Pre-clinical mouse models of cancer immunotherapy

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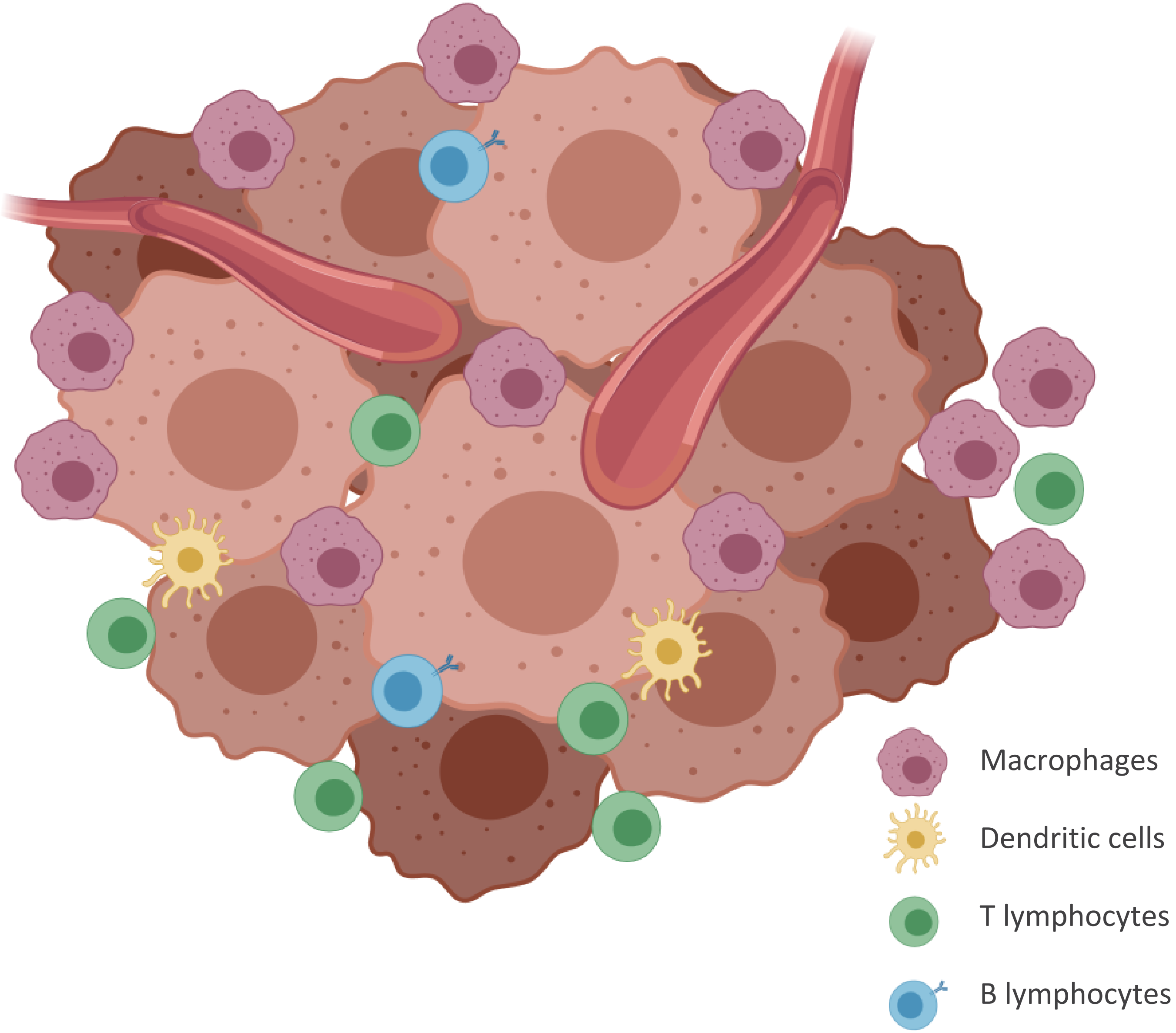
CURES START HERE

The tumor microenvironment is complex



Cell

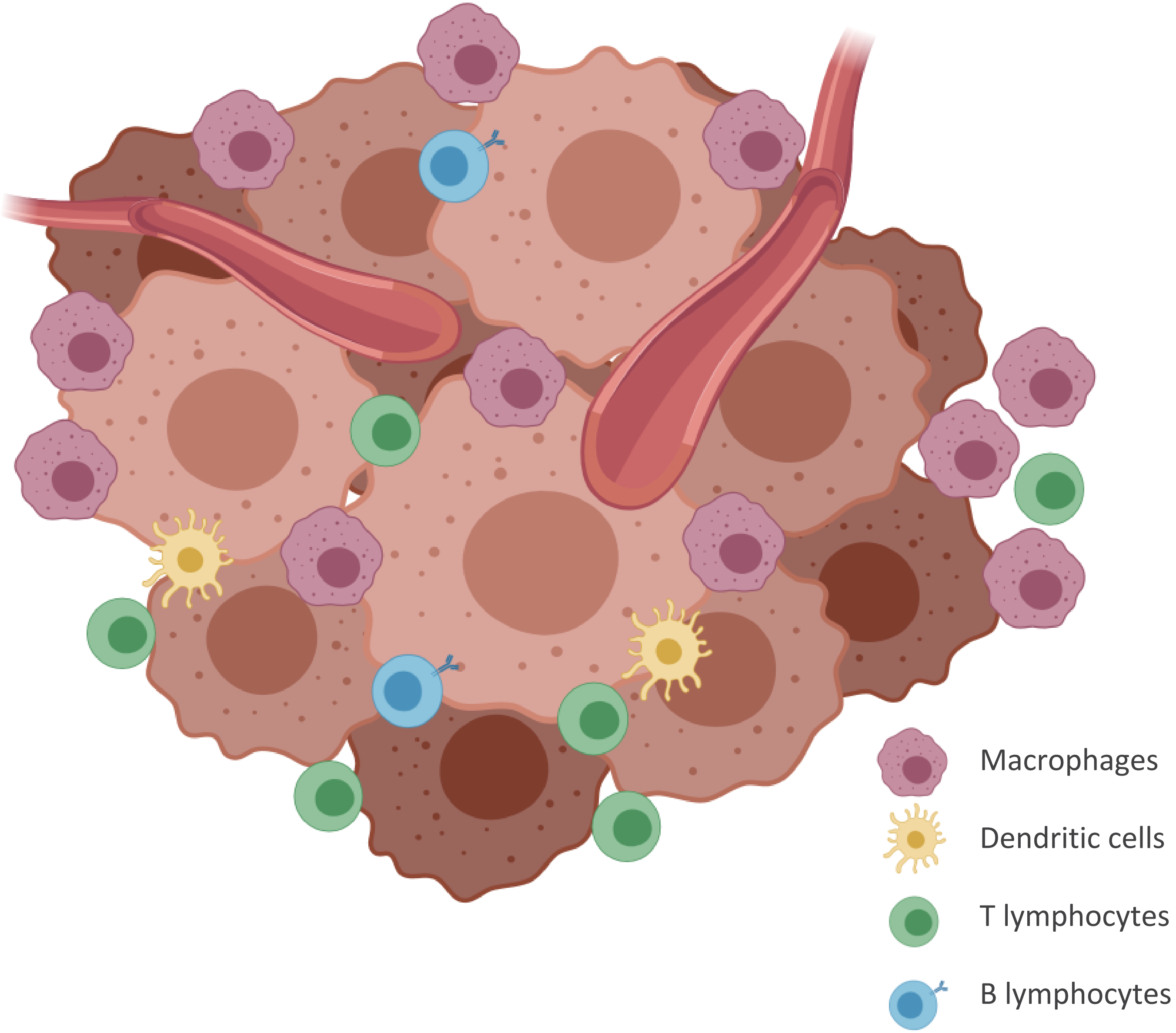
Leading Edge
Review

Hallmarks of Cancer: The Next Generation

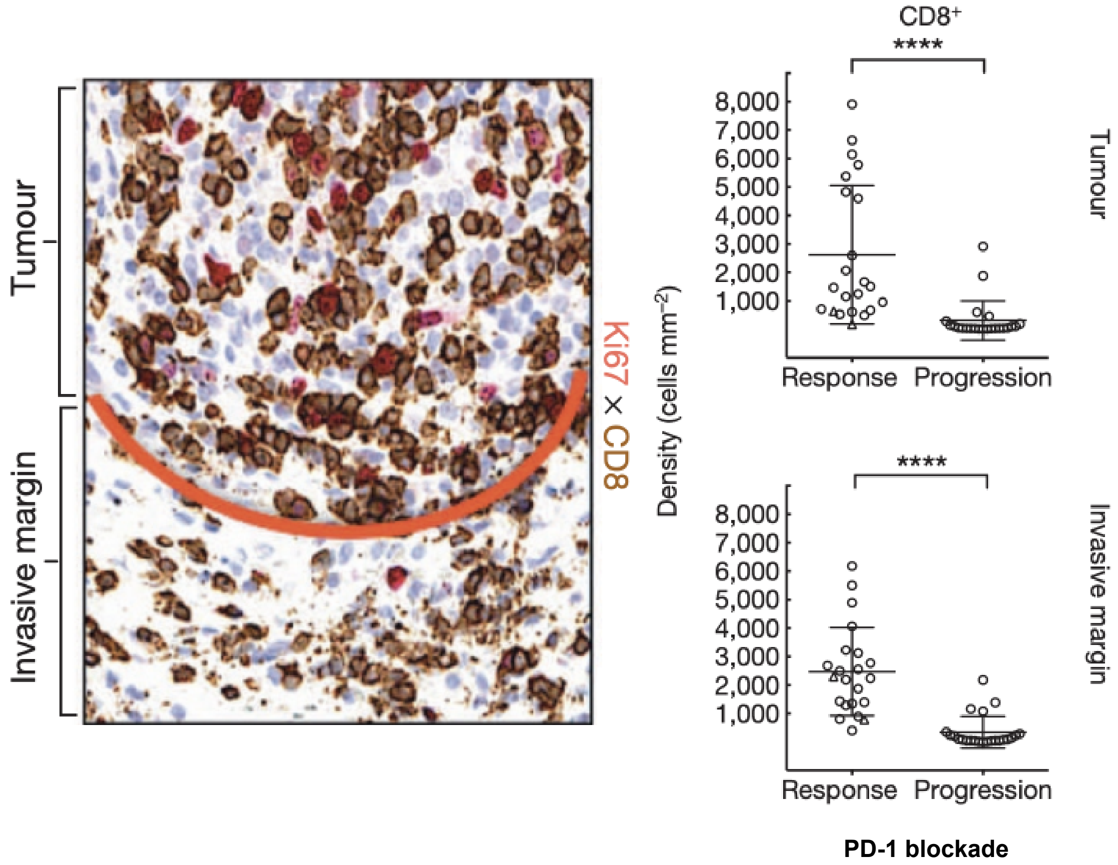
Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]

An Emerging Hallmark: Evading Immune Destruction

Evading T cell-mediated antitumoral immunity

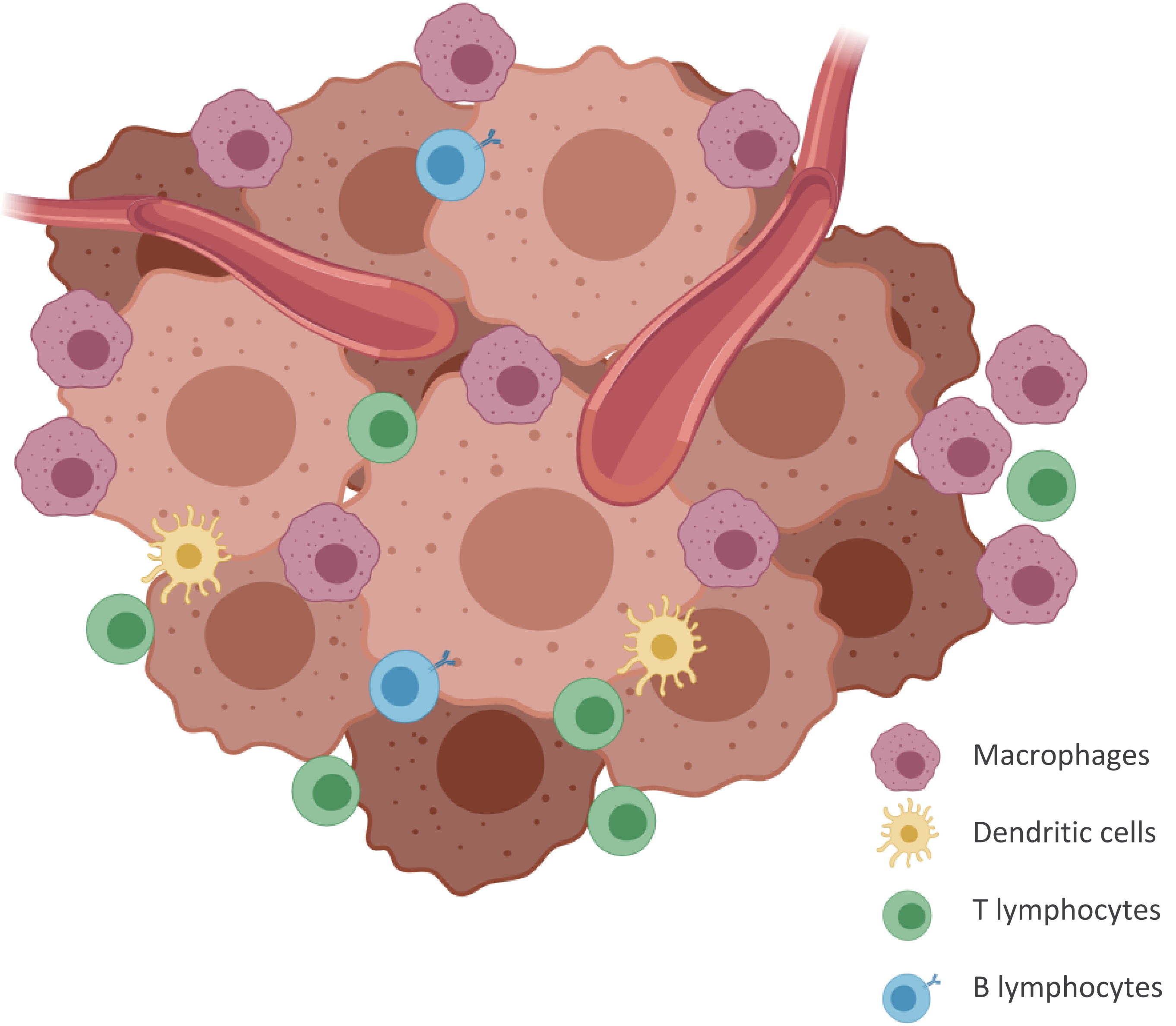


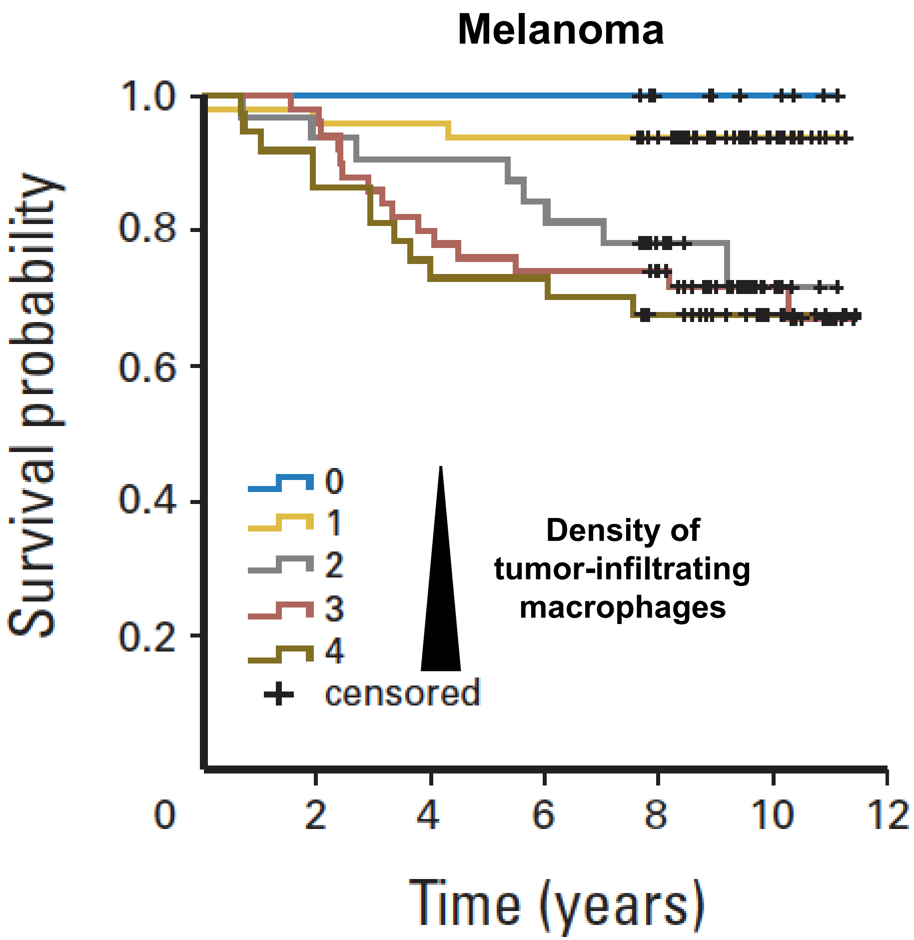
Melanoma



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 easily 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

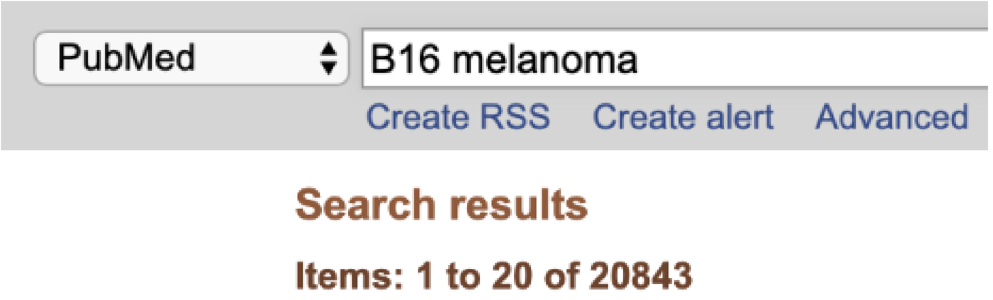
3) Patient-derived xenografts (PDX)

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

Mouse models of cancer

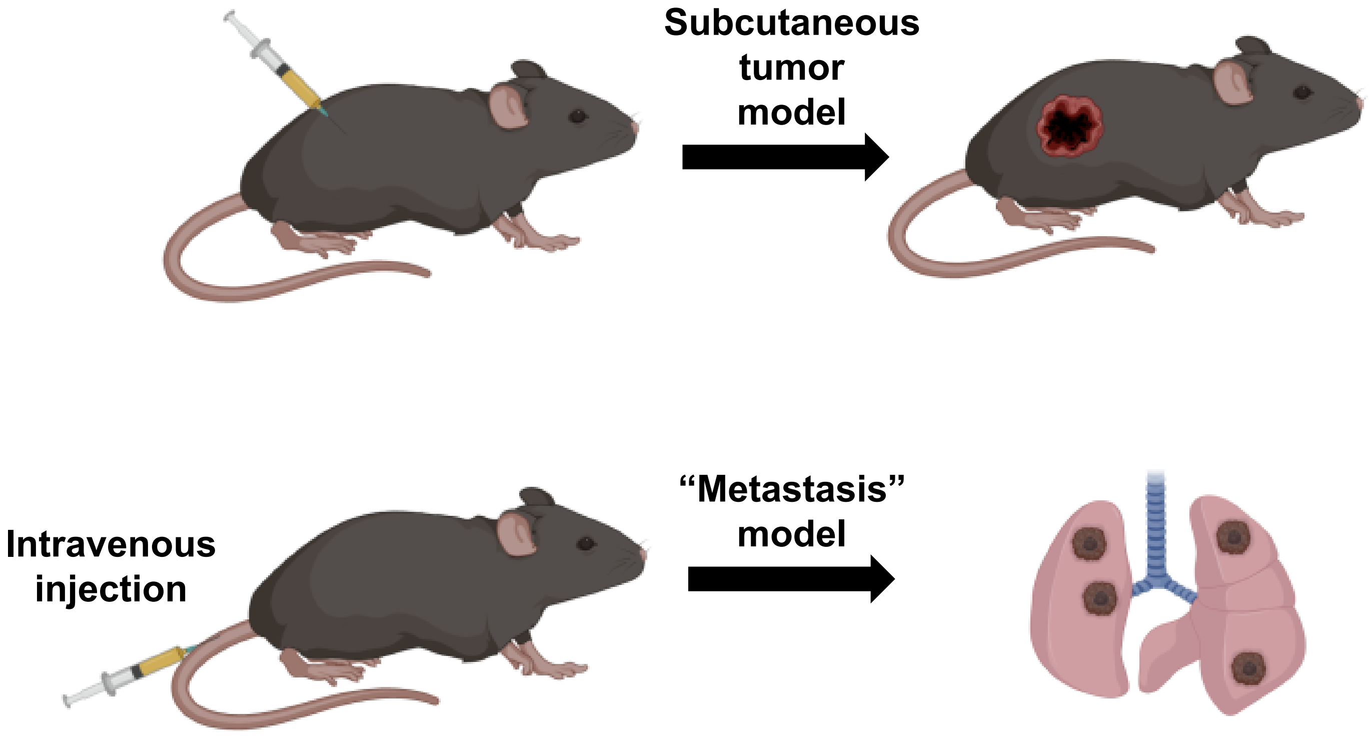
1) Mouse tumor cell line implantation in immunocompetent mice

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

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

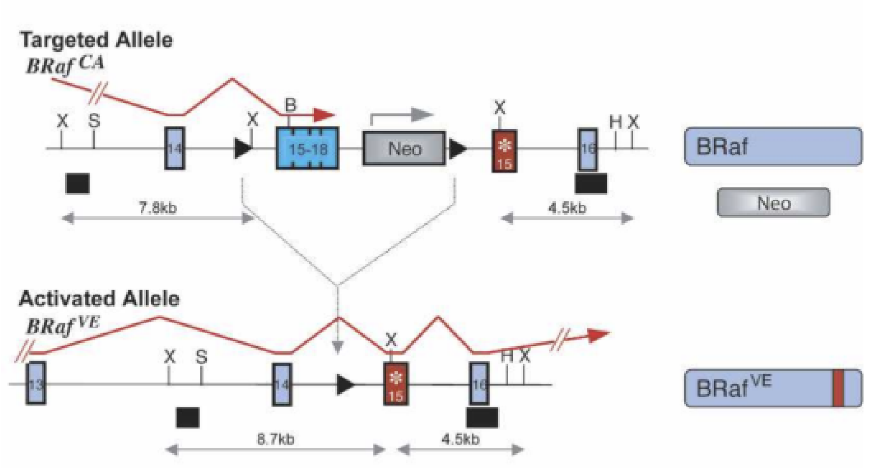
2) Genetically-engineered mouse (GEM) model of cancer

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

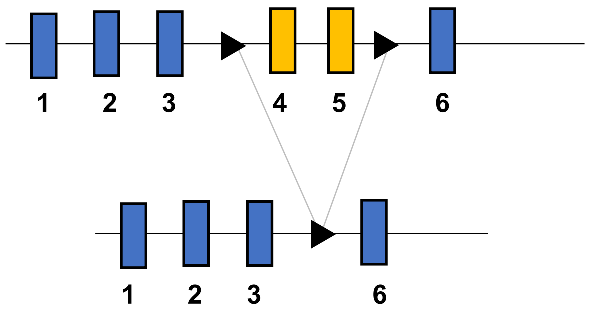
➔ Example: the BRAF/PTEN melanoma model

The BRAF/PTEN melanoma model

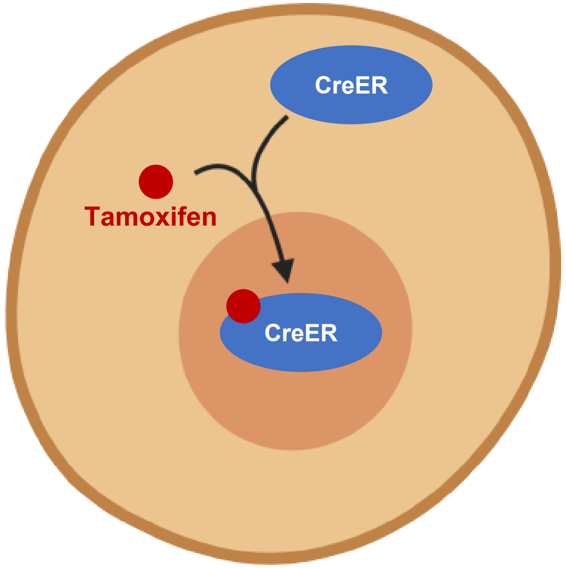
BRAF^{CA}
(Cre-activated BRAF^{V600E})

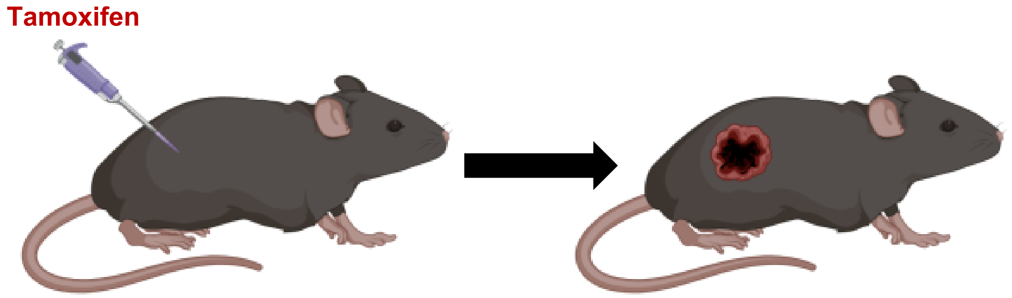


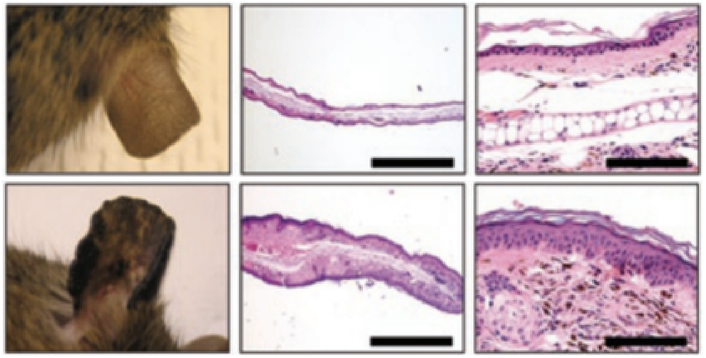
PTEN$^{fl/fl}$
(Cre-activated PTEN deletion)



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







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

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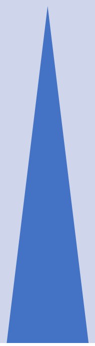
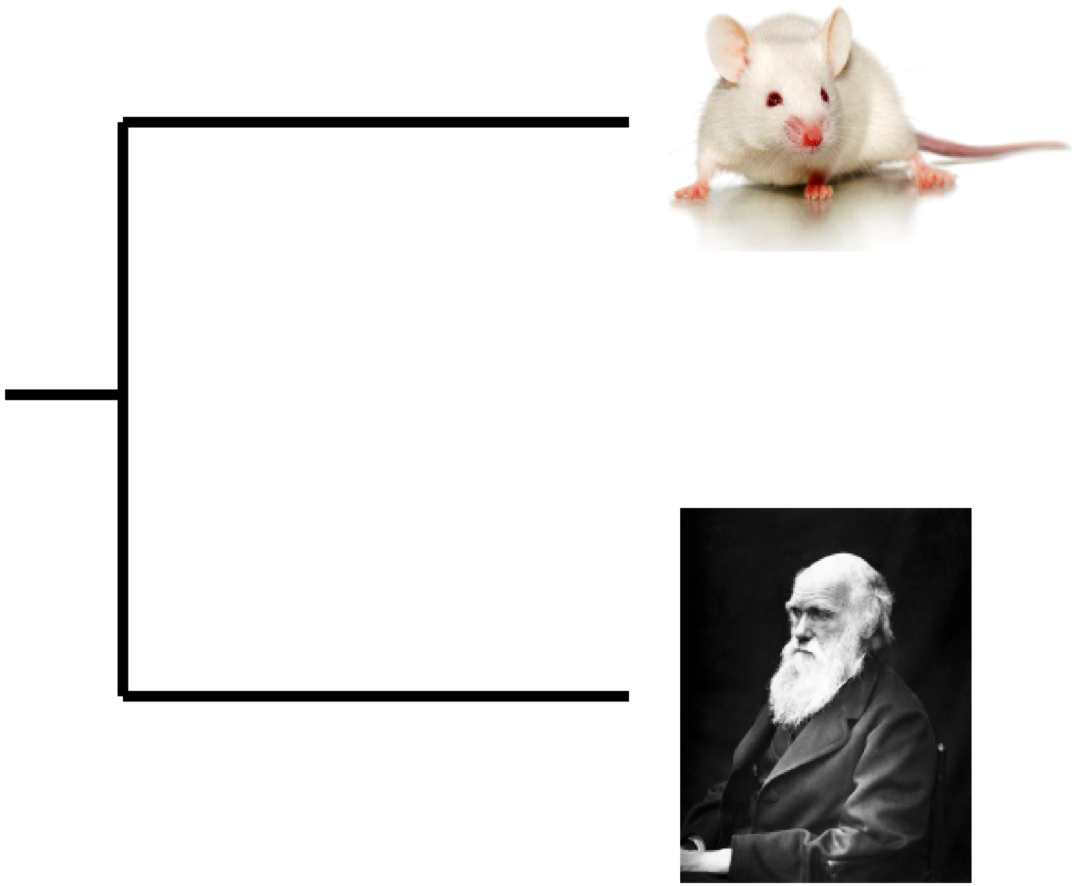
Mouse tumor implants	- Simple and reproducible - Genetic engineering	- Genetically homogenous - Artificial implantation - Mouse, not human	
GEM	- Well-defined oncogenic mutations, representative of human cancer - Oncogenesis	- Low mutational burden and immunogenicity - Mouse, not human	

Mice are not humans

Most recent common ancestor

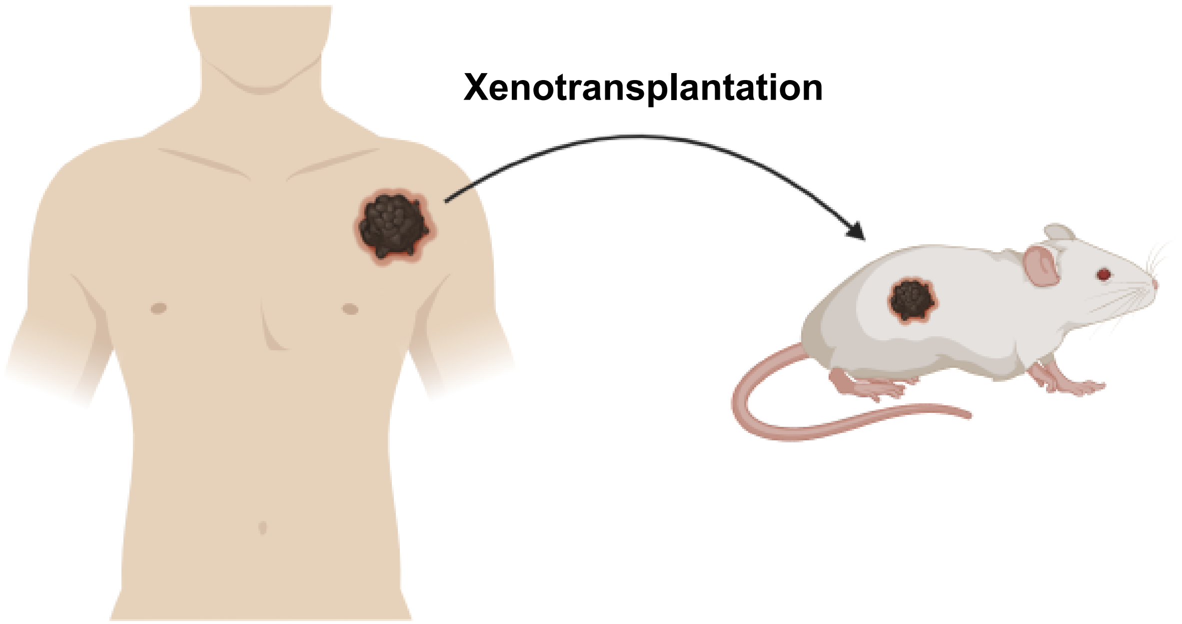
90 million years ago



Each human is unique

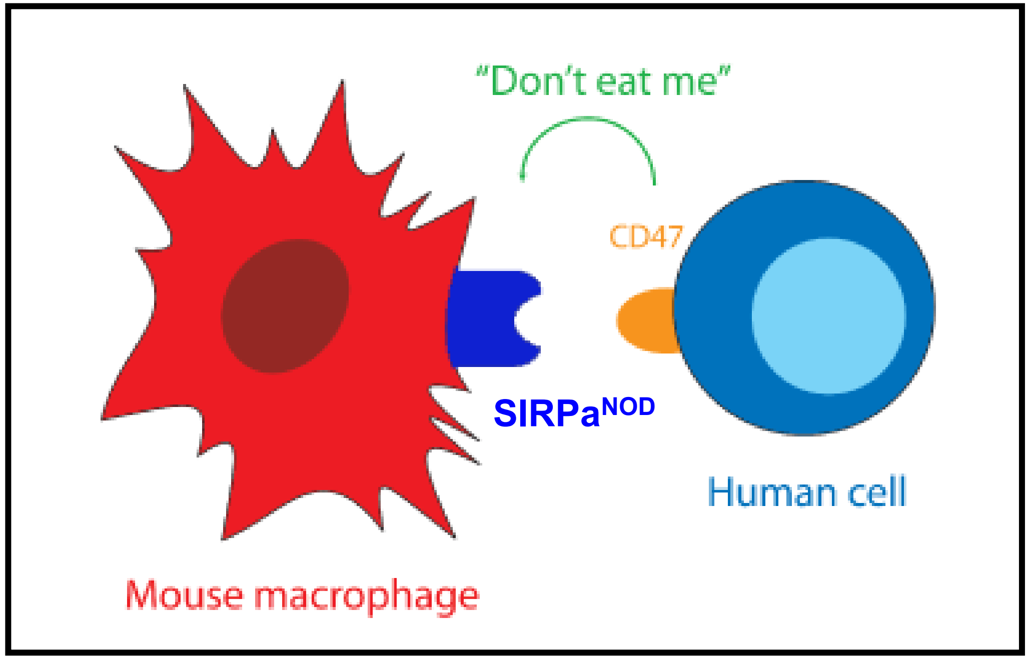
Mouse models of cancer

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

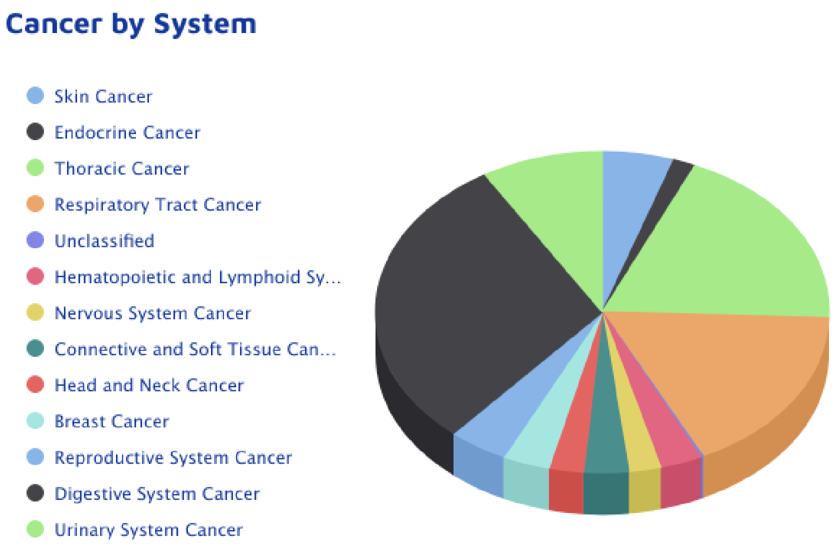
PDX FINDER

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

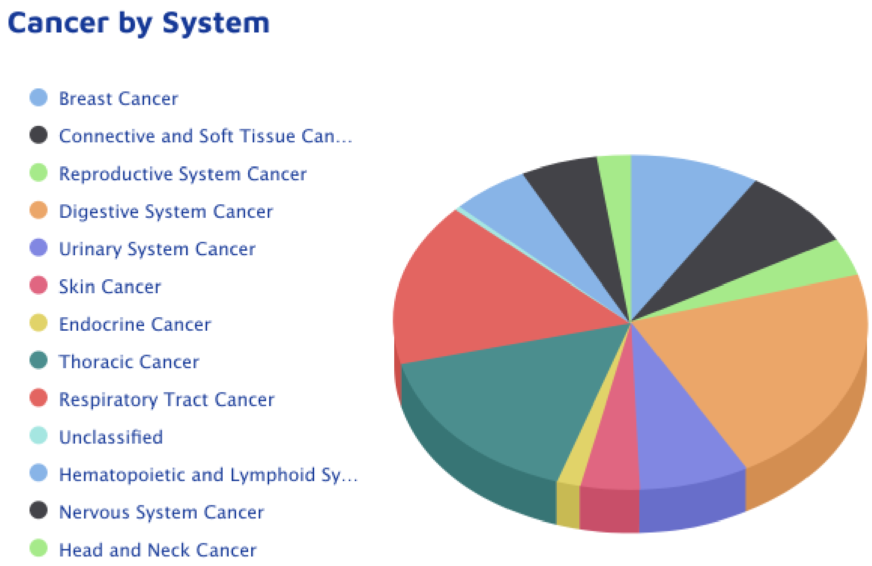
charles river

459 PDX models

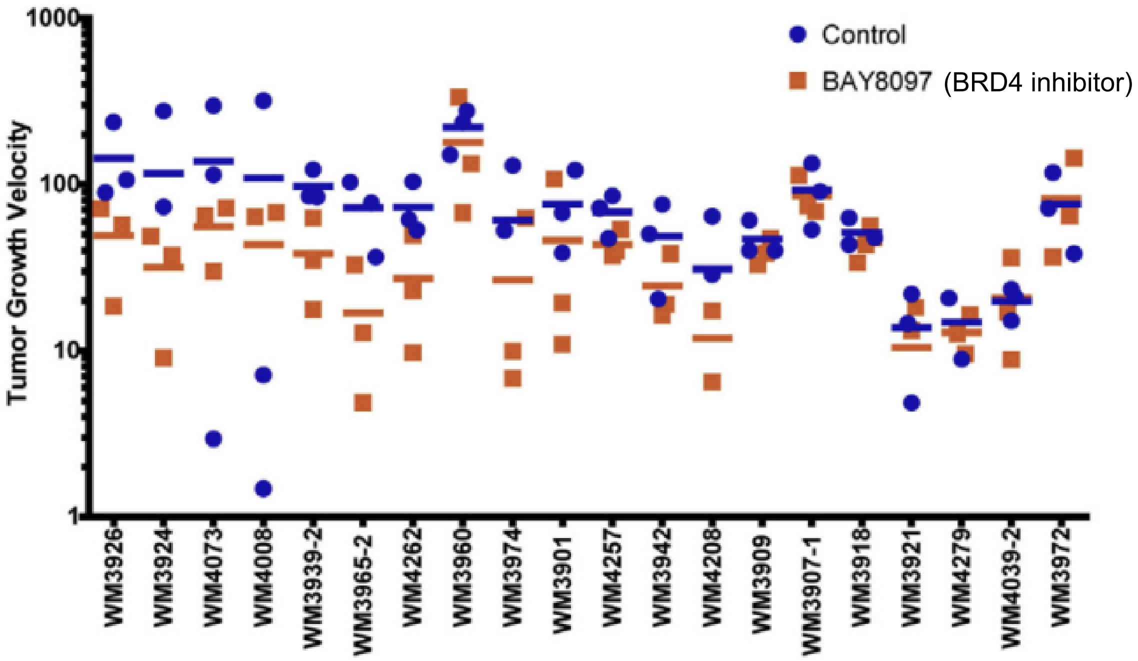


The Jackson Laboratory

406 PDX models



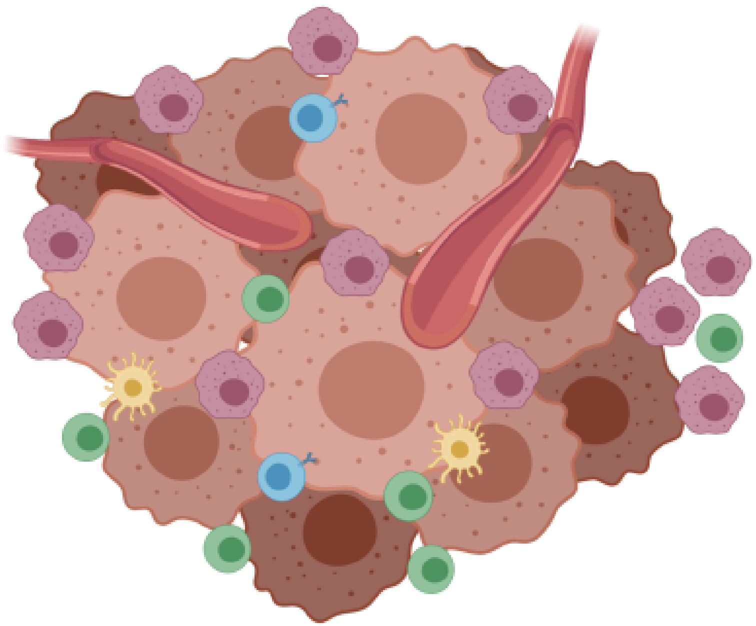
Melanoma PDX “pre-clinical” trial



Krepler et al, Cell Reports 2017

PDX lack a functional immune system

Patient tumor microenvironment



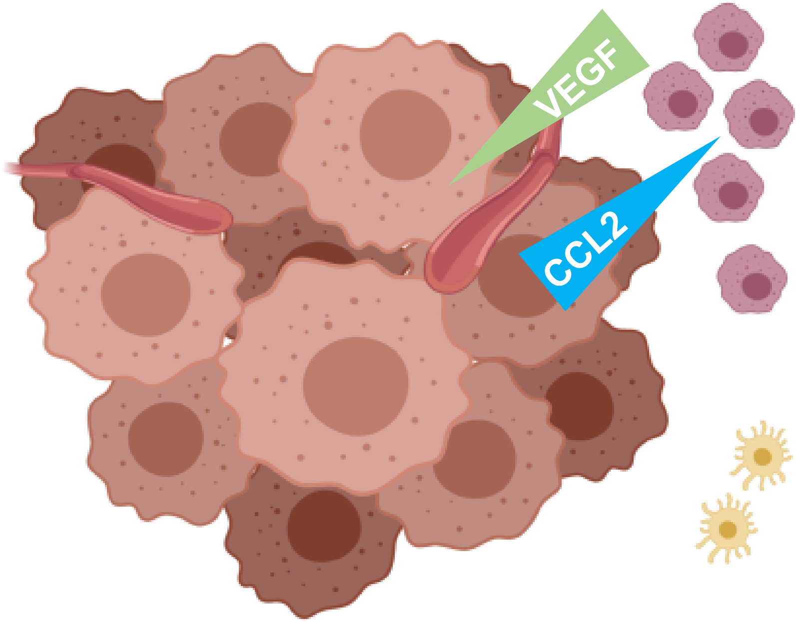
Macrophages

Dendritic cells

T lymphocytes

B lymphocytes

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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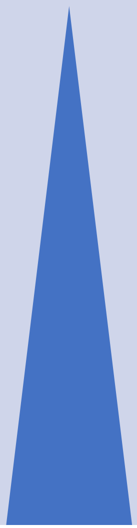
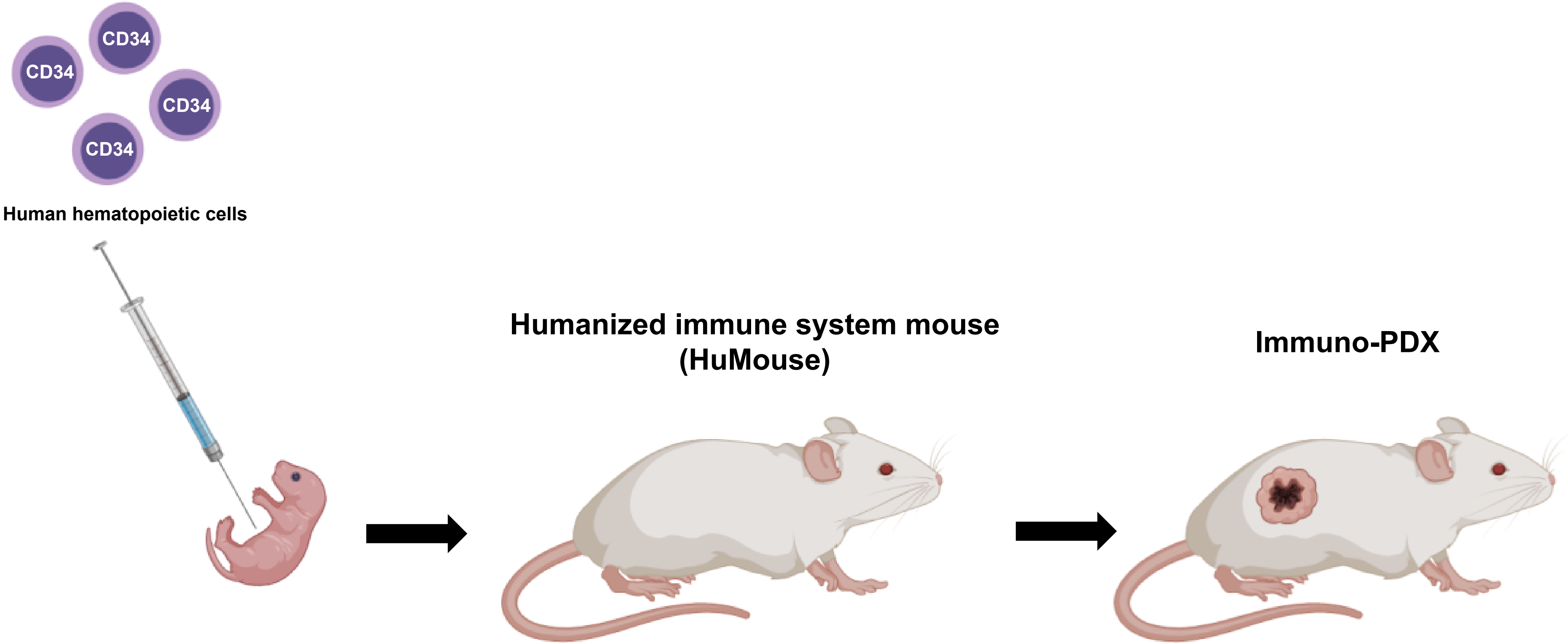
Pros and cons

	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)

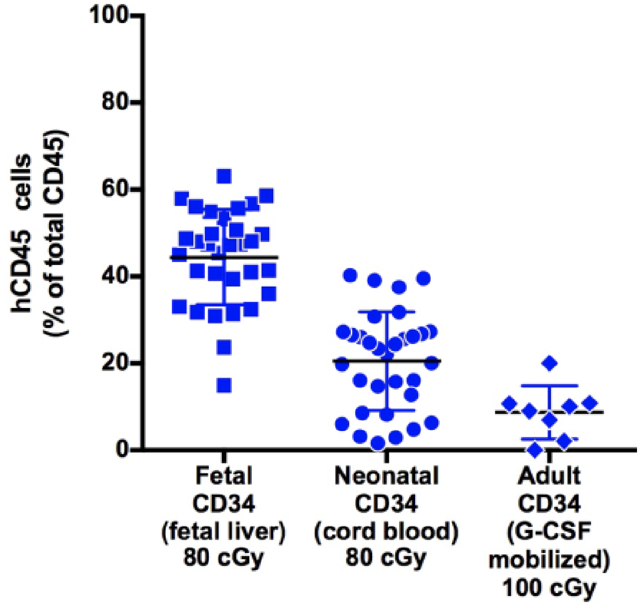


How to generate a HuMouse?

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. Orthotopic hematopoietic cell transplantation

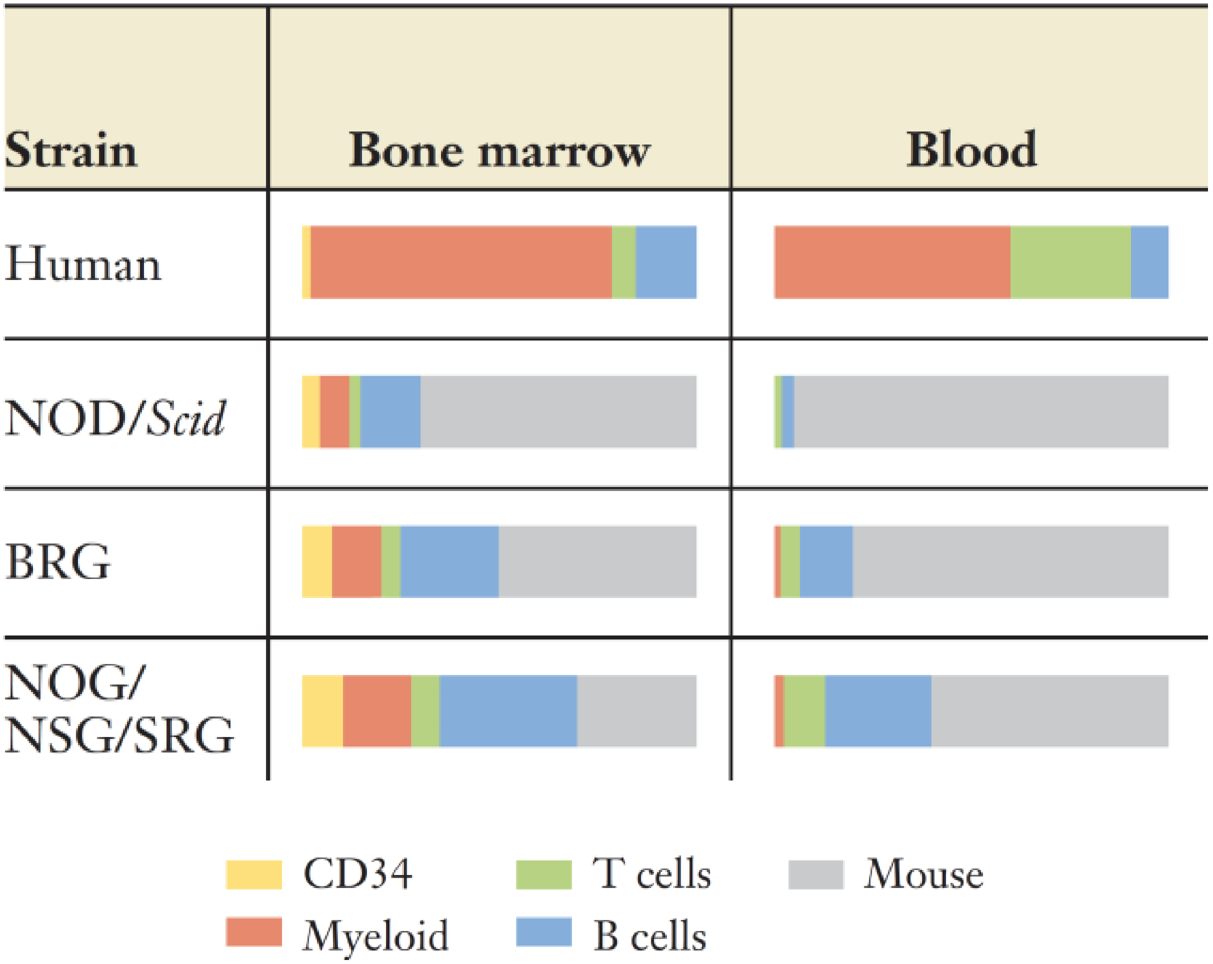
- Intravenous injection in adult mice

- 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

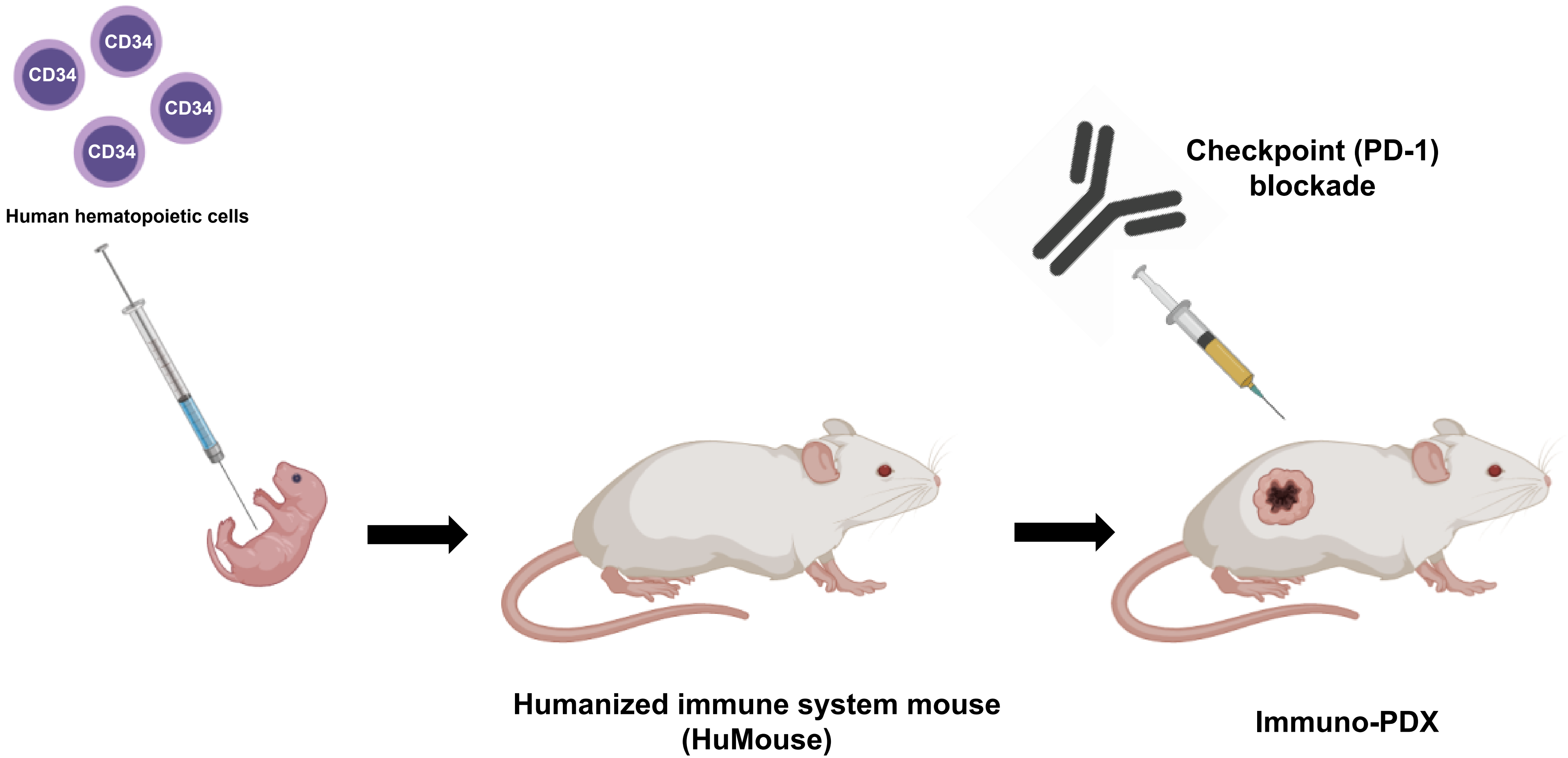
Traggiai et al, Science 2004

How to generate a HuMouse?



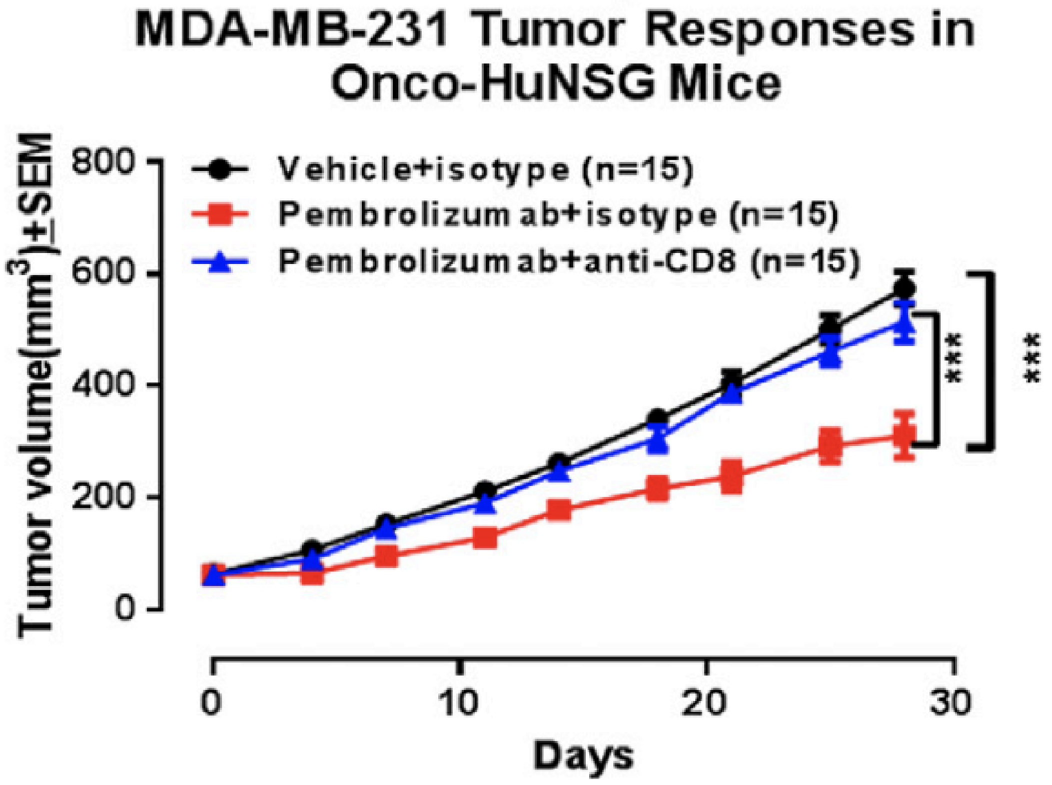
Rongvaux et al, Ann Rev Immunol 2013

Modeling checkpoint inhibition in solid tumors



Modeling checkpoint inhibition in solid tumors

Breast cancer cell line

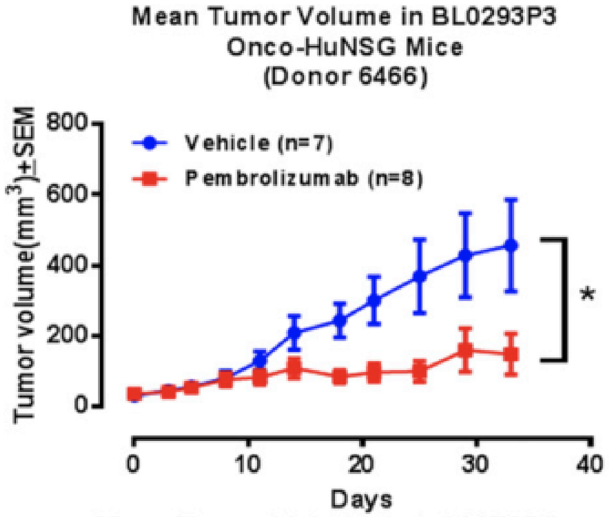


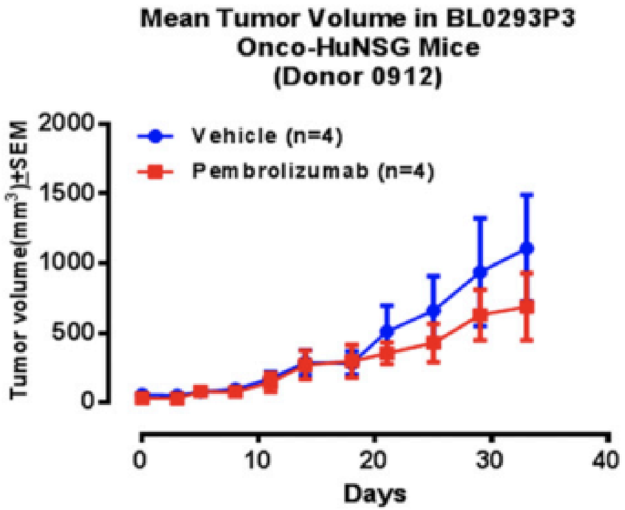
Wang et al, FASEB J 2018

Modeling checkpoint inhibition in solid tumors

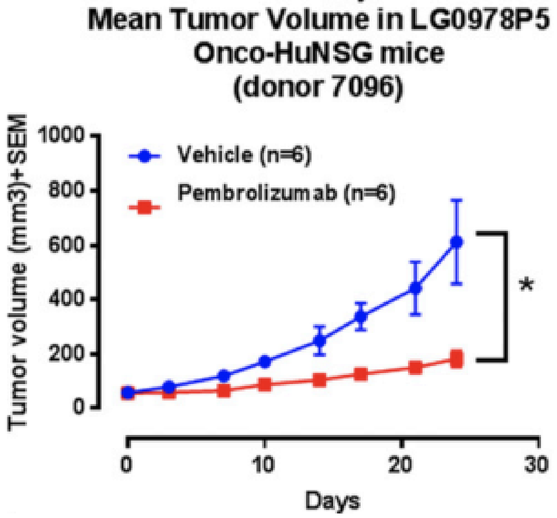
PDX - hematopoietic cell donor variation

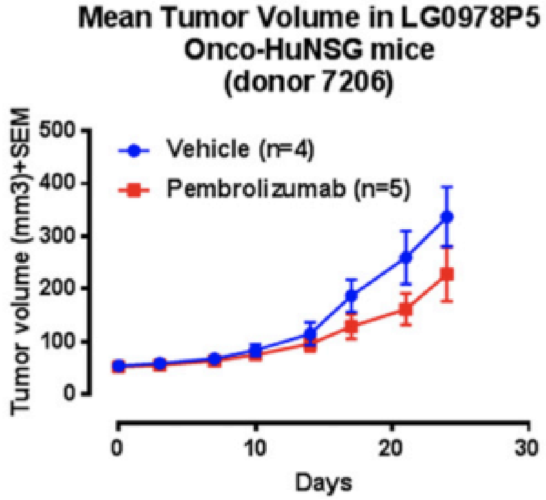
Bladder cancer PDX





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

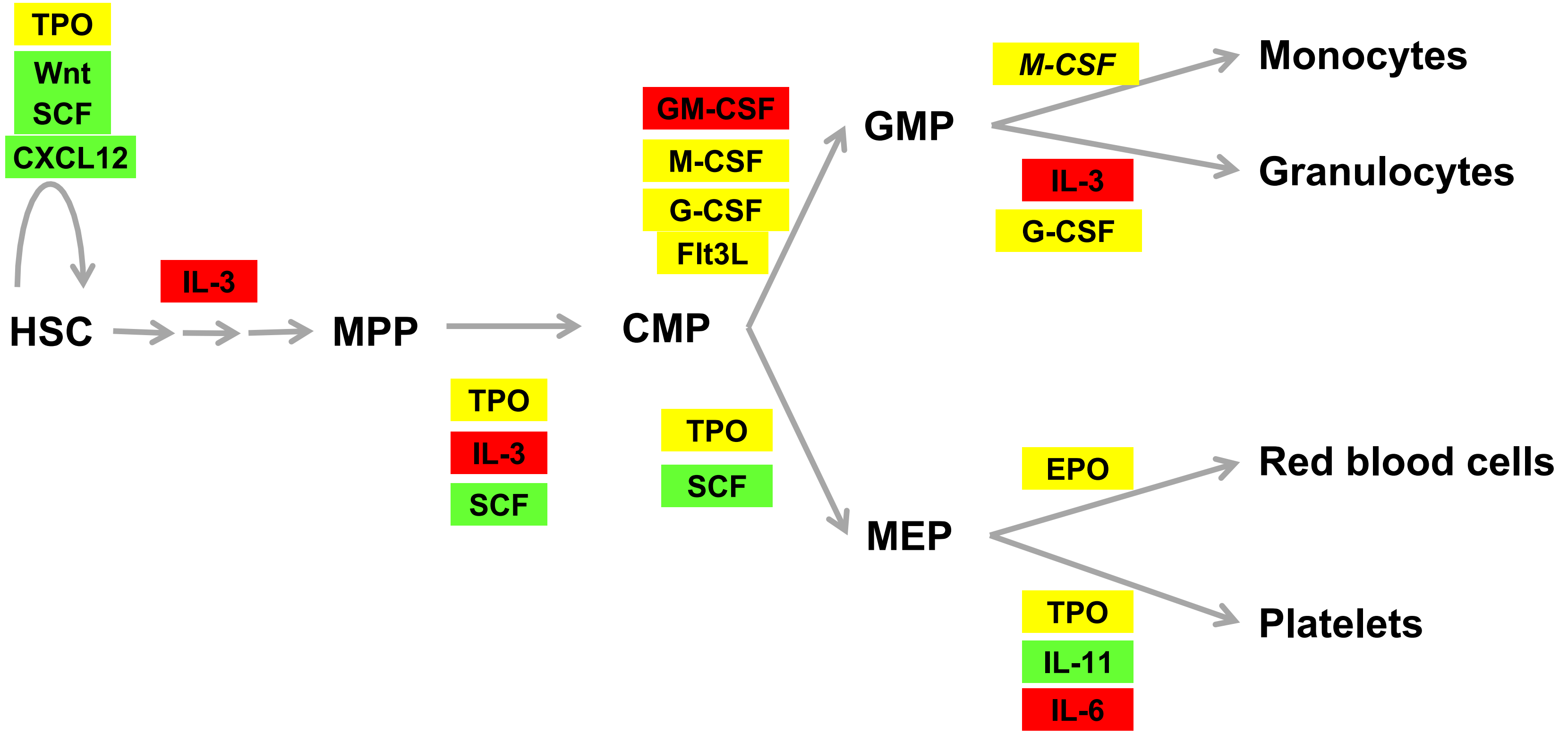
➔ Next-gen HuMice: HLA expression, additional cytokines, restore lymphoid structures

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

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

81-100% a.a. identity
61-80% a.a. identity
<60% a.a identity



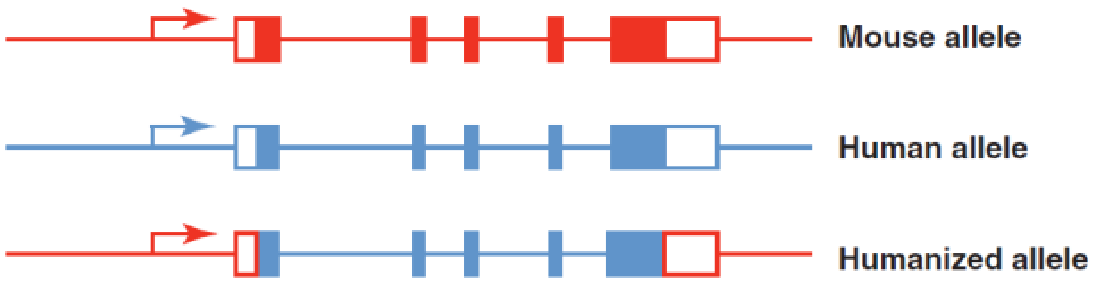
Rongvaux et al, Ann Rev Immunol 2013

How to generate a HuMouse?

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

M-CSF$^{h/h}$ **I**L-3/GM-CSF$^{h/h}$	**Myeloid development**
Sirpα$^{h/m}$	**Phagocytic tolerance**
TPO$^{h/h}$	**Longterm maintenance of functional HSCs**
RAG2$^{-/-}$ IL2R**G**amma$^{-/-}$	**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?

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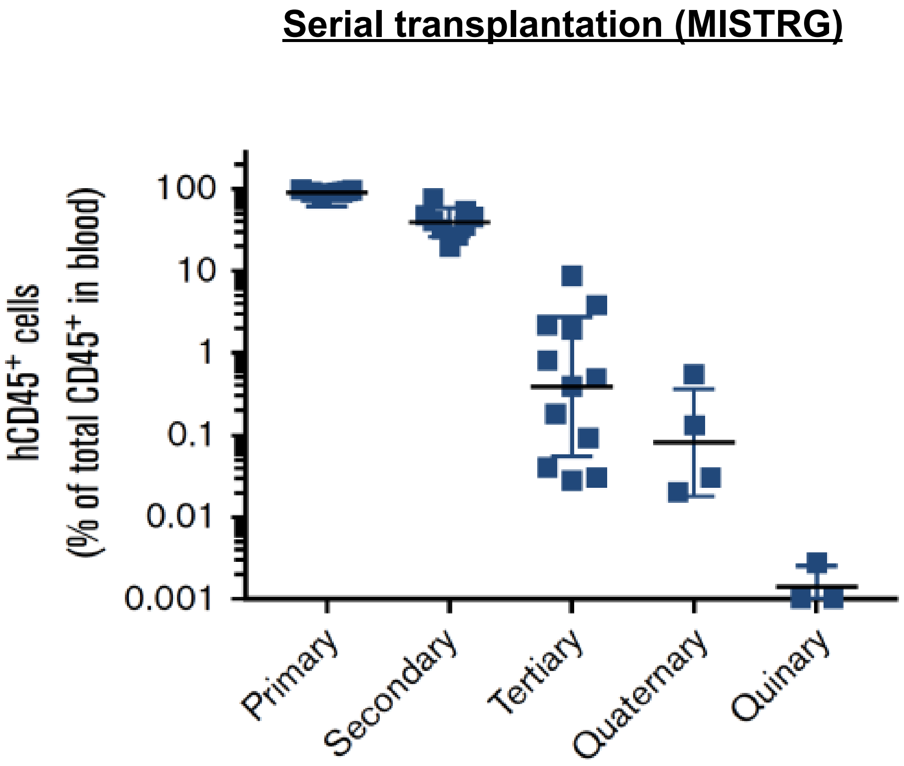
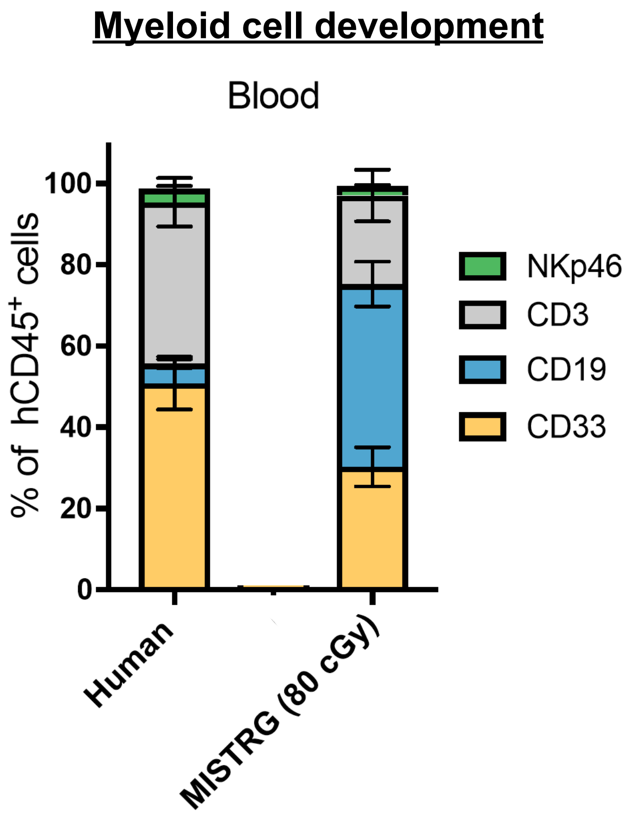
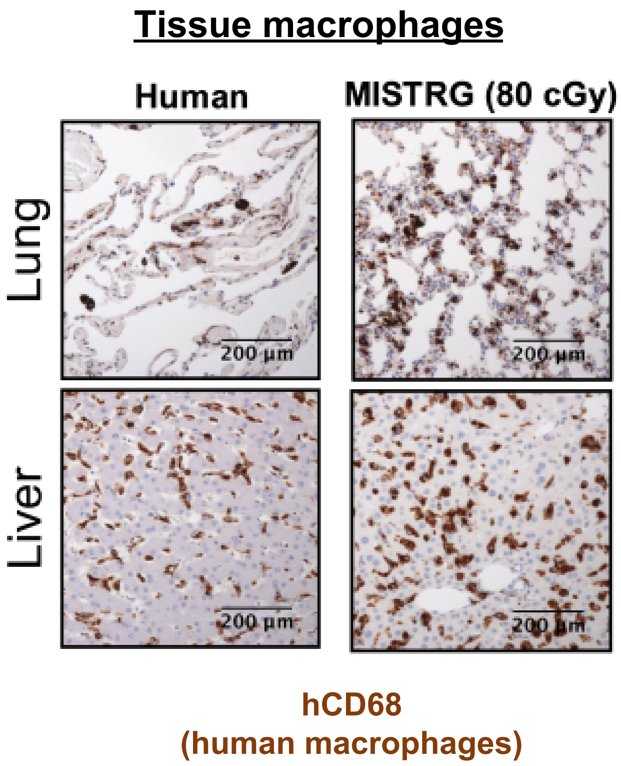
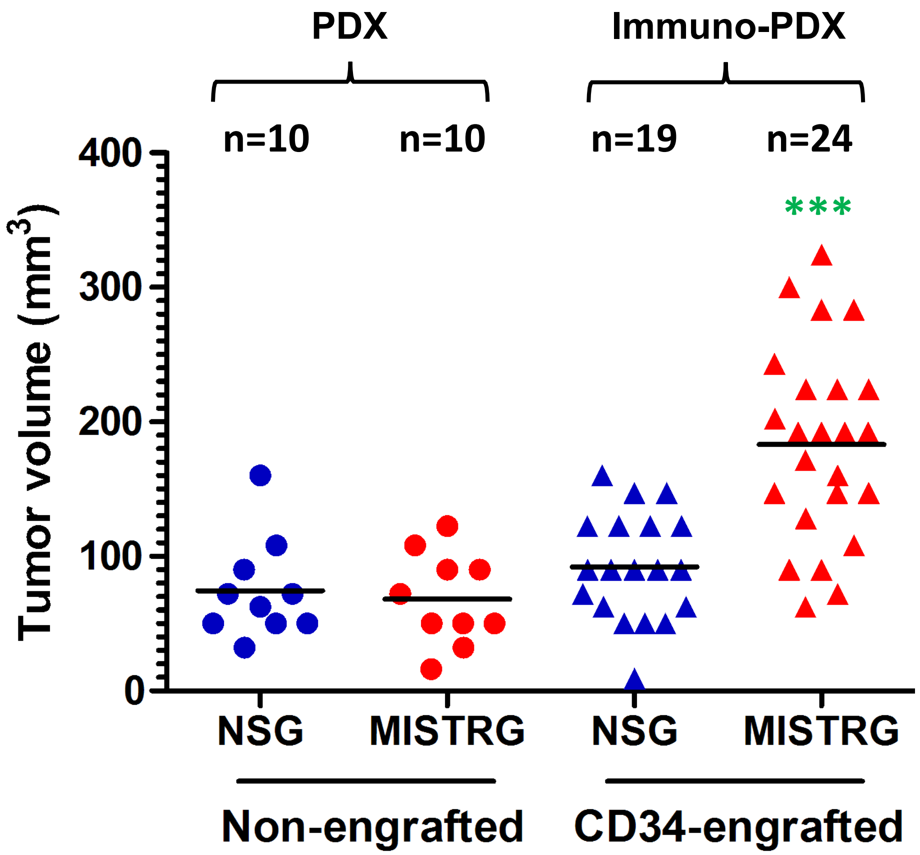






Sippel et al, Blood Advances 2019

Studying macrophage function in a solid tumor

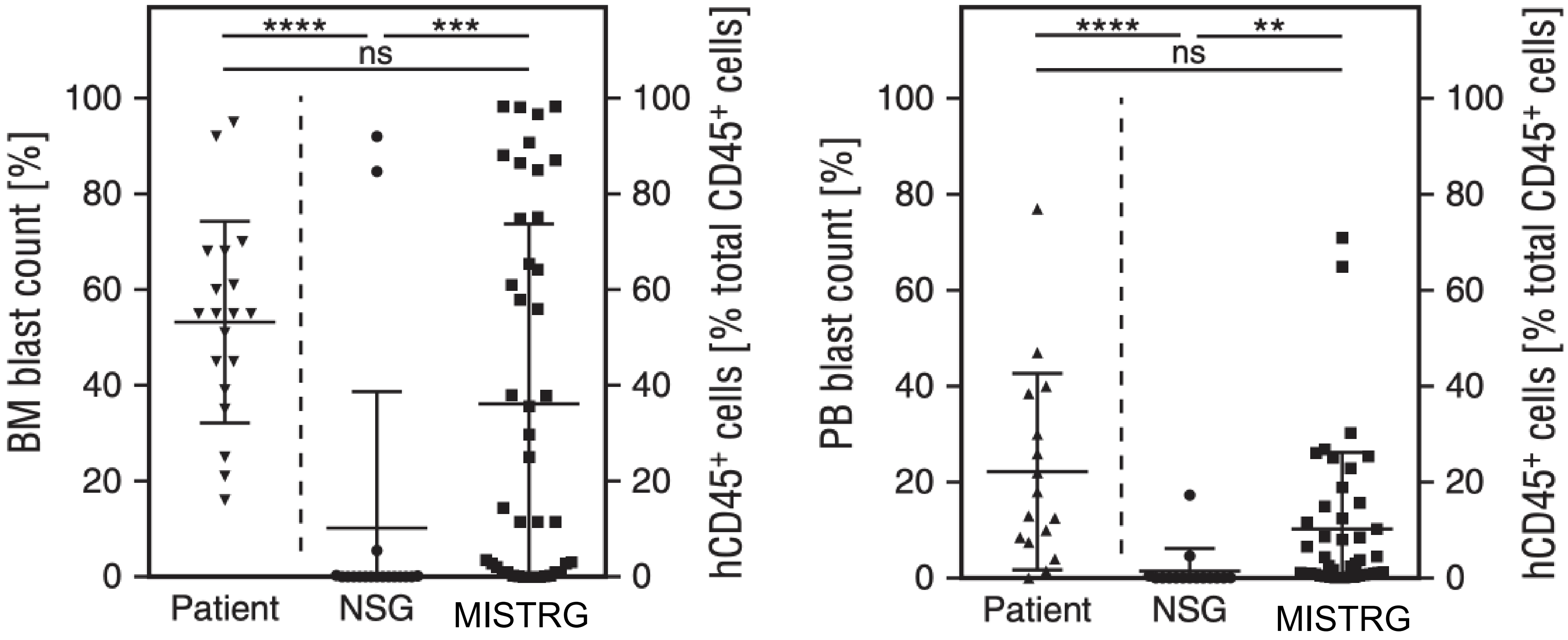


One-way ANOVA $p<0.0001$
*** $p<0.05$ vs. all other group
(Tukey post-hoc test)

Rongvaux et al, Nat Biotech 2014

Transplantation of human hematopoietic diseases

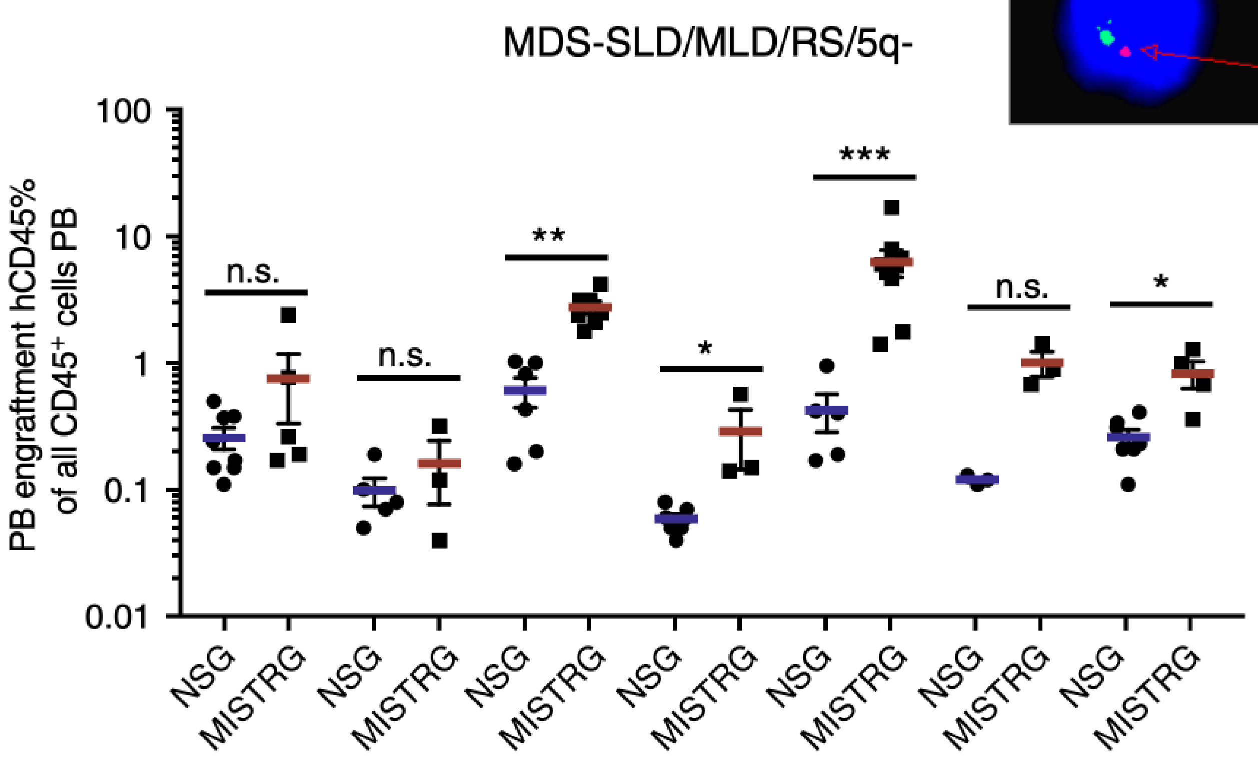
Acute myeloid leukemia (AML) – inv16 “good risk” AML

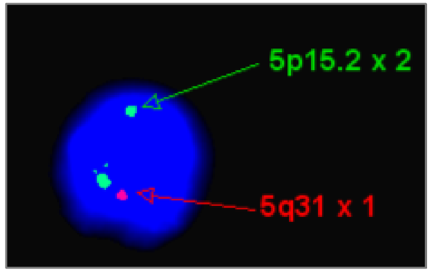


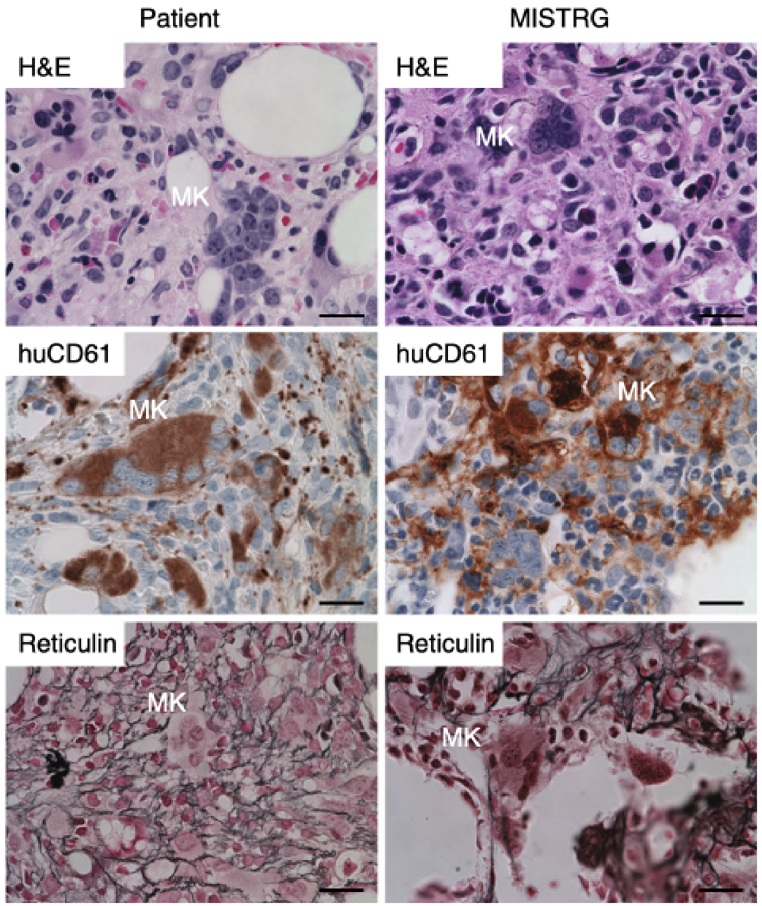
Ellegast et al, Blood 2016

Transplantation of human hematopoietic diseases

Myelodysplastic syndromes (MDS)





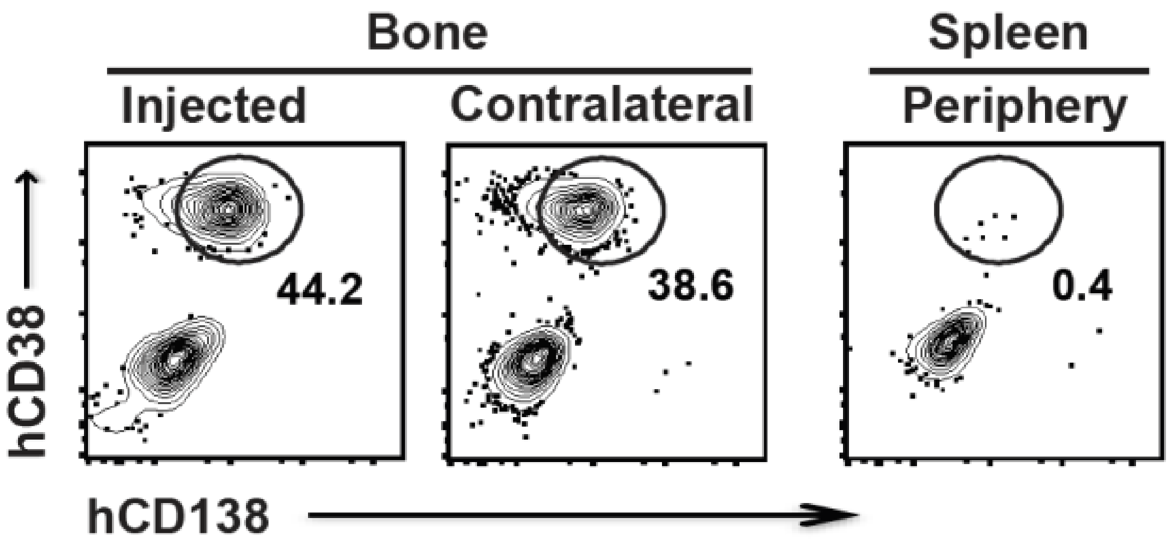


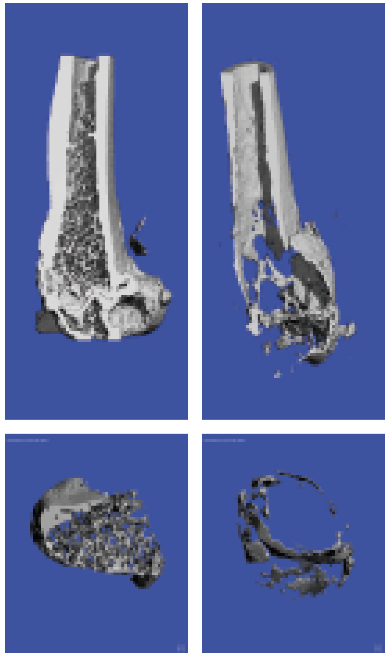
Song et al, Nat Comm 2019

Transplantation of human hematopoietic diseases

Multiple myeloma

M-CSF$^{h/h}$
IL3/GM-CSF$^{h/h}$
Sirpα$^{h/m}$
TPO$^{h/h}$
RAG2$^{-/-}$
IL2RGamma$^{-/-}$
IL6$^{h/h}$



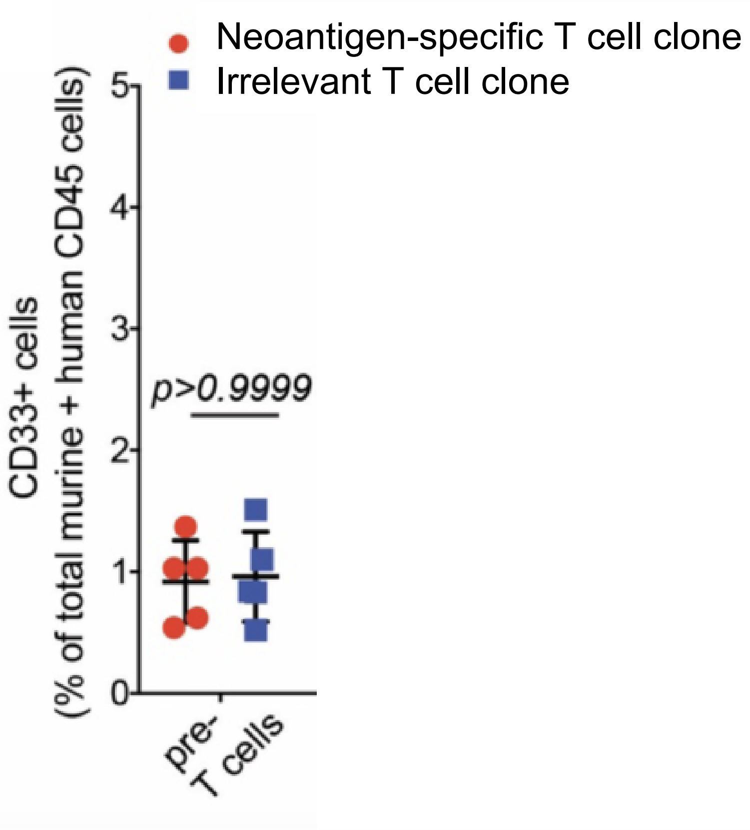


Das et al, Nat Medicine 2016

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



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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

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

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Annual Review of Immunology

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

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