

Humanized mice to study cancer-immune system interface

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SITC Cancer Immunotherapy Winter School
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Presenter Disclosure Information

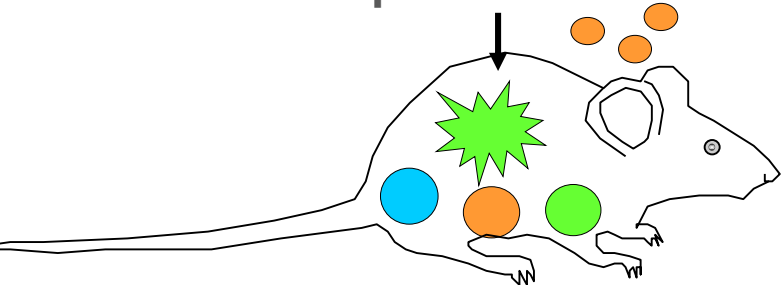
Karolina Palucka, MD, PhD

The following relationships exist related to this presentation:

Merck : Consulting, grant support

Using humanized mice to study basic immunology

Adoptive T cell transfer



**NOD-SCID $\beta 2m^{-/-}$
mouse
Adult $CD34^{+}$ HPCs**

Palucka et al., Blood 2003

Yu et al., Blood 2008

Yu et al., Immunity 2013

Yu et al., JI 2014

Graham et al., Vaccine 2016

Aymeric, Yu et al., Science Immunology 2017

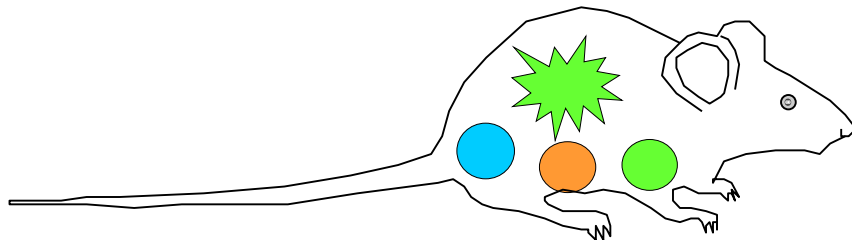
Aspord C, J Exp Med 2007

Pedroza-Gonzalez, J Exp Med 2011

Wu et al, Cancer Immunol Res 2013

Wu et al, Cancer Res 2018

Endogenous T cells



**NOD-SCID $\gamma c^{-/-}$ or $Rag2^{-/-}\gamma c^{-/-}$
mouse
Fetal/cord blood $CD34^{+}$ HPCs**

MISTRG: Human M-CSF/IL-3,

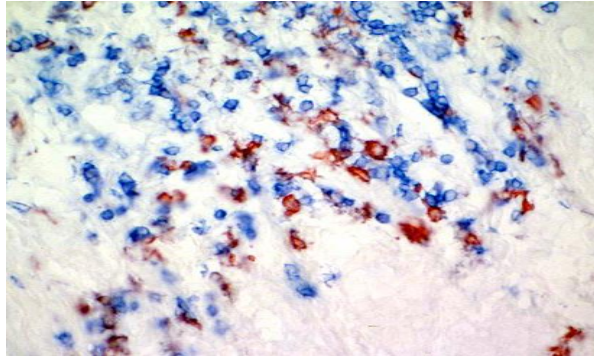
GM-CSF/SCF/SIRPa/TPO

NSG and NSG-SGM3:SCF/GM-CSF, IL-3

Rongvaux et al, Nat Biotech 2014

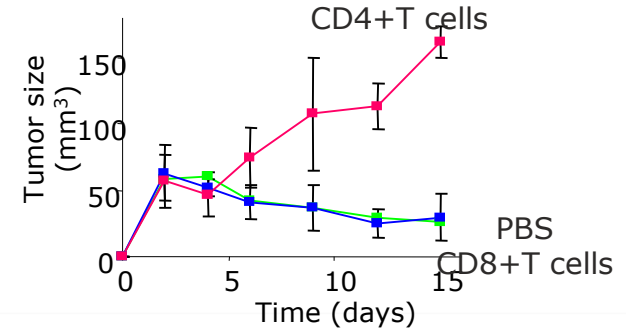
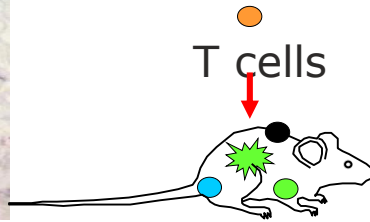
Wang et al FASEB J 2018

Breast cancer subverts dendritic cell maturation to induce Th2 cells promoting cancer progression



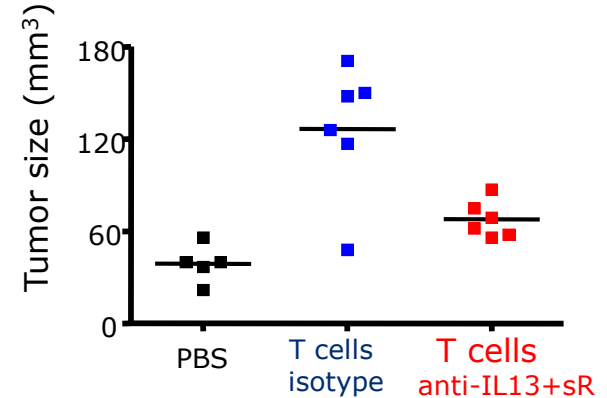
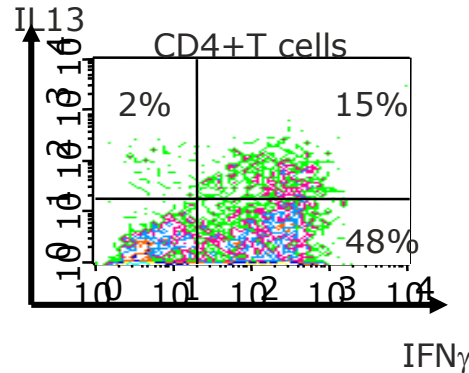
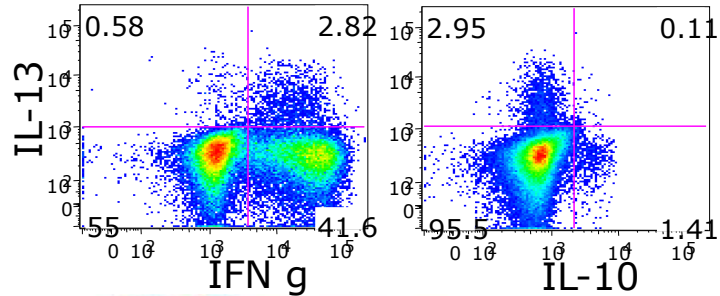
Bell et al, J Exp Med 1999

From patients to 1st generation Onco-Humice back to patients

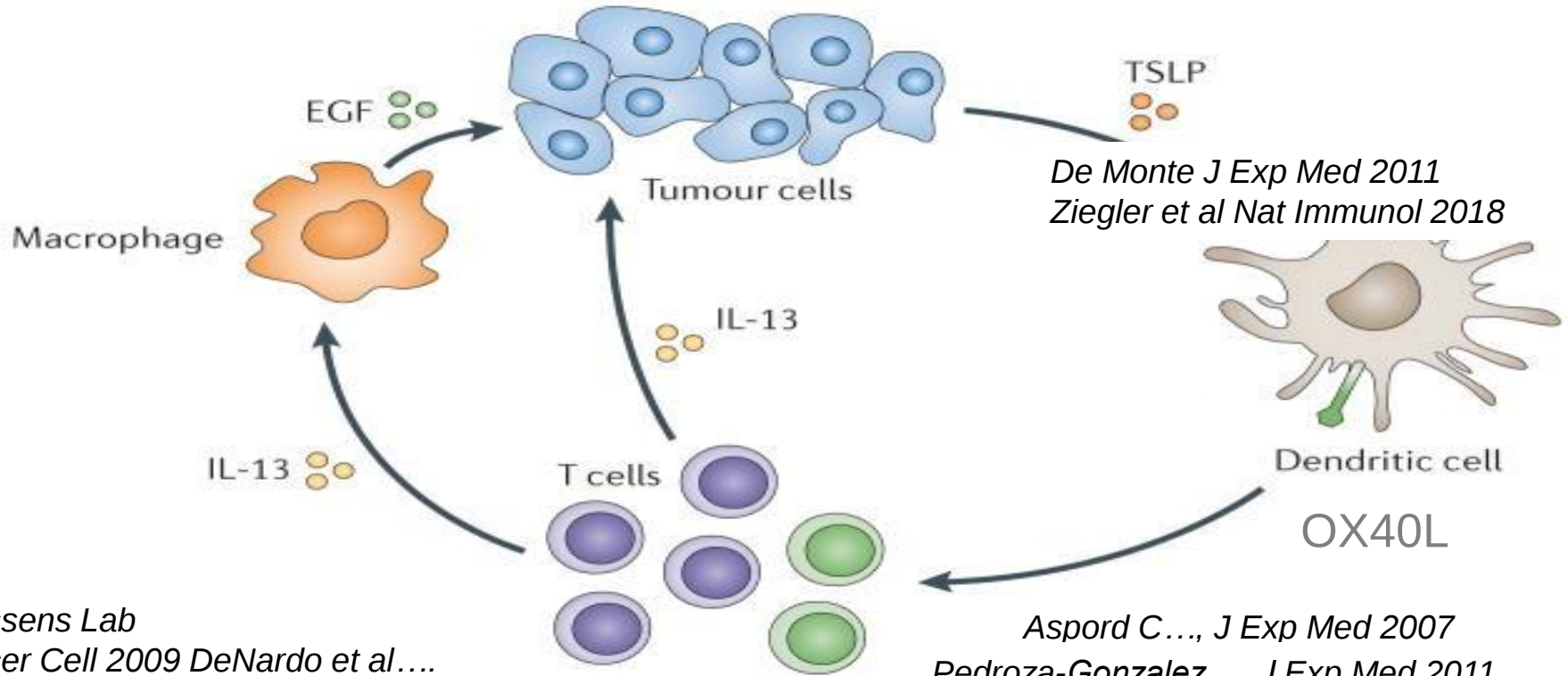


CD4+T cells

CD83+ mature DCs



Corrupted dendritic cells drive tumor promoting Th2 inflammation

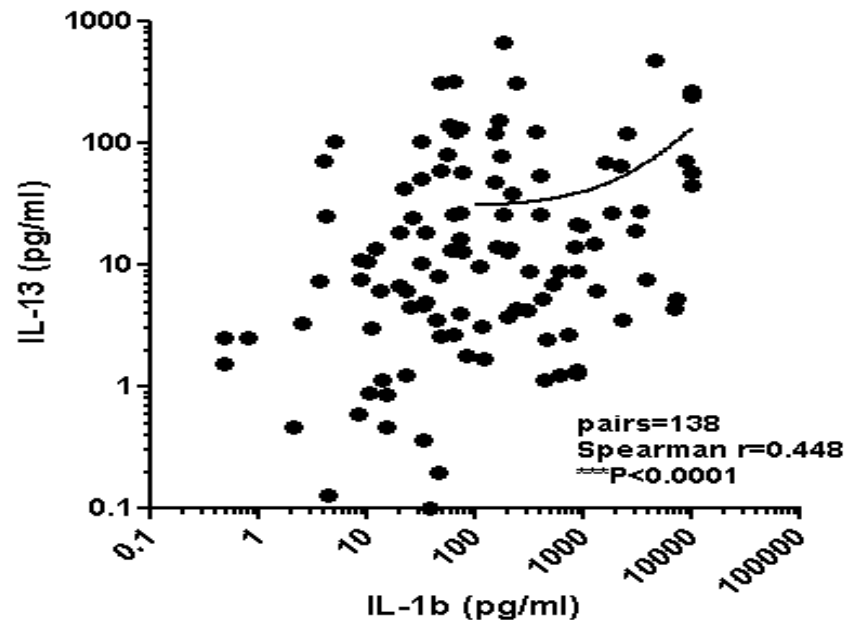
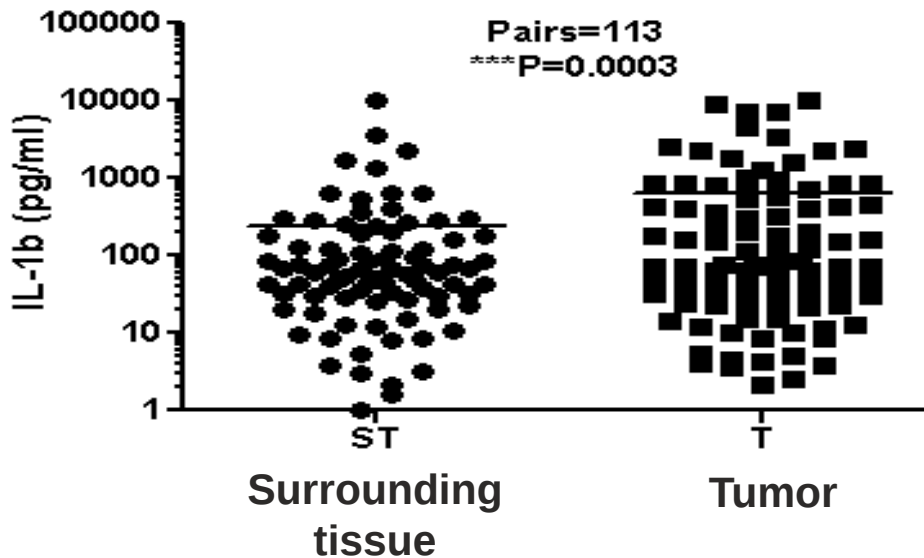


De Monte J Exp Med 2011
Ziegler et al Nat Immunol 2018

Coussens Lab
Cancer Cell 2009 DeNardo et al....
Cancer Discovery 2011
Cancer Immunol Res 2015

Aspord C..., J Exp Med 2007
Pedroza-Gonzalez..., J Exp Med 2011
Wu Te-Chia.... Cancer Immunol Res 2014
Wu, et al Cancer Res 2018

IL-1 β upregulation in patients breast cancer correlates with pathogenic cytokine IL-13



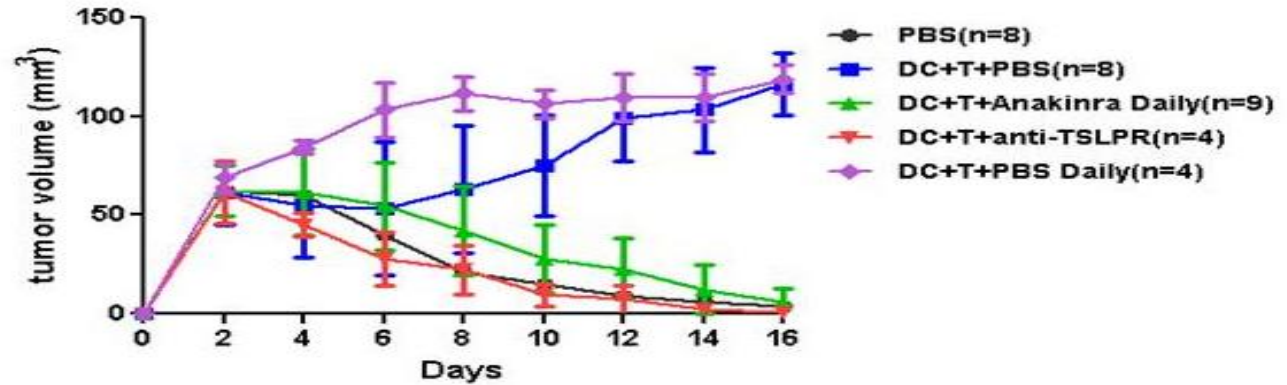
IL-1 blockade in experimental tumors :

Anakinra prevents IL-13 production and breast cancer progression

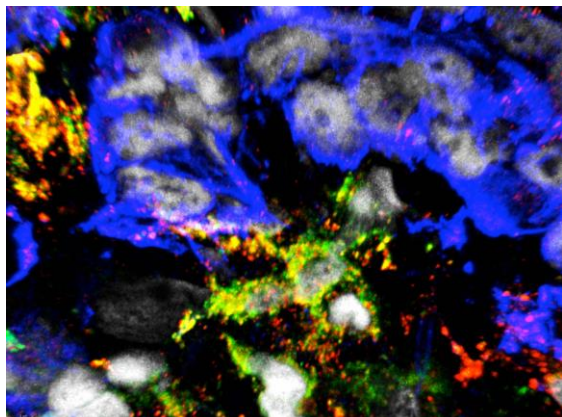


Inject human DCs,
T cells
Plus Anakinra
or PBS daily

Analysis



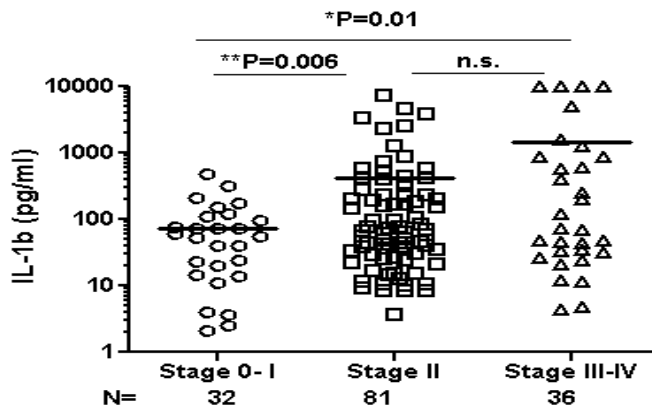
IL1 blockade in patients: Attenuating tumor promoting inflammation in breast cancer



Nucleus Cytokeratin CD11c IL1b

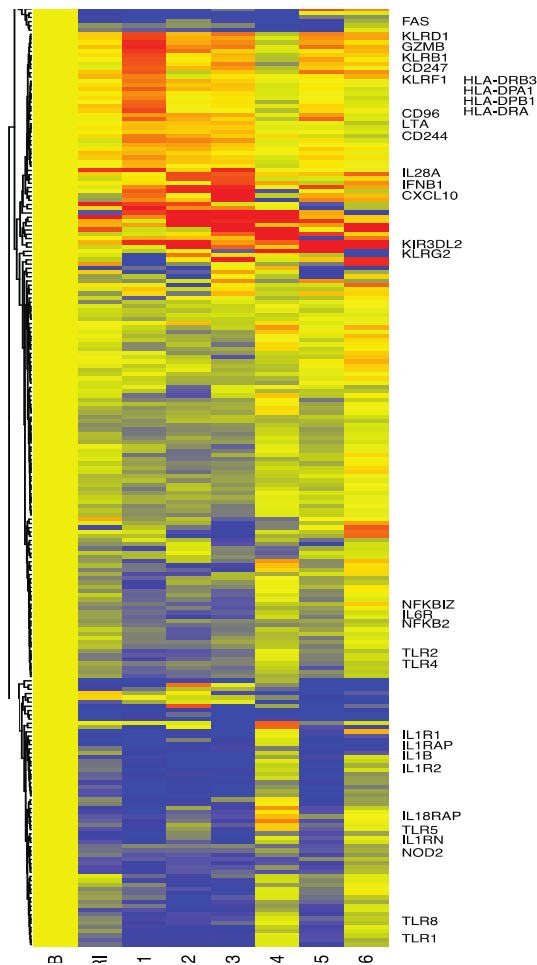
Exploratory clinical trial
(IRB 012-099)

Combination of IL-1 blockade with
chemotherapy in metastatic
TNBC



Joyce O'Shaughnessy
Robin Young
Virginia Pascual
Romain Banchereau
Clinical Team
Patients

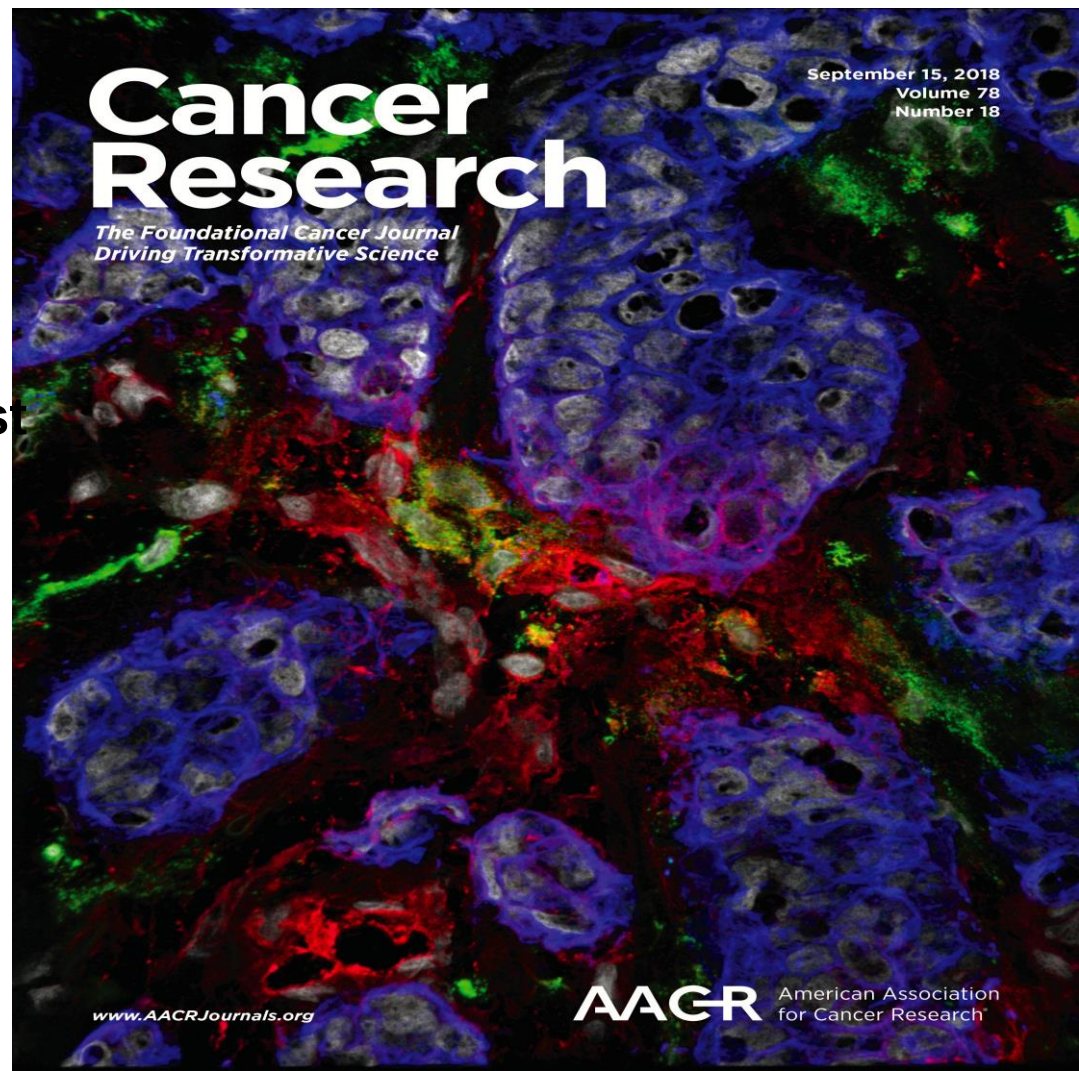
Blood transcriptome



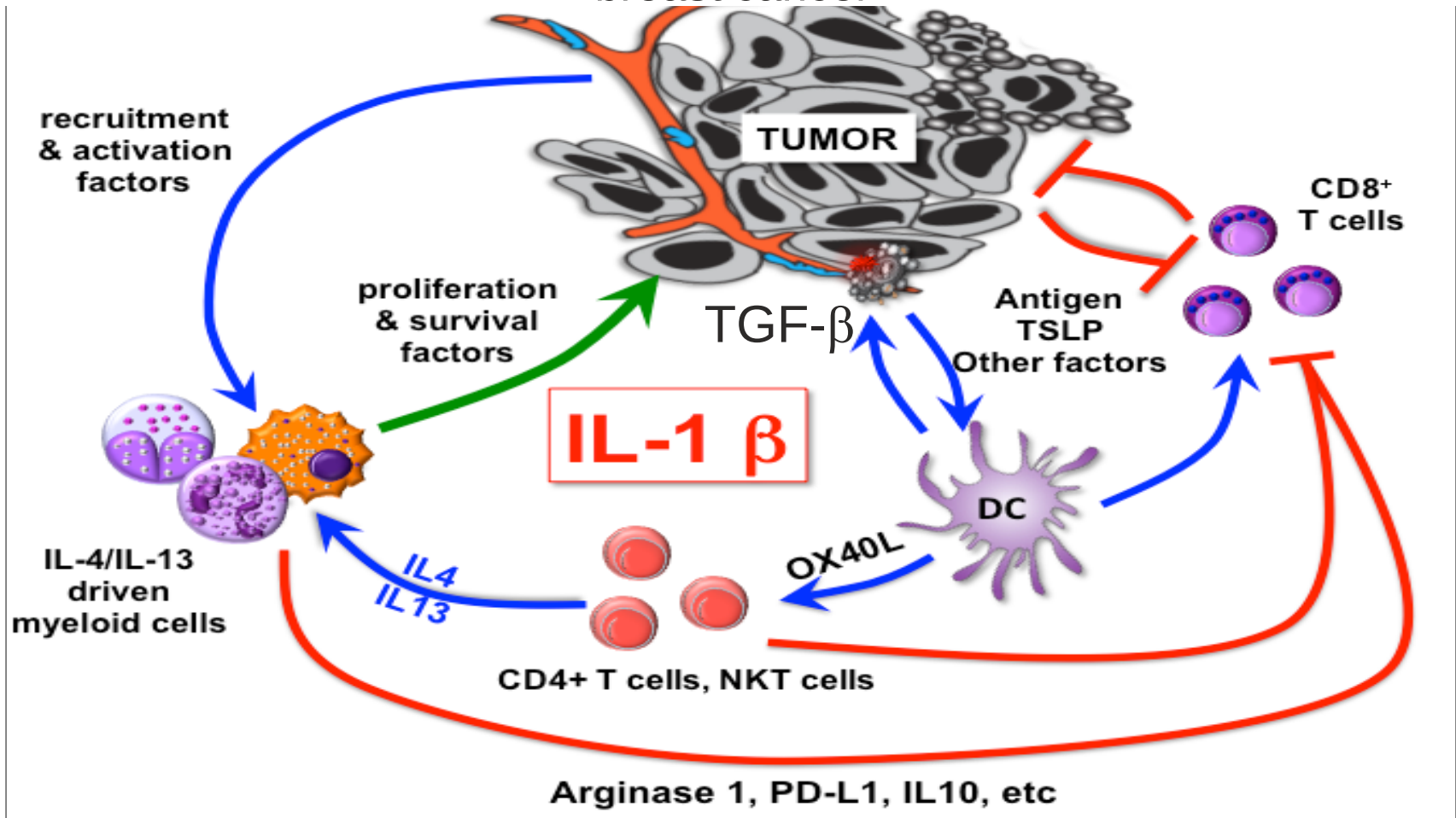
Wu et al. Cancer Res 2018

IL1 Receptor Antagonist Controls Transcriptional Signature of Inflammation in Patients with Metastatic Breast Cancer

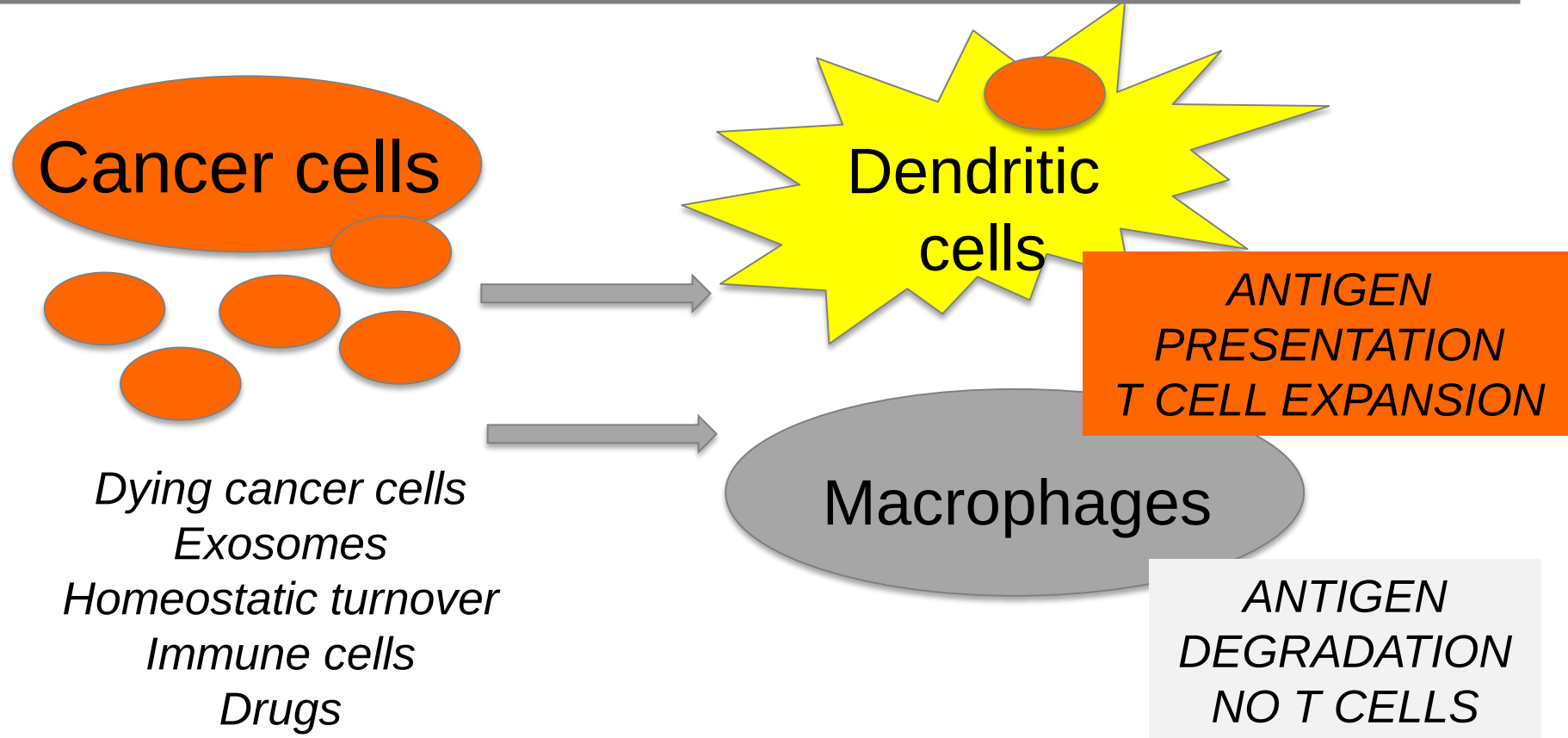
TGF β
Cancer cell
IL1 β
nuclei



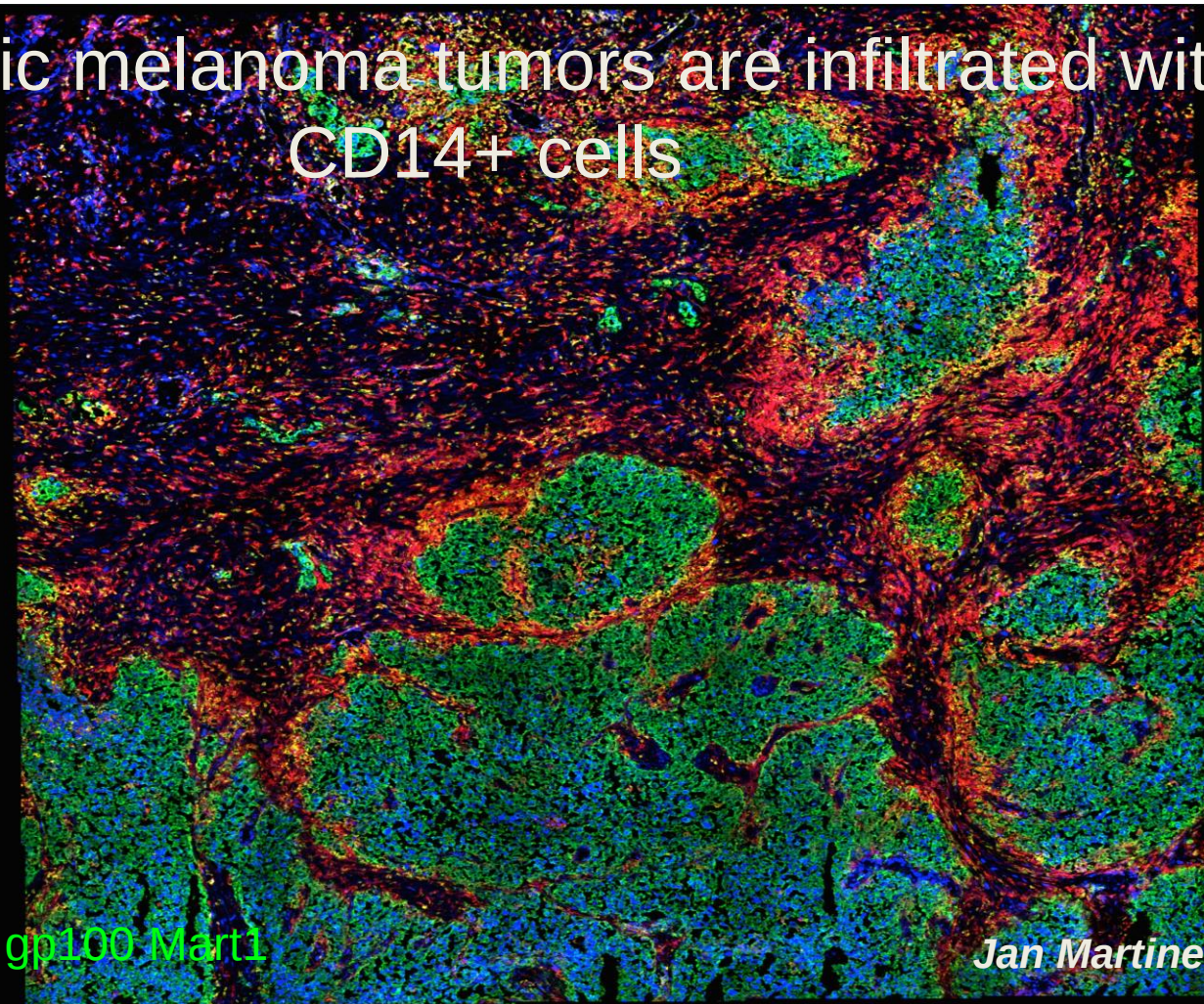
Crosstalk between IL-1 β and TGF- β drives pathogenic inflammatory loops in breast cancer



Different antigen presenting cells in the tumor determine the fate of antigen



Metastatic melanoma tumors are infiltrated with CD14+ cells



Mel 59818

DAPI CD3 CD14 gp100 Mart1

Jan Martinek

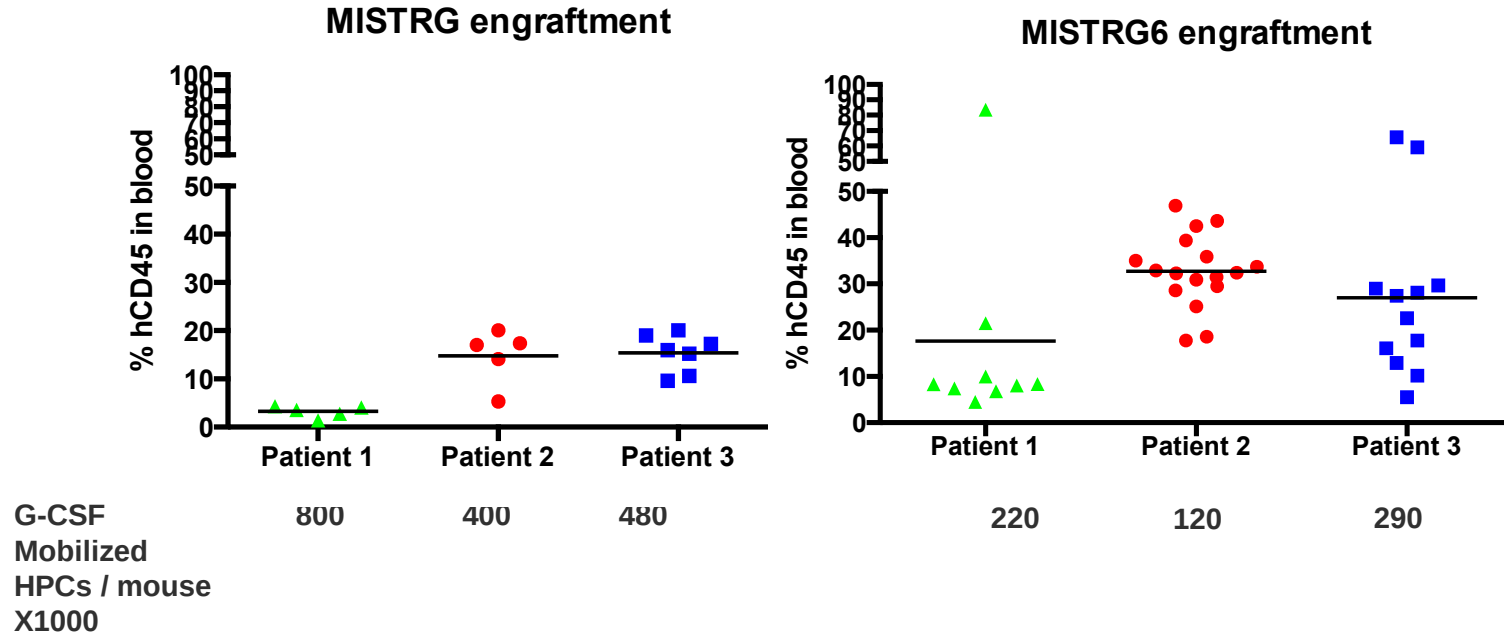
200 μ m

In vivo models of human myeloid cells and human cancer

- MISTRG for the encoded proteins M-CSF (CSF-1), IL-3/GM-CSF, SIRPα and TPO in the *Rag2-Il2rg*- background (Rongvaux, Martinek, Palucka... et al, Nat Biotech 2014).
CSF-1-dependent myeloid compartment
- NSG-SGM3 strain, an immunodeficient strain that expresses transgenes for human SCF and GM-CSF/IL-3 (Billerbeck et al., Blood 2011; Coughlan et al., Stem Cells Dev 2016).
CSF-1-independent myeloid compartment
- Other models in works

Autologous in vivo model of human melanoma:

Humanized MISTRG-6 mice efficiently support
Patient mobilized hematopoietic progenitor cells engraftment



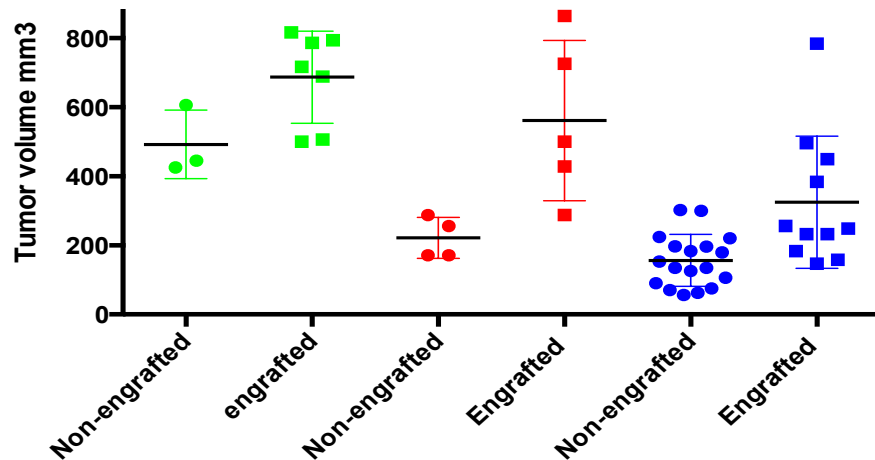
UNPUBLISHED

Jan Martinek, Michael Chiorazzi

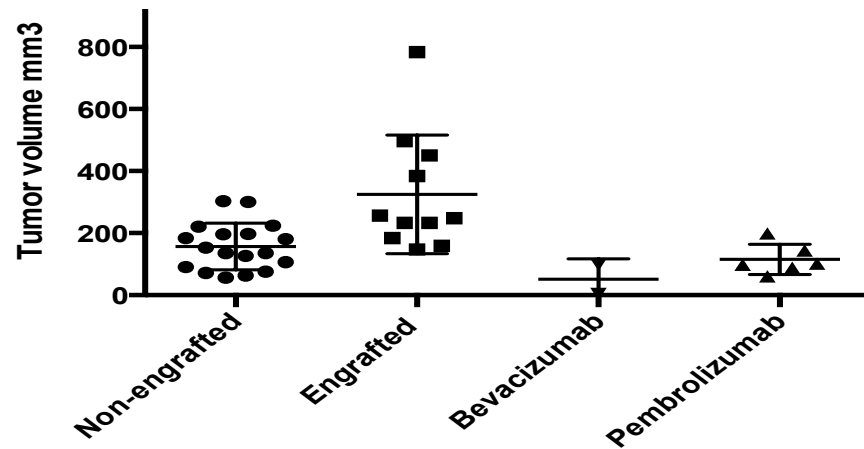
Autologous in vivo model of human melanoma:

Humanized MISTRG-6 mice support
tumor development and drug response

The impact of humanized host on tumor growth



Treatment response

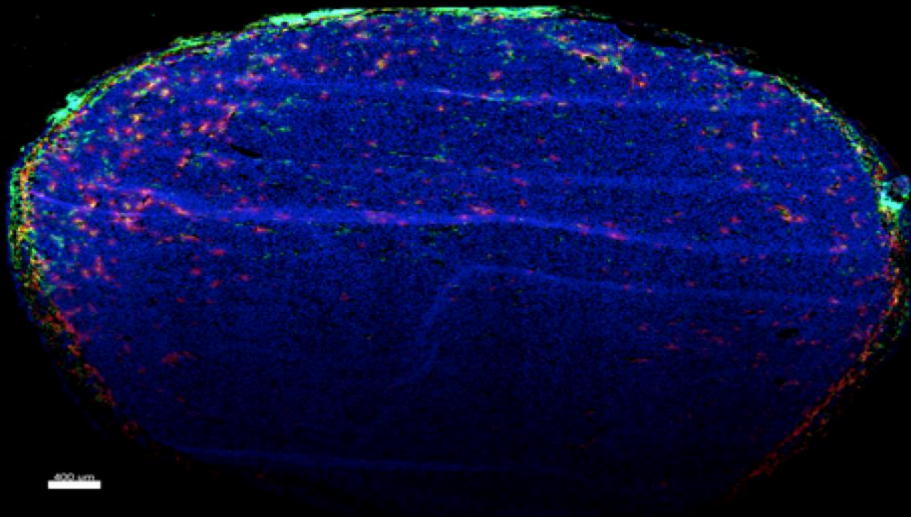


MISTRG-6 mice were engrafted with 200k peripheral blood mobilized hCD34+ cells; engraftment assayed at 9 weeks. PDX cell suspension injected at 9 weeks (1 million cells); tumors collected at 14 weeks.

Jan Martinek, Michael Chiorazzi

UNPUBLISHED

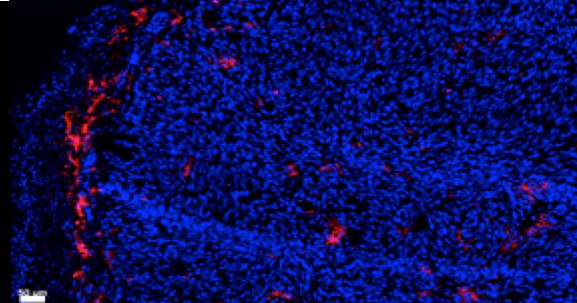
Immune infiltrate in untreated tumors



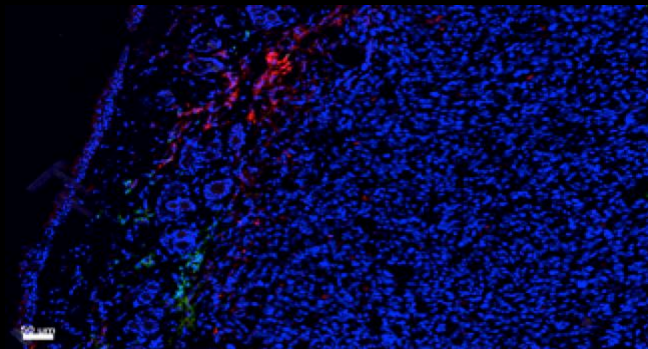
DAPI hCD45 mCD45

Patient 3

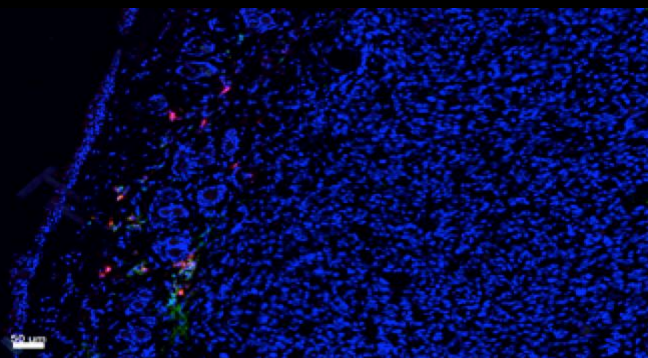
DAPI
hCD45



DAPI
CD14
CD3



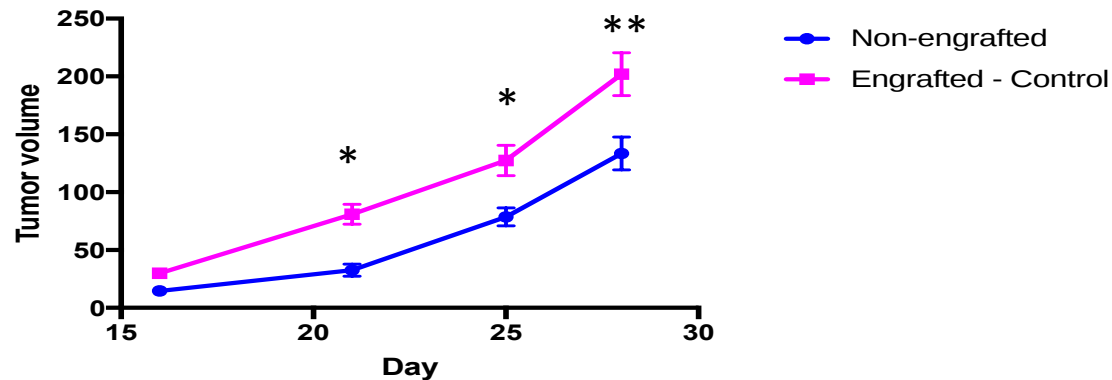
DAPI
CD8
CD3



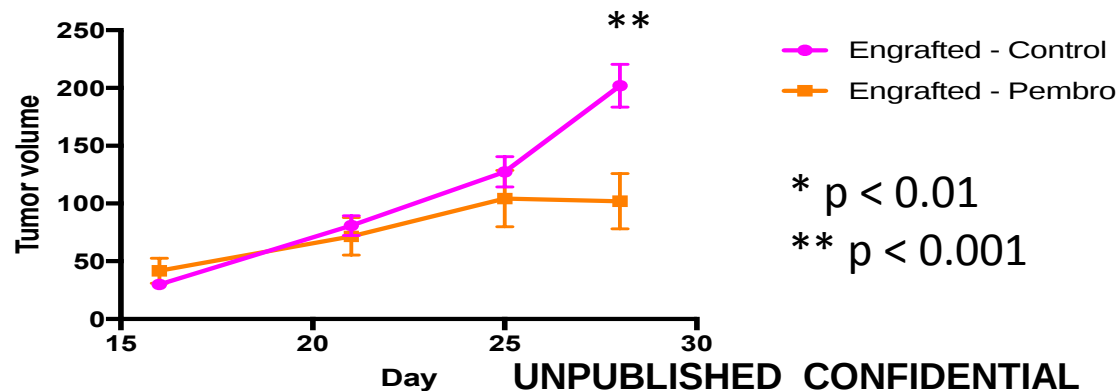
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Treatment related changes in immune infiltrate

Patient 3



Patient 3

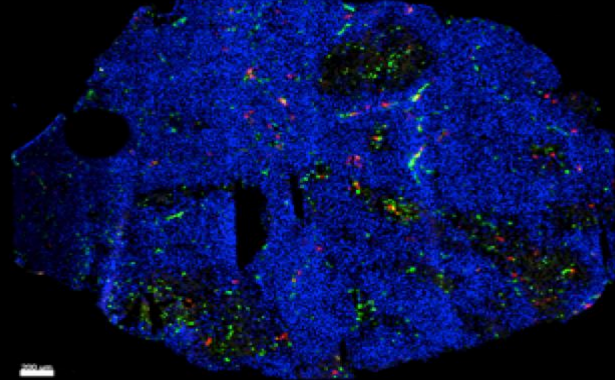


* $p < 0.01$

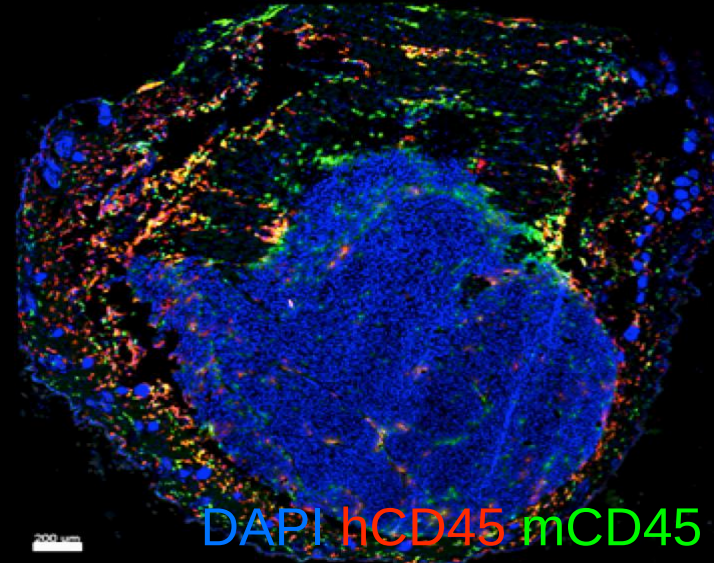
** $p < 0.001$

UNPUBLISHED_CONFIDENTIAL

Patient 3 Untreated



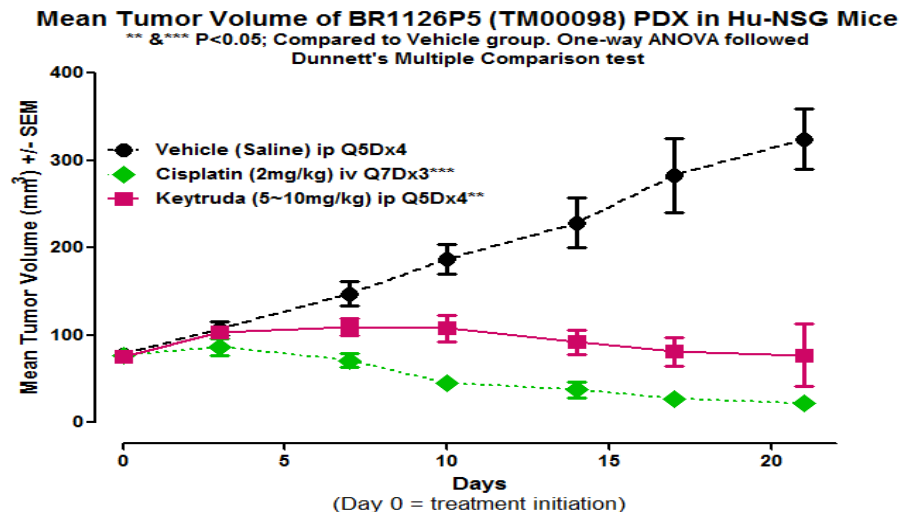
Patient 3 Pembrolizumab



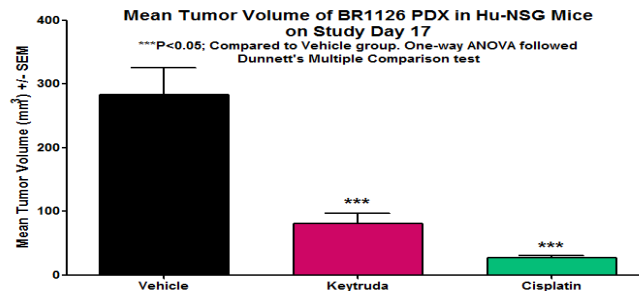
DAPI hCD45 mCD45

Using humanized mice to study checkpoint inhibitors:

Pembrolizumab and Cisplatin Inhibit Growth of the breast cancer BR1126 PDX Model in Hu-CD34 NSGTM PDX Mice

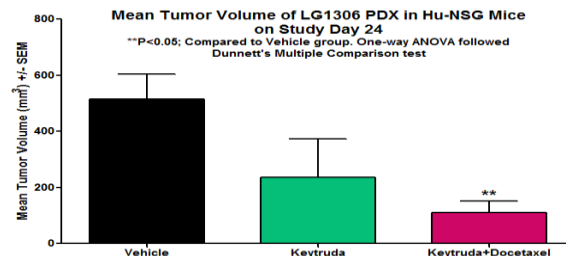
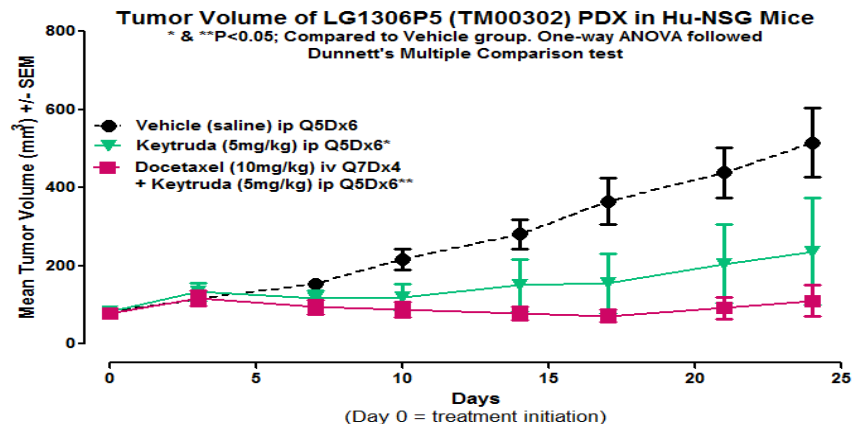


- HuCD45+ in Hu-NSG mice: >25%
- BR1126 PD-L1 surface expression: 56.9%



HLA match	CD34 ⁺ HPC donor		
Tumor	1	2	3
BR1126	HLA-C, DPA1	HLA-A,DQA1, DPB1, DPA1	HLA-C, DPA1

Efficacy Results of Pembrolizumab +/- Docetaxel on lung cancer LG1306 PDX Tumors in Hu-NSGTM Mice

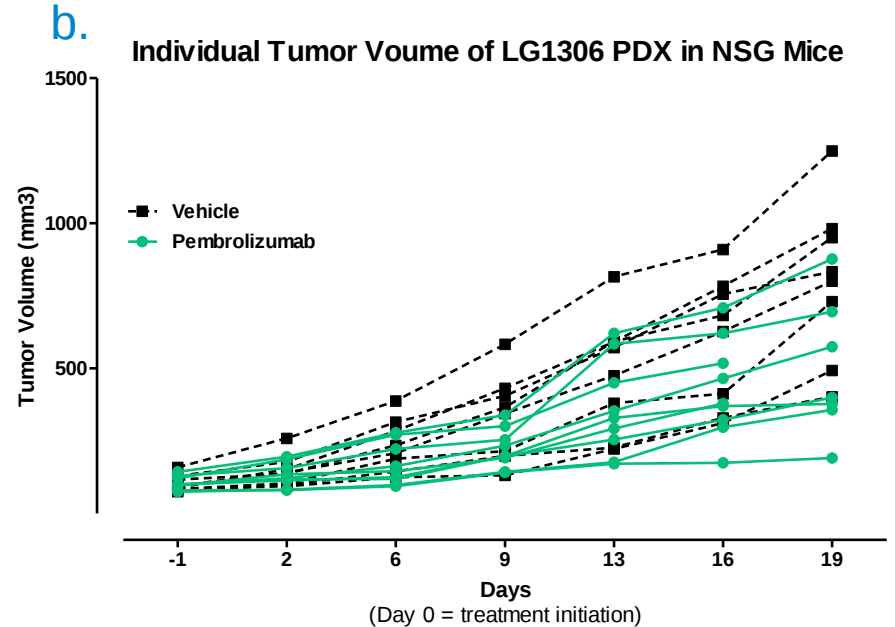
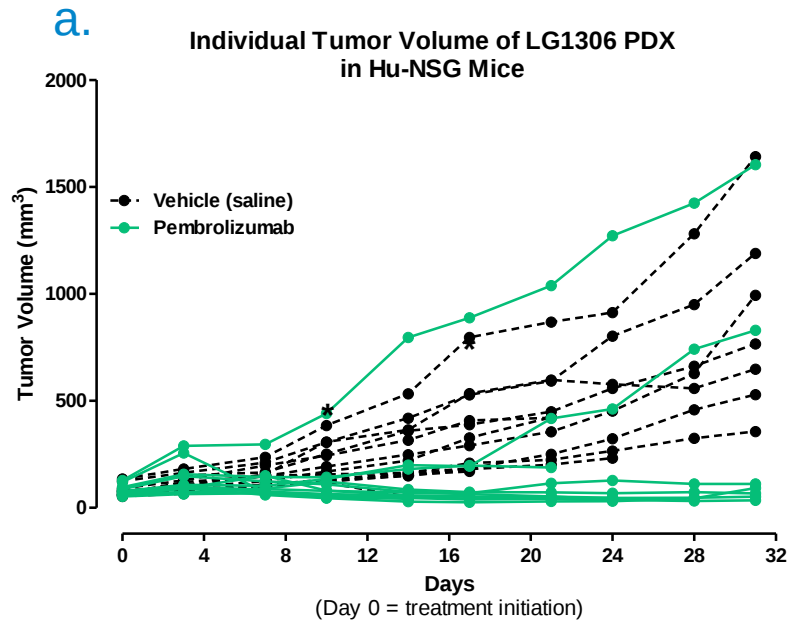


- Two or more CD34 donors
- Fresh tumor tissue engraftment
- HuCD45+ more than 20%
- LG1306 PD-L1 surface expression: 89.1%

HLA match	CD34 ⁺ HPC donor	
Tumor	1	2
LG1306	HLA-DRB4, DQA1, DQB1	No match

*Jim Keck Lab, JAX Sacramento
Wang et al FASEB J 2018*

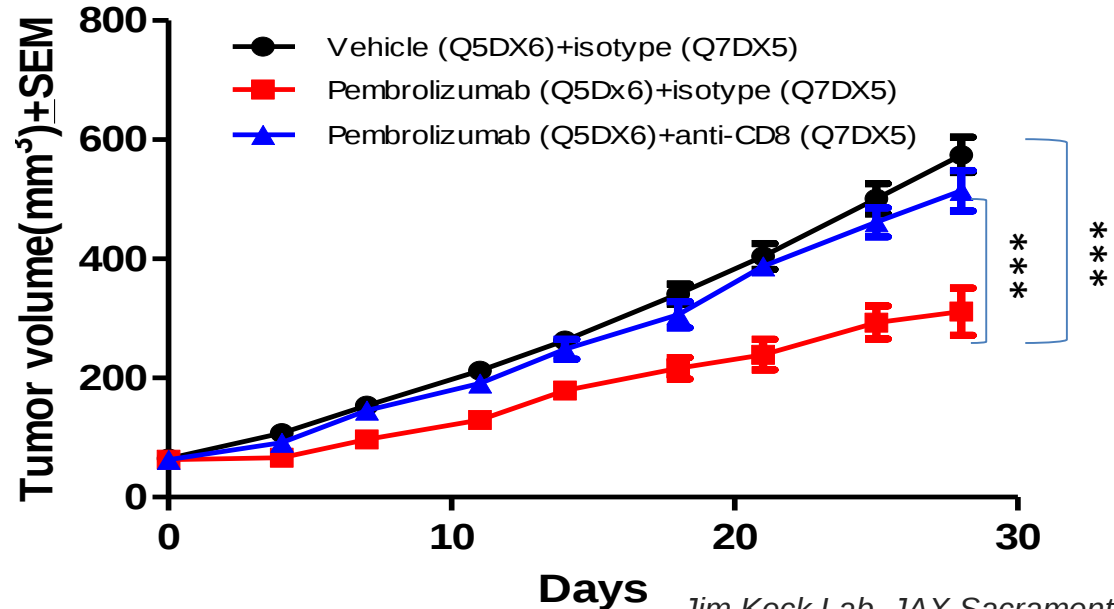
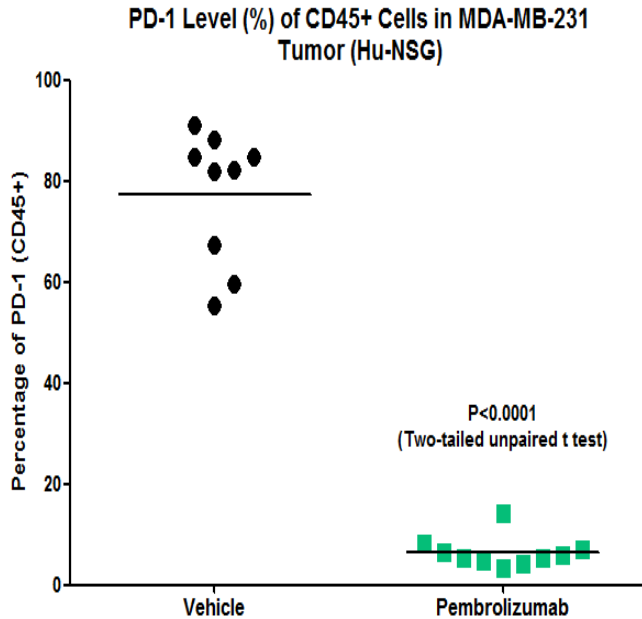
Mechanisms: Efficacy of Pembrolizumab Depends on Human Immune Cells



*Jim Keck Lab, JAX Sacramento
Wang et al FASEB J 2018*

Mechanisms: Efficacy of PD-1 blockade in humanized NSG mice bearing breast cancer CDX is CD8⁺ T Cell Dependent

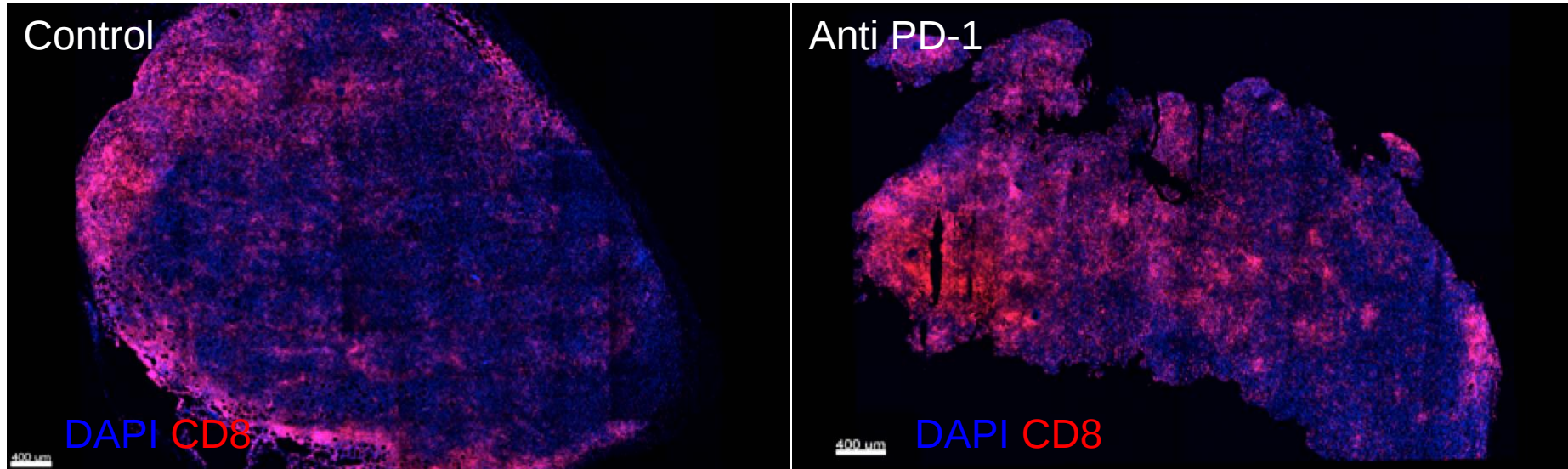
MDA-MB-231 Tumor Response in Hu-NSG Mice



*** $P < 0.0001$; two-way ANOVA followed by Bonferroni post-tests.

Jim Keck Lab, JAX Sacramento
Wang et al FASEB J 2018

Redistribution of CD8 infiltrate after anti PD-1 treatment



*Jim Keck Lab, JAX Sacramento
Jan Martinek, Palucka Lab, JGM Connecticut
Wang et al FASEB J 2018*

Using humanized mice to study checkpoint inhibitors: targeting HDAC and PD1

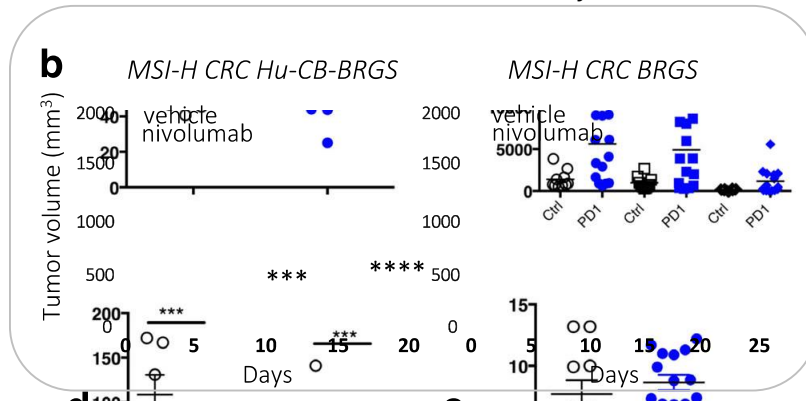
Characterization of immune responses
to anti-PD-1 mono and combination
immunotherapy in hematopoietic
humanized mice implanted with tumor
xenografts



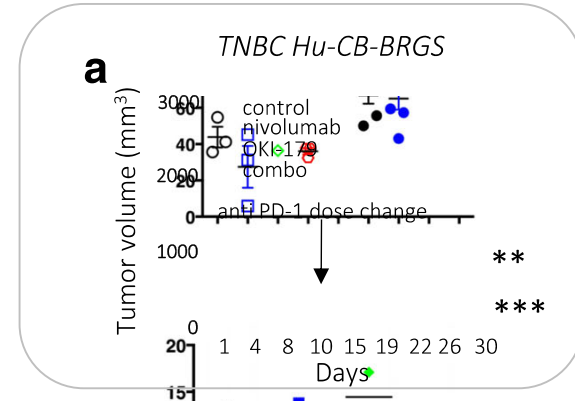
Capasso et al. *Journal for ImmunoTherapy of Cancer* (2019) 7:37
<https://doi.org/10.1186/s40425-019-0518-z>

A. Capasso^{1†}, J. Lang^{3†*}, T. M. Pitts^{1,2}, K. R. Jordan³, C. H. Lieu^{1,2}, S. L. Davis^{1,2}, J. R. Diamond^{1,2}, S. Kopetz²,
J. Barbee³, J. Peterson³, B. M. Freed⁶, B. W. Yacob¹, S. M. Bagby¹, W. A. Messersmith^{1,2}, J. E. Slansky^{2,3},
R. Pelanda³ and S. G. Eckhardt²

Treatment response in humanized host but not in the
absence of the human immune system



Combination therapy



Using humanized mice to study mechanisms of action of biologics

Humanized Mice as a Model for Aberrant Responses in Human T Cell Immunotherapy

The Journal of Immunology, 2014, 193: 587–596.

Nalini K. Vudattu,^{*1} Frank Waldron-Lynch,^{*1} Lucy A. Truman,^{*} Songyan Deng,^{*} Paula Preston-Hurlburt,^{*} Richard Torres,[†] Maurice T. Raycroft,[‡] Mark J. Mamula,[‡] and Kevan C. Herold^{*,‡}

CD34+ HPCs humanized mice treated with Ipi

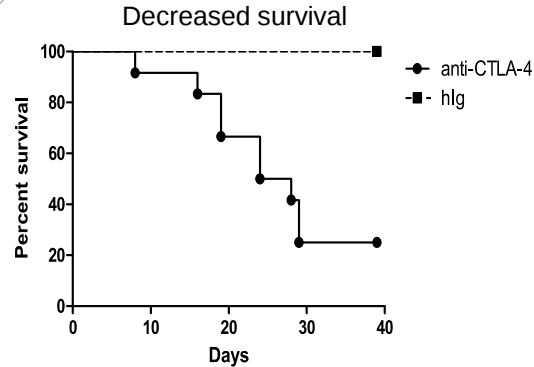
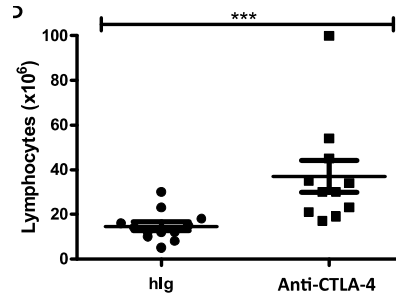


FIGURE 1. Systemic effects of ipilimumab (anti-CTLA mAb) treatment on humanized mice. Mice treated with ipilimumab (circles) had median survival of 26 d (versus hlg [squares]; $p = 0.0002$, $n = 12$ in each group).

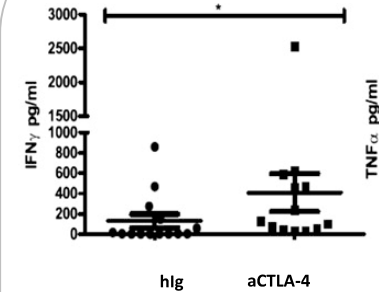
Increased spleen cellularity



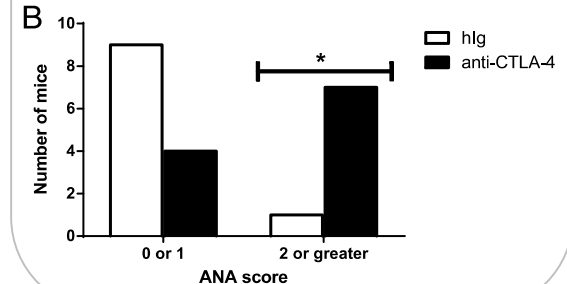
Anti-CTLA-4 hlg



Skewed CD4+ T cell cytokines



Enhanced titers of auto-antibodies

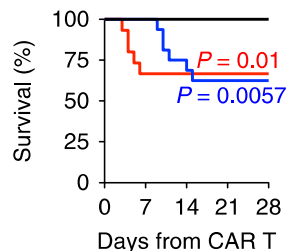
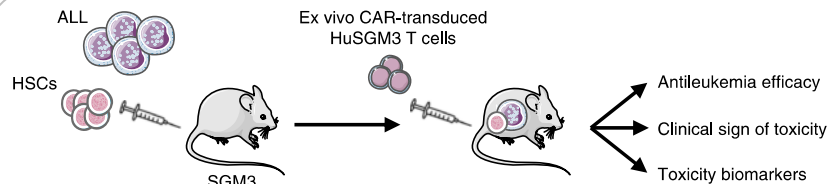
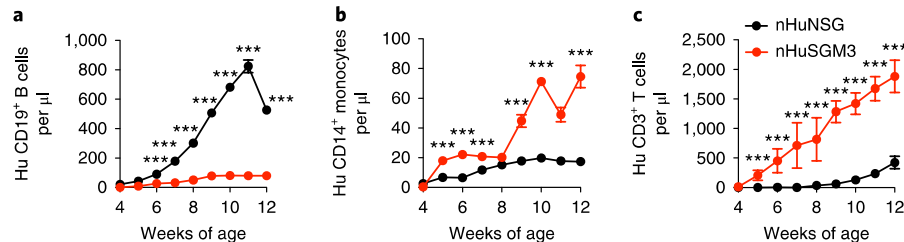


Using humanized mice to study mechanisms of toxicity of cell therapies

Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells

Margherita Norelli^{1,2}, Barbara Camisa¹, Giulia Barbiera³, Laura Falcone¹, Ayurzana Purevdorj¹, Marco Genua³, Francesca Sanvito⁴, Maurilio Ponzoni⁴, Claudio Dogliani⁴, Patrizia Cristofori⁵, Catia Traversari⁶, Claudio Bordinon^{2,6}, Fabio Ciceri^{2,7}, Renato Ostuni³, Chiara Bonini^{2,8}, Monica Casucci¹ and Attilio Bondanza^{1,2*}

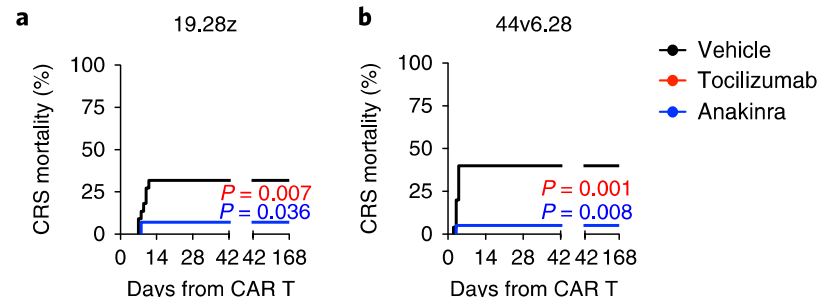
NATURE MEDICINE | VOL 24 | JUNE 2018 | 739–748 | www.nature.com/naturemedicine



— 44v6/19.28z in SGM3

— 44v6.28z in HuSGM3

— 19.28z in HuSGM3



Using humanized mice to study cancer vaccines

Peptide plus adjuvant DC vaccine

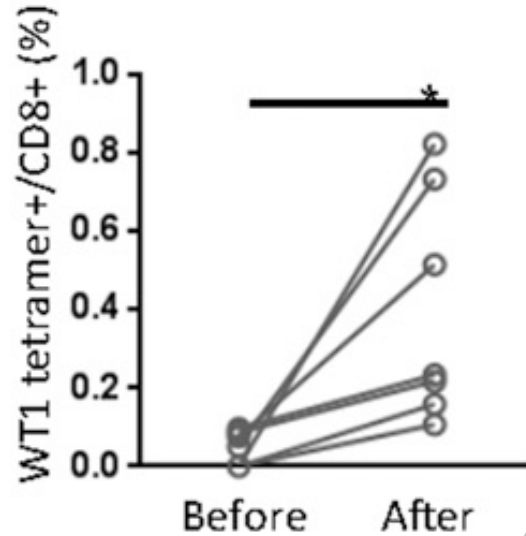
Induction of WT1-specific human CD8⁺ T cells from human HSCs in HLA class I Tg NOD/SCID/IL2rgKO mice

Yuhō Najima,¹ Mariko Tomizawa-Murasawa,¹ Yoriko Saito,¹ Takashi Watanabe,² Rintaro Ono,¹ Toshiki Ochi,³ Nahoko Suzuki,¹ Hiroshi Fujiwara,³ Osamu Ohara,² Leonard D. Shultz,⁴ Masaki Yasukawa,³ and Fumihiko Ishikawa¹

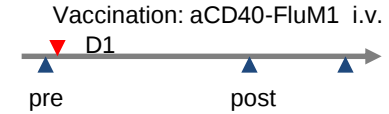
(*Blood*. 2016;127(6):722-734)

Key Points

- Vaccination using WT1 peptides and dendritic cells induced WT1-specific human CTLs in vivo in HLA class I Tg NSG mice.
- Human HSCs transduced with human TCR genes generated HLA class I-restricted, WT1-specific CTLs in vivo.



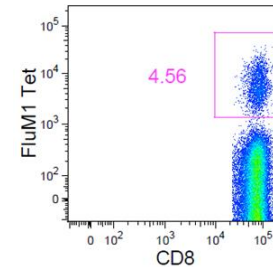
DC targeting in vivo



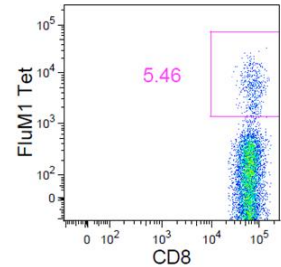
CD8 T cell Response in vivo

aCD40-FluM1

Mouse #1

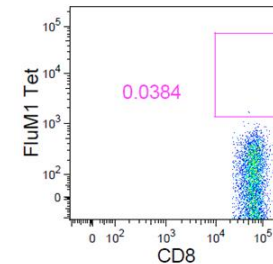


Mouse #2

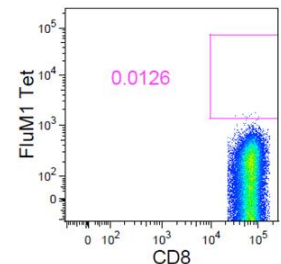


IgG4-FluM1 + aCD40

Mouse #3



Mouse #4



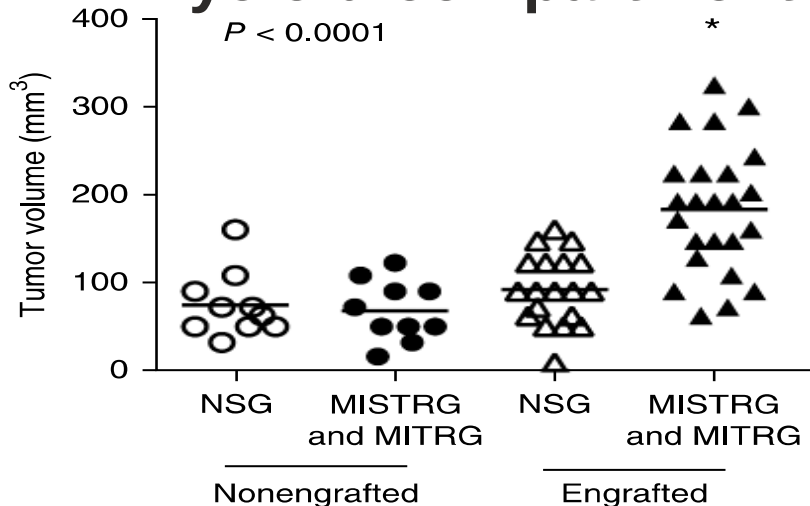
Graham...Yu et al., Vaccine 2016

In vivo models of human myeloid cells and human cancer

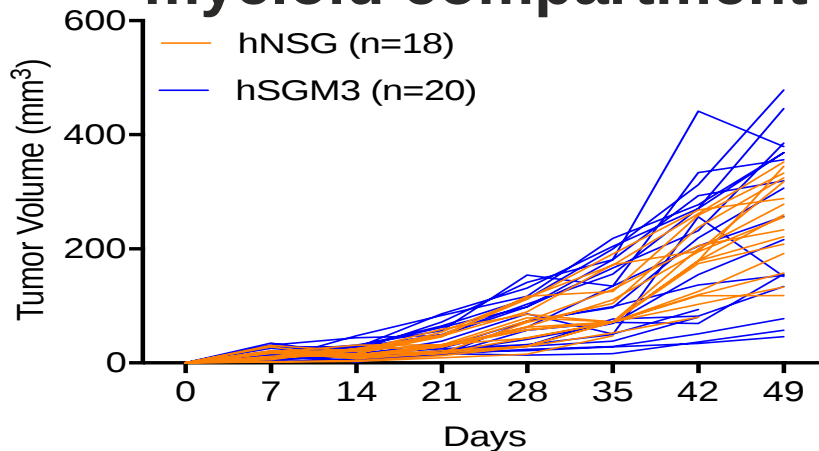
- MISTRG for the encoded proteins M-CSF (CSF-1), IL-3/GM-CSF, SIRPα and TPO in the *Rag2-Il2rg*- background (Rongvaux, Martinek, Palucka... et al, Nat Biotech 2014).
CSF-1-dependent myeloid compartment
- NSG-SGM3 strain, an immunodeficient strain that expresses transgenes for human SCF and GM-CSF/IL-3 (Billerbeck et al., Blood 2011; Coughlan et al., Stem Cells Dev 2016).
CSF-1-independent myeloid compartment
- Other models in works

Human myeloid cells differentially impact progression of “primary” experimental Me275 melanoma tumors

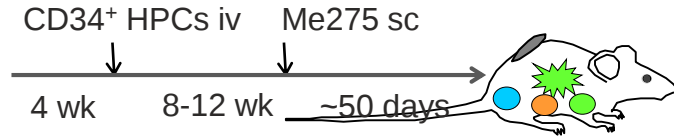
CSF-1 dependent myeloid compartment



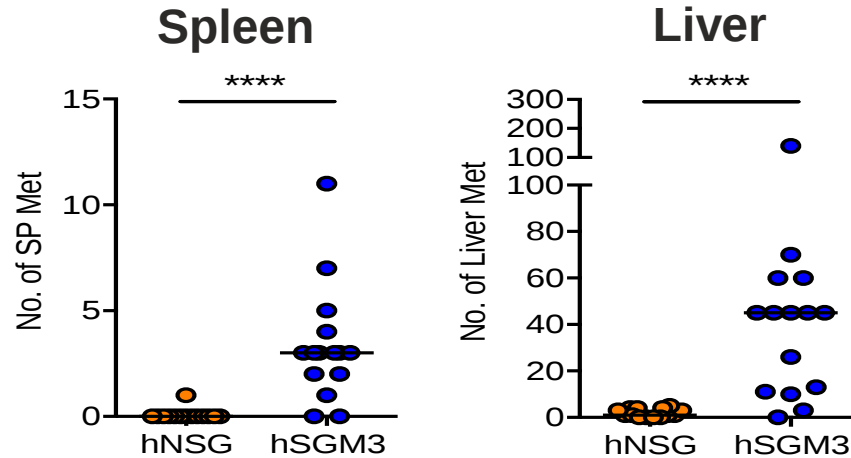
CSF-1 independent myeloid compartment



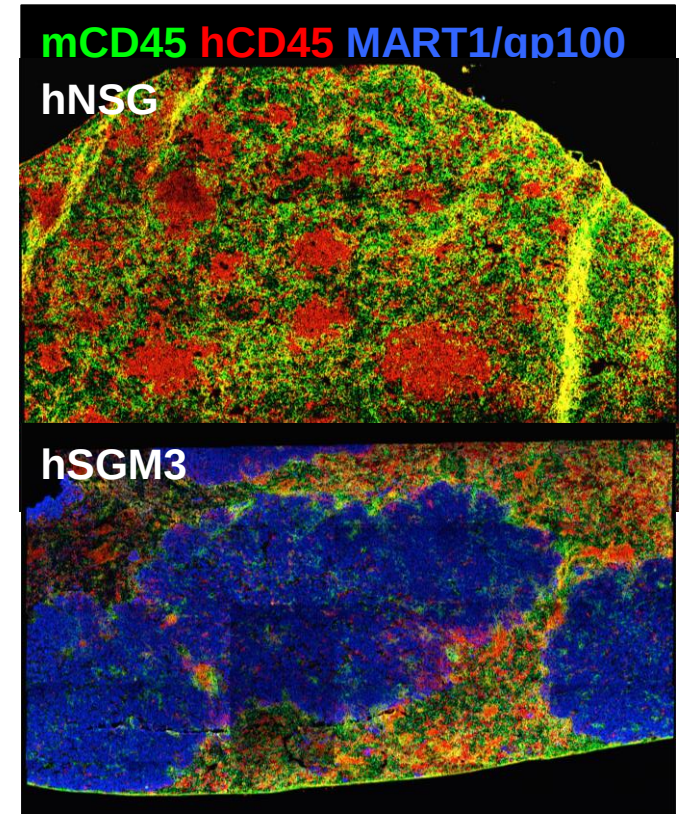
Humanized (h)NSG-SGM3 mice display macroscopic tumors in visceral organs



Macroscopic metastasis

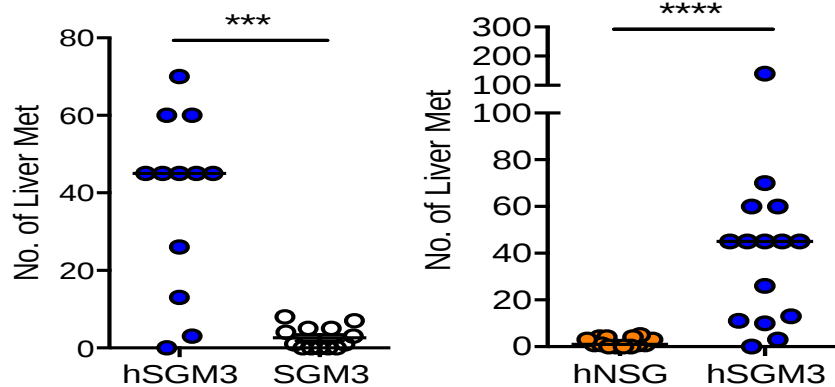


Yu et al, submitted

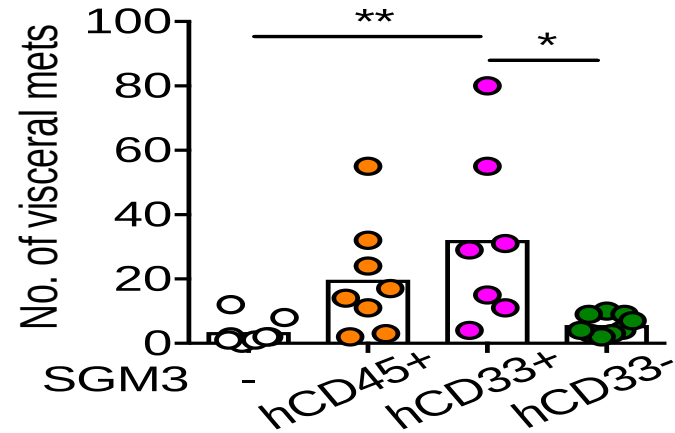


Human CD33+ Myeloid Cells Support Melanoma Metastatic Colonization of Distant Organs

Humanized NSG-SGM3 mice support melanoma metastasis in distant organs



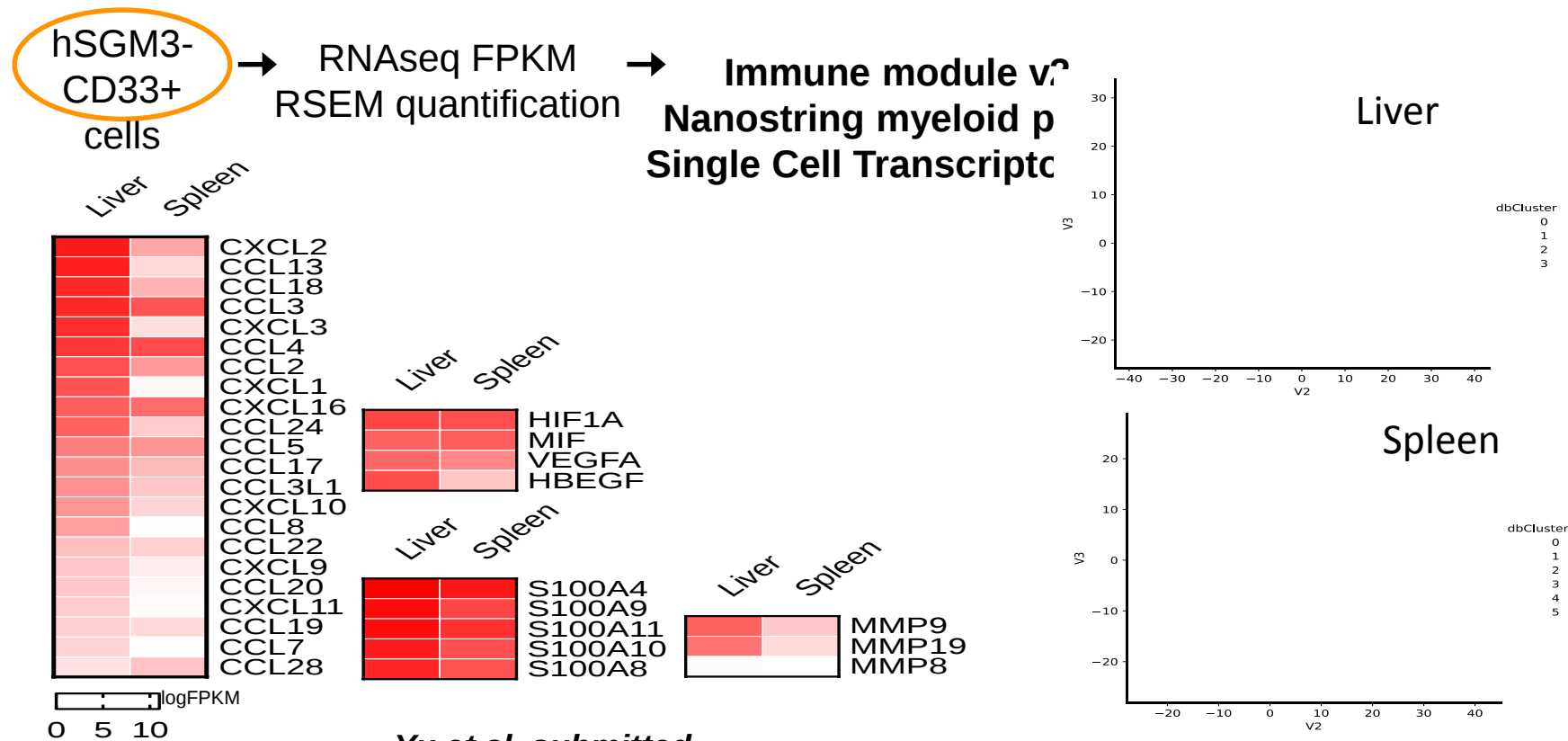
Pro-metastatic activity is mediated by human CD33+ myeloid cells



Purify human leukocytes (CD45+)
Purify human myeloid cells (CD33+)
Adoptive transfer to non-humanized SGM3

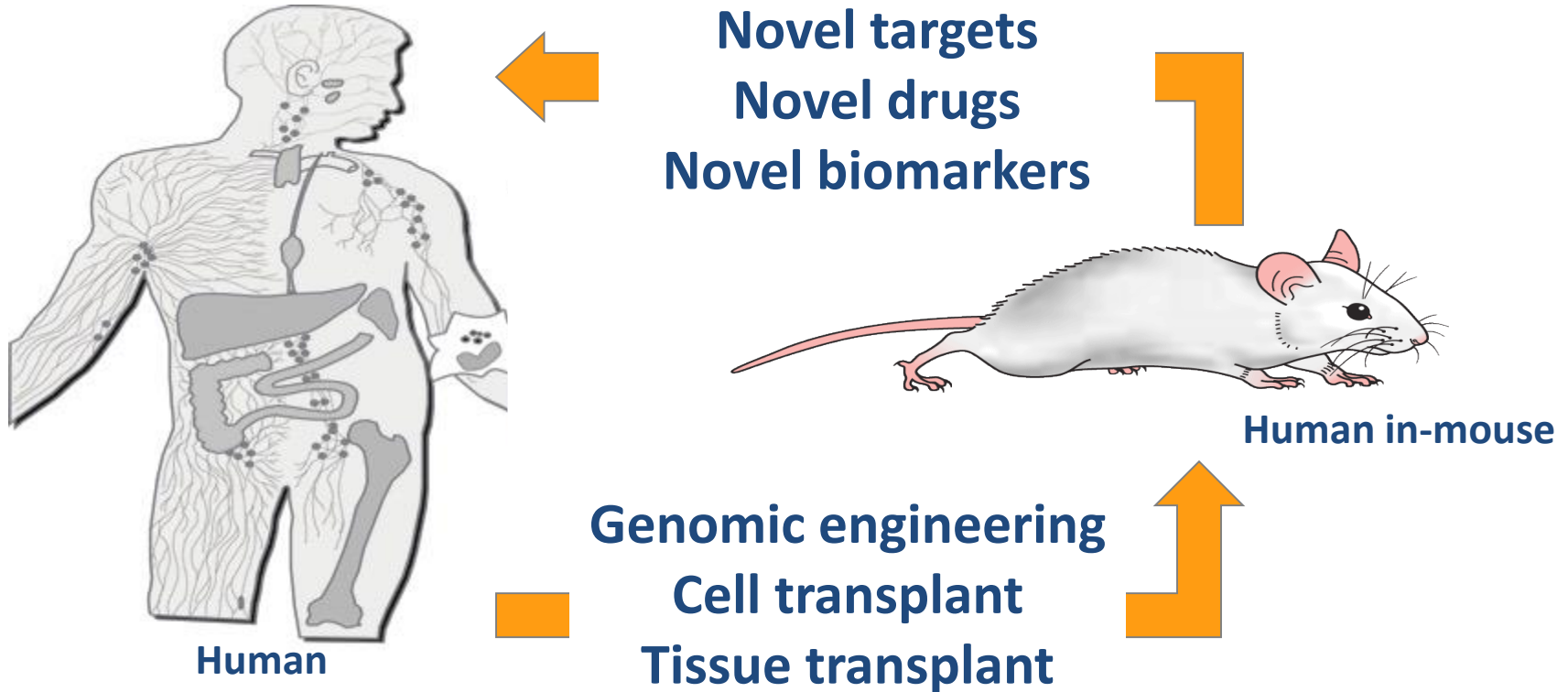
Yu et al, submitted

Tissue specific composition and transcriptional profiles of pro-metastatic CD33+ myeloid cells



Yu et al, submitted

Emerging Model for Human Immunotherapy



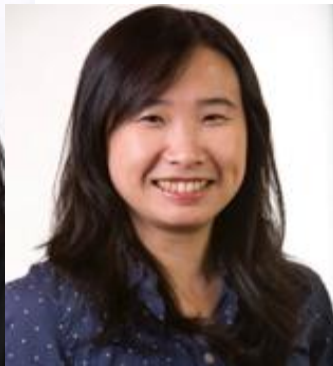
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