Humanized mice models of oncoimmunology

Karolina Palucka, MD, PhD

The Jackson Laboratory for Genomic Medicine

Farmington, CT

SITC Cancer Immunotherapy Winter School

February 20, 2019

The Jackson Laboratory

Leading the search for tomorrow's cures

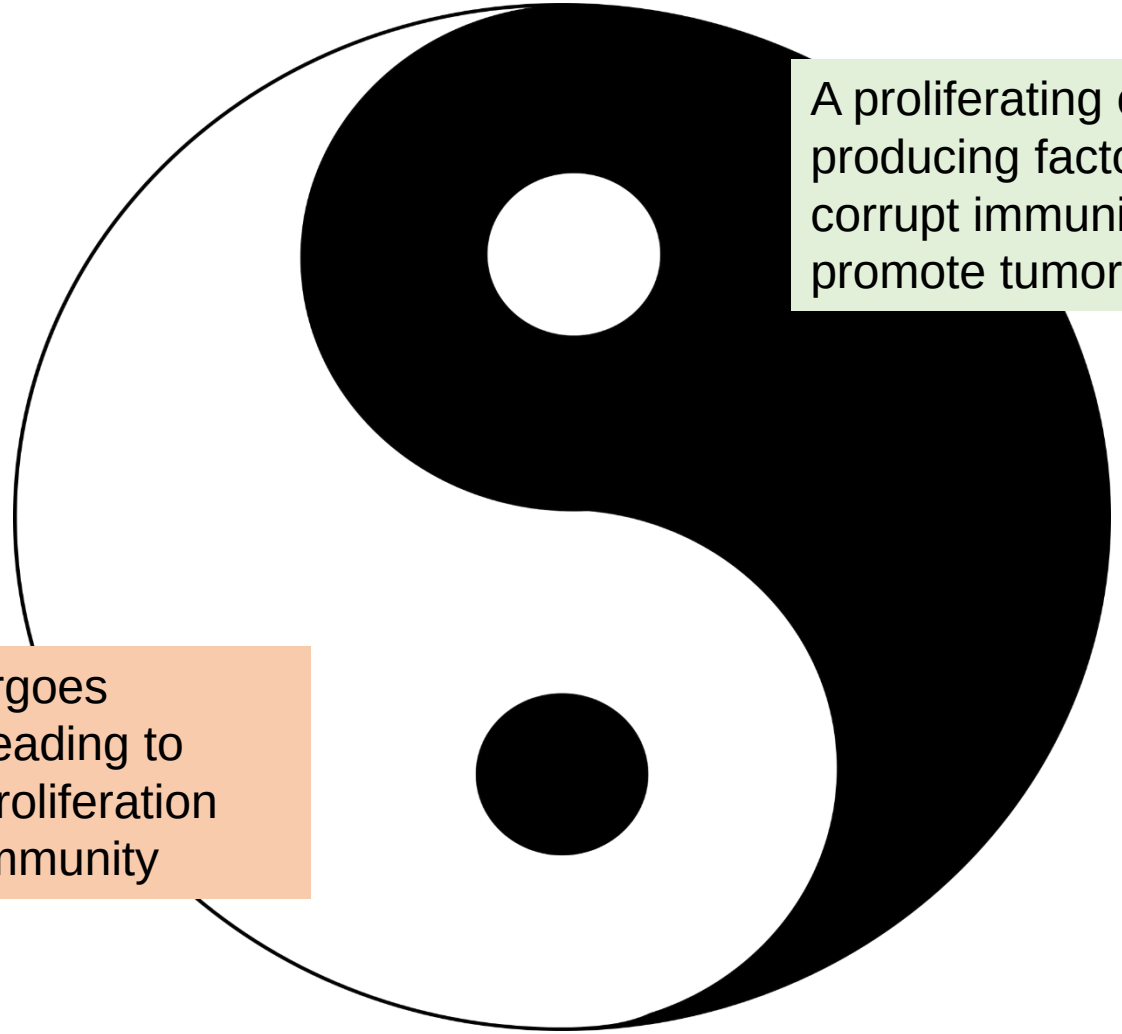
Presenter Disclosure Information

Karolina Palucka, MD, PhD

The following relationships exist related to this presentation:

Merck : Consulting, grant support

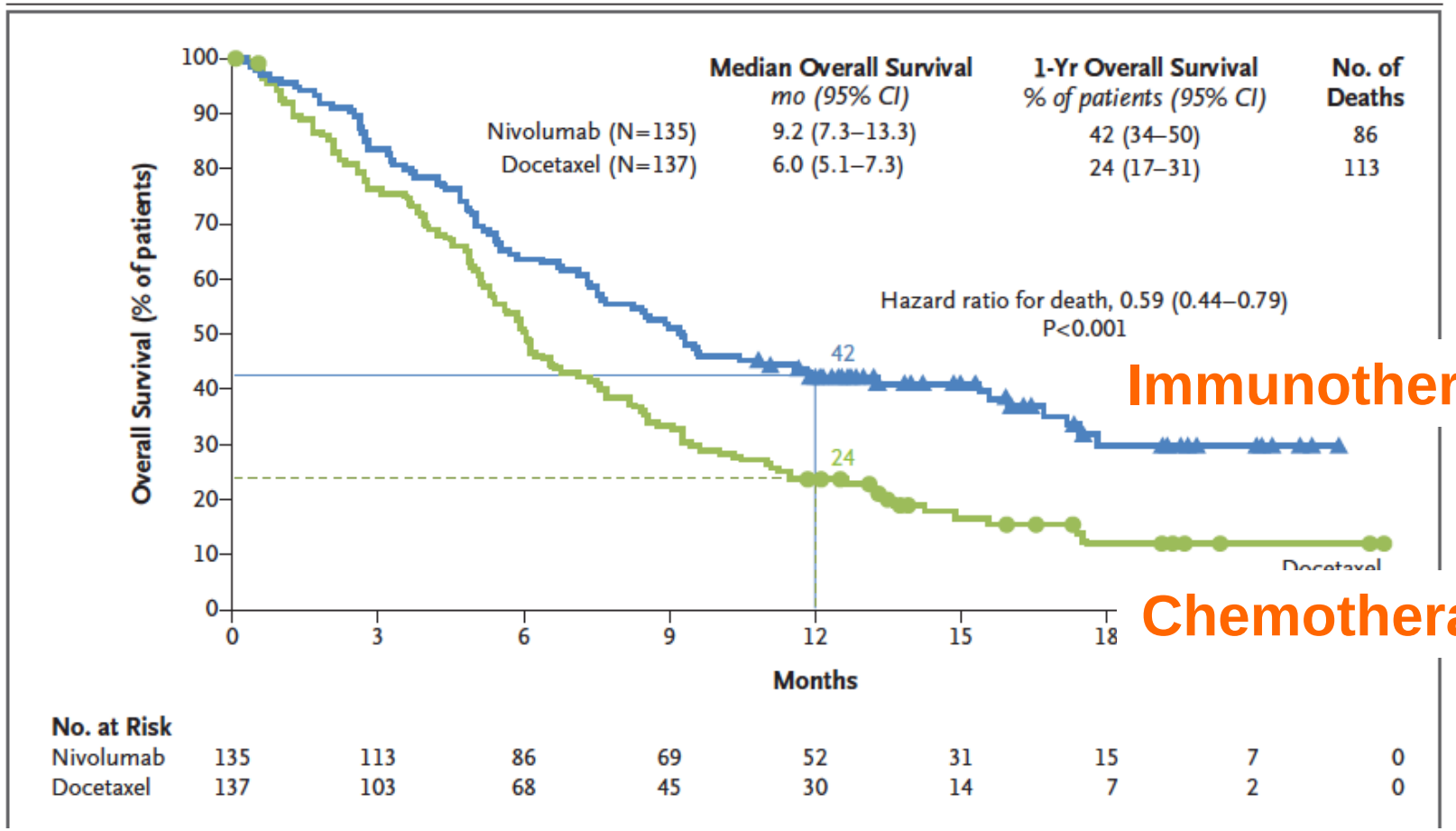
Cancer: mutant cell that expands and corrupts Immunity



A proliferating cell producing factors that corrupt immunity and promote tumor growth

A cell undergoes mutations leading to unabated proliferation triggering immunity

Fighting corruption:
Immunotherapy via blockade of T cell inhibitory pathways



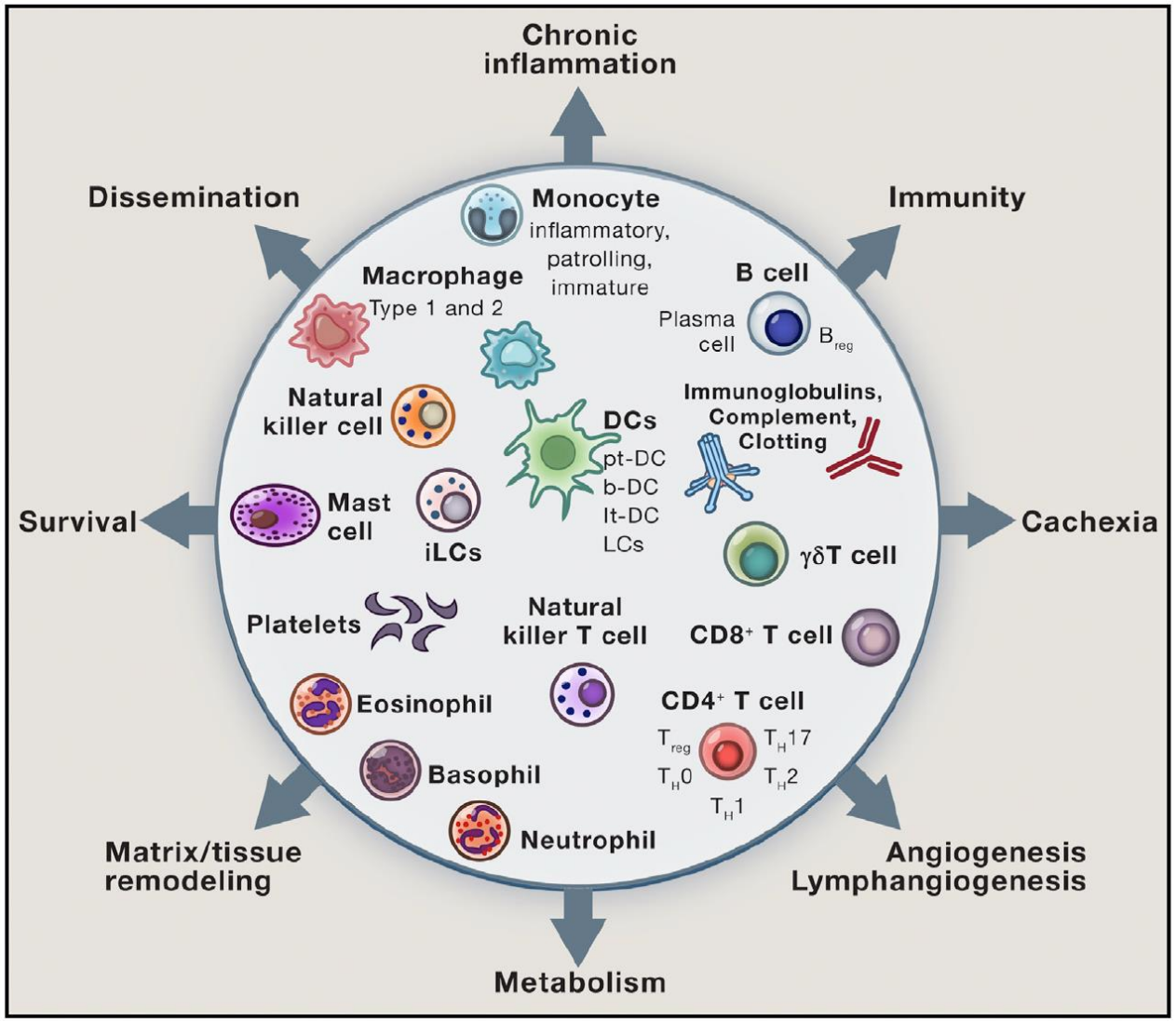
Immunotherapy

Chemotherapy

Squamous-Cell Non–Small-Cell Lung Cancer

N Engl J Med 2015;373:123-35.
DOI: 10.1056/NEJMoa1504627

How do we unravel the mechanisms of resistance and toxicity in genetically complex, multicellular environments and heterogeneous hosts?



The Basis of Oncoimmunology
Palucka & Coussens, Cell 2016

Pre-clinical models

In vitro:

1. **Standard 2D cultures**
2. **3D cultures: organoids, spheroids, printed tissues**

In vivo:

1. **Mice: syngeneic, GEMMs, xenografts, humanized**
2. **Non-human primates**
3. **Canine models**

There is no perfect model

Non-humanized mouse models for oncoimmunology

Model	Key features	Pros	Cons
Transplantable tumors Syngeneic mice	Ectopic transplanted tumors immunocompetent inbred mice	Rapid tumor growth Reproducibility Simple monitoring	Genetically homogenous Rapid growth w/o chronic inflammation
Carcinogen-induced	“Natural” oncogenesis	Genetically diverse Heterogenous Closer to human	Time and resource dependent Difficult to monitor Poorly defined genetic alteration
GEMMs	Well-defined genetic alteration	Heterogenous with respect to onset, progression and histology Closer to human	Low mutational load Multiple concurrent transformation events leading to overwhelmed host

Adapted from Zitvogel et al Nat Rev Cancer 16; 759

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

Javier Mestas and Christopher C. W. Hughes[1]

J Immunol 2004; 172:2731-2738; ;
doi: 10.4049/jimmunol.172.5.2731
http://www.jimmunol.org/content/172/5/2731

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

	Mouse	Human	Notes	Refs.
Hemotopoiesis 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
Hemotopoietic stem cells	c-*kit*^{high}, *flt-3*$^{-}$	c-*kit*^{low}, *flt-3*$^{+}$		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	Pseudogene	Widely expressed		
Sialic acid Neu5GC expression	Widespread	Absent	Binds pathogens	92
CD33	Expressed on granulocytes	Expressed on monocytes	Binds sialic acids	93
Leukocyte defensins	Absent	Present	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-γ and LPS	Induced by IFN-α/β, IL-4^{+} anti-CD23		17
CD4 on macrophages	Absent	Present		96
Predominant T cells in skin and mucosa	γ/δ TCR (dendritic epidermal T cells—DETC)	α/β TCR		40
γ/δ 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, Rae1β	MIC A, MIC B, ULBP	NK activating Rs	98
fMLP receptor affinity	Low	High		99
FcαRI	Absent	Present		21
FcγRIIA, 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

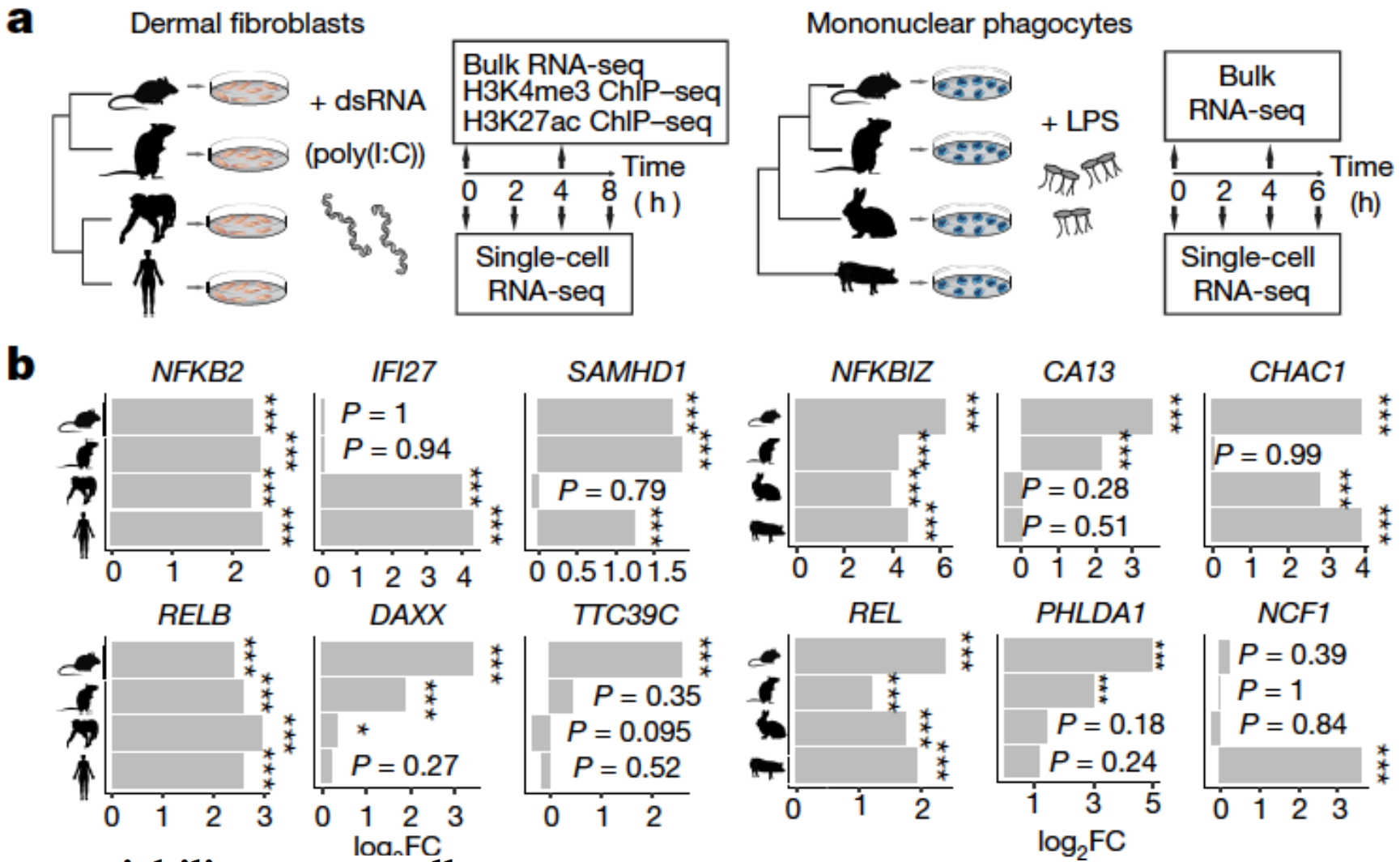
Ig CDR-H3 region	Shorter, less diverse	Longer, more diverse		100
BLNK deficiency	IgM^{high} B cells in periphery	No peripheral B cells		25, 26
Btk deficiency	Normal pre-B and immature B	Blocks pro-B to pre-B transition		28
λ5 deficiency	"leaky" block at pro-B to pre-B transition	Blocks pro-B to pre-B 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 γ_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 γ_c deficiency	Phenocopies γ_c deficiency		31
Effect of IL-7R deficiency	Blocks T and B cell development	Only blocks T cell development		35, 36
ZAP70 deficiency	No $CD4^+$ or $CD8^+$ T cells	No $CD8^+$ T but many nonfunctional $CD4^+$	Related to *syk* level?	37, 38
Caspase 8 deficiency	Embryonic lethal	Viable—immunodeficiency		62, 63
Caspase 10	Absent	Present		62
IFN-α promotes Th1 differentiation	No	Yes	Mutant stat2 in mice	44
Th expression of IL-10	Th2	Th1 and Th2		51
IL-4 and IFN-γ expression by cultured Th	Either/or	Sometimes both		
CD28 expression on T cells	On 100% of $CD4^+$ and $CD8^+$	On 80% of $CD4^+$, 50% of $CD8^+$		54
ICOS deficiency	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^+ 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, eotaxin-2/3	Absent	Present	Chemokines	66, 67
MRP-1/2, lungkine, MCP-3	Present	Absent	Chemokines	66, 67
IFN-γ 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+ T	No	Yes	Memory T only	75–77
CD58 (LFA-3)	Absent	Present	CD2 ligand	82
T cell dependence on CD2-ligand interactions	Low	High		82
CD2 ligand interaction	Lower affinity, with CD48	Higher affinity, with CD58		82
CD40 on EC	Absent	Present		83, 84
Vascularized grafts tolerogenic?	Yes	No		5
Microchimerism induces graft tolerance?	High success rate	Low success (expts. in non-human primates)		7
Passenger leukocytes	Account for graft immunogenicity	Do not account for graft immunogenicity		6

Response divergence across species in innate immune response



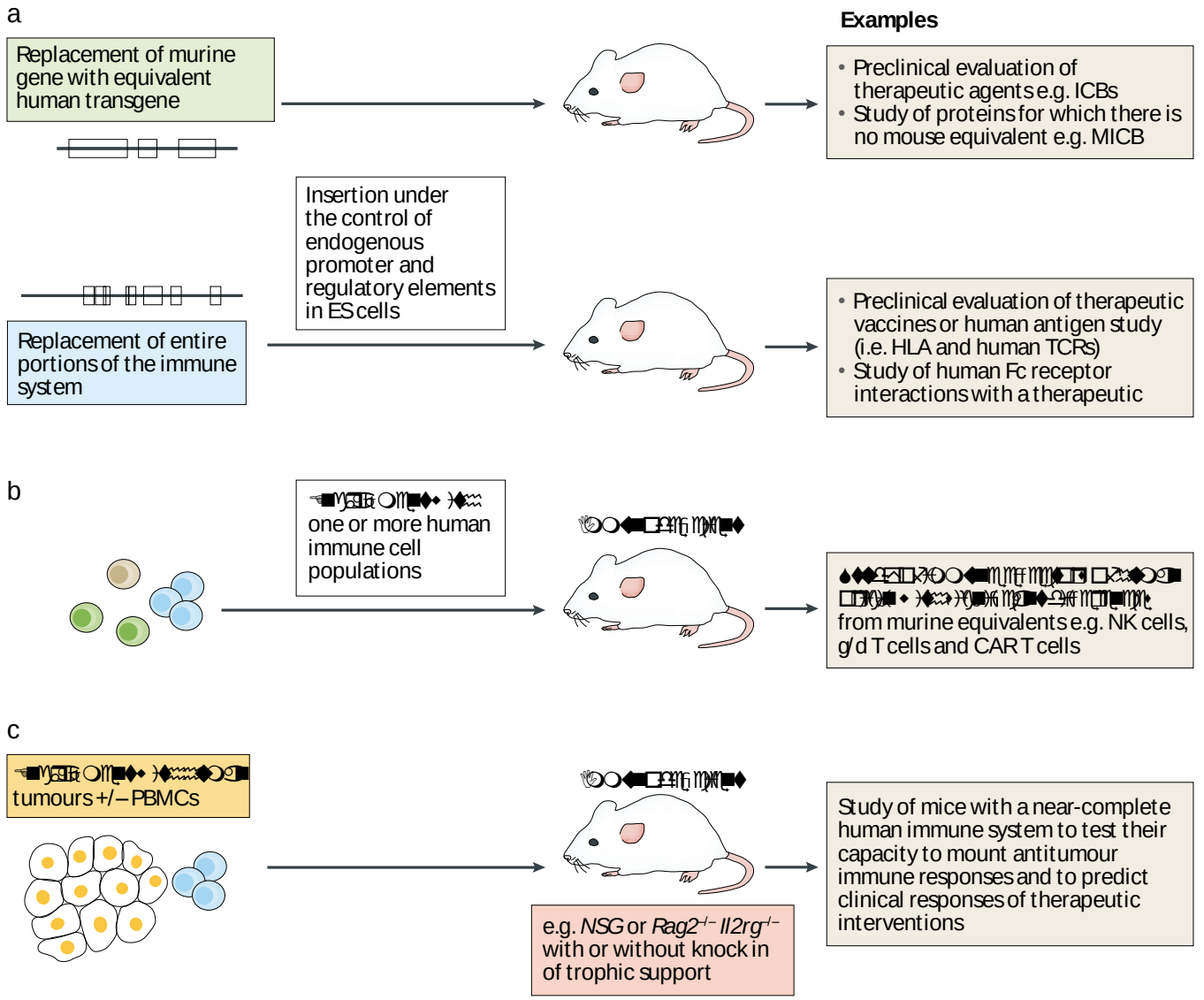
Gene expression variability across cells and species shapes innate immunity

Tzachi Hagai[1,2], Sarah A. Teichmann[1]

https://doi.org/10.1038/s41586-018-0657-2

NATURE | www.nature.com/nature

Humanized mouse models for oncoimmunology

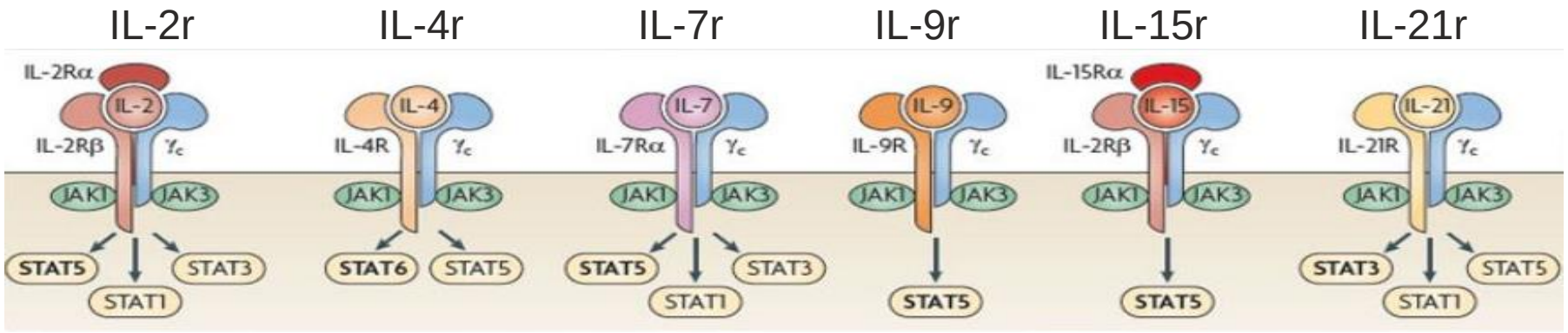


Adapted from Zitvogel et al Nat Rev Cancer 16; 759

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.**

Targeting the IL-2r Common Gamma Chain prevents mouse T, B and NK Cell Development

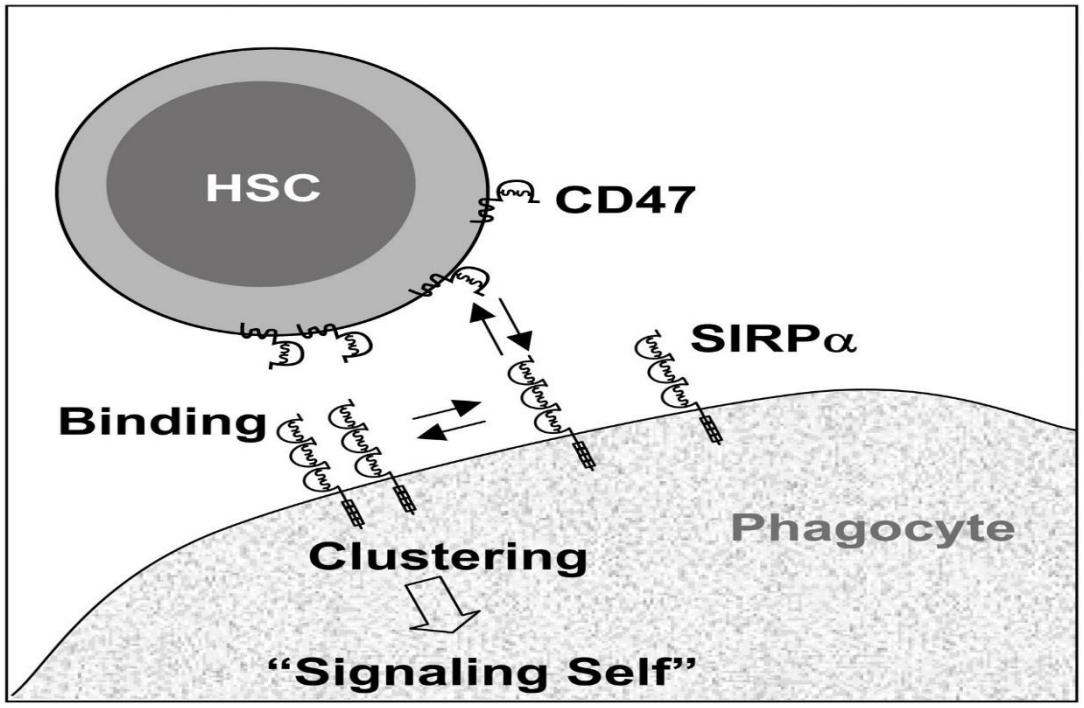


IL2r common gamma chain targeted by 4 different groups and combined with *scid*, *Rag1*^{null}, or *Rag2*^{null} on different genetic backgrounds

L Shultz et al (2007) Nat Rev Immunol 7:118
Y Rochman et al. 2009. *Nat Rev Immunol* 9:480
M Noguchi et al (1993) Cell 73:147

Courtesy of L. Shultz

NSG mice:
Expression of Human-Like SIRPα Polymorphism by NSG Macrophages Protects Human HSCs from Phagocytosis

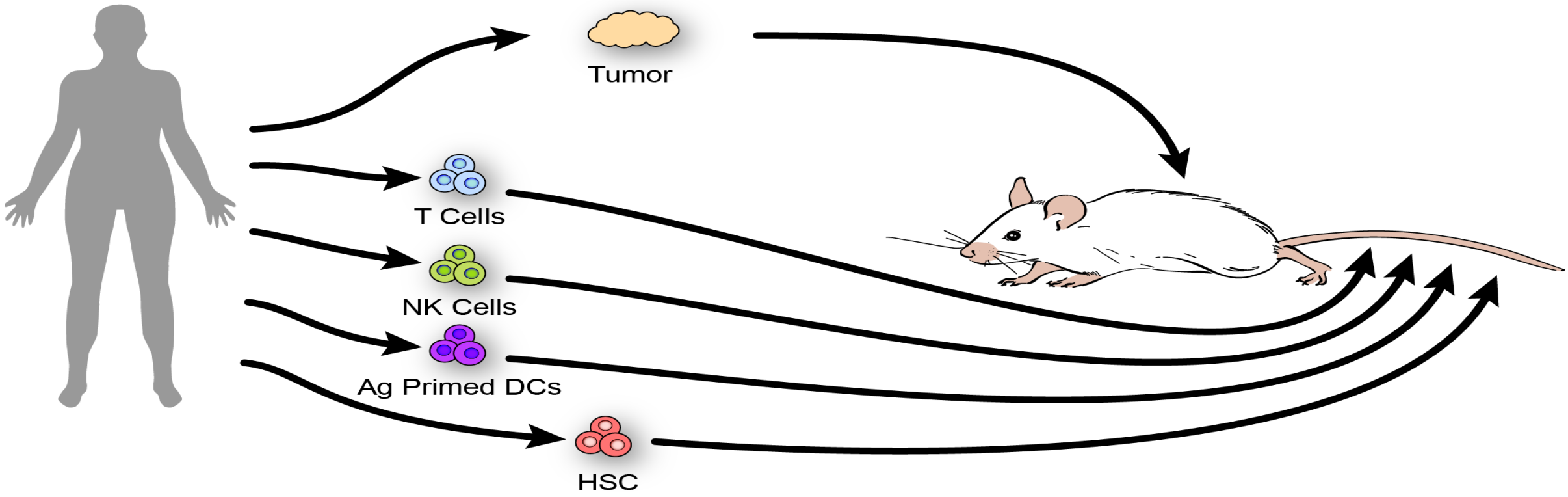


Adapted from Subramanian et al (2006)

- Binding of CD47 to SIRPα triggers SIRPα clustering.
- Phosphorylation at the cytoplasmic tail ultimately signals "self" > inhibition of phagocytosis

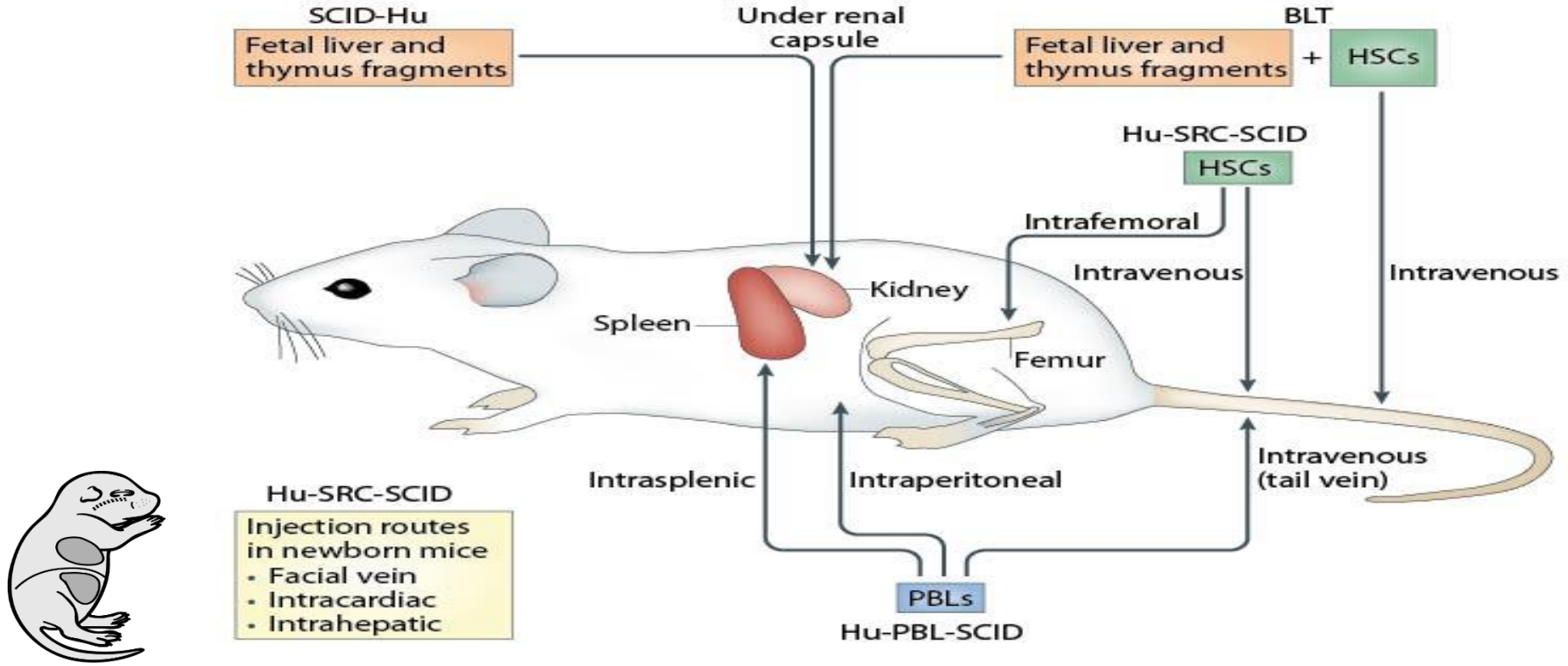
Courtesy of L. Shultz

Modeling Human Tumor Immunotherapy in immunodeficient [NSG] Mice



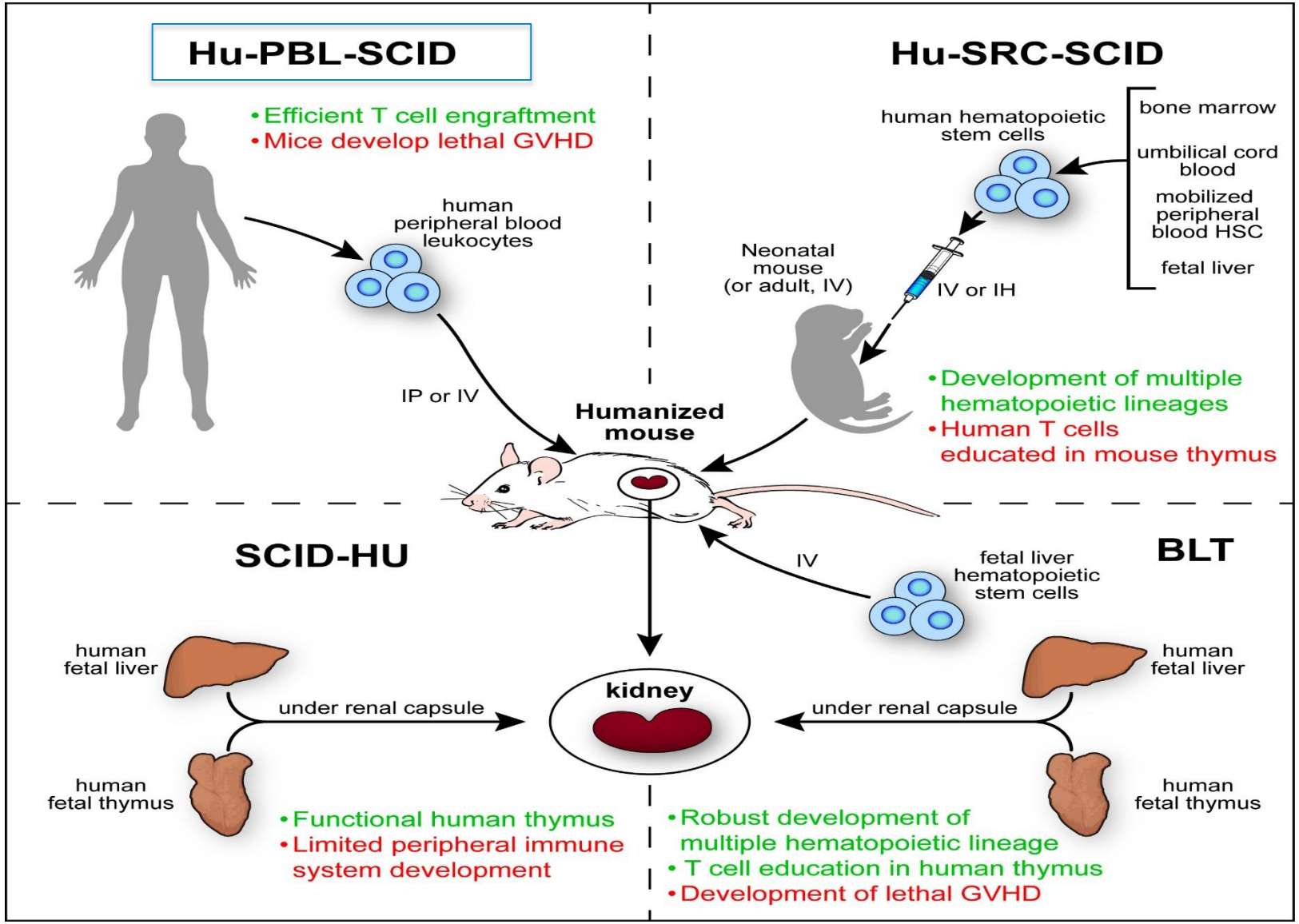
Courtesy of L. Shultz

NSG Mice Support Engraftment With Human Hematopoietic Cells and Tissues



L Shultz et al (2012) Nat Rev Immunol 12:786

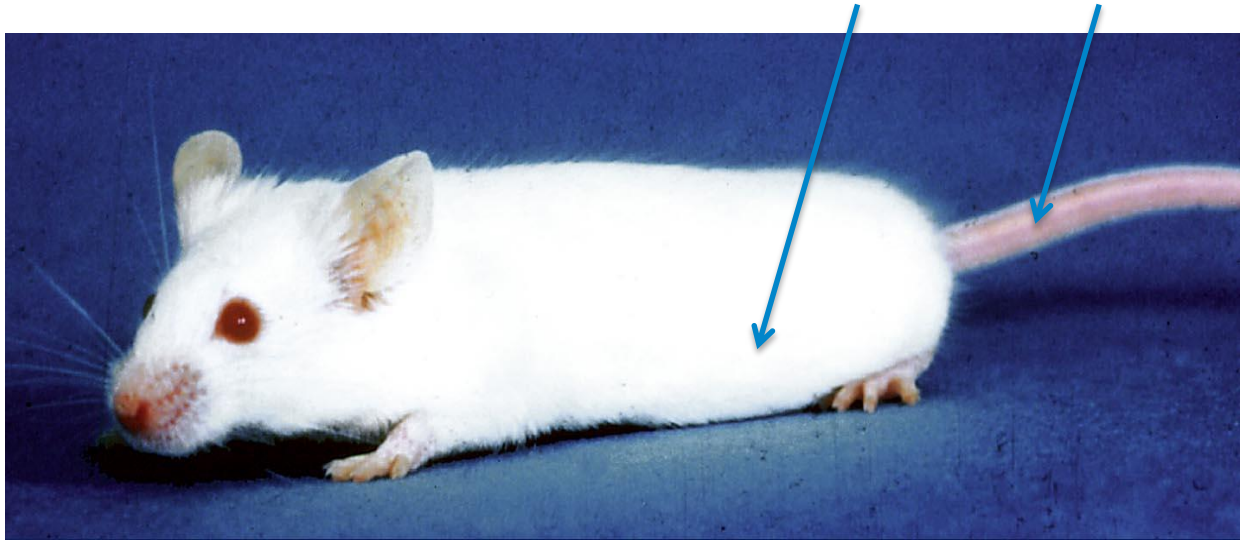
Hematolymphoid Engraftment Methods



Courtesy of L. Shultz

Engraftment of NSG Mice with Human PBMC

i.v. or i.p. injection of human PBMC

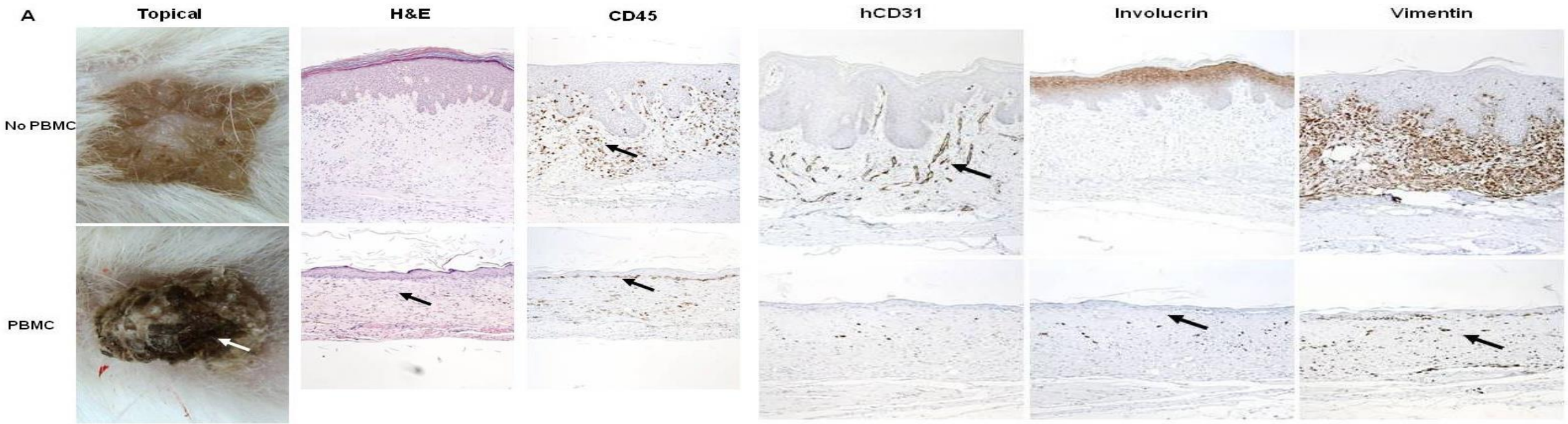


Human T cell function can be analyzed for 4-6 week prior to development of lethal xenogeneic GVHD

T Pearson et al (2008) Curr Prot Immunol 15:21

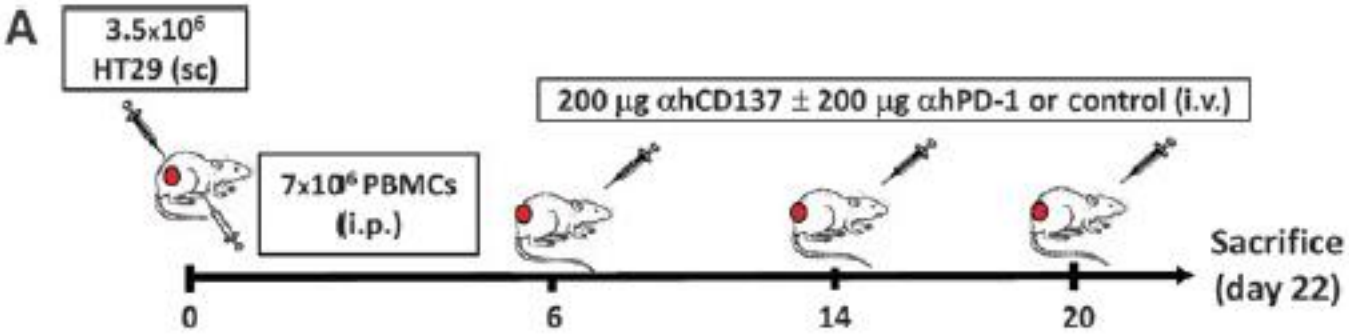
Courtesy of L. Shultz

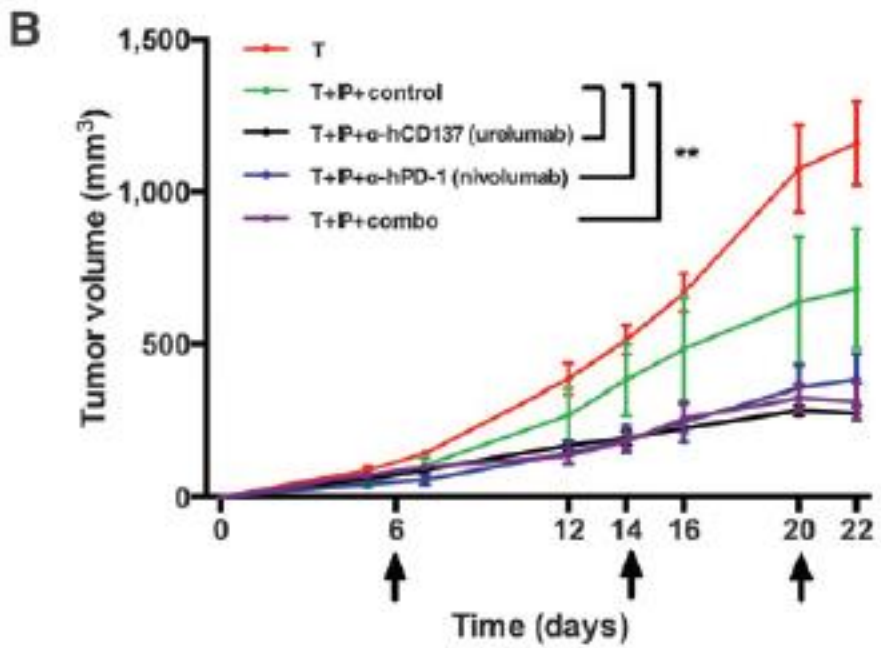
Human Skin Allograft Rejection in PBMC model

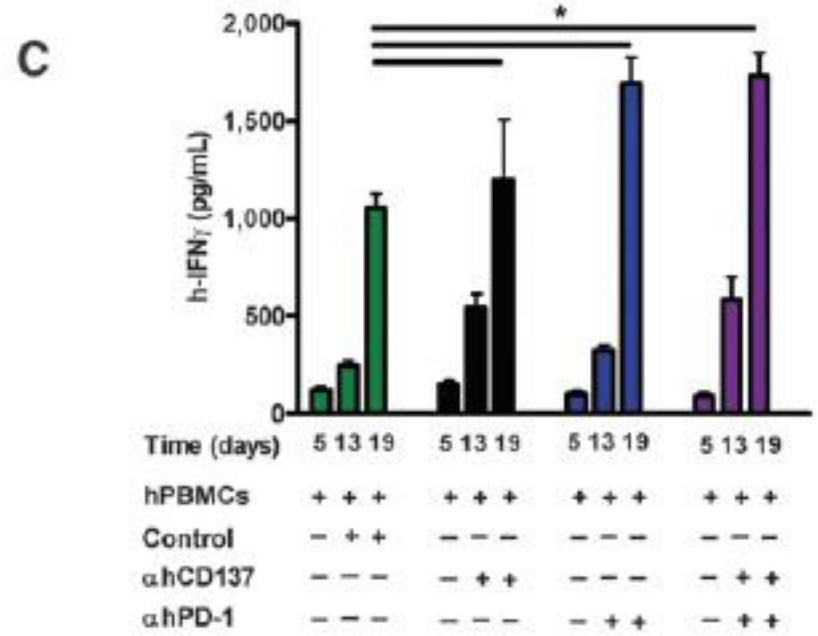


Split thickness human skin grafts were transplanted on NSG mice treated with anti-Gr-1mAb to reduce mouse granulocyte and macrophage activity. Four weeks later mice were left untreated (top panel) or were injected with 20 x 10^6 allogenic human PBMC (bottom panel). Allografts were evaluated 4 wk following PBMC injection W Racki et al (2010) Transplantation 89:527

Colon cancer tumor rejection mediated by human allogeneic PBMC in a model of combination immunotherapy





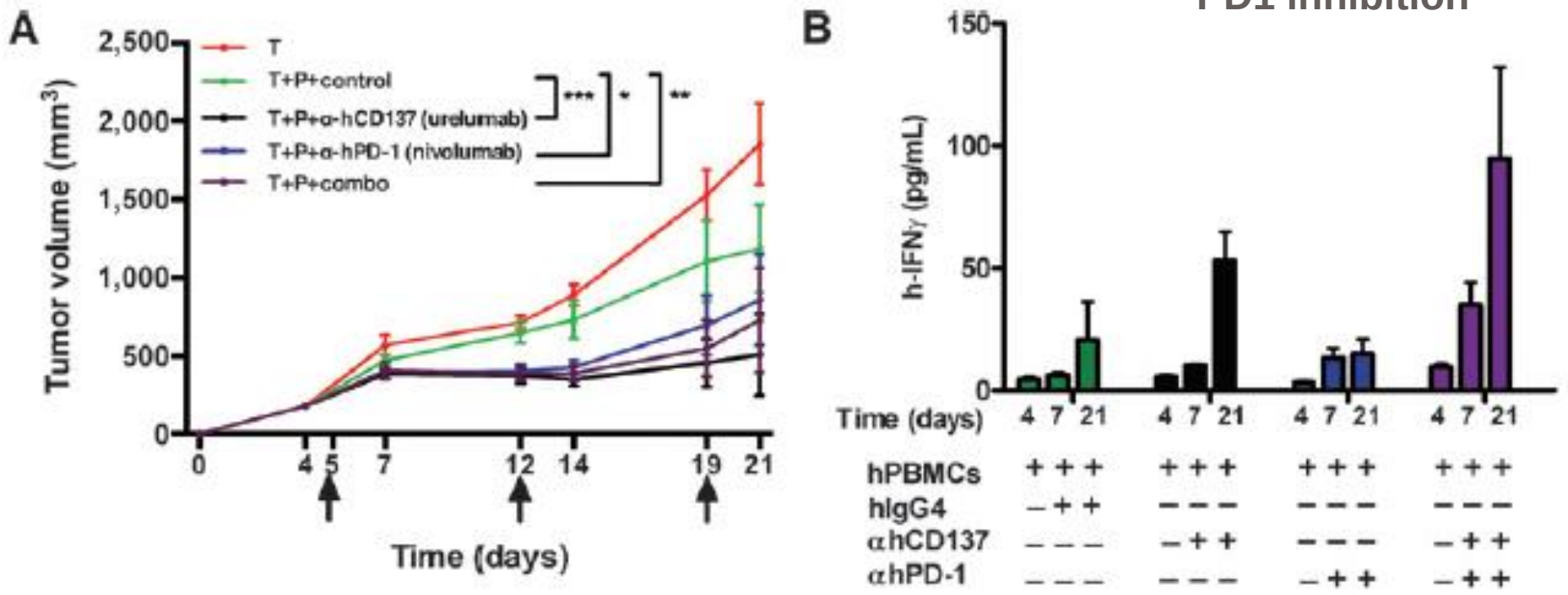


CD137 agonist to boost T cells
PD1 inhibition

Sanmamed et al Cancer Res 75 (17) 2015

Gastric cancer tumor rejection mediated by autologous PBMC in a model of combination immunotherapy

CD137 agonist to boost T cells
PD1 inhibition



Sanmamed et al Cancer Res 75 (17) 2015

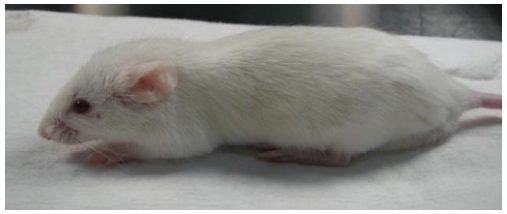
Xenogeneic GVHD Mediated by Human PBMC

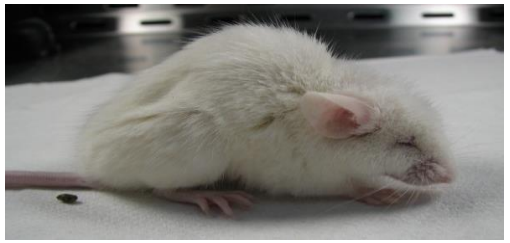
Intravenous Injection of 20 x 10^6 Human PBMC

(NSG) Mice

+ or - radiation

Follow for engraftment and disease





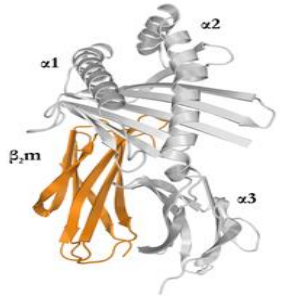
-hair loss/erythema
-hunched posture
-weight loss
-death

Courtesy of L. Shultz

Reduced Xenogeneic GVHD in NSG Mice lacking Murine MHC Class I and II Molecules

Mouse MHC class I knockouts

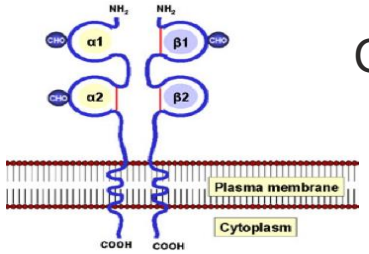
NSG *(β2M)*^{null}
NSG *(KD)*^{null}



MA King et al (2009)
Clin Exp Immunol 157:104

Mouse MHC class II knockouts

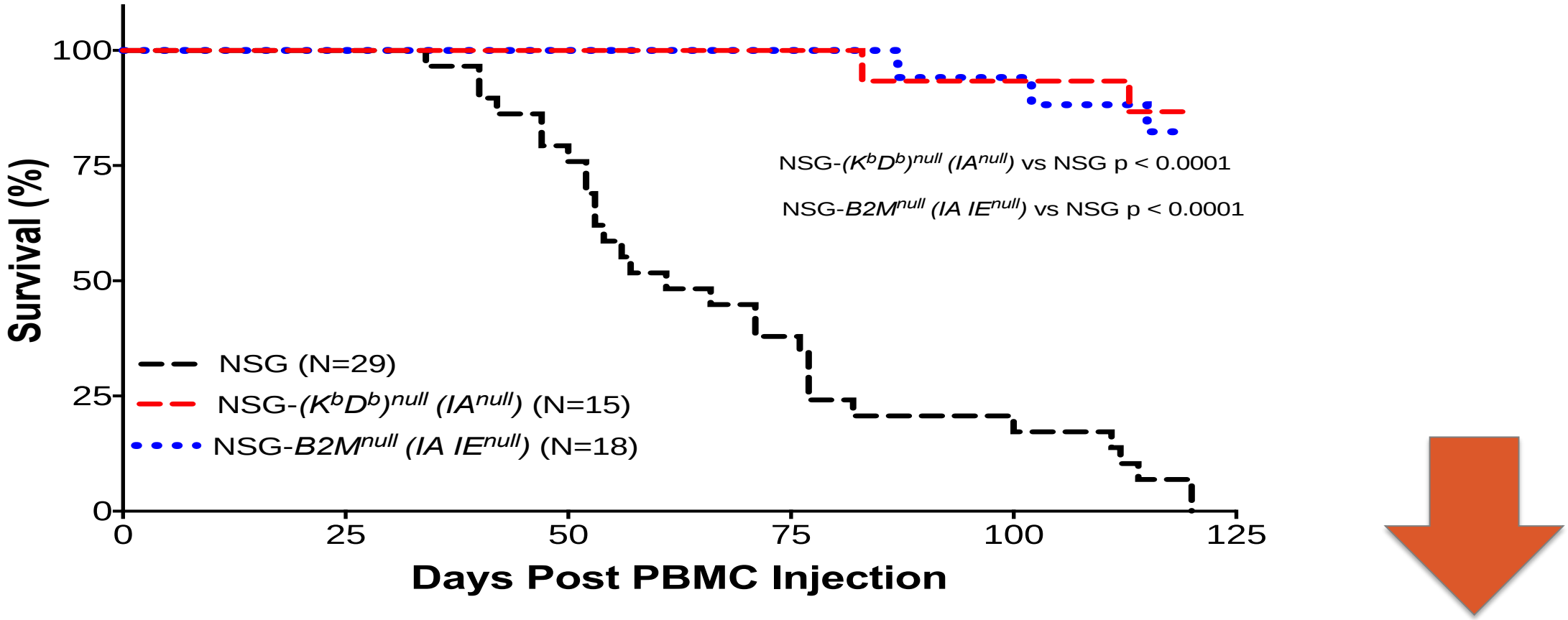
NSG *(I-A)*^{null}
NSG *(I-A/I-E)*^{null}



L Covassin et al (2011)
Clin Exp Immunol 166:269

Courtesy of L. Shultz

NSG-*(KD)^{null}* (*IA^{null}*) and NSG-*B2M^{null}(IA/IE)^{null}* Mice show Increased Survival Following Injection with Human PBMC

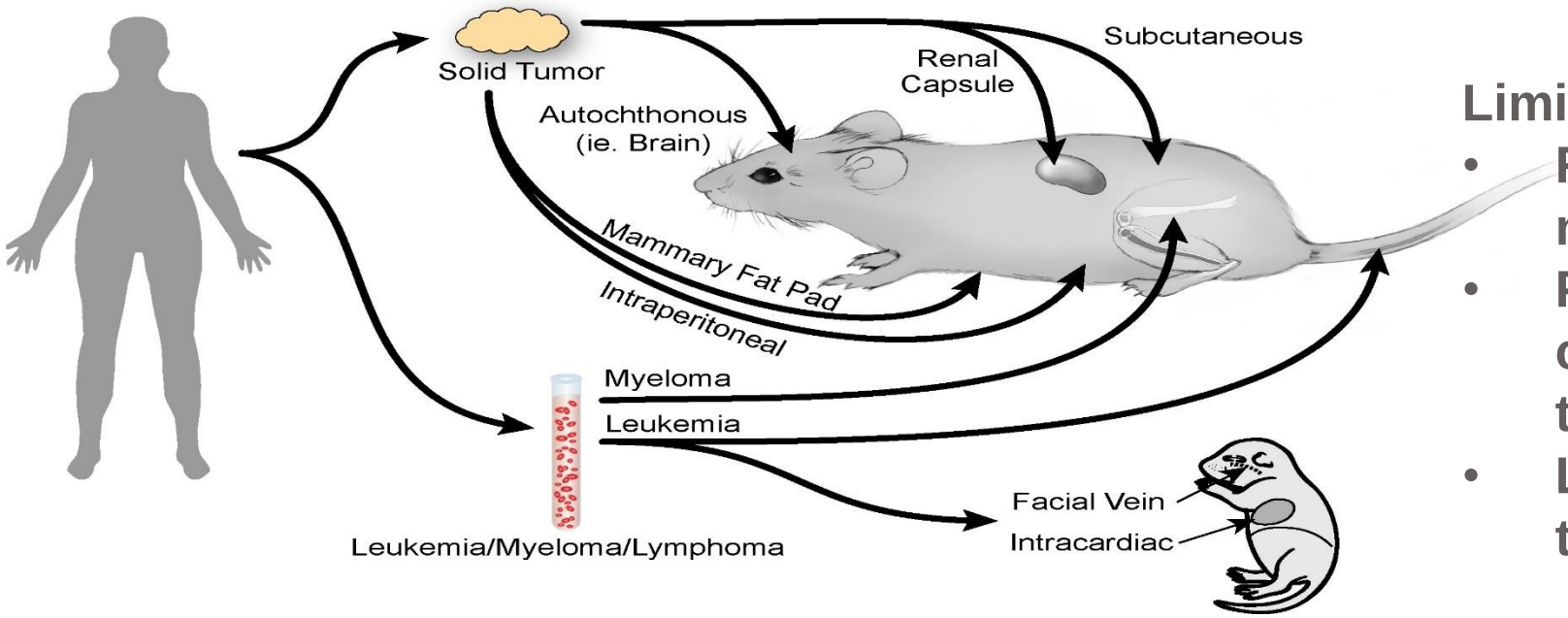


8-12 week-old mice were injected IP with 1 x 10^7 human PBMC

Autologous models combined with PDX tumors

Brehm et al. FASEB Journal 33, 2019

Patient-Derived Xenografts (PDX)



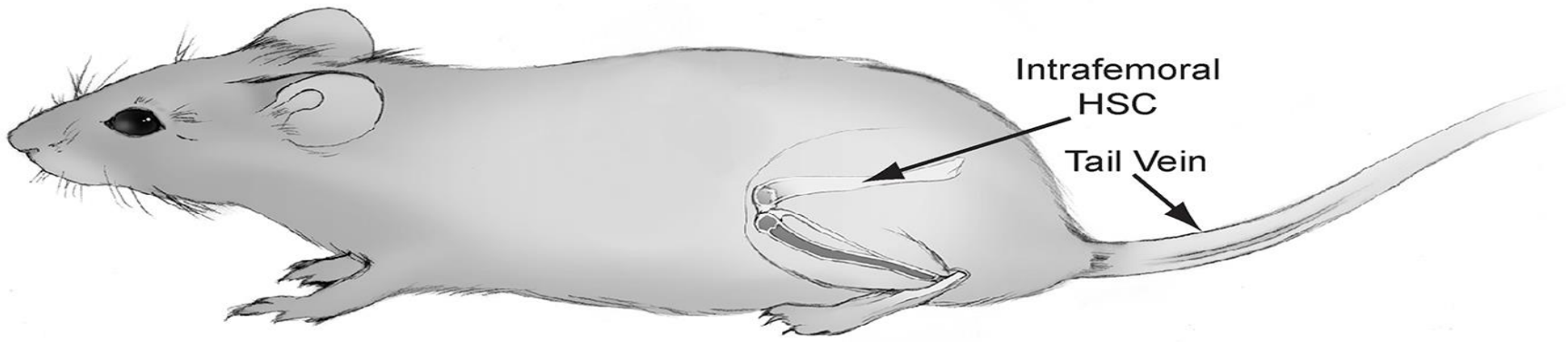
Limitations:

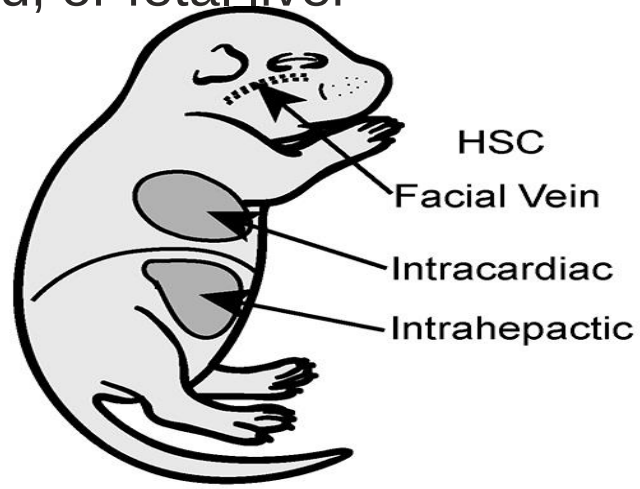
- **Replacement of stroma with mouse cells**
- **Pre-existing infiltrate that cannot be maintained over time**
- **Lack of systemic immune cells that can be attracted to tumor**

Courtesy of L. Shultz

Engraftment of NSG Mice with Human Hematopoietic Stem Cells

Human HSC source: Umbilical cord blood, bone marrow, mobilized, or fetal liver

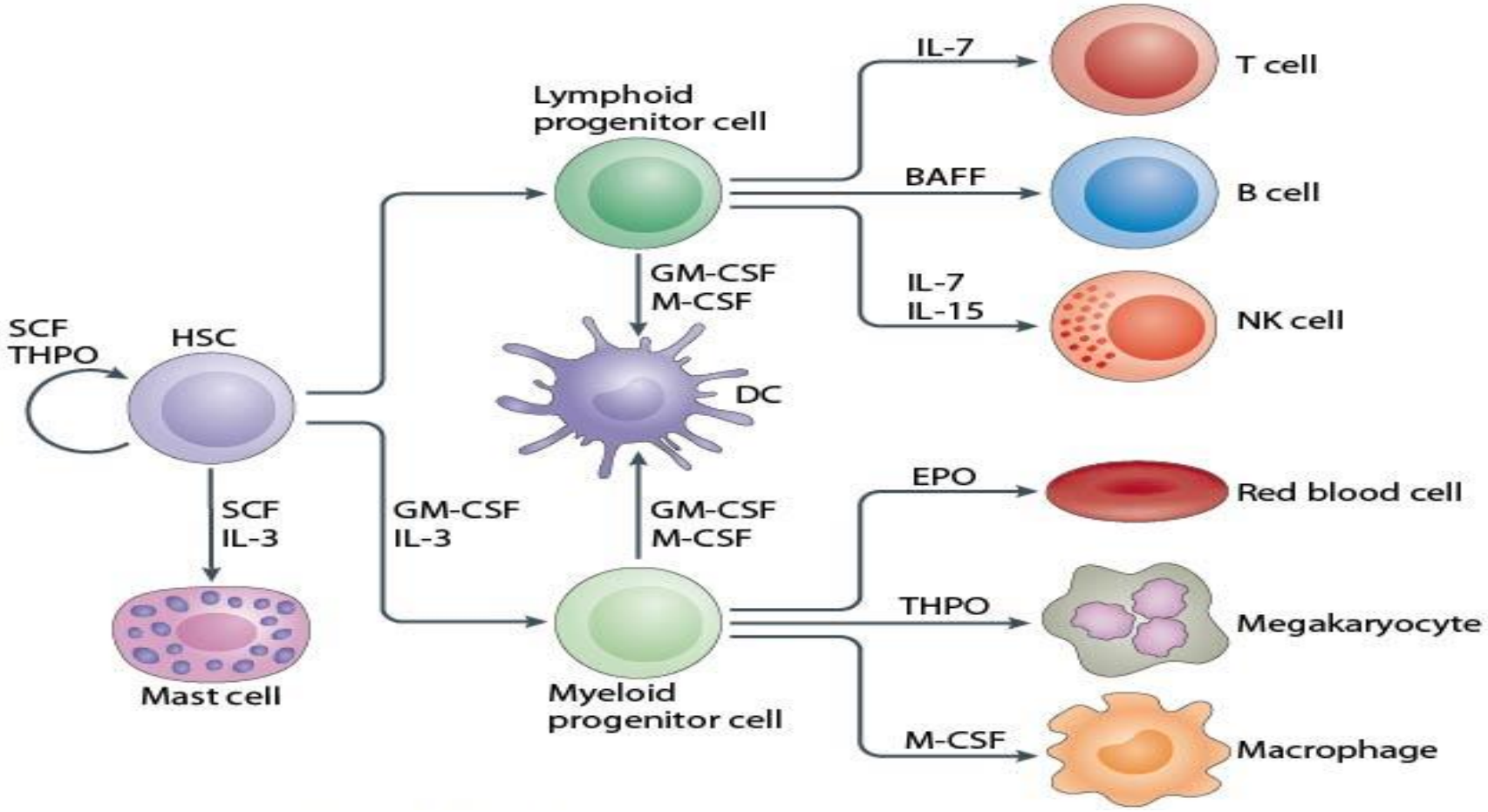




X-Ray dose 250cGy 100cGy

T Pearson et al (2008) Current Protocols Immunol. 15:21

Human Cytokines are Required for the Differentiation of Human HSC into Multiple Cell Lineages



NC Walsh et al (2017) Annu Rev Pathol 12:187

Human Cytokines Expressed in Humanized Mice Support Human HSC Differentiation

Human cytokine(s)	Cell populations targeted
Membrane-bound SCF	Hematopoietic stem cells (HSC), mast cells
SCF, IL-3, GM-CSF (SGM3)	HSC, myeloid cells, mast cells
BAFF	B cells
Thrombopoietin	HSC, platelets
IL2	T cells and NK cells
IL-6	Plasma cells
IL7	T cells
IL15	NK cells
FLT3L	Dendritic cells
CSF1	Macrophages

Courtesy of L. Shultz

Combination of multiple humanized alleles

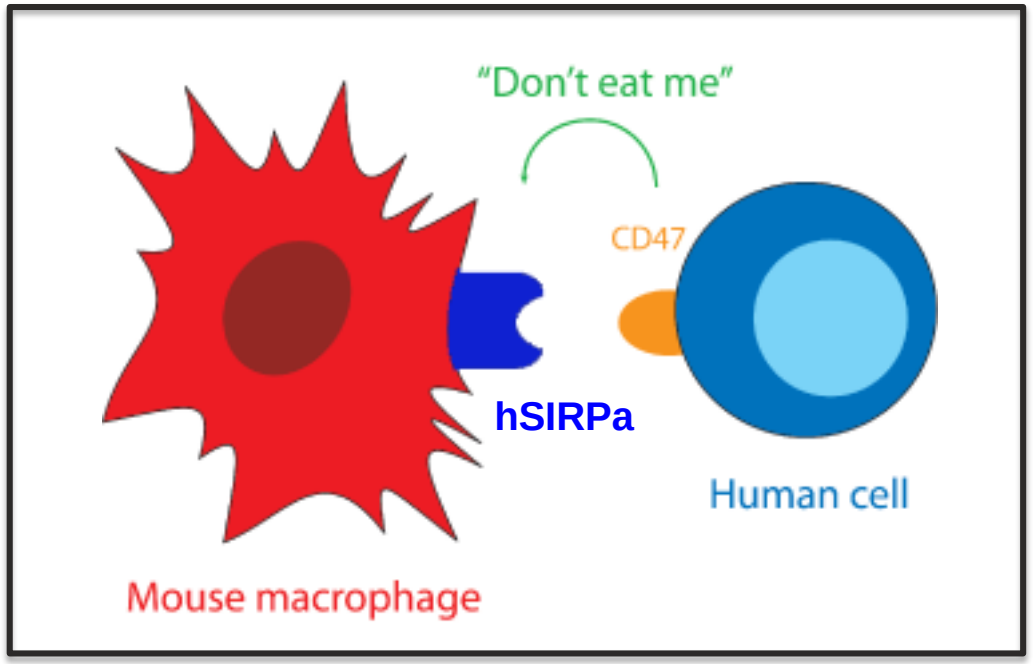
M-CSF$^{h/h}$
IL-3/GM-CSF$^{h/h}$
hSirpα^{tg}
TPO$^{h/h}$
RAG2$^{-/-}$
IL2RGamma$^{-/-}$

} Myeloid development

Phagocytic tolerance

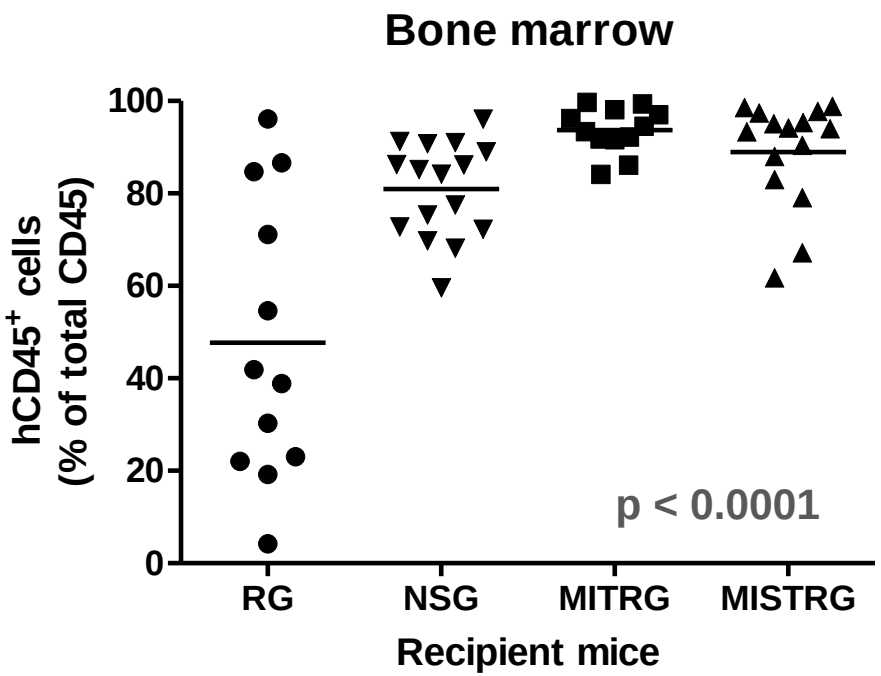
Longterm maintenance of functional HSCs

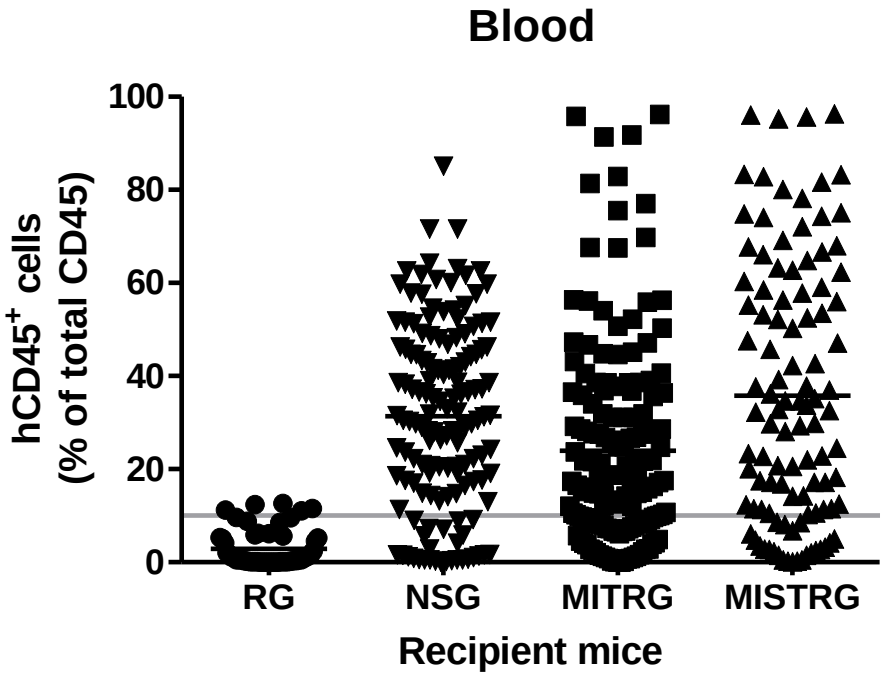
} Immunosuppression (no mouse T, B, NK cells)



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

MI(S)TRG mice are highly permissive for human hematopoiesis





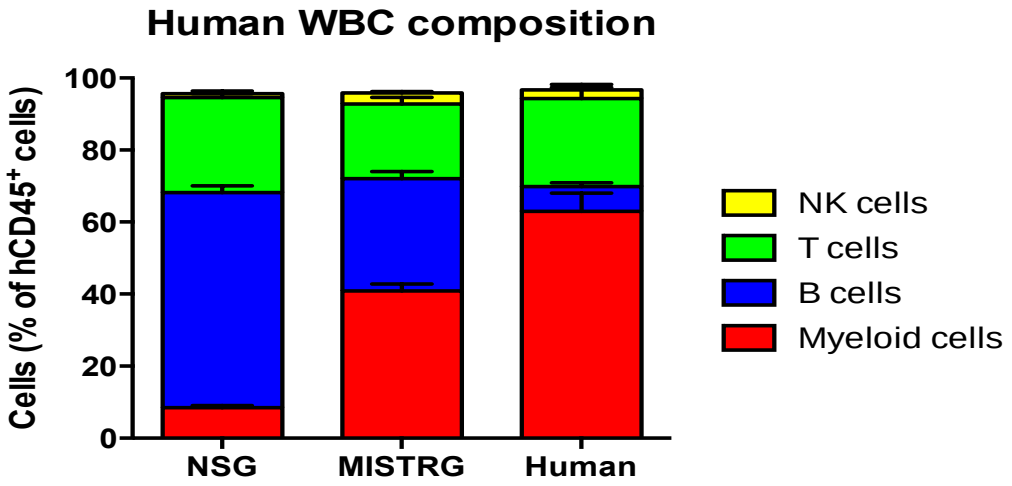
p-value : One-way ANOVA

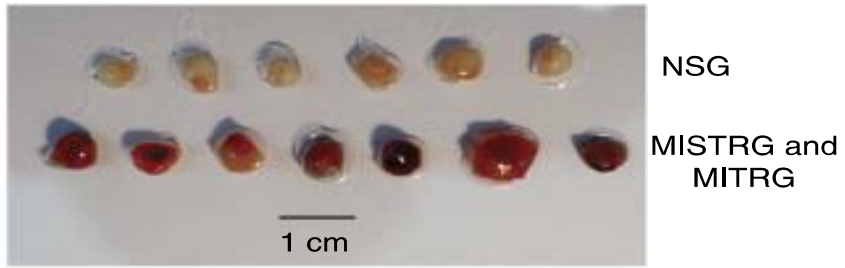
19 independent fetal liver samples
n= 56-155 mice/group
7-9 weeks post-transplantation

Courtesy of R. Flavell

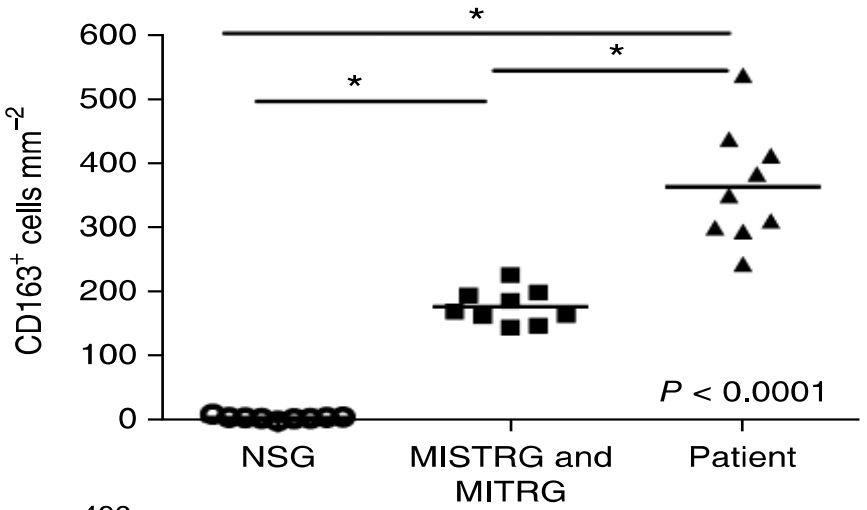
Development and function of human innate immune cells in a humanized mouse model

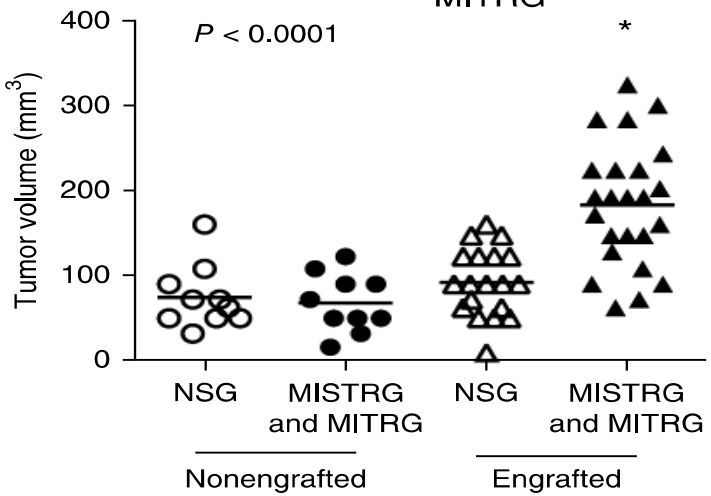
Anthony Rongvaux[1,10], Tim Willinger[1,10], Jan Martinek[2,3], Till Strowig[1,9], Sofia V Gearty[1], Lino L Teichmann[4,5], Yasuyuki Saito[6], Florentina Marches[2], Stephanie Halene[7], A Karolina Palucka[2], Markus G Manz[6] & Richard A Flavell[1,8]





Human melanoma



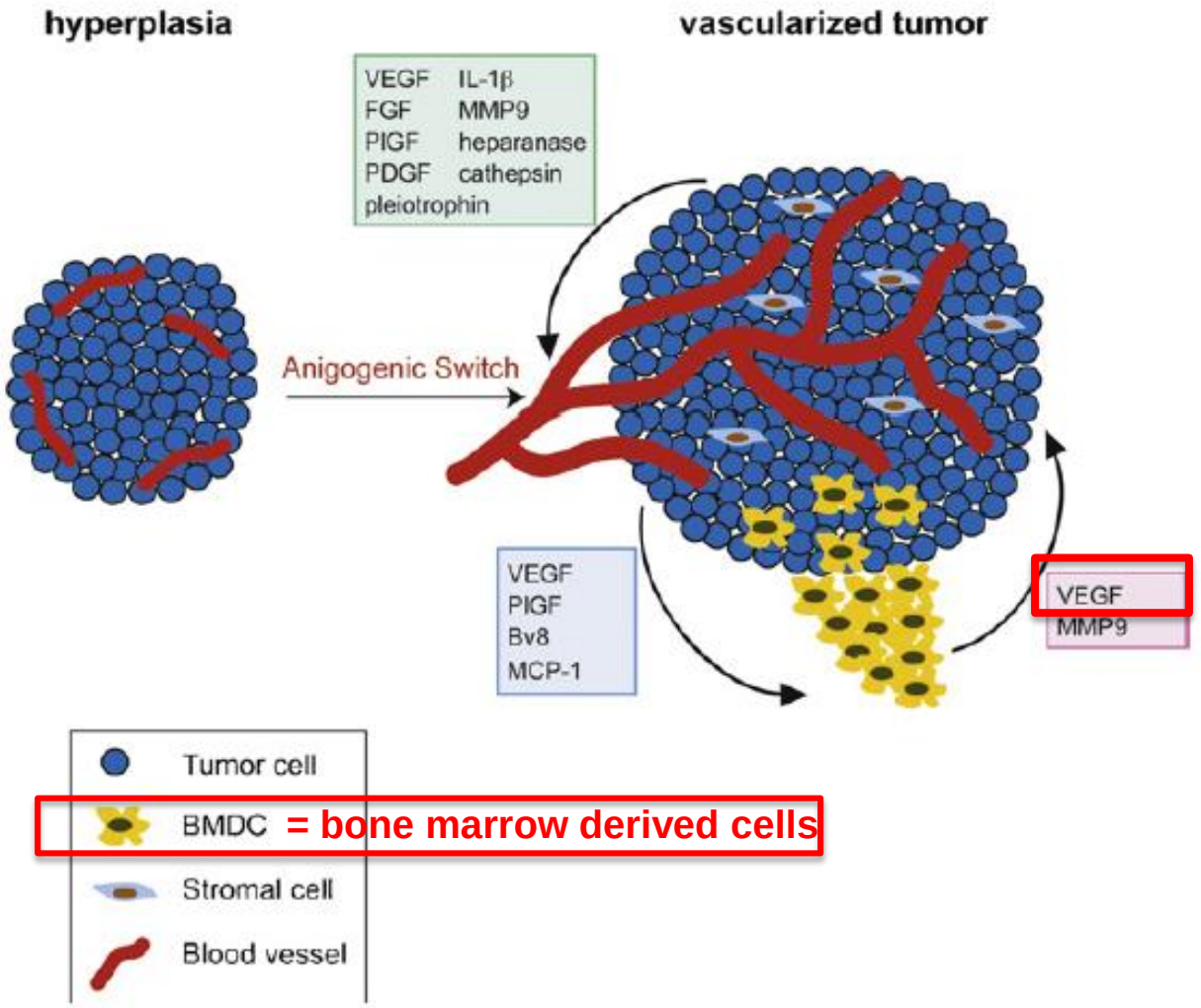


NATURE BIOTECHNOLOGY ADVANCE ONLINE PUBLICATION

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

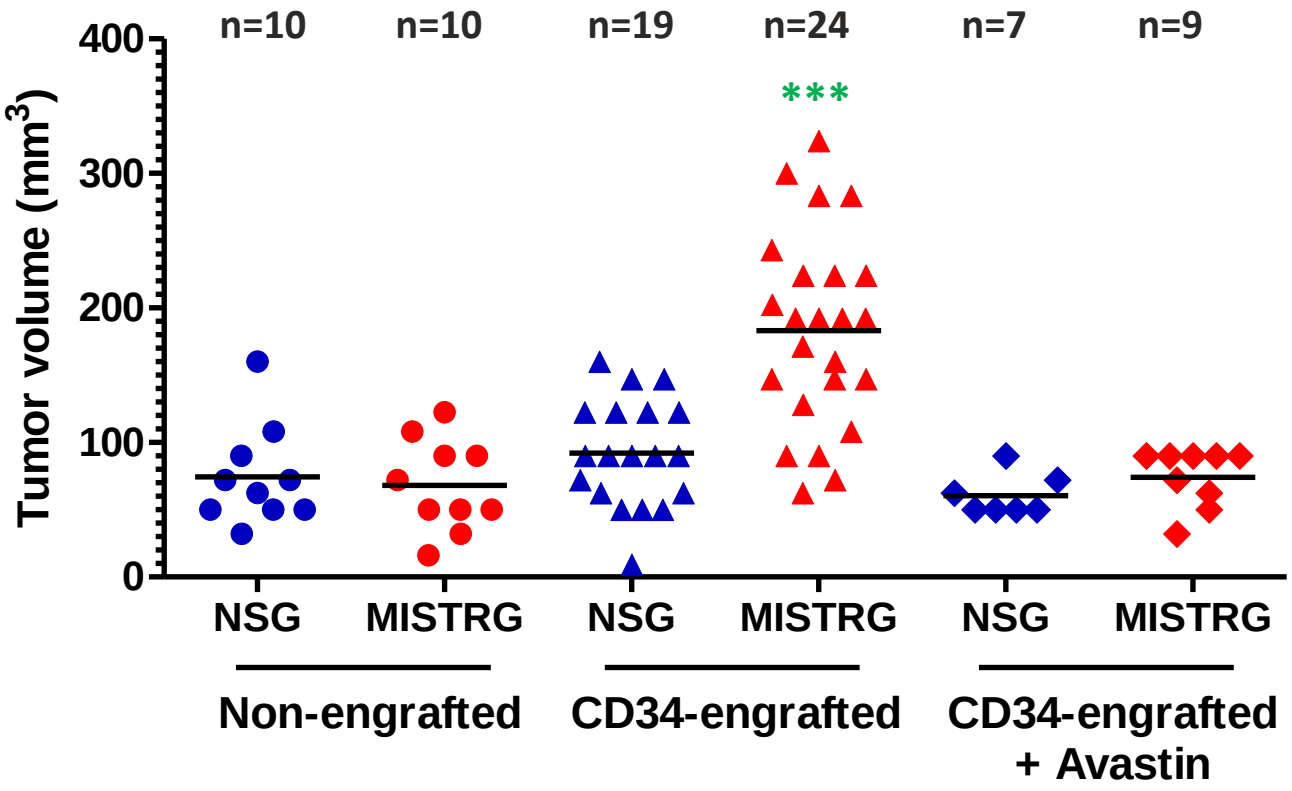
The angiogenic switch

- Mediated by pro-angiogenic factors (VEGF, …)
- Transition from hyperplasia to tumor progression and malignancy
- Role of inflammation in the tumor microenvironment



Baeriswyl et al, 2009

Tumor growth in MISTRG requires human VEGF



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

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

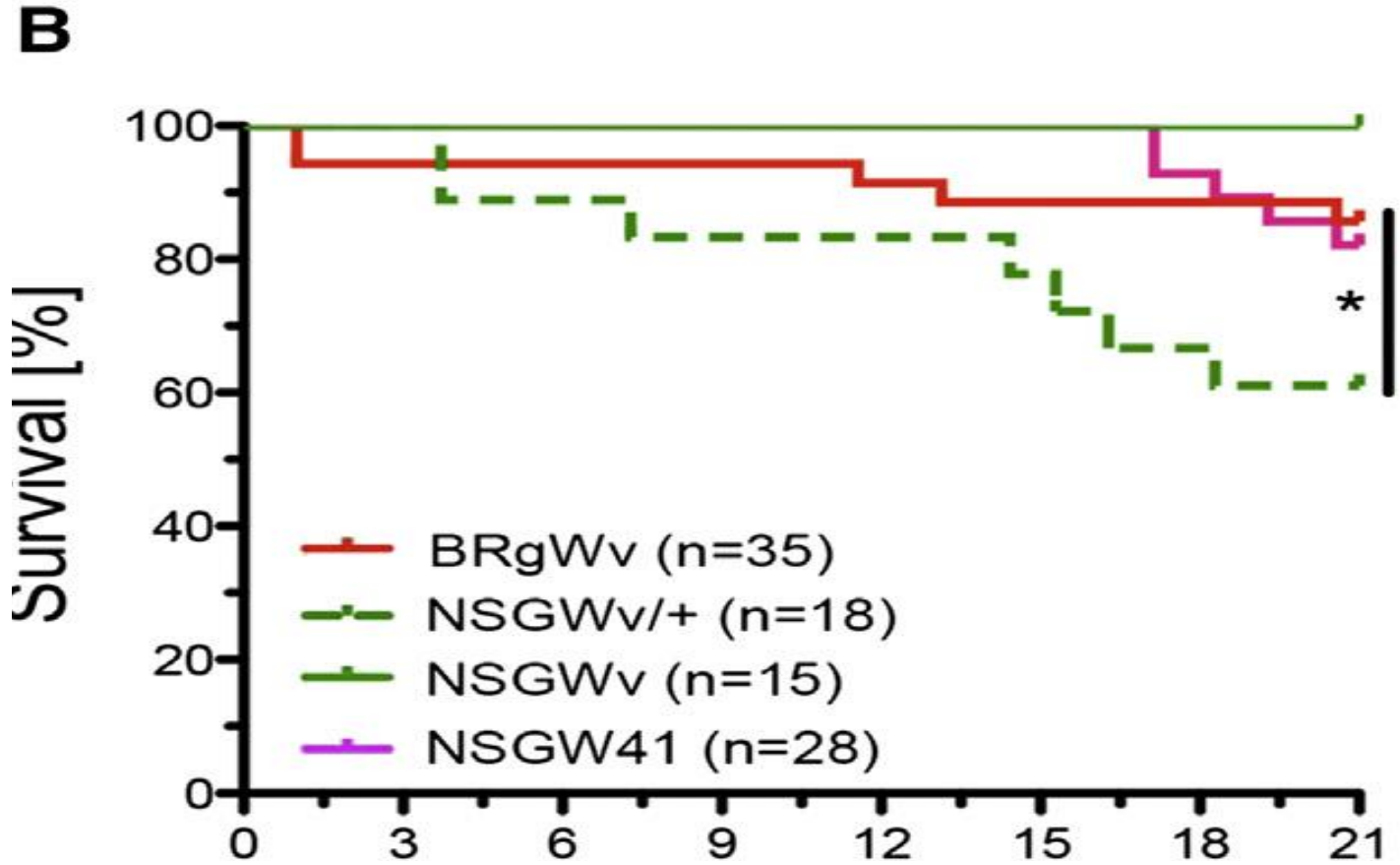
- next generation MISTRG mice with IL15&IL15Ra [R. Flavell]
- MISTRG6 for B cell malignancy *Nat Med Nov 2016* [M. Dhodapkar]
- NSG with mouse kit mutant (Kit^{w41}) for engraftment *Cell Stem Cell 2014* [S. Rahmig]
- BAFF for improved antibody responses [R. Pelanda]
- NSG-SGM3 with CSF1-tg for macrophages and IL2-tg for NK cells [D. Greiner]
- NSG-FcRg-ko for IVIG *Cell Rep. 2015* [I. Schwab]
- Human thymus reconstruction [M. Brehm, M. Sykes]

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

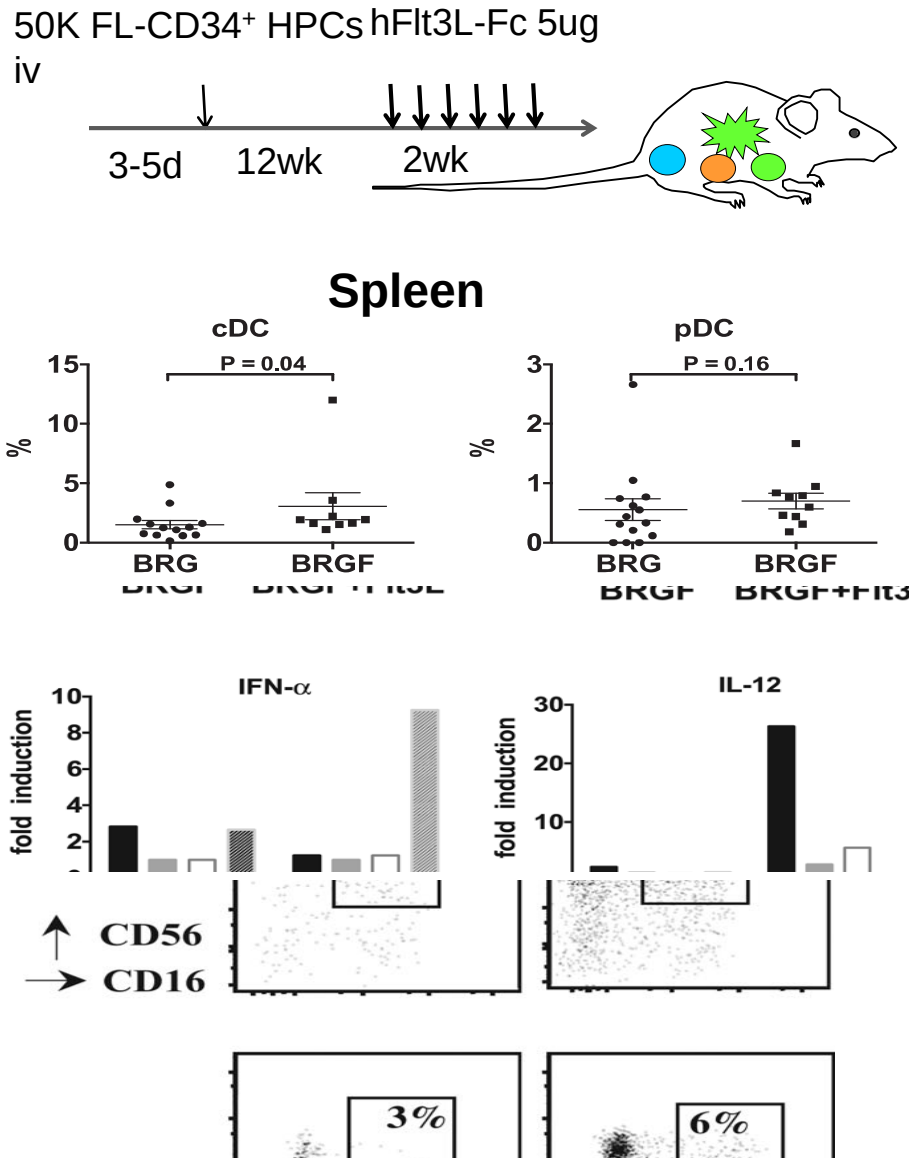


Cosgun et al., Cell Stem Cell 2014

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



Humanized mice: Current challenges and opportunities

- **Engraftment with HPCs**
  Lack of human cytokines impairs HSC growth & differentiation
  Source of HPCs: fetal tissues, bone marrow, blood,
  Autologous models: 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

Next Generation of Humanized Mice

CRISPR editing of the host and of human cells

iPS cells to create autologous models

Genetic editing for 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

Thanks to our patients
Thanks to funding organizations

KP Lab:
Chun Yu
Florentina Marches
Jan Martinek
Patrick Metang
Pierre Authie
……

Lenny Shultz
Jim Keck and colleagues at JAX Sacramento
Susie Airhart

Richard Flavell
Anthony Rongvaux
Michael Chiorazzi

Jacques Banchereau

https://ocg.cancer.gov/programs/HCMI
oncologymodels.org
http://tumor.informatics.jax.org/mtbwi/pdxSearch.do;jsessionid=23644E4F8468C119FF68A70AA64AFA34
karolina.palucka@jax.org