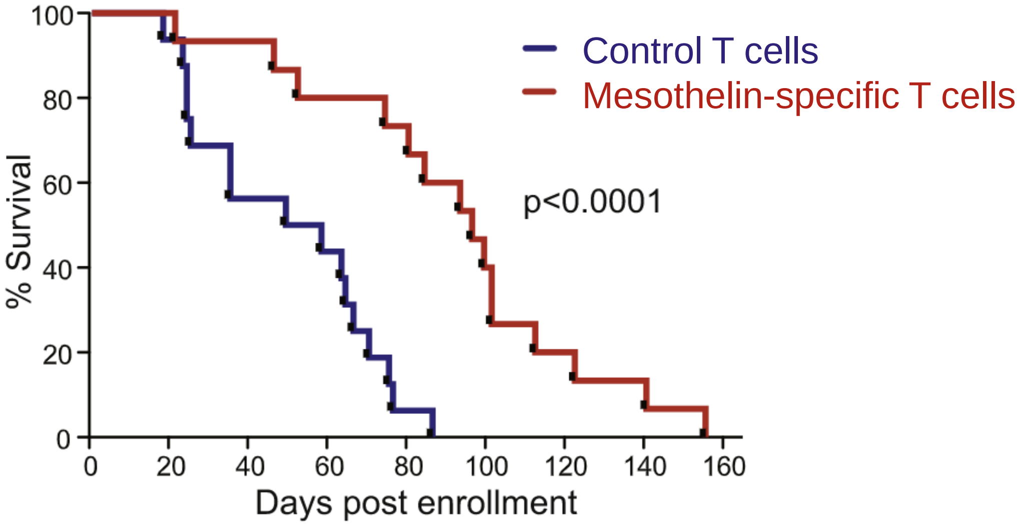
Application of a GEM pancreas cancer model

$Kras^{LSL\text{-}G12D/+}$ $Trp53^{LSL\text{-}R172H/+}$ $p48^{Cre/+}$ Mice

Adoptive
T cell transfer

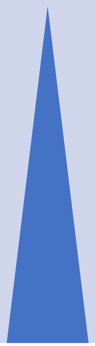
Pancreas cancer:





Stromnes et al, Cancer Cell 2015

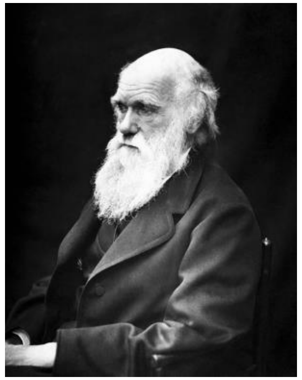
Pros and cons

	Pros	Cons	Cost
Mouse tumor implants	- Simple and reproducible - Genetic engineering	- Genetically homogenous - Artificial implantation - Mouse, not human	
GEM	- Well-defined oncogenic mutations, representative of human cancer - Oncogenesis	- Low mutational burden and immunogenicity - Mouse, not human	

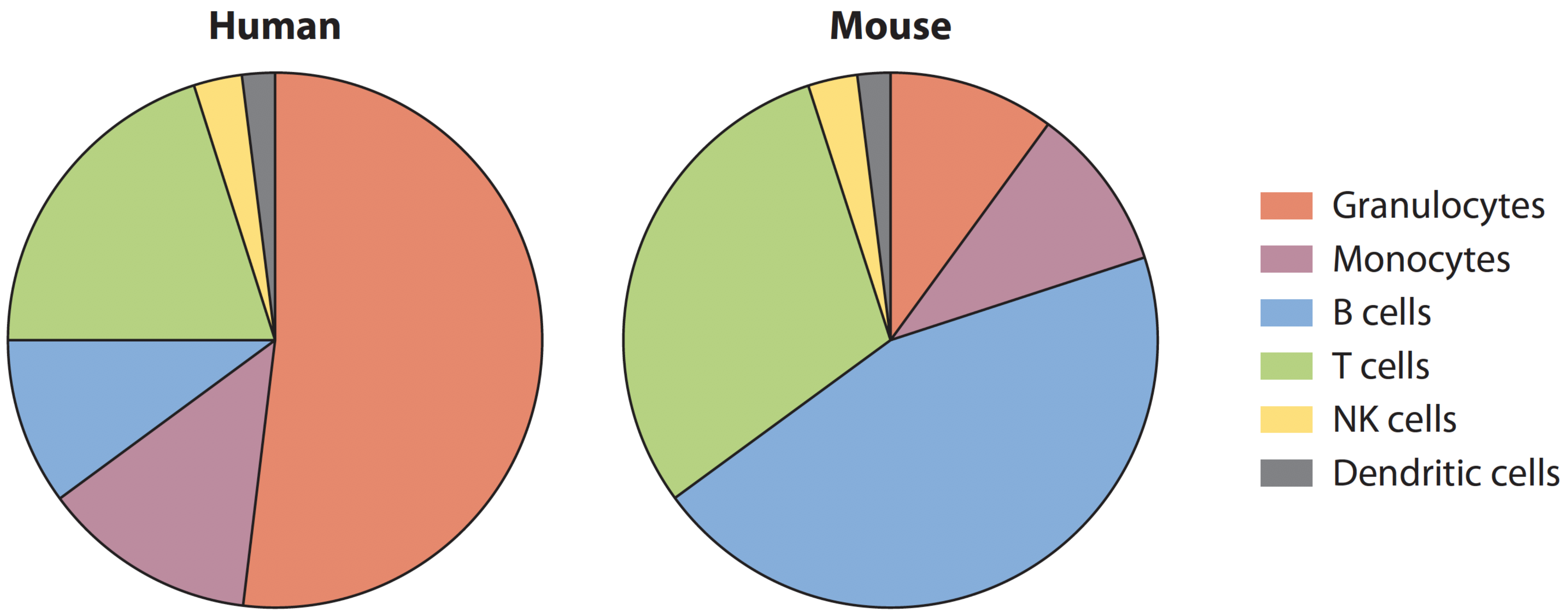
Mice are not humans

Most recent common ancestor

90 million years ago



Human and mouse white blood cell composition

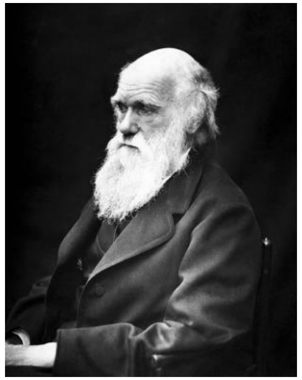


Rongvaux et al, Ann Rev Immunol 2013

Mice are not humans

Most recent common ancestor

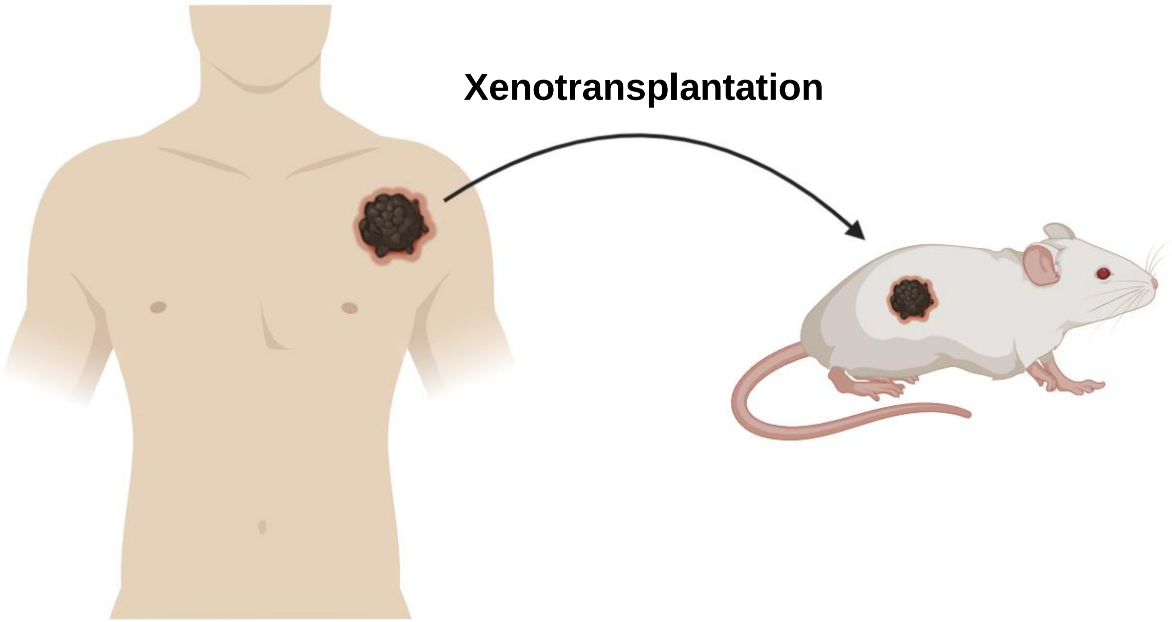
90 million years ago



All humans are different

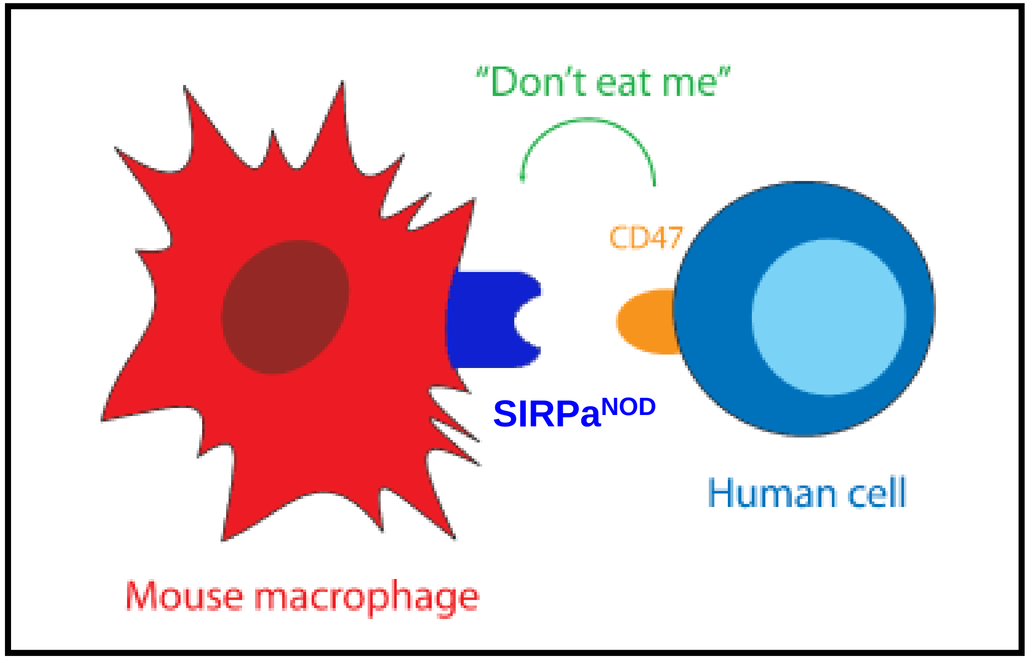
Mouse models of cancer

3) Patient-derived xenografts (PDX)



Immunodeficient "NSG" recipient mice

NOD	Phagocytic tolerance (SIRPα polymorphism)
Scid	T and B cell deficiency
IL2R**G**amma$^{-/-}$	NK cell deficiency



PDX repositories

pdxfinder.org

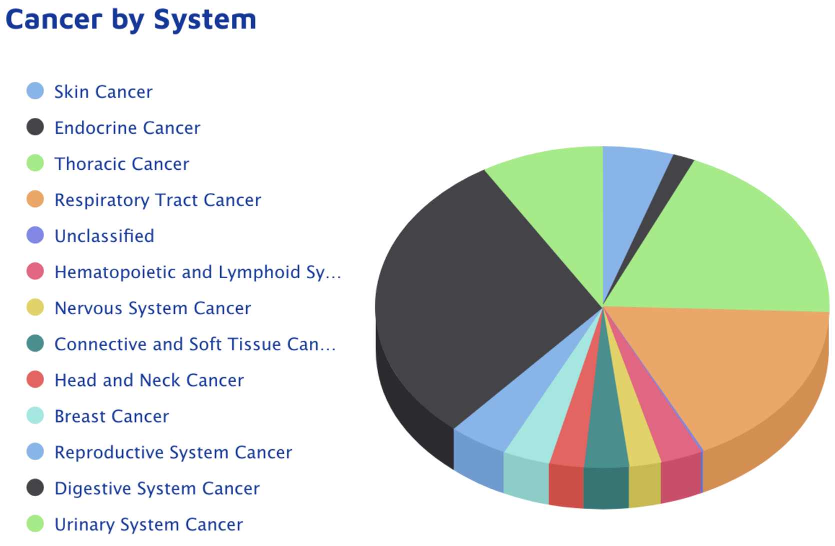
PDX FINDER

PROVIDERS

- **639** Candiolo Cancer Institute - Colorectal
- **459** Charles River Laboratories
- **406** The Jackson Laboratory
- **316** MD Anderson Cancer Center
- **298** Patient-Derived Models Repository
- **256** Wistar/MD Anderson/Penn
- **119** Washington University in St. Louis
- **94** Princess Margaret Living Biobank
- **76** Candiolo Cancer Institute-Gastric Cancer

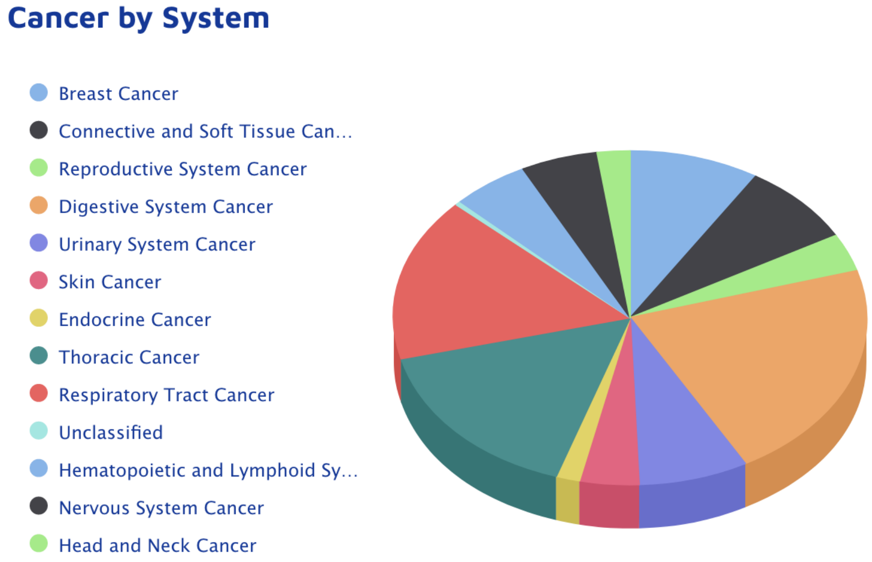
charles river

459 PDX models

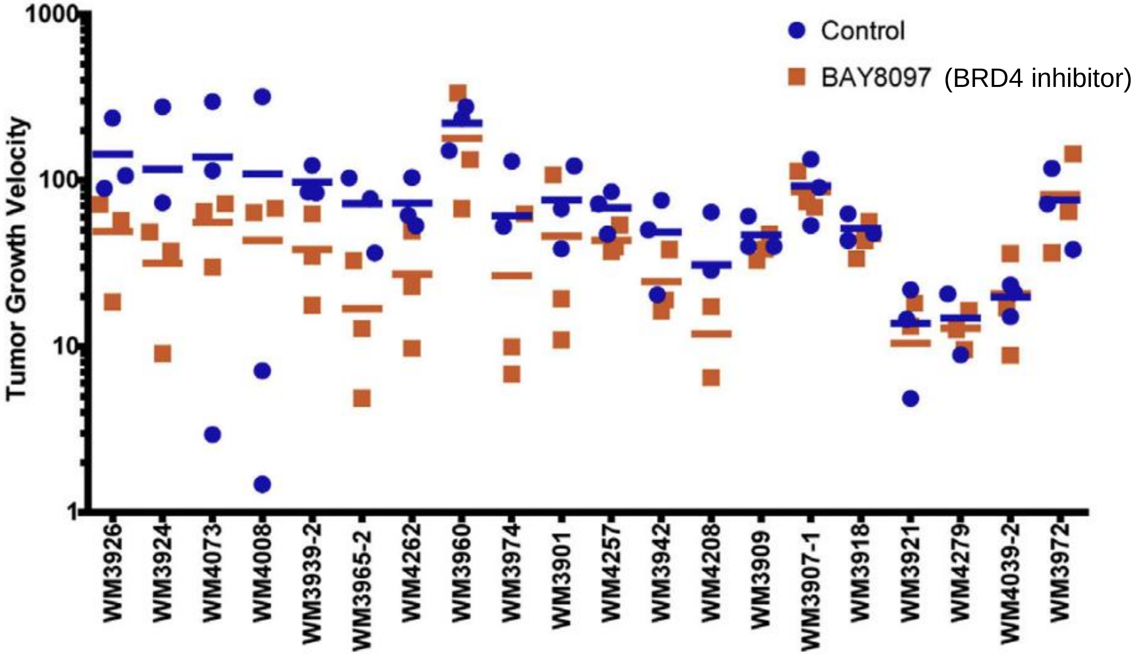


The Jackson Laboratory

406 PDX models



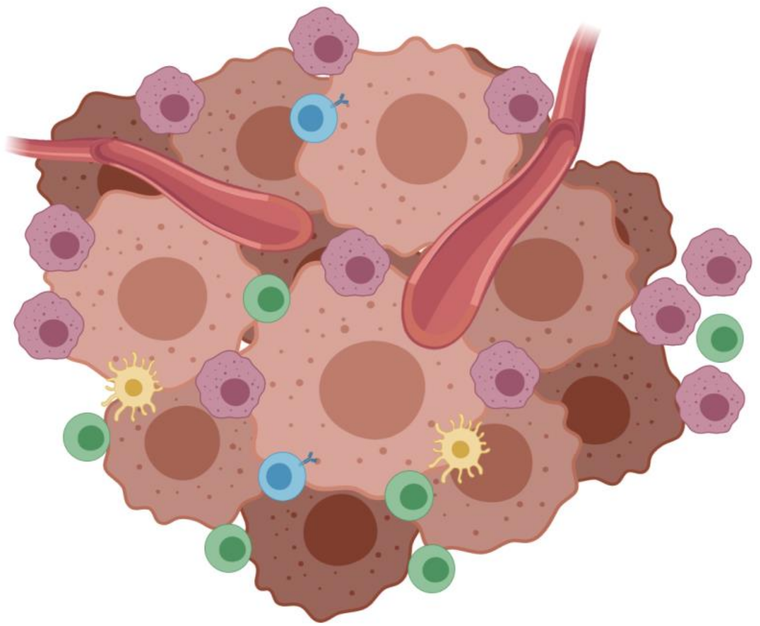
Melanoma PDX “pre-clinical” trial

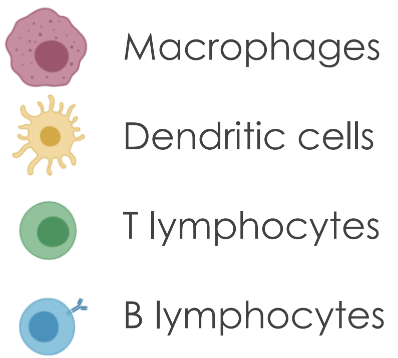


Krepler et al, Cell Reports 2017

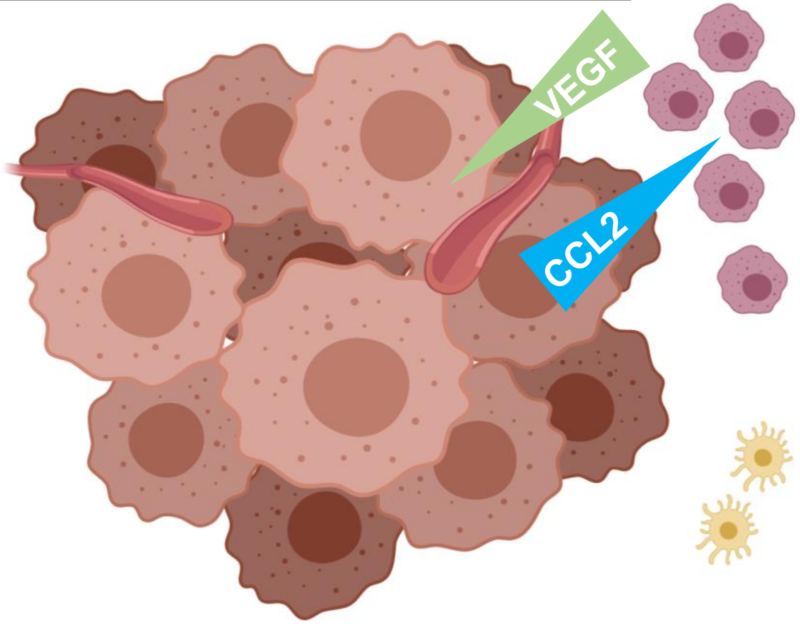
PDX lack a functional immune system

Patient tumor microenvironment





PDX tumor microenvironment



CCL2:
(35% a.a. conservation)

```
               10        20        30        40        50
Mouse_ MQVPVMLLGLLFTVAGWSIHVLAQPDAVNAPLTCCYSFTSKMIPMSRLES
       :.:   :: ::   : .    ::::::.:::.::::.::.. : . :: :
Human_ MKVSAALLCLLLIAATFIPQGLAQPDAINAPVTCCYNFTNRKISVQRLAS
               10        20        30        40        50

               60        70        80        90       100
Mouse_ YKRITSSRCPKEAVVFVTKLKREVCADPKKEWVQTYIKNLDRNQMRSEPT
       :.:::::.::::::.: : . .:.:::::..:::  . .::. : ..  :
Human_ YRRITSSKCPKEAVIFKTIVAKEICADPKQKWVQDSMDHLDK-QTQTPKT
               60        70        80        90

              110       120       130       140
Mouse_ TLFKTASALRSSAPLNVKLTRKSEANASTTFSTTTSSTSVGVTSVTVN

Human_ ------------------------------------------------
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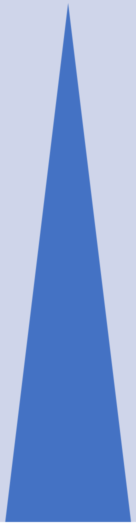
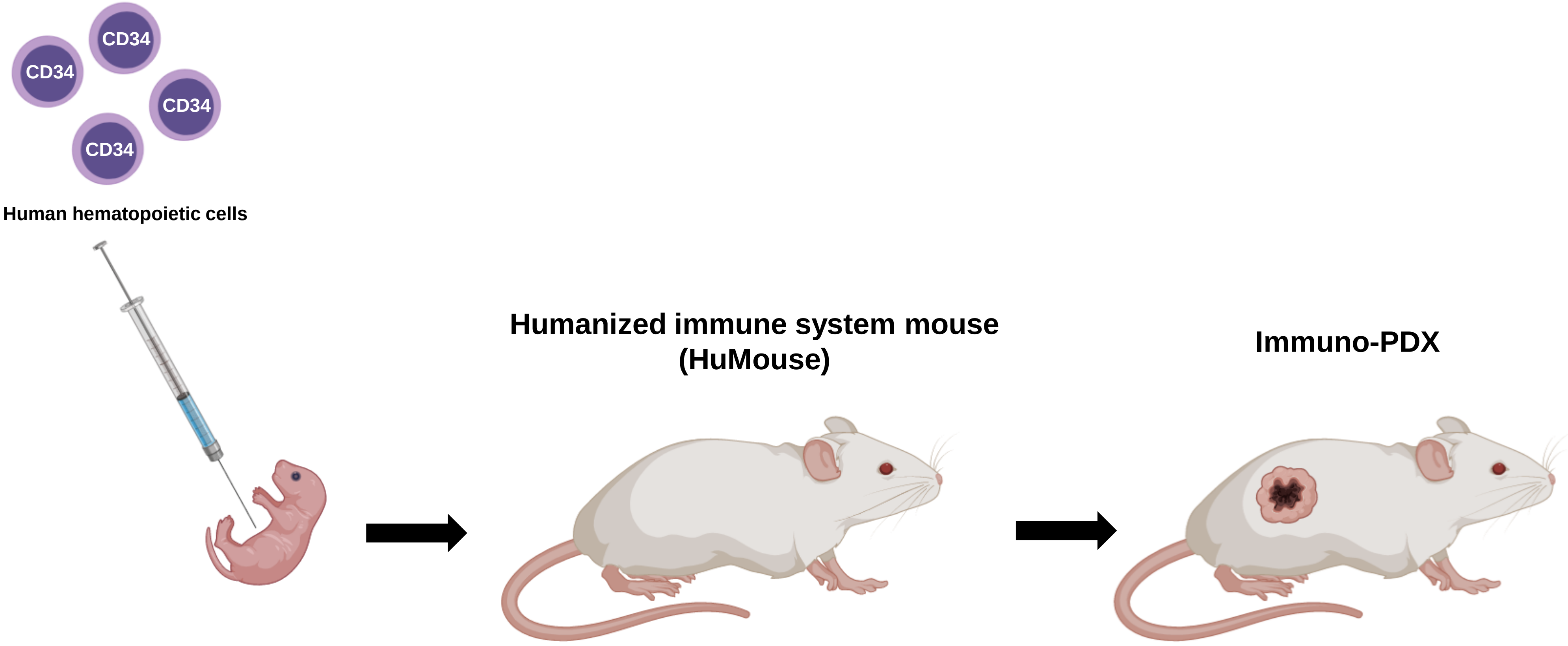
Pros and cons

	Pros	Cons	Cost
Mouse tumor implants	- Simple and reproducible - Genetic engineering	- Genetically homogenous - Artificial implantation - Mouse, not human	
GEM	- Well-defined oncogenic mutations, representative of human cancer - Oncogenesis	- Low mutational burden and immunogenicity - Mouse, not human	
PDX	- Representative of human cancer diversity	- Immunodeficiency	

Mouse models of cancer

4) PDX in mice with a humanized immune system (immuno-PDX)

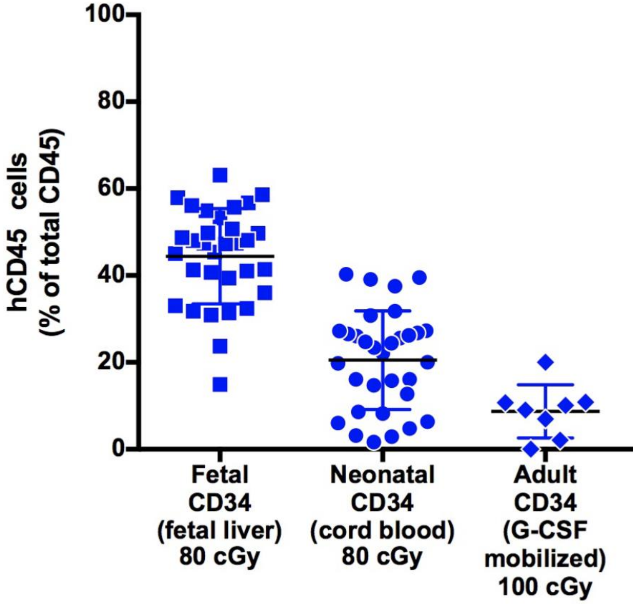


How to generate a HuMouse?

A. Source of human hematopoietic cells

- Peripheral blood mononuclear cells (PBMCs)
  → B and T cells only are maintained
  → Xeno-graft vs host disease (xGVHD)

- CD34^{+} hematopoietic stem and progenitor cells (HSPCs)
  → give rise to all blood cell types
  → sustained hematopoiesis for entire life
  → several sources of HSPCs: fetal, newborn, adult



Sippel et al, Blood Advances 2019

How to generate a HuMouse?

B. Recipient mice

		Balb/c
NOD	Phagocytic tolerance	SIRPa$^{h/h}$
Scid	T and B cell deficiency	RAG2$^{-/-}$
IL2RGamma$^{-/-}$	NK cell deficiency	IL2RGamma$^{-/-}$

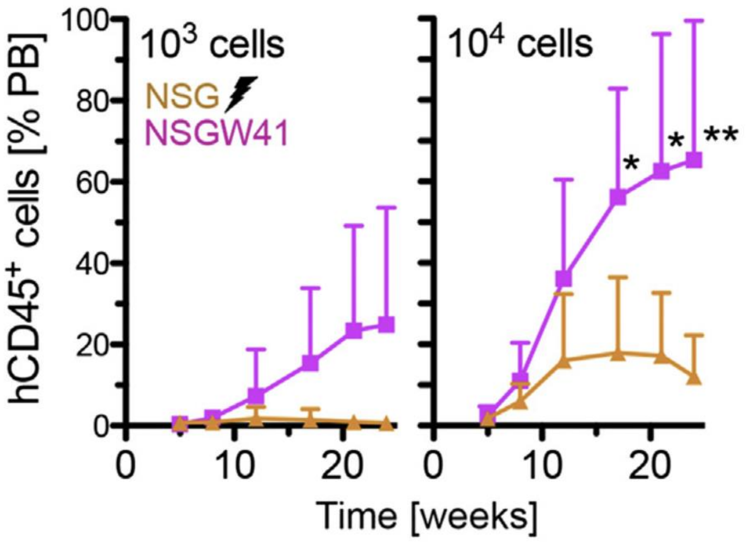
How to generate a HuMouse?

C. Opening the niche (pre-conditioning)

Irradiation

"Genetic" pre-conditioning

NOD	Phagocytic tolerance
Scid	T and B cell deficiency
IL2R**G**amma$^{-/-}$	NK cell deficiency
cKit-**W**41	Mouse HSPC deficiency



Cosgun et al, Cell Stem Cell 2014

How to generate a HuMouse?

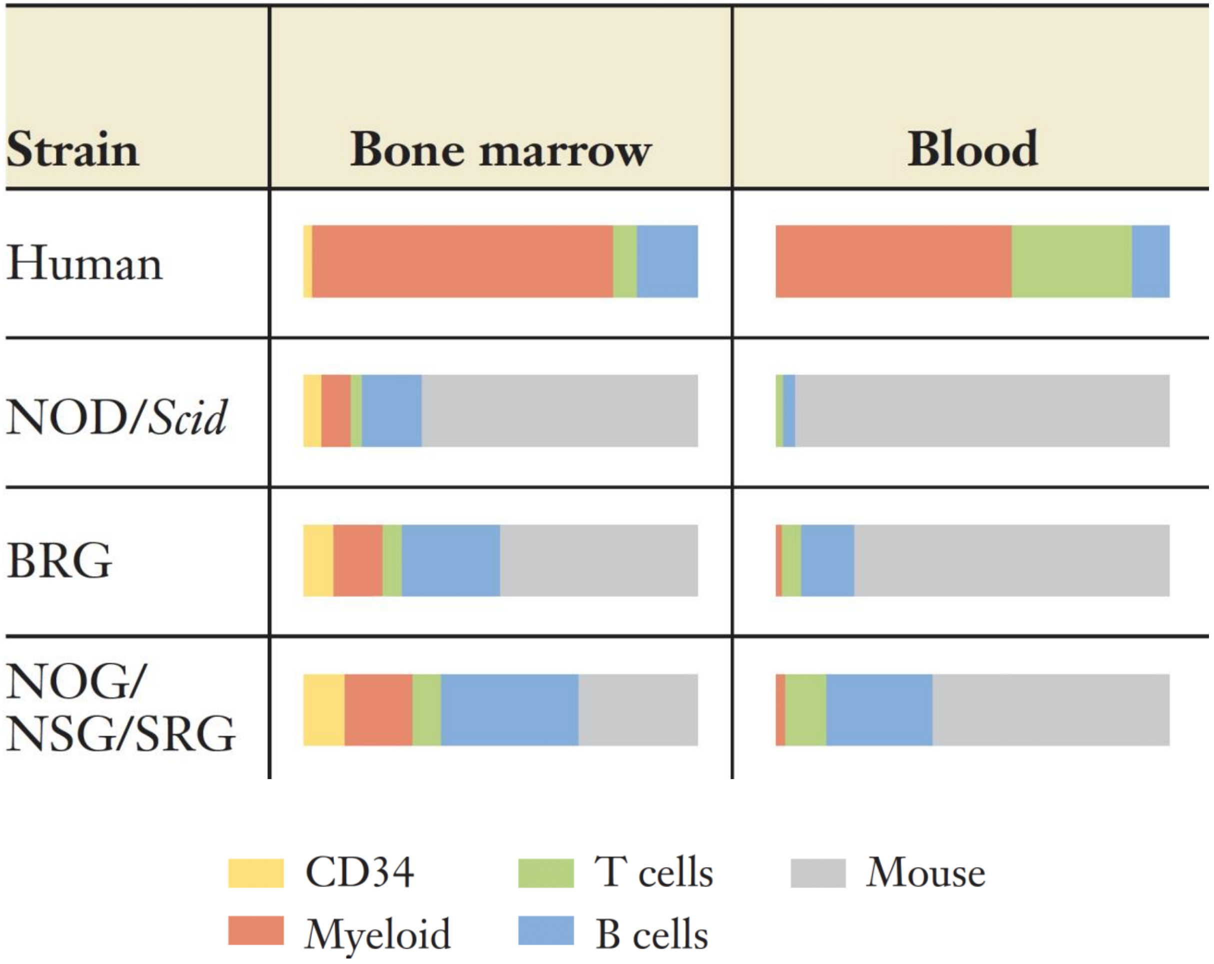
D. Orthotopic hematopoietic cell transplantation

- Intravenous injection in adult mice
- Intrafemoral injection in adult mice
- Intrahepatic injection in newborn mice
  - → the liver is a natural site of hematopoiesis until day 3-4
  - → newborns naturally support the expansion of hematopoiesis

Traggiai et al, Science 2004

How to generate a HuMouse?

E. Support for graft differentiation (cross-reactive cytokines)

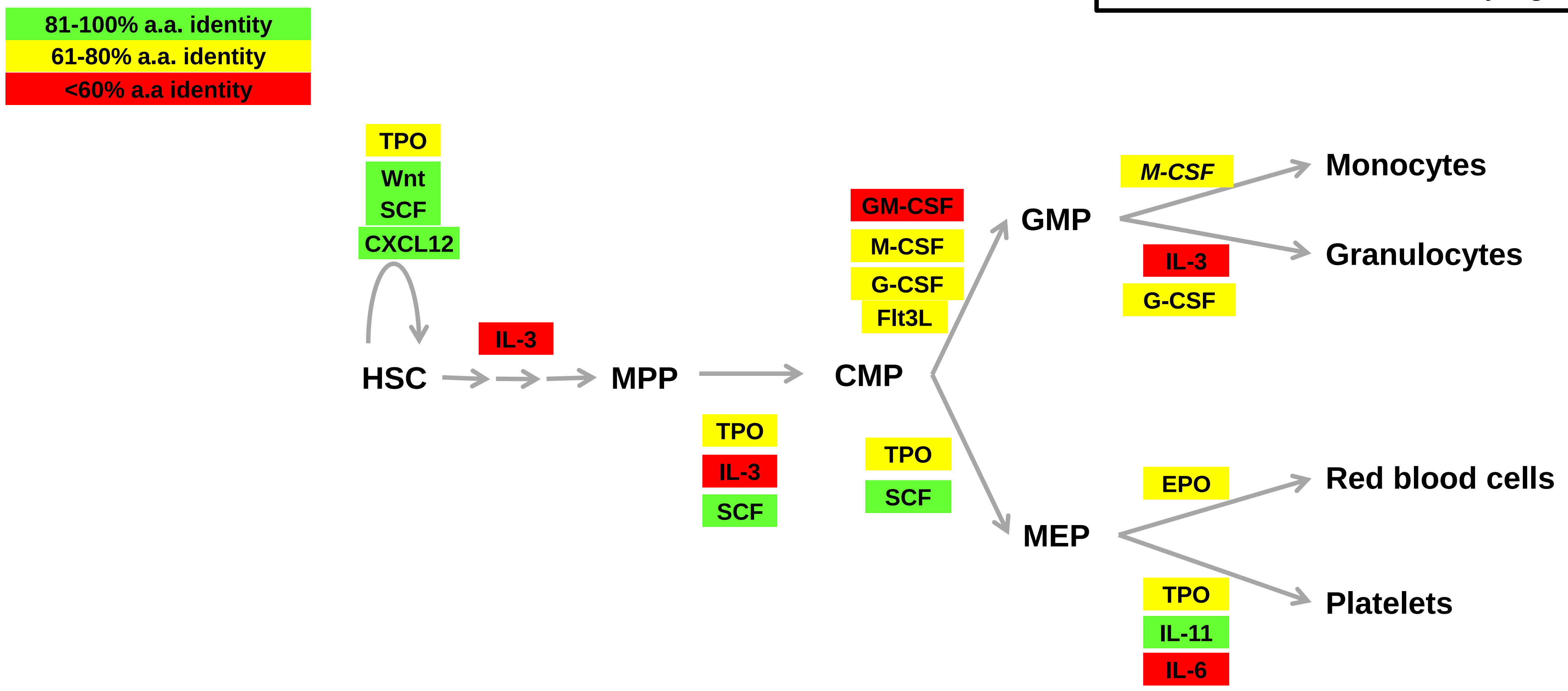


Rongvaux et al, Ann Rev Immunol 2013

How to generate a HuMouse?

HSC: Hematopoietic stem cell
MPP: Multipotent progenitor
CMP: Common myeloid progenitor
GMP: Granulocyte macrophage progenitor
MEP: Megakaryocyte erythrocyte progenitor
CDP: Common dendritic cell progenitor

E. Support for graft differentiation (cross-reactive cytokines)



Rongvaux et al, Ann Rev Immunol 2013

How to generate a HuMouse?

E. Support for graft differentiation (cross-reactive cytokines)

NOD	**Phagocytic tolerance**
Scid **IL2RGamma$^{-/-}$**	**Immunosuppression (no mouse T, B, NK cells)**
pCMV-**hSCF^{tg}**	**Longterm maintenance of functional HSCs**
pCMV-**hGM-CSF^{tg}** pCMV-**hIL3^{tg}**	**Myeloid development**

→ Transgenic overexpression of human cytokines

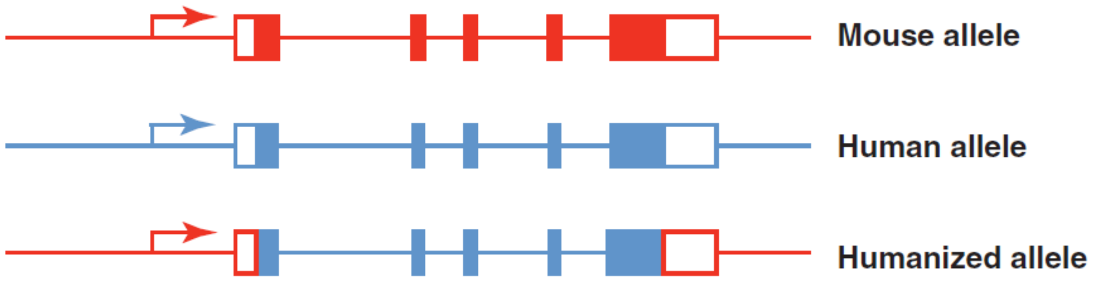
Nicolini, Leukemia 2004
Billerbeck et al, Blood 2011

How to generate a HuMouse?

E. Support for graft differentiation (cross-reactive cytokines)

M-CSF$^{h/h}$ IL-3/GM-CSF$^{h/h}$	Myeloid development
Sirpα$^{h/m}$	Phagocytic tolerance
TPO$^{h/h}$	Longterm maintenance of functional HSCs
RAG2$^{-/-}$ IL2RGamma$^{-/-}$	Immunosuppression (no mouse T, B, NK cells)

→ Knockin replacement (mouse to human) of cytokine-encoding genes (Velocigene)



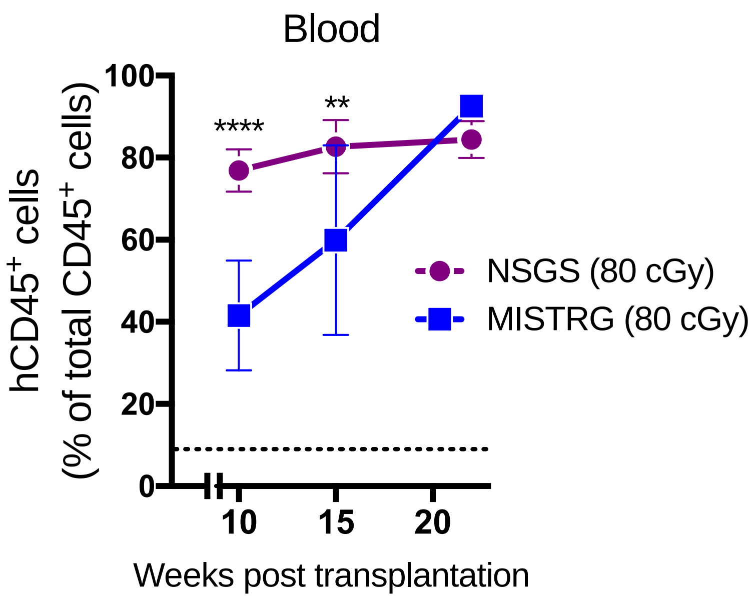
Valenzuela et al, Nat Biotech 2003
Rongvaux et al, Nat Biotech 2014

How to generate a HuMouse?

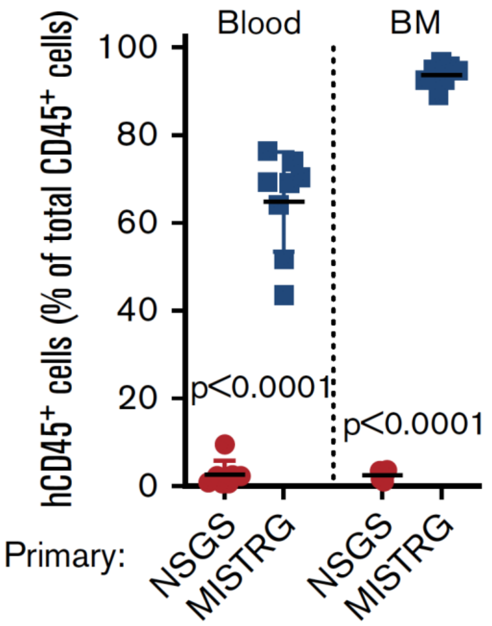
E. Support for graft differentiation (cross-reactive cytokines)

NSG-SGM3 (“NSGS”) vs. MISTRG

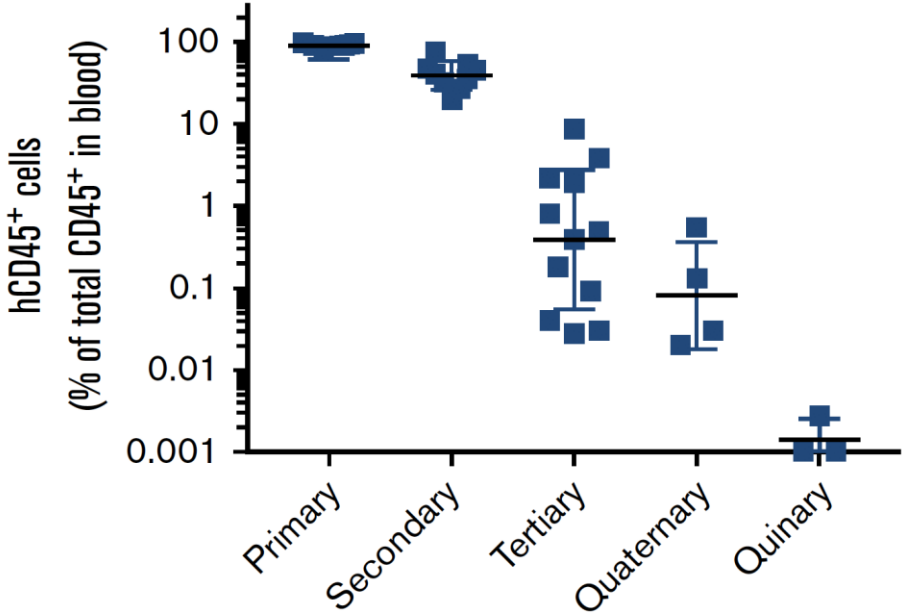
Overall engraftment



Secondary transplantation



Serial transplantation (MISTRG)



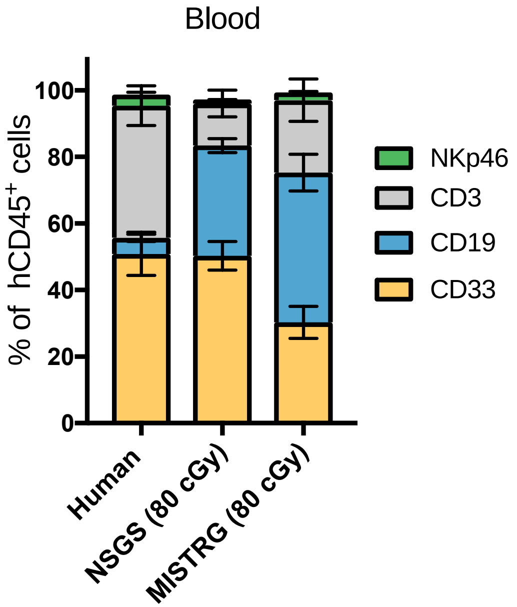
Sippel et al, Blood Advances 2019

How to generate a HuMouse?

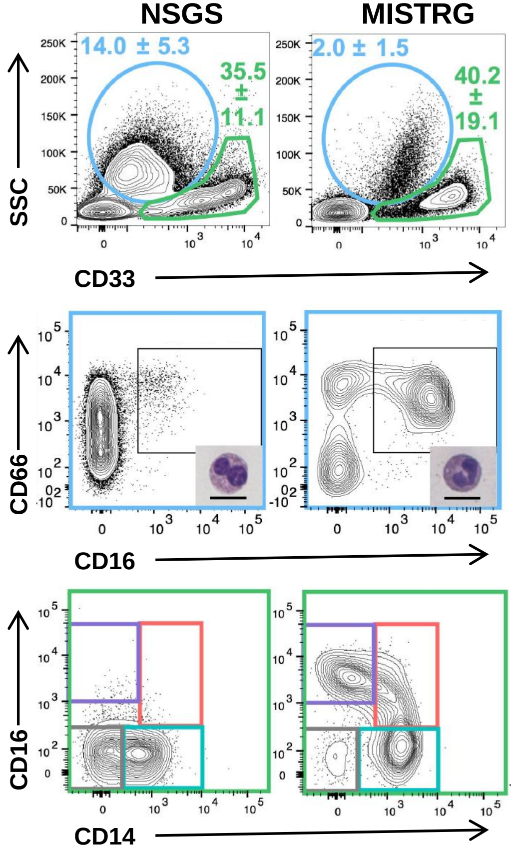
E. Support for graft differentiation (cross-reactive cytokines)

NSG-SGM3 (“NSGS”) vs. MISTRG

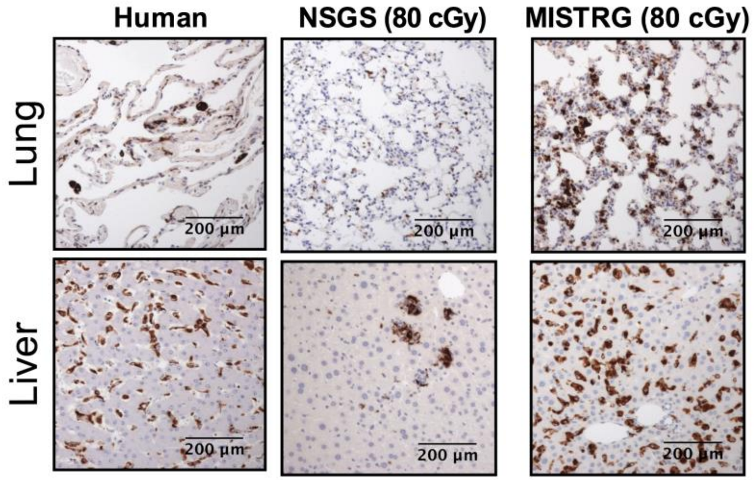
Myeloid cell development



Granulocyte/monocyte maturation



Tissue macrophages

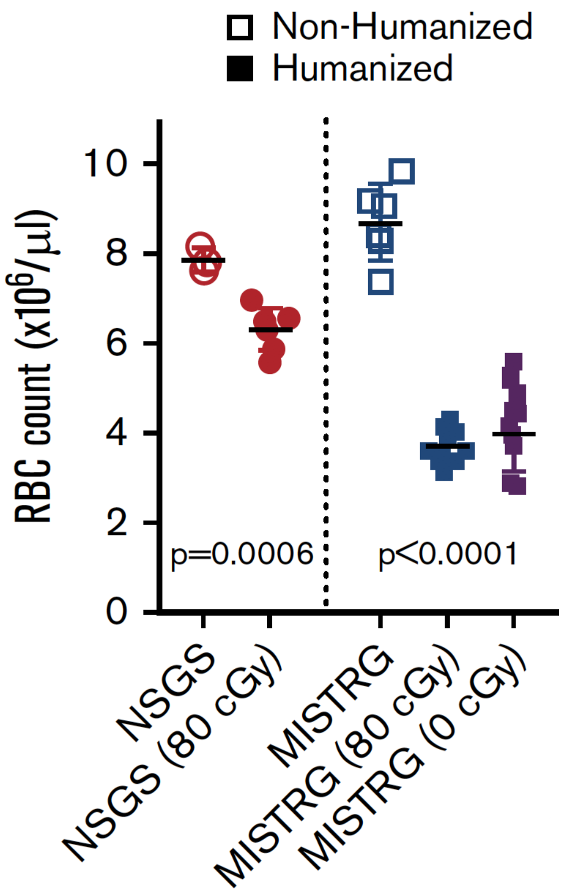


hCD68
(human macrophages)

Sippel et al, Blood Advances 2019

How to generate a HuMouse?

F. Graft vs. host innate and adaptive tolerance

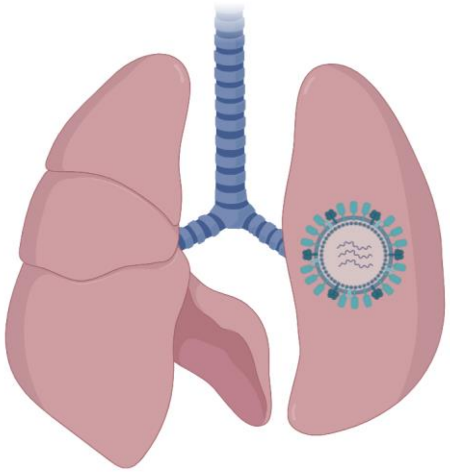
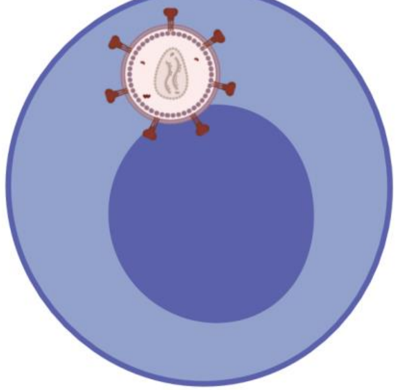
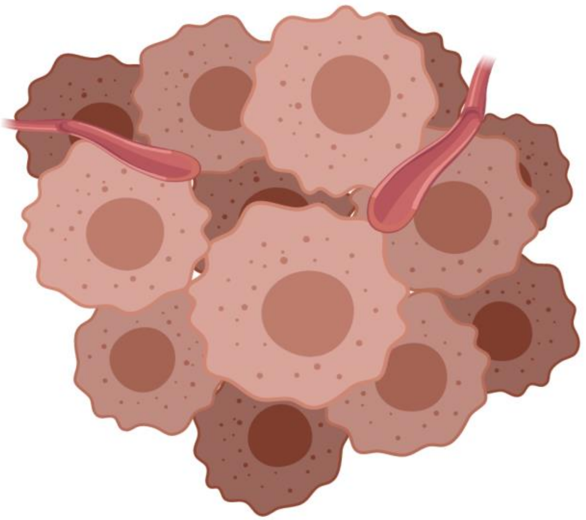


→ Mouse red blood cells phagocytosed by human macrophages

Sippel et al, Blood Advances 2019

How to generate a HuMouse?

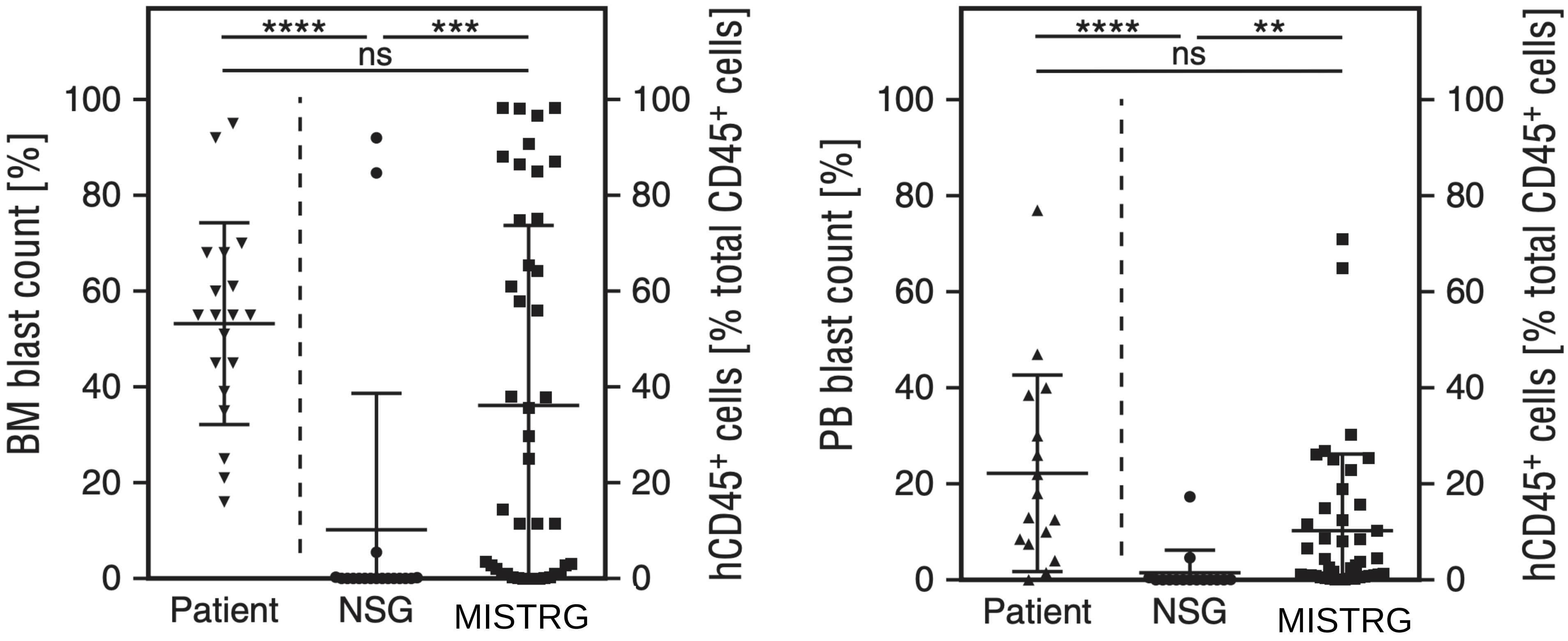
G. Reactivity of effector mechanisms on target cells/tissues

	Influenza infection	HIV infection	Human tumor (PDX)	
				
Target cells:	Mouse lung	Human T cells	Human cancer cells	Mouse vasculature
Cross-reactivity of human immune cells:	✗	✓	✓	✗

Rongvaux et al, Ann Rev Immunol 2013

Transplantation of human hematopoietic diseases

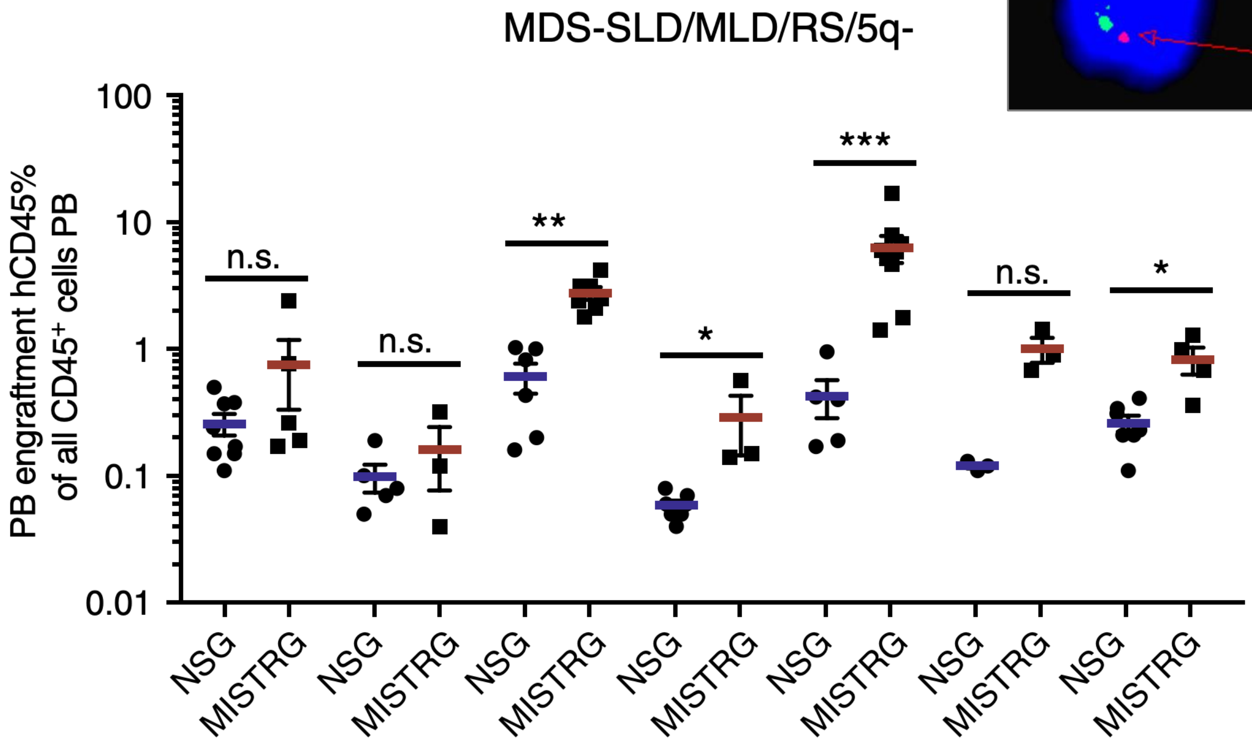
Acute myeloid leukemia (AML) – inv16 "good risk" AML

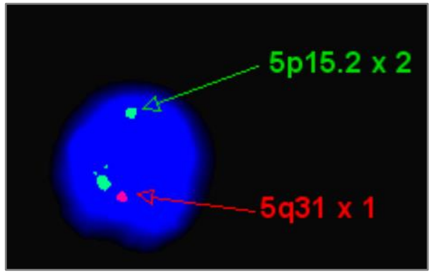


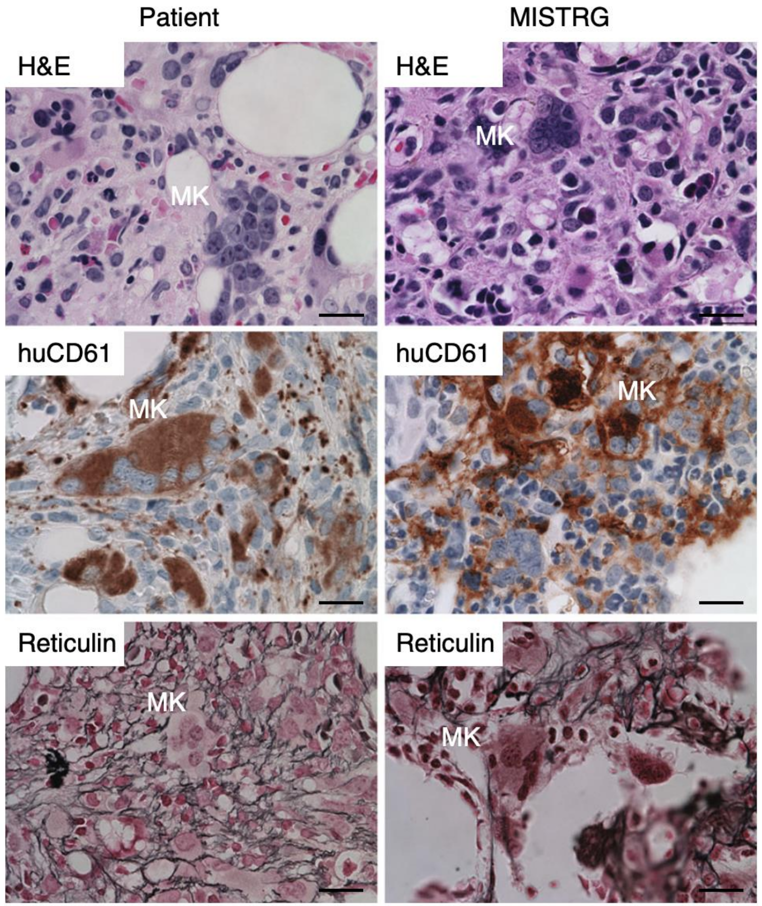
Ellegast et al, Blood 2016

Transplantation of human hematopoietic diseases

Myelodysplastic syndromes (MDS)





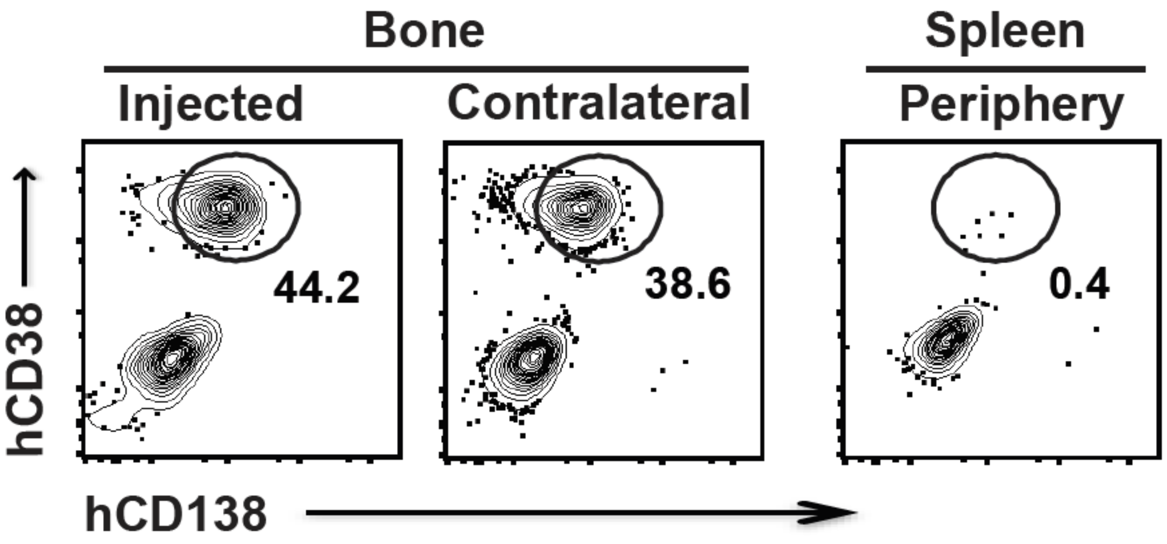


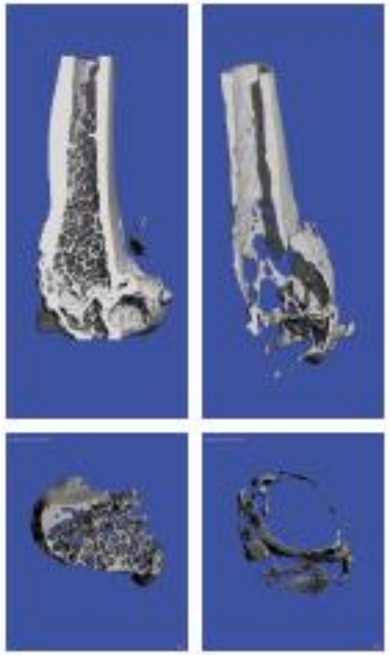
Song et al, Nat Comm 2019

Transplantation of human hematopoietic diseases

Multiple myeloma

M-CSF$^{h/h}$
IL3/GM-CSF$^{h/h}$
Sirpα$^{h/m}$
TPO$^{h/h}$
RAG2$^{-/-}$
IL2R**G**amma$^{-/-}$
IL**6**$^{h/h}$



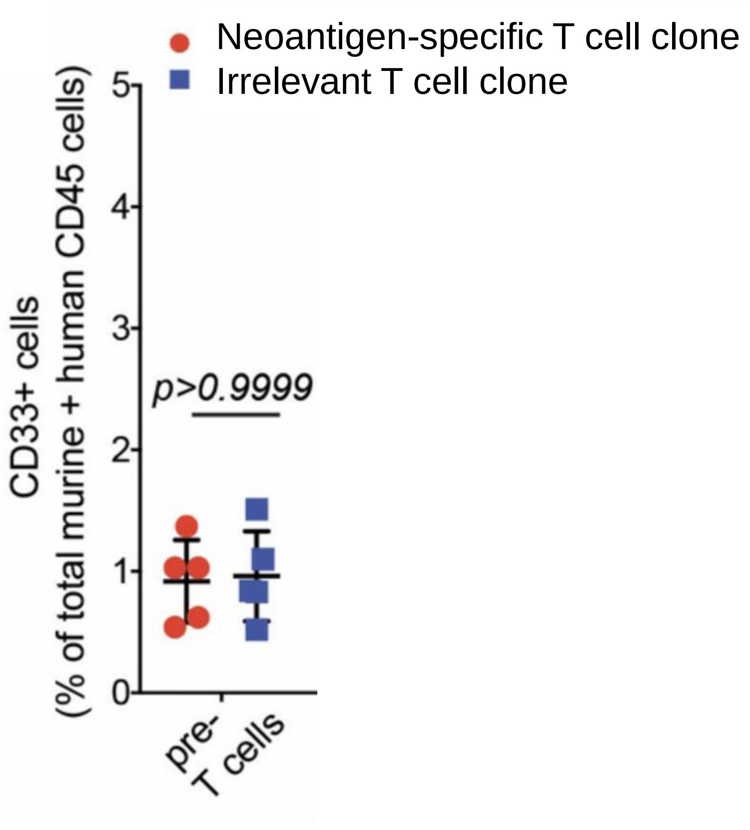


Das et al, Nat Medicine 2016

Modeling adoptive T cell therapy of AML

Effective clearance of leukemia

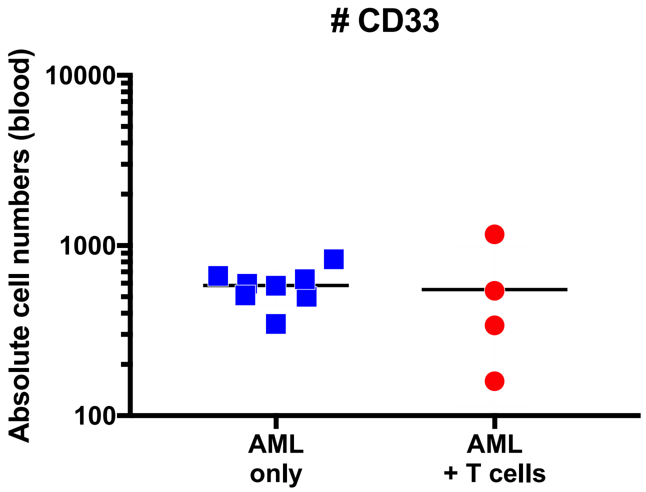
Primary AML transplanted in MISTRG mice
“Good risk” AML – Core binding factor (CBF) fusion protein
T cells specific for a neoantigen in the fusion protein

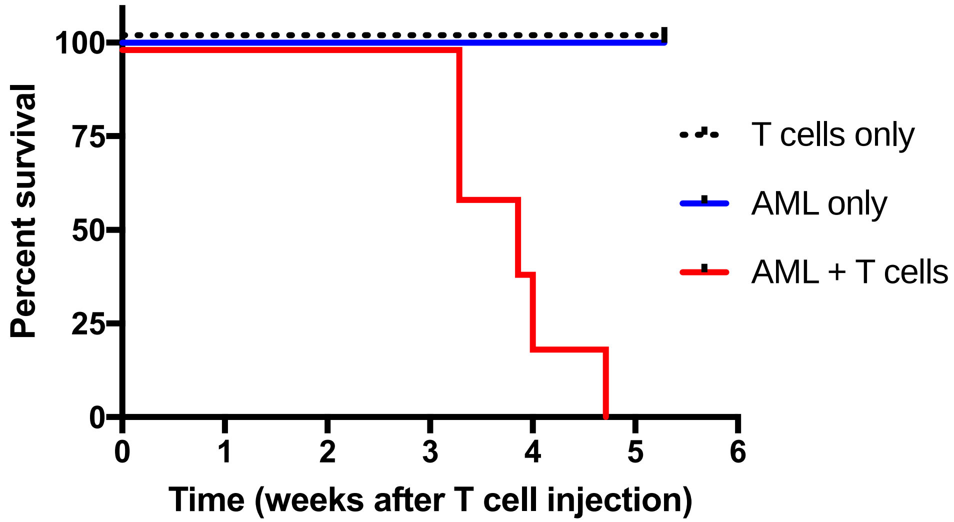


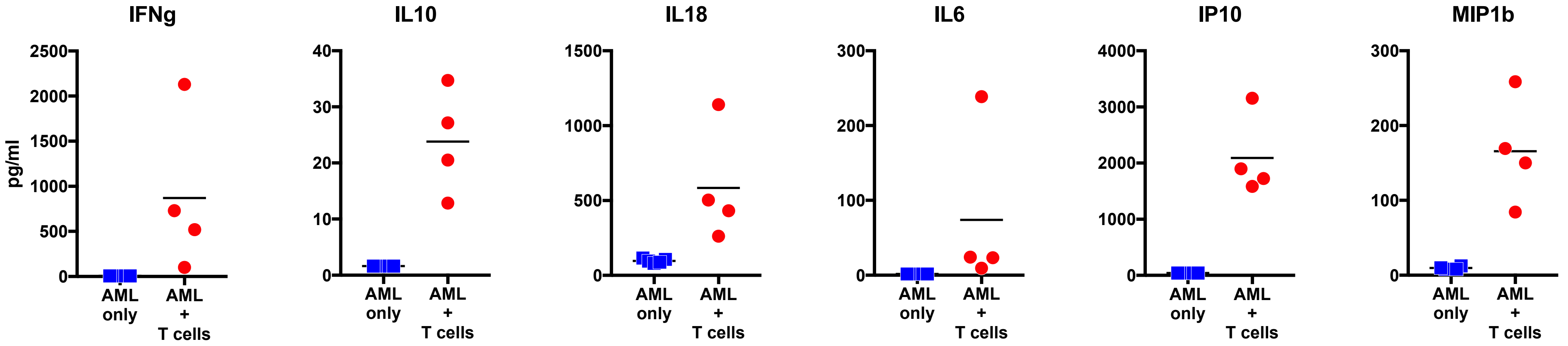
Modeling adoptive T cell therapy of AML

Cytokine release syndrome

Primary AML in MISTRG mice
T cells specific for a cancer/testis antigen

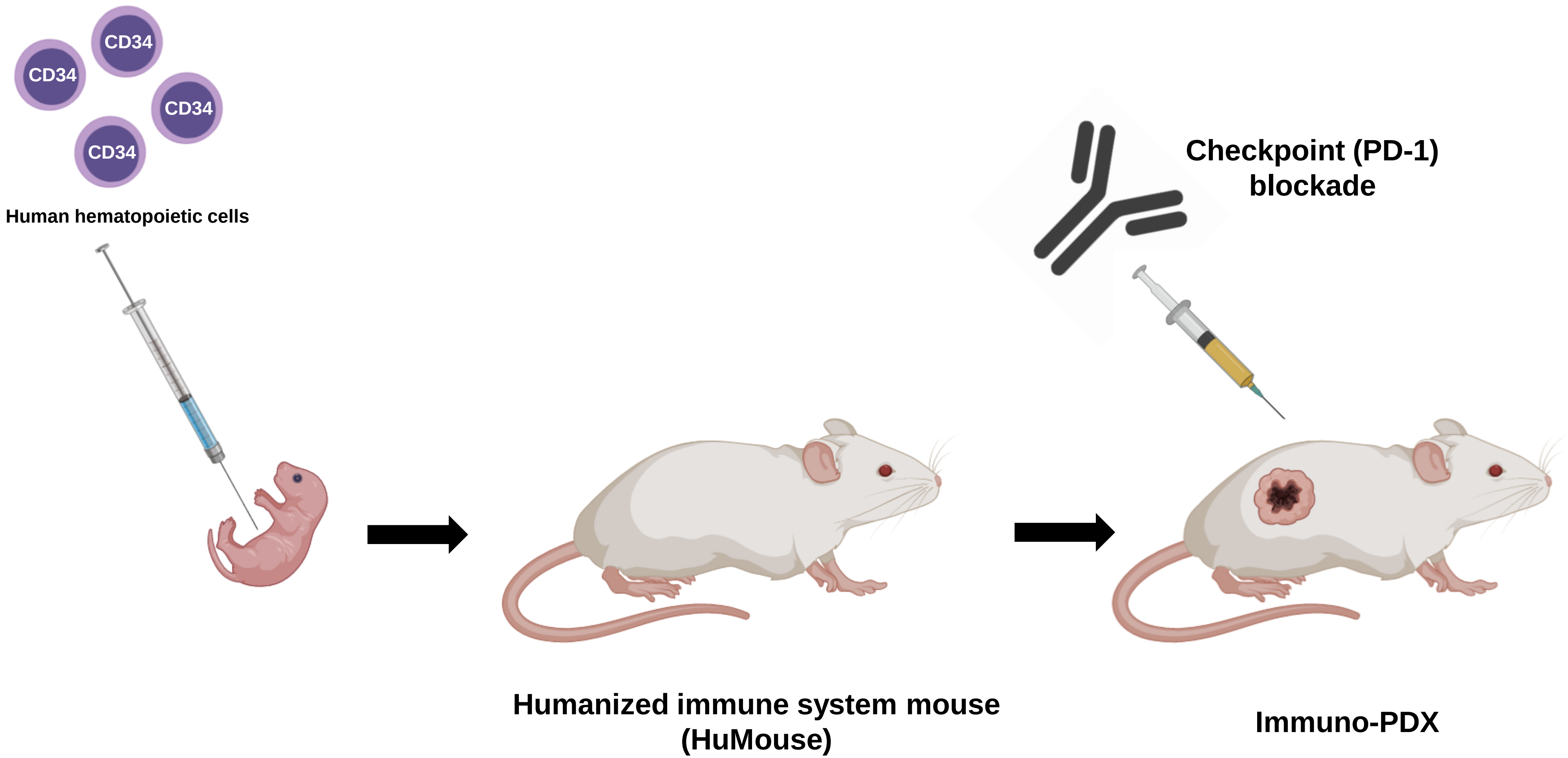






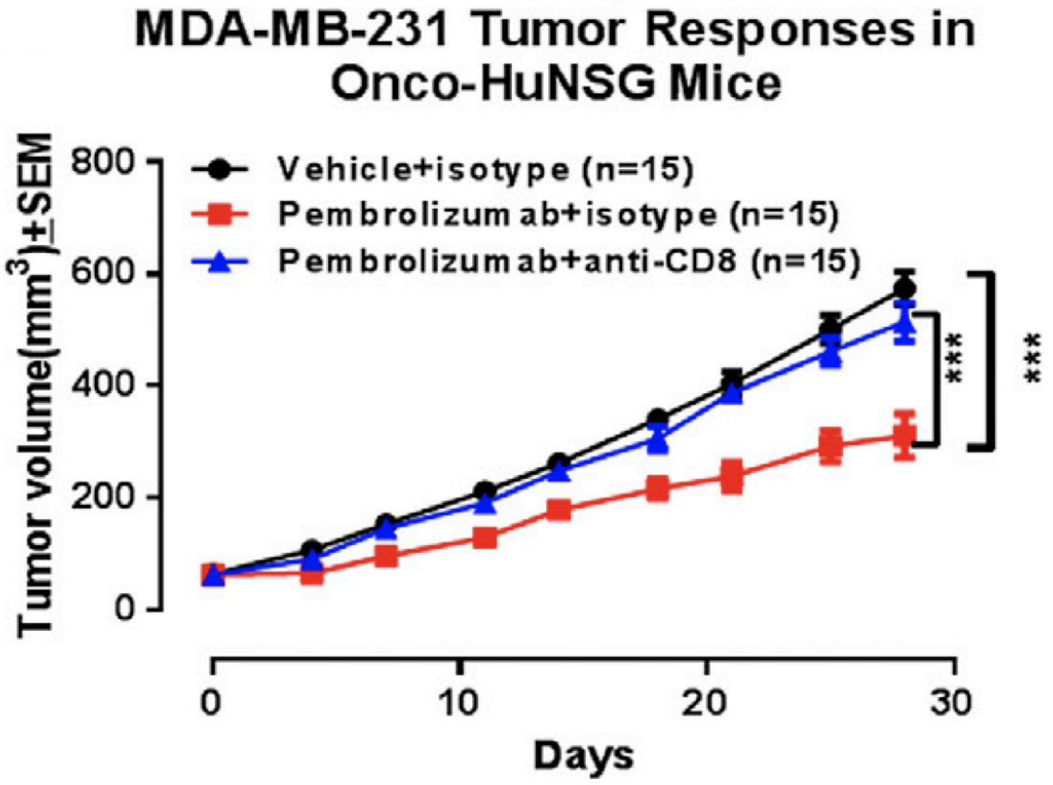
Denise Buenrostro

Modeling checkpoint inhibition in solid tumors



Modeling checkpoint inhibition in solid tumors

Breast cancer cell line



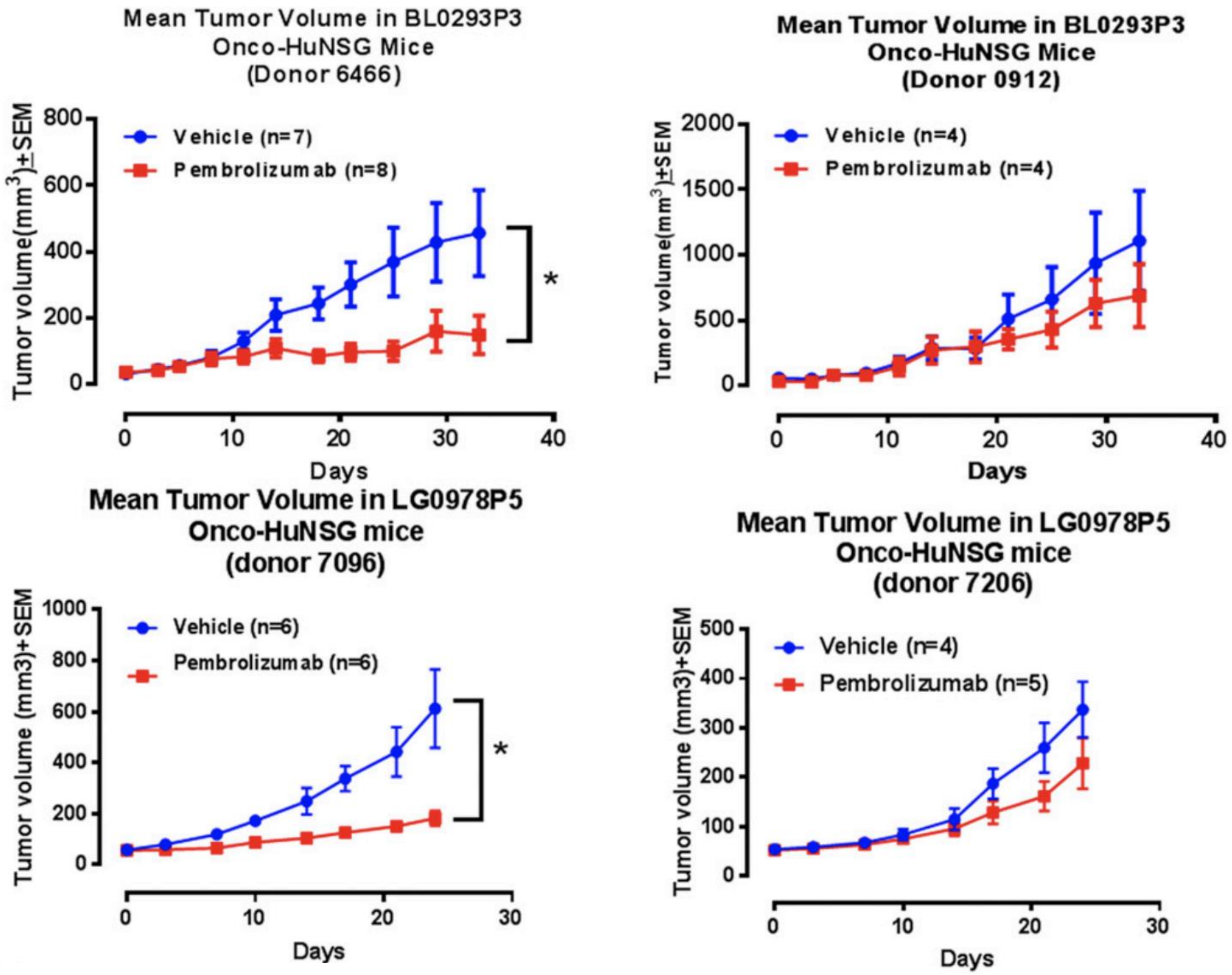
Wang et al, FASEB J 2018

Modeling checkpoint inhibition in solid tumors

PDX - hematopoietic cell donor variation

Bladder cancer PDX

Non small cell lung cancer PDX



Wang et al, FASEB J 2018

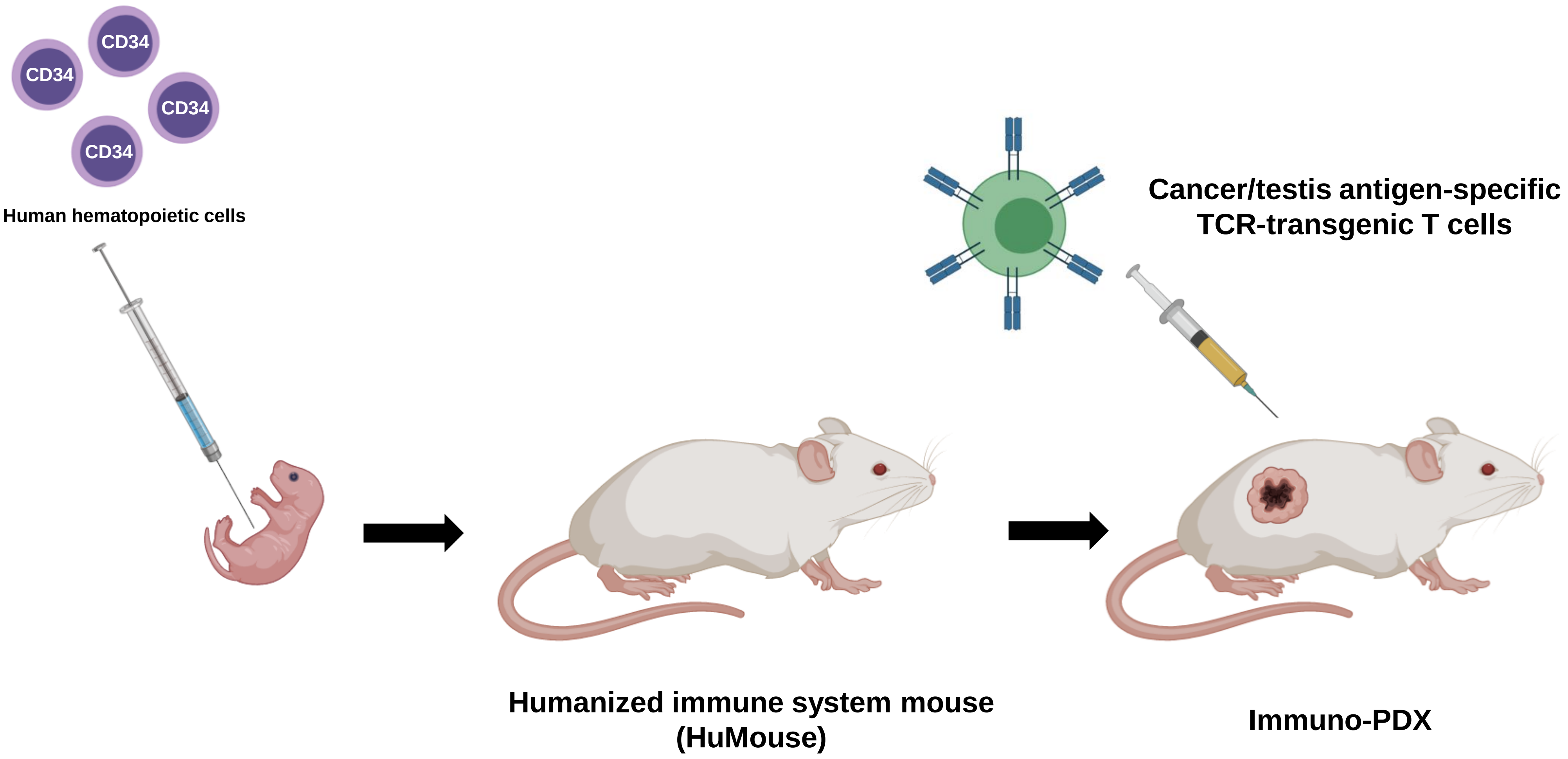
T cells in humanized mice

- Human T cells develop in the mouse thymus
  - → Tolerance (no xeno-GVHD)
  - → Abnormal TCR repertoire selection
- CD4/CD8, Treg, naïve/effector/memory subsets are normal
- Normal response ex-vivo to polyclonal stimulation (proliferation, cytokines)
- Defective structure of secondary lymphoid structure

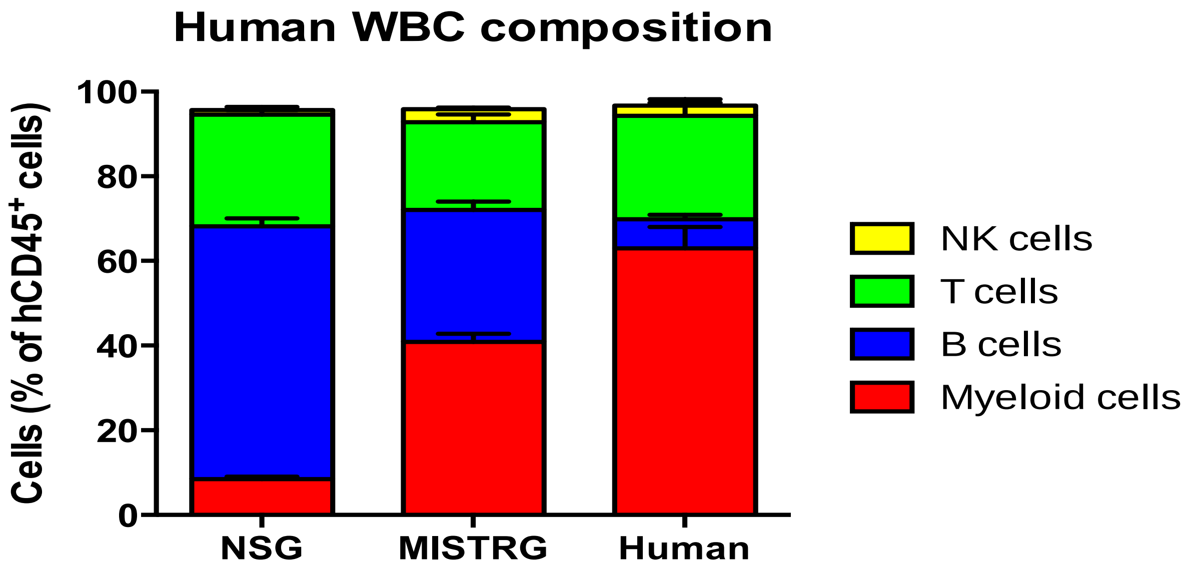
➔ Generally, low efficiency of de novo adaptive immune responses

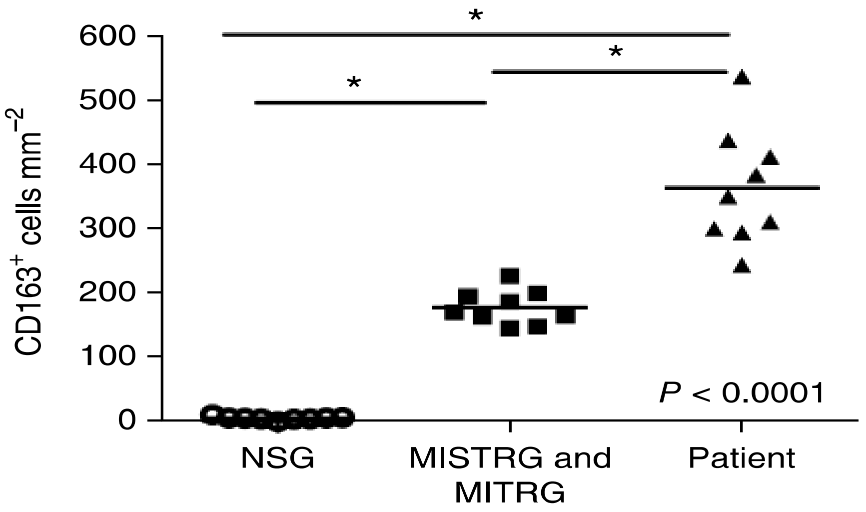
➔ Next-gen HuMice: HLA expression, additional cytokines, restore lymphoid structures

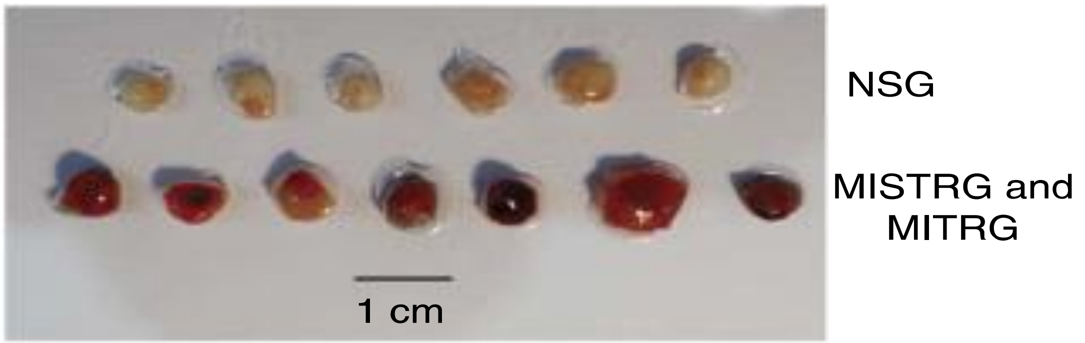
Modeling adoptive T cell therapy in solid tumors



Studying macrophage function in a solid tumor

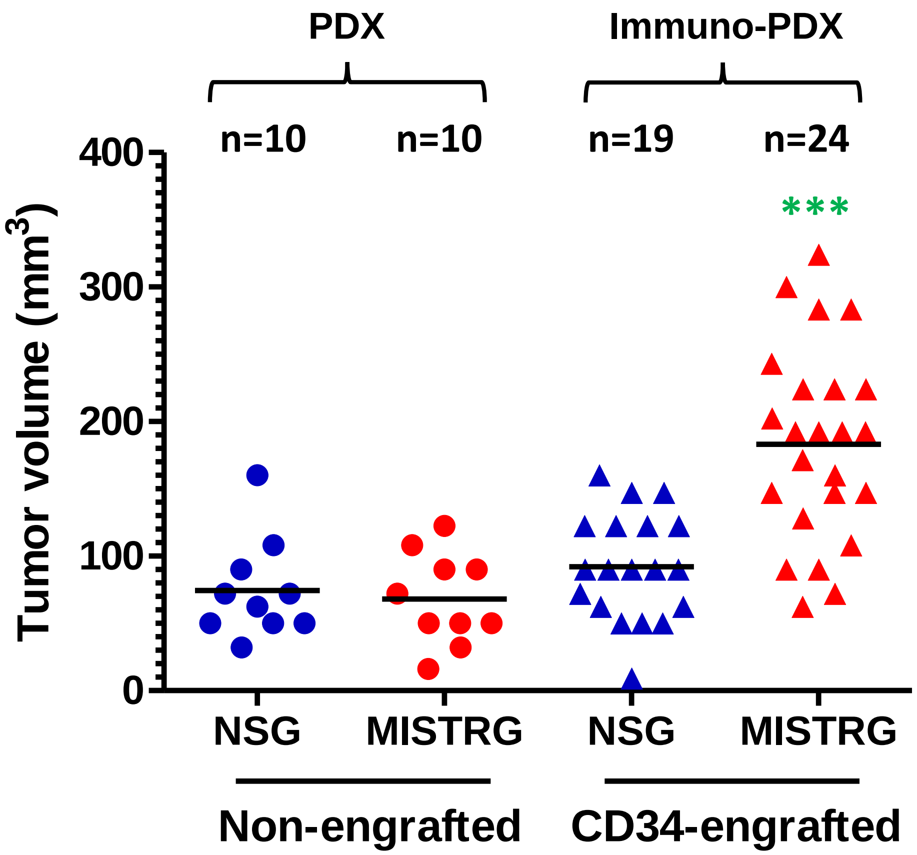






Rongvaux et al, Nat Biotech 2014

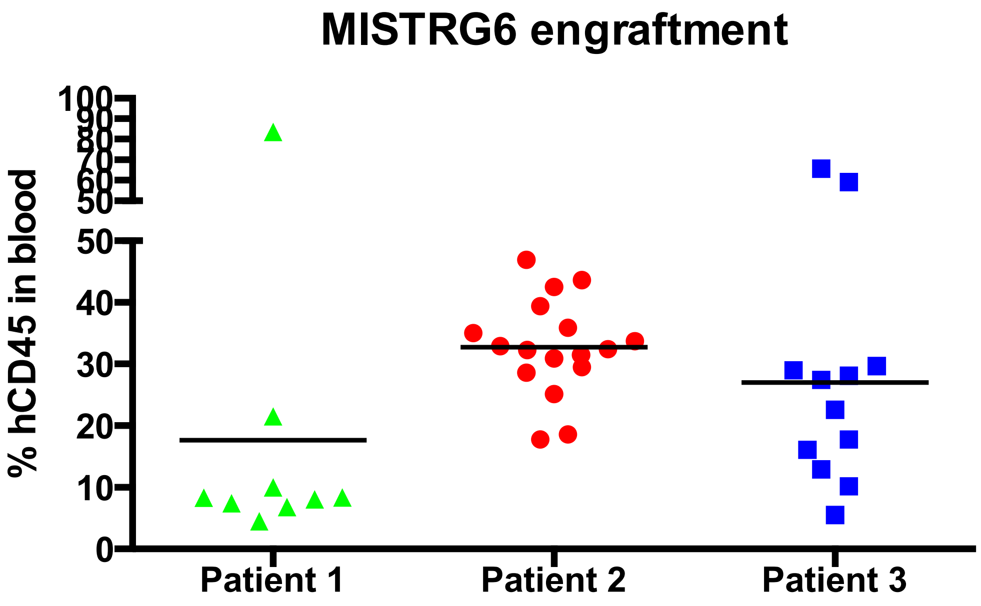
Studying macrophage function in a solid tumor

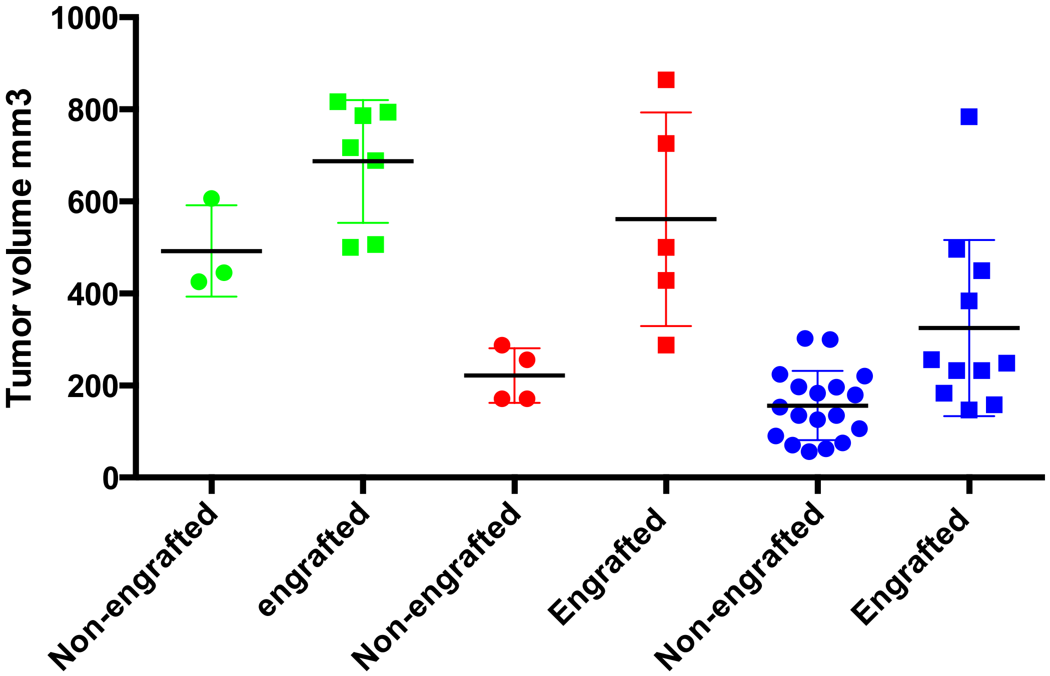


One-way ANOVA p<0.0001
*** p<0.05 vs. all other group
(Tukey post-hoc test)

Rongvaux et al, Nat Biotech 2014

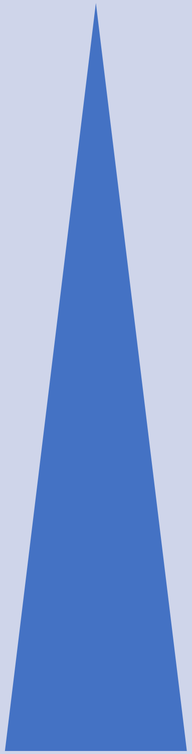
A fully patient-derived immuno-PDX model





Michael Chiorazzi (Yale)
Jan Martinek (Jax)

Pros and cons

	Pros	Cons	Cost
Mouse tumor implants	- Simple and reproducible - Genetic engineering	- Genetically homogenous - Artificial implantation - Mouse, not human	
GEM	- Well-defined oncogenic mutations, representative of human cancer - Oncogenesis	- Low mutational burden and immunogenicity - Mouse, not human	
PDX	- Representative of human cancer diversity	- Immunodeficiency	
Immuno-PDX	- Representative of human cancer diversity - Human immune system is somewhat function - Recapitulate some aspects of human immunity and response to immunotherapy	- Human donor variability (immune responses) - Human immune system functionally incomplete - Mismatch immune/tumor donor - Prototypes: need extensive development and validation	

Conclusions

- Consider strengths and weaknesses of each model
- Optimize and validate each disease model
- Start with the simplest possible experiments
- Confirm results in independent models and in human

Example #1

- Good risk AML
- Antigen-specific T cell clone

➔ Effective killing of the leukemia in vivo?

Example #2

- B cell leukemia
- CAR19 T cell therapy
- Cytokine release syndrome

➔ What is the role myeloid cells? Which subsets produce cytokines?

Example #3

- Pancreas cancer patients → samples available for PDX
- Checkpoint inhibition
- Combination with a new drug

➔ Can we use HuMice to predict response in patients?
(the company will pay for the mice)

Resources

Cell

Preclinical Mouse Cancer Models: A Maze of Opportunities and Challenges

Chi-Ping Day,[1] Glenn Merlino,[1,*] and Terry Van Dyke[2,*]

rongvaux@fredhutch.org

@RongvauxLab

nature REVIEWS CANCER

OPINION

Interrogating open issues in cancer precision medicine with patient-derived xenografts

Annette T. Byrne, Denis G. Alférez, Frédéric Amant, Daniela Annibali, Joaquín Arribas, Andrew V. Biankin, Alejandra Bruna, Eva Budinská, Carlos Caldas, David K. Chang, Robert B. Clarke, Hans Clevers, George Coukos, Virginie Dangles-Marie, S. Gail Eckhardt, Eva Gonzalez-Suarez, Els Hermans, Manuel Hidalgo, Monika A. Jarzabek, Steven de Jong, Jos Jonkers, Kristel Kemper, Luisa Lanfrancone, Gunhild Mari Mælandsmo, Elisabetta Marangoni, Jean-Christophe Marine, Enzo Medico, Jens Henrik Norum, Héctor G. Palmer, Daniel S. Peeper, Pier Giuseppe Pelicci, Alejandro Piris-Gimenez, Sergio Roman-Roman, Oscar M. Rueda, Joan Seoane, Violeta Serra, Laura Soucek, Dominique Vanhecke, Alberto Villanueva, Emilie Vinolo, Andrea Bertotti and Livio Trusolino

ANNUAL REVIEWS

Annual Review of Immunology

Human Hemato-Lymphoid System Mice: Current Use and Future Potential for Medicine

Anthony Rongvaux,[1] Hitoshi Takizawa,[3] Till Strowig,[1] Tim Willinger,[1] Elizabeth E. Eynon,[1] Richard A. Flavell,[1,2,*] and Markus G. Manz[3,*]