이투데이 바이오포럼 2019

21st May, 2019

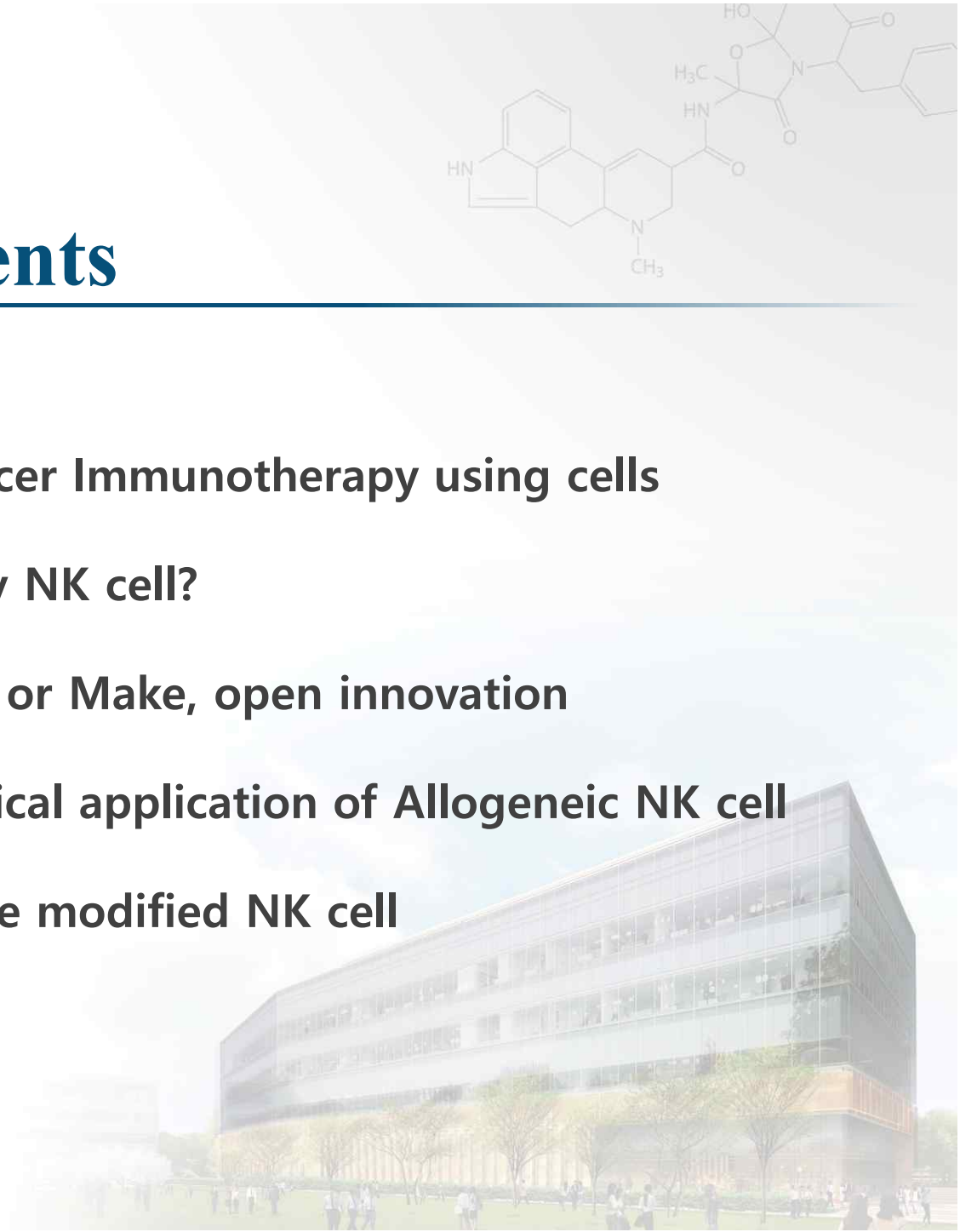
Gene modified allogeneic cells,

면역항암제의 대안,
동종 NK세포를 이용한 유전자치료제

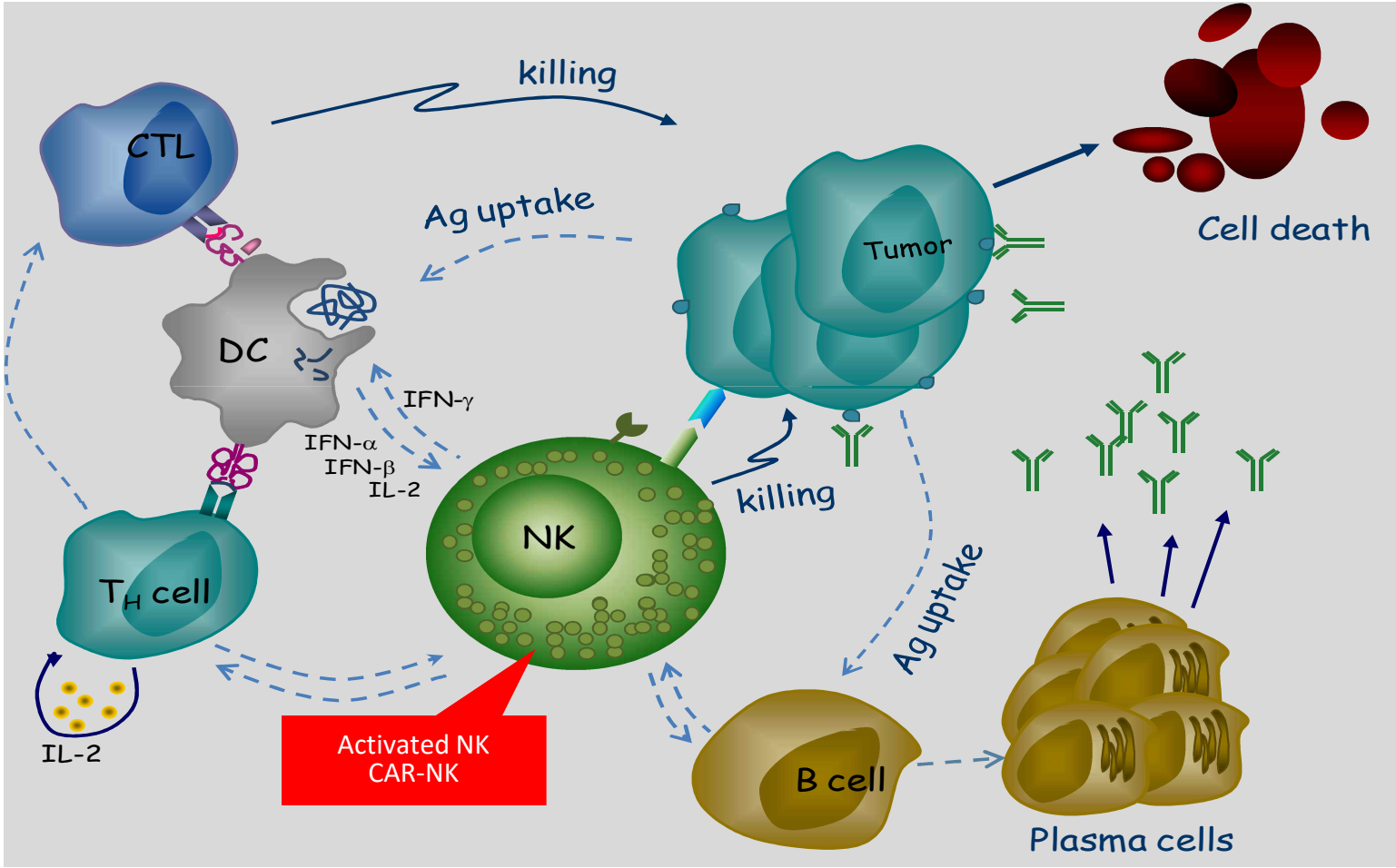
Yu-Kyeong Hwang, Ph. D

GC 녹십자랩셀

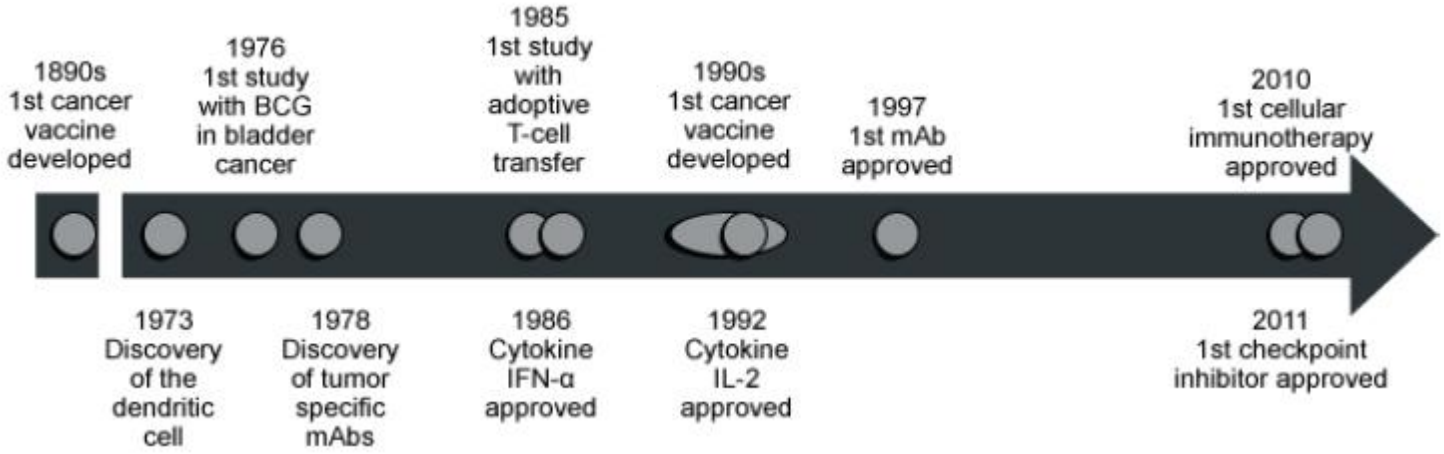
Contents



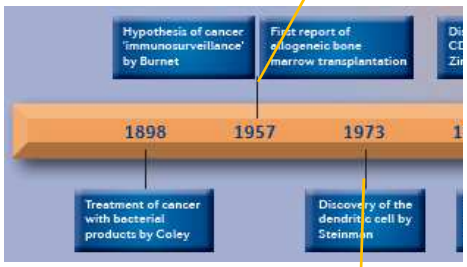
Cancer immunotherapy



항암면역치료의 역사

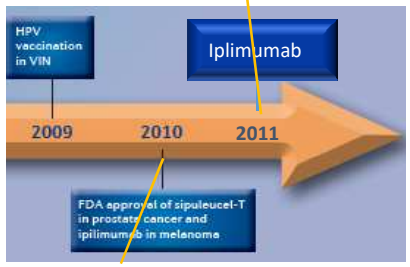


First rep



Disco

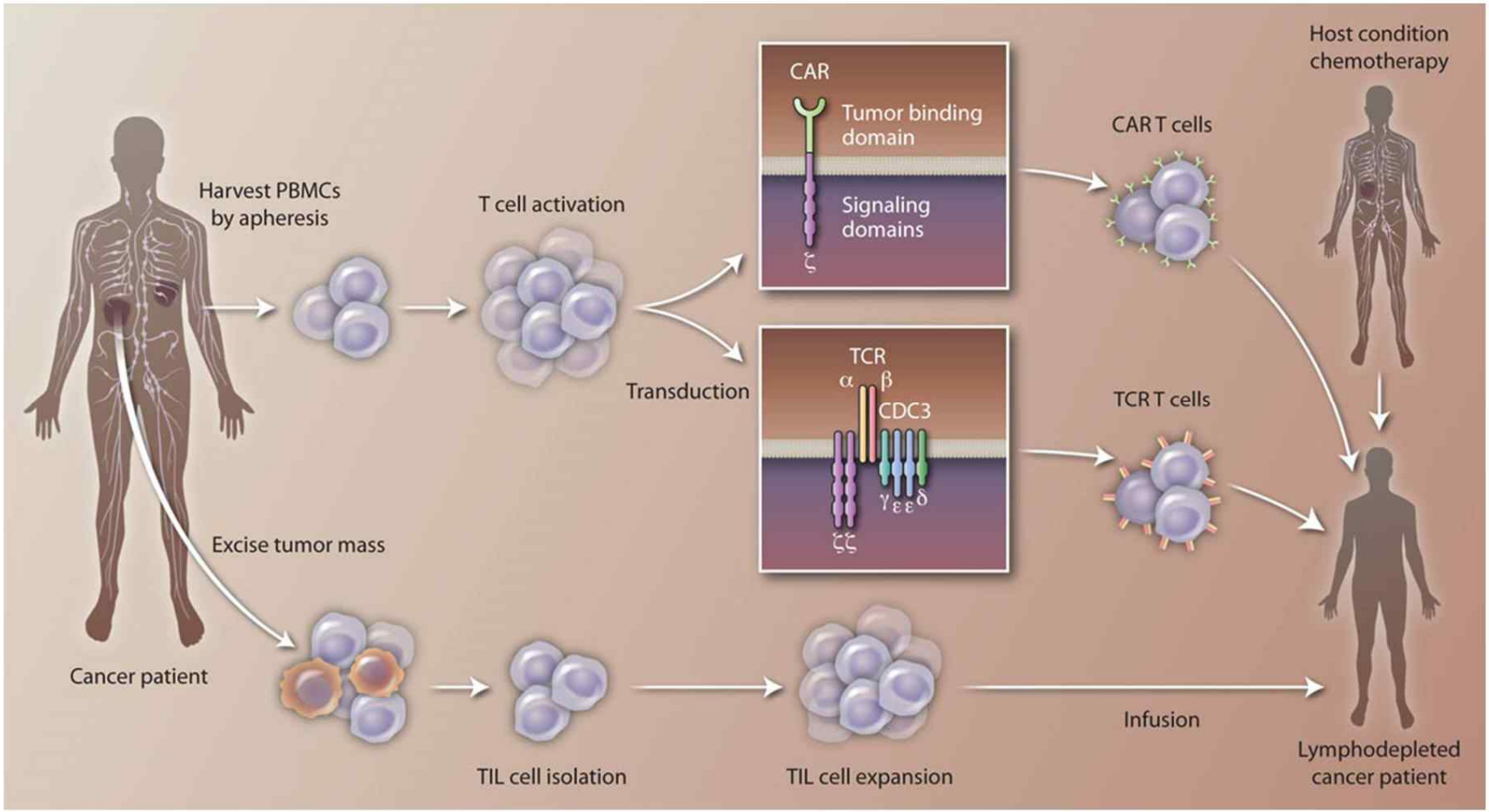
Immune check point inhibitor



proval of PROVENGE

Chimeric Antigen Receptor – T cell

Cellul



June et al, Sci Trans Med 2015

새로운 기술의 등장 (CAR-T), 그리고 문제점

항암면역세포치료제로서 막강한 효력: 악성 혈액암에 대한 치료 효과 입증 (6~8% → 80 ~ 90%)

산업화 기대: Novartis의 Kymriah 품목허가 (2017.08)

"新 패러다임" 노바티스 CAR-T 승인, '가격은 5.3억'

기사입력 : 2017-08-31 08:27 | 수정 : 2017-08-31 08:38

가- 가+

바이오스펙테이터 김성민 기자

'티사젠렉류셀-T' 재발/불응성 B세포 급성 림프구성백혈병(ALL) 어린이 및 젊은 성인(children & young adults) 대상

경제성 이슈

- ☐ 환자 자신의 T세포를 사용: 대량 생산이 어려움
- ☐ 고비용 (5억 이상)

대안이 필요함

안전성 이슈

- ☐ 지나치게 높은 활성
- ☐ Cytokine storm

산업 노바티스 '킴리아' 유사 셀레틱스 치료제 임상시험 중단

메디컬투데이 손성우(mipi306@mdtoday.co.kr) 기자
입력일 : 2017-09-05 21:08:17

[메디컬투데이 손성우 기자] 노바티스사의 최근 승인을 받은 킴리아(Kymriah)와 유사한 유전자 변형 항암 치료제를 개발중인 프랑스 세포치료전문업체인 셀레틱스(Cellectis)사가 한 환자가 사망한 후 혈액종양에 대한 조기 단계 두 종의 임상시험 중단 처분을 받았다.

산업 '주노테라퓨틱스' 개발중 백혈병 치료요법 사망자 발생... 개발 중단

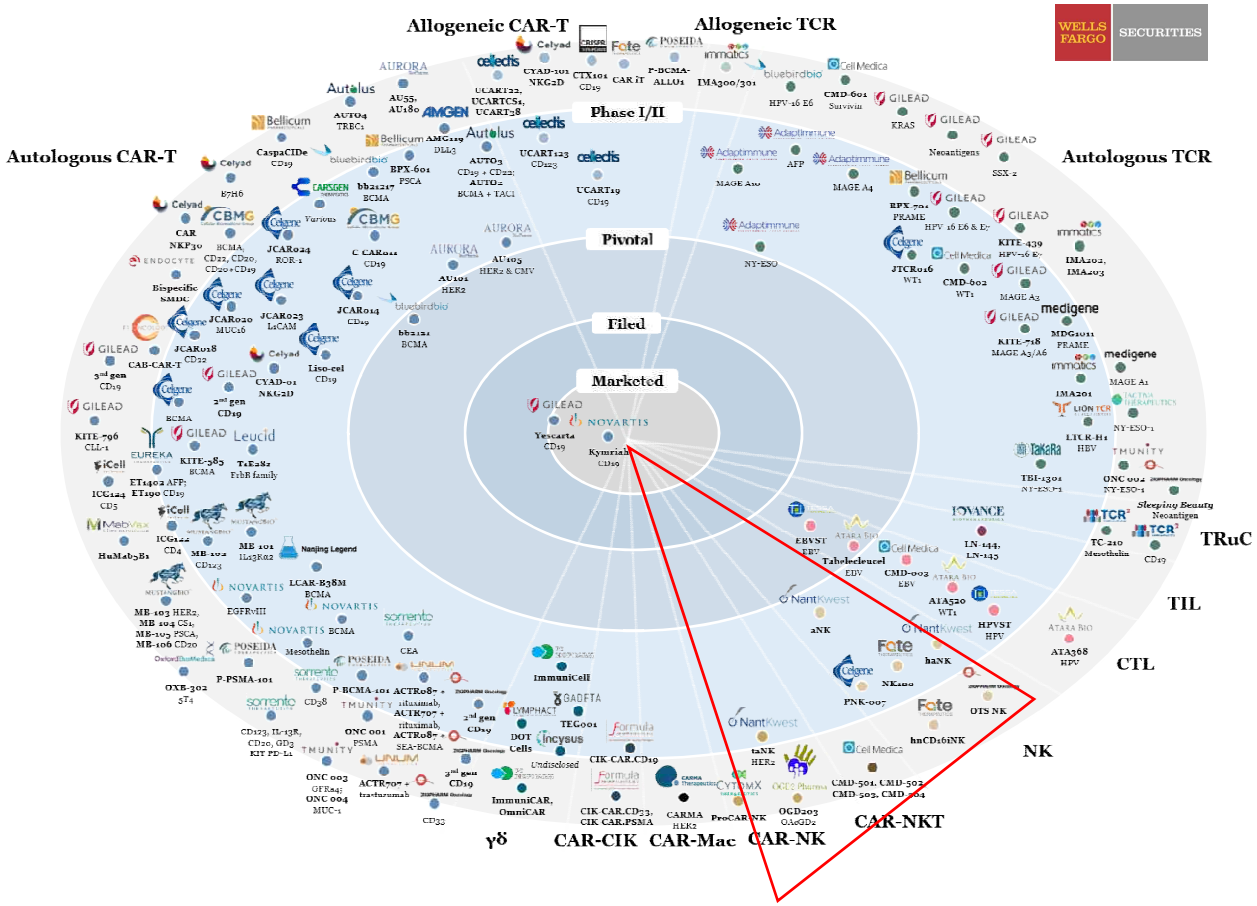
메디컬투데이 강현성(ds1315@mdtoday.co.kr) 기자
입력일 : 2017-03-03 06:15:02

[메디컬투데이 강현성 기자] 주노테라퓨틱스(Juno Therapeutics)사가 개발중인 백혈병 치료요법에 대한 임상시험에서 일부 환자에서 사망이 발생 주노사가 이 같은 치료법 개발을 중단하기로 했다.

3일 주노사는 CAR015 라는 CAR-T 라는 기법을 사용한 전도 유망한 새로운 계열의 면역치료요법에 대한 드물지만 치명적인 혈액암인 재발성급성임파구성백혈병(relapsed acute lymphoblastic leukemia)을 앓는 성인 환자를 대상으로 한 임상시험에서 5명이 뇌 부종으로 사망 해 이 같은 치료법 개발을 중단하기로 했다라고 밝혔다.

Cellular I-O Landscape

- Highly competitive, rapidly evolving, relatively immature landscape
- Investors are turning attention to solutions to the challenging patient-specific autologous business model
- NK cell segment is still small, and no OTS programs are beyond proof of concept trials
- Cellularity, Allogene & Fate have substantial war chests to pursue OTS therapies

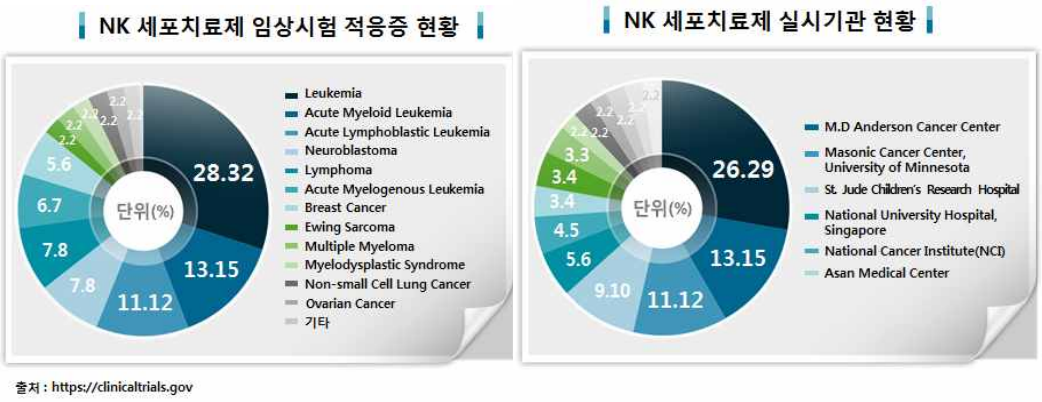


Alternatives to CAR-T, NK cell

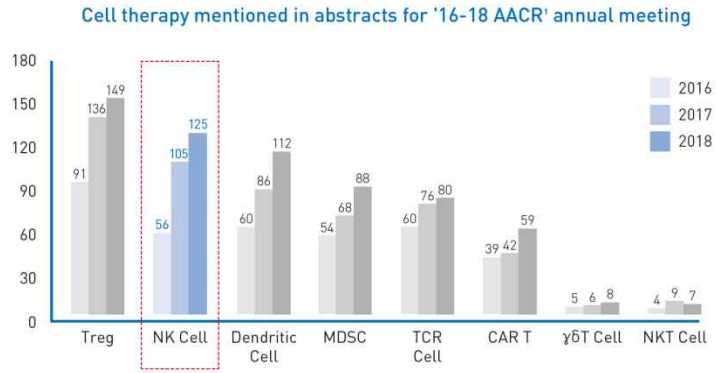
Natural Killer Cell

- Can recognize abnormal cell (like cancer) and delete from body.
- Can be used **without GvHD** in allogeneic transplantation.
- Evaluated it's **anti-cancer effect** and **safety** through HSCT.
- Is useful in a **commercial aspect**, compared to autologous product.
- AACR 2017, NK cell research presentation the was most increased area.
  : **NK cell (88% increase)** vs T cell (7.7% increase)
- Company No. : > 10 in 2010, 17 in 2017.

Natural Killer Cell



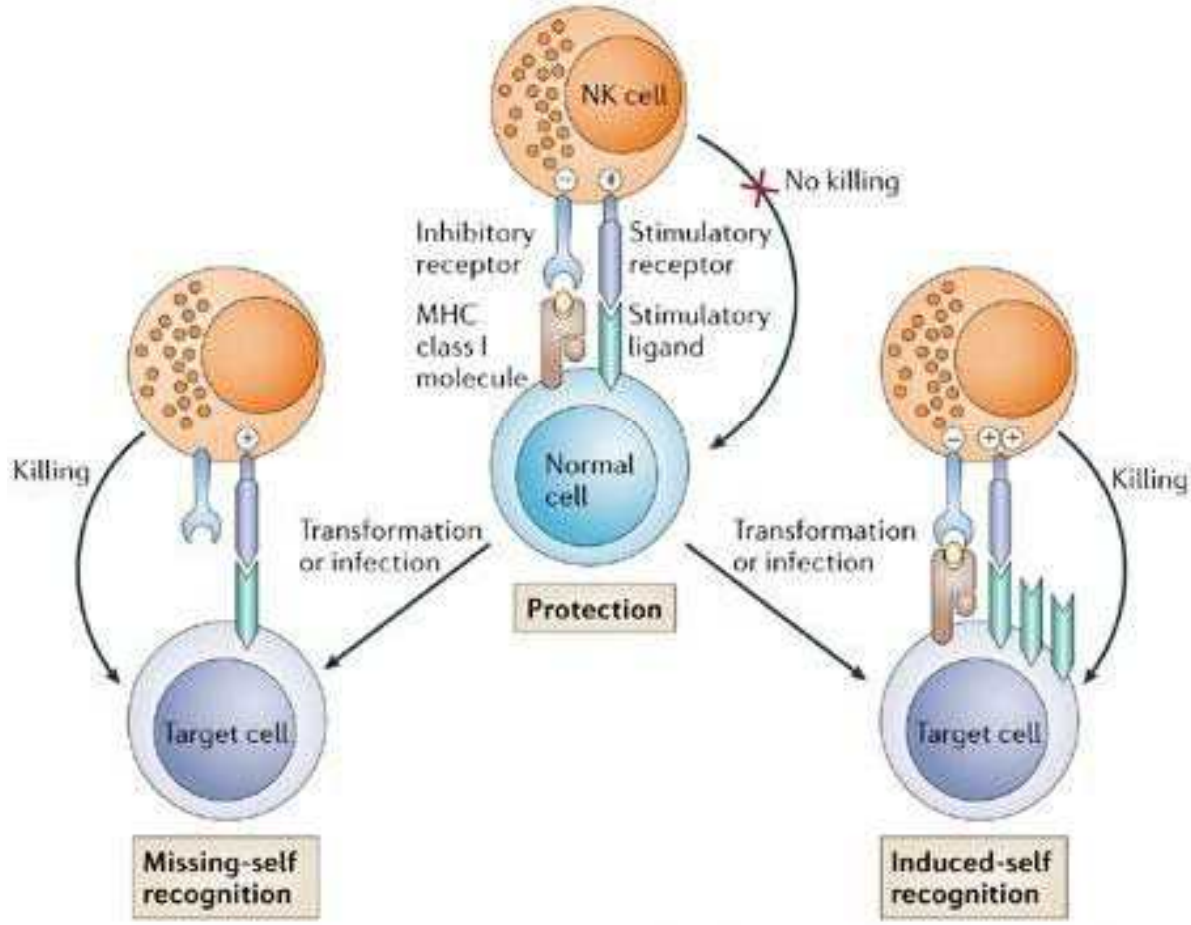
출처 : https://clinicaltrials.gov



Cancer immunotherapy with NK cells

- NK cells were initially described in 1975 and were originally defined as innate effector lymphocyte (Int. J. Cancer 1975, 16:230–239/Eur J Immunol 1975, 5: 112–117). They make up about 5% up to 15% of the total lymphocyte circulating population in normal healthy subjects.
- NK cells are specialized lymphocytes that provide a first line of immune defense against viral infections and cancer. They are considered to influence both innate and adaptive immune host defenses.
- Depressed NK cell activity and populations are associated with a wide variety of diseases including various cancers, hepatitis, AIDS, chronic fatigue syndrome, various immunodeficiency syndromes, and certain autoimmune diseases.
- Mouse NK cell studies demonstrated that NK cells could inhibit graft-versus-host disease (GVHD) and promote graft-versus-tumor (GVT) effects (J Clin Invest. 1998, 101:1835–42).
- Patients with AML who underwent haploidentical stem cell transplantation (HI-SCT) in which KIR-ligand mismatch prevailed in the graft-versus-host direction showed improved disease-free survival (DFS) and reduced GVHD (Science, 2002, 295:2097–2100).
- The usage of NK cells has been proposed in human cancer immunotherapy and treatments with these cells have been recently entered clinical trials targeting various cancer types.

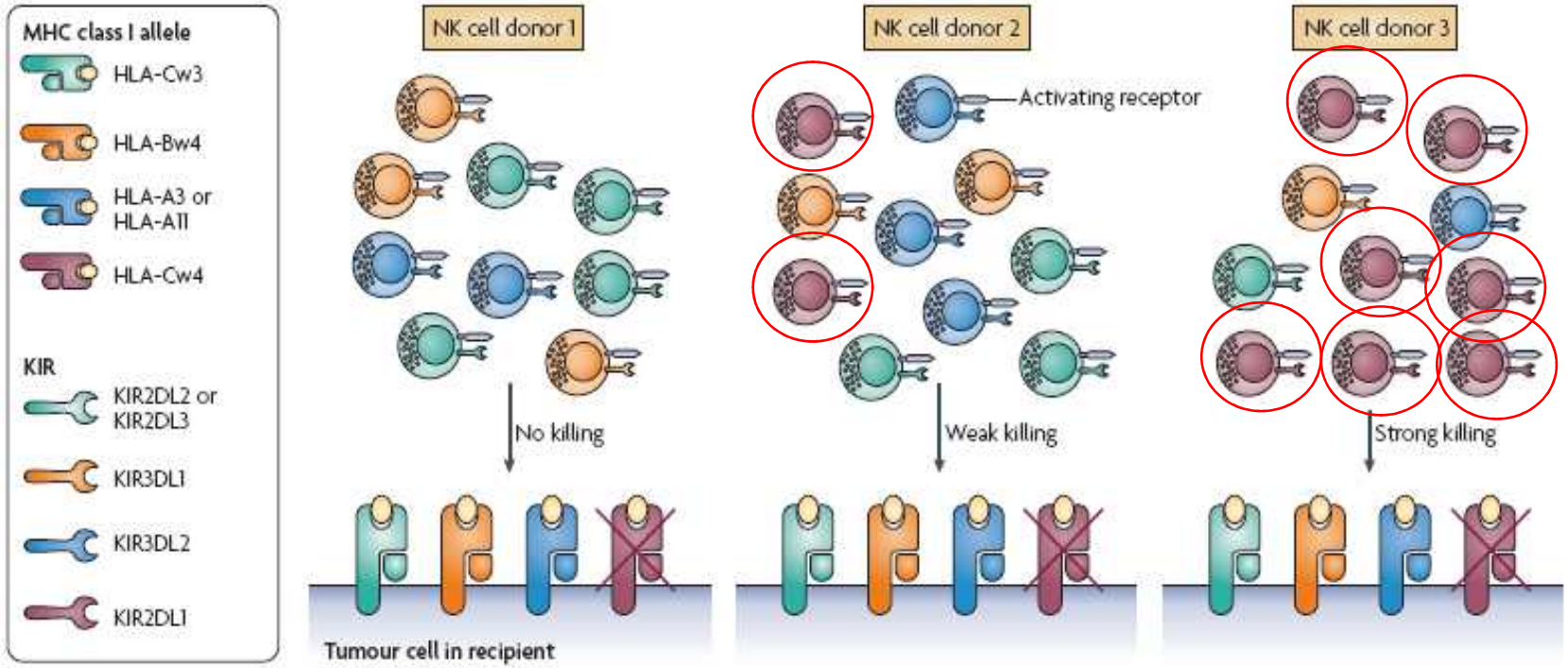
Target recognition by NK cells



Nat Rev Immunol. 2007 May;7(5):329-39.

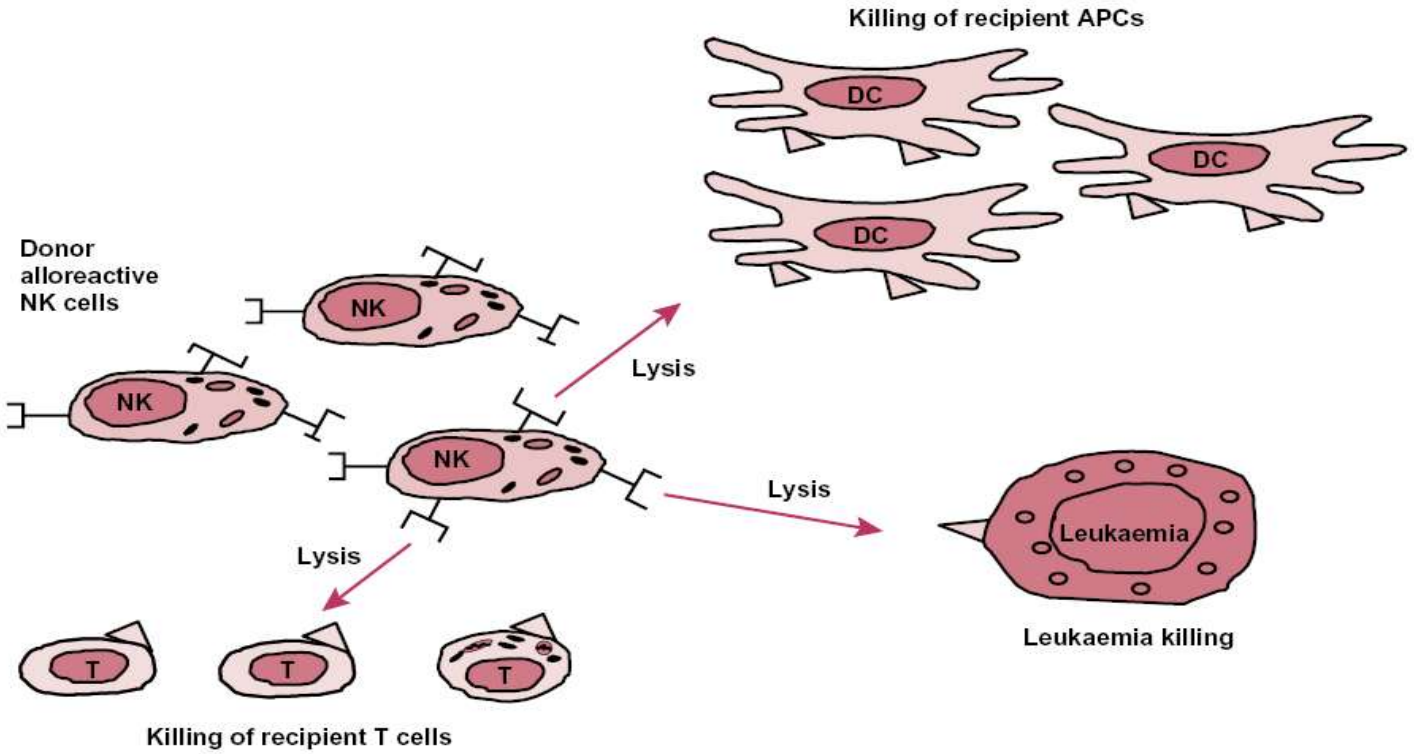
Prospects for the use of NK cells in immunotherapy of human cancer.

Ljunggren HG, Malmberg KJ.
Centre for Infectious Medicine, Department of Medicine, Karolinska Institute, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. hans-gustaf.ljunggren@ki.se



Role of NK cells in killing recipient immune cells

Haploidentical bone marrow hematopoietic stem cells transplantation (HSCT) in leukemia patients



Ruggeri et al Science 2002; 295:2097-2100

Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia

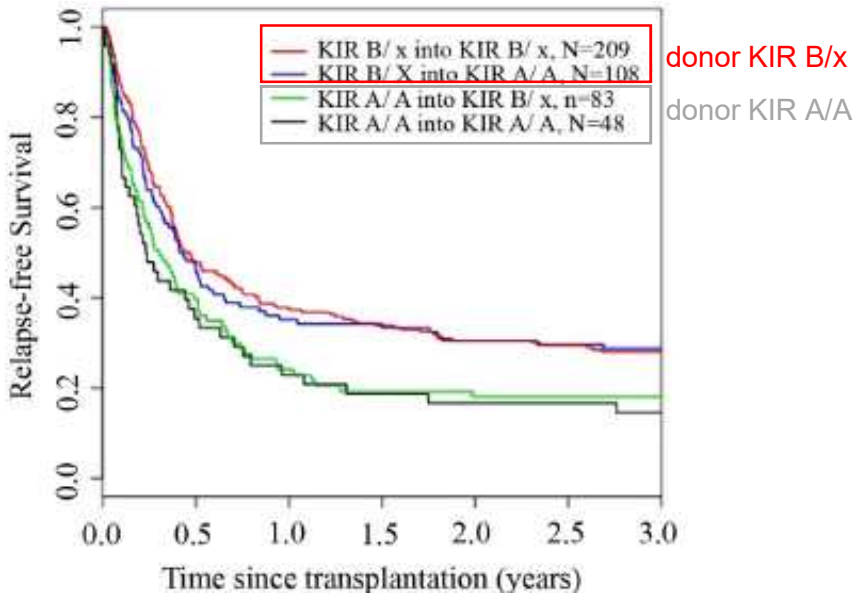
Blood (2009) 113:726-732

- Acute myelogenous leukemia patients after HSCT: 448
- NK helps implantation, reduce GVHD (graft-versus-host disease), reduce the recurrence of the leukemia.
- KIR haplotype

KIR haplotype A	KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4
KIR haplotype B	KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, KIR3DS1, KIR2DL5

Group A	A/A; 2A KIR haplotype
Group B	B/x; at least 1 B haplotype

- KIR genotype of donor influence the survival (3 year) of AML in URD (unrelated donor) HCT
- KIR A/A(21%) vs KIR B/x(31%)



donor KIR B/x

donor KIR A/A

Published in final edited form as:
Biol Blood Marrow Transplant. 2010 April ; 16(4): 533–542. doi:10.1016/j.bbmt.2009.11.022.

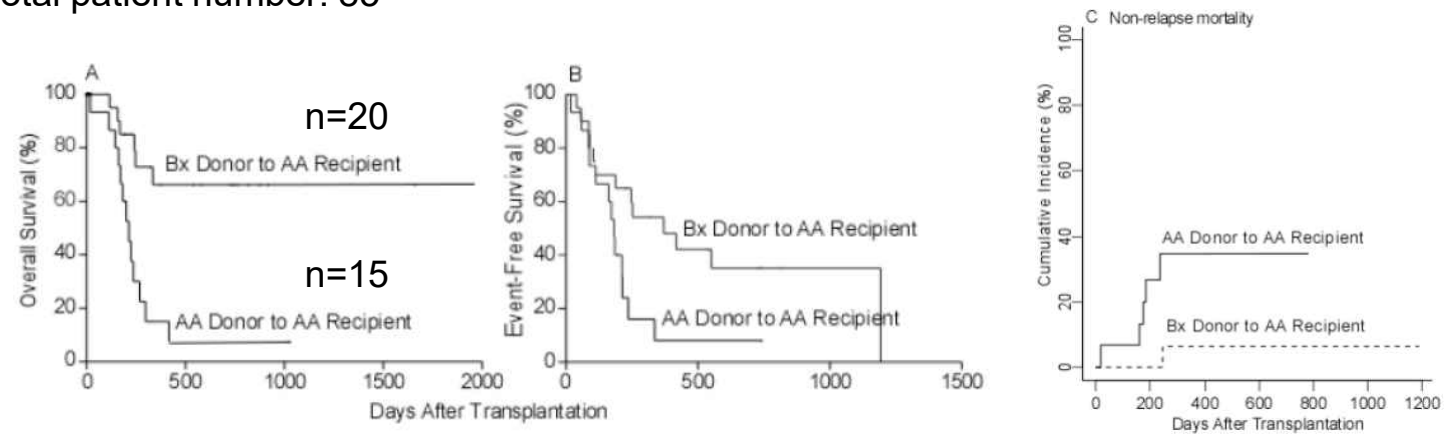
Improved survival with inhibitory Killer Immunoglobulin Receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation

Heather J. Symons, MD, MHS[1,3], **M. Sue Leffell, PhD**[2], **Nancy D. Rossiter**[2], **Marianna Zahurak, MS**[1], **Richard J. Jones, MD**[1], and **Ephraim J. Fuchs, MD**[1]

[1]Department of Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD, USA

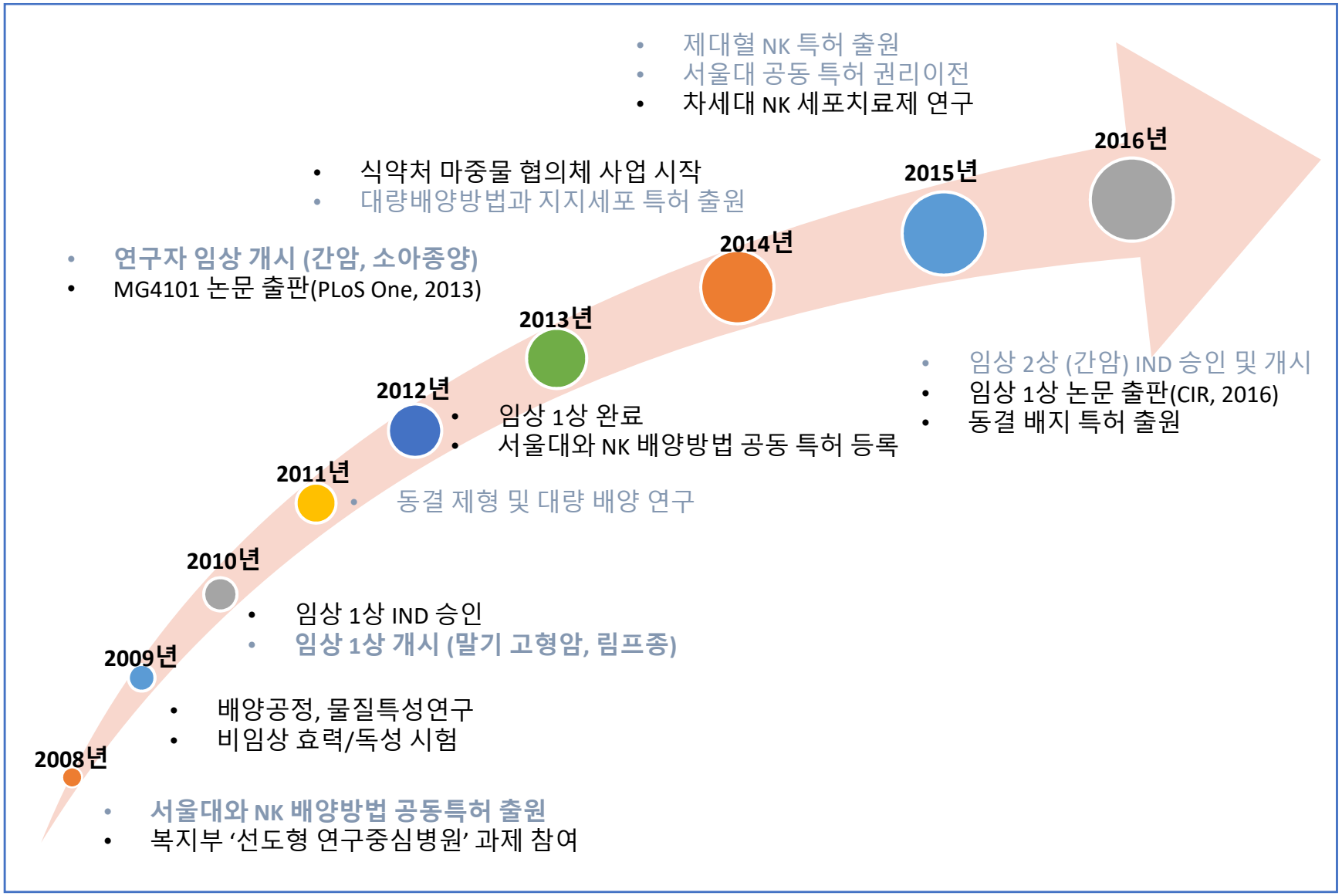
[2]Department of Medicine, Johns Hopkins University, Baltimore, MD, USA

Total patient number: 85



Open Innovation
: Buy or make?

NK 세포치료제 (MG4101) 개발 경위



녹십자랩셀 - 서울대학교 병원 공동 기술 개발

[보건복지부] 선도형 특성화연구사업, 선도형 세포치료연구사업단 참여

- 10대 질병정복 메디클러스터 구축과제 (2006. 12. ~ 2016. 11.)
- 연구성과물의 공동출원 약정(서울대병원/녹십자랩셀)

"자연살해세포의 증식방법" 특허출원 (서울대병원/녹십자랩셀 공동)

- **KR** 2008-0074069 (2008.07.29)
- **PCT**/KR2009/008-0074069 (2008.07.29)
- **JP** 2011-521023 (2009.07.29)
- **CN** 2009-80130121 (2009.07.29)

GC 녹십자랩셀

업무제휴 계약 체결 (2011.06.23)

- 공동 특허권에 대한 양자의 권리의무 규정

SNUH 서울대학교병원 SEOUL NATIONAL UNIVERSITY HOSPITAL

업무제휴 계약서에 대한 추가 합의서 체결 (2015.12.22)

- 기술료 확정 및 공동특허권의 랩셀 단독소유 합의

녹십자랩셀 – 목암연구소 공동 기술 개발

"자연살해세포의 제조방법" 특허출원 (목암연구소/녹십자랩셀 공동)

- **PCT**/KR2012/011114 (2012.07.27)
- **KR** 2014-7020547 (2014.07.22)
- **JP** 2014-548657 (2014.06.23)
- **AU** 2012-354438 (2014.06.19)
- **EP** 2012-859499 (2014.06.20)
- **US** 14/367,813 (2014.06.20)
- **CN** 2012-80070546 (2014.06.20)
- **IN** 5356/CHENP/2014 (2014.06.20)

GC 녹십자랩셀

MOGAM Biotechnology Institute

기술실시계약 체결 (2011.06.23)

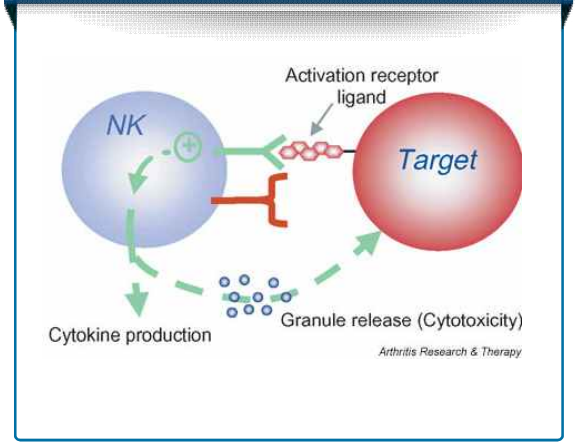
- 공동 특허권에 대한 양자의 권리의무 규정

기술실시계약서에 대한 추가 합의서 체결 (2017.10.)

- 녹십자 랩셀의 특허절차 단독 진행 및 단독 실시권 합의

Allogeneic NK cells

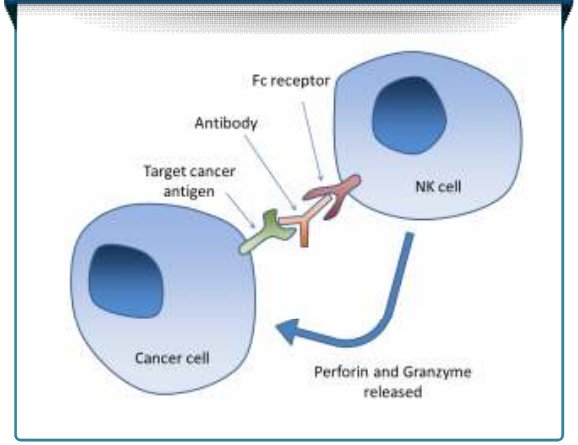
Direct killing



Mass Production

- Feeder cell system
- **Cell source: PBMC, CBMC**
- Bioreactor

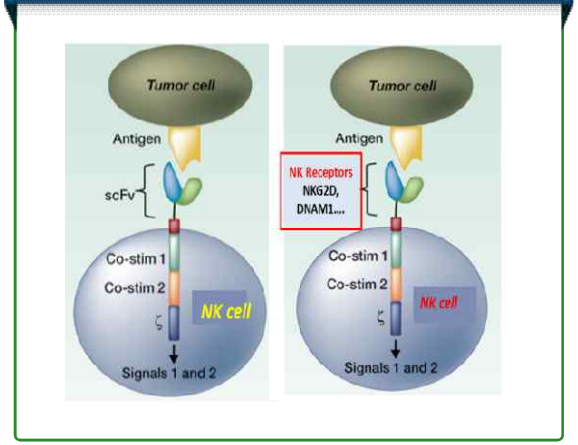
Antibody-mediated killing



Targeting

- **Targeting antibody** (Rituxan, Herceptin, Cetuximab etc.)
- **ICI**: anti-PD-L1, PD-1 etc.

Targeted-activated killing



Proliferation, survival

- scFV-CAR-NK
- NCR-CAR-NK
- NK specific intra-cellular domain

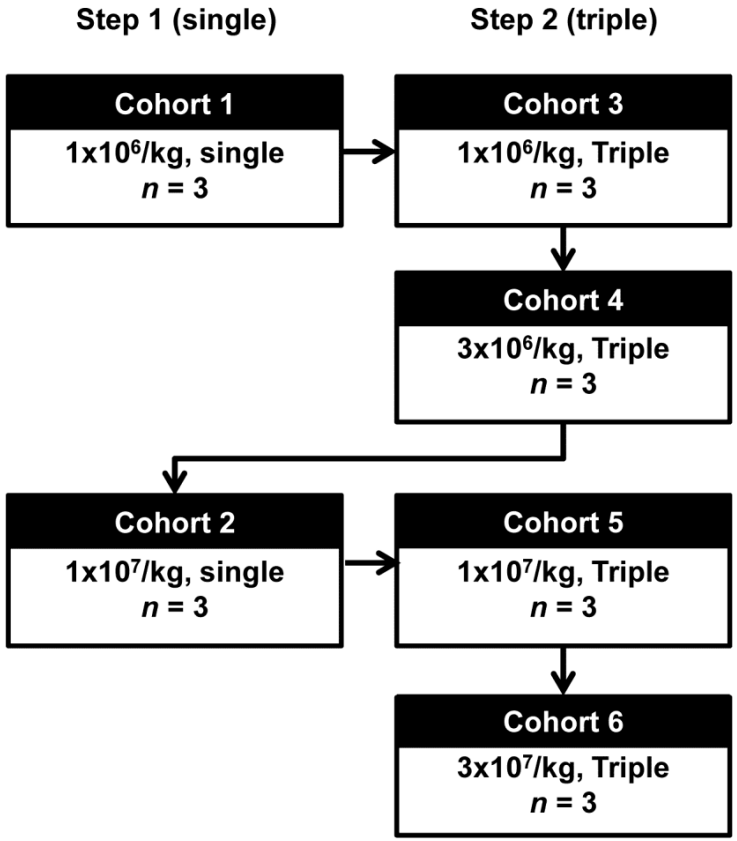
→ ***Highest efficiency & safety***

Clinical application of Allogeneic NK cell

Allogeneic NK cell: Phase I trial (NK cell stand alone)

First-in-Human trial with **expanded random donor NK cell.**

Title: A Phase I, single center, non-comparative, dose-escalation, single and repeated dose studies of MG4101



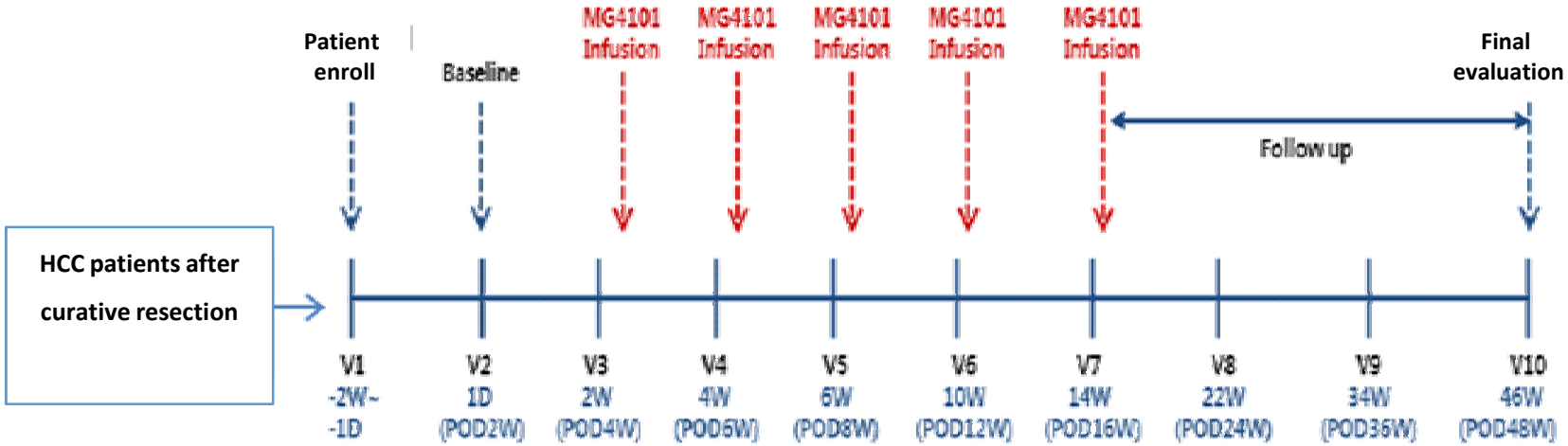
	Dose	Type of cancer
Single injection	1 x 10^6/kg	DLBCL Renal cell cancer Thymic carcinoma
	1 x 10^7/kg	Ovarian cancer Maxillary cancer Sigmoid colon cancer
Triple injection	1 x 10^6/kg	Maxillary sinus, adenoid cystic cancer Bladder cancer Tongue cancer
	3 x 10^6/kg	NK T cell Lymphoma Nasopharyngeal cancer NSCLC
	1 x 10^7/kg	Renal cell cancer Esophageal cancer triple primary cancer Ovarian cancer
	3 x 10^7/kg	Adenoid cystic carcinoma of salivary gland Papillary thyroid cancer NSCLC

Cancer Immunol Res. 2016 Jan 19

Allogeneic NK cell: IIT, Hepatectomy (NK cell stand alone)

Patients: HCC after resection, modified UICC T3 stage

- Treatment: 5qw 3 x 10^7cells / kg
- No. of Patients: 5 Patients
- Study site : Samsung Medical Center, Transplantation



Unpublished data

Allogeneic NK cell: Case study, r/rAML, 3rd party NK cell (HSCT + NK cell)

- Patients: Refractory, relapsed AML, 3 patients
- Phase 1, Single center, open
- Pre-treatment: allogeneic HSCT, lymphodepletion by fludarabine, Cyclophosphamide before 3rd party MG4101 treatment
- Dose: 3x10^7/kg ~ 9x10^7/kg, 3~4 days interval, 6 times
- Results: complete dosing of 3rd party NK cell for 3 patients.

  → No AE related with NK cell

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9[1)~]
Days	Screening	1	8	11	15	22	25	29	113~
Allo HSCT		V							
NK cell infusion			V	V	V	V	V	V	
Blood test	V		V		V	V		V	
Marrow test								V	V
Tumor response								V	V
Survival								V	V
Vital			V	V		V	V	V	
AE		V	V	V	V	V	V	V	V[2)]

Unpublished data

Allogeneic NK cell: IIT, r/rAML, NK with conditioning (lymphodepletion + NK cell + IL-2)

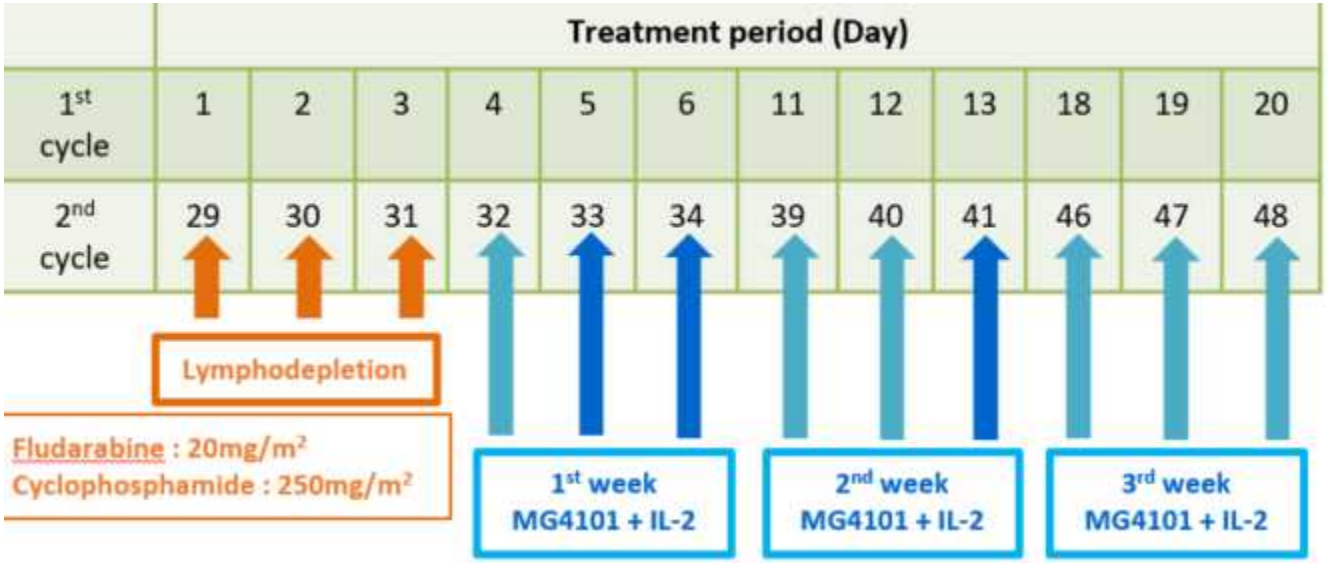
Open, Single center, Single-arm, Phase II, Pilot study for monitoring the safety and efficacy of MG4101(Ex vivo-expanded Allogeneic Natural killer cells) and Interleukin-2(IL-2) combination following lymphodepletion in patients with refractory or relapsed AML

Endpoint

Primary : Overall Response Rate

Secondary : CR rate, duration of CR, overall survival, HLA-KIR research, Immunological Assays

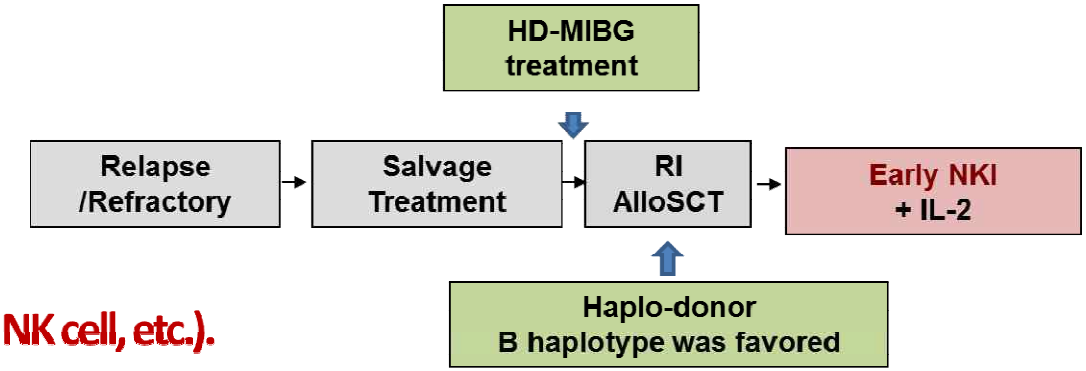
Patient no. 13 patients



Unpublished data

Allogeneic NK cell: IIT, high rish Neuroblastoma, Haplo NK (HSCT + NK cell + IL-2)

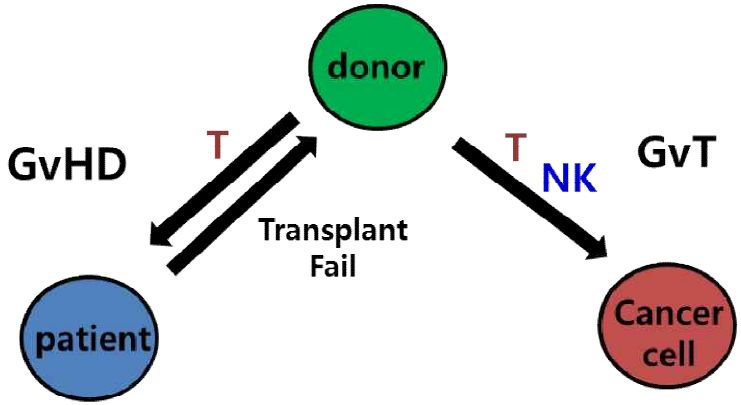
- **Title:** Haploidentical Stem Cell Transplantation and NK Cell Therapy in Patients With High-risk Solid Tumors
- **Patients:** Neuroblastoma
- **Outcome:** Immune reconstitution
  - Rapid immune reconstitution (T cell, NK cell, etc.).



– ***Ex vivo* expanded donor-derived NK cell (MG4101)**
  - 3×10^7 cells /kg on days 2, 9, and 16

– **IL-2 treatment after NKI**
  - $1 \times 10^6/m^2$/day

    on days 2 / 4 / 6, 9 / 11 / 13, and 16 / 18 / 20



Unpublished data

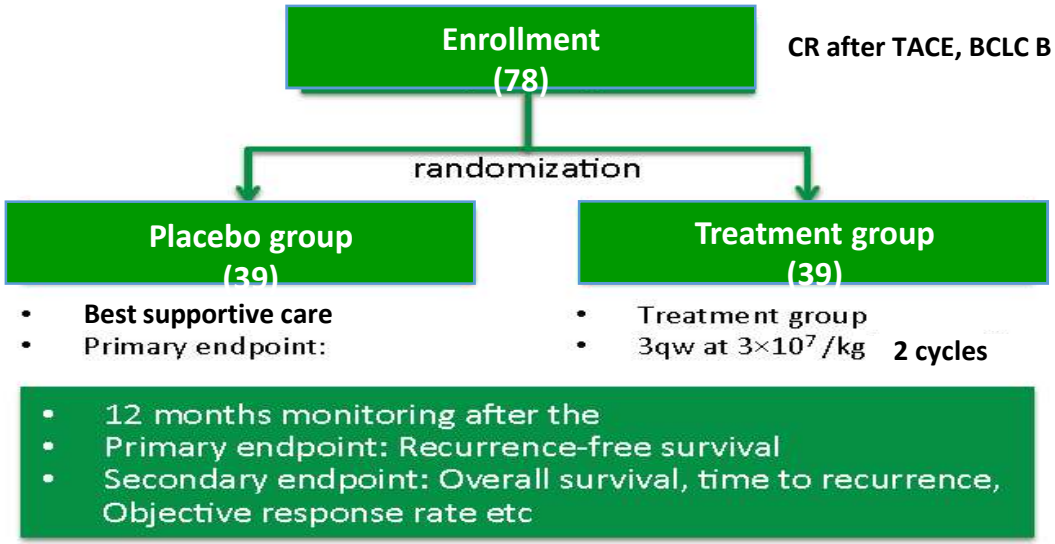
Allogeneic NK cell: Phase IIa trial, HCC-TACE (NK cell stand alone)

- **Title:** A Study of MG4101 (Allogeneic Natural Killer Cell) for Intermediate-stage of Hepatocellular Carcinoma

- **Purpose:** evaluate the safety and efficacy of MG4101 (allogeneic Natural killer cells) in patients with hepatocellular carcinoma (HCC) after trans arterial chemoembolization (TACE).

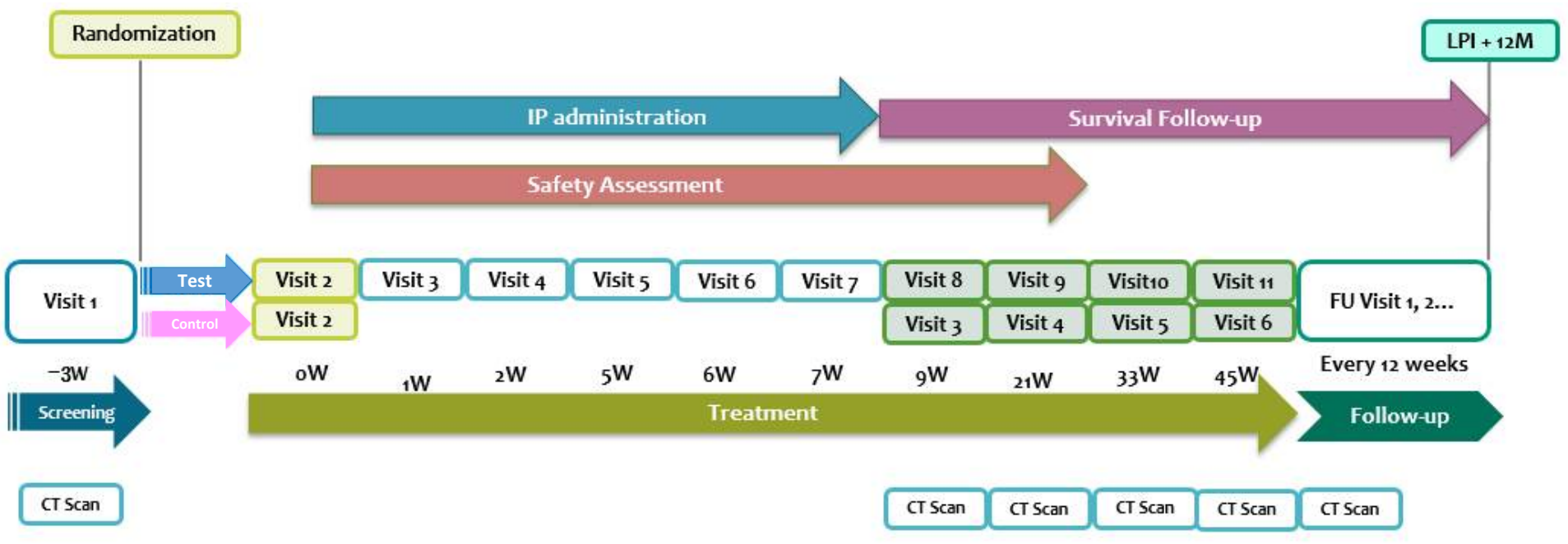
- **Primary outcome:** Time To progression [Time Frame: every 12 weeks, up to the time of death or 18 months]

- **Estimated Enrollment:** 78



Allogeneic NK cell: Phase IIa trial, HCC-TACE (NK cell stand alone)

Study Design	Endpoint	Study Site
Phase Ⅱa, Randomized, Open	Primary: Time to Progression Secondary: Progression-free survival, Overall Survival, Tumor Markers	Local, Multiple



Unpublished data

Allogeneic NK cell: Phase I/IIa, Lymphoma, Rituxan combi. (Antibody + NK cell)

Study Title

Multi-center, Open-label, ***Phase I/IIa*** Clinical trial to evaluate

the ***efficacy and safety*** of **MG4101** plus **Rituximab**

in ***patient with relapsed/refractory Non-Hodgkin's lymphoma of B-cell Origin***

Study Objectives

Phase I: RP2D

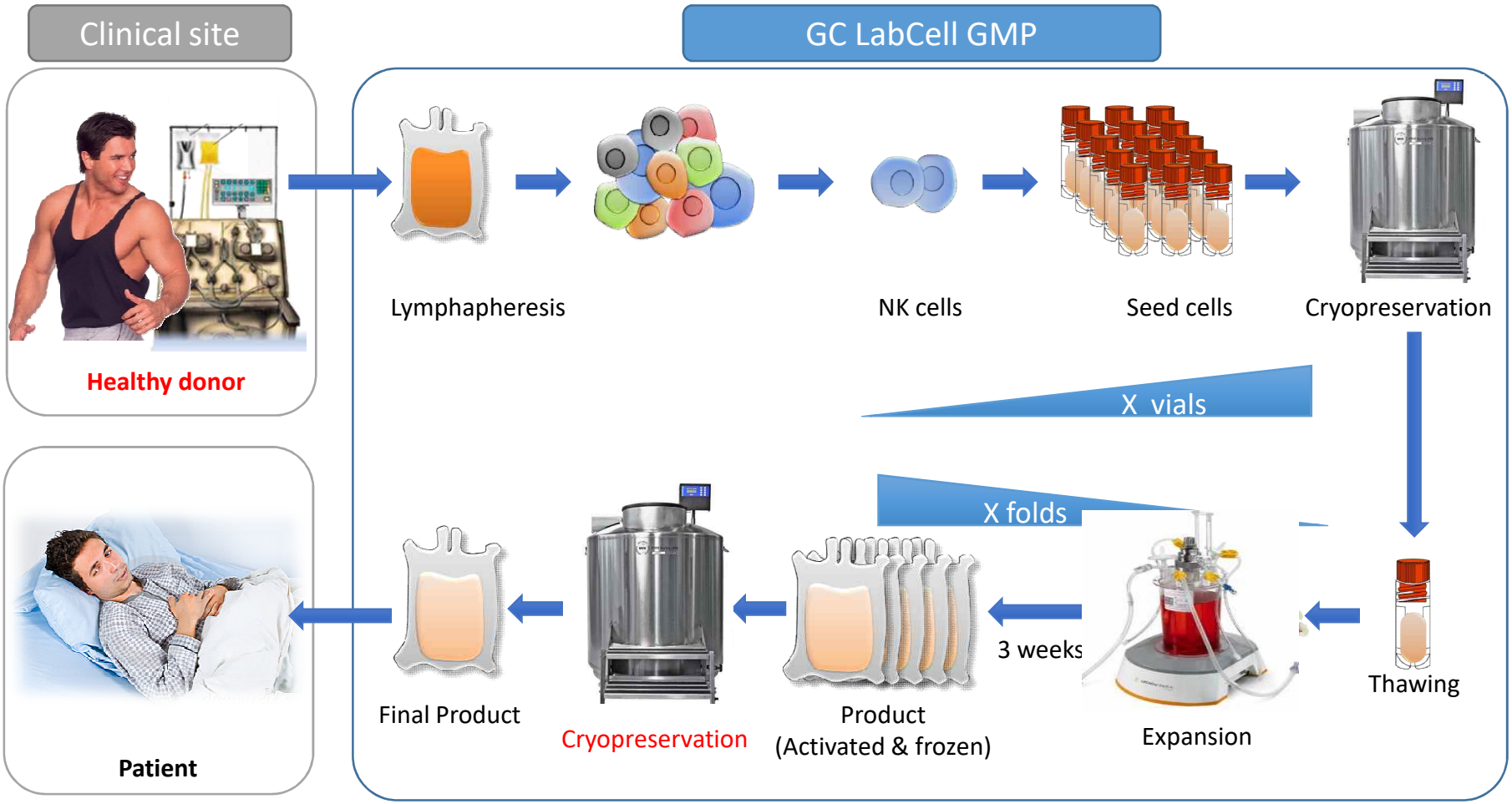
To determine the dose of MG4101 to be studied in Phase IIa for patients with NHL

Phase IIa: Proof of Concept

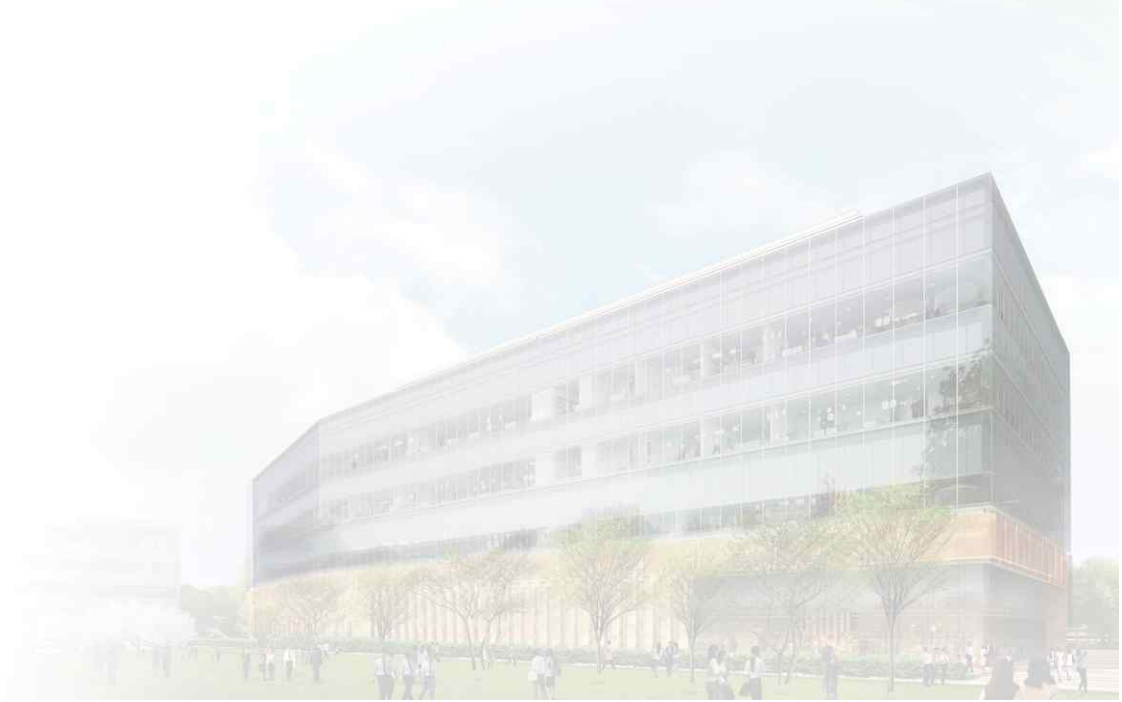
RP2D of MG4101 in combination with Rituximab (under pre-conditioning and IL-2) is efficacious in patients with Relapsed and Refractory NHL of B Cell origin (either indolent FL or DLBCL)

Unpublished data

Allogeneic NK cell: Manufacturing of PBNK

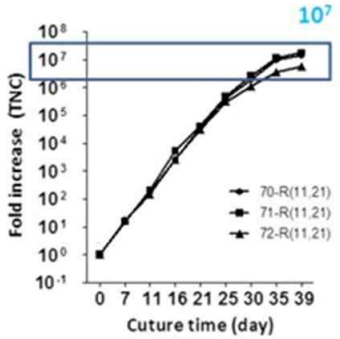


Gene modified NK cell



Allogeneic NK cell: Manufacturing of cord blood-NK

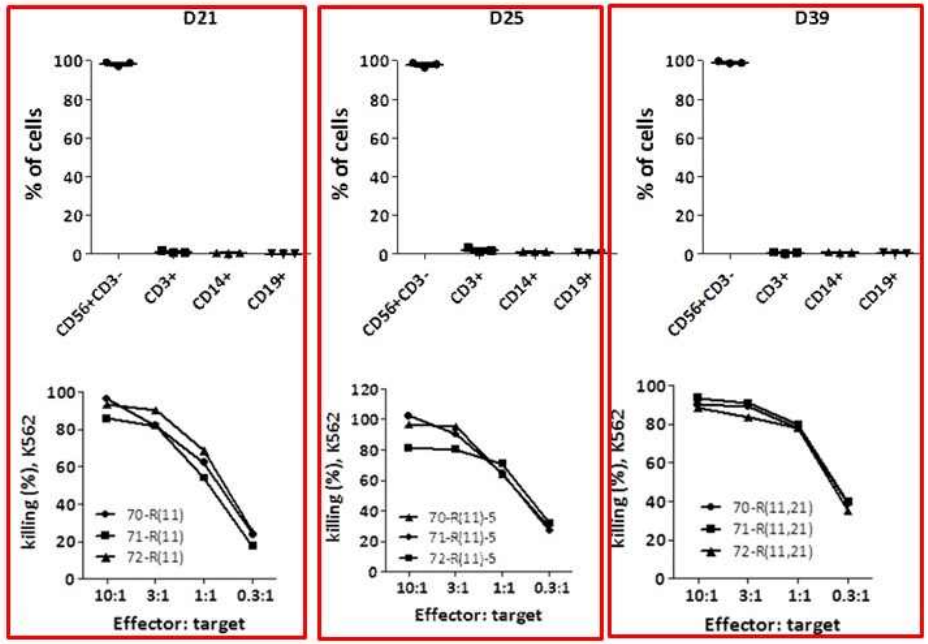
Expansion



- 10^7-fold of cell expansion from cord blood-derived NK cells for 35 days, with good viability and cytotoxicity.
- During 35 days, the log phase of growth is maintained (total 3 times stimulation)
- Cytotoxicity and purity are maintained.

Purity

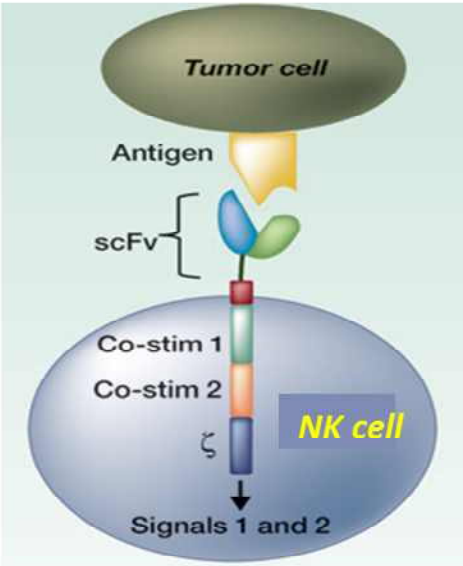
Cytotoxicity



Unpublished data

Gene Modified NK cell: CAR-NK

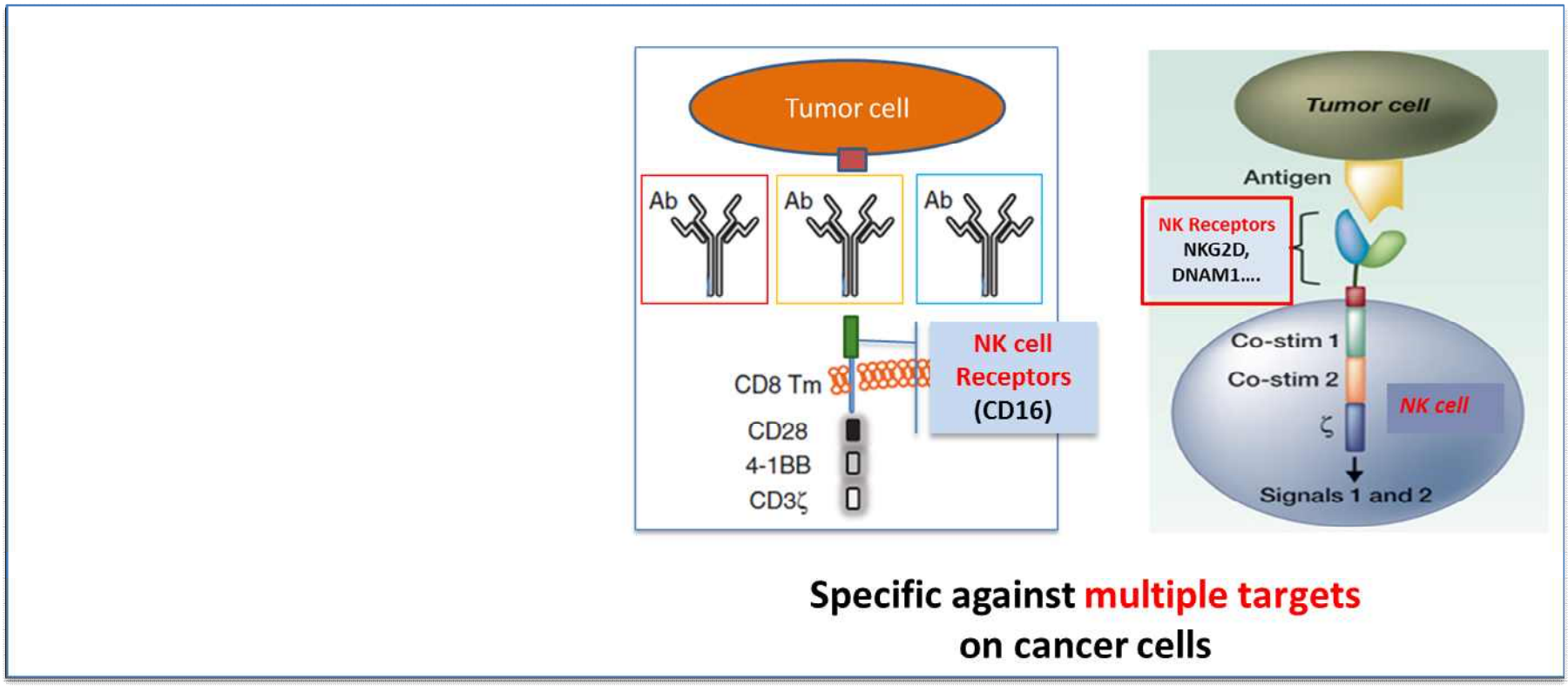
	CAR-NK	
target	scFv	
Internal sequence	NK specific stimulation signal	
Production	Allogeneic, Off-the-Shelf	



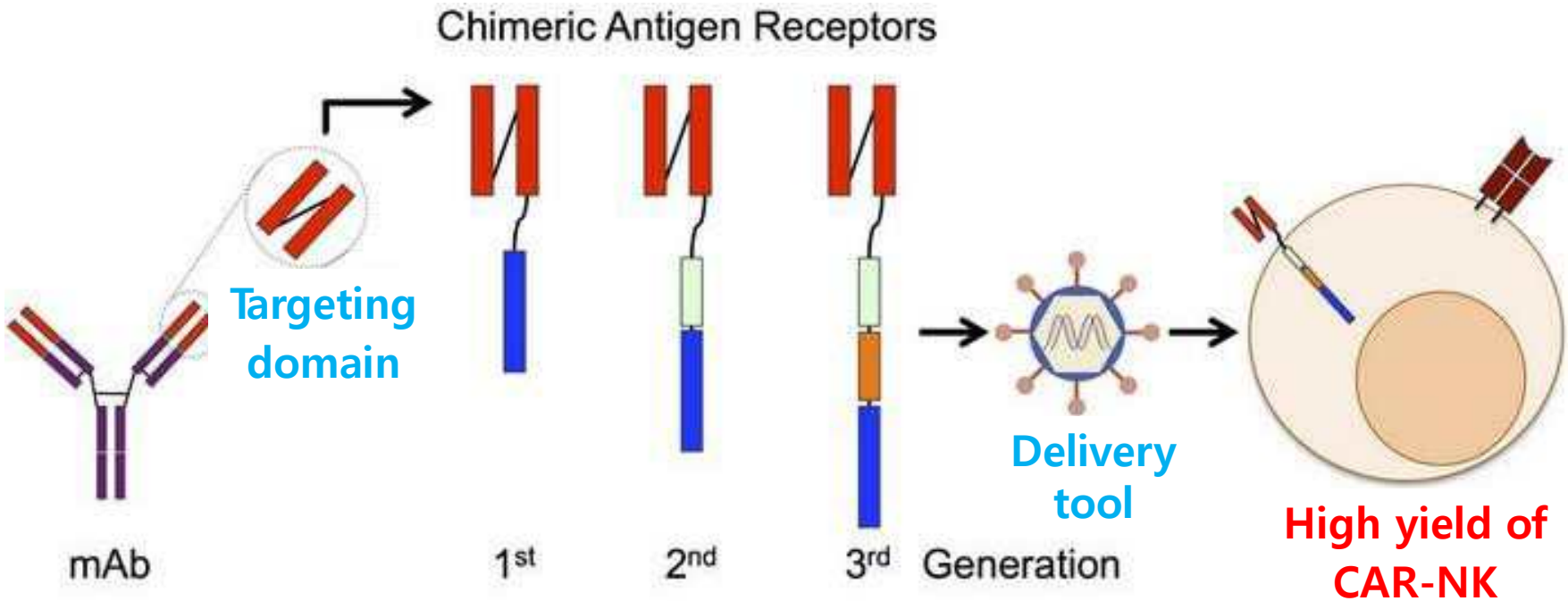
Specific against only one target on cancer cells

Gene Modified NK cell: Universal-NK

		Universal-NK
target		NK specific receptor
Internal sequence	NK specific stimulation signal	
Production	Allogeneic, Off-the-Shelf	

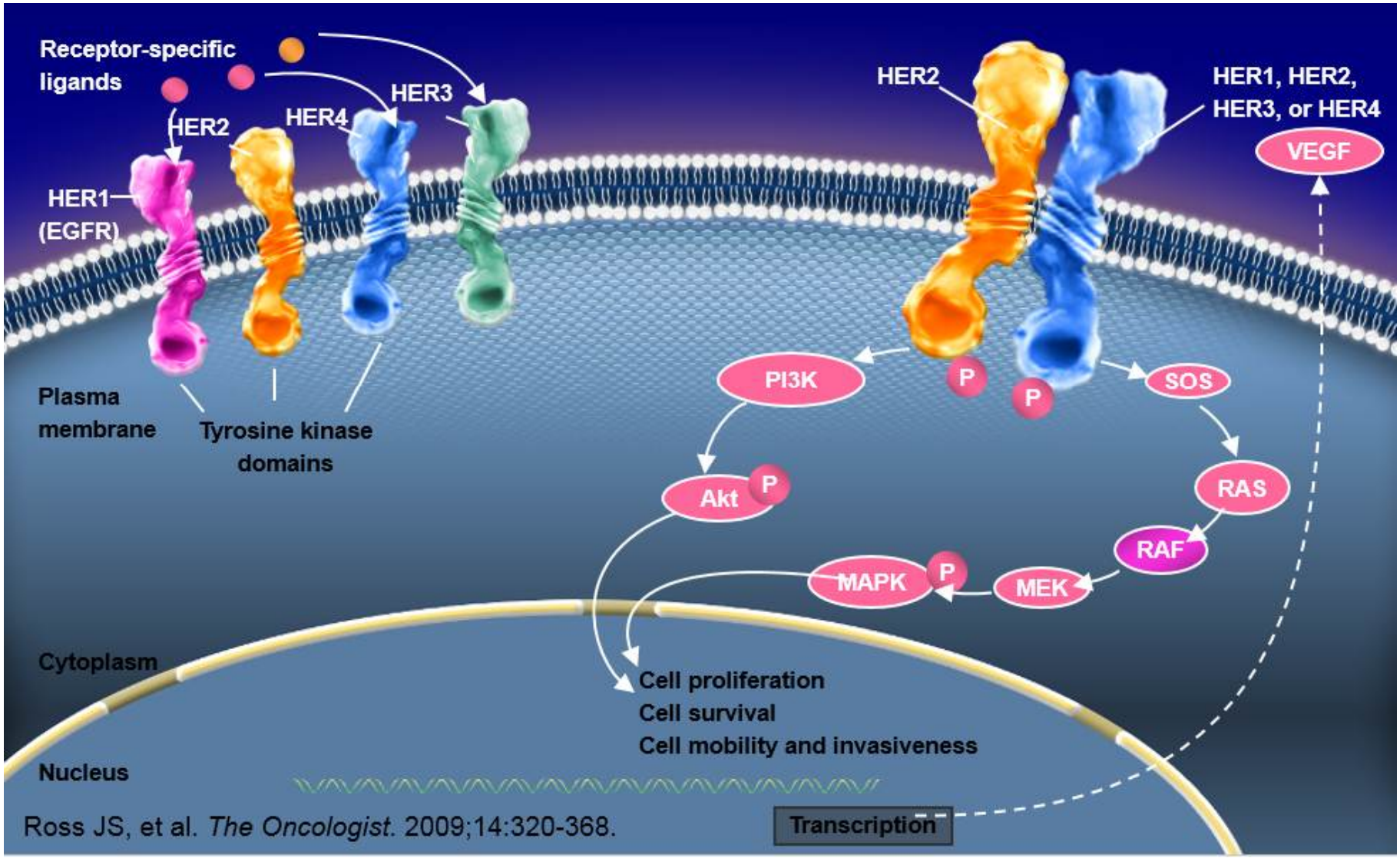


Gene Modified NK cell: Key technology



NK-specific signaling molecule

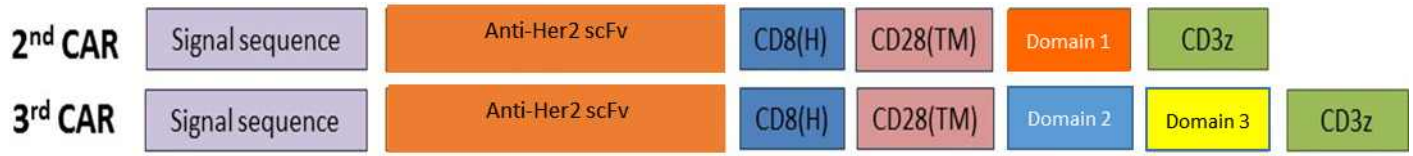
Gene Modified NK cell: scFv-CARs



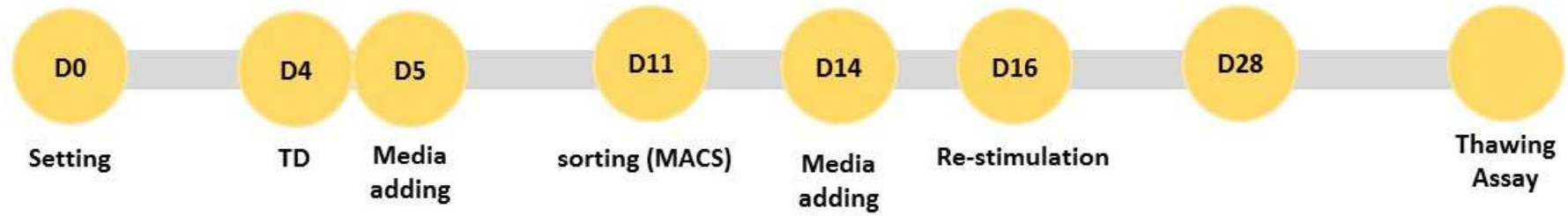
HER2 signaling is related to Breast cancer/ Gastric cancer/ Colon cancer…..

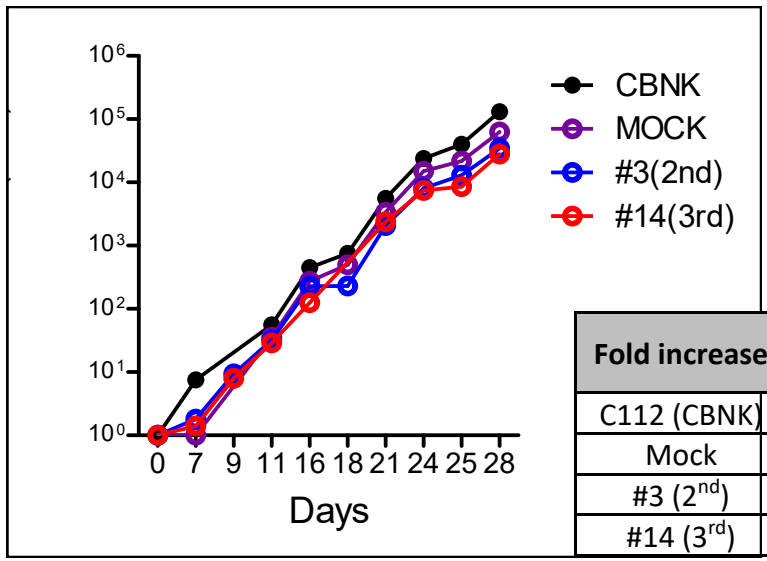
Gene Modified NK cell: scFv-CARs (HER2-CAR)

Construction of HER2 CAR-NK

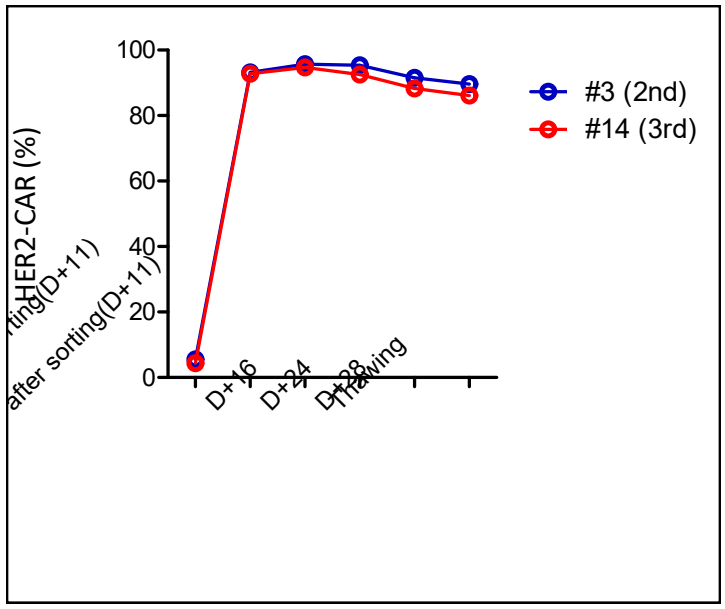


❖ 실험 요약





Fold increase	D28 (harvest)
C112 (CBNK)	131025.6
Mock	63073.5
#3 (2nd)	34391.7
#14 (3rd)	27942.7

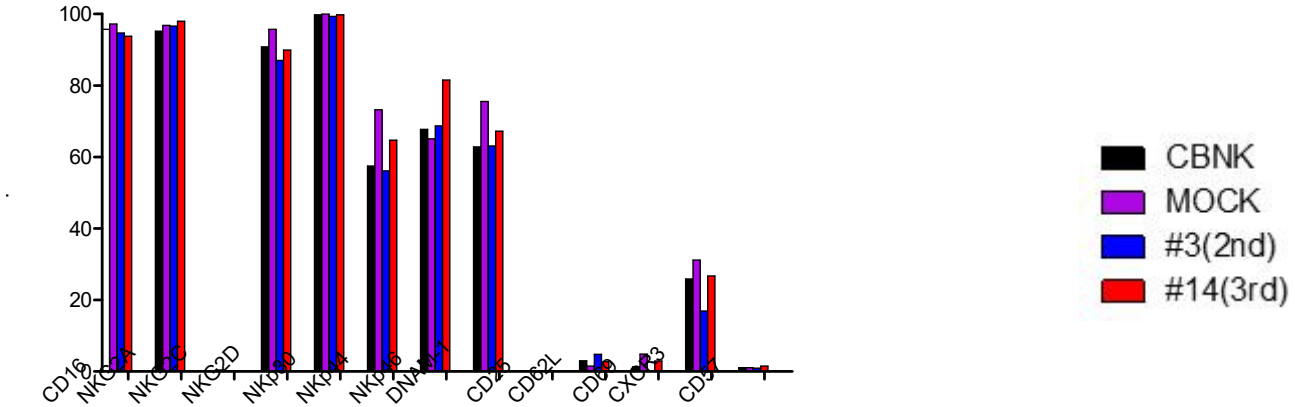


Unpublished data

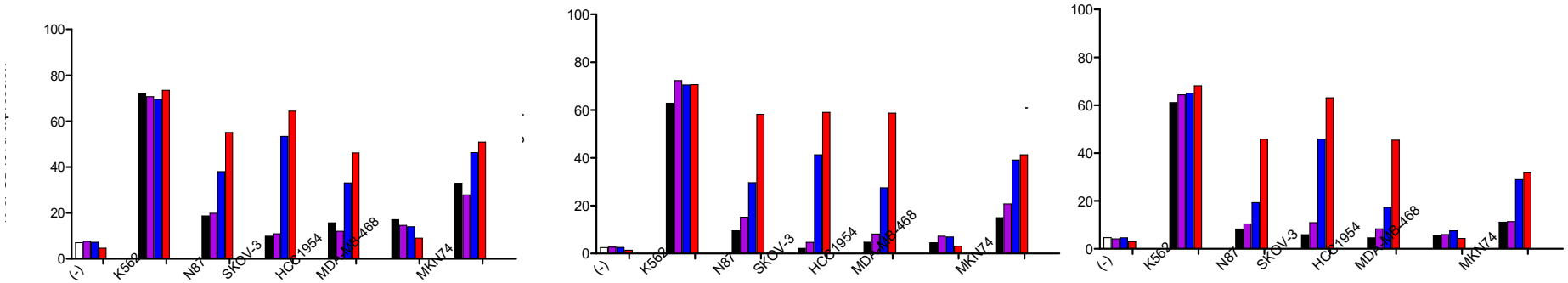
Gene Modified NK cell: scFv-CARs (HER2-CAR)

Characterization of HER2 CAR-NK (IncuCyte analysis)

❖ **Phenotype (thawing)**



❖ **CD107a expression & cytokine release (thawing)**

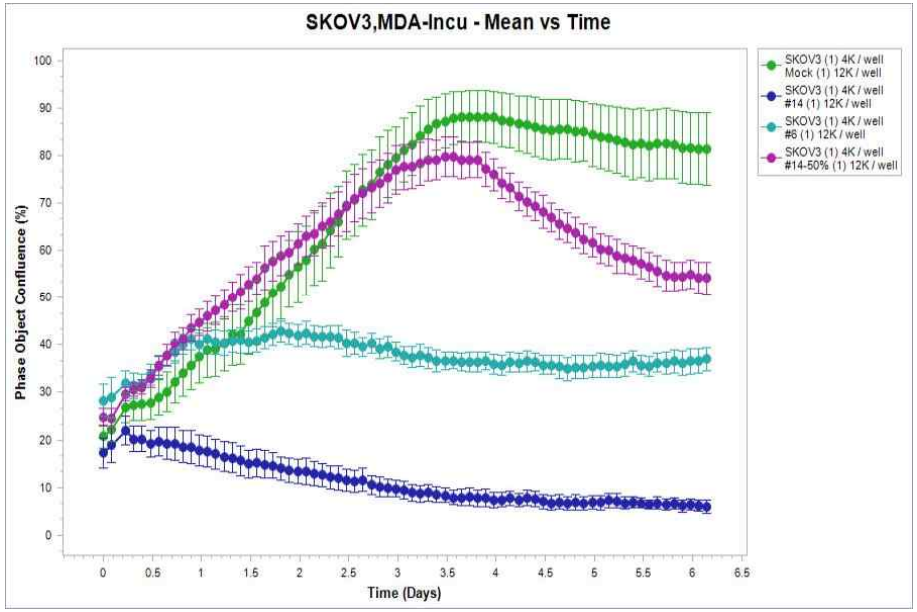


Unpublished data

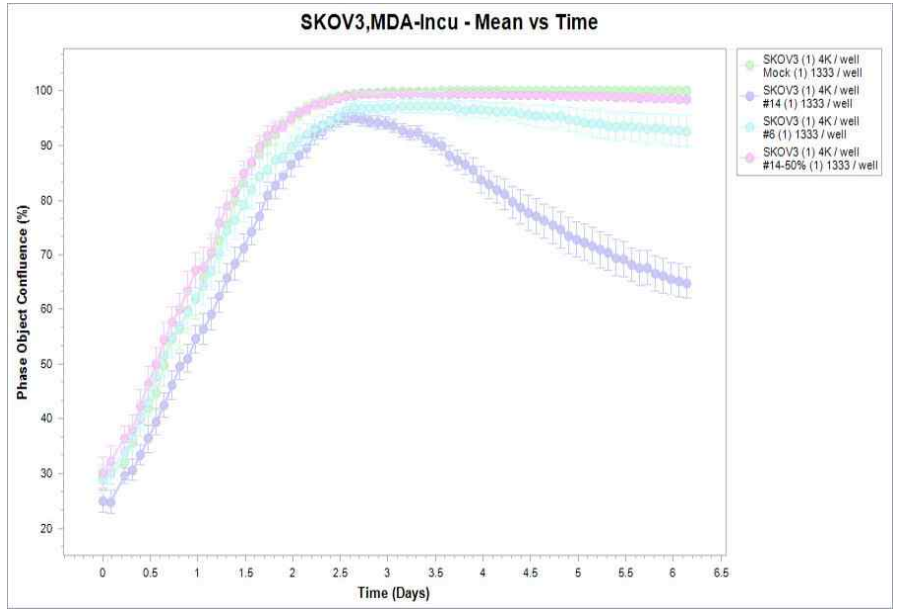
Gene Modified NK cell: scFv-CARs (HER2-CAR)

In vitro Efficacy of HER2 CAR-NK (IncuCyte analysis)

SKOV3-Confluence (%)



E:T ratio = 3:1



E:T ratio = 0.3:1

: mock

: #14 (Her2 scFv-3rd generation)

: #6 (Her2 scFv-2nd Generation)

: #14 + mock

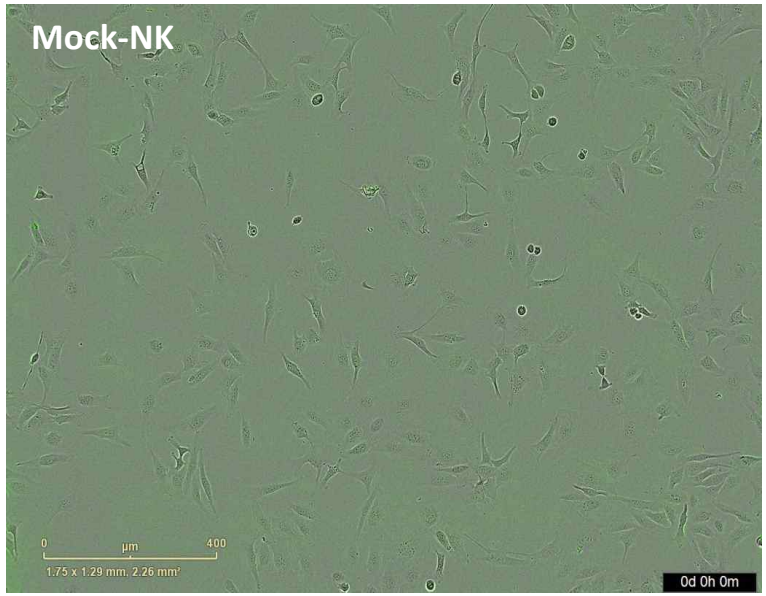
Unpublished data

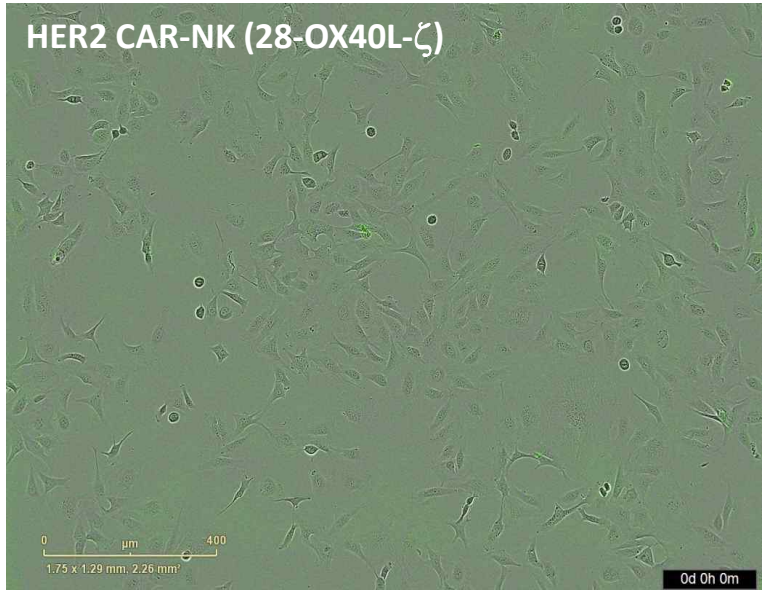
Gene Modified NK cell: scFv-CARs (HER2-CAR)

Killing activity of HER2 CAR-NK (IncuCyte analysis)

Long-term killing activity (InCucyte™)

Target: SKOV3
(E : T ratio = 0.3 : 1)



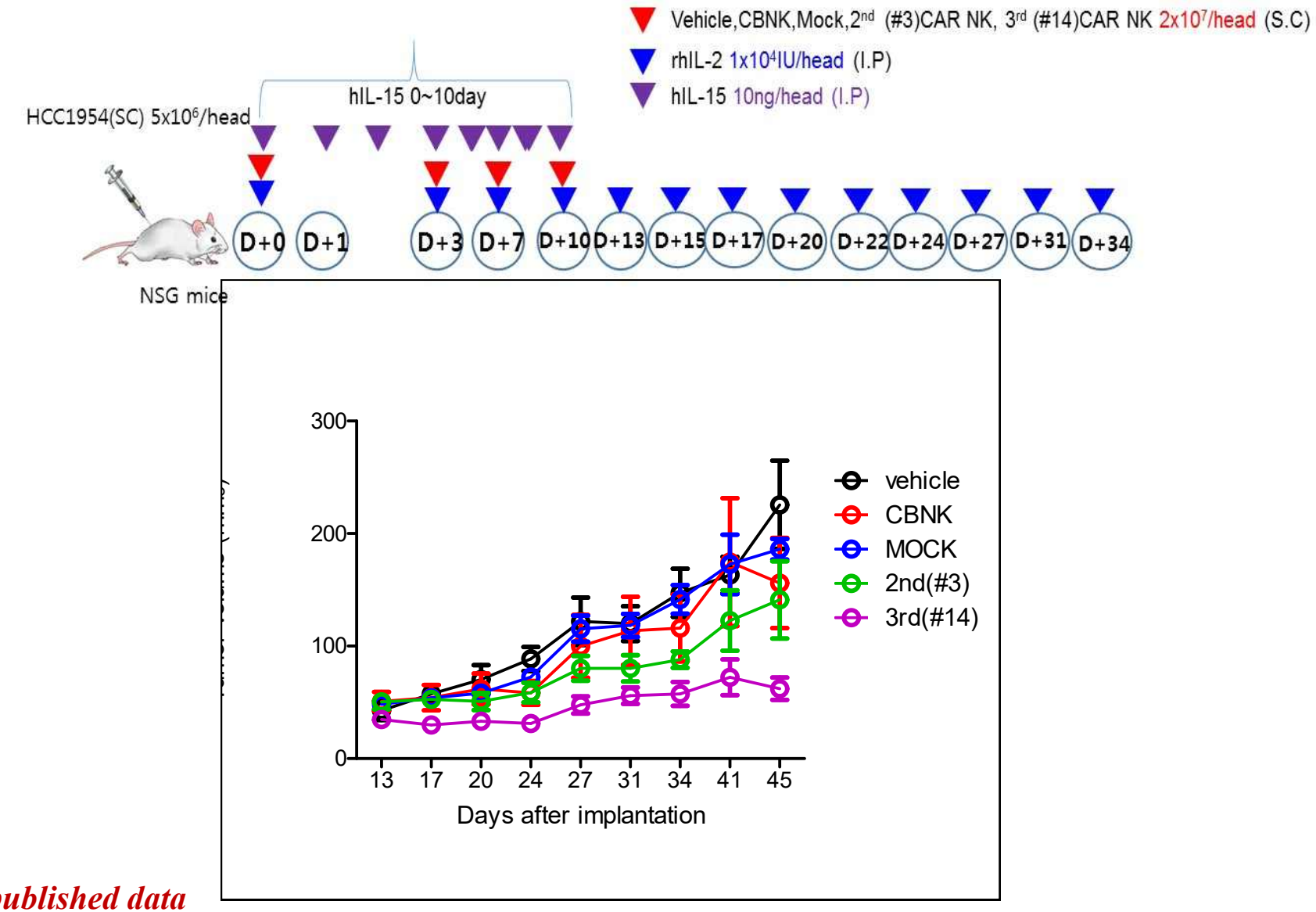


NK killing activity (BioStation)

Unpublished data

Gene Modified NK cell: scFv-CARs (HER2-CAR)

In vivo Efficacy of HER2 CAR-NK



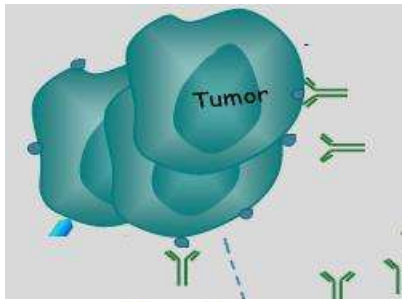
Unpublished data

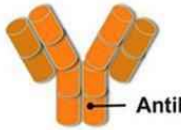
Gene Modified NK cell: CD16 based Universal CARs

Unpublished data

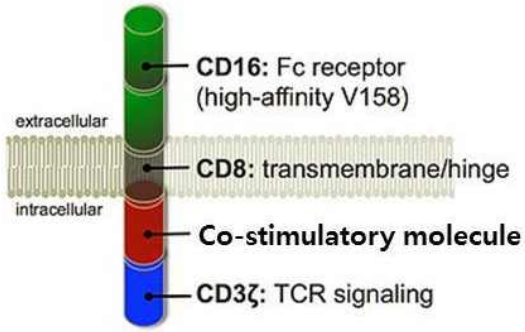
NK lymphocytes expressing a CD16-CAR exert antibody-dependent cancer cell killing.

→ Applicable to any type of cancers if tumor specific Abs are available



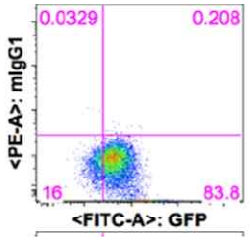


Antibody: Specific to any target on tumor

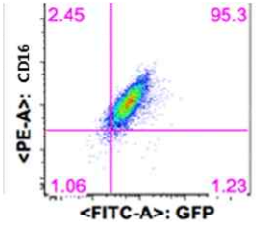


CD16 expressing Universal T or NK

CD16-CAR-Lentivirus	Promoter1	CD16-costim	Promoter 2	GFP
Mock-Lentivirus	Promoter1	mock	Promoter 2	GFP

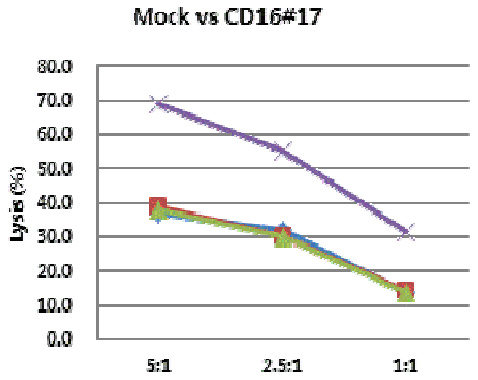


Mock virus infected NK cells



#17 CAR virus infected NK cells

CD3- CD56+ gated NK cells

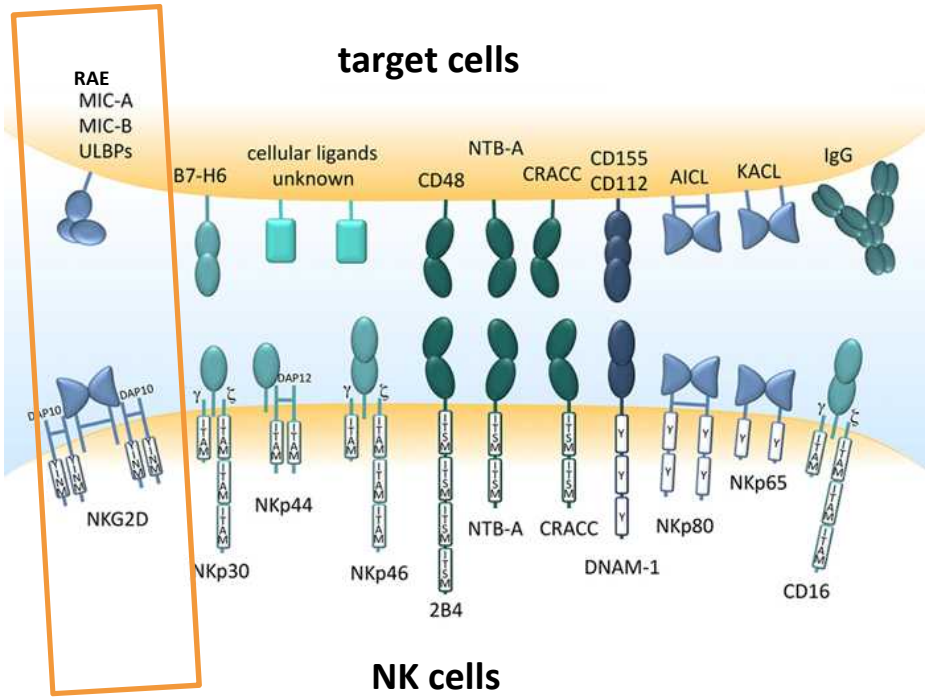


Mock virus
+ control IgG
+ Rituxan

CAR virus
+ control IgG
+ Rituxan

Gene Modified NK cell: NKG2D based Universal CARs

Activating NK cell receptors and their ligands



Expression of NKG2D ligands on human tumor cells	
Tumor Type	**Reference**
Carcinoma	
• *Ovarian*	(40, 43, 148-151)
• *Bladder*	(152)
• *Breast*	(40, 153-155)
• *Lung*	(40, 156, 157)
• *Hepatocellular*	(158)
• *Colon*	(40, 41)
• *Renal*	(40, 159, 160)
• *Prostate*	(40, 161)
Leukemia	
• *AML*	(39, 162-164)
• *CML*	(39, 165, 166)
• *CLL*	(167, 168)
Lymphoma	(169)
Multiple Myeloma	(138, 170)
Melanoma	(42, 171, 172)
Ewing's Sarcoma	(56, 173)
Glioma	(38, 108)
Neuroblastoma	(104)

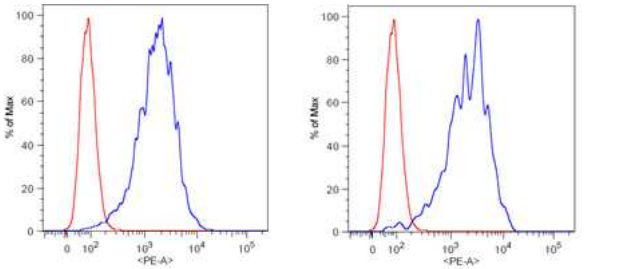
< Frontiers in Bioscience 17, 1418-1432, January 1, 2012 >

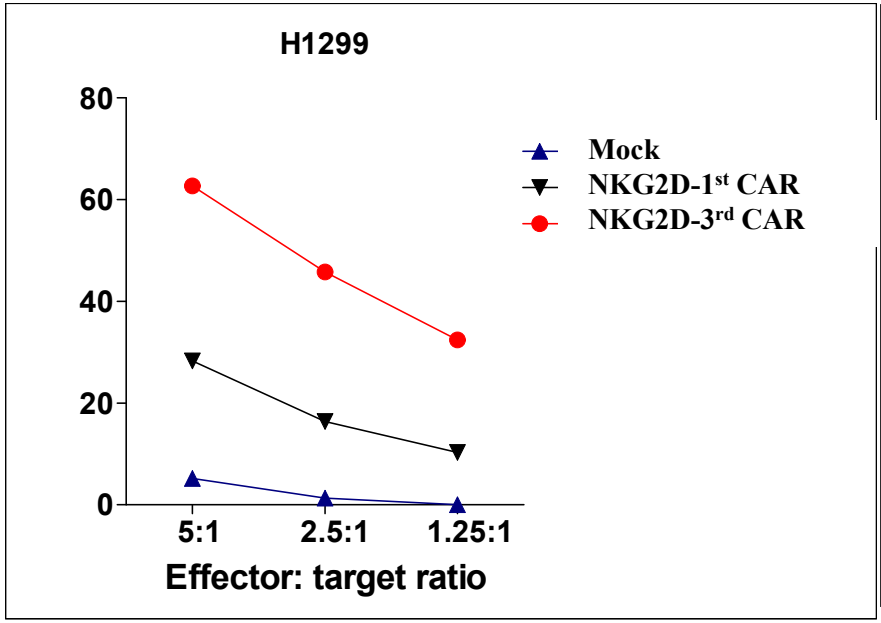
< Cancer Immunity (2013) 13, 8 P.Spear et al.>

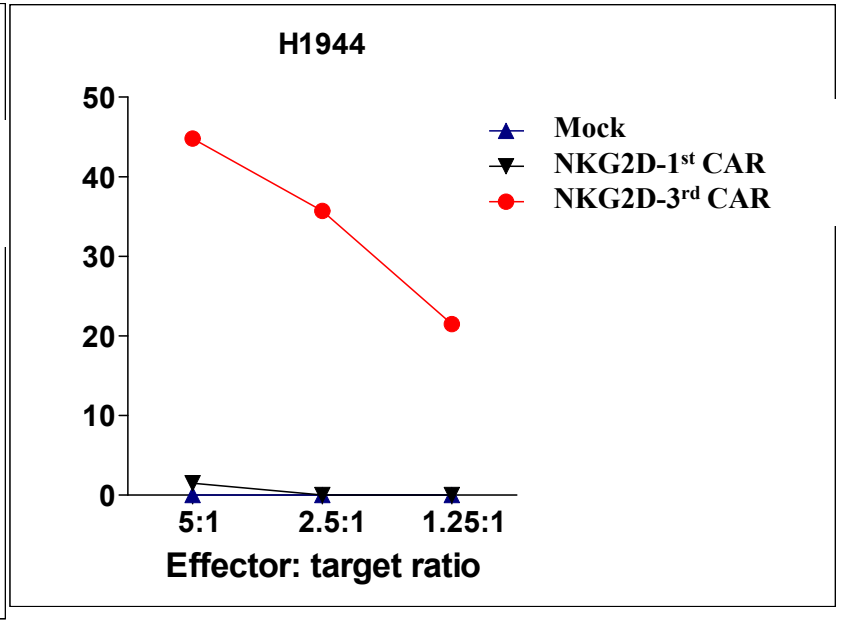
Gene Modified NK cell: NKG2D based Universal CARs

Unpublished data

NKG2D CAR expression on NK cells







유전자 가위를 이용하여 면역원성이 제거된 Long-lasting NK (Log-NK) 개발

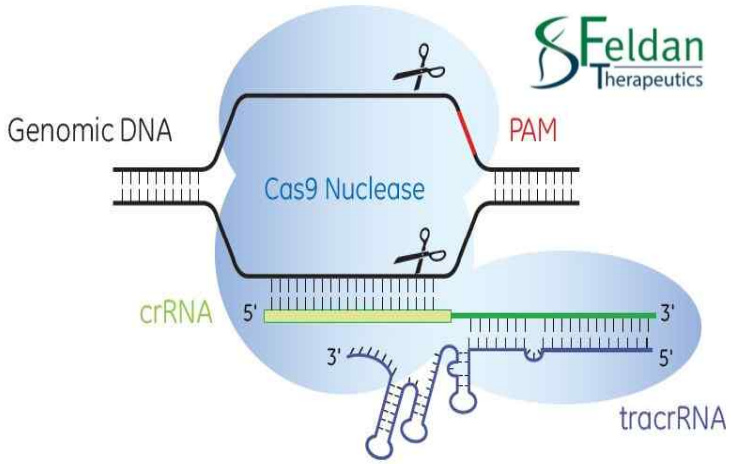
기능 저하 유전자가 제거된 Log-iGVT-NK 개발

유전자 가위 기술 적용

Log-NK

NKG2A
CBLB
CISH
GSK3

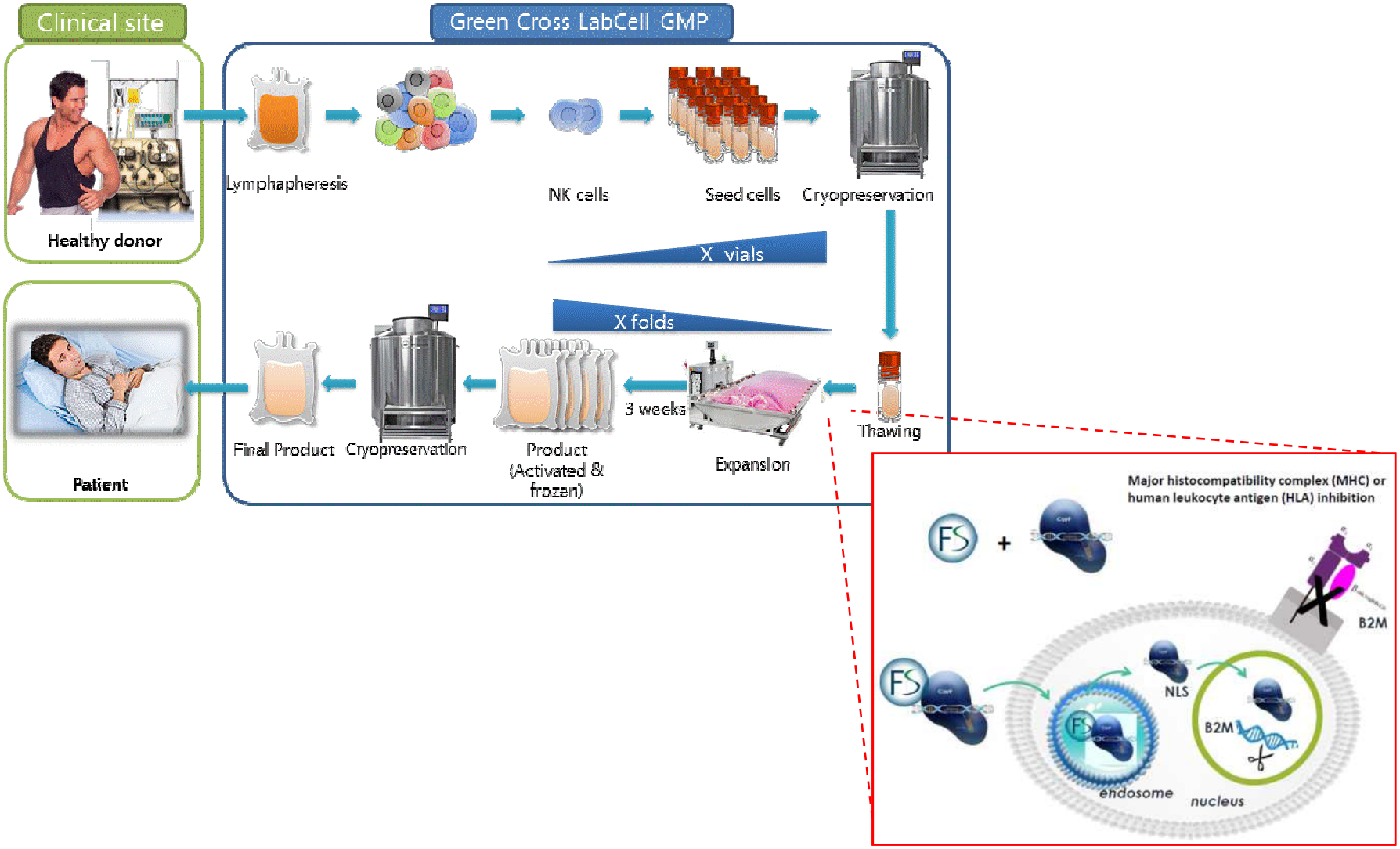
Inhibitory molecules



Log-iGVT-NK

~~*NKG2A*~~
~~*CBLB*~~
~~*CISH*~~
~~*GSK3*~~

Long-lasting & iGVT NK (Log-iGVT-NK) 생산 공정



Pipelines of GCLC

Classification	Project	Indication	상업화 임상시험					개발 국가
			Research	Preclinical	Phase 1	Phase 2	Phase 3	
NK Cell	MG4101 (Allogeneic NK)	HCC after TACE						국내
NK+Ab	MG4101+Rituxan	Lymphoma						국내
CB-NK	제대혈 유래 Allogeneic NK	간절제술						국내
Engineered NK	HER2 CAR-NK	Solid cancer						국내 및 해외
	OGD2-CAR NK	Pediatric Neuroblastoma						국내 및 해외
	B7-H3 CAR-NK	Solid cancer						국내 및 해외
	NKG2D-CAR NK	Solid / blood cancer						국내 및 해외
	CD16-CAR NK	Solid / blood cancer						국내 및 해외
Stem Cell	편도유래줄기세포	부갑상선 기능저하증						국내
	기능강화줄기세포	항염증(건선)						국내

Classification	Project	Indication	연구자주도 임상시험	개발 국가
NK Cell	MG4101 (Allogeneic NK)	Acute Myeloid Leukemia		국내

Thank you!