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Ex vivo-expanded allogeneic natural killer cell for cancer therapy

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Green Cross LabCell

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Overview of Allogeneic NK Cell Therapy : MG4101

Key Highlights of MG4101

- GMP-compliant, Large-scale expanded **natural killer (NK) cells for allogeneic transfusion purpose**.
- Expanded from normal healthy donor-derived peripheral blood mononuclear cells.
- Highly cytotoxic and cytokine-producing NK cells with anti-tumor activity against a variety of cancer types.
- Safety proven through Phase I clinical trial in patients with lymphoma or solid tumors which was completed in Dec. 2012.
- General use for cancer patients but more favorably for patients with higher grade of KIR-Ligand mismatch.
- Currently in two separate Phase II clinical trials against childhood patients with high-risk solid tumors following haplo-identical hematopoietic stem cell transplantation, and patients with hepatocellular carcinoma after curative resection, respectively.

Introduction of NK cells

Cancer immunotherapy with NK cells

- **NK cells**
  - defined as innate effector lymphocyte (Eur J Immunol 1975, 5: 112–117).
  - 5% up to 15% of the total lymphocyte in normal healthy subjects.
  - provide a first line of immune defense against viral infections and cancer.
  - influence both innate and adaptive immune host defenses.

- **Decreased cell number and weak activity of NK cells**
  - cause various cancers, hepatitis, AIDS, chronic fatigue syndrome, various immunodeficiency syndromes, and certain autoimmune diseases.

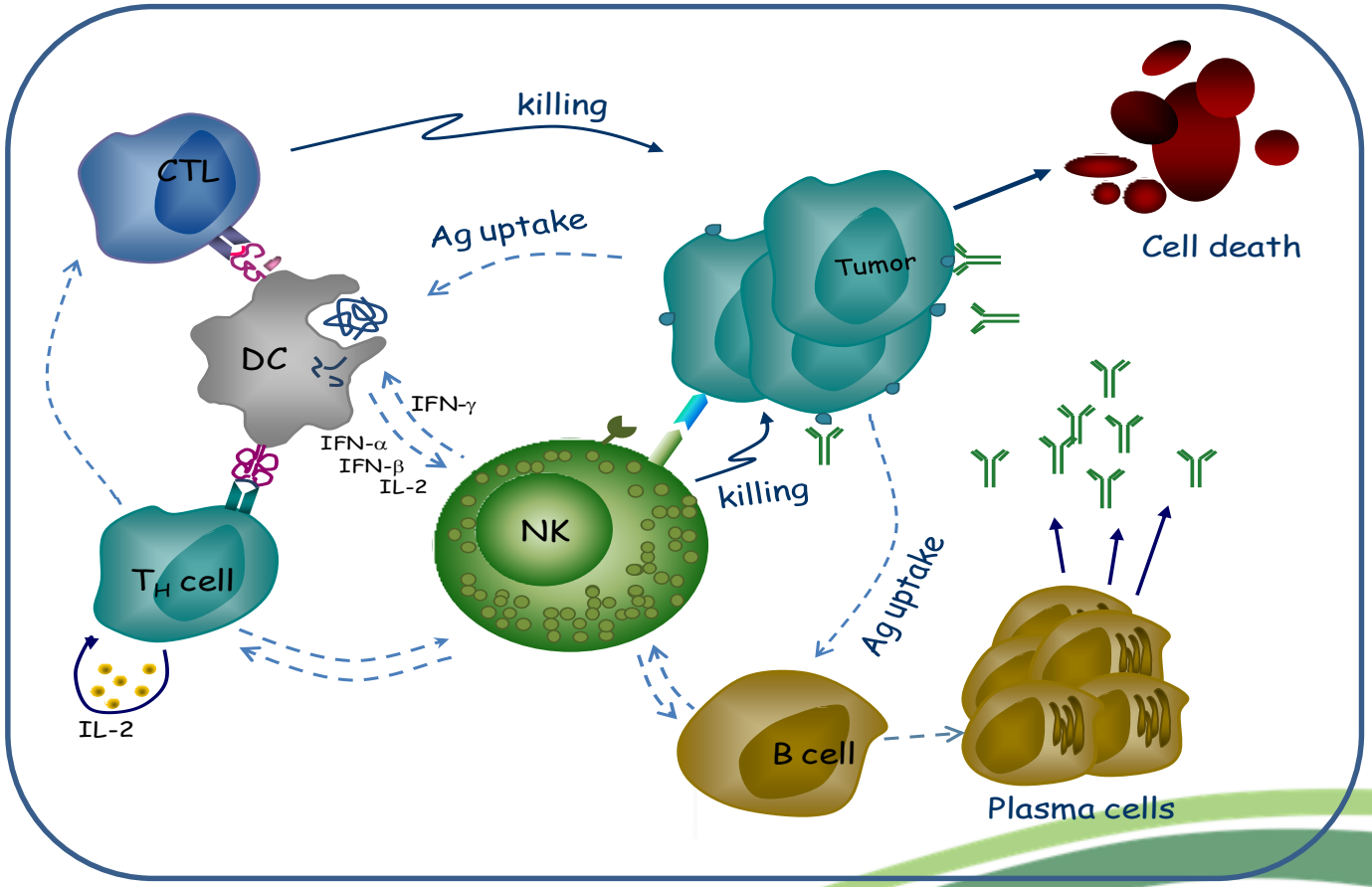
- **NK cell studies in mouse mode**
  - NK cells do not induce graft-versus-host disease (GVHD)
  - promote graft-versus-tumor (GVT) effects (J Clin Invest. 1998, 101:1835–42).

- **NK cells therapy has been recently entered clinical trials of various cancer types.**

- **Allogeneic NK cell therapy**
  - 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).

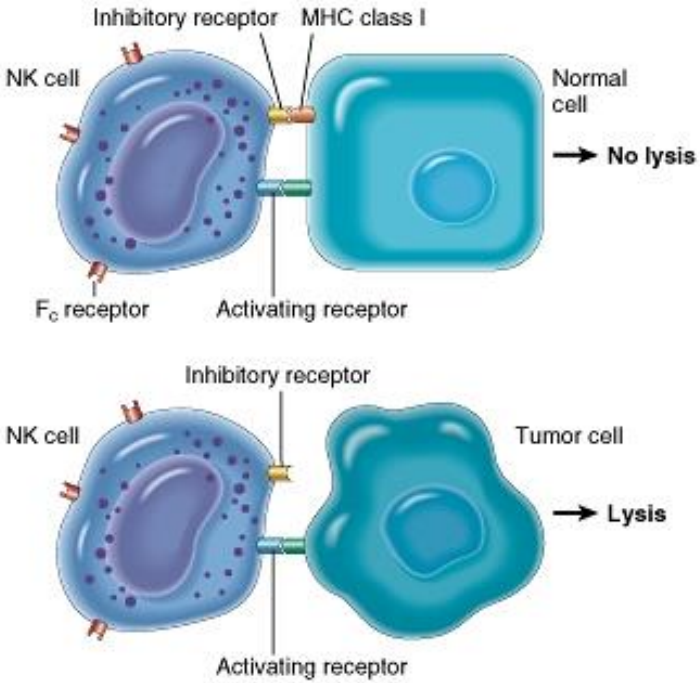
Therapeutic potential of NK cells

Immune modulation & anti-tumor effects



Regulation of NK cell effector function

- In contrast to T cells and DCs, NK cells have antigen-independent cytolytic activity against tumor cells.
- NK cells sense the balance of expression between activating and inhibitory molecules at the surface of interacting cell.
- The sum of signals from inhibitory and activating receptors determines the effector function of NK cells (tolerance or activation).



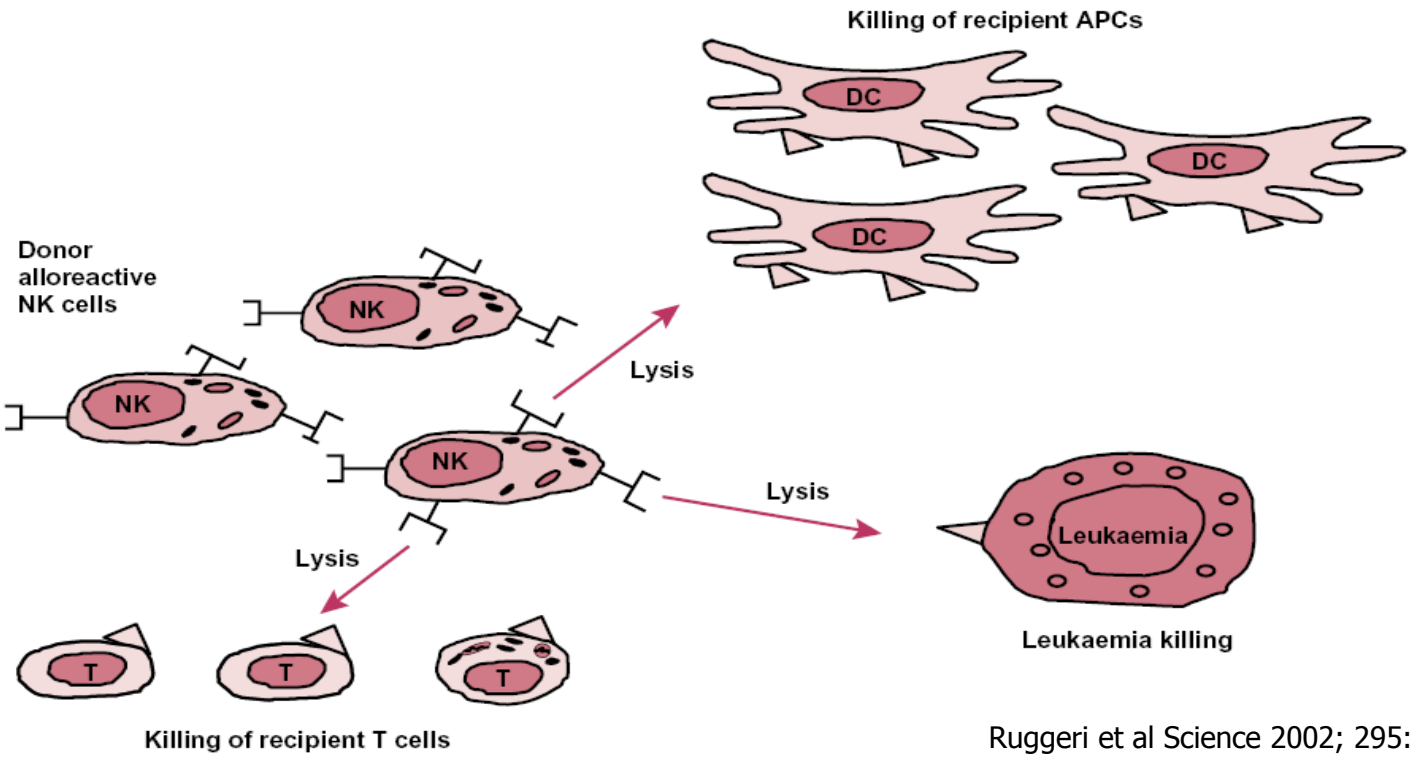
Clinical relevance of NK cells to human diseases

Affected protein	Gene mutated	Protein function	Disease	NK cell biology of disease	Infectious susceptibility
NK cell activating receptors and/or ligands					
CD16 (FcγRIIIa)	*FCGR3A*	Activation induced by IgG binding resulting in ADCC and IgG-independent cytotoxicity	NK cell deficiency due to CD16 functional impairment	Impaired cytotoxicity; mutant alleles *FCGR3A*230A, FCGR3A*230G*	Upper respiratory infections, HSV, EBV, VZV
Killer cell immunoglobulin-like receptor 3DS1	*KIR3DS1*	Activation of NK cell responses through recognition of HLA class I molecules on target cells	AIDS (HIV infection)	Protective effect of *KIR3DS1* in combination with the *HLA-B Bw4-80Ile* allele against the progression to AIDS	Multiple infections
			Hepatocellular carcinoma (HCV infection)	Protective effect of *KIR3DS1* in combination with the *HLA-B Bw4-80Ile* allele against the development of hepatocellular carcinoma	Unknown
			Cervical cancer	Increased risk of developing cervical neoplasia associated with the presence of *KIR3DS1* in combination with the absence of ligand for the inhibitory *KIR2DL1* (HLA-C2) and *KIR3DL1* (HLA-B Bw4)	HPV
NK cell p30-related protein (NKp30)	*NCR3*	Activation of NK cell responses through recognition of ligand(s) on target cells	Malaria (*Plasmodium falciparum* infection)	Increased risk of developing mild malaria attack associated with the *NCR3*-412C* allele	Unknown
Signaling lymphocyte activation molecule–associated protein (SAP)	*SH2D1A*	Receptor-mediated cell activation	X-linked lymphoproliferative syndrome (XLP)	Impaired cytotoxicity (through specific 2B4-CD48 interaction; allele *SH2D1A*507T*)	EBV
Phosphatidylinositol glycan class A	*PIGA*	Biosynthesis of glycosylphosphatidylinositol-anchored proteins (including CD48)	Paroxysmal nocturnal hemoglobinuria	Decreased NK cell number and impaired cytotoxicity (multiple mutant alleles)	Multiple infections
NK tumor recognition molecule	*NKTR*	Recognition and lysis of target tumor cells	Von Hippel–Lindau syndrome	Impaired cytotoxicity	Unknown
NK cell inhibitory receptors and/or ligands					

Nature Immunology, 2008, 9(5):486-494

Role of NK cells in killing recipient immune cells in leukemia after HSCT

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

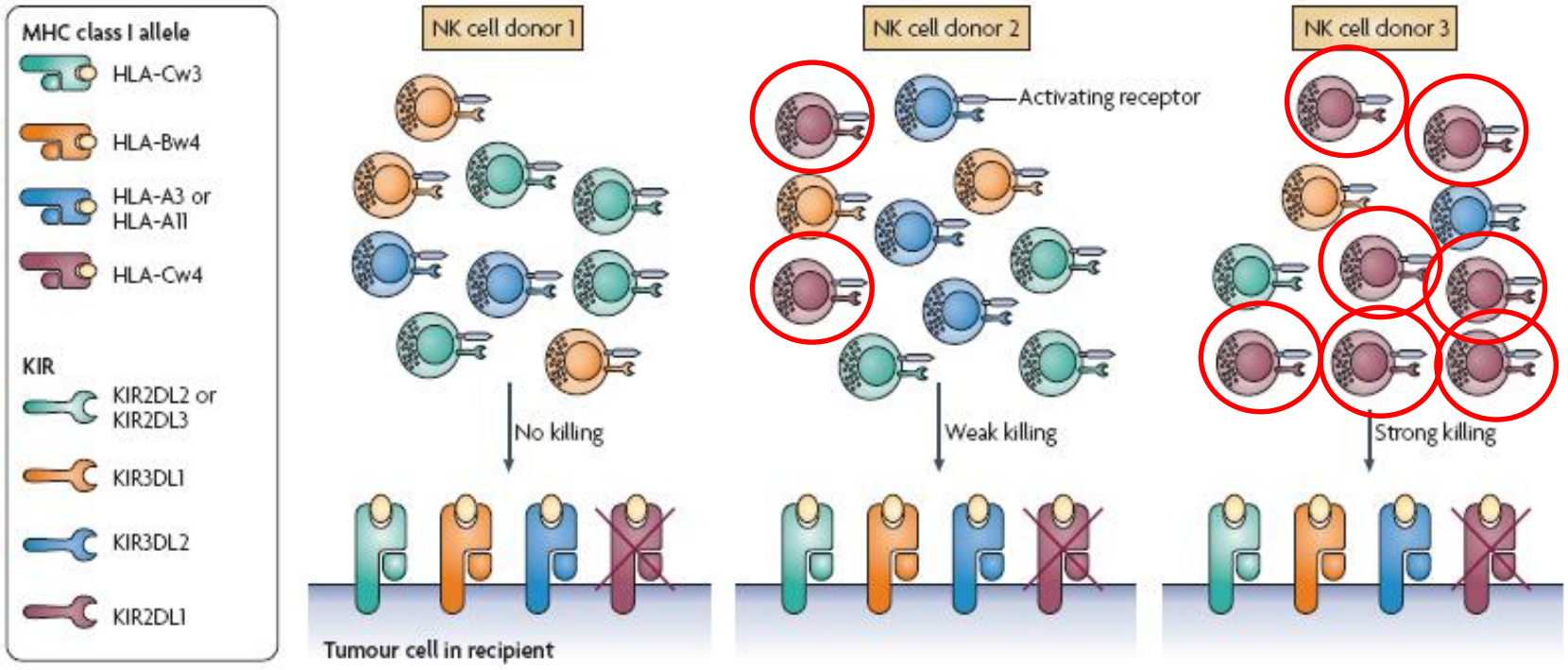


Ruggeri et al Science 2002; 295:2097-2100

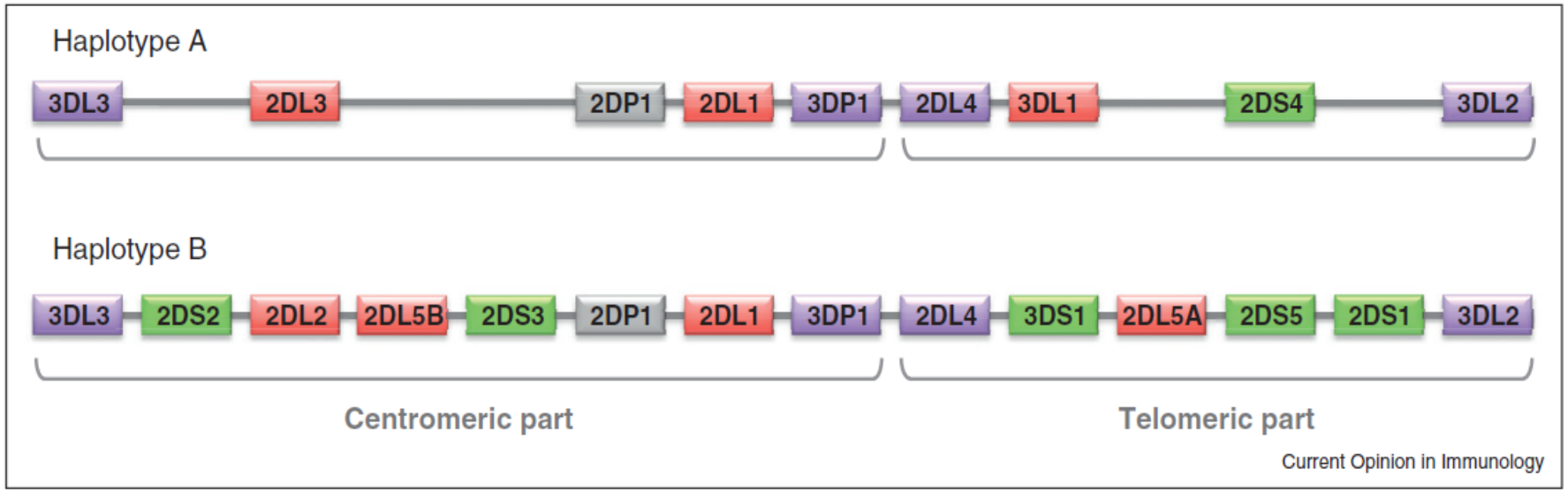
Nat Rev Immunol. 2007 May;7(5):329-39.

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



Schematic representation of haplotypes A and B at the KIR locus



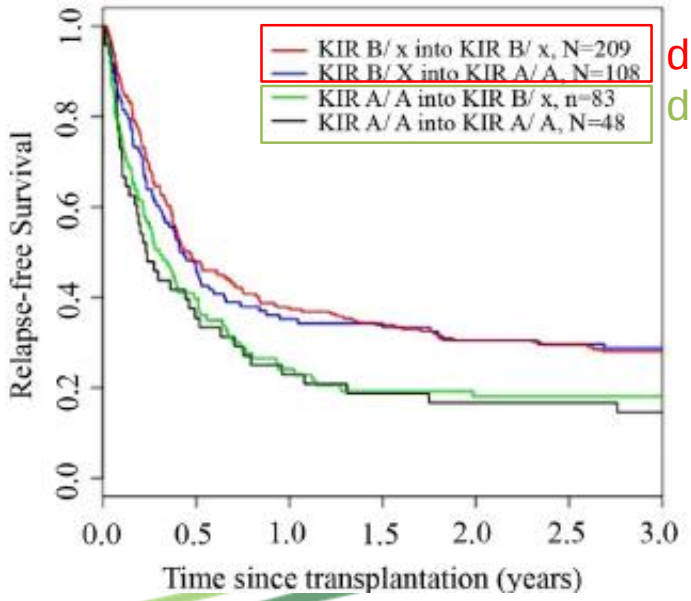
KIR locus is located on the human chromosome region 19q13.4. Two examples of haplotypes A and B are depicted. Pseudogenes are indicated with grey boxes, **activating receptor genes are in green**, and inhibitory receptor genes in red. Conserved genes, which can encode activating or inhibitory receptor or be pseudogenes, are in purple boxes. Each centromeric haplotype fragment can combine with any telomeric haplotype fragment, giving rise to a high diversity of KIR haplotypes.

Curr Opin Immunol (2012) Jan 19

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

Blood (2009) 113:726-732

- Patients: 448 AML (Acute myelogenous leukemia) patients who received allogeneic hematopoietic cell transplantation.
- NK cell helps the implantation of hematopoietic cell, and to reduce the GVHD (graft-versus-host disease) and leukemic recurrence.
- Three-year overall survival was significantly higher after transplantation from a KIR B/x donor (31% [95% CI: 26-36] vs 20% [95% CI: 13-27]; P = .007).
- 30% improvement in the relative risk of relapse-free survival with B/x donors compared with A/A donors (RR: 0.70 [95% CI: 0.55-0.88]; P = .002).
- B/x donors were associated with a higher incidence of chronic graft-versus-host disease (GVHD; RR: 1.51 [95% CI: 1.01-2.18]; P = .03).



donor KIR B/x

donor KIR A/A

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

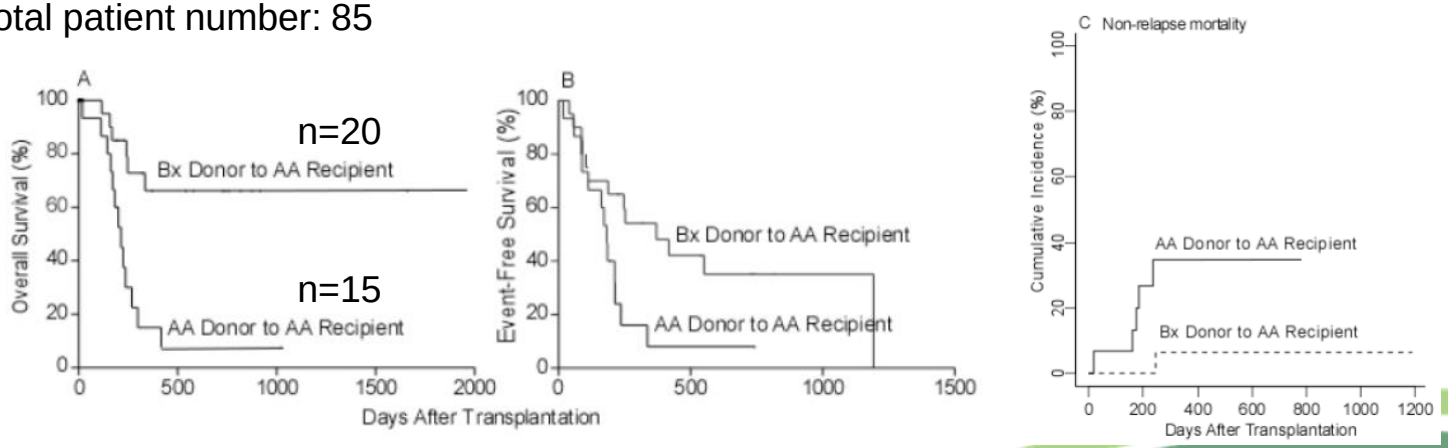
Improved survival with inhibitory Killer Immunoglobulin Receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation

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



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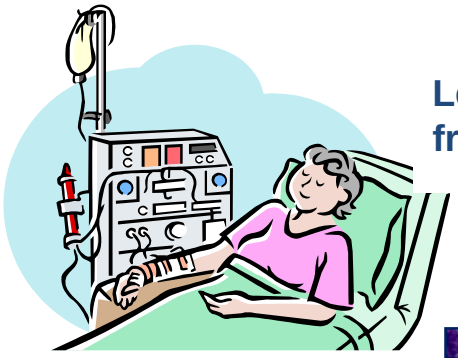
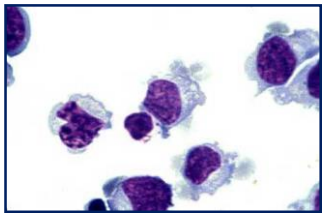
Characteristics of Allogeneic NK cells (MG4101)

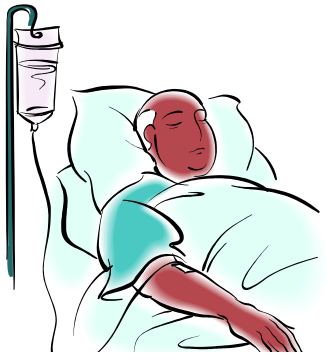
Concept of allogeneic NK cell, MG4101



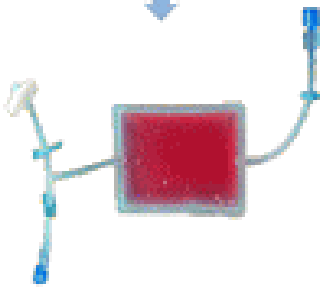
Leukapheresis
from a healthy donor



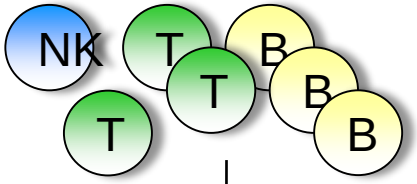
Ex vivo NK expansion



Adoptive transfer to a patient

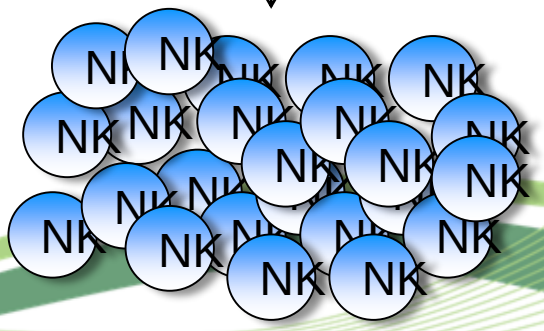


Quality control

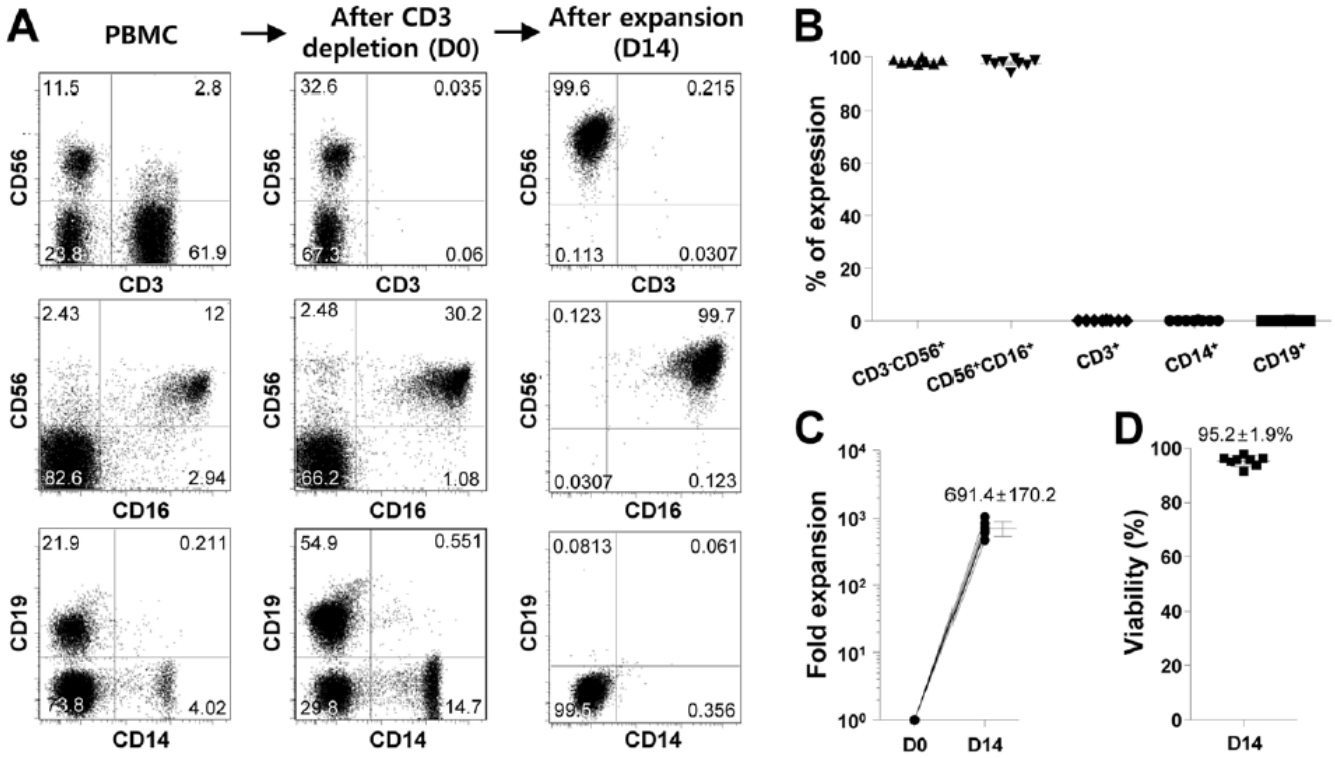


Isolation

Selective
amplification



Characteristics of Large-scale GMP-expanded NK cells



A-B. Representative FACS dot plots and data analysis, C. Fold expansion of NK cells, D. Cell viability (n=8)

PLoS One. 2013;8(1):e53611

Phenotype of activated NK cells after expansion

Analysis by flow cytometry

before and after NK cell expansion

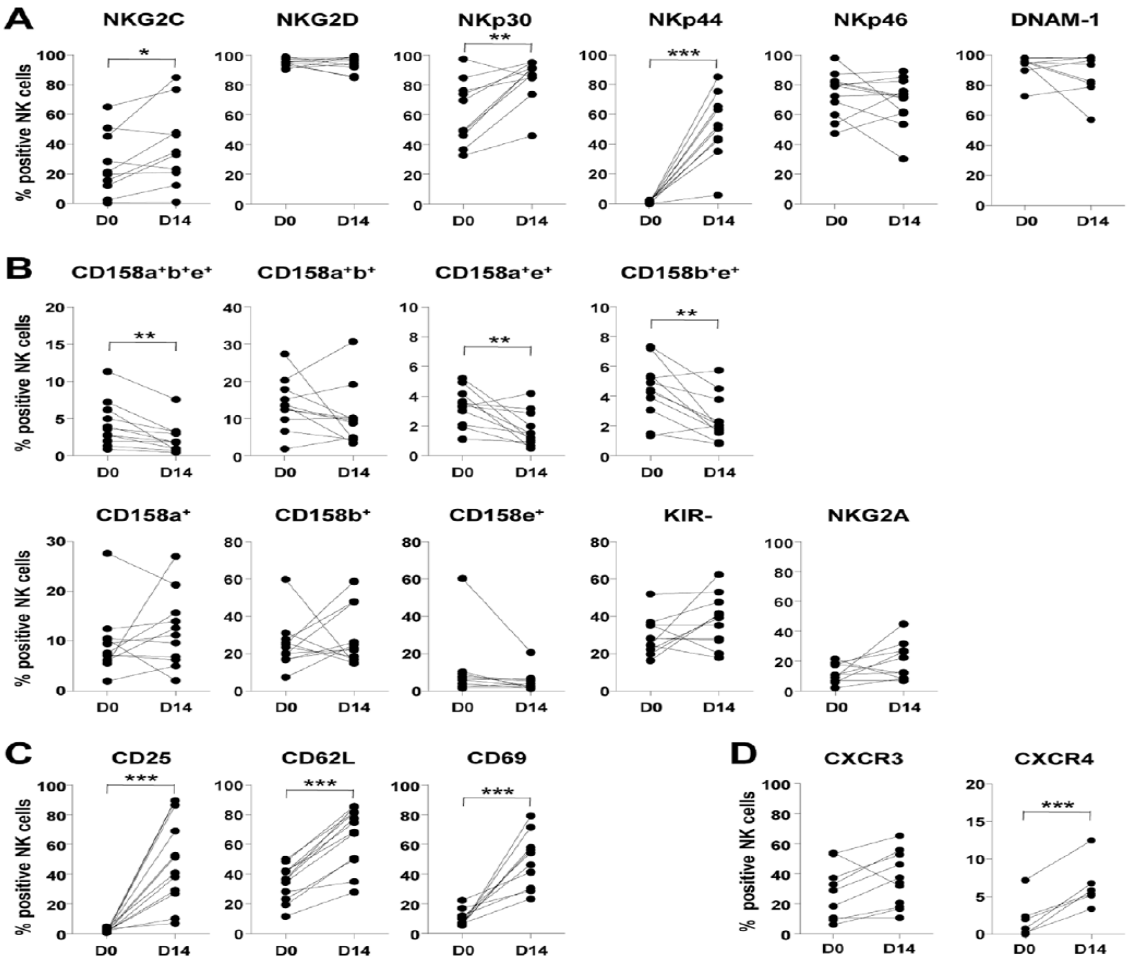
(D0 vs. D14, n = 10 ~ 12)

(A) activating receptors

(B) inhibitory receptors (KIRs)

(C) activation markers

(D) chemokine receptors



PLoS One. 2013;8(1):e53611

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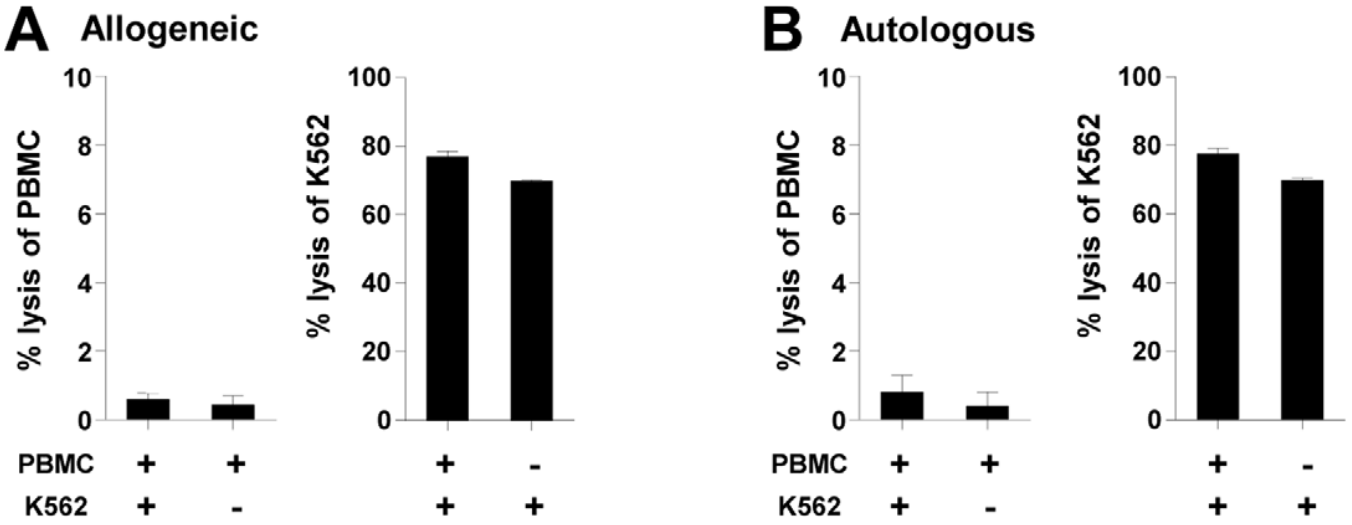
Efficacy of MG4101

Tumor-specific cytotoxicity of MG4101

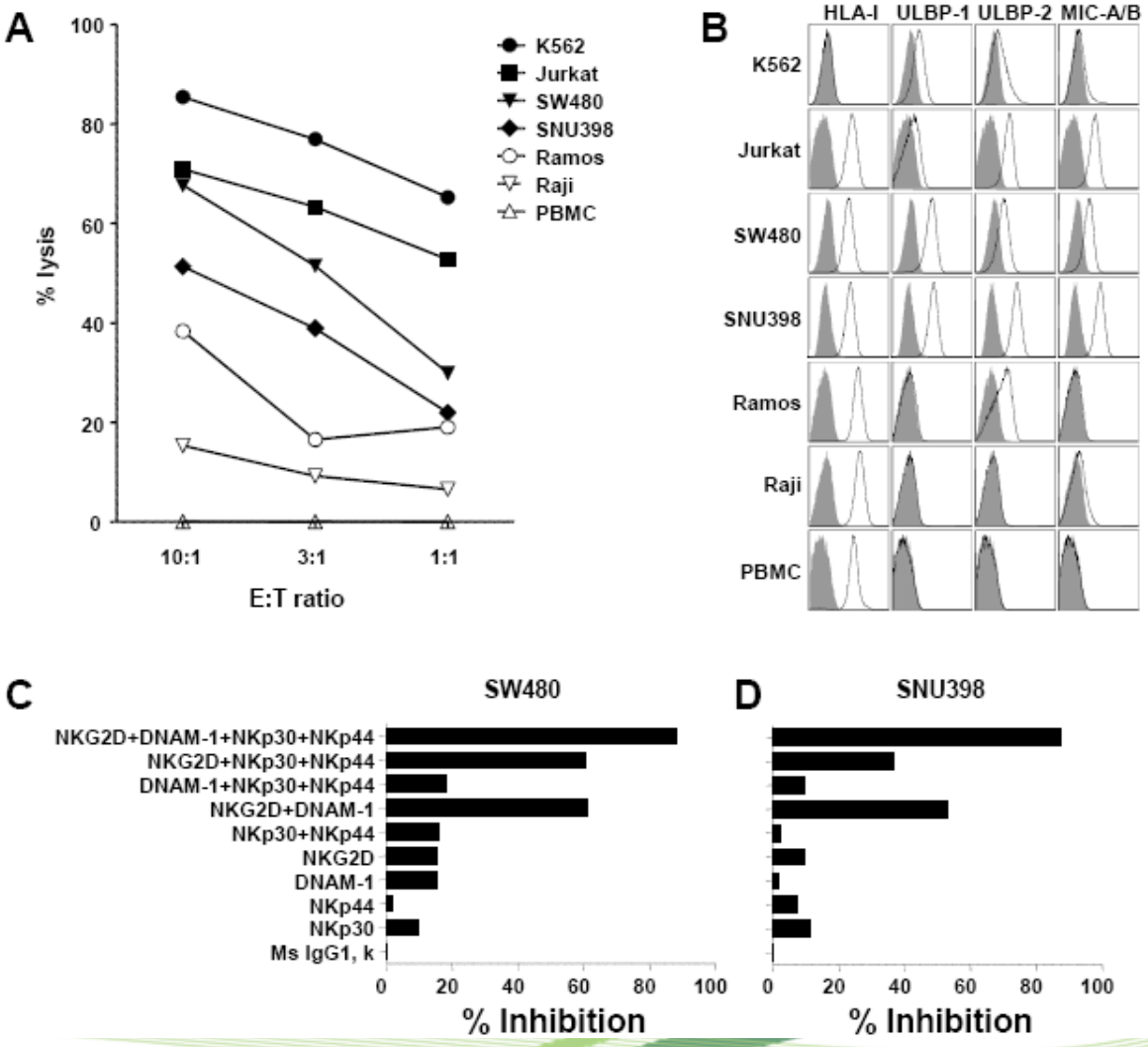
(*in vitro* killing activity in the co-culture system)



MG4101 effectively discriminated tumor cells from allogeneic normal PBMCs and selectively killed transformed cells, confirming that MG4101 prepared from unrelated healthy donors can be used for the treatment of cancer patients in allogeneic settings.

PLoS One. 2013;8(1):e53611

NKG2D ligand-mediated NK cell cytotoxicity

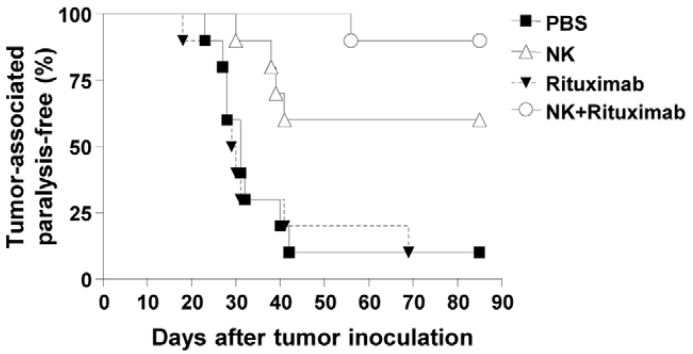


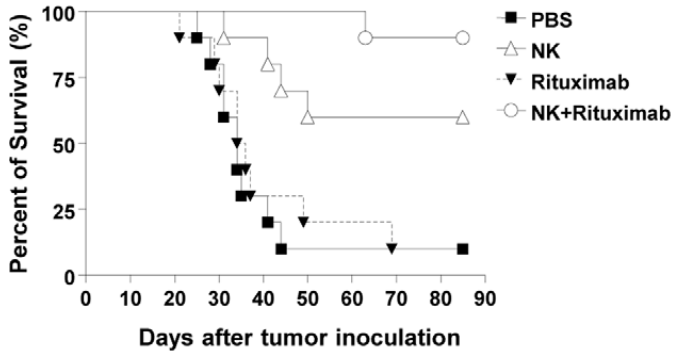
PLoS One. 2013;8(1):e53611

Summary of *in vivo* efficacy of MG4101 in preclinical cancer models

- Immune competent mouse models
  - Syngeneic tumor models for neuroblastoma
- Immune deficient mouse models
  - Xenogeneic tumor models for lymphoma, glioblastoma, ovarian cancer, and HCC.
- MG4101-treated groups showed reduced tumor mass, alleviated symptoms, and improved survival compared with control groups.
- MG4101 showed significantly enhanced anti-cancer activities in the lymphoma model when combined with low dose Rituxan which itself exerted no therapeutic efficacy as a single agent.

PLoS One. 2013;8(1):e53611





Preclinical Safety study

Pre-Clinical toxicity studies

- **Toxicology - Single dose**
  - SCID mice, IV
  - No severe adverse effects
  - NOAEL: > 2.5 x 10^7 cells/head
- **Toxicology - Repeated dose**
  - SCID mice, IV, 6 times repeat
  - No severe adverse effects
  - NOAEL: > 5 x 10^6 cells/head

Manufacturing of Allogeneic NK cells

Ex vivo expansion of Allogeneic NK cells
(Set up of efficient manufacturing system)

- Expansion : over several thousands folds for 14-21 days
- Purity : more than 98 percent
- Contamination-free closed culture process using commercialized plastic bag
- Storage : stable for 72 hours in the cold storage condition

  w/o loss of viability and activity
- Set up cryopreservation technology for final product
- Applications: NK cell therapeutics, CAR-NK cell etc.

Mass production with cryopreservation technology: reduction of the production cost !

Manufacture of Allogeneic NK cells

Clinical site

Green Cross LabCell GMP

Healthy donor

Leukapheresis

Isolation of NK cells

Cryopreservation

Thawing

Expansion

Product (Freezed)

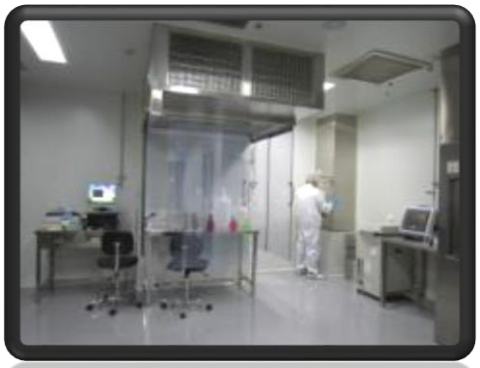
Cryopreservation

Final Product

Patient

GMP Conditions for Production

- Manufacturing sites : Yongin, South Korea
- Clean culture rooms, Cell storage room, Support room, QC rooms
- Clean Class : class 100 ~ class 100,000
- Clinical-GMP Permission obtained from KFDA in 2010,

Be a pioneer in cancer immunotherapy through allogeneic NK cell…

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