Dissecting disease mechanisms and human biology in humanized mice

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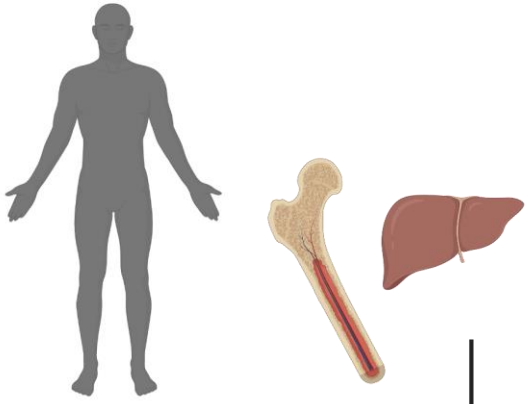
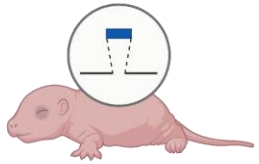
Generation & evolution of humanized mice



1. Human hematopoietic stem/progenitor cells (CD34⁺ cells)

(fetal liver, cord blood, adult BM/blood)

2. Transplantation into humanized mice



3. Hematopoietic development and immune function

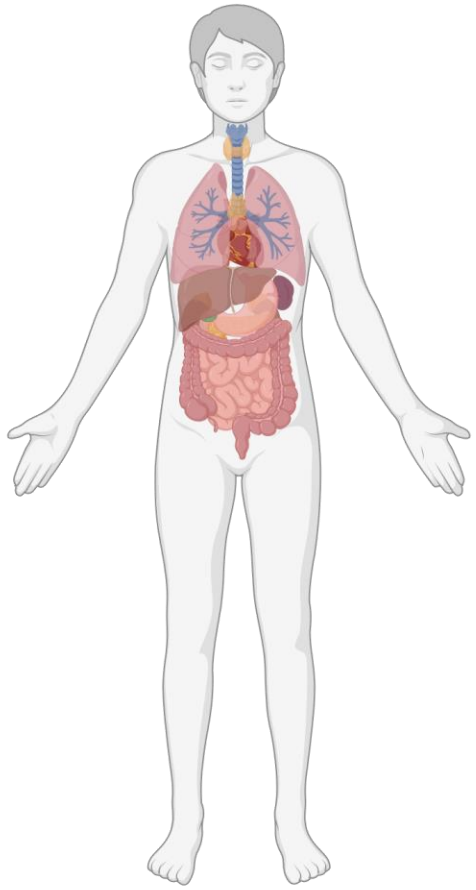
Strain	Bone marrow	Blood
Human		
NOD/ SCID		
BRG		
NOG/ NSG/SRG		
BLT		

CD34 T cells Mouse
Myeloid B cells

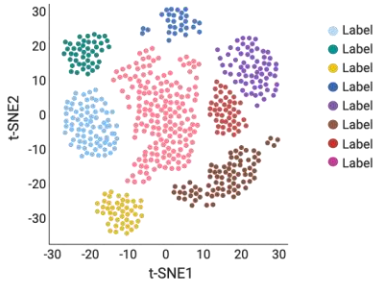
Traggiai et al., Science, 2004
Rongvaux et al., Annu. Rev. Immunol., 2013

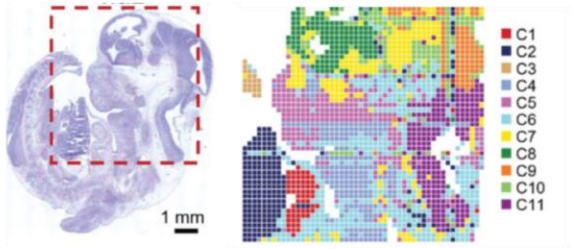
Advances in genomic and proteomic technologies and bioinformatics have improved our ability to study humans.

Patient



Multi-omics





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In vivo perturbations

Humanized mice

- Cause & effect
- HSPCs+/- genes
- Mechanism

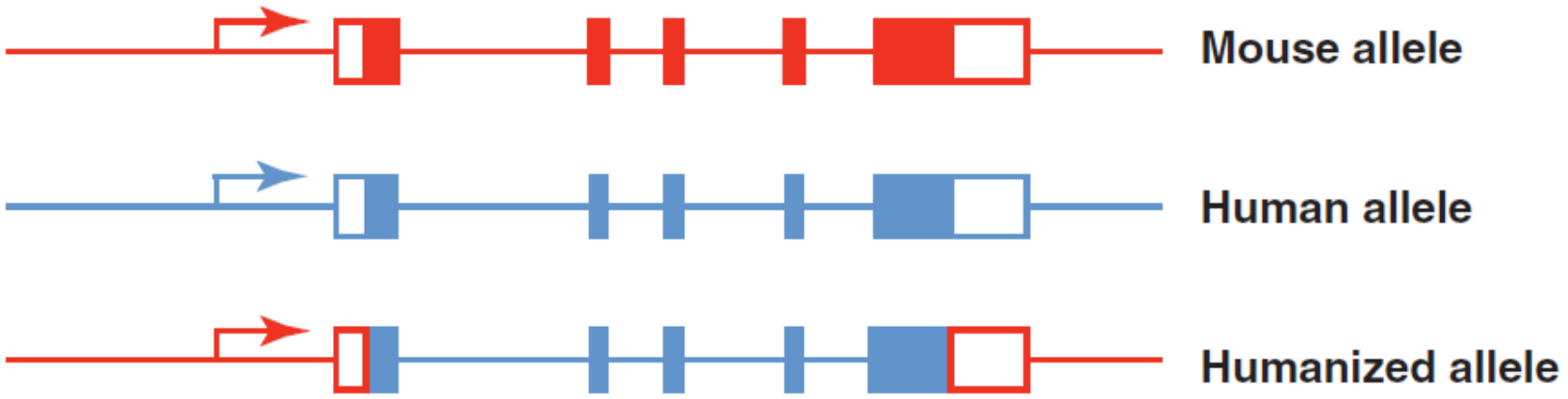
Perturbations in vivo help bridge the human data to causality.

Genetic humanization of cytokine-encoding genes

Rationale: Factors/cytokines species specific- so humanize the key factors

Method: Velocigene technology

In collaboration with Regeneron Pharmaceuticals



Willinger *et al*, Trends Immunol., 2011

Improvement of humanized mice by human cytokine knock-in gene replacement

MISTRG, multi cytokine KI

Rongvaux et al., Nat. Biotech 2014
Song et al., Nat Comm, i

Rag2 KO
Il2rg (γ_c) KO

Human M-CSF KI

Rathinam et al., Blood 2011

Monocytes/macrophages

Human IL-3/GM-CSF KI

Willinger et al., PNAS 2011

Alveolar macrophages
Immune responses lung

Human SIRPa

Strowig et al., PNAS 2011
Deng et al., Nature 2015

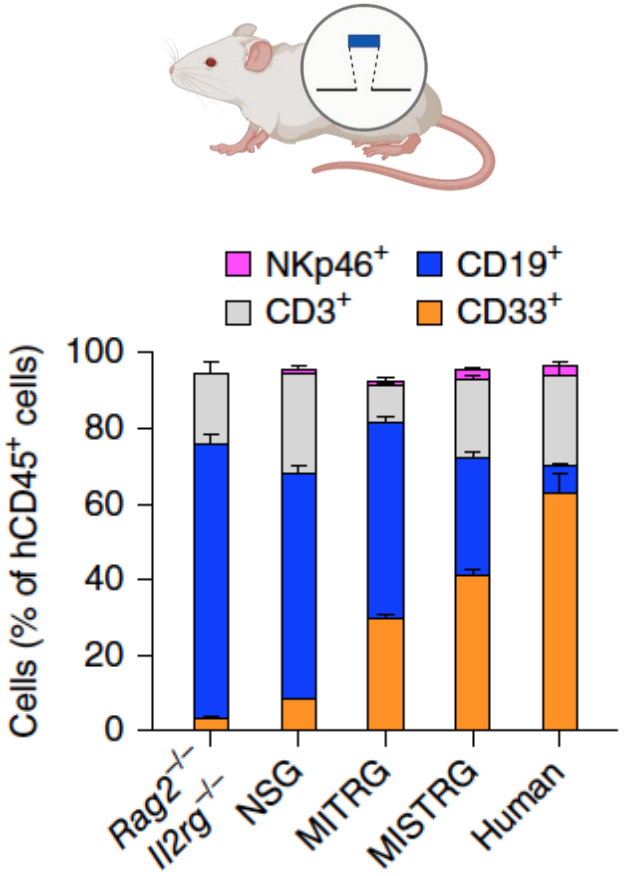
Lymphoid cell engraftment
Adaptive immune response

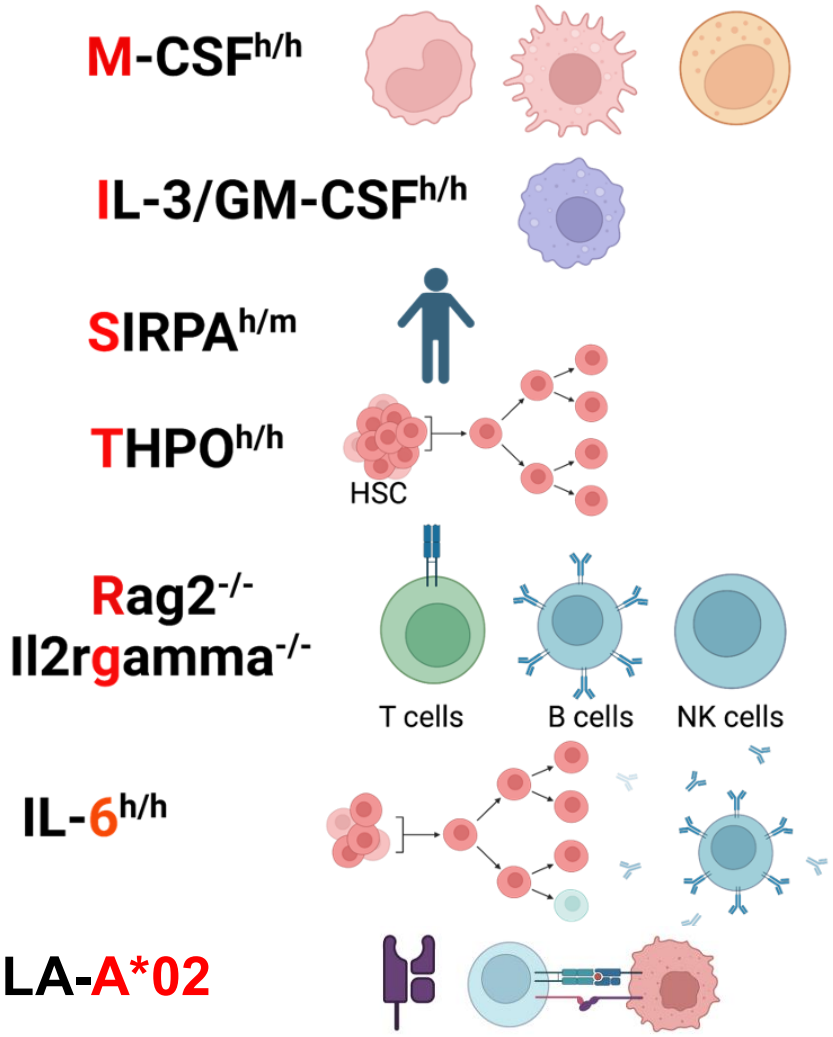
Human TPO KI

Rongvaux et al., PNAS 2011

Hematopoietic stem cells
Multilineage differentiation

MISTRG6-A2 supports a diverse human immune system

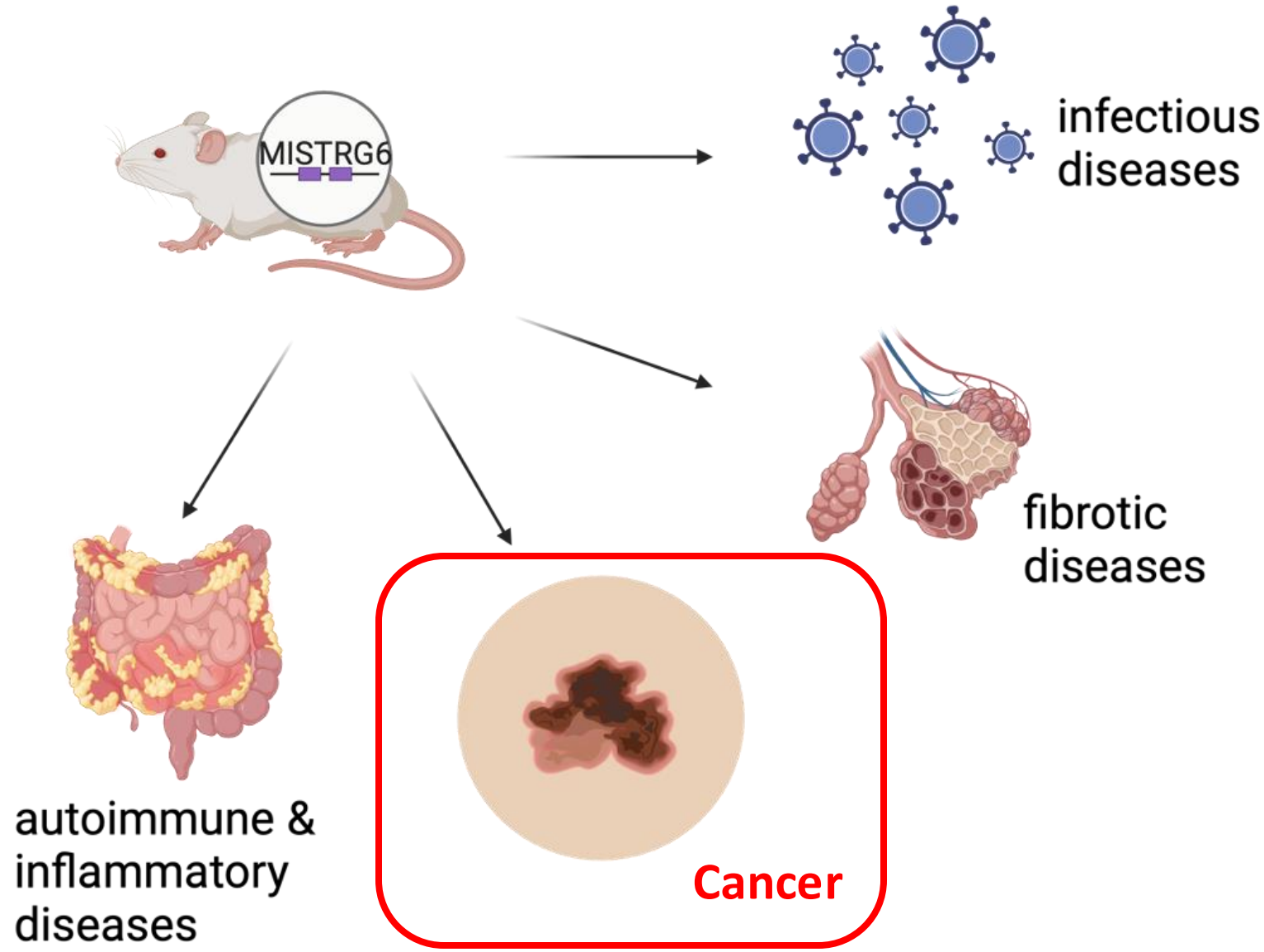




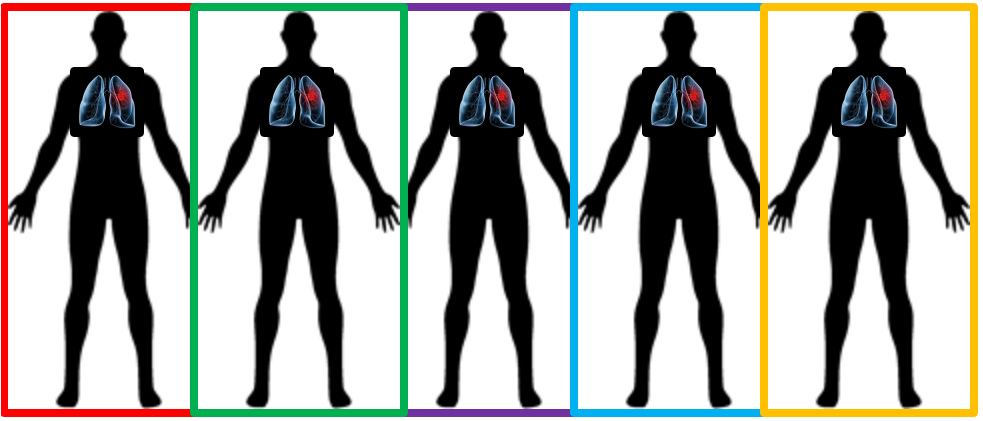
- Monocytes
- Tissue macrophages
- NK cells (via IL15)
- Alveolar macrophages
- Xeno-tolerance
- Maintenance of hematopoietic stem cells (HSC)
- Immuno-deficiency
- No mouse T, B, NK cells
- More efficient hematopoiesis
- B cell response
- Peptide presentation on human MHC I

Rongvaux et al., Nature Biotechnology, 2014
Das et al., Nature Medicine, 2016
Yu et al., Blood, 2017
Chiorazzi et al., JITC, 2023

Humanized mice have been instrumental in understanding disease mechanisms in multiple contexts.

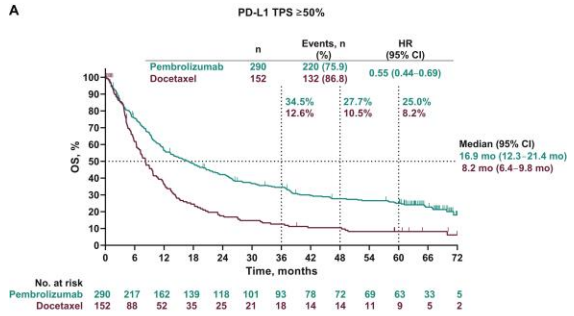


Individuals respond differently to TME-targeting drugs



Our ability to predict outcomes is limited by a lack of pre-clinical models recapitulating the human tumor microenvironment

Therapy →

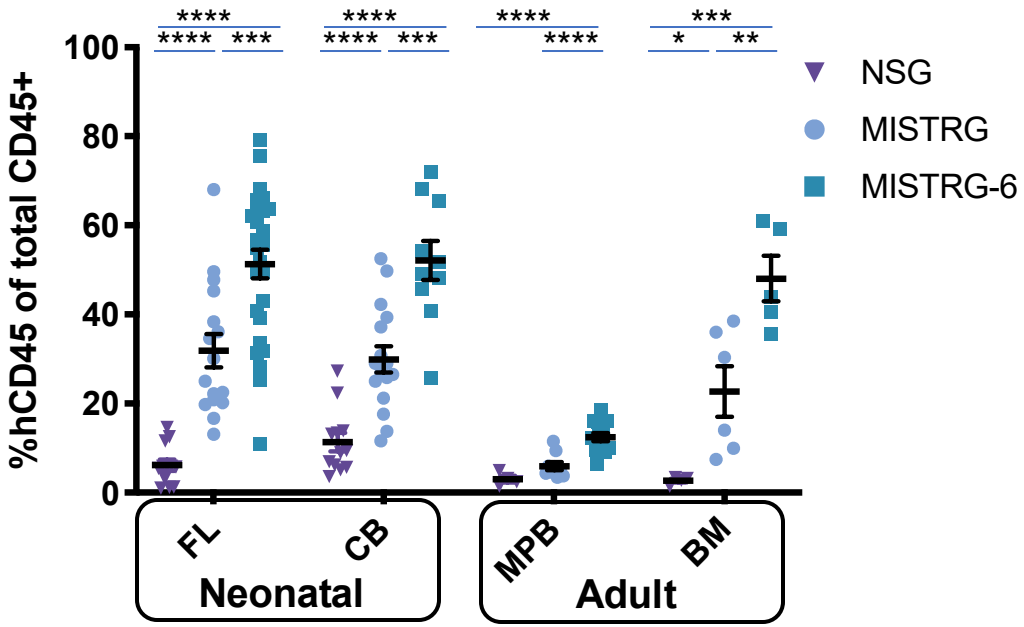


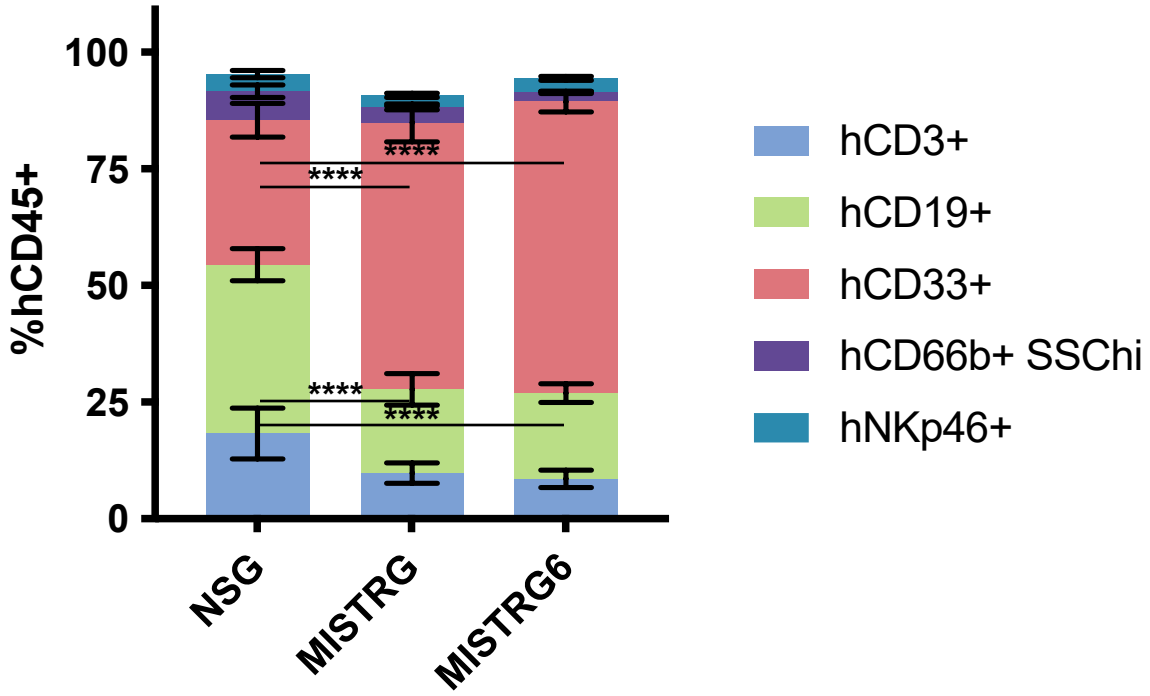
Outcome

- Genetics:
  - Host
  - Tumor
- Environment/Physiology:
  - Infections – HPV, HIV, SARS-Cov-2
  - Microbiota

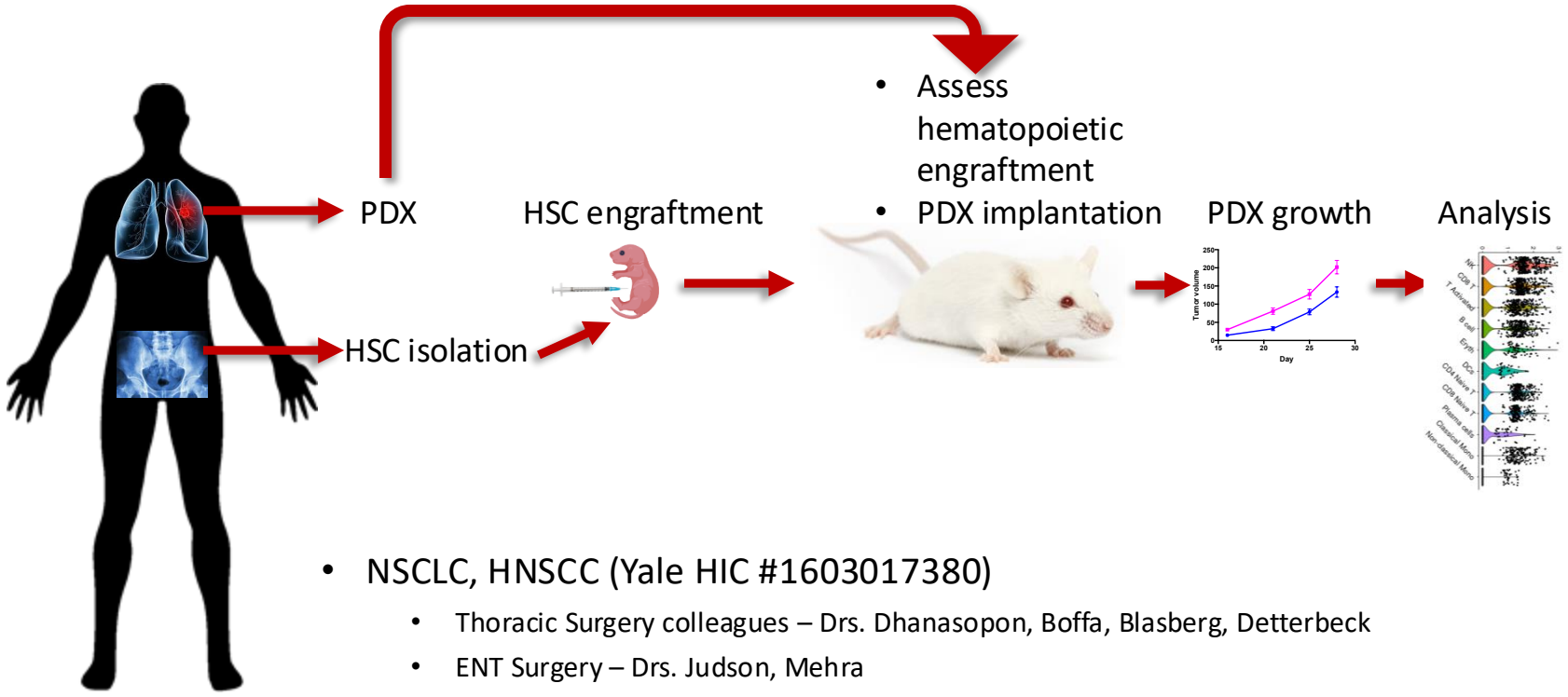
- Local Tumor Microenvironment (TME):
  - Cell types
  - Cell states
  - Cell interactions
  - Soluble factors

Humanization of the IL-6 locus enhances human hematopoietic engraftment in MISTRG6 mice





Autologous humanized PDX studies

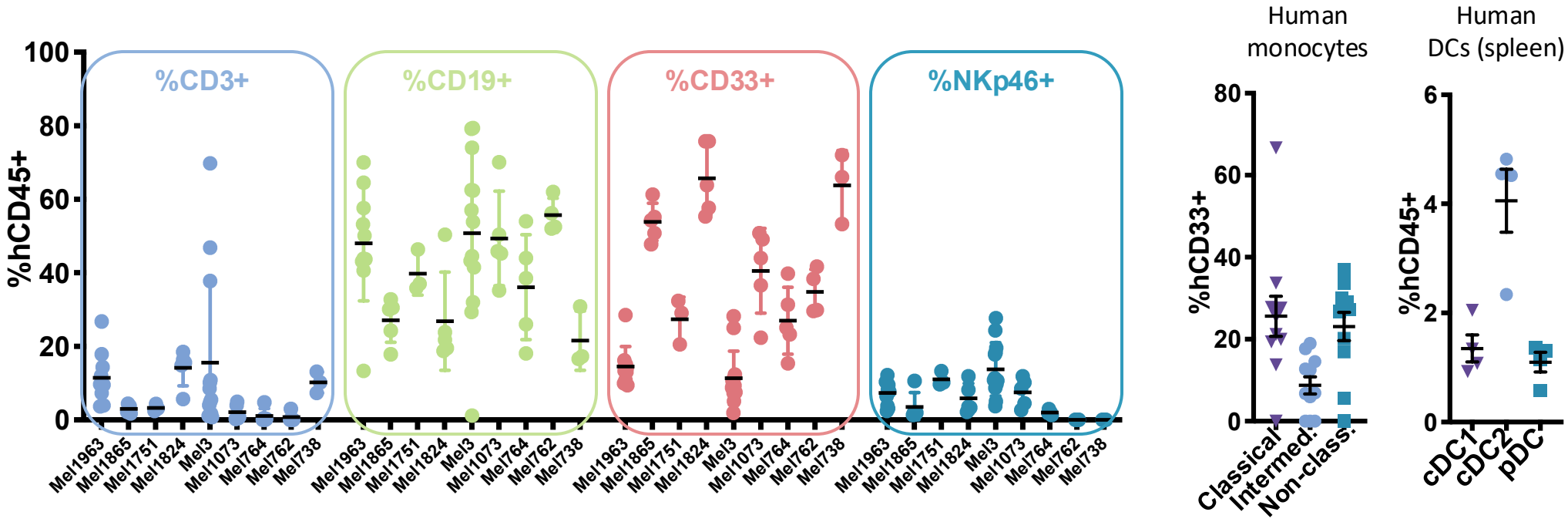


- NSCLC, HNSCC (Yale HIC #1603017380)
  - Thoracic Surgery colleagues – Drs. Dhanasopon, Boffa, Blasberg, Detterbeck
  - ENT Surgery – Drs. Judson, Mehra
- Melanoma patients from vaccine trial with Karolina Palucka's lab (Jackson Labs)
- Melanoma and pancreatic cancer patients with Ryan Fields' lab (WashU)

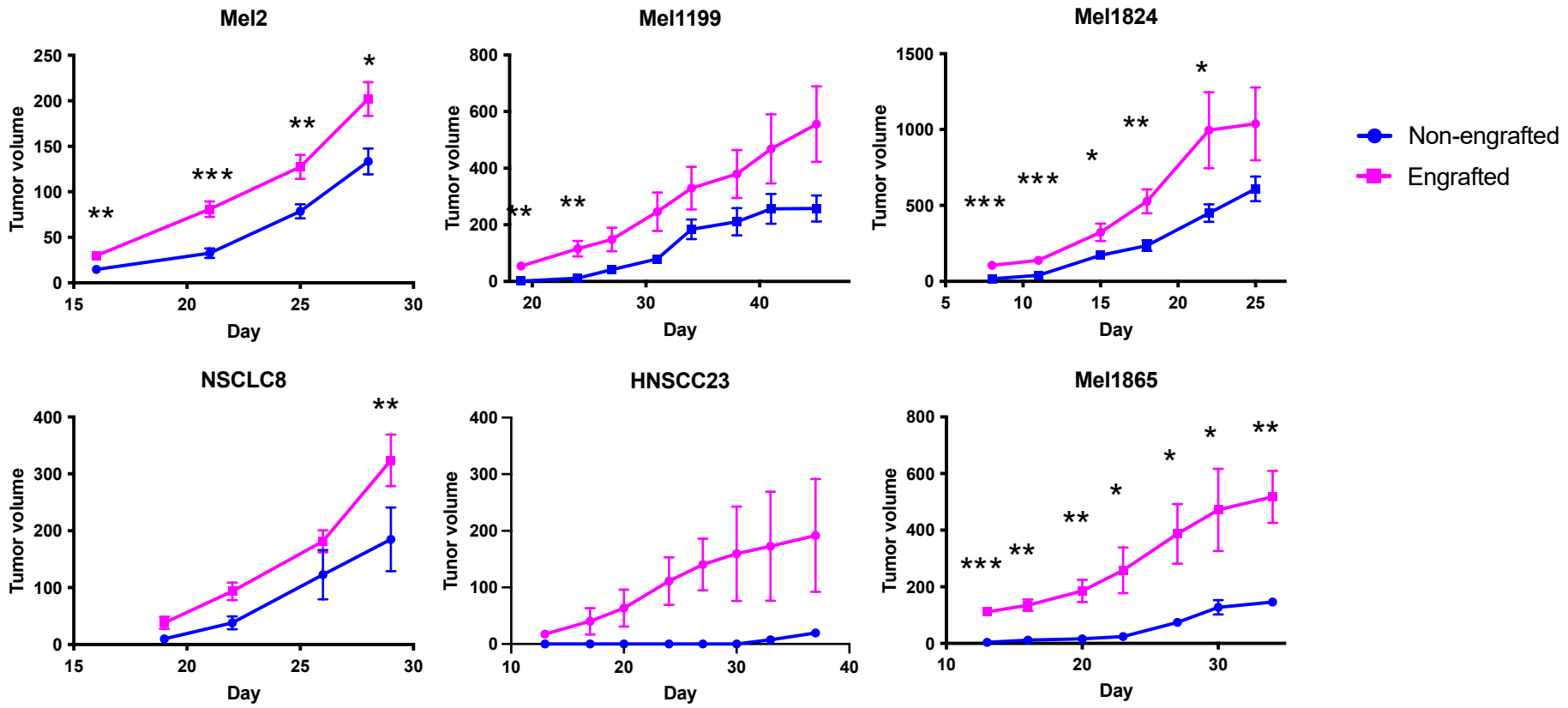
Prospective collection of tumor tissue and BM aspirate for autologous PDX studies

Patient	Age	Sex	Tumor Type	Diagnosis	CD34+ cell #	PDX?
1	65	F	NSCLC	Stage IA adenocarcinoma	<100k	
2	73	F	NSCLC	Stage IA adenocarcinoma	150k	
3	82	F	NSCLC	Stage IA squamous cell carcinoma	3.9 million	Yes
4	56	M	NSCLC	Stage IIB large cell carcinoma	4 million	
5	70	F	NSCLC	Mucoepidermoid carcinoma	5.1 million	
6	57	F	NSCLC	Adenosquamous carcinoma	7 million	
7	70	M	NSCLC	Stage IB adenocarcinoma	6.5 million	Yes
8	62	F	NSCLC	Stage IA adenocarcinoma	6 million	Yes
9	79	M	NSCLC	Stage IIA squamous cell carcinoma	3.7 million	Yes
10	71	M	NSCLC	Stage IA adenocarcinoma	14 million	
11	63	F	NSCLC	Stage IA adenocarcinoma	8.2 million	
12	79	F	NSCLC	Stage IV squamous cell carcinoma	2.7 million	Yes
13	54	F	NSCLC	Stage II adenocarcinoma	5.0 million	
14	57	M	NSCLC	Stage IV NSCLC	2.5 million	
15	72	M	NSCLC	Stage IV adenocarcinoma	3.6 million	Yes
16	74	M	NSCLC	Stage IV adenocarcinoma	3.4 million	
17	73	M	NSCLC	Stage IV neuroendocrine tumor	6.6 million	
18	72	F	NSCLC	Stage IV NSCLC	4 million	
19	79	F	NSCLC	Stage IV NSCLC	2.7 million	Yes
20	78	F	HNSCC	Stage II HNSCC of lateral tongue	1 million	Yes
21	63	F	HNSCC	Stage IVa HNSCC of buccal mucosa	2 million	Yes
22	46	F	Melanoma	Stage IV melanoma	6 million	
23	70	F	Melanoma	Stage IIIc melanoma	250k	
24	62	M	Melanoma	Stage IV melanoma	2.2 million	
25	67	M	Melanoma	Melanoma	1.8 million	
26	61	M	Melanoma	Stage IIIc melanoma	100k	
27	34	M	Melanoma	Melanoma	1.9 million	
28	61	F	Melanoma	Stage IV melanoma	1 million	
29	57	M	Melanoma	Stage IV melanoma	2.5 million	Yes
30	55	F	Melanoma	Melanoma	2 million	
31	42	M	Melanoma	Stage IV melanoma	4.8 million	Yes
32	22	M	Melanoma	Stage IIIc melanoma	3 million	
33	52	F	Melanoma	Stage IIIb melanoma	4 million	Yes
34	65	M	Melanoma	Stage IV melanoma	n/a	
35	71	F	Melanoma	Melanoma	500k	
36	53	M	Melanoma	Stage IIIc melanoma	600k	
37	71	M	Melanoma	Melanoma	80k	
38	65	M	Melanoma	Stage IIIc melanoma	1.5 million	
39	73	M	Melanoma	Stage IIIb melanoma	3.4 million	
40	65	M	Melanoma	Stage IIIb melanoma	1.8 million	
41	43	F	Melanoma	Stage IIIc melanoma	3.6 million	
42	50	F	Melanoma	Stage IIIc melanoma	7 million	
43	42	M	Melanoma	Stage IIIc melanoma	3.6 million	Yes
44	54	F	PDAC	Pancreatic adenocarcinoma	1.2 million	
45	48	M	PDAC	Pancreatic adenocarcinoma	2.5 million	Yes
46	48	M	Melanoma	Stage IV melanoma	2.1 million	Yes
47	67	M	PDAC	Pancreatic adenocarcinoma	1.6 million	Yes
48	85	F	PDAC	Pancreatic adenocarcinoma	6.8 million	Yes
49	80	M	Melanoma	Stage IIIc melanoma	1.95 million	Yes
50	75	F	Melanoma	Stage IIIb melanoma	5.5 million	
51	51	M	Melanoma	Stage IIIc melanoma	8.5 million	Yes
52	74	M	Melanoma	Stage IV melanoma	5 million	Yes
53	35	M	Melanoma	Stage IIIc melanoma	6.1 million	
54	81	M	Melanoma	Stage IIIb melanoma	3 million	
55	63	M	Melanoma	Stage IIIc melanoma	3.1 million	Yes
56	35	M	Melanoma	Stage IIId melanoma	3 million	
57	60	M	Melanoma	Stage IV melanoma	1.4 million	
58	64	F	Melanoma	Stage IV melanoma	2.5 million	
59	57	M	Melanoma	Stage IIIc melanoma	4.5 million	
60	51	F	Melanoma	Stage IIIc melanoma	2.6 million	
61	58	F	Melanoma	Stage IV melanoma	2.4 million	
62	48	M	Melanoma	Stage IIIc melanoma	1.76 million	Yes
63	68	M	Melanoma	Stage IV melanoma	4 million	Yes
64	30	M	Melanoma	Stage IIIc melanoma	12 million	
65	79	M	Melanoma	Stage III melanoma	2.8 million	Yes
66	47	M	Melanoma	Stage IIIc melanoma	2.4 million	
67	42	M	Melanoma	Stage IIIb melanoma	4.6 million	Yes
68	83	M	Melanoma	Stage IIId melanoma	2.8 million	
69	33	M	Melanoma	Stage IIId melanoma	4.8 million	
70	58	M	Melanoma	Stage IIIc melanoma	1.8 million	
71	57	F	Melanoma	Stage IIIc melanoma	820k	

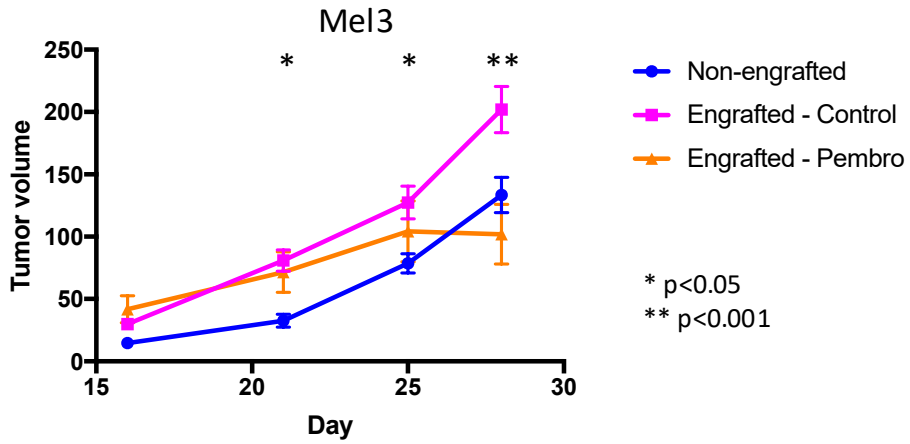
MISTRG6 mice support development of human immune subsets from patient HSPCs



Autologous PDXs display enhanced growth in MISTRG6 mice

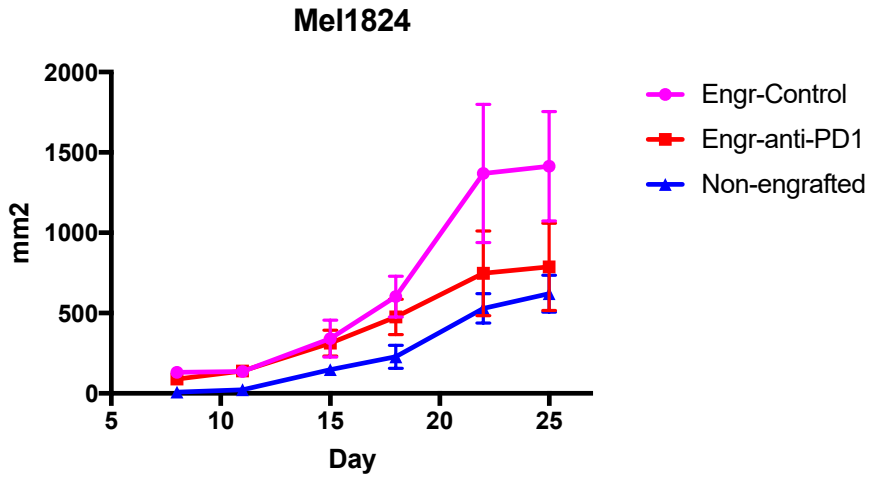


Anti-PD1 antibodies are active in autologous MISTRG6 mice

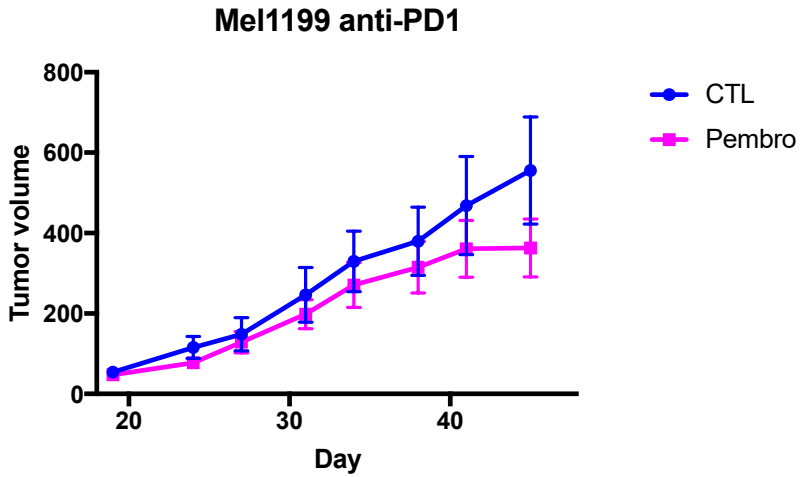


Non-engrafted
Engrafted - Control
Engrafted - Pembro

* p<0.05
** p<0.001

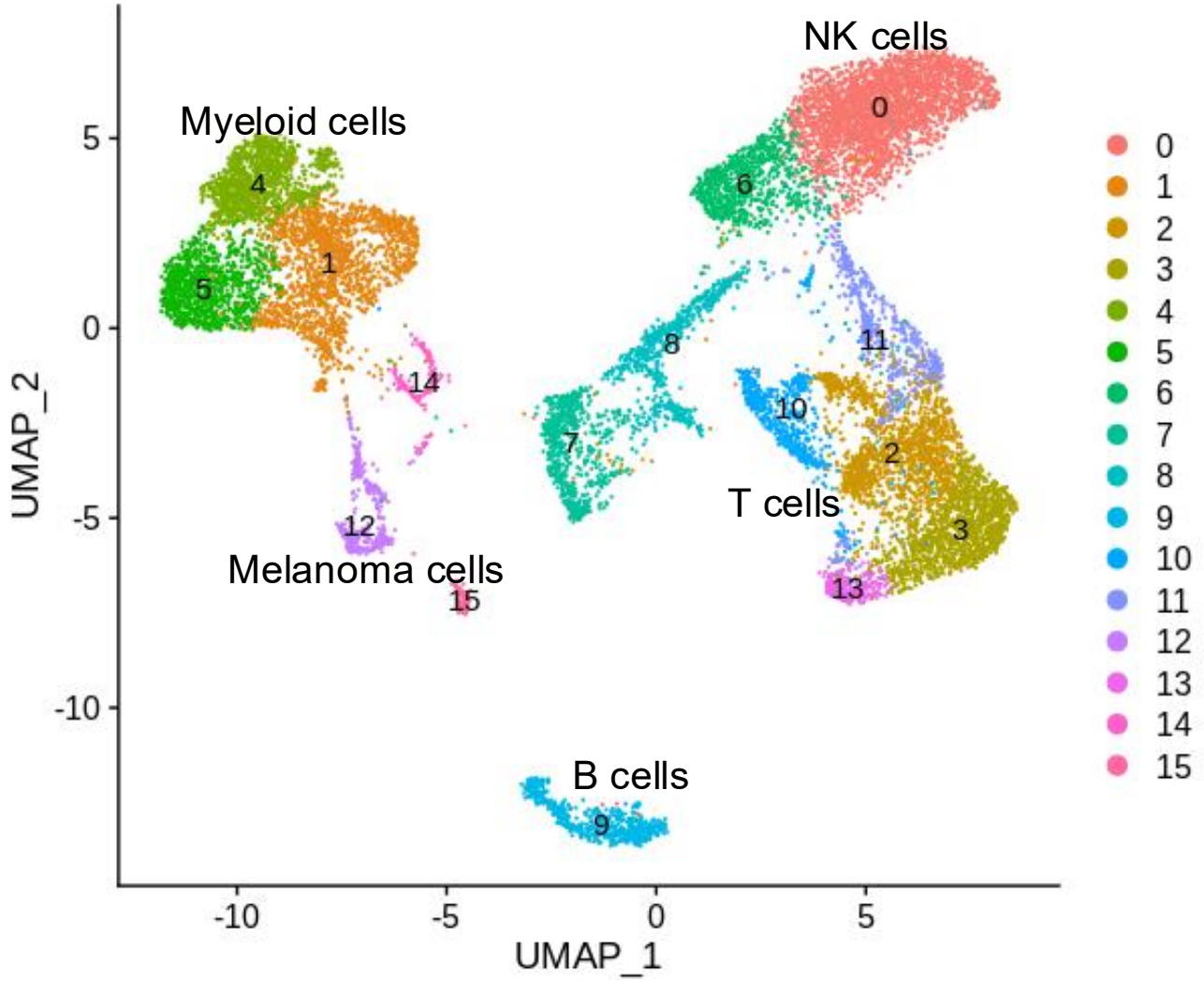


Engr-Control
Engr-anti-PD1
Non-engrafted



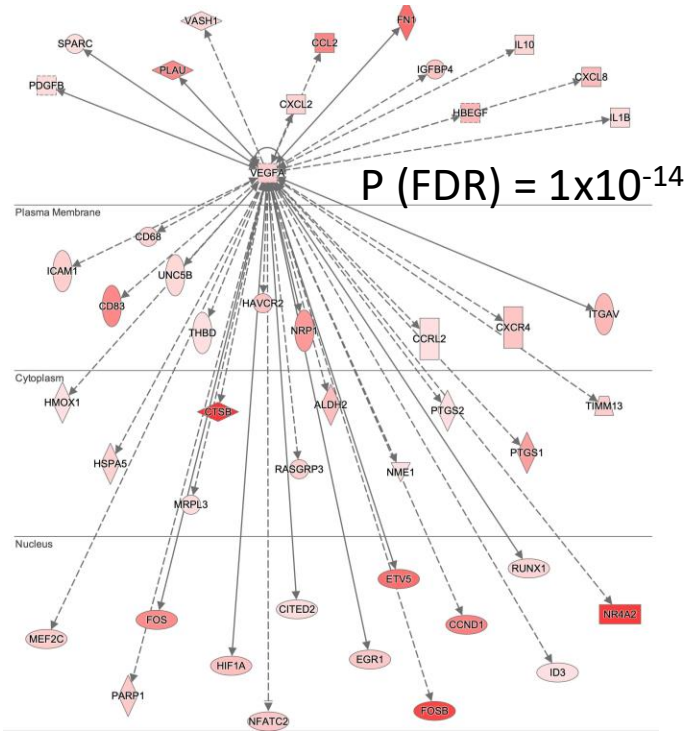
CTL
Pembro

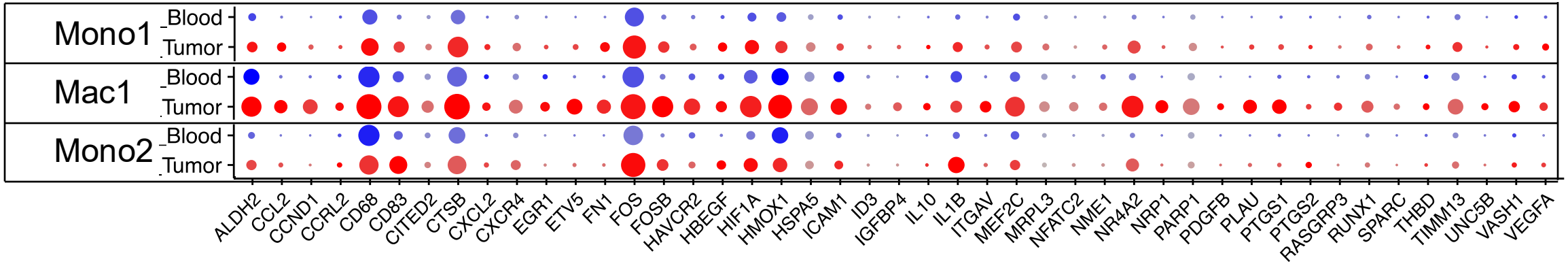
Single cell genomics to characterize the autologous MISTRG6 TME



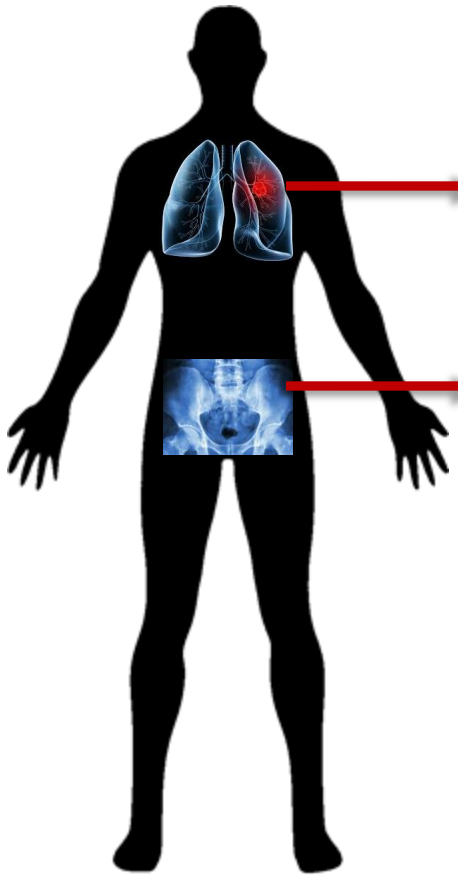
0: NK cells 1
1: Myeloid 1 (monocytes)
2: CD4 T cells 1 (activated)
3: CD4 T cells 2 (naïve)
4: Myeloid 2 (macrophages)
5: Myeloid 3 (monocytes)
6: NK cells 2
7: Cycling lymphocytes 1
8: Cycling lymphocytes 2
9: B cells
10: CD4 T cells 3
11: CD8 T cells 1 (activated)
12: Melanoma cells 1
13: CD8 T cells 2 (naïve)
14: Melanoma cells 2 (cycling)
15: Melanoma cells 3

Ingenuity Pathway Analysis (IPA) identifies VEGF-A as key upstream regulator in TME



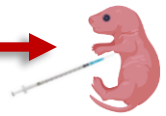


Testing influence of VEGF-A on tumor growth in autologous models



PDX generation

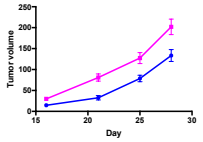
HSC isolation



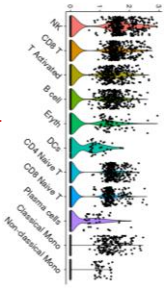
Control

Bevacizumab
(anti-VEGF)

Randomize
to drug therapies

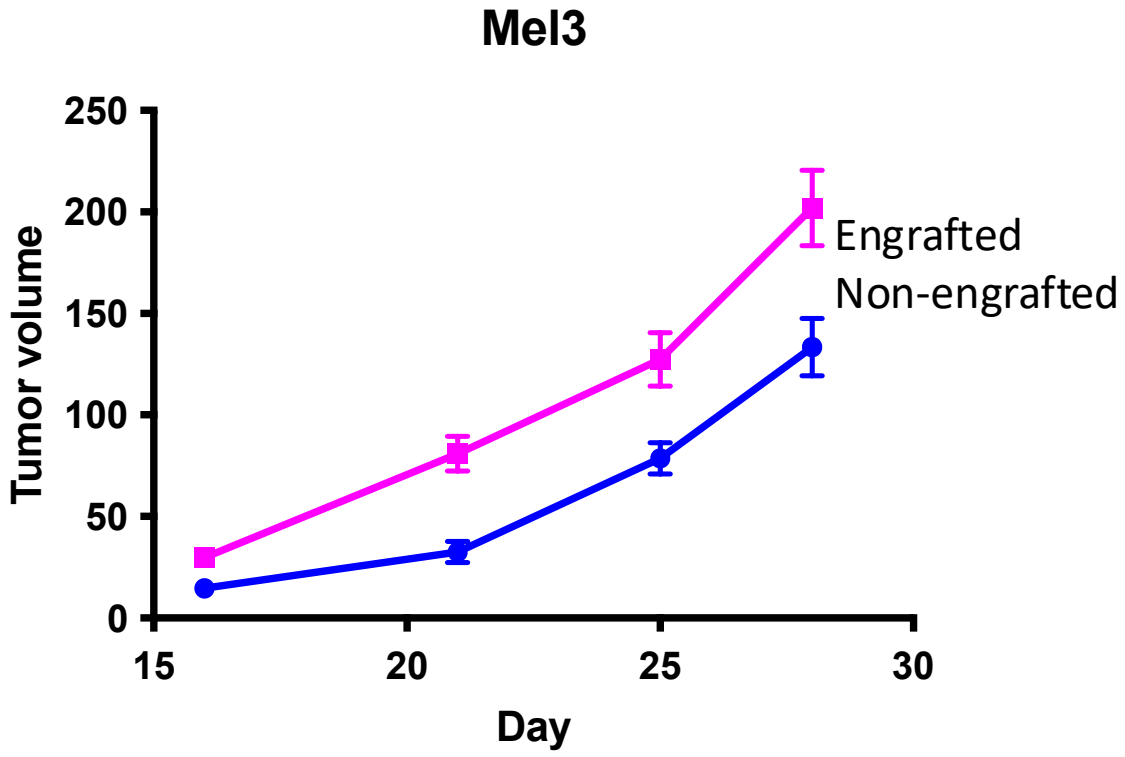


PDX growth

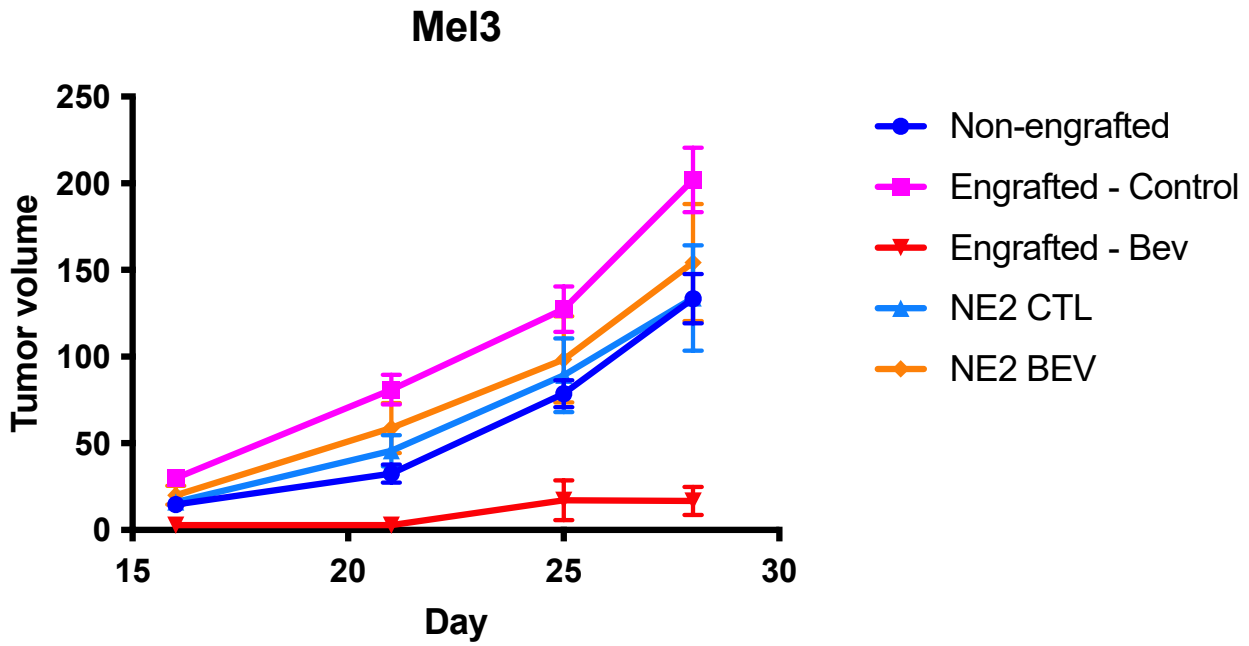


Analysis

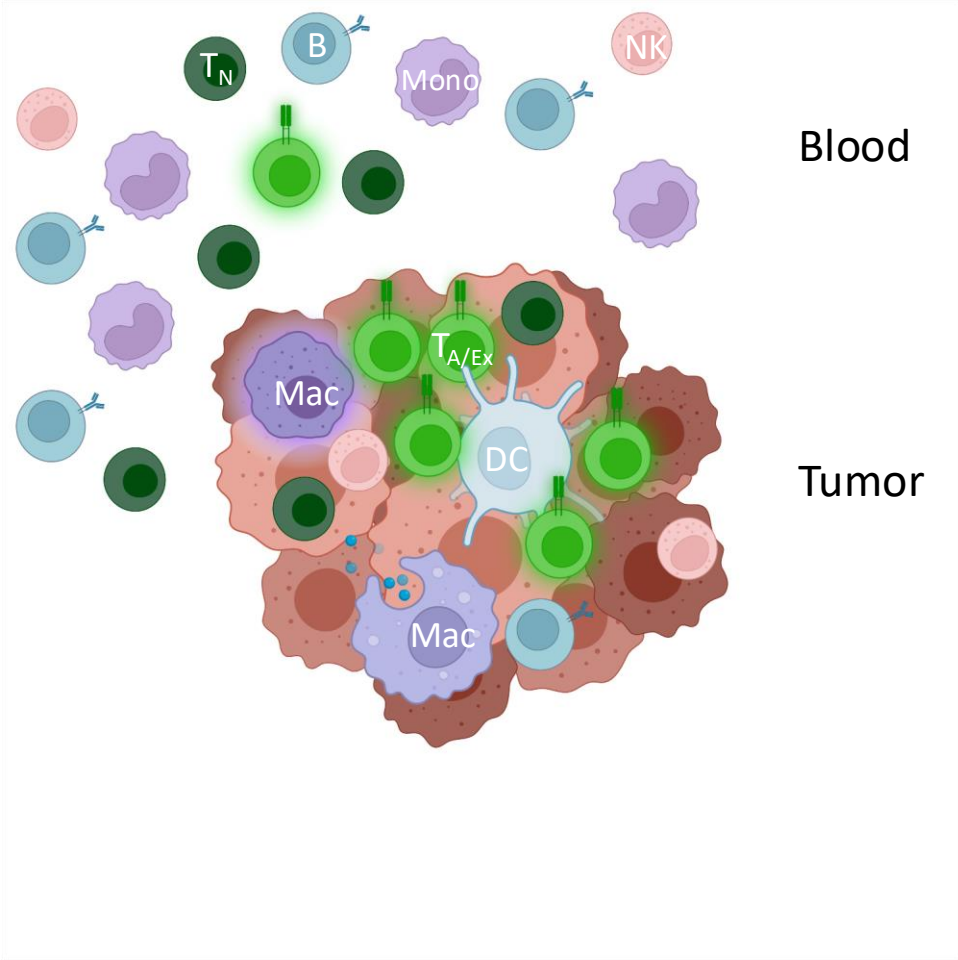
VEGF-A inhibition with bevacizumab abrogates enhanced tumor growth in autologously-engrafted MISTRG6 mice



VEGF-A inhibition with bevacizumab abrogates enhanced tumor growth in autologously-engrafted MISTRG6 mice

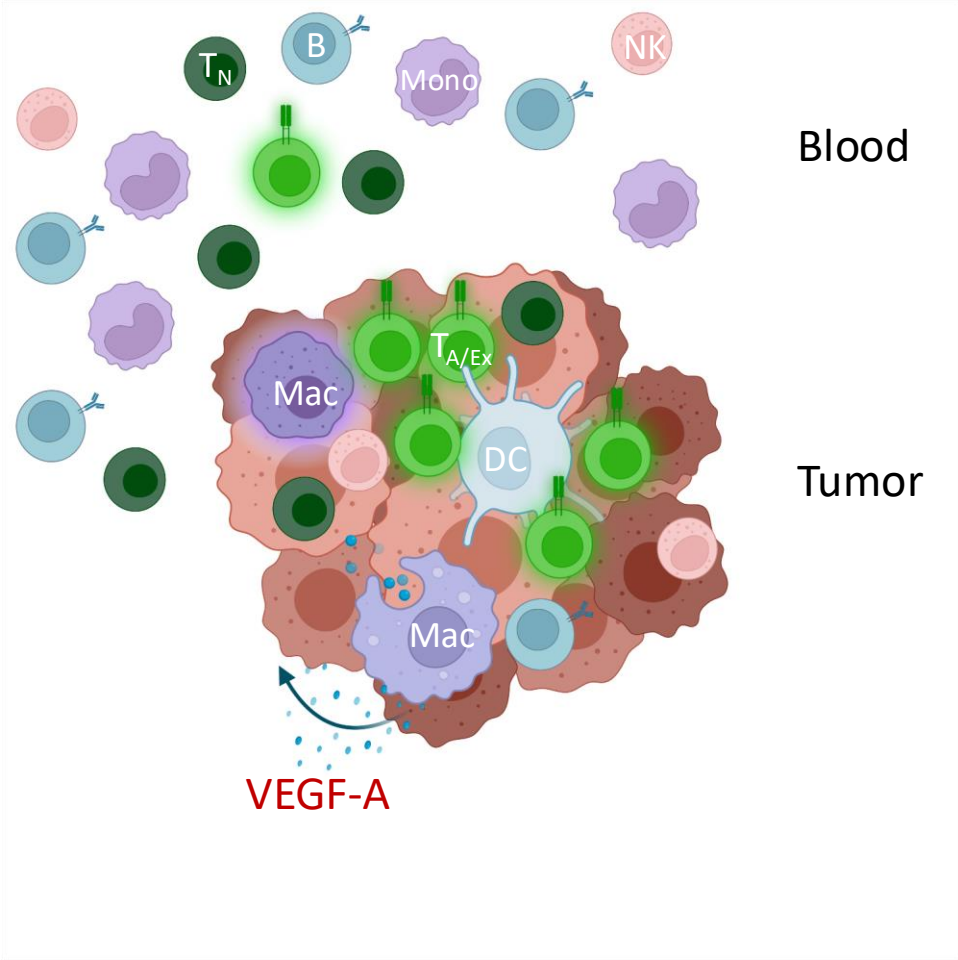


The autologous MISTRG6 TME recapitulates major features of human tumors for study of human cellular circuits



The autologous MISTRG6 TME recapitulates major features of human tumors for study of human cellular circuits

- Fully genetically matched human system bearing innate and adaptive immune cells from an individual patient
- T cell activation/exhaustion in TME
- Monocyte/macrophage transition from blood to tumor
- Presence of innate immune cells: DCs, macrophages, NK cells in TME
- Response to therapies

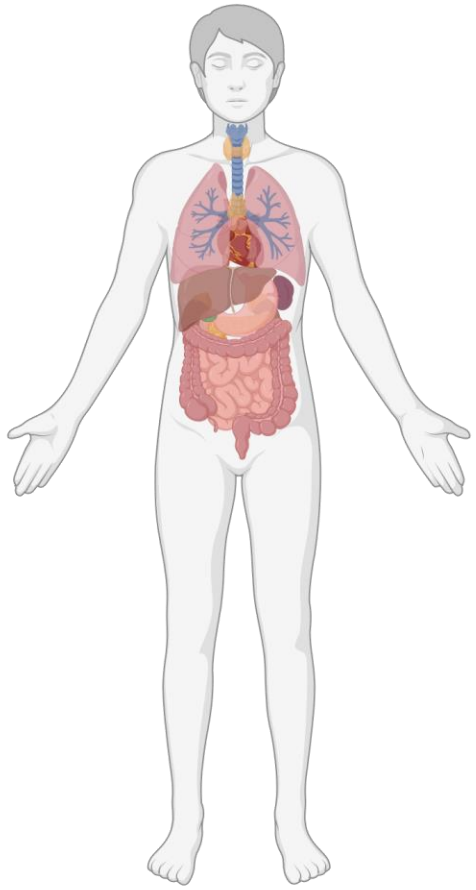


<u>Limitations</u>:

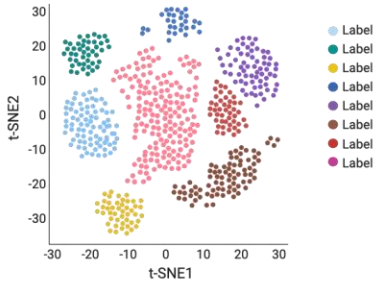
- Lifespan
- T cell education
- Irradiation
- HSC availability
- PDX generation

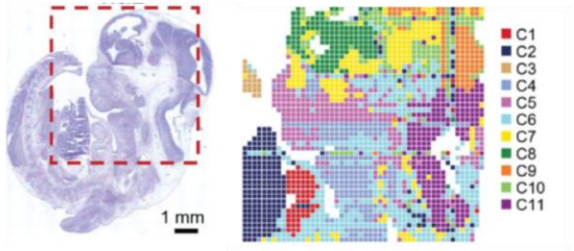
Advances in genomic and proteomic technologies and bioinformatics have improved our ability to study humans.

Patient



Multi-omics





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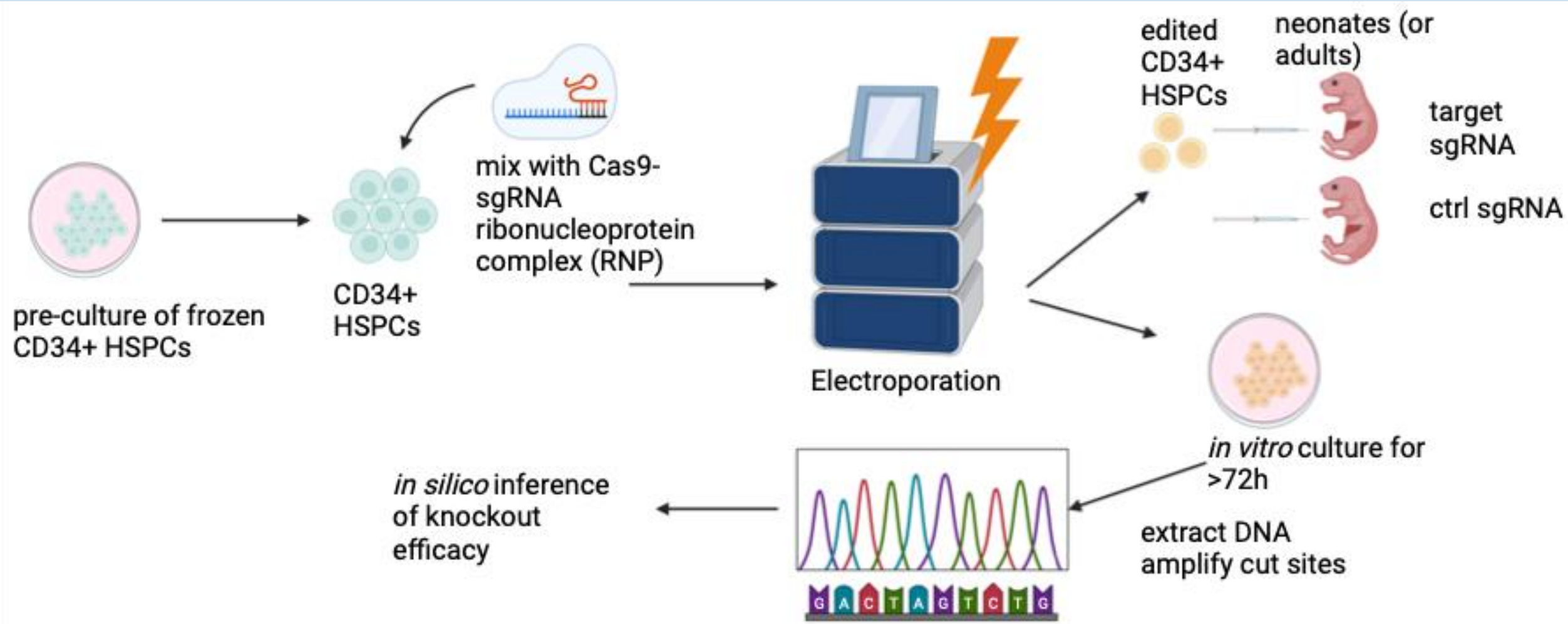
In vivo perturbations

Humanized mice

- Cause & effect
- HSPCs+/- genes
- Mechanism

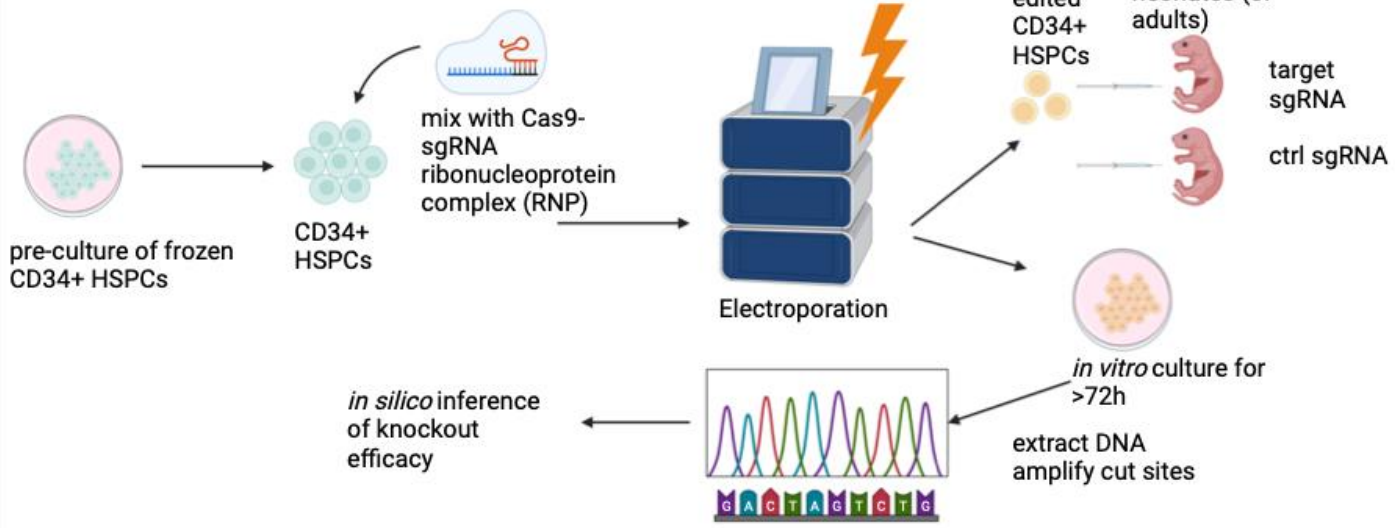
Perturbations in vivo help bridge the human data to causality.

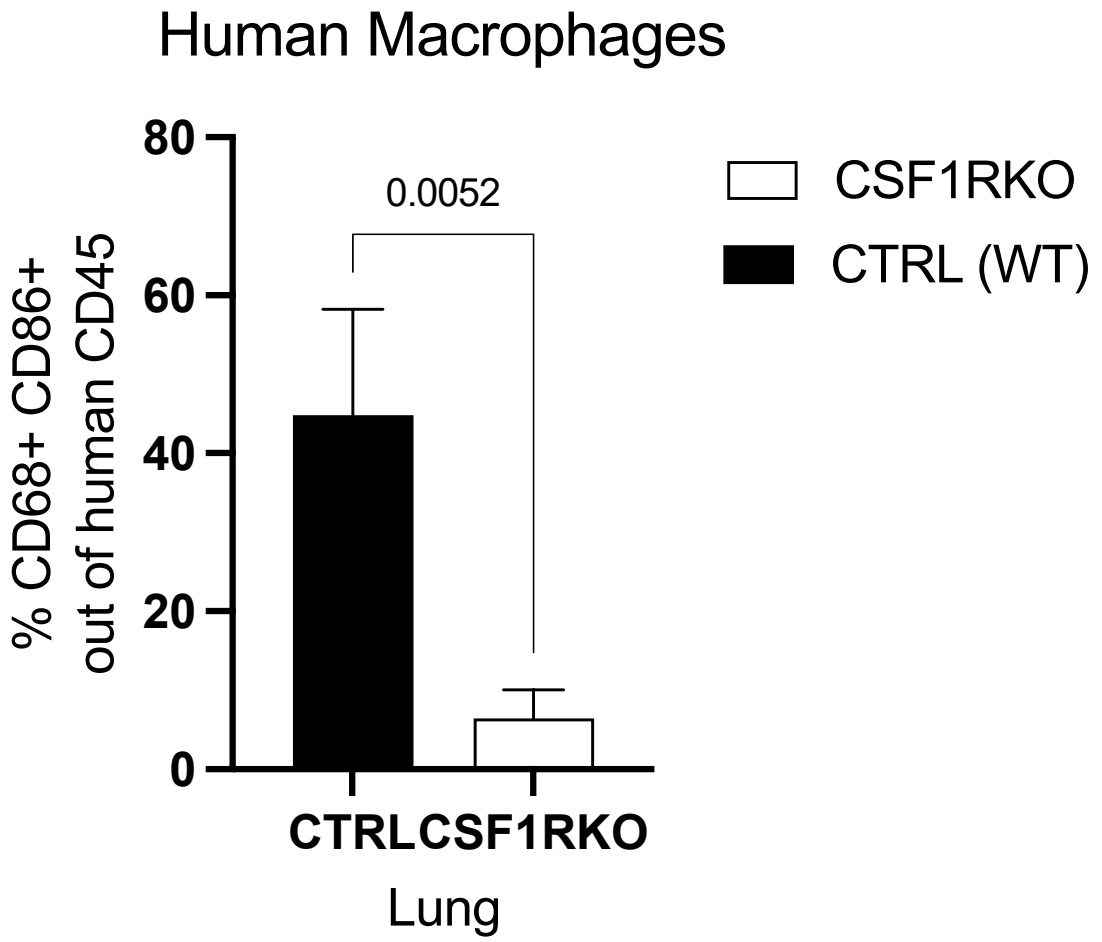
Mice engrafted with HSPCs edited for loss of *CSFR1* do not have monocyte-derived macrophages.



with Tianli Xiao and Liang Shan

Mice engrafted with HSPCs edited for loss of *CSFR1* do not have monocyte-derived macrophages.

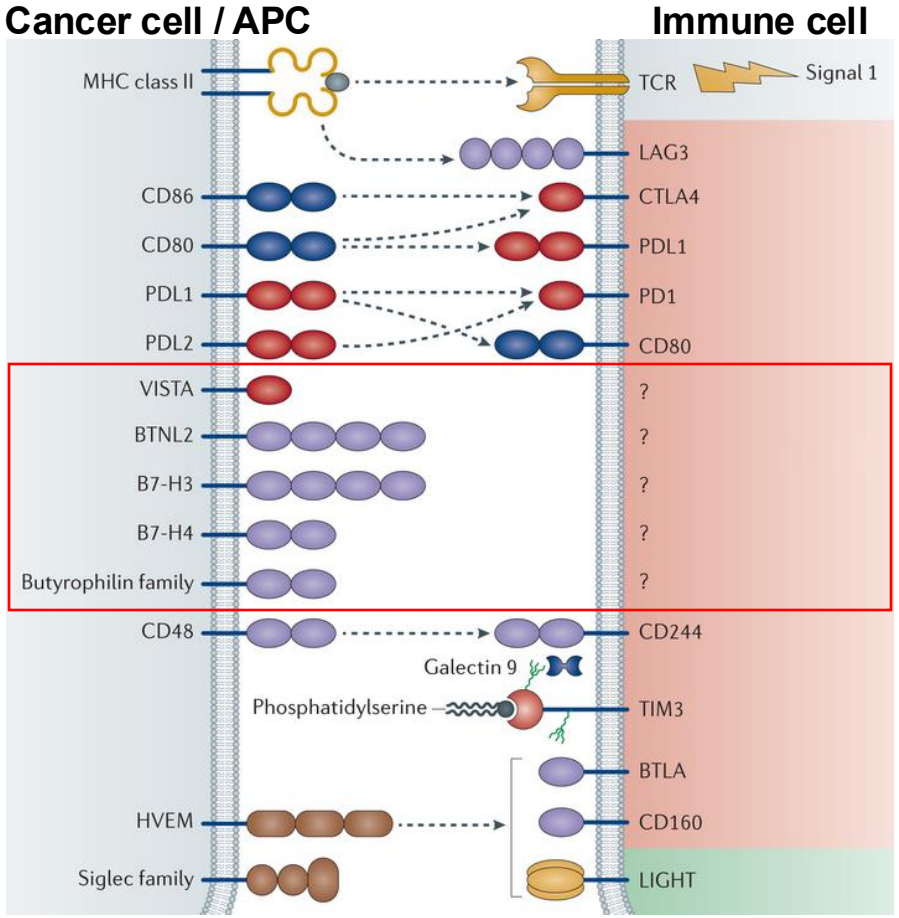




with Tianli Xiao

Ongoing projects

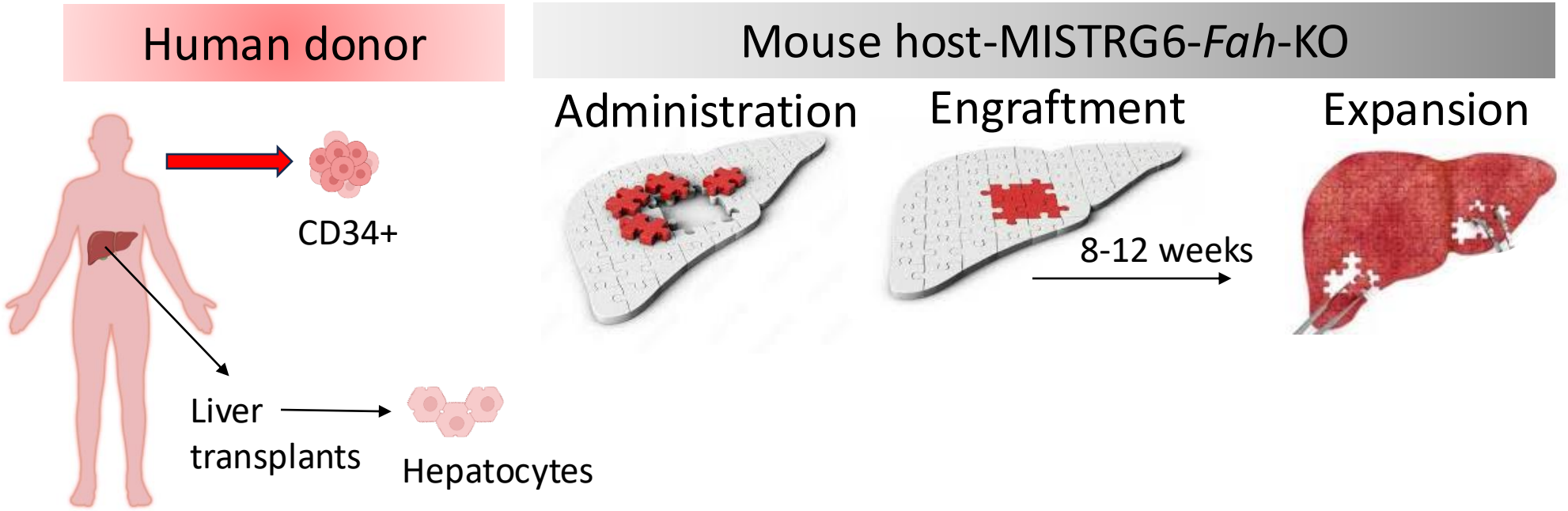
1. In search of novel innate immune checkpoints



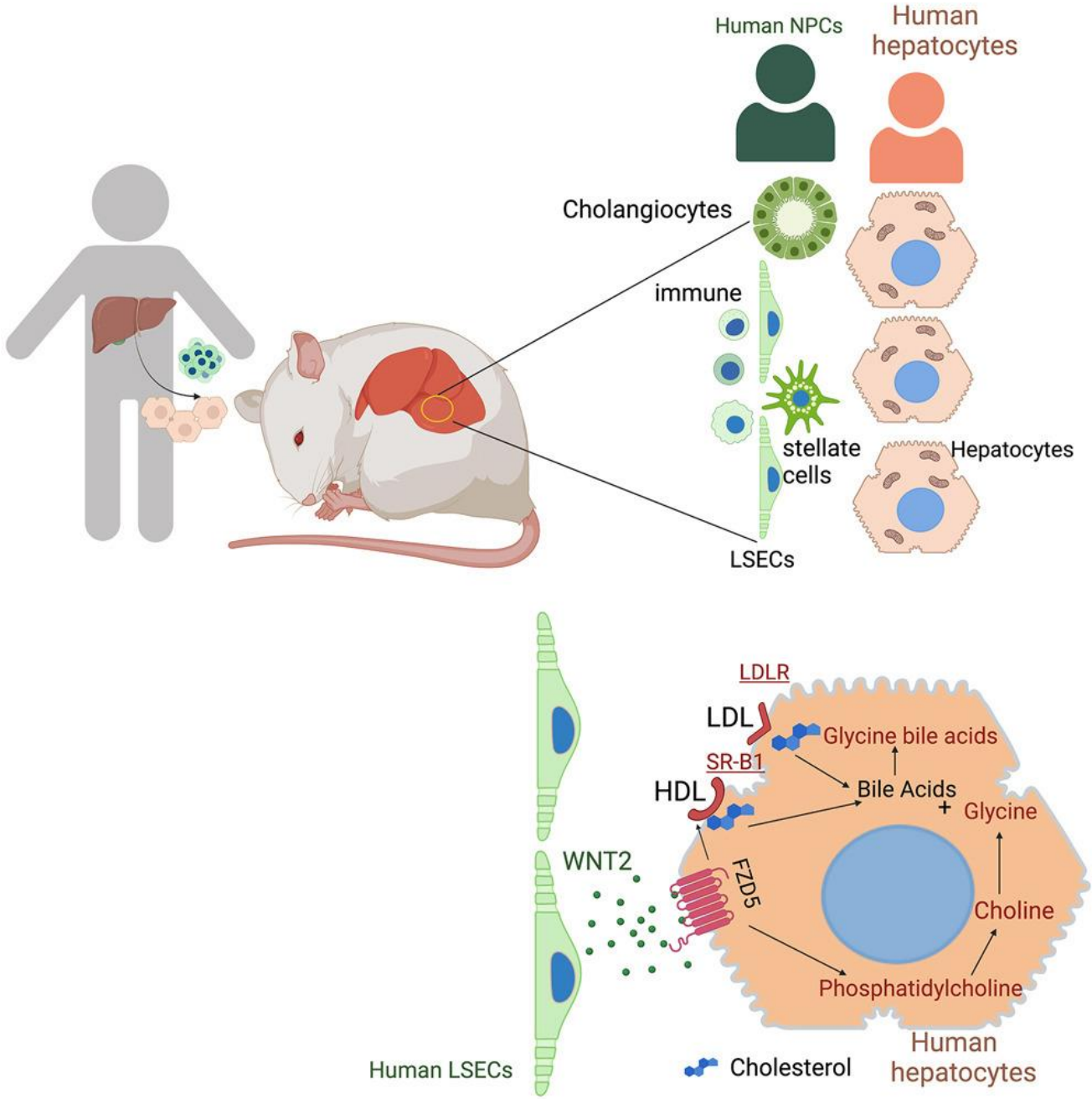
(Mahoney *et al.*, *Nat Rev Drug Discov*, 2015)

Takeshi Ito

Combined Immune cell and and hepatocyte humanization

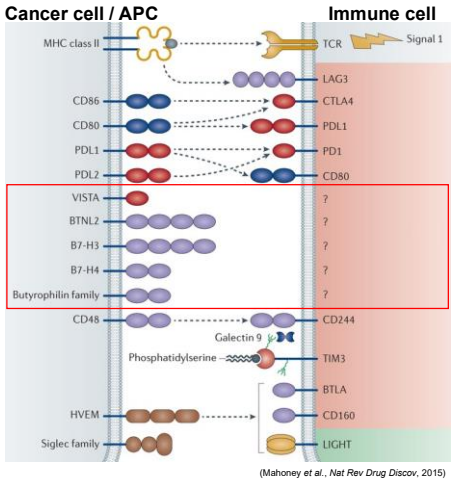


Kaffe et al, Cell 2023



Kaffe et al, Cell 2023

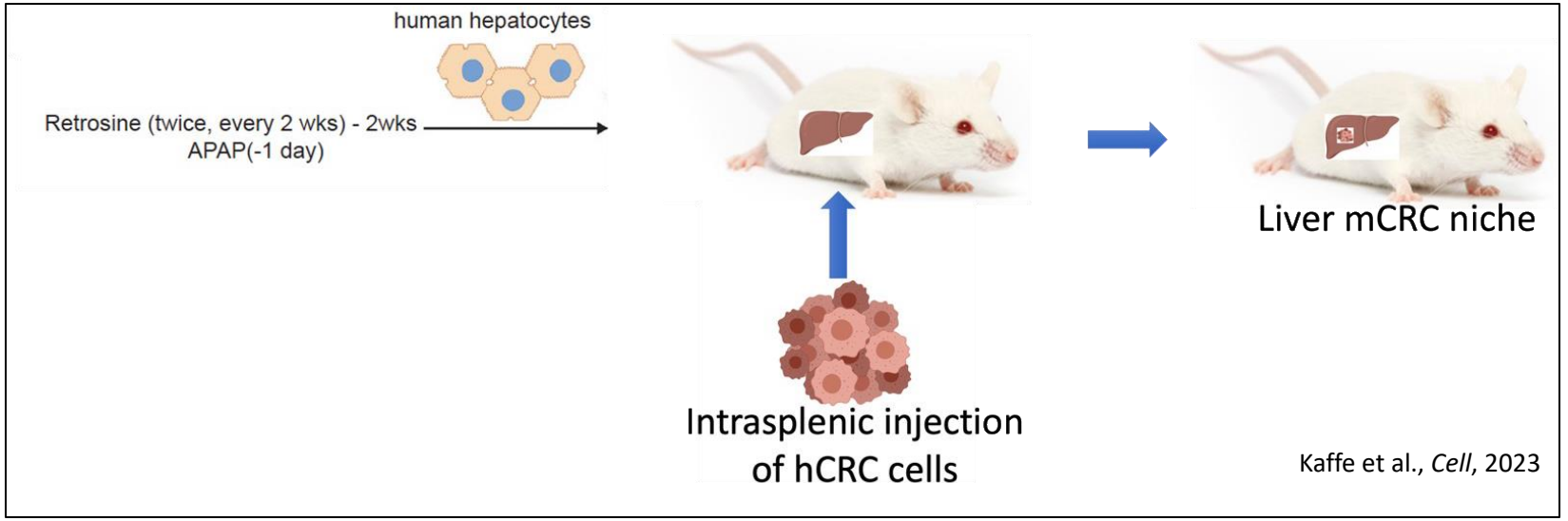
Ongoing projects



(Mahoney *et al.*, *Nat Rev Drug Discov*, 2015)

Orthotopic- humanized models

Humanized liver model of metastatic CRC



Kaffe et al., *Cell*, 2023

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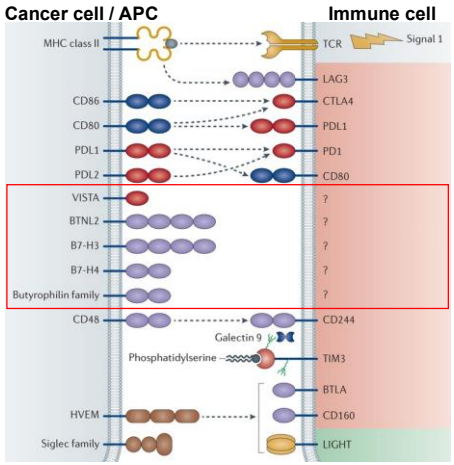
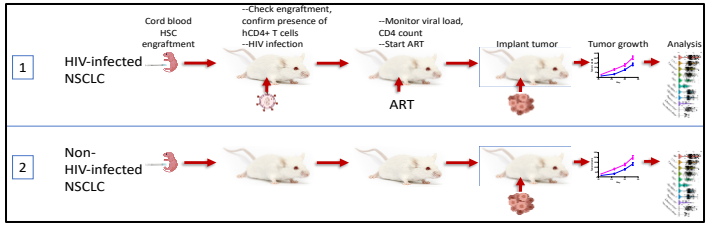
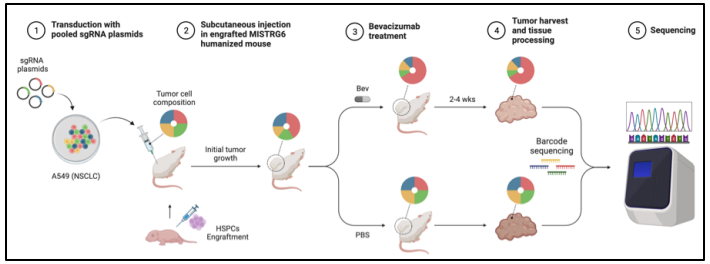
Ongoing projects

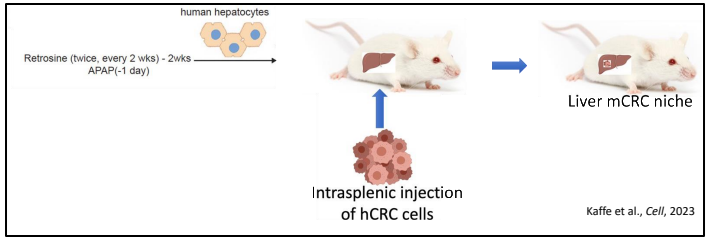
1. In search of novel innate immune checkpoints
2. Pre-clinical modeling of HIV-associated NSCLC
3. In vivo CRISPR screening for novel anti-VEGF co-therapies
4. Humanized liver model of mCRC



(Mahoney *et al.*, *Nat Rev Drug Discov*, 2015)







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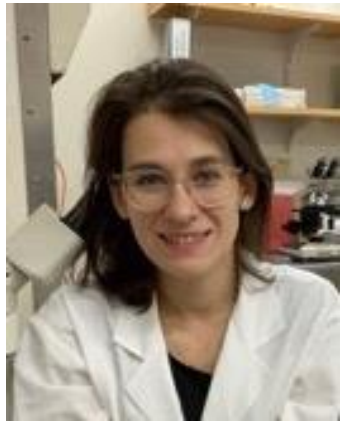

Esen Sefik

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Thank you!

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