Studying Antitumoral Immunity in the New Generation of Humanized Mice

Richard A. Flavell
Yale University School of Medicine

October 2018
The evolution of humanized mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bone marrow</th>
<th>Blood</th>
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<tbody>
<tr>
<td>Human</td>
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<tr>
<td>BRG</td>
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<td>NOG/NSG/SRG</td>
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<tr>
<td>BLT</td>
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</tbody>
</table>

- **CD34**
- **T cells**
- **Myeloid**
- **B cells**
- **Mouse**
The tumor microenvironment

Tumor = cancer cells + other cell types (up to 50% of the tumor volume)

What is the role of immune cells in the tumor microenvironment?

In particular: what is the role of myeloid cells?
Generation of humanized mice

Human hematopoietic stem cells (CD34+ cells)
(cord blood, fetal liver, adult HSCs, in the future: iPSC-derived cells)

Transplantation into immunocompromised mice

8-12 weeks Hematopoietic development and immune function
Cytokines supporting myeloiesis

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

TPO KI: Rongvaux et al, PNAS, 2011
Genetic humanization of cytokine-encoding genes

Velocigene technology
In collaboration with Regeneron Pharmaceuticals

Willinger et al, Trends Immunol., 2011
Combination of multiple humanized alleles

- M-CSF<sup>h/h</sup>
- IL-3/GM-CSF<sup>h/h</sup>
- hSirpa<sup>tg</sup>
- TPO<sup>h/h</sup>
- RAG2<sup>-/-</sup>
- IL2R<sup>Gamma</sup>-/-

### Myeloid development

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

**4 groups of mice compared:**
- RG
- NSG* (∼ SRG)
- MITRG
- MISTRG

* NSG = NOD Scid IL2Rγ<sup>-/-</sup>

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Rongvaux et al., Nat. Biotech., 2014
MI(S)TRG mice are highly permissive for human hematopoiesis

Bone marrow

Blood

p-value: One-way ANOVA

19 independent fetal liver samples
n= 56-155 mice/group
7-9 weeks post-transplantation
Increased frequency myeloid cells in the blood of MISTRG

Myeloid cells (hCD33+) in the blood

P<0.0001

Human WBC composition

n = 67-122 (mice)

n = 8 (human)
Increased density of myeloid cells in tissues of MISTRG

hCD68 staining (Myeloid cell marker)

NSG | MISTRG
---|---
Lung
Liver
Colon

---

20 μm
Three subsets of monocytes in MISTRG

Three subsets of monocytes are defined in humans

<table>
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<tr>
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<th>CD14^{dim}CD16⁺</th>
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<tr>
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<tr>
<td>Response to viruses</td>
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<td>+</td>
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</table>

Human blood
_(gated on myeloid CD33⁺ cells)_
Three subsets of monocytes in MISTRG

Three subsets of monocytes are defined in humans:

<table>
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<tr>
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<th>CD14^+CD16^-</th>
<th>CD14^+CD16^+</th>
<th>CD14^{dim}CD16^+</th>
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<tbody>
<tr>
<td>Phagocytosis</td>
<td>+++</td>
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<tr>
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<td>+++</td>
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<td>+</td>
</tr>
<tr>
<td>Response to viruses</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Monocytes subsets from human and from MISTRG are comparable based on the expression of specific markers (CD11b, CD115, CD62L, CX3CR1)

Relative distribution of monocyte subsets (among hCD33^+ monocytic cells)

Phagocytosis

Response to LPS

Response to viruses
Systemic hIL6 production 48 h after Listeria monocytogenes infection

Serum cytokines (2 days post Listeria infection)

- NSG
- MISTRG

$p = 0.0002$
MISTRG Mesenchymal Stromal Cells Express Human M-CSF, GM-CSF, and TPO at physiologic levels

MSC (Mesenchymal Stromal Cells): Patient-specific MSC co-transplanted with patient MDS CD34+ cells improve engraftment. (Medyouf et al., Cancer Cell 2014 (14): 824-837)
### Summary #1

#### MISTRG:
- Synergistic effect of multiple gene humanizations
- Highly permissive for human hematopoiesis
- Support myeloid development
- Functional innate immune responses against pathogens

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<tr>
<td>Human</td>
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<td>NOD/SCID</td>
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<tr>
<td>MISTRG</td>
<td><img src="chart11" alt="Chart" /></td>
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</tbody>
</table>

- **CD34**
- **T cells**
- **Myeloid**
- **B cells**
- **Mouse**
Hypothesis

Human cells → Cytokines → Other human cell types

Myeloid cells → IL-15 → NK cells
Increased human IL-15/IL-15Rα expression in MISTRG mice

Increased expression of IL-15/IL-15Rα in MI(S)TRG... particularly in CD14⁺CD16⁺ monocytes
MISTRG mice support the development of human NK cells

These cells express NK cell-specific markers (CD16, perforin, CD94, CD161, KIRs) → *Bona fide* human NK cells
Summary #2

Mouse cytokines

- hTPO
- hIL-3
- hGM-CSF
- hM-CSF

Human Monocytes/macrophages

- IL-15/IL-15Rα

Human NK cells

Mouse cells

Mouse cytokines
Tissue-resident T cells, NK cells & cancer immunotherapy in SRG-15 humanized mice

Dietmar Herndler-Brandstetter
Liang Shan
Cagan Gurer
Targeted genomic replacement of mouse \( \text{IL15} \)

\[ \text{Sirpa}^{h/m} \]
\[ \text{Rag2}^{-/-} \]
\[ \text{Il2rg}^{-/-} \]
\[ \text{Il15}^{h/m} \]

IL-15 protein identity (human vs. mouse): 73%

Valenzuela et al., Nat Biotechnol 2003

VelociGene® technology (Regeneron Pharmaceuticals)
Human intraepithelial lymphocytes (IELs) develop in the small intestine of SRG-15 mice

Denton et al., *Mucosal Immunol* 2012

Herndler-Brandstetter, Shan et al., (2017) *PNAS*
Human CD8\(^+\) IELs express CD8\(\alpha\alpha\) and the tissue-resident markers CD69 and CD103.
SRG-15 mice support human NK cell development in multiple tissues

14 weeks p.e.
Efficient development of cytotoxic CD56\textsuperscript{dim} CD16\textsuperscript{+} human NK cells in SRG-15 mice

Spleen

Blood

16 weeks p.e.

7 weeks p.e.
viSNE plots showing high-dimensional cytometry data of NK cells acquired by time-of-flight (CyTOF)

hCD56\text{bright}^{\text{HR5}} \quad \text{hCD16}^{-}

SRG-15
(n=9)

Human
(n=20)

hCD56\text{dim}^{\text{HR5}} \quad \text{hCD16}^{+}

SRG-15
(n=9)

Human
(n=20)

8 of 33 parameters are shown
ViSNE plots showing analogy of NK cell subsets between SRG-15 mice and humans
Human NK cells in SRG-15 mice kill MHC-I-deficient tumor cells \textit{in vivo}

Raji cells (MHC-I sufficient)  
K562 cells (MHC-I deficient)  
1:1

Collect splenocytes

0h  
24h

Specific lysis (K562 : Raji)

SRG-15 N/E  
SRG  
SRG-15

N/E: not engrafted with hCD34$^+$ cells
NK cell-based cancer immunotherapy in SRG-15 mice

Rituximab: mechanism of action

ADCC: Antibody-dependent cellular cytotoxicity

CD16 (FcγRIII)

NK cell-based cancer immunotherapy in SRG-15 mice

Raji cells s.c. (Luciferase\(^+\))

Rituximab (RTX; anti-human CD20 mAb)

0 7 10-14 26-30 days

+D-Luciferin i.p.

Analysis by IVIS
Human NK cells in SRG-15 mice inhibit tumor growth following treatment with Rituximab
Summary & Perspectives

- Targeted insertion of human *Sirpa* and *Il15* improves the development, maturation and function of human NK cells

- Tissue-resident human CD8 T cells develop in the small intestine of SRG-15 mice

- Human NK cells in SRG-15 mice infiltrate human tumor xenografts and inhibit tumor growth following cancer immunotherapy with Rituximab

- SRG-15 mice may serve as an *in vivo* platform for the preclinical screening of novel candidates for cancer immunotherapy
Patient-derived humanized mice to study antitumoral immune responses

In collaboration with:

Karolina Palucka
Jan Martinek

1. Engrafted MISTRG mouse (CD34+ cells)
   (intra-hepatic injection in irradiated newborns)

2. Human melanoma s.q.
Model of human tumor/macrophage interaction \textit{in vivo}

\textit{In collaboration with Dr. Karolina Palucka and Jan Martinek (Baylor University)}

1. Engrafted mouse
\textit{(intra-hepatic injection in irradiated newborns)}

2. Human melanoma cell line (Me290) s.q.

- Do human myeloid cells infiltrate the tumor?
- Do they affect tumor growth?
- By which mechanisms?
Macrophages support tumor growth

Macrophages promote tumorigenesis

→ Strong correlation between high density of macrophages infiltrating the tumor and poor patient prognosis, in several types of cancer

Human melanoma

CD163: Human macrophages
Human myeloid cells infiltrate the tumor in MISTRG

CD163: human macrophages

**Density of CD163+ cells**

- NSG
- MISTRG
- Patient

- DAPI
- CD163
- HLA-DR
- Overlay

**p < 0.0001**
Tumors promote M2 macrophage differentiation

The M1/M2 paradigm

- **M1 Macrophage**
  - TNF-α
  - IL-12
  - Promote Th1 response

- **M2 Macrophage**
  - IL-1β
  - Growth factors, ex. VEGF

<table>
<thead>
<tr>
<th>Acute infection</th>
<th>Tumor</th>
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<tr>
<td>Pro-inflammatory</td>
<td>Anti-tumoral</td>
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<tr>
<td>Anti-inflammatory</td>
<td>Tumor-support</td>
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<tr>
<td>Tissue-repair/remodeling</td>
<td>Vascularization</td>
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</table>

➔ Tumors promote the differentiation of “M2-like” for their own advantage
M2-like phenotype of tumor-infiltrating macrophages

MISTRG

Patient

CD163: Human macrophages
CD206: M2 marker
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
Increased vascularization of tumors in MISTRG

mCD31 staining
Mouse endothelial cells
3 mice/group shown

**NSG**
No human myeloid infiltrate

**MISTRG**
Human myeloid infiltrate
Human hematopoietic cells in MISTRG support tumor vascularization and growth

Non-engrafted mice
(i.e., no hCD34+ cell transplantation)

hCD34+ cells-engrafted mice
Tumor growth in MISTRG requires active VEGF

One-way ANOVA p<0.0001
*** p<0.05 vs. all other group
(Tukey post-hoc test)
Summary #3

- MISTRG recapitulates the infiltration of the tumor by myeloid cells

- Support vascularization and tumor growth

- Relevant for drug testing (the response to drug is affected by the presence immune cells)
Human hematopoietic cells in MISTRG support tumor metastasis

Non-engrafted MISTRG + sq melanoma

Engrafted MISTRG + sq melanoma

Liver metastasis:
- 0/6 mice
- 2/4 mice

Spleen metastasis:
- 0/6 mice
- 4/4 mice
Patient-derived humanized MISTRG mice

In the context of cancer:

1. Fetal liver-derived hematopoietic cells + tumor cell line *(allogeneic – current model)*

2. Fetal liver-derived hematopoietic cells + primary tumor *(allogeneic)*

3. Patient-derived hematopoietic cells + primary tumor from same patient *(autologous)*

4. Patient-derived hematopoietic cells + primary tumor + effector T cells from same patient *(autologous)*
Autologous Humanized Mouse Modeling

- NSCLC (here at Yale)
- Melanoma patients from vaccine trial with Karolina Palucka’s lab (Jackson Labs)
- Melanoma and pancreatic cancer patients with Ryan Fields’ lab (WashU)
Humanization of IL-6 locus enhances engraftment in MISTRG-6

- M-CSF$^h/h$ – support human myeloid development
- IL-3 / GM-CSF$^h/h$ – support human myeloid development
- hSirpα transgene – phagocytic tolerance
- TPO$^h/h$ – supportive HSC niche
- Rag2$^{-/-}$ – immunodeficient mouse background
- IL-2Rγ$^{-/-}$ – immunodeficient mouse background
- IL-6 – improved engraftment

Rongvaux, Willinger et al., Nat. Biotech., 2014
Das et al., Nat. Med., 2016

Enhanced engraftment of hCD45$^+$ cells in peripheral blood of MISTRG-6
Autologous studies in melanoma -- engraftment

Engraftment of autologous hCD34+ cells from melanoma patients. 200,000 - 250,000 hCD34+ cells were engrafted into newborn MISTRG-6 mice after 150 rads of irradiation. Peripheral blood engraftment (fraction of hCD45+ cells per total mCD45+ plus hCD45+ cells) was assayed at week 7.

Michael Chiorazzi (YCC) with Jan Martinek, Karolina Palucka’s lab (JAX)
Tumor growth is enhanced in autologous humanized mice compared with non-engrafted
Spontaneous metastases and associated immune infiltrate detected in autologous humanized mice

<table>
<thead>
<tr>
<th>Mouse #</th>
<th>Engr?</th>
<th>Liver</th>
<th>Spleen</th>
<th>LN</th>
<th>Kidney</th>
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</table>

Table summary of numbers of observed autologous metastases in various organs of autologously-engrafted MISTRG-6 mice. H&E staining of mouse liver showing metastatic deposits.
Human immune infiltrate in autologous humanized mice

Autologous hematopoietic cells infiltrate MISTRG-6-grown melanoma PDX tumors.

Green: Pmel, Mart1
Red: hCD45

Michael Chiorazzi (YCC) with Jan Martinek, Karolina Palucka’s lab (JAX)
Investigating anti-PD1 and anti-VEGF mechanisms in autologous humanized mice

In process: scRNAseq to characterize identities of cells infiltrating and transcriptional states

Michael Chiorazzi (YCC) with Jan Martinek, Karolina Palucka’s lab, JAX
HIC #1603017380 – Collecting NSCLC samples and autologous bone marrow aspirate

• Consent patients with NSCLC scheduled for surgical resection for collection of tumor tissue, bone marrow aspirate, peripheral blood at the time of surgery

• Use these materials to produce:
  – Patient-derived xenograft lines from primary tissue
  – CD34+ HSCs from bone marrow aspirate
    • Engraft both into MISTRG-6 mice to investigate tumor/immune system interaction, drug sensitivity, immune infiltrate
HIC #1603017380 – Collecting NSCLC samples and autologous bone marrow aspirate

NSCLC patients undergoing resection

Tumor sample
- Tumor DNA (KRAS testing), baseline IHC
- Implant into F1 mice
  - Monitor for tumor growth
    - Passage to F2 mice
      - Cryopreserve PDX lines

Bone marrow aspirate
- Isolate CD34+ cells
  - Cryopreserve

Peripheral blood
- Isolate PBMCs

Transfer to recipient MISTRG mice engrafted with same patient’s HSCs, PBMCs

Assay for tumor growth, immune infiltration, drug sensitivity

Michael Chiorazzi
HIC #1603017380 – Patients Enrolled and BM aspirate collected

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
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<td>&lt;100k</td>
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<td>Stage IA squamous cell carcinoma</td>
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<td>56</td>
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<td>Stage IIB large cell carcinoma</td>
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<td>Adenosquamous carcinoma</td>
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<td>70</td>
<td>M</td>
<td>Stage IIB adenocarcinoma</td>
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<tr>
<td>8</td>
<td>62</td>
<td>F</td>
<td>Stage IA adenocarcinoma</td>
<td>6 million</td>
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<td>9</td>
<td>79</td>
<td>M</td>
<td>Stage IIA squamous cell carcinoma</td>
<td>3.7 million</td>
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<td>17</td>
<td>74</td>
<td>M</td>
<td>Stage IV adenocarcinoma</td>
<td>3.4 million</td>
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</tbody>
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* PDX established

Michael Chiorazzi
HIC #1603017380 – Successful PDX growth

Patient 3
- Tumor volume over time for Patient 3 with markers for different days.

Patient 8
- Tumor volume over time for Patient 8 with markers for different days.

Patient 9
- Tumor volume over time for Patient 9 with markers for different days.

Autologous Engraftment
- Graph showing % hCD45+ markers for different cell types.

NSCLC-8 Pilot
- Graph comparing tumor volume for non-engrafted and engrafted conditions.

Michael Chiorazzi
Genomic and neoantigen studies in melanoma and pancreatic adenocarcinoma

Ryan Fields Lab – Washington University, St. Louis
Karolina Palucka lab – Jackson Laboratories
Flavell lab – Yale University
Genomic and neoantigen studies in melanoma and pancreatic adenocarcinoma
The future of humanized mice in cancer immunotherapy development

- Understand the fundamental mechanisms of human innate immunity in cancer
- Patient-specific humanized mice
- Generate novel humanized mice with longer lifespan
- Generate novel humanized mice with improved adaptive immunity
- Understand the fundamental mechanisms of antitumoral adaptive immunity
- Develop and test in vivo novel candidate immunotherapies
Michael Chiorazzi
Thanks

- Michael Chiorazzi
- Yunjiang Zheng
- Dietmar Herndler-Branstetter
- Liang Shan
- Anthony Rongvaux

- Karolina Palucka, JAX
- Jan Martinek, JAX
- Florentina Marches, JAX

- Ryan Fields, WashU
- Bradley Krasnick, WashU

- Carla Weibel
- Scott Gettinger
- Katie Politi
- Dan Boffa
- Frank Detterbeck
- Justin Blasberg
- Heather Lazowski
- Cindy Bensley
- Ed Kaftan
- Roy Herbst
MISTRG-6

- M-CSF<sup>h/h</sup> – support human myeloid development
- IL3 / GM-CSF<sup>h/h</sup> – support human myeloid development
- hSirpα transgene – phagocytic tolerance
- TPO<sup>h/h</sup> – supportive HSC niche
- IL-6<sup>h/h</sup> – supportive HSC niche, enhanced engraftment
- Rag2<sup>-/-</sup> – immunodeficient mouse background
- IL2Rγ<sup>-/-</sup> – immunodeficient mouse background

1A. Human WBC composition

1B. Human hematopoietic engraftment

Rongvaux, Willinger et al., Nat. Biotech., 2014
Yunjiang Zheng, unpublished data
Limited reconstitution of adult CD34 cells in current humanized mouse models


100,000 to 500,000 adult mobilized CD34 cells per mice 10-16 weeks post engraftment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Obtained CD34⁺ cells</th>
<th>CD34⁺ cells/mouse</th>
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<tr>
<td>CP18</td>
<td>7,000,000</td>
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<td>3,500,000</td>
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<td>CP36</td>
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<td>90,000</td>
</tr>
<tr>
<td>CP38</td>
<td>1,200,000</td>
<td>100,000</td>
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</table>

Liang Shan
Enhanced engraftment of hCD45$^+$ cells in peripheral blood (7 weeks post engraftment)

- 100,000 fetal liver CD34+ cells
- 150 rads irradiation

Yunjiang Zheng
Robust reconstitution of human CD45+ cells in MISTRG-6

Yunjiang Zheng
Representation of human hematopoietic lineages in periphery blood of engrafted MISTRG-6
Engraftment of adult CD34+ cells in MISTRG-6 peripheral blood

Engrafted with 50,000 adult mobilized CD34+ cells

Engrafted with 100,000 adult bone marrow aspirate CD34+ cells
Enhanced engraftment of hCD45\(^+\) cells in \textit{peripheral blood} of MISTRG-6 (5-7 weeks post engraftment)

Mice are engrafted with 100,000 fetal liver, 50,000 cord blood, 100,000-180,000 adult mobilized, or 120,000 adult BM CD34+ cells, with 150 Rads irradiation.