Humanized models to study immunity and to accelerate the development of new solutions for human health

Fondation Mérieux Conference Center
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Next generation humanized mouse models for normal and malignant hematopoiesis

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Talk outline

1. **Introduction of next generation humanized “cytokine” mice**
2. Normal hematopoiesis
3. Malignant hematopoiesis

Humanized Mice

In an ideal model of a humanized mouse, the human graft would fully replace the endogenous mouse hematopoietic and immune system in both space and function.

Requirements

1. Tolerance by the host
2. Physiologic location and migration of cells
3. Full support by the host environment

Achievements and Limitations with Humanized Mice

Achievements

- in situ development of human B cells, T cells, DCs
- some lymphoid organ structuring
- infection with human pathogens (e.g. HIV, EBV, HTLV-1, Salmonella typhi)
- therapy and targeting *in vivo* possible (gene therapy, antibody therapy)

Limitations

- limited availability of hematopoietic stem cell grafts
- variable efficacy of engraftment
- questionable T (and B) cell selection and limited maintenance
- very limited adaptive immune responses
- limited myeloid cell and NK cell differentiation
- limited hematopoietic stem cell maintenance

Key Issues to Address

1.) To establish an "off the shelf" standardized human HSC resource for "larger-scale", industrial use of humanized mice

a) HSC expansion

b) huES cell and iPS -> huHSCs

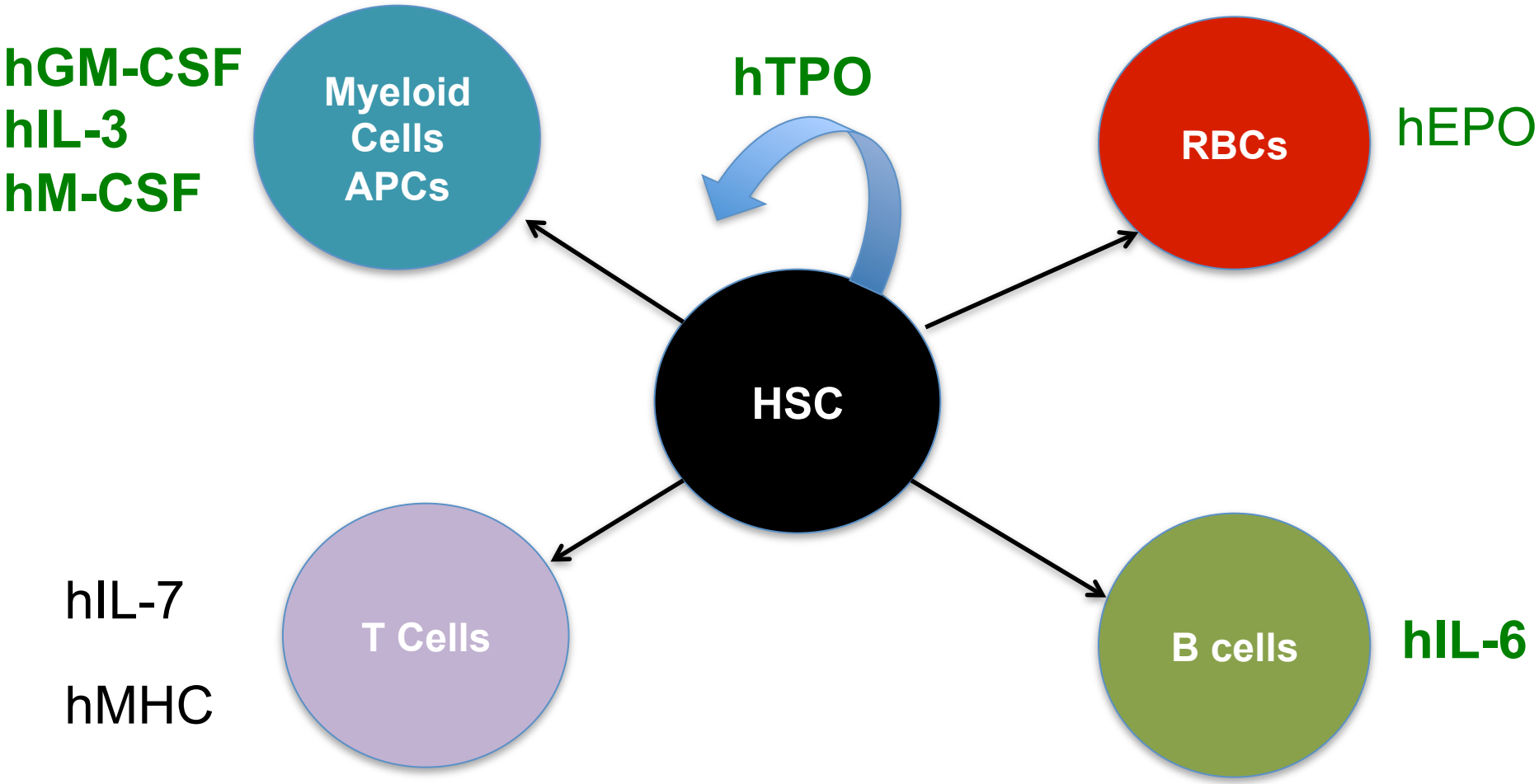
2.) To improve human cell maintenance and immune system development and function

a) implementation of human stromal cells

b) **mouse genetic background modification**

Genetic Humanization of Mice

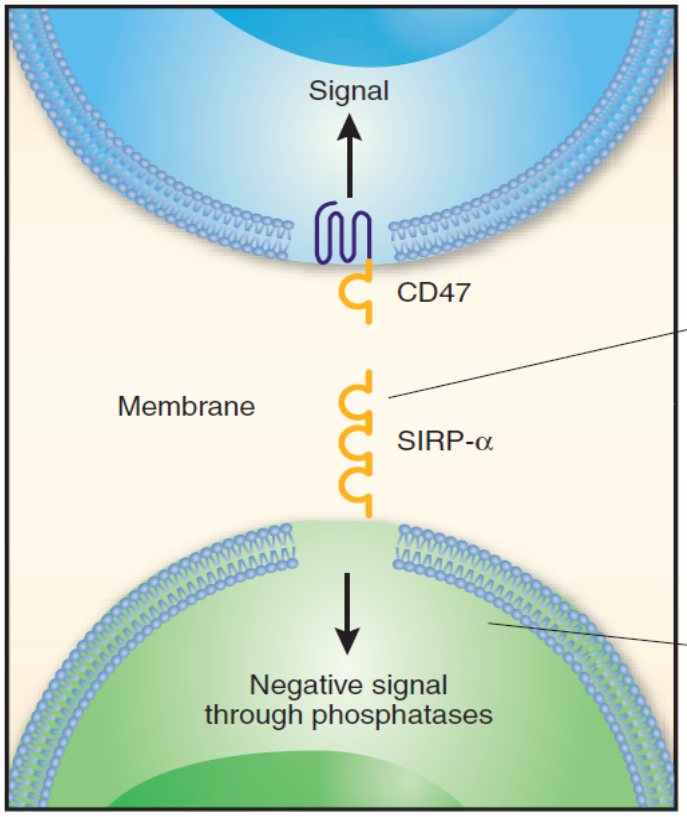
Targets for replacement of mouse with human components



Engraftment and Survival: **hSIRPα**

SIRPα-CD47–mediated signals induce macrophage tolerance

Hematopoietic cell

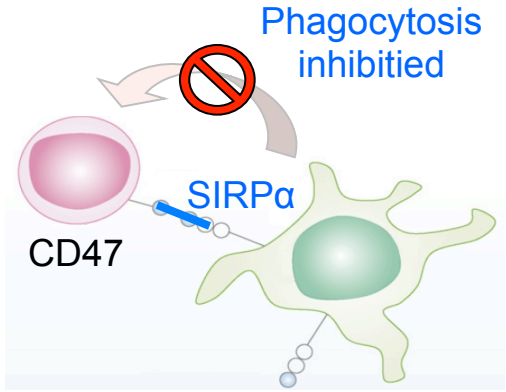


Macrophage

SIRPα
IgV domain
binds CD47

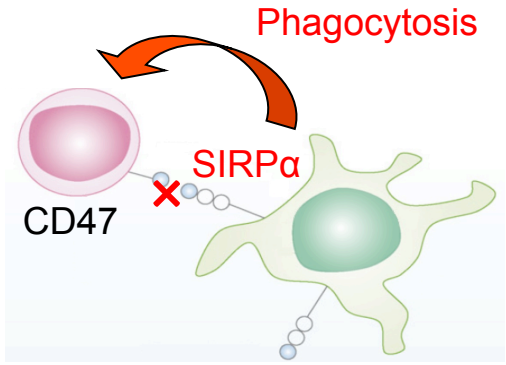
"no action" signal
transmitted

NOD.*SCID* or NSG



Engraftment

NOD.NOR-*Idd13.SCID*



No engraftment

Takenaka et al., Nature Immunology 2007
Takizawa and Manz, News & Views Nature Immunology 2007

Example: human TPO to improve human HSC support

- **TPO** - required for HSC expansion and maintenance

  e.g. Fox N et al., J Clin Invest. 2002 Aug;110(3):389-94.

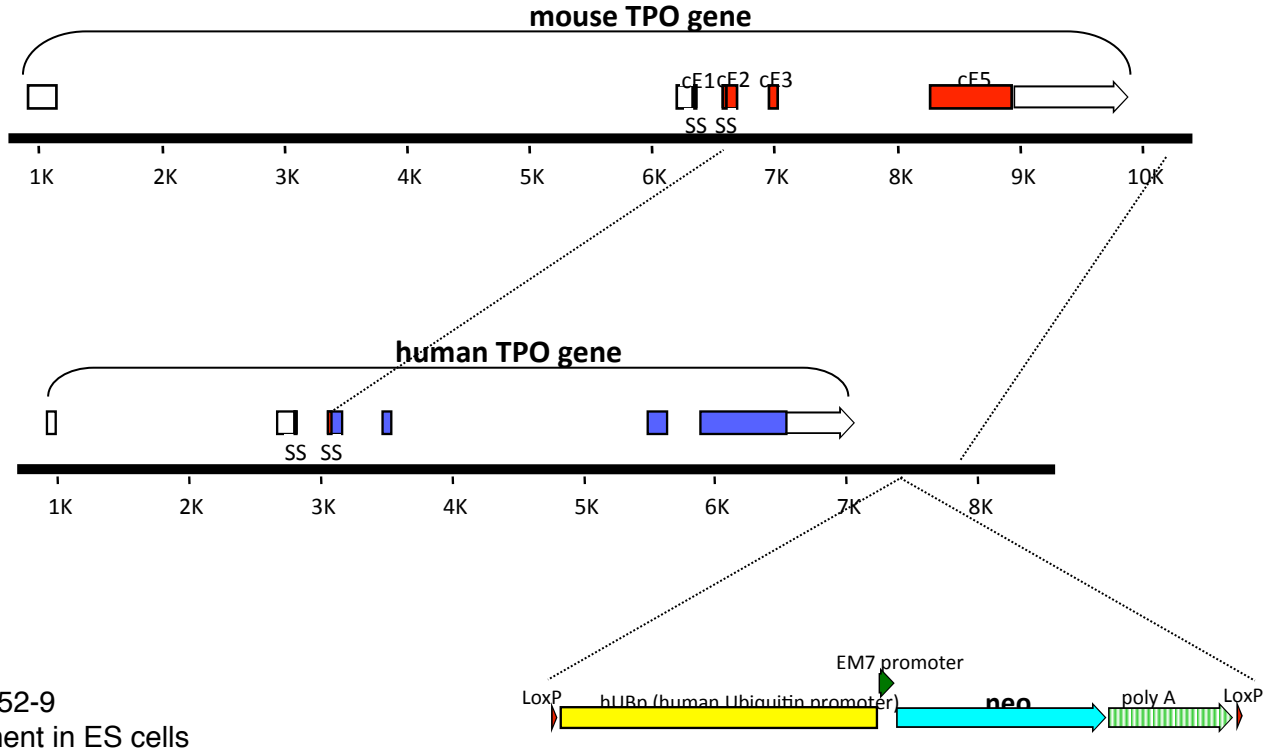
  Yoshihara H et al., Cell Stem Cell. 2007 Dec 13;1(6):685-97.

- mTPO binds to human receptor with low affinity (~1:10)
- mTPO KO mice viable and fertile

→ TPO humanization possibly supportive for human HSC maintenance

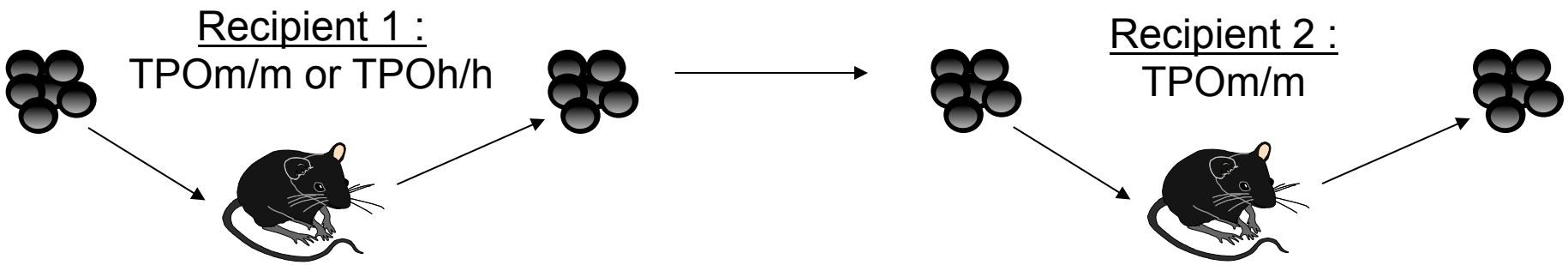
Regeneron: Velocigene

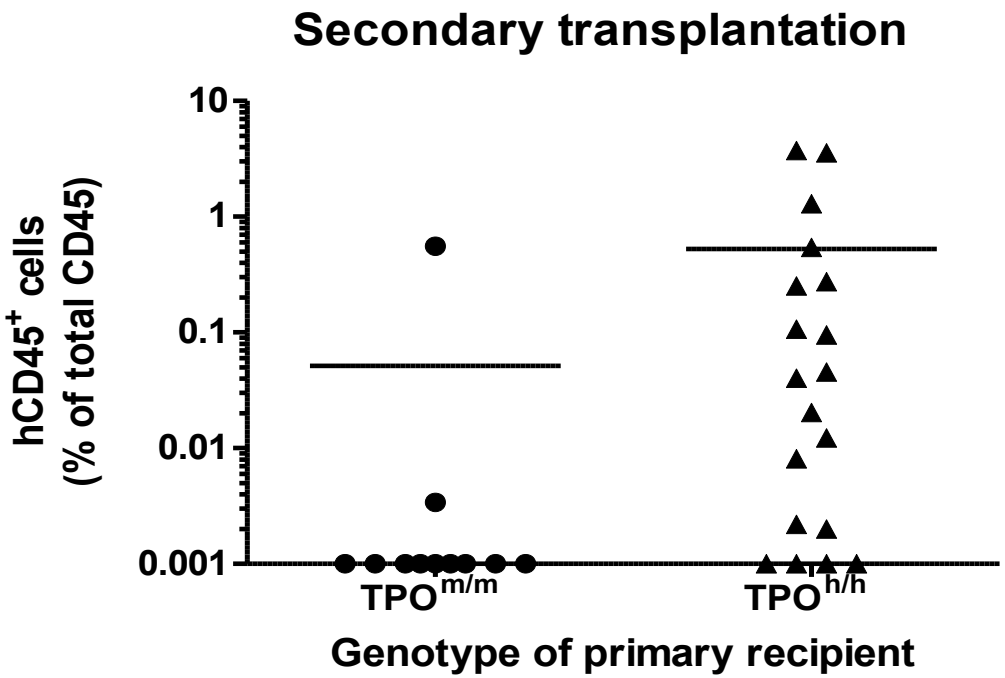
Nat Biotechnol 2003 v21, 652-9
BACvec, targeted replacement in ES cells



Transgenic expression of human TPO supports human HSCs

Human stem and progenitor cells: TPOm/m *vs.* TPOh/h





2011 Feb 8;108(6):2378-83 PNAS

Genetically Improved Humanized Mice

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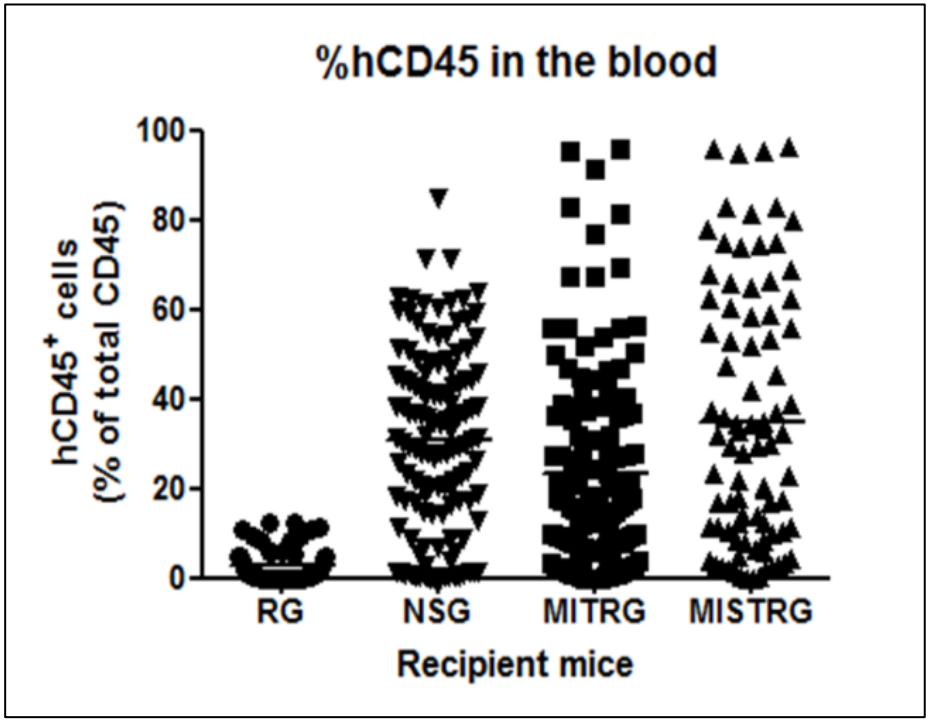
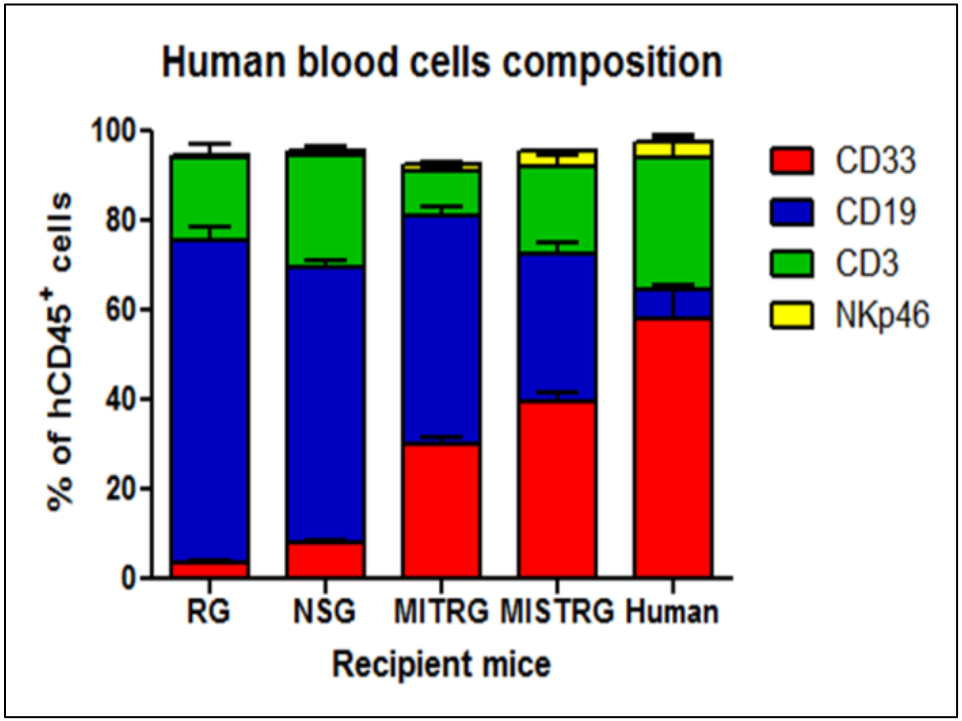
Human peripheral blood composition in MISTRG mice



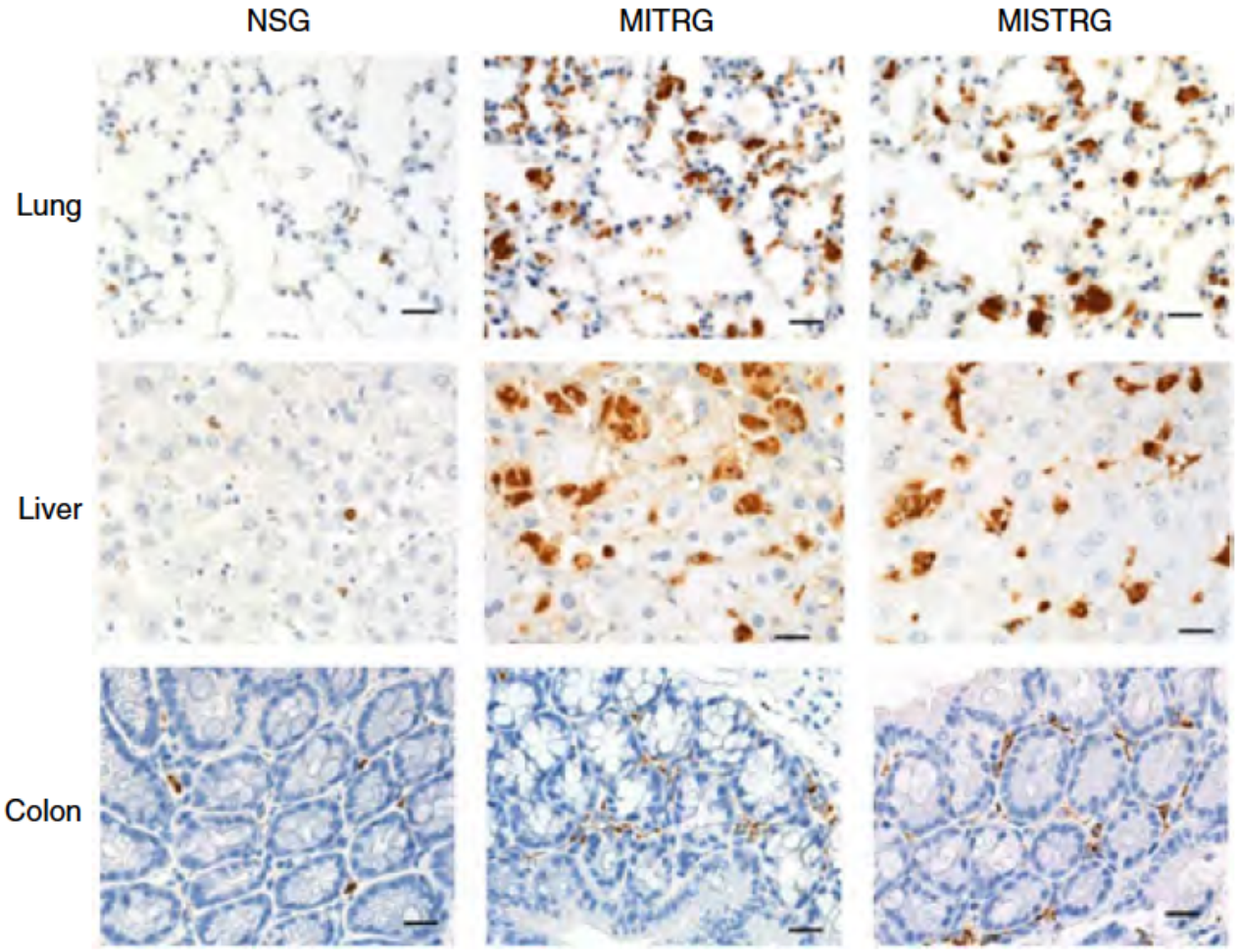


Peripheral blood chimerism week 8 post-transplantation of CD34+ cells into sublethally irradiated newborn animals

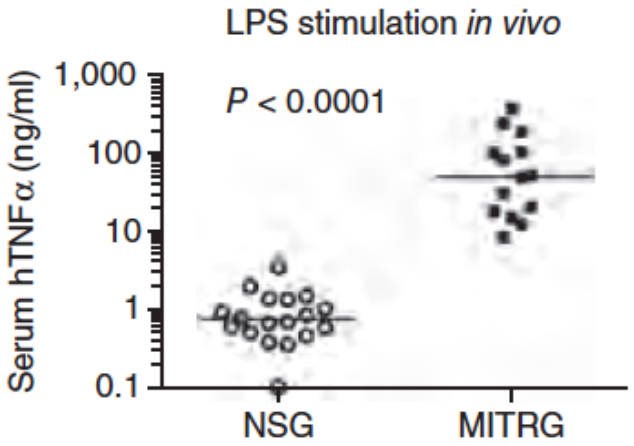
Rongvaux et al., 2014

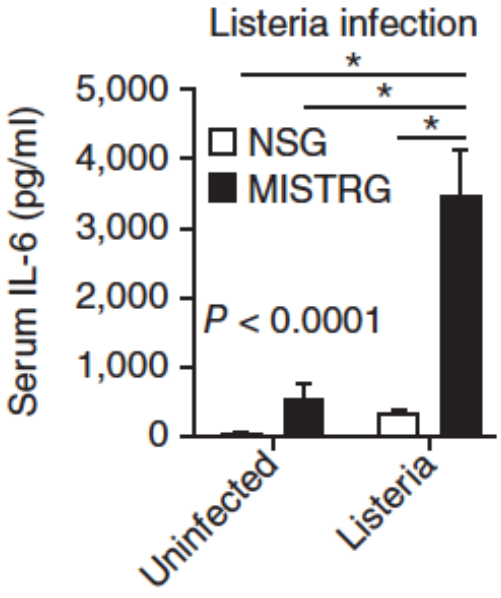
nature biotechnology

MISTRG mice support human macrophage development



Human CD68+ macrophages in tissues (week 8)



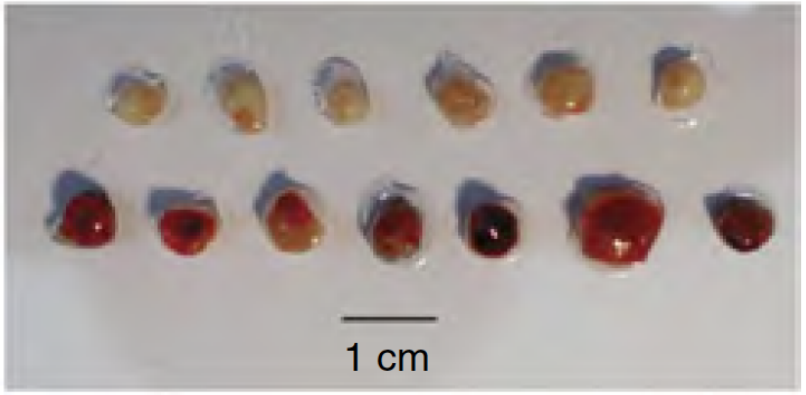


Rongvaux et al., 2014

nature biotechnology

Human macrophages in MISTRG mice infiltrate human melanoma

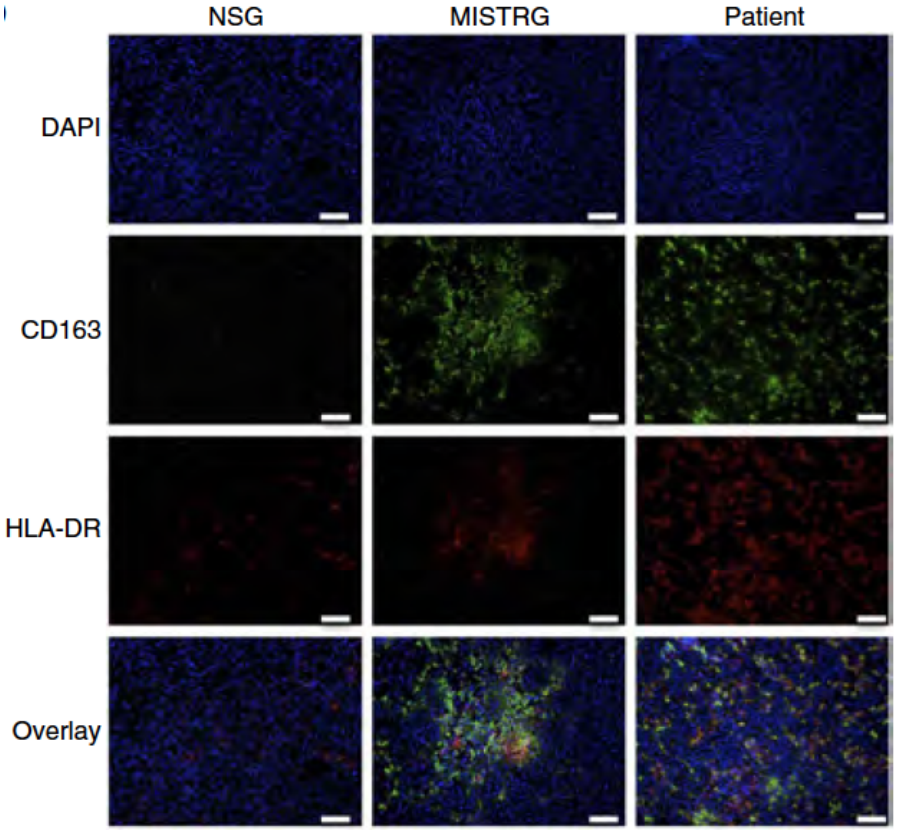
HuMelanoma Me290



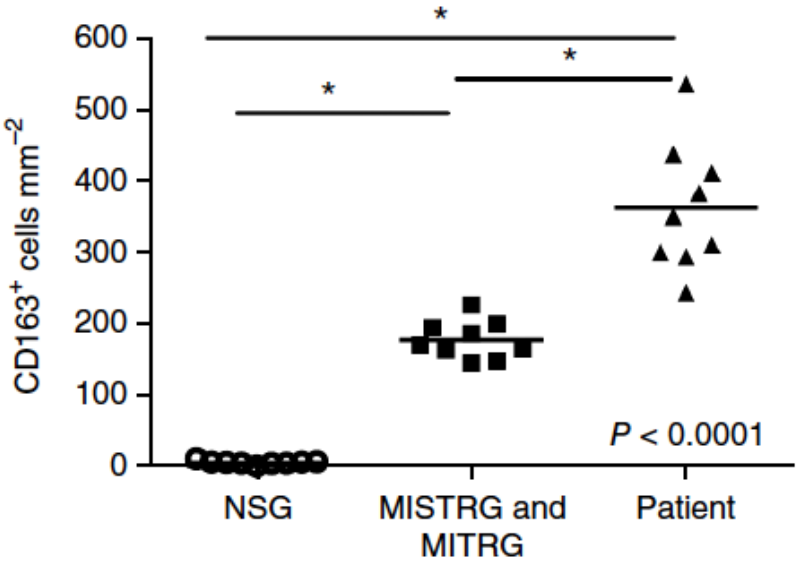
NSG

MISTRG and MITRG

HuMelanoma Me290 histology



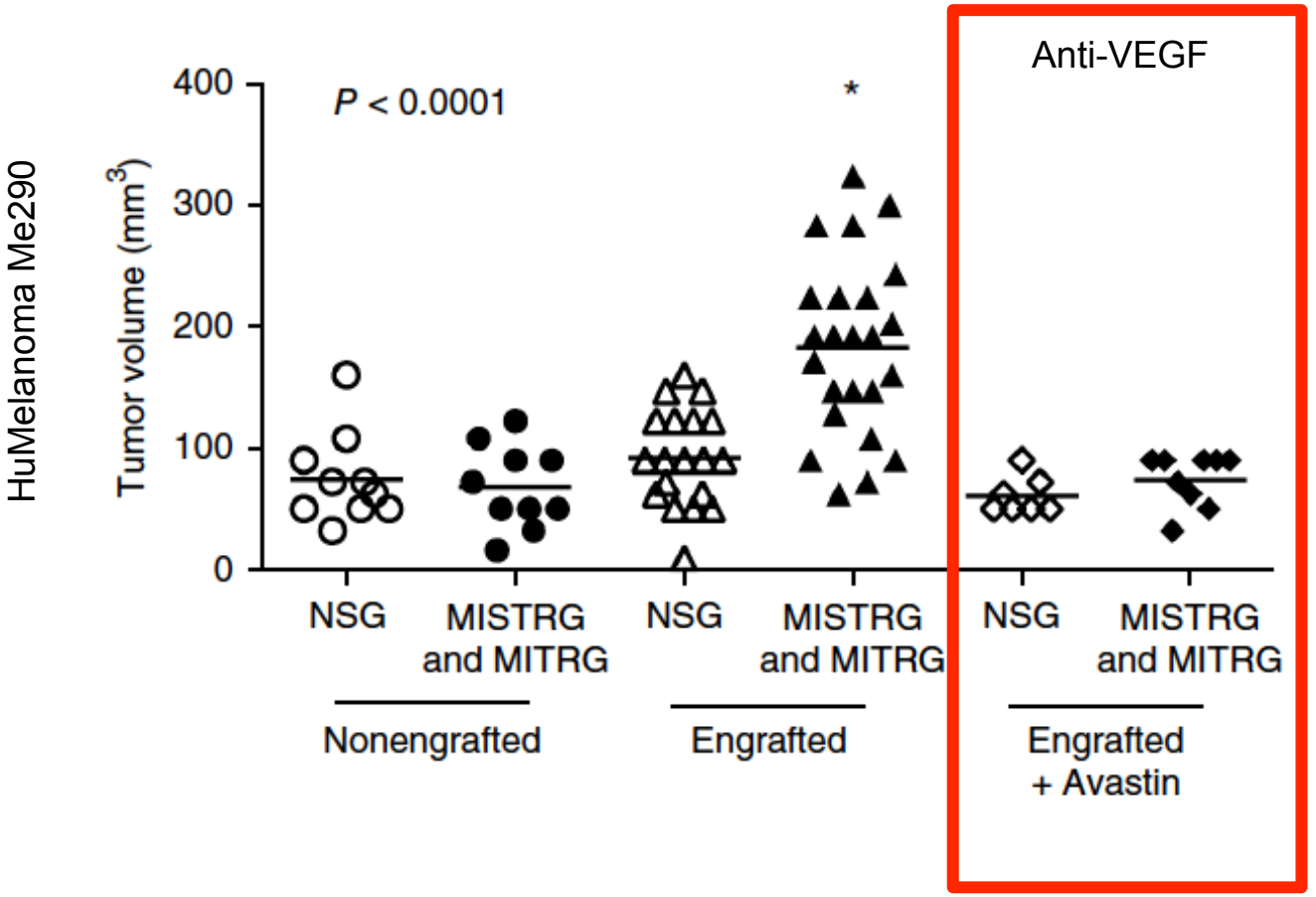
M2-like macrophages in tumors



Rongvaux et al., 2014

nature biotechnology

Human macrophages support human melanoma development in MISTRG mice via a VEGF-dependent pathway

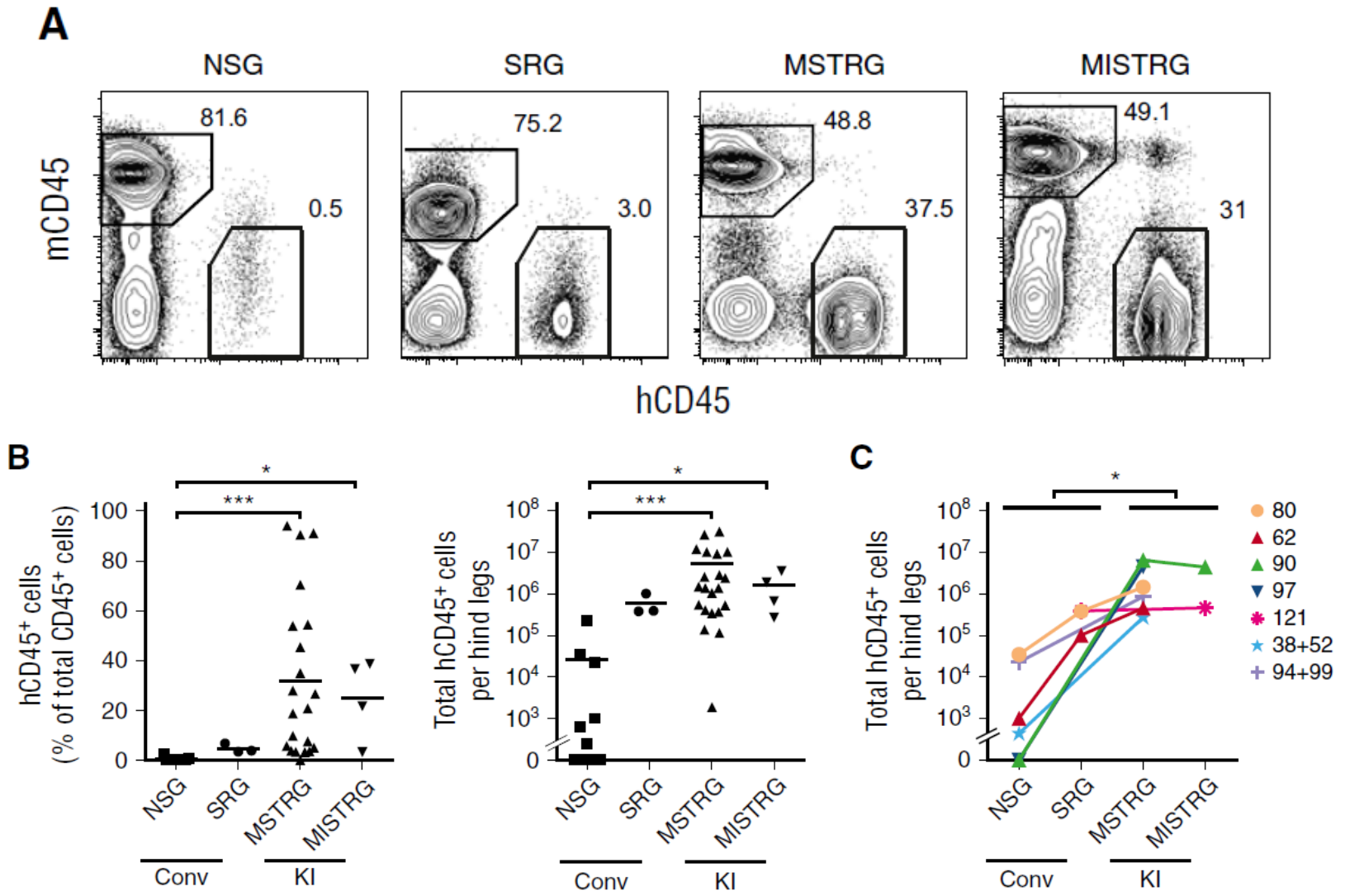


Rongvaux et al., 2014

nature biotechnology

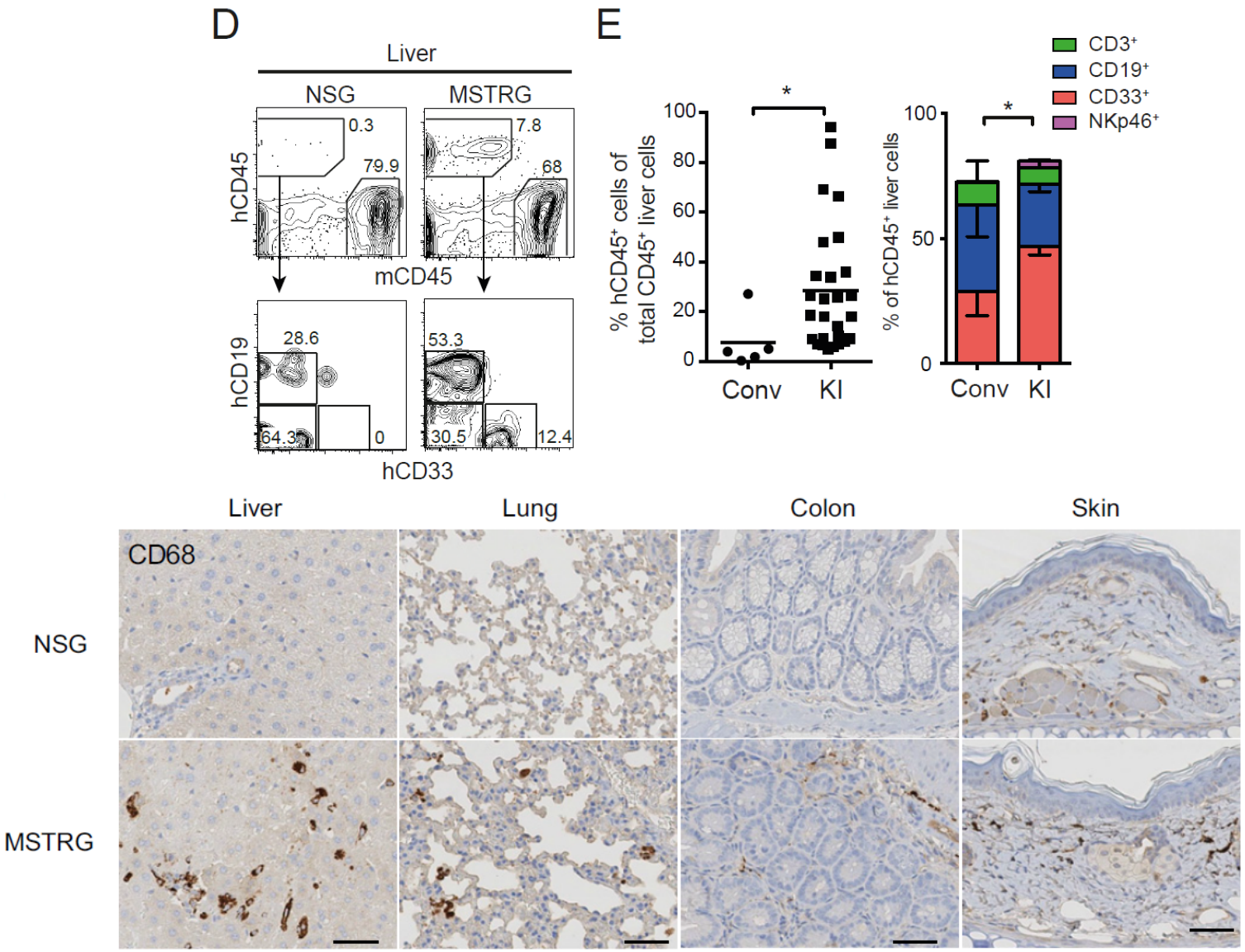
Increased engraftment of mobilized human peripheral blood CD34+ in MISTRG mice

300‘000-500‘000 **mobilized** human peripheral blood CD34+ cells / newborn mouse



Saito and Ellegast, Blood 2016

MISTRG mice promote myeloid differentiation of mobilized human PB CD34+



Saito and Ellegast et al., Blood 2016

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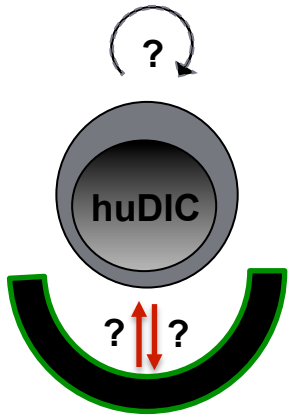
1. Introduction of next generation humanized “cytokine” mice
2. Normal hematopoiesis
3. **Malignant hematopoiesis**

Engraftment of human hematopoietic stem cell neoplasms in NSG/NOG mice

Lymphoid	**ALL**	**++++**
	MM	**+---**
Myeloid	**AML**	**++--**
	MDS	**+----**
	CML-CP	**+----**
	MPN	**+----**



Mouse DIC Niche → Improved human LIC Support?

---- : need for an improved humanized mouse model

Humanized mouse models for defined disease entities

e.g. TB
- hSIRPα
- hTPO
- hGM/IL-3
- hM-CSF
- hMHC

e.g. Malaria
- hSIRPα
- hTPO
- hEPO
- hLiver

e.g. HIV, EBV
- hSIRPα
- hTPO
- hIL-7
- hMHC

e.g. human lymphoid neoplasm Mouse
- hIL-7
- hIL-15
- hTSLP
- hBM-Stroma

e.g. human myeloid neoplasm Mouse
- MISTRG
- hEPO
- hIL-6
- and/or c-kit KO mouse
- hBM-Stroma

Theocharides et al., Haematologica 2016

Engraftment of human hematopoietic stem cell neoplasms in NSG/NOG mice

Lymphoid	ALL	++++
	MM	+---
Myeloid	AML	++--
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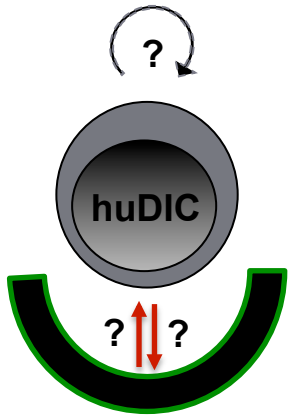

Mouse DIC Niche
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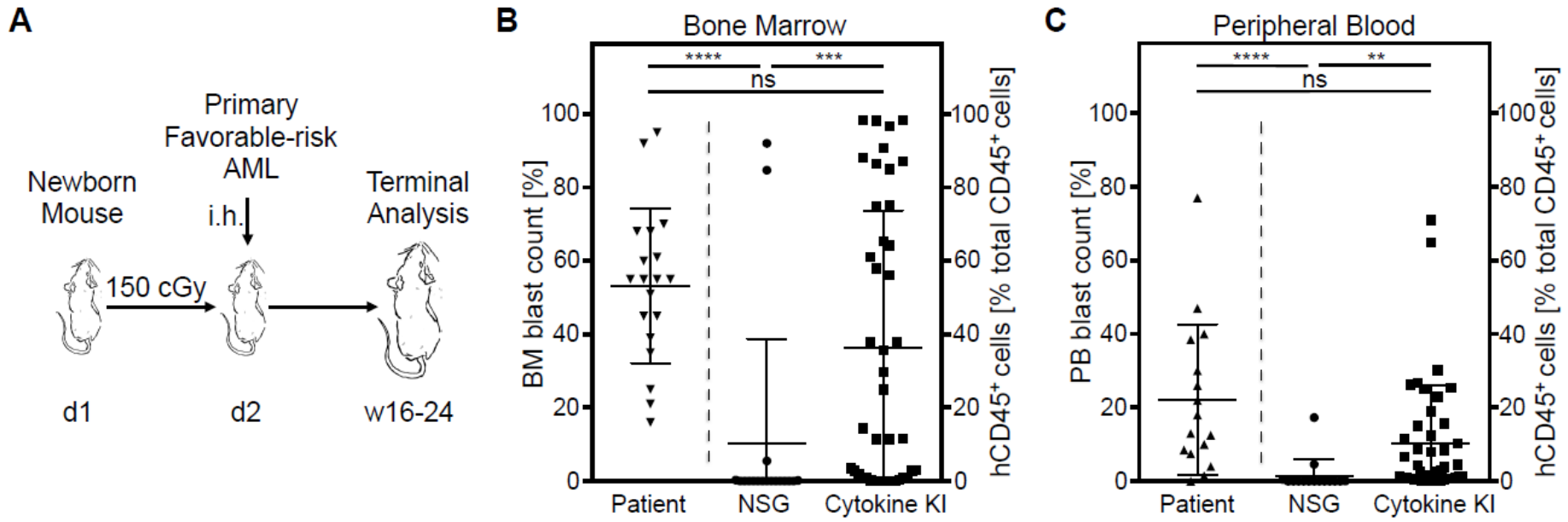
“Favorable-Risk” AML in MISTRG-Mice

- about 40-50% of AML engraft in NSG (J Dick and others)
- those AML have poorer clinical outcomes
- **How about the “favorable-risk” AMLs that do not or poorly engraft?**

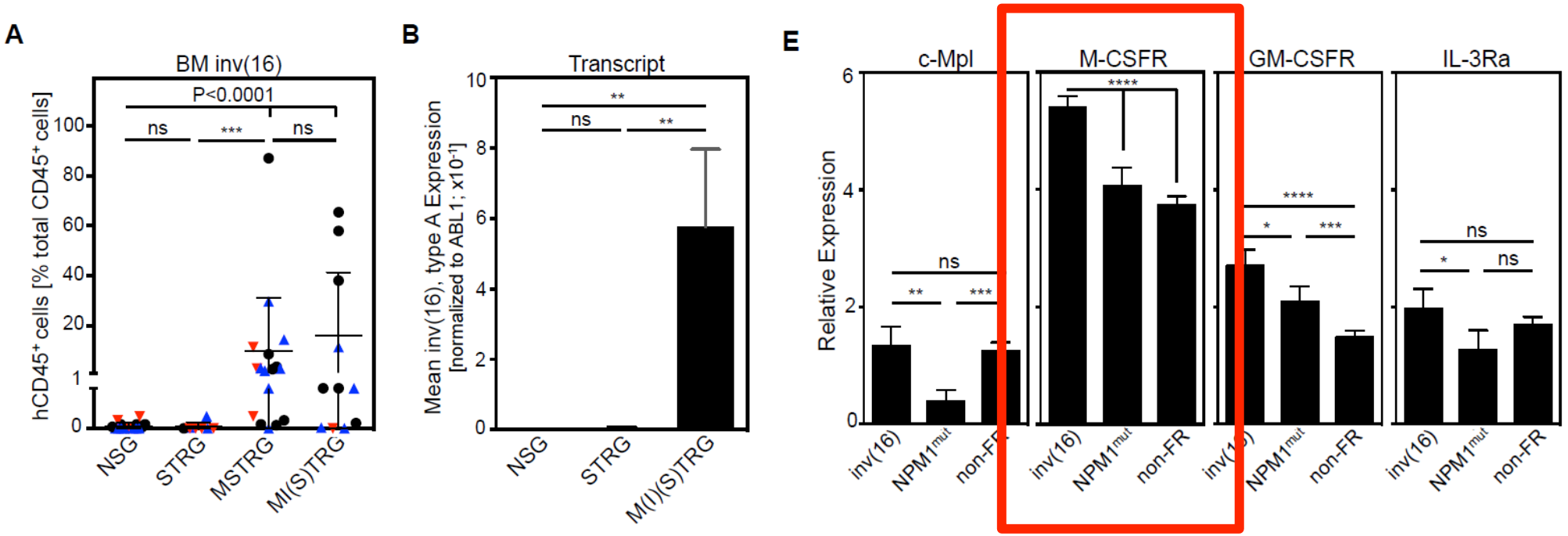
→ inv(16), CBF-MYH11 AML



Ellegast et al., Blood 2016

“Favorable-Risk” AML in MISTRG-Mice

→ inv(16)



- Humanized cytokine knock-in mice support engraftment of human favorable-risk AML, maintenance of clonal stability
- Engraftment and gene-enrichment analysis suggest M-CSF dependency of inv(16) AML
- Dependency of inv(16) AML on M-CSF might provide a basis for therapy optimization studies

Ellegast et al., Blood 2016

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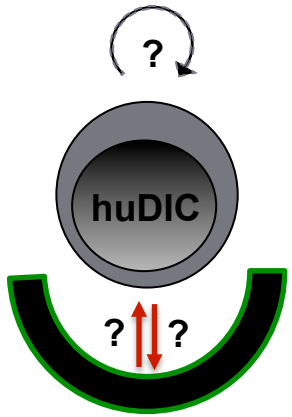

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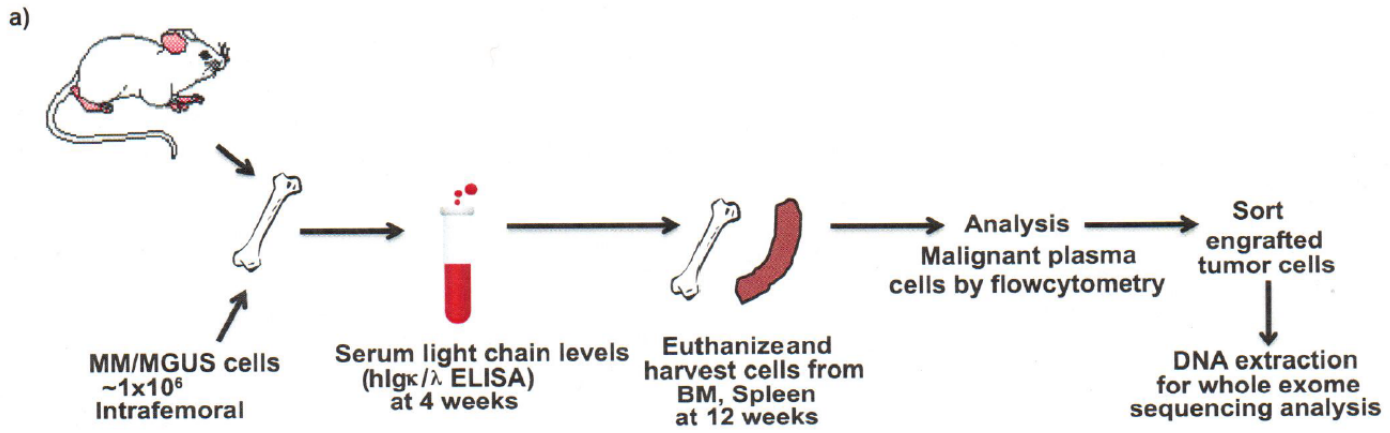
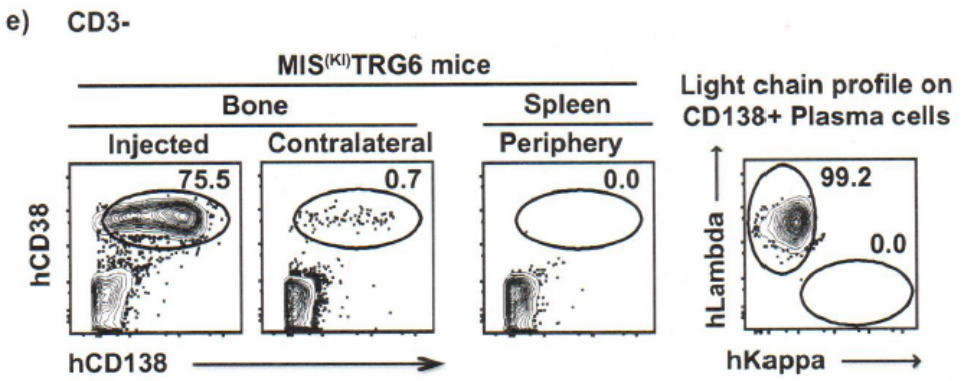
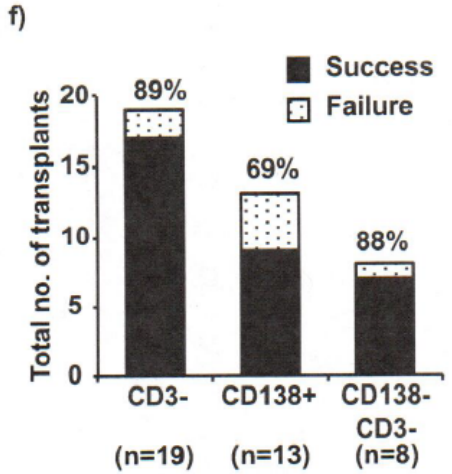
Monoclonal gammopathy and Multiple Myeloma in MISTRG-6 Mice







→ **High success of Myeloma and MGUS engraftment, maintenance of clonal diversity**

→ **New approach to investigate entire spectrum of plasma cell neoplasms**

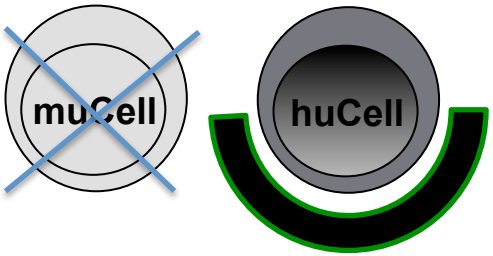
Das R et al., Nat Med 2016

Complementary Approaches

Kit Regulates HSC Engraftment across the Human-Mouse Species Barrier

Kadriye Nehir Cosgun,[1,9] Susann Rahmig,[1,9] Nicole Mende,[1] Sören Reinke,[1] Ilona Hauber,[2] Carola Schäfer,[2] Anke Petzold,[3] Henry Weisbach,[1,10] Gordon Heidkamp,[4] Ariawan Purbojo,[5] Robert Cesnjevar,[5] Alexander Platz,[6] Martin Bornhäuser,[7] Marc Schmitz,[3] Diana Dudziak,[4] Joachim Hauber,[2] Jörg Kirberg,[8] and Claudia Waskow[1,*]

Cell Stem Cell *15*, 227–238, August 7, 2014 ©2014 Elsevier Inc. 227

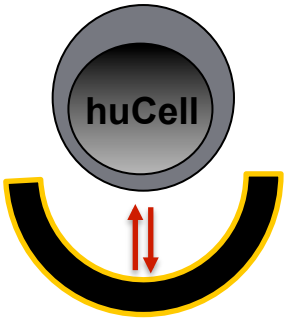


Creation of "HSC space" by genectic mouse HSC reduction
(Kit Muations)

Myelodysplastic Cells in Patients Reprogram Mesenchymal Stromal Cells to Establish a Transplantable Stem Cell Niche Disease Unit

Hind Medyouf,[1,11,13,*] Maximilian Mossner,[2] Johann-Christoph Jann,[2] Florian Nolte,[2] Simon Raffel,[3] Carl Herrmann,[4,5] Amelie Lier,[3] Christian Eisen,[3] Verena Nowak,[2] Bettina Zens,[1,3] Katja Müdder,[1,3] Corinna Klein,[1,3] Julia Obländer,[2] Stephanie Fey,[2] Jovita Vogler,[2] Alice Fabarius,[2] Eva Riedl,[6] Henning Roehl,[7] Alexander Kohlmann,[8] Marita Staller,[8] Claudia Haferlach,[8] Nadine Müller,[2] Thilo John,[9] Uwe Platzbecker,[10] Georgia Metzgeroth,[2] Wolf-Karsten Hofmann,[2] Andreas Trumpp,[1,3,11,12,*] and Daniel Nowak[2,12]

Cell Stem Cell *14*, 824–837, June 5, 2014 ©2014 Elsevier Inc.



Fully human/artificial niche?

Engineering of a functional bone organ through endochondral ossification

Celeste Scotti[a,1], Elia Piccinini[a,1], Hitoshi Takizawa[b,1], Atanas Todorov[a], Paul Bourgine[a], Adam Papadimitropoulos[a], Andrea Barbero[a], Markus G. Manz[b], and Ivan Martin[a,2]

PNAS | March 5, 2013 | vol. 110 | no. 10 | 3997–4002

Summary

- **Humanized mice are continuously improved to support “full” human hematopoietic development**

  Main remaining challenges

  – HSC expansion/maintenance
  – appropriate adaptive immune responses
  – red blood cell and platelet development

- **Humanized mice -as already available- have proven value for studying**
  – Normal hematopoietic development
  – Biology and therapy of human specific lymphotropic viral infections
  – Biology and therapy of human hematopoietic malignancies

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REGENERON

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Till Strowig
Jon Alderman
Madhav Dhodapkar
Rituparna Das

Markus G. Manz

Alexandre Theocharides
Veronika Lysenko
Hitoshi Takizawa
Chiara Borsotti
Rouven Müller
Yasuyuki Saito
Jana Ellegast
Steffen Böttcher

Sean Stevens
Cagan Gurer
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