ขอเชิญประชุมวิชาการ ครั้งที่ 5

มหาวิทยาลัยมหิดล
คณะแพทยศาสตร์
โรงพยาบาลรามาธิบดี

29 ปีปลูกถ่ายไขกระดูกรามาธิบดี
Ramathibodi Stem Cell Transplantation Conference

21 กรกฎาคม 2561

ณ ห้องประชุมอรรถสิทธิ์ เวชชาชีวะ ชั้น 5 ศูนย์การแพทยสิริกิติ์
คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

CAR T Cell against B Cell Malignancies: Preclinical Studies

Pa-thai Yenchitsomanus

Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT)
Faculty of Medicine Siriraj Hospital
Mahidol University

Agenda

1. Cancer immunotherapy.
2. Cellular immunotherapy for cancer.
3. Chimeric antigen receptor (CAR) T cells.
4. CAR T cells against B cell malignancies.
5. Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT).

***Science*'s editors have chosen cancer immunotherapy as Breakthrough of the Year for 2013,**

a strategy that harnesses the body's immune system to combat tumors.

Cancer Immunotherapy

Treatment that uses certain parts of the immune system to fight cancer.

Types of Immunotherapy

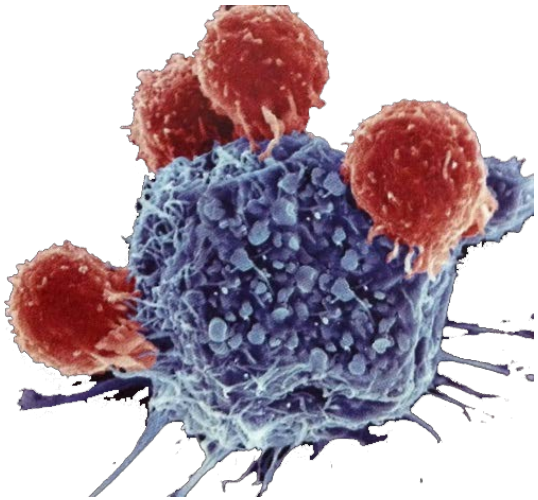
Passive immunotherapy:

- Administration of monoclonal antibodies targeting to tumor-specific or over-expressed antigens.

Active immunotherapies:

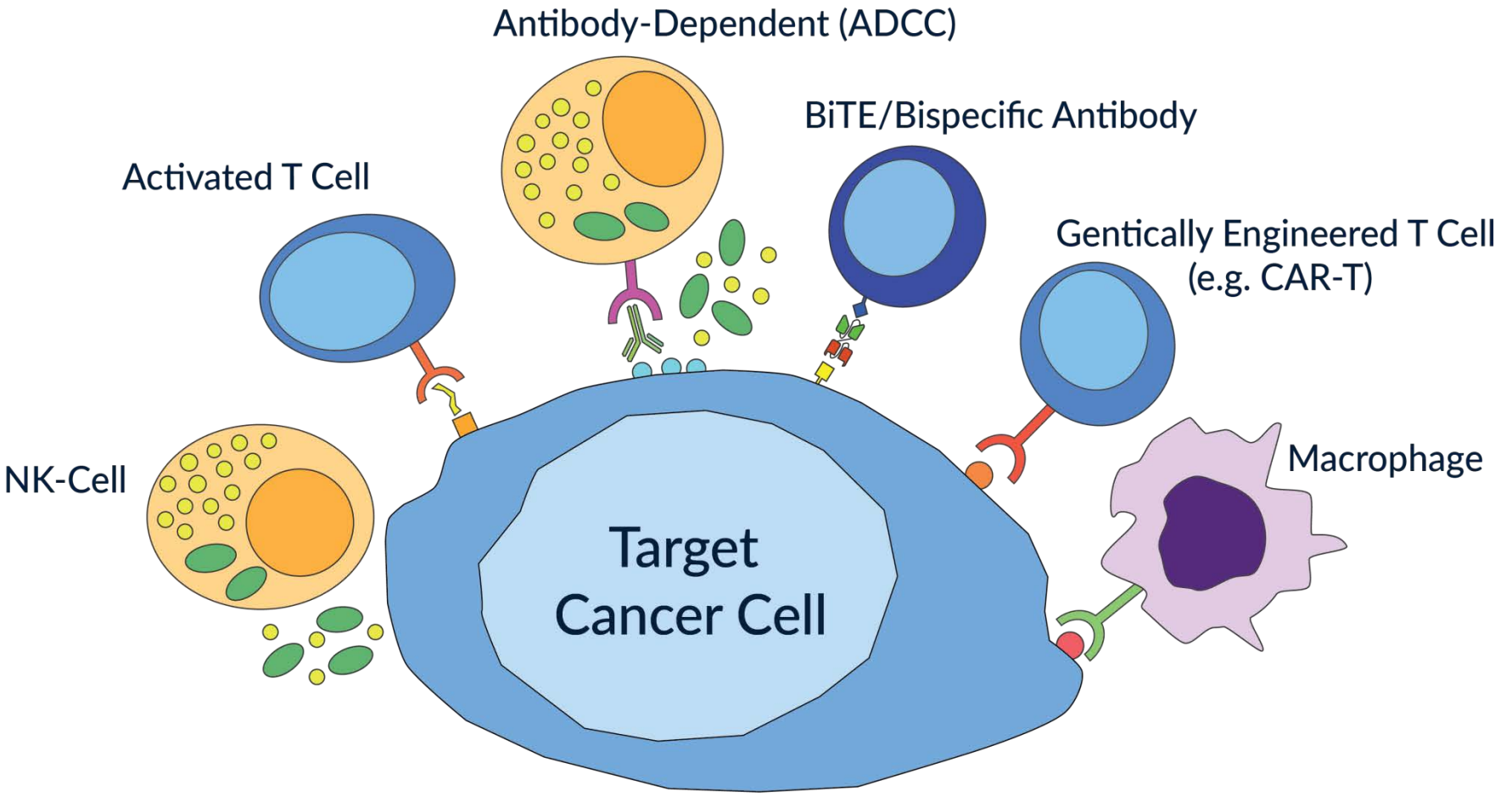
- Cytokines: IL-2/ IFNs/ TNFα
- Cancer (Ag) vaccines
- Tumor-derived antigen presenting cells (APC)
- Dendritic cell (DC) vaccine
- Tumor-specific cytotoxic lymphocytes (CTL)
- Chimeric antigen receptor (CAR) T-cells
- Other cell-based therapies (e.g. NK, NKT, γδ Cells)

Cellular Immunotherapy



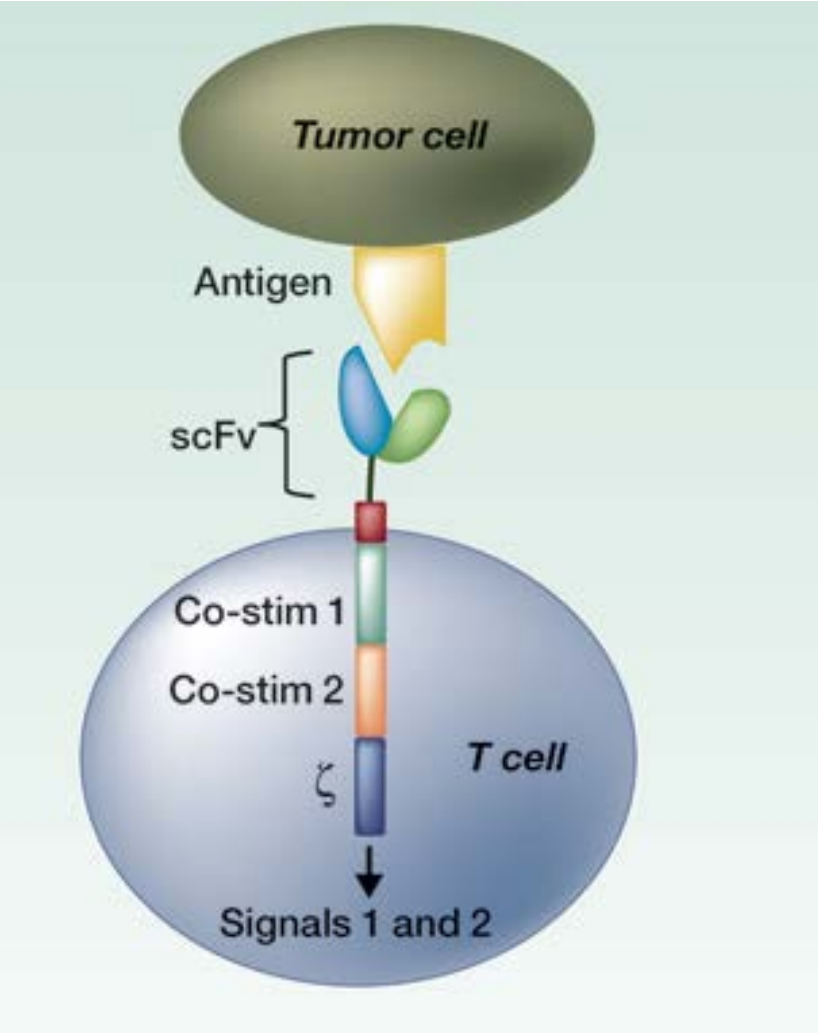
- Training/engineering the patient's immune cells to combat cancer cells.
- The patient's immune cells are used as 'living drug'.

Cellular Immunotherapy for Cancer



https://www.aceabio.com/applications/cancer-immunotherapy/

Chimeric Antigen Receptor (CAR) T Cells

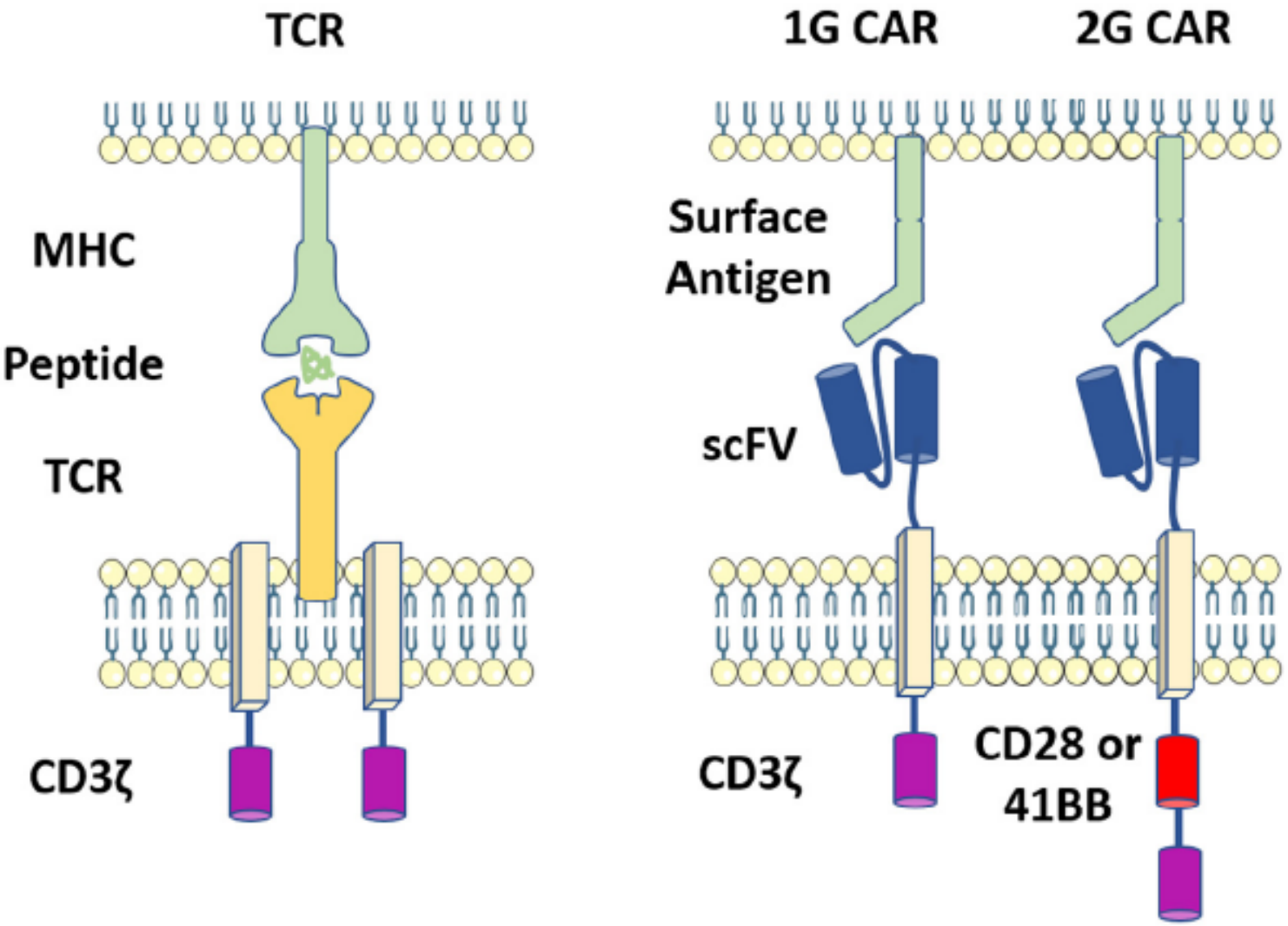


The engineered T-cells that their receptors are modified by fusing T-cell receptor with scFv, targeting to tumor specific antigen.

- **MHC-independent antibody engagement and induction of signaling.**
- **Proliferation, cytokine production, cytotoxic function, tumor lysis.**

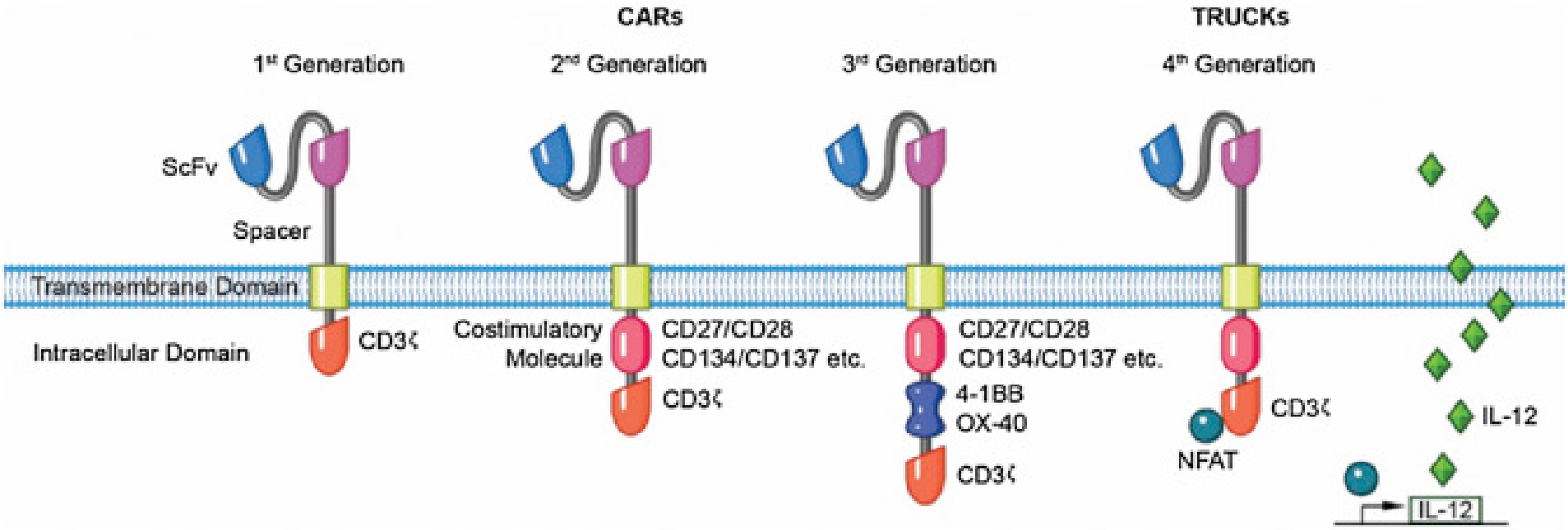
https://seekingalpha.com/article/4062480-car-t-cell-therapy-means-lot-one-two-new-drug-approvals

Antigen recognition by T cells.



K. Perica et al. Biol Blood Marrow Transplant 24 (2018) 1135–1141.

Evolution of CAR Design



A.J. Smith et al. Journal of Cellular Immunotherapy 2 (2016) 59-68

Chimeric Antigen Receptor (CAR) T Cell Therapy

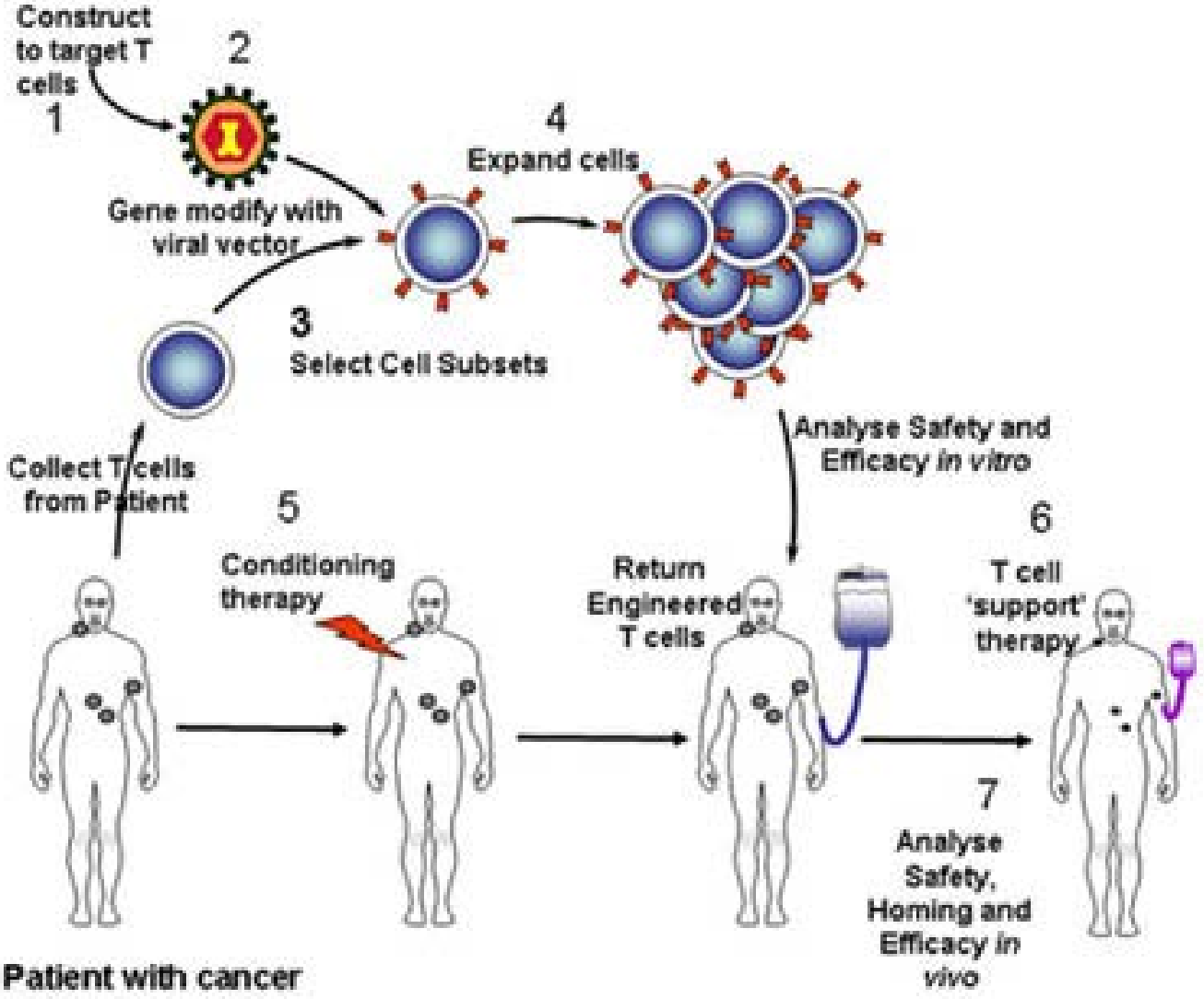
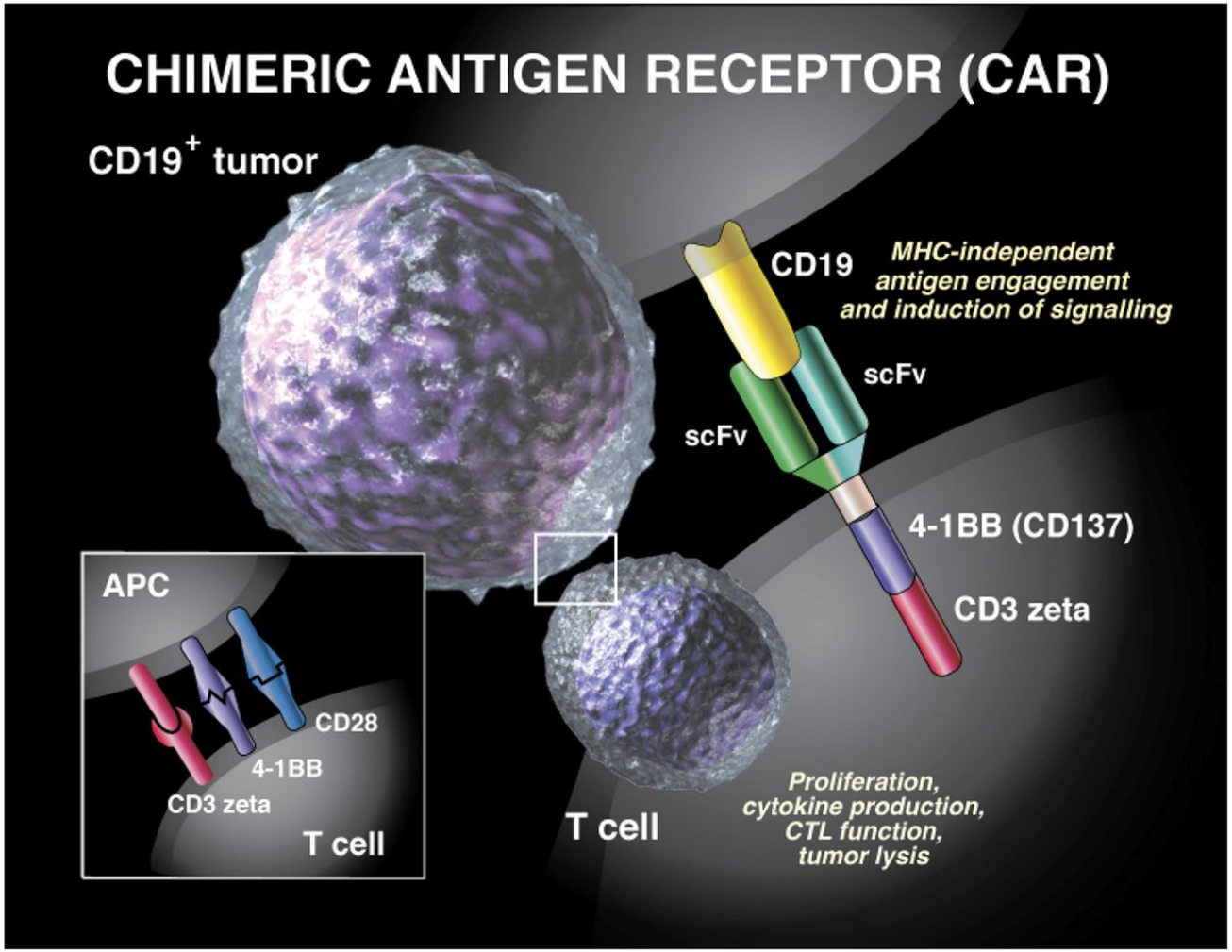


Figure illustrates the processes involved in Engineered T cell therapy. The ATTACK project will optimise the indicated aspects in model systems to inform future clinical trial design.

http://ashlandhumanbio.blogspot.com/2014/07/immunotherapy.html

CD19 CAR T-cells is first and most successful CAR T-cells for treatment of acute lymphoblastic leukemia (ALL).



Shannon L. Maude, et al. Blood 2015 125:4017-4023.

The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med. 2011 August 25; 365(8): 725–733.

Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia

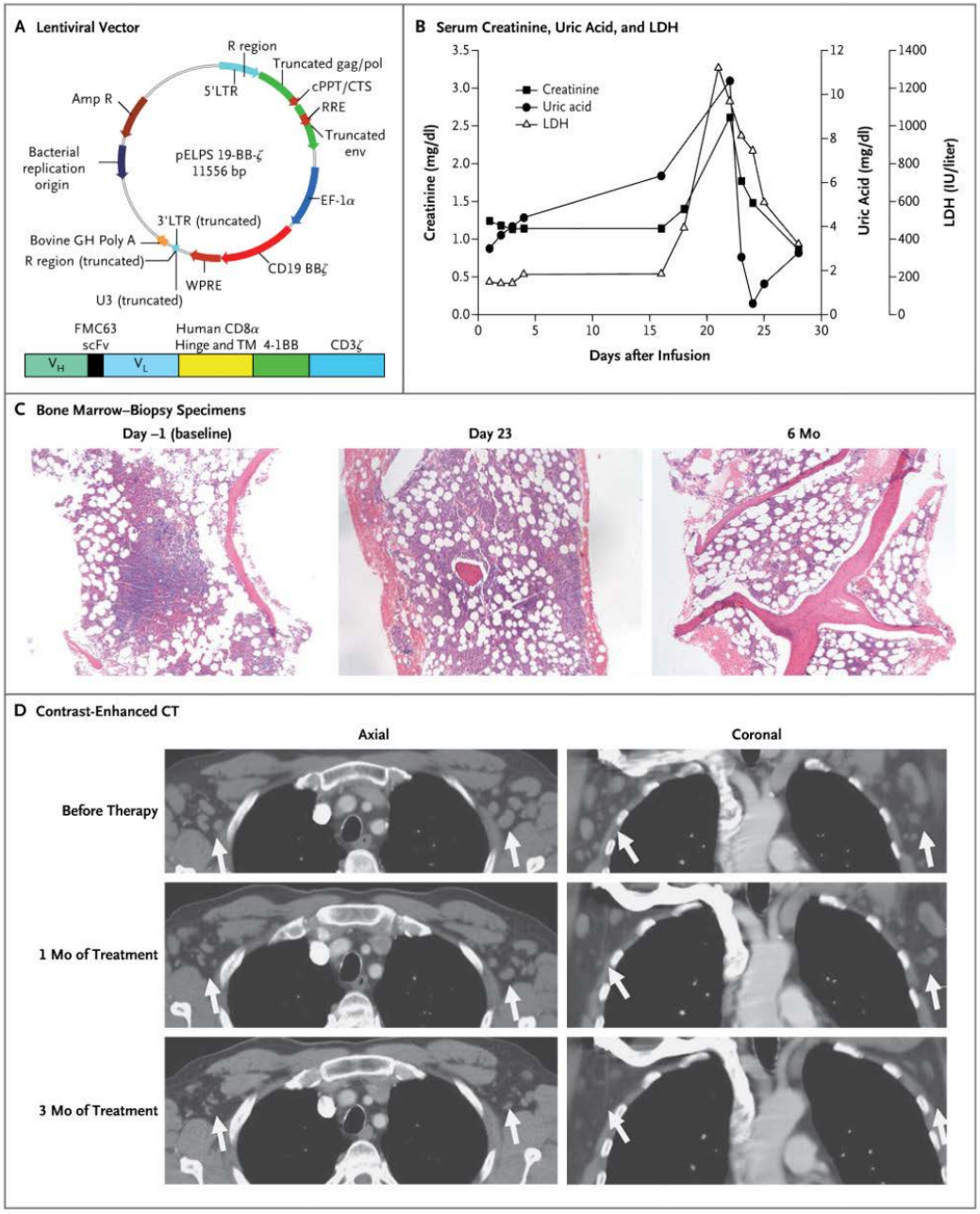
David L. Porter, Bruce L. Levine, Michael Kalos, Adam Bagg, and Carl H. June

Abramson Cancer Center, the Department of Medicine, the Department of Pathology and Laboratory Medicine, and the Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia.

A lentiviral vector expressing a chimeric antigen receptor with specificity for the B cell antigen CD19, coupled with CD137 (a costimulatory receptor in T cells [4-1BB]) and CD3- zeta signaling domains.

A specific immune response was detected in the bone marrow, accompanied by loss of normal B cells and leukemia cells that express CD19. Remission was ongoing 10 months after treatment. Hypogammaglobulinemia was an expected chronic toxic effect.

Clinical Response in the Patient.



The NEW ENGLAND JOURNAL of MEDICINE

Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Shannon L. Maude, M.D., Ph.D., Noelle Frey, M.D., Pamela A. Shaw, Ph.D., Richard Aplenc, M.D., Ph.D., David M. Barrett, M.D., Ph.D., Nancy J. Bunin, M.D., Anne Chew, Ph.D., Vanessa E. Gonzalez, M.B.A.,

N ENGL J MED 371;16 NEJM.ORG OCTOBER 16, 2014

A total of 30 children and adults received CTL019. Complete remission was achieved in 27 patients (90%), including 2 patients with blinatumomab-refractory disease and 15 who had undergone stem-cell transplantation.

On August 30, 2017, Novartis receives first ever FDA approval for a CAR-T cell therapy, Kymriah™ (CTL019), for children and young adults with B-cell ALL that is refractory or has relapsed at least twice.

- **Therapy showed an 83% (52/63) overall remission rate in this patient population with limited treatment options and historically poor outcomes.**
- **Novel approach to cancer treatment is the result of pioneering CAR-T cell therapy collaboration with University of Pennsylvania.**

https://www.novartis.com/news/media-releases/novartis-receives-first-ever-fda-approval-car-t-cell-therapy-kymriahtm-ctl019

Yescarta (axicabtagene ciloleucel), a CD19 CAR T cells, is the second CAR T cell therapy approved by the FDA (on October 18, 2017) and the first for certain types of non-Hodgkin lymphoma (NHL).

Axicabtagene, was initially developed at NCI by Dr. Steven Rosenberg, and his colleagues. It was later licensed to a private company, Kite Pharma, for further development and commercialization.

https://www.cancer.gov/news-events/cancer-currents-blog/2017/tisagenlecleucel-fda-childhood-leukemia

Characteristics of Approved CAR T Cell Products

Company	Novartis	Gilead (Kite Pharma)
Product name	Tisagenlecleucel	Axicabtagene ciloleucel
Academic license	Uni. of Penn.	NCI
Binding domain (all murine scFV)	FMC63	FMC63
Indications	Pedr. ALL <26 yr of age	DLBCL, tFL, PMBCL
Spacer domain	CD8α	CD28
Transmembrane domain	CD8α	CD28
Stimulatory domain	4-1BB-CD3ζ	CD28-CD3ζ
Starting cell population selection	None	None
Final CD4/CD8 ratio	Variable	Variable
Ablation technology	None	None
Viral vector	Lentivirus	Gamma retrovirus
Outcomes		
CR rate	ALL 83%	NHL 54%
Grade 3-5 cytokine release	48%	13%
Grade 3-5 neurotoxicity	15%	28%
Average LOS on study	NS	14 days
Projected demand in the United States	400-600 patients	4000-6000 patients

K. Perica et al. Biol Blood Marrow Transplant 24 (2018) 1135–1141.

CAR T cell therapy efficacy in patients with refractory/relapsed (R/R) CD19+ hematologic malignancies.

- In R/R ALL, minimal residual disease (MRD)-negative complete remission (CR) rates were 60–93%.
- In R/R non-Hodgkin lymphoma (NHL), best overall response rates (ORR) were 53–82%.
- In R/R CLL patients, reported ORR were 57–74%.

J. Gauthier, C.J. Turtle Current Research in Translational Medicine 66 (2018) 50–52.

Limitations of CAR T-cells

Toxicities

- On-target/off-tumor toxicities
- Cytokine release syndrome
- Neurotoxicity

Tumor microenvironment

- Presence of MDSCs & Treg in tumor
- Immunosuppressive agents

Relapses

- Poor CAR T-cell persistence
- Antigen loss

On-target/off-tumor toxicities

- **The ideal target antigen is restricted to the tumor cell.**
- **Unfortunately, most targets of CAR T cells have shared expression on normal tissues and some degree of “on-target/off-tumor” toxicity occurs through engagement of target antigen on nonpathogenic tissues.**
- **The severity of reported events has ranged from manageable lineage depletion (B-cell aplasia) to severe toxicity (death).**

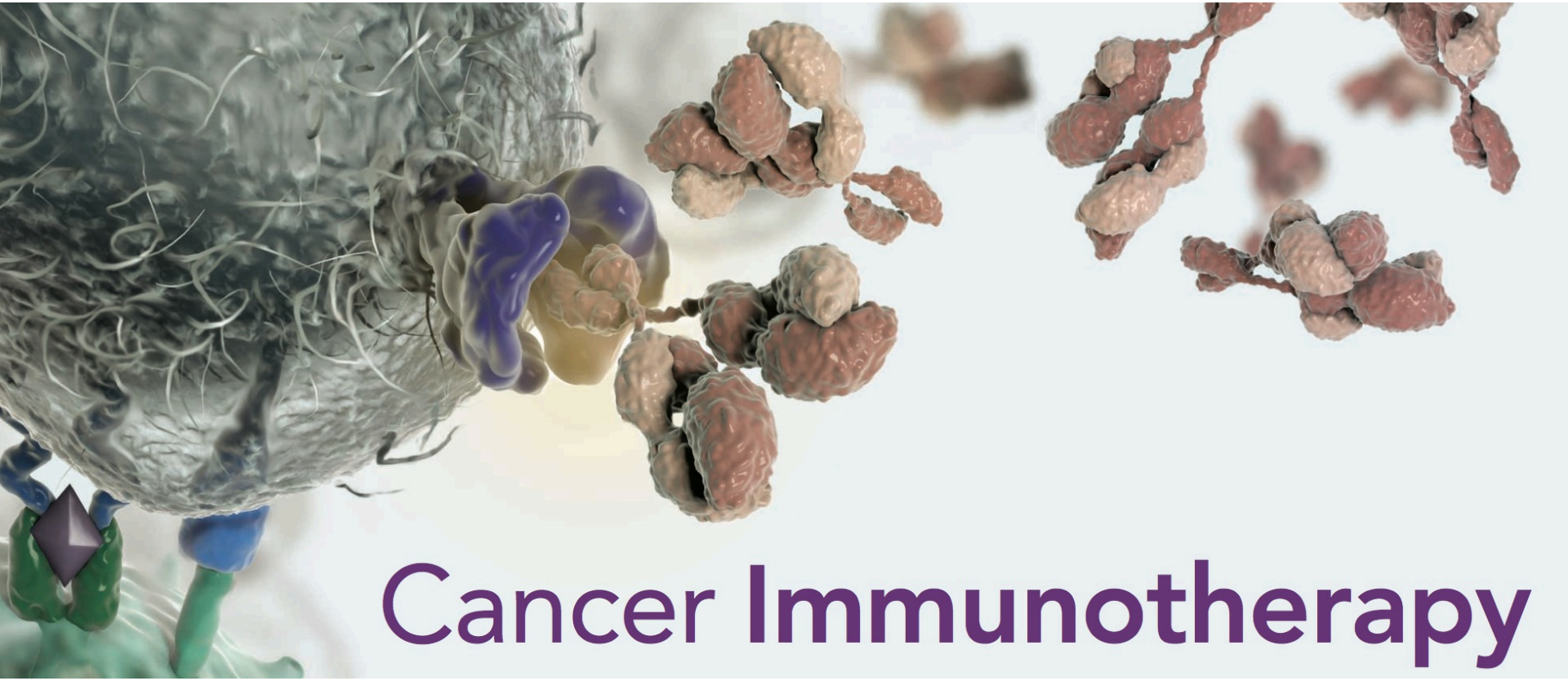
Cytokine release syndrome (CRS)

- **a variety of systemic symptoms and signs, such as fever, hypotension, capillary leak, coagulopathy and occasionally multiorgan failure.**
- **may differ between distinct CAR-T cell products, but generally occurs within a few days after CAR-T cell infusion.**
- **In most clinical trials for ALL, incidences of CRS above 70% and severe CRS 15%.**

Neurotoxicity

- commonly presents with delirium, headache, decreased level of consciousness, or speech impairment.
- focal neurologic deficits, seizures, and acute cerebral edema are infrequent.
- incidence varies across studies, 13 – 63% in ALL, and 7% – 31% in NHL.
- usually develops after the onset of CRS and can present after its resolution.
- in almost all cases, neurotoxicity and CRS are reversible.

Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT)



Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT) Research Team

SiCORE-CIT Research Plan on Cellular Immunotherapy for Cancer

[Conceptualization: 2013 – 2014]

4 Working Phases:

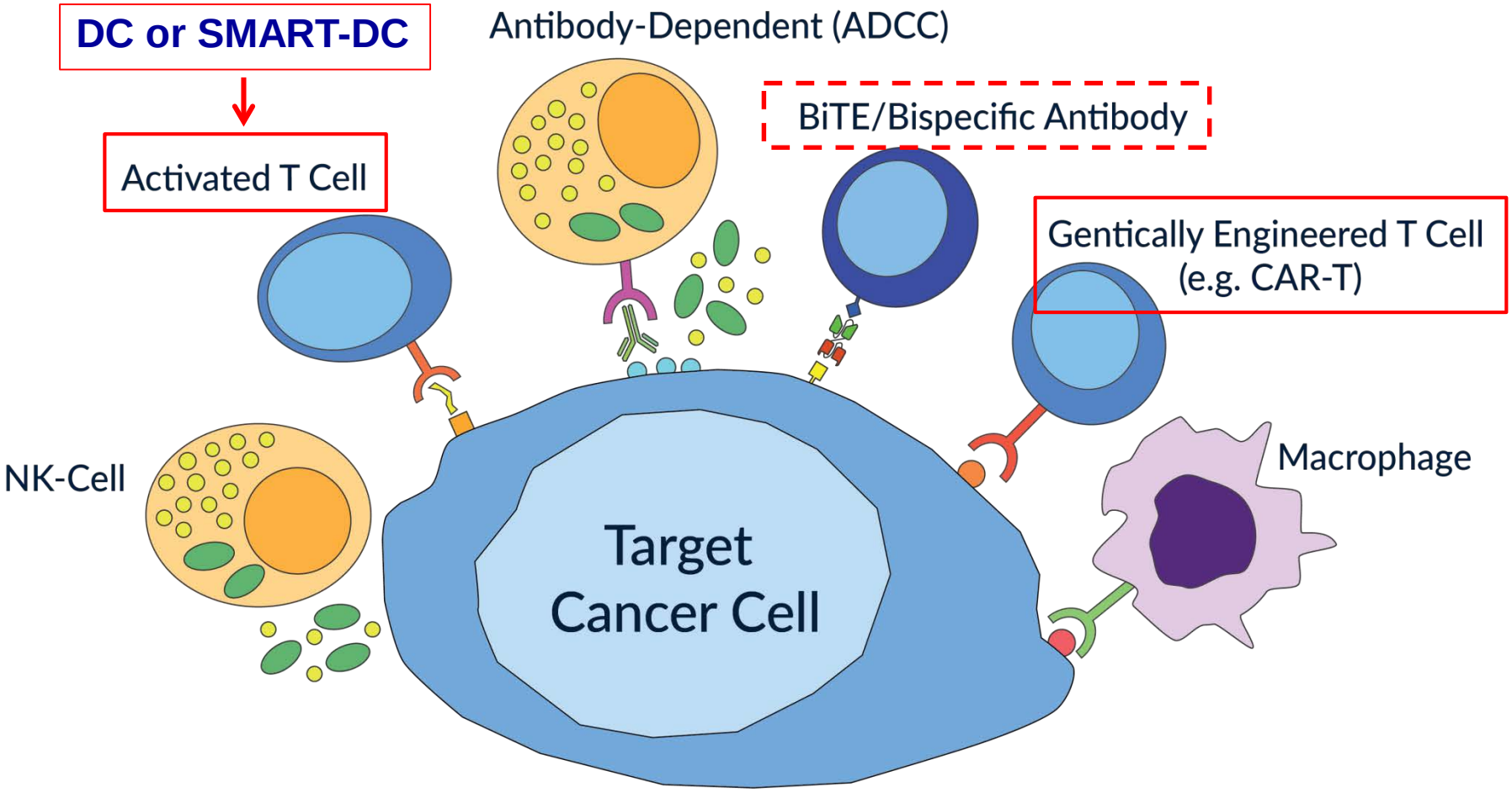
Phase I: Pre-clinical *in vitro* studies (2015-2017)

Phase II: *In vivo* (animal model) and *ex vivo* (patients' samples) studies (2018-2019)

Phase III: Clinical trials (2020-2021)

Phase IV: Clinical services (2022 onwards)

Cellular Immunotherapy for Cancer



https://www.aceabio.com/applications/cancer-immunotherapy/

Three Technology Platforms of Cellular Immunotherapy for Cancers

1. **Dendritic cell (DC) - based Immunotherapy**

   1.1 Activation of T-cells by DC pulsed with tumor lysate or RNA

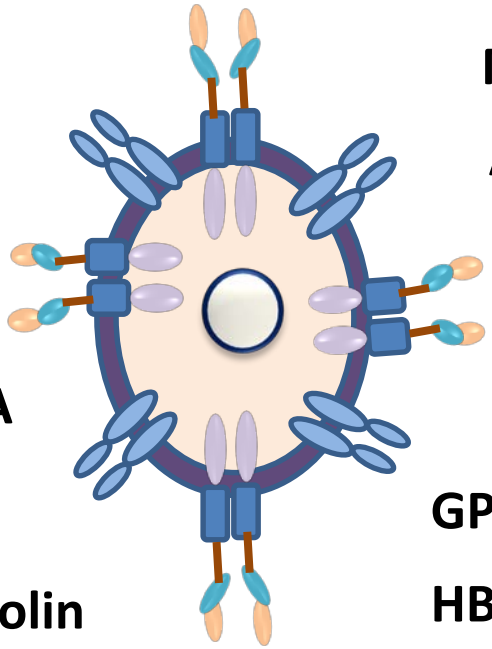
   1.2 Activation of T-cells by **S**elf-differentiated **M**yeloid-derived **A**ntigen-presenting-cells **R**eactive against **T**umor-DC (**SMART-DC**)

2. **Chimeric Antigen Receptor (CAR) T-cell Therapy**
   - Retinoblastoma
   - Leukemia
   - Cholangiocarcinoma
   - Hepatocellular carcinoma
   - Breast cancer
   - Ovarian cancer
   - Colorectal cancer

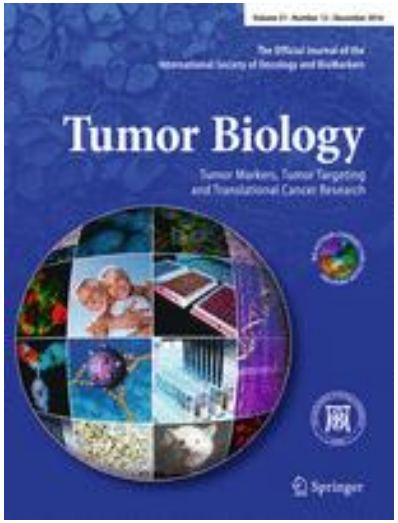
3. **Bispecific T-cell Engagers (BiTE) / Bispecific Antibodies**
   - Recombinant BiTE
   - Cell-secreting BiTE

Development of CAR T therapy for cancers

Target antigens for CAR T cells



- Retinoblastoma — GD2
- B-cell malignancies — CD19, BCMA
- Breast and ovarian cancers — Frα, Nucleolin, Trop2
- Cholangiocarcinoma (CCA) — Mucin1, ANXA2, CD133
- Hepatocellular carcinoma (HCC) — GPC3, HBs Ag, HCV Ag



Original Article

Tumor Biology

Enhanced cytotoxic activity of effector T-cells against cholangiocarcinoma by dendritic cells pulsed with pooled mRNA

Tumor Biology
October 2017: 1–13

DOI: 10.1177/1010428317733367
journals.sagepub.com/home/tub
SAGE

Mutita Junking[1], Janya Grainok[1,2], Chutamas Thepmalee[1,3], Sopit Wongkham[4,5] and Pa-thai Yenchitsomanus[1]

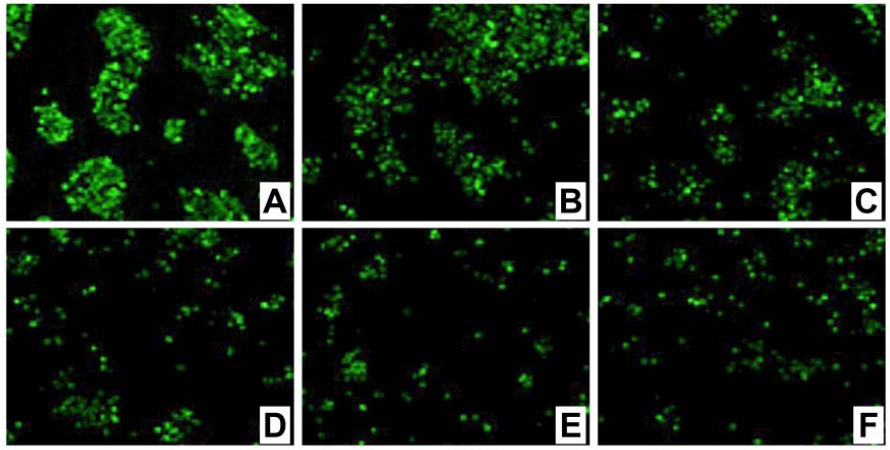
Tumour Biol. 2017 Oct;39(10):1010428317733367.

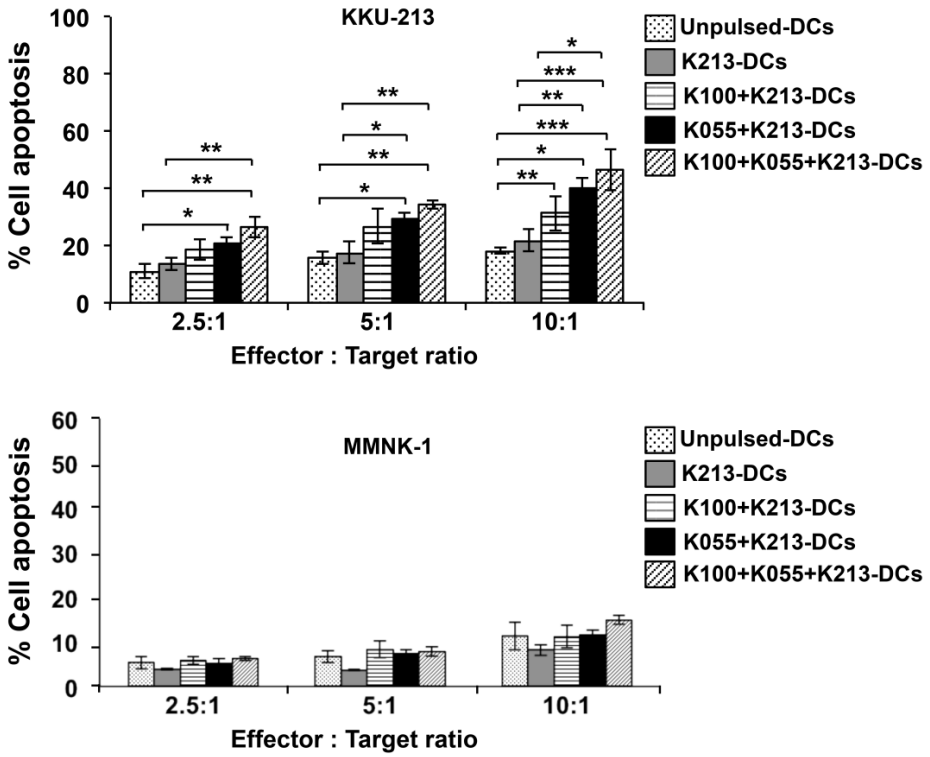
Dr. Mutita Junking

Janya Grainok (M.Sc. Student)

Chutamas Thepmalee (Ph.D. Student)

Enhanced Cytotoxic Activity of Effector T-cells against Cholangiocarcinoma by Dendritic Cells Pulsed with Pooled mRNA





HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 0, NO. 0, 1–9
https://doi.org/10.1080/21645515.2018.1431598

human VACCINES & IMMUNOTHERAPEUTICS

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

Inhibition of IL-10 and TGF-β receptors on dendritic cells enhances activation of effector T-cells to kill cholangiocarcinoma cells

Chutamas Thepmalee[a,b,c], Aussara Panya[a,d], Mutita Junking[a], Thaweesak Chieochansin[a], and Pa-thai Yenchitsomanus[a]

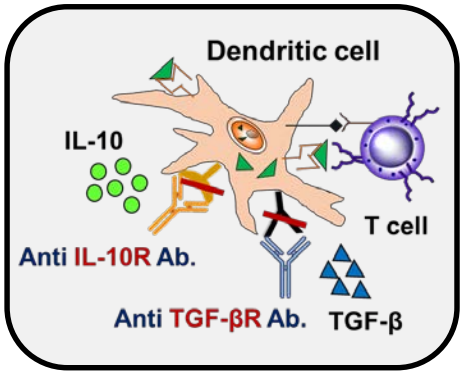
Chutamas Thepmalee (Ph.D. Student)

Dr. Aussara Paya

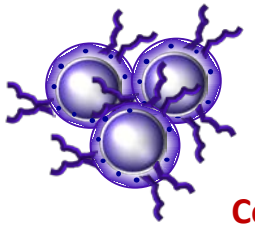
Dr. Mutita Junking

Dr. Thaweesak Chieochansin

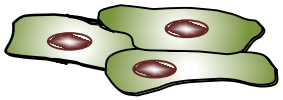
Inhibition of IL-10 and TGF-β receptors on dendritic cells enhances activation of effector T-cells to kill cholangiocarcinoma cells.



Activated effector T-cells



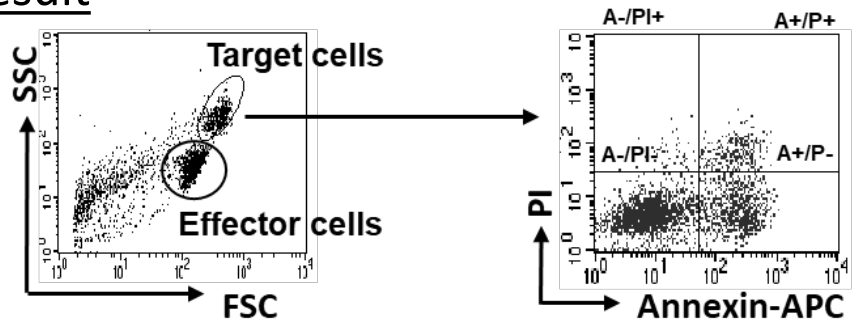
Co-culture



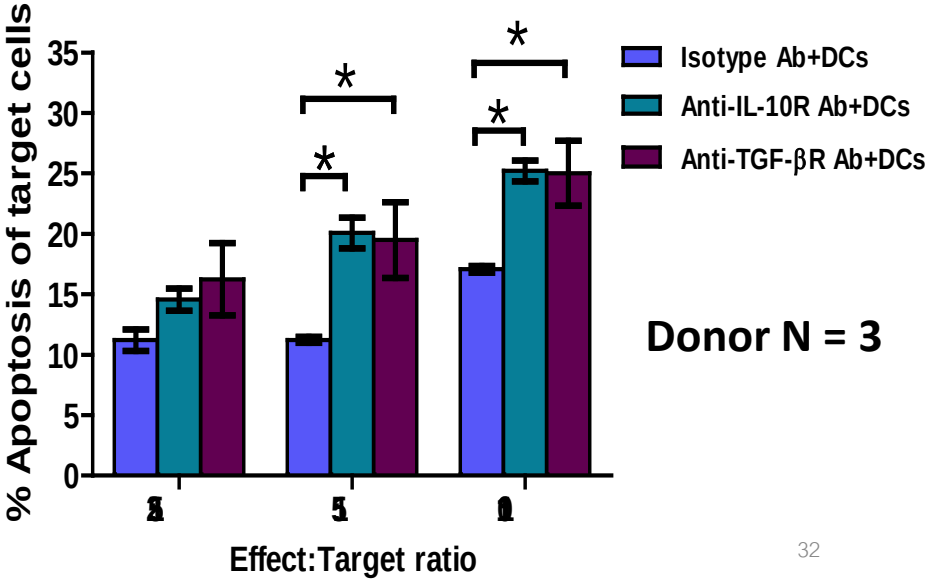
CCA cell line

Cytolytic activity by killing assay using AnnexinV-PI staining

Result

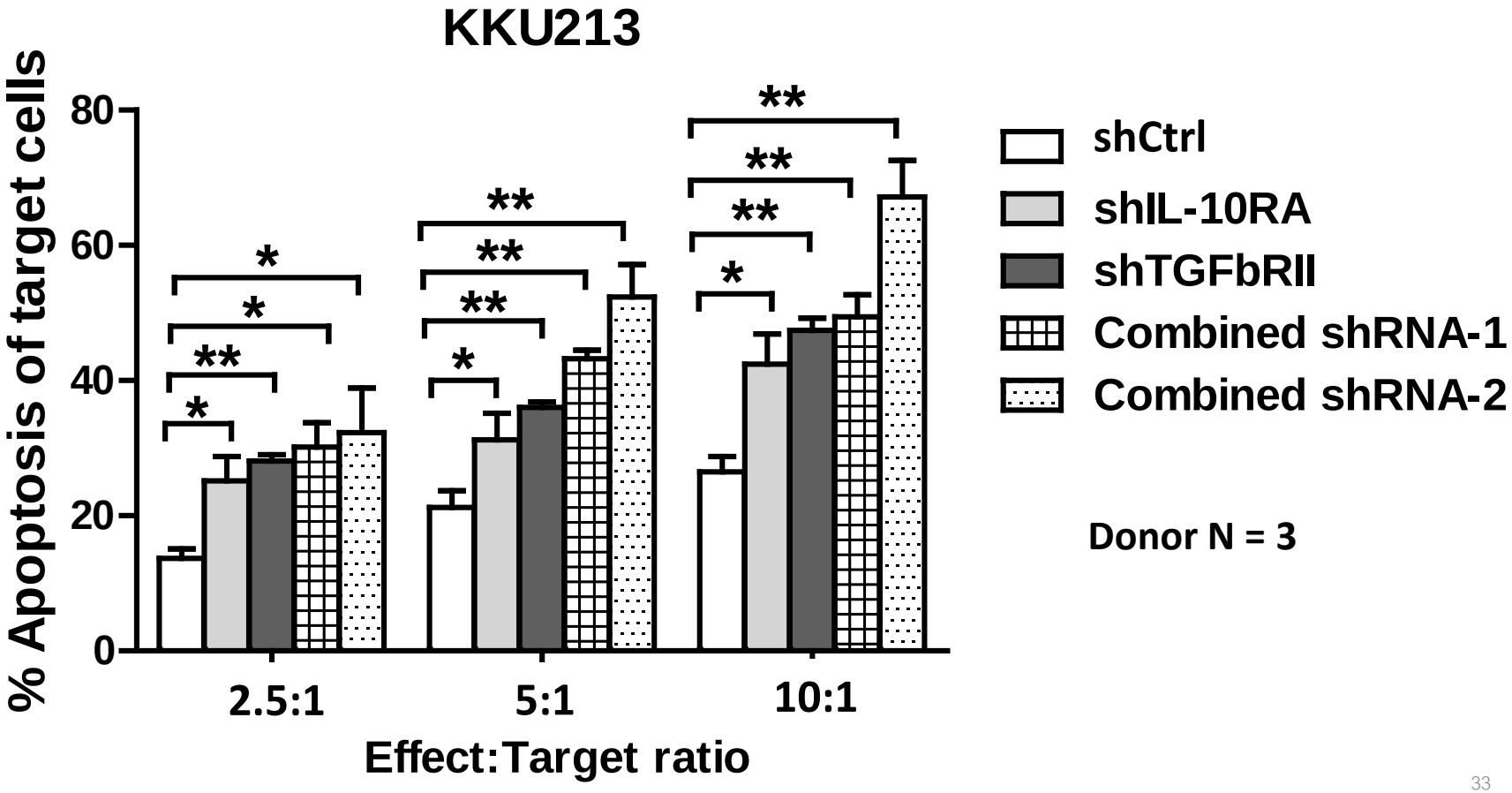


Cytolytic activity of effector T-cells



Suppression of IL-10 and TGF-β receptors by shRNAs improve SD-DC function to activate effector T cells to kill CCA cells.

Results



Cytotoxic Activity of Effector T-cells against Cholangiocarcinoma Enhanced by Self-differentiated Monocyte-derived Dendritic Cells

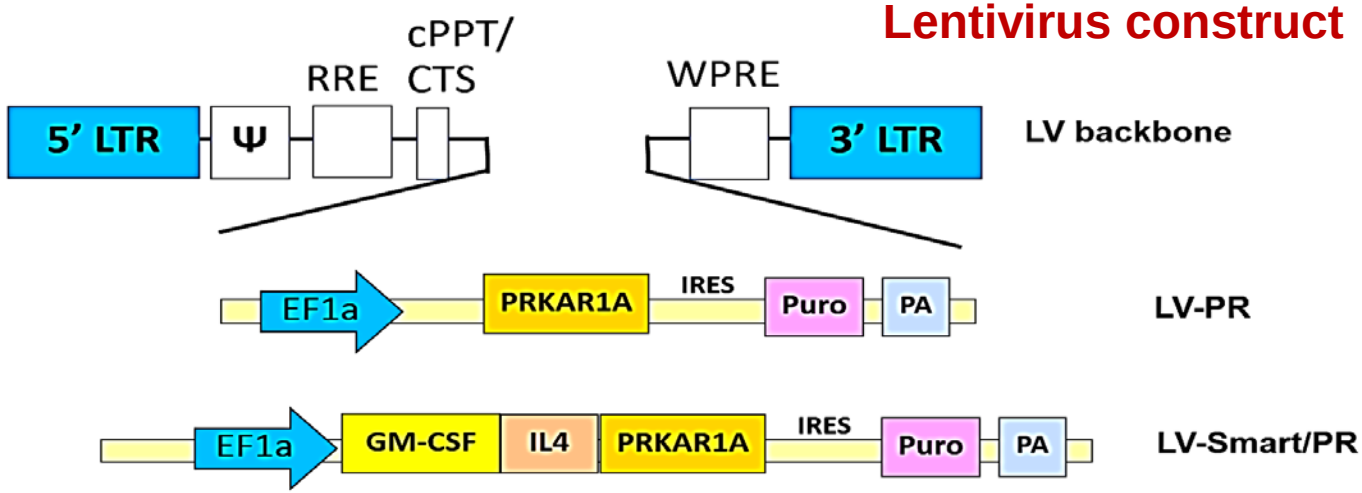
Aussara Panya[1,2], Chutamas Thepmalee[2,3], Jatuporn Sujjitjoon[2], Nattaporn Phanthaphol[2,3], Mutita Junking[2], Nunghathai Sawasdee[2], Sopit Wongkham[4], Pa-thai Yenchitsomanus[2]*

Cancer Immunology, Immunotherapy

Self-differentiated Myeloid-derived Antigen-presenting-cells Reactive against Tumor-DC (SMART-DC)

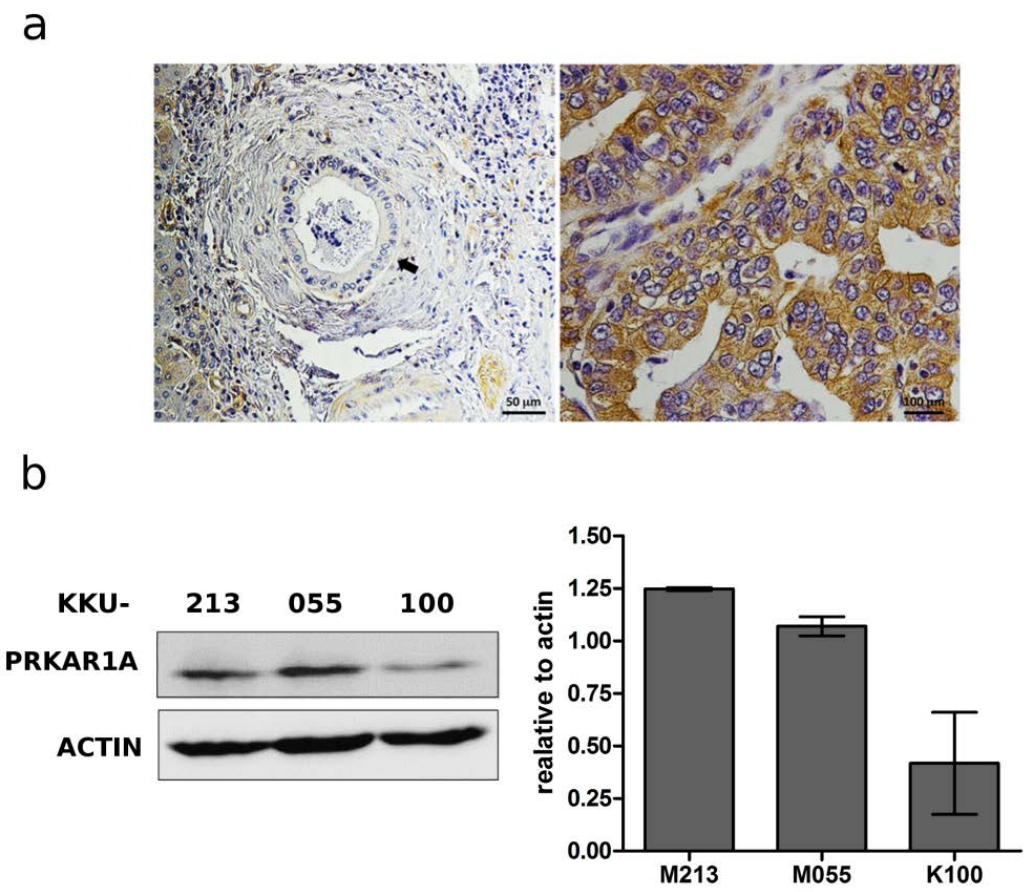
Dr. Aussara Paya

Lentivirus construct



cAMP-dependent protein kinase type I-alpha regulatory subunit (PRKAR1A)

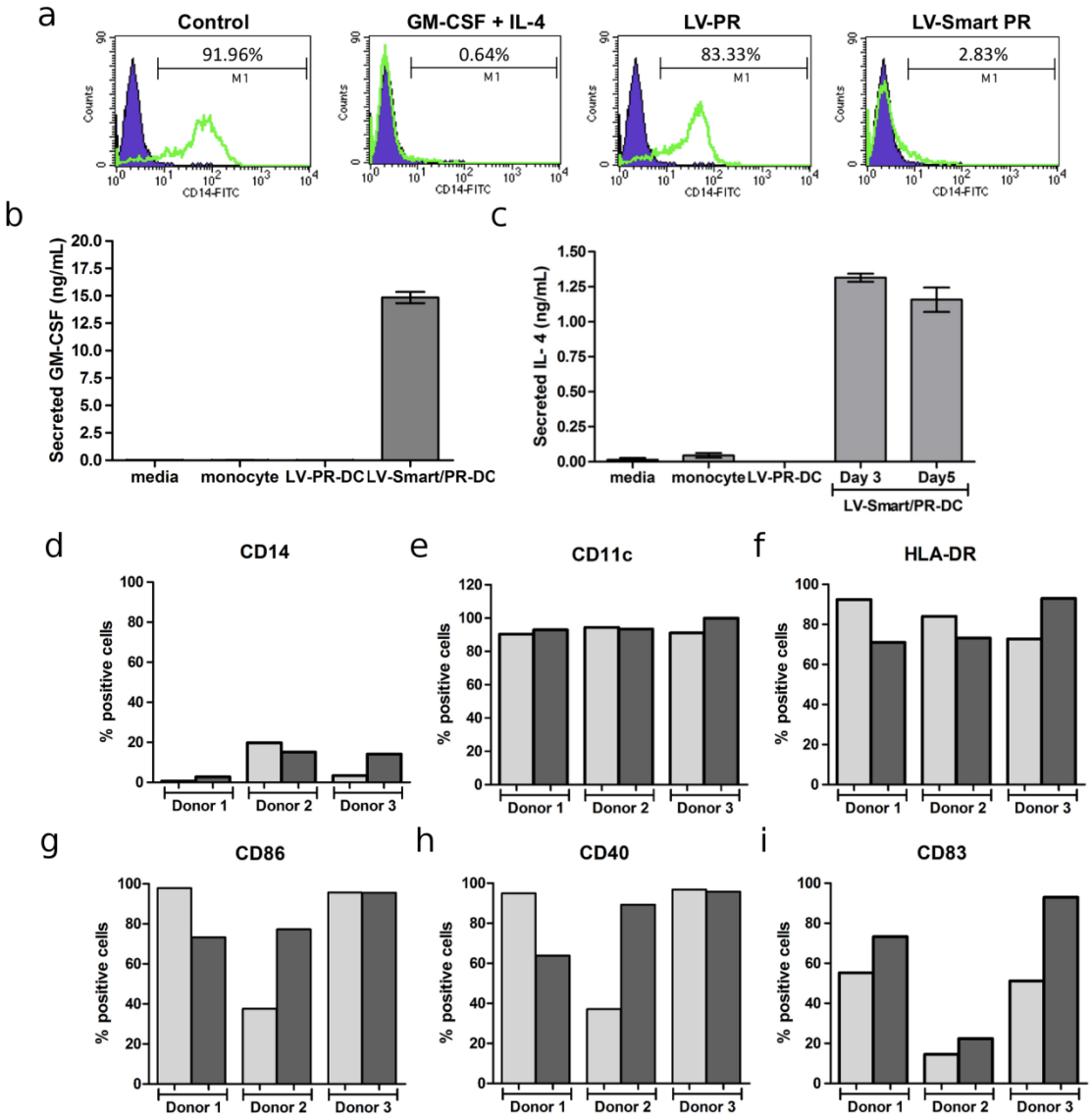
PRKAR1A expression in CCA tissue and cell lines.



(a) Immunohistochemistry showing PRKAR1A expression level in normal bile duct tissue and CCA tissue.

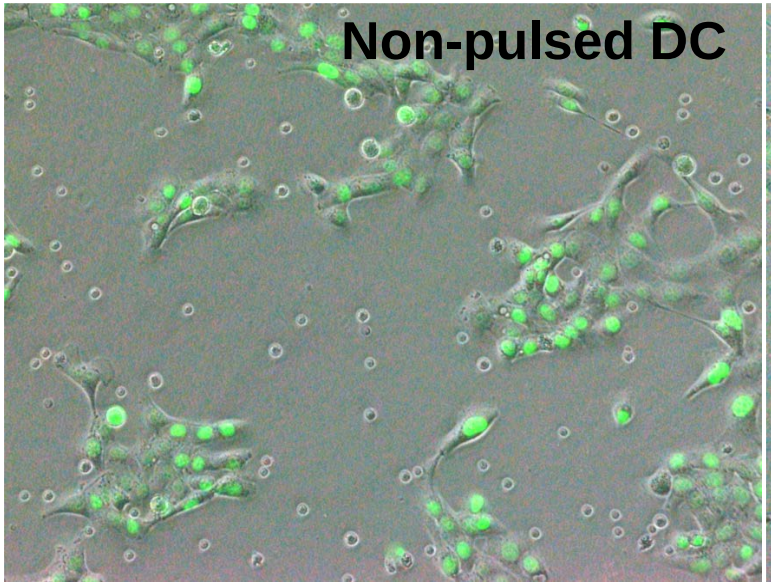
(b) Western blot analysis showing PRKAR1A expression in CCA cells; KKU-213, KKU-055, and KKU-100.

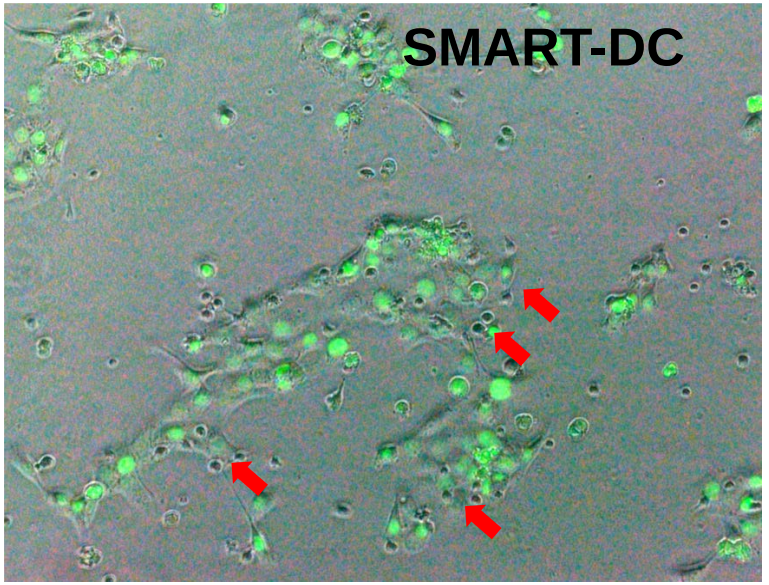
Lentivirus transduction to produce Smart-DC from monocytes.



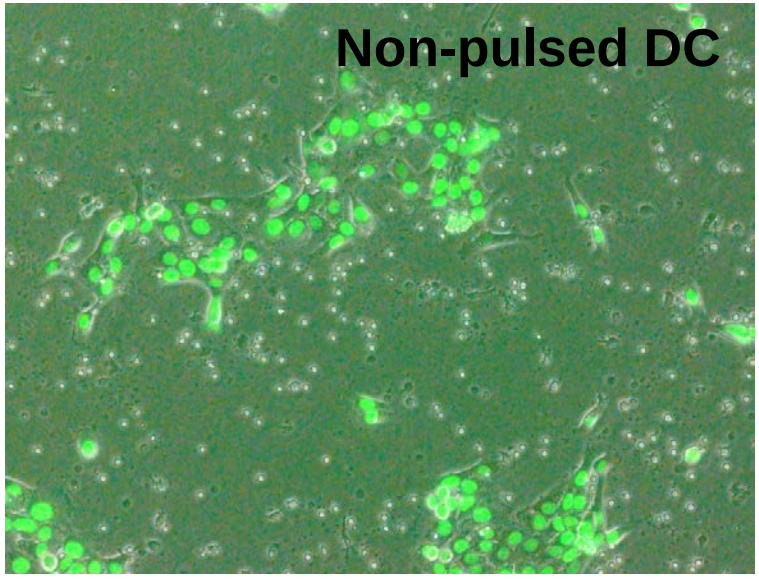
SMART-DC function on activating effector T-cells to kill CCA cells

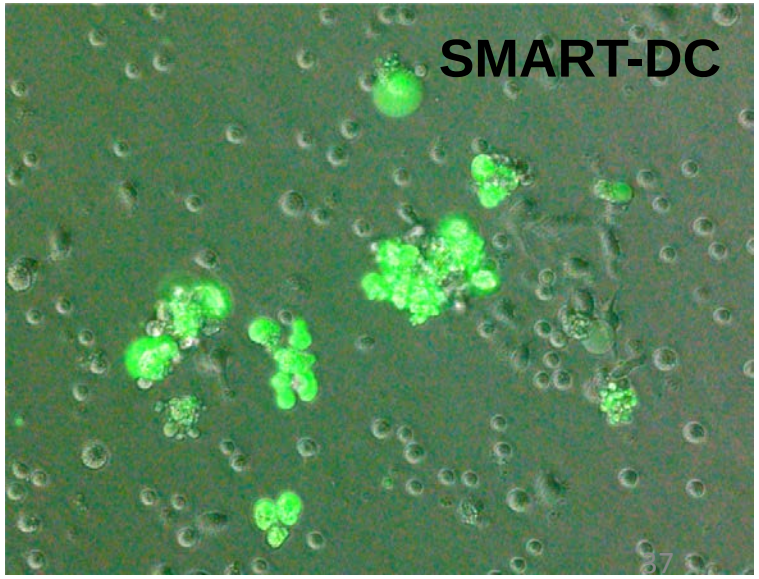
3 hrs after co-culture





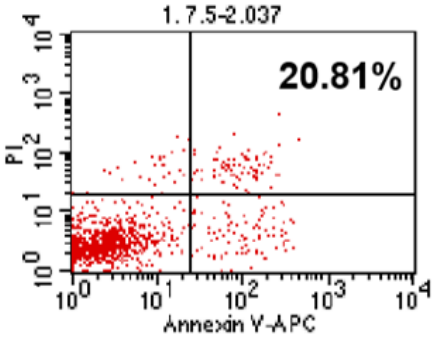
24 hrs after co-culture



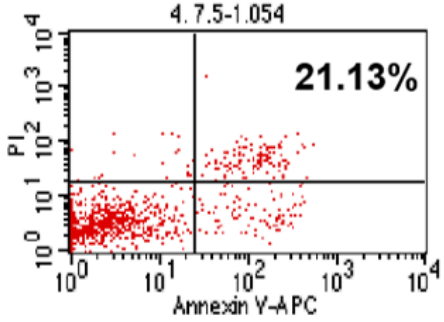


SMART-DC function on activating effector T cells to kill CCA cells

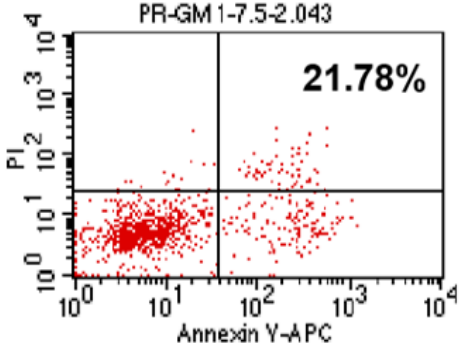
Non pulsed-DC



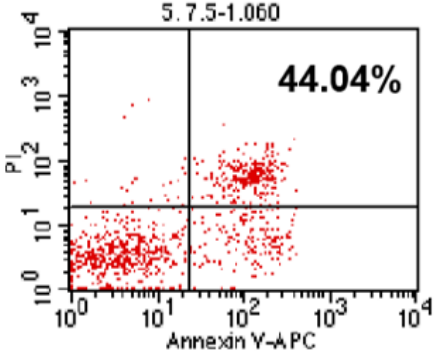
Pulsed-DC

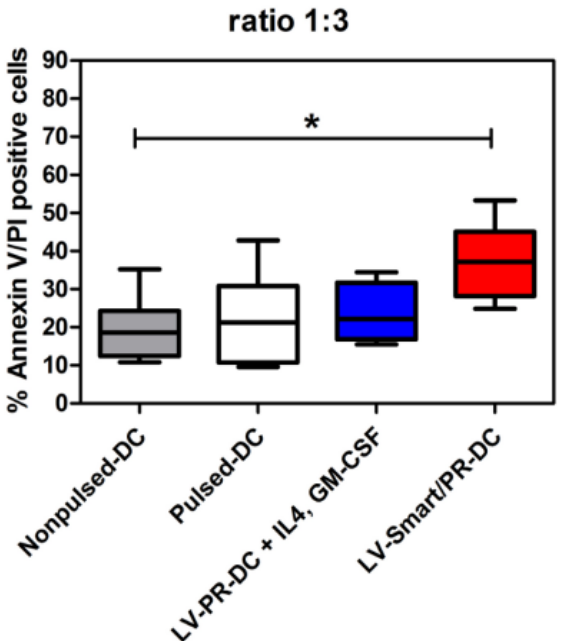


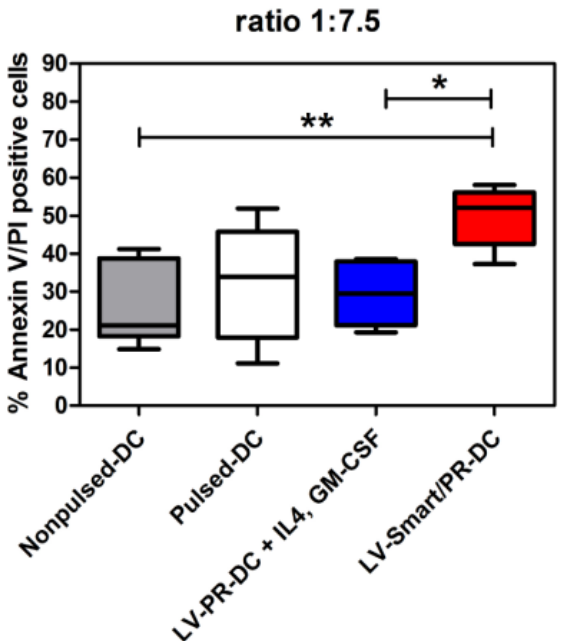
LV-PR-DC

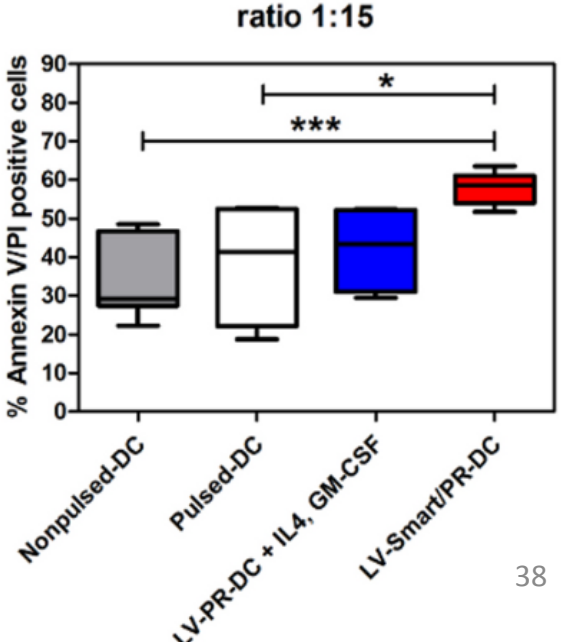


LV-Smart/PR-DC



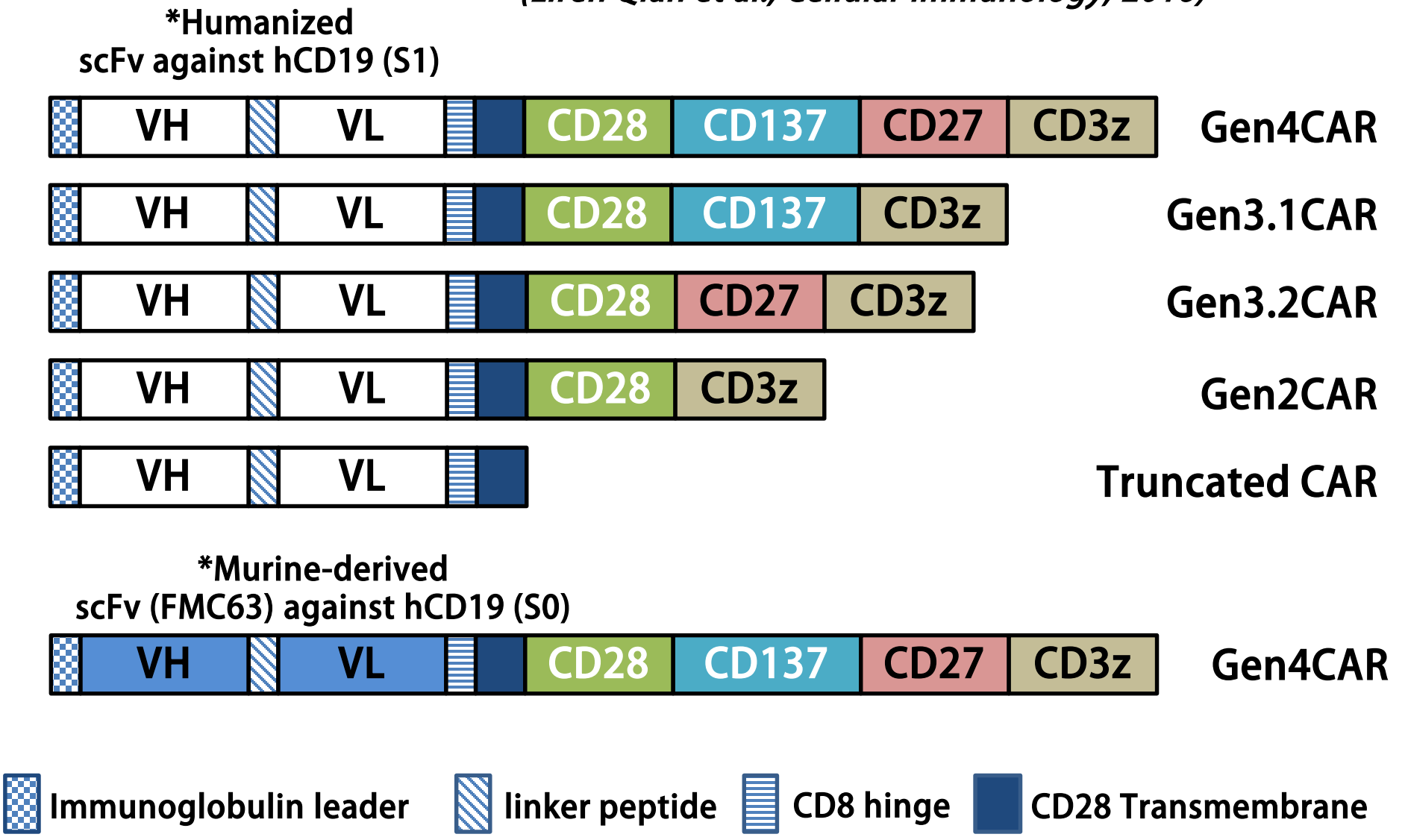






Maps of different generations of CAR constructs

*(Liren Qian et al., Cellular Immunology, 2016)

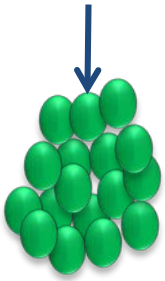


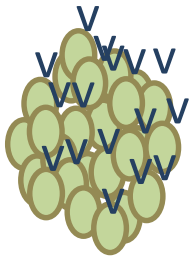
Preliminary data of an *in vitro* CD19-CAR-T killing assay

Target cells overexpressing GFP or mCherry

scFv-CD19 CAR T

Raji (+)
K562 (-)
HeLa-CD19 (+)
HeLa (-)

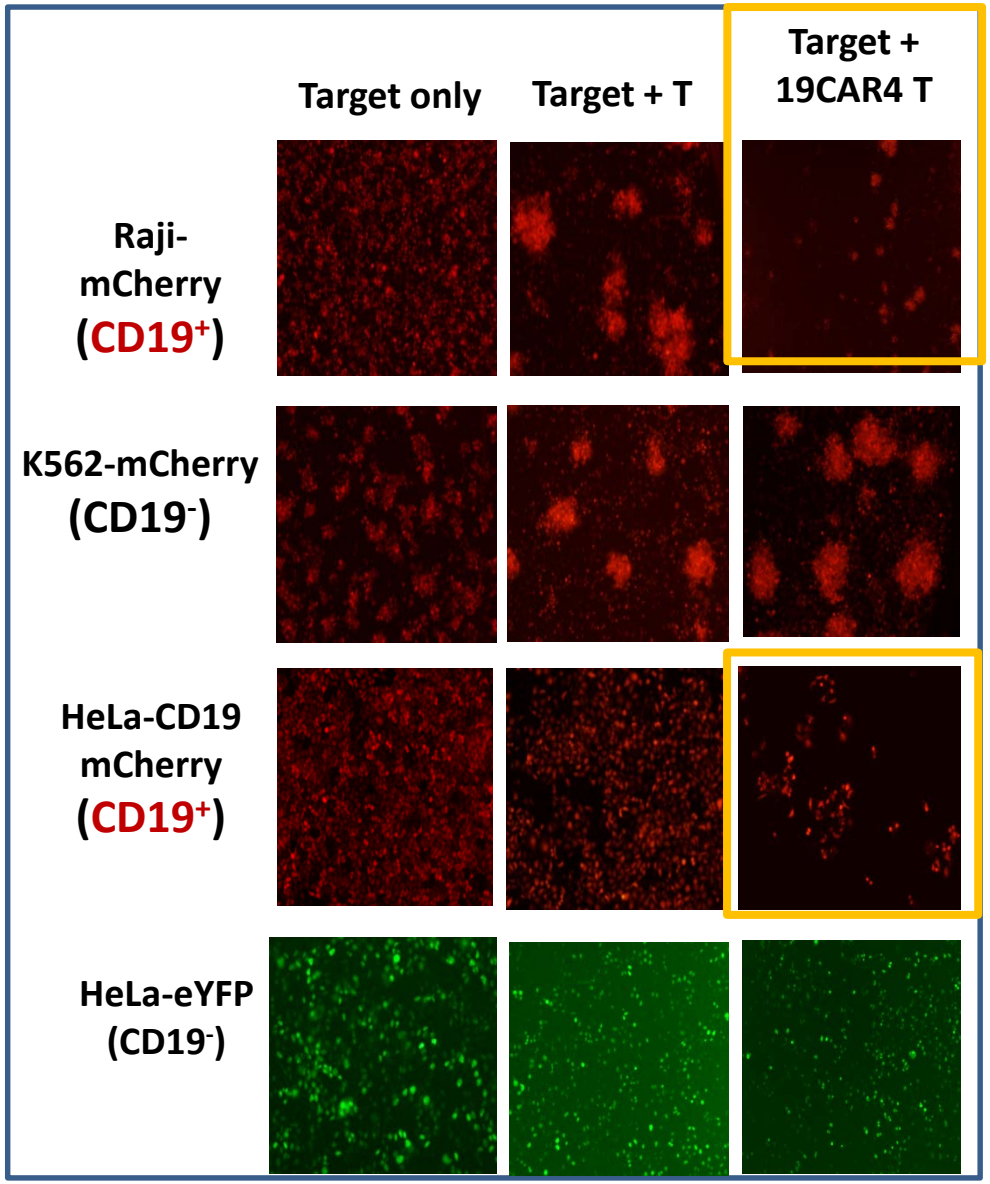




Co-culture

E:T ratio = 1:1

Monitoring tumor cell death under fluorescence microscope



Raji cell line (human lymphoblast; B lymphocytes; Burkitt's lymphoma)

Take Home Message

CAR T-cell therapy for leukemia/lymphoma has transformed previously incurable diseases into durable remissions for many patients; it is potential to be developed for treatment of other cancers.

"True success is not in the learning, but in its application to the benefit of mankind"

H.R.H. Prince Mahidol