Development of regenerative immunotherapy with induced pluripotent stem cell technology

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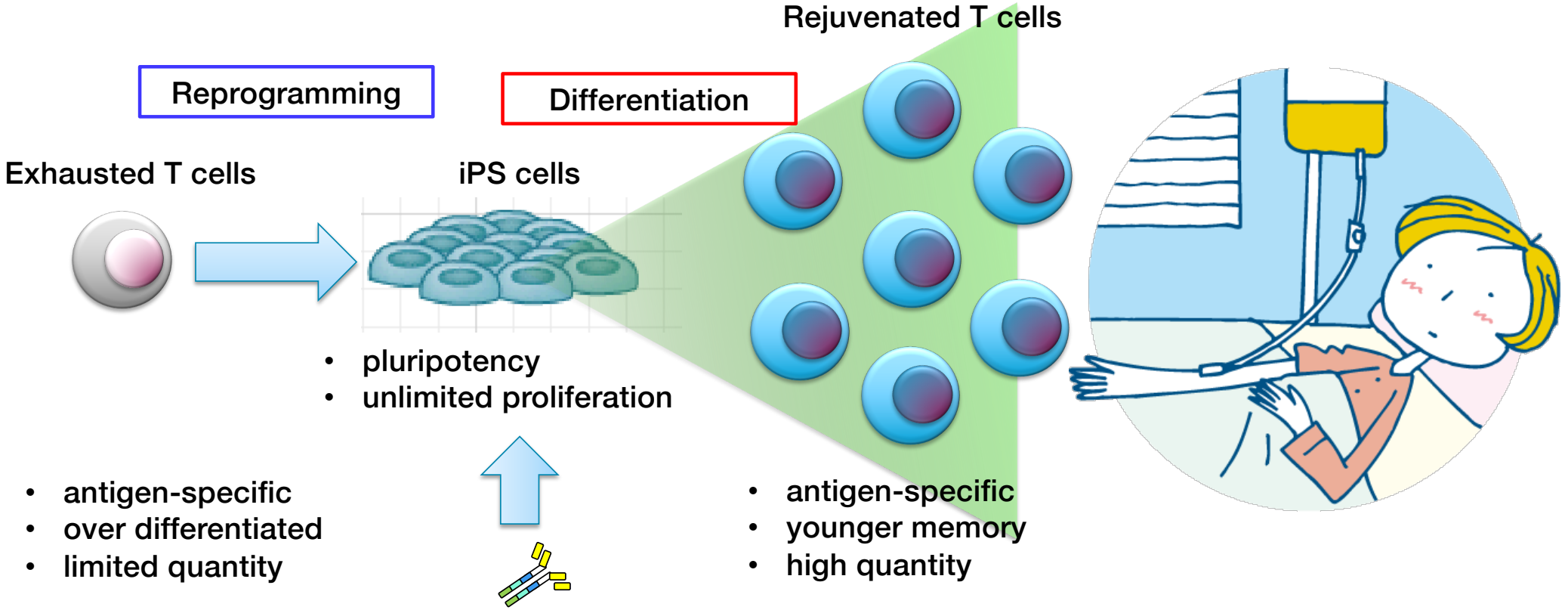
Regeneration gives youth and clonality to anti-cancer immune cells

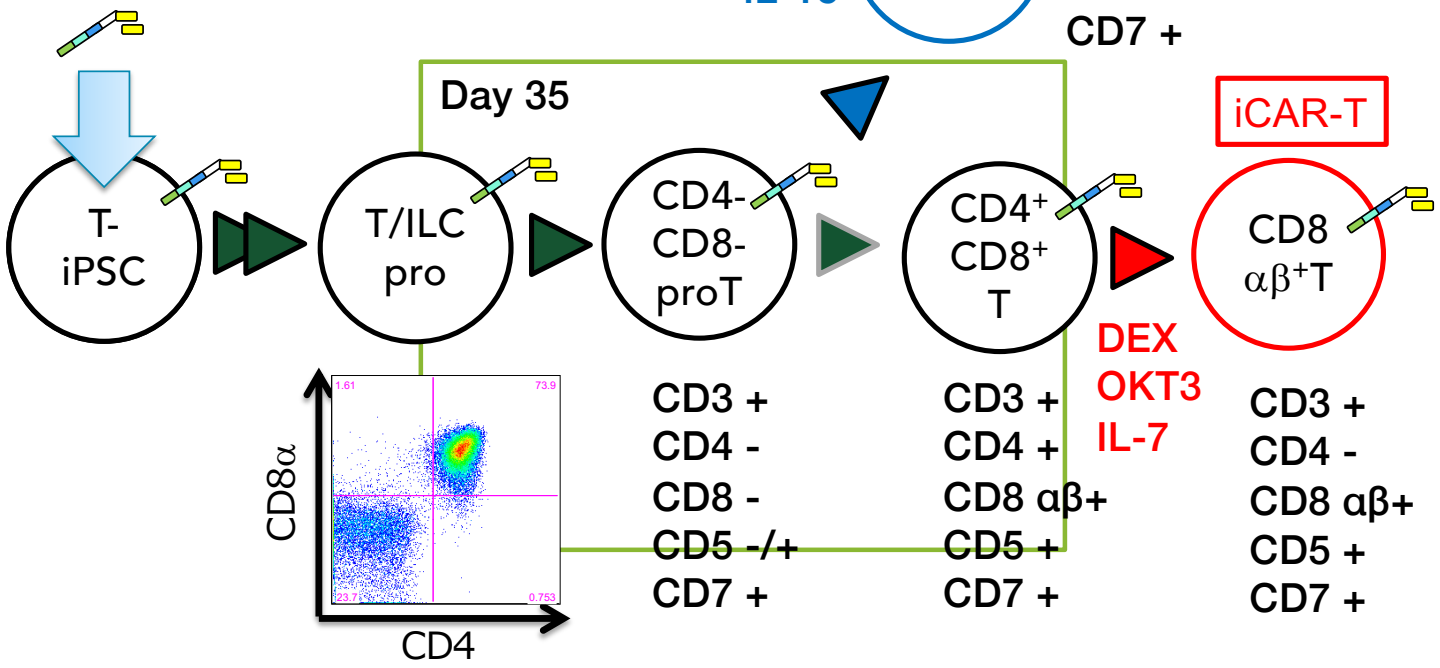


Conceptual image of regenerative T cell immunotherapy
from Minagawa A et al., ***Cell Stem Cell*** 2018

2D differentiation of human PSCs generates TCR-expressing immune cells that resemble peripheral T or ILC/NK

- **2D T cell differentiation in PSC/OP9-DL1 cell coculture**
  Schmitt TM et al, Nat Immunol. 2004

Chimeric Antigen Receptor (CAR)



Innate Lymphoid Cell-like :

Nishimura T et al.,
Cell Stem Cell 2013

Ueda N et al.,
Stem Cell Reports 2018

Ueda T et al.,
Cancer Science 2020

Adaptive T Cells :
Cytotoxic T Lymphocytes

Maeda et al.,
Cancer Research 2016

Minagawa A et al.,
Cell Stem Cell 2018

Iriguchi S et al.,
Nat Commun 2021

Wang et al.,
Nat Biomed Eng 2021

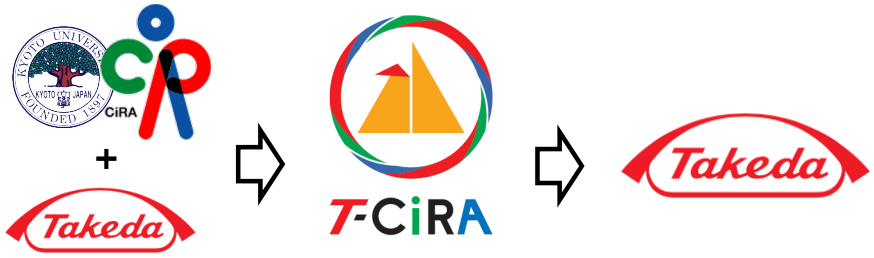
Kawai et al.,
Mol Ther 2021

The right iPSC-immune cell in the right place at clinical setting

Direct and multiple injections to localized tumor site

iPSC-derived	Receptor modification	NK receptors	Homing to tumor site	Persistency (survival in vivo)	Injection	GVHD risk
NK	CAR	present	Poor	Relatively short	Local	No - Less
CD8αβ T	TCR CAR	absent	Better	Relatively long	Systemic	Possible

Intravenous and multiple systemic injections to remote and disseminated tumor site



Ueda T., unpublished data

HLA-homozygous allogeneic iPSCs for clinical development of immunotherapy

- Currently available iPSCs distributed by CiRA Foundation

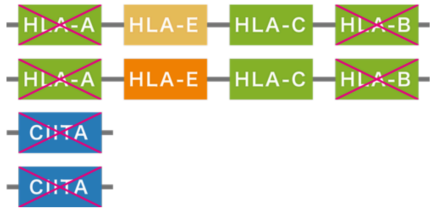
HLA Haplotype identical		Peripheral Blood	Cord Blood
1st frequent	17% of JPN	1 released (4 clones)	2 released (5+4 clones)
2nd frequent	9% of JPN	1 released (4 clones)	1 released (4 clones)
3rd frequent	8% of JPN	1 released (5 clones)	
4th frequent	5% of JPN	1 released (1 clones)	

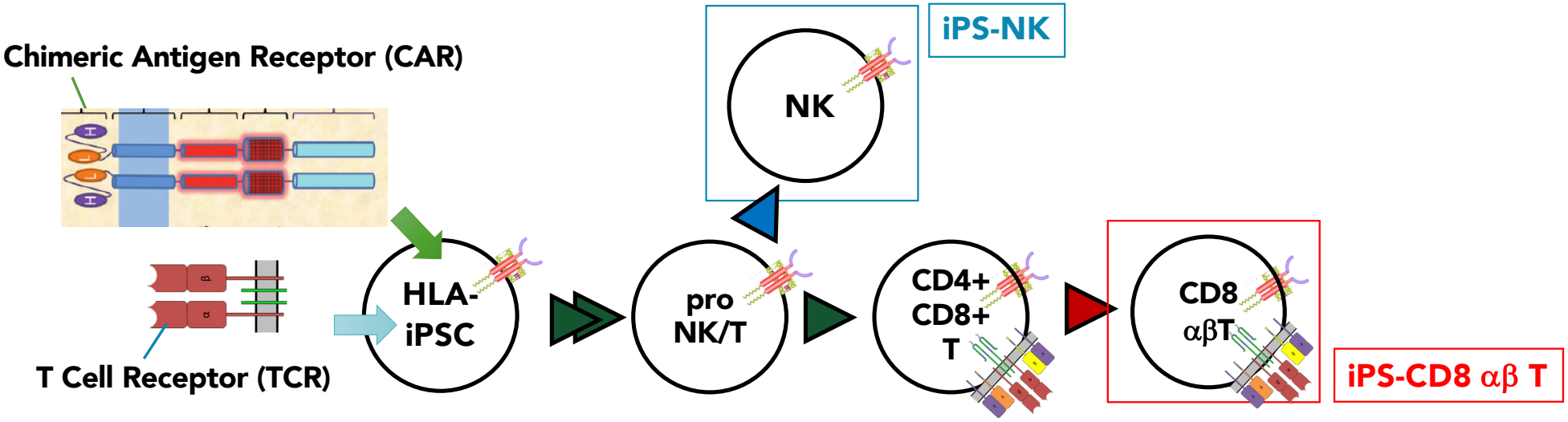
Targeted Disruption of HLA Genes via CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility

Huaigeng Xu,[1,3] Bo Wang,[1,3] Miyuki Ono,[1,2] Akihiro Kagita,[1] Misato Nishikawa,[1] Masaki Nomura,[1] Fumiyo Kitaoka,[1] Tor Shin Kaneko,[1,*] and Akitsu Hotta[1,2,4,*]

Cell Stem Cell, 2019

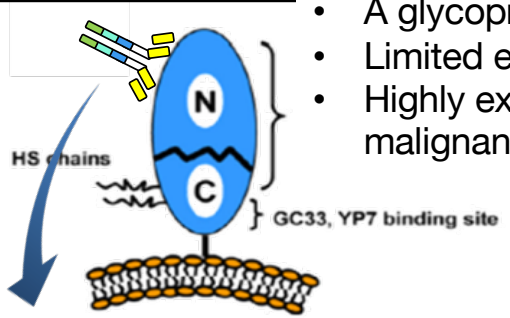
7 iPSC lines may cover most of the global population





Utilizing anti-GPC3 iCAR-ILC/NK for treatment of ovarian cancer patients

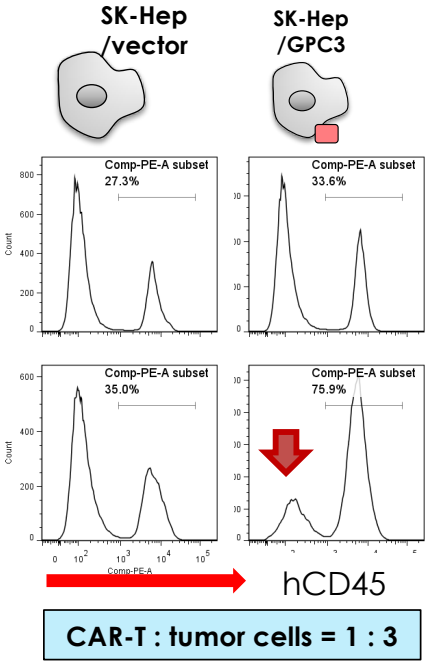
- **Glypican-3 (GPC3)**
  - A glycoprotein anchored on the cellular membrane that connects functional proteins and their receptors
  - Limited expression in fetal liver, and **no expression in normal tissues**
  - Highly expressed in hepatocellular carcinoma, **ovarian cancer**, lung squamous cell carcinoma, malignant melanoma, and pediatric malignant tumors.



- **3rd gen. CAR**

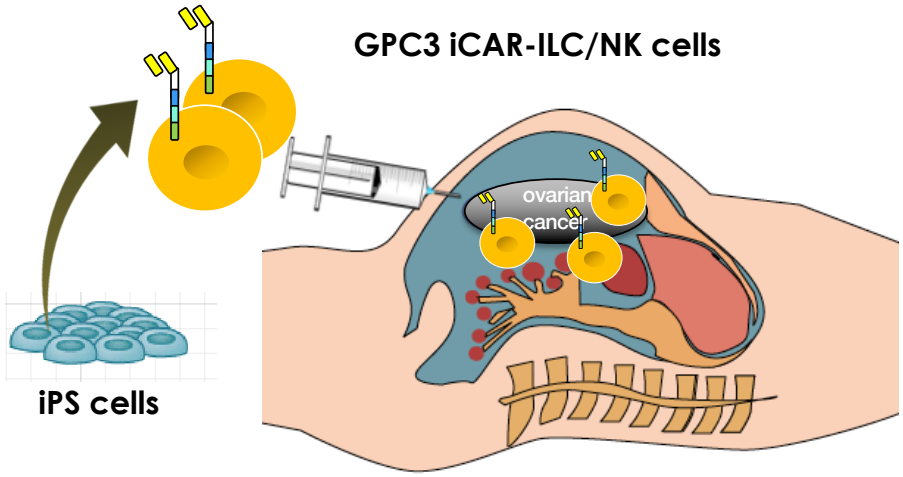
non-transduced T cells

GPC3 CAR-T cells



CAR-T : tumor cells = 1 : 3

- **Targeting ovarian cancer by iCAR-ILC/NK cells**

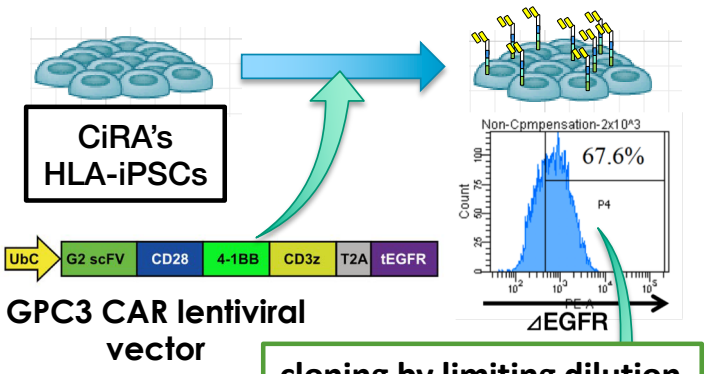


- **Peritoneally disseminated ovarian cancer** may be treated by local and multiple injections of CAR-expressing iPS-NK cells

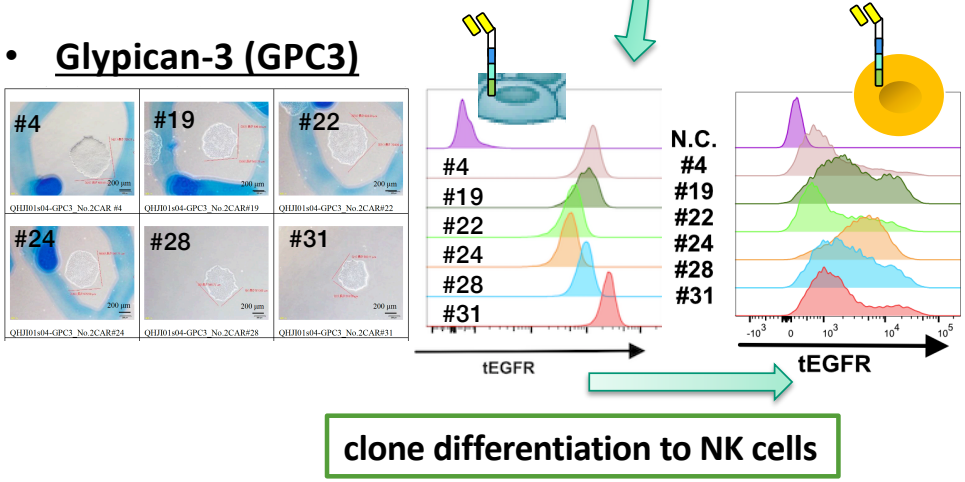
Ueda T et al., Cancer Science, 2020

Clinical Cell Production (1)
CAR-transduced iPSC Master Cell Bank Generation

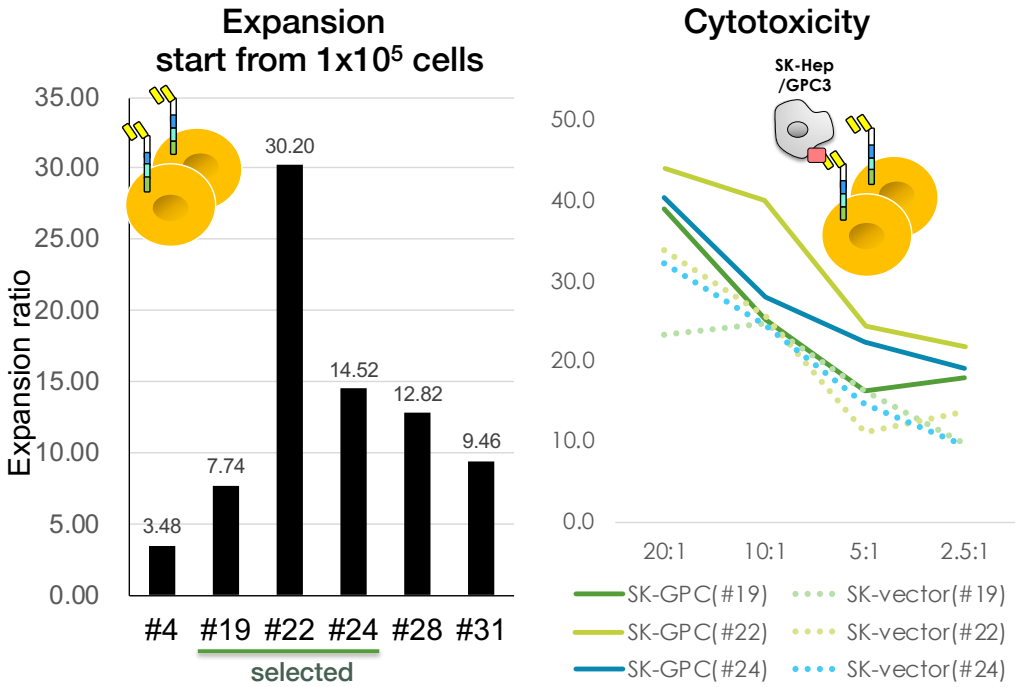
- **Glypican-3 (GPC3)**



- **Glypican-3 (GPC3)**



- **Functional screening of CAR-iPSC clone for MCB**



Ueda T et al., Cancer Science, 2020

Clinical Cell Production (2)
GMP-processing for anti-GPC3 iCAR-ILC/NK induction

- **Manufacturing strategy**

CiRA's iPSC QHJI01s04 from FiT

CAR lentiviral vector

CAR-iPS cells (Seed cell stock) Clone #22

CAR-iPS cells **(Master/Working Cell Bank)**

11Days

iCAR-HPC*

21Days

iCAR-LPC**

Patient's PBMC feeder

14Days

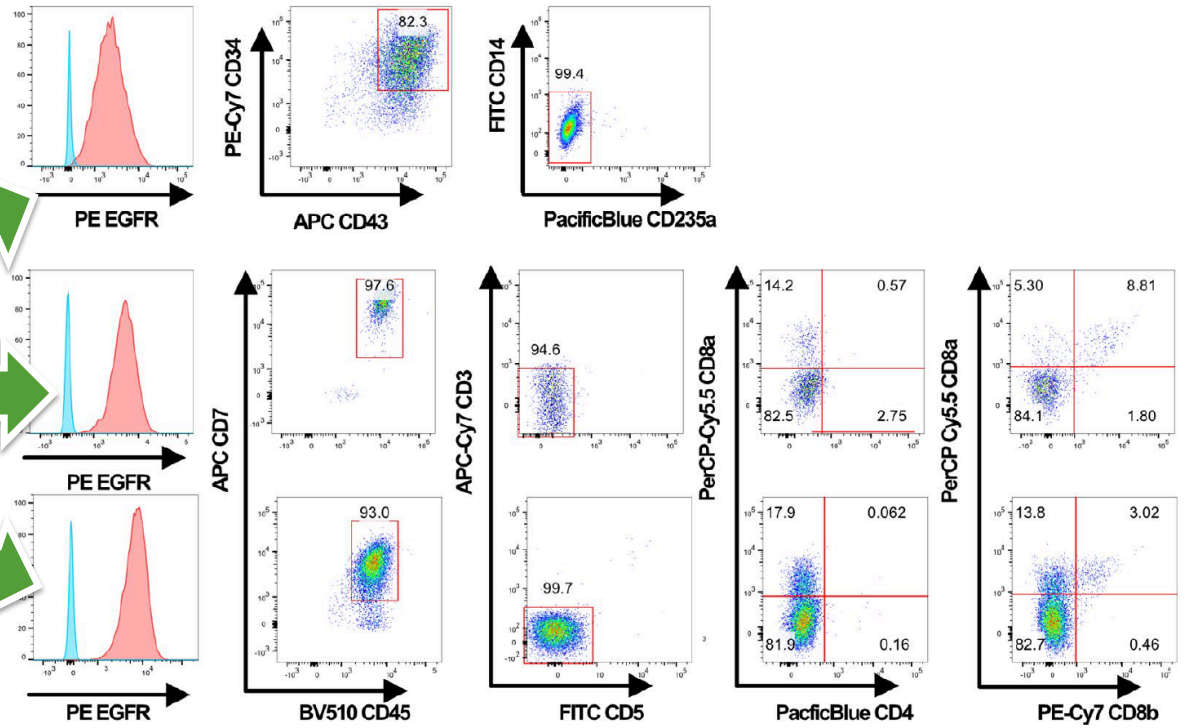
iCAR-ILC/NK

1Day

Frozen iCAR-ILC/NK

*hematopoietic progenitor cells, **lymphoid progenitor cells

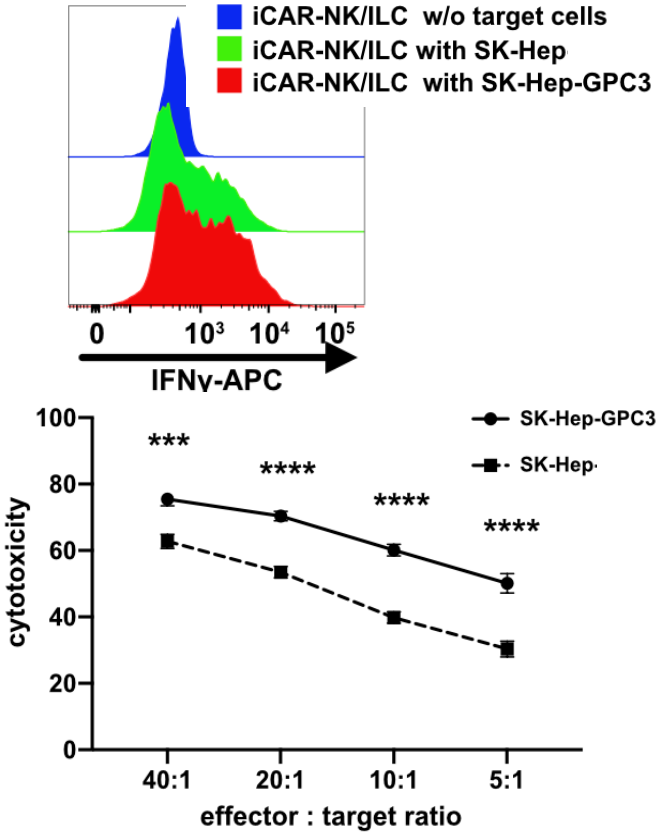
- **FCM phenotyping of differentiating HPC, LPC, and ILC/NK cells**



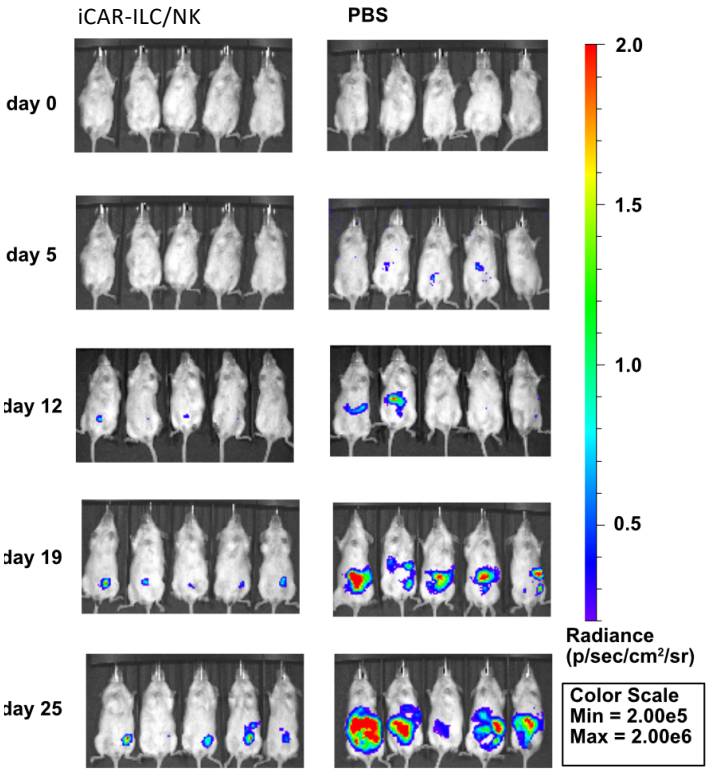
Ueda T et al., Cancer Science, 2020

CAR-dependent and -independent iCAR-ILC/NK effector functions contributed to therapeutic effects

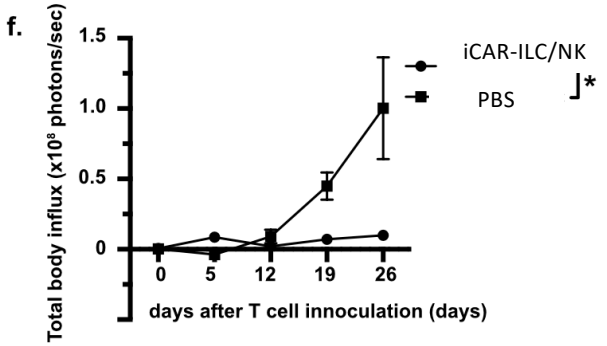
in vitro

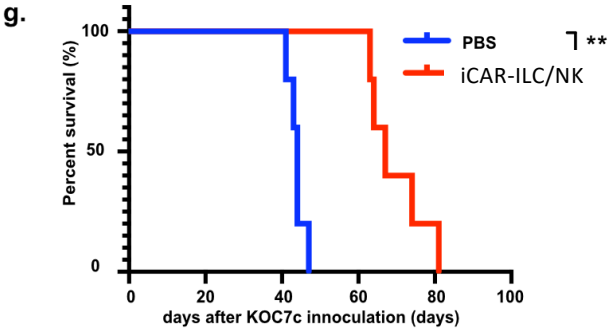


in vivo



Koc7c ovarian cancer intraperitoneally inoculated NSG mice





Ueda T et al., Cancer Science, 2020

Non-clinical safety test (1): in vivo acute toxicity test of iCAR-ILC/NK

in vivo toxicity test

- **schedule**

  0 3 7 10 14 17 21 (days)

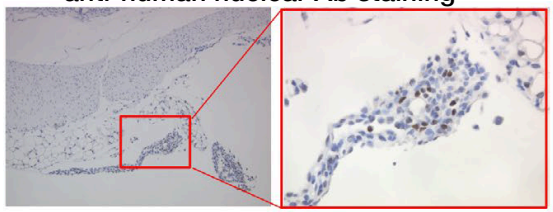
  iCAR-ILC/NK

  1.0×10^7 i.p. x 6 times x 12♀NSG mice

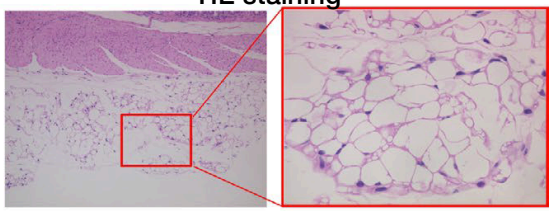
- **general status and lab data**
  - No body weight change to control
  - No laboratory data change to control
- **histology @ day 21**
  - No organ weight change to control
  - Infiltrated human cells to omentum around stomach(4), spleen(4), and pancreas(2) in 12 mice

omentum

anti-human nuclear Ab staining



HE staining



- **test items**

Lab data

AST	RBC
ALT	HGB
LDH	HCT
ALP	MCV
T-CHO	MCH
TG	MCHC
PL	Retic
T-BIL	PLT
GLU	WBC
BUN	
CRE	
TP	
ALB	
A/G	

Organs for histology

Cerebrum	Bronchs	Gall bladder
Cerebellum	**Lung**	Pancreas
Spinal code	Tongue	**Kidney**
Sciatic nerve	Esophagus	Bladder
Eyes	Stomach	Ovary
Optic nerve	Duodenum	Uterus
Herder grand	Jejunum	Vagina
Pituitary	Ileum	Mammal grand
Thyroid	Cecum	Sternum
Parathyroid	Colon	Femoral bone
Adrenal grand	Rectum	Femoral muscle
Spleen	**Submandibular grand**	Skin
Heart	**Sublingual grand**	Thymus
Aorta	**Liver**	Lymph nodes

Organs in bold ~~letter~~ were weighed at sacrifice

Ueda T et al., Cancer Science, 2020

Summary

- CD8αβ cytotoxic T cells and NK cells were induced from HLA-homozygous allogenic iPS cells in a clinically relevant manipulation protocol; both are thought to be candidates of an immune-cell therapy platform.

- Anti-GPC3 iCAR-ILC/NK produced by a clinically relevant manipulation protocol showed therapeutic efficacy and safety in non-clinical tests using an animal model

- Preparation for a clinical trial targeting GPC3-expressing ovarian cancer by the iCAR-ILC/NK is ongoing.

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