



# Clinical Trial of NK Cell

## Lymphoma

### The Safety and efficacy of Autologous Natural Killer cell infusions for the Treatment of Malignant Lymphoma : Phase II trial

NKBIO, 2006

NK Therapy	
Patients	50 patients with Relapsed or refractory malignant lymphoma Previously untreated NK/T cell lymphoma
NK cell infusion Autologous activated NK cell	2 x 10 <sup>9</sup> cells/100 mL IV x 5 times
Duration	between 1 <sup>st</sup> Mar. 2006 and 31 Dec. 2006
Results	Intermediate Tumor Response Assessment Power CR reached
	QOL (quality of life) Excellent
	Reduced non-hematologic complications Side effects Very few

**“As a result of phases II trial, all 50 patients treated with NK cell therapy showed CR (Complete Response)”**

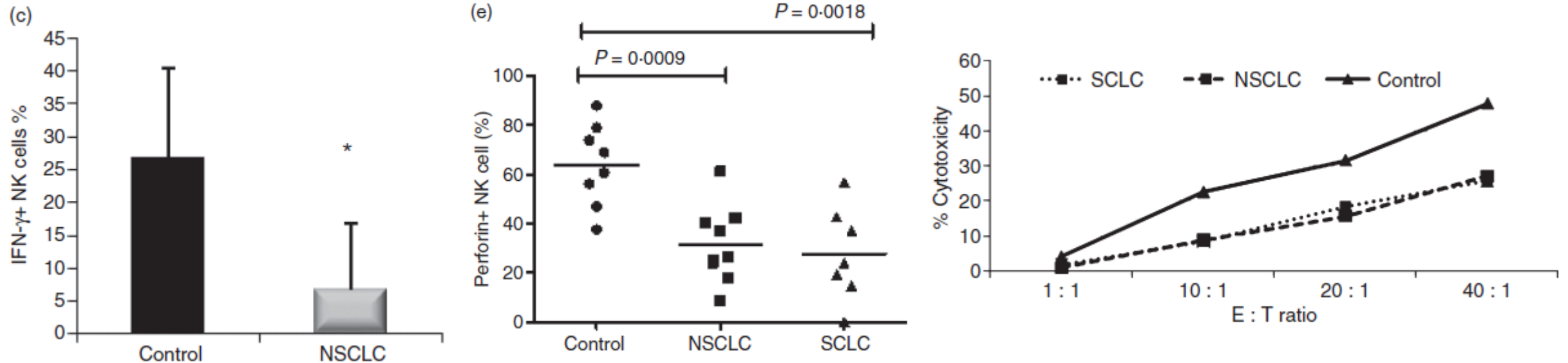


# Clinical Trial of NK Cell

## Lung cancer

### Increased killer immunoglobulin-like receptor expression and functional defects in natural killer cells in lung cancer

Immunology, 2011



Cytolytic NK cells and granzyme B expression was reduced in Lung cancer patient, and due to the reduction in NK cell's ability to stimulate T cell's reaction, **IFN- $\gamma$  production was reduced**



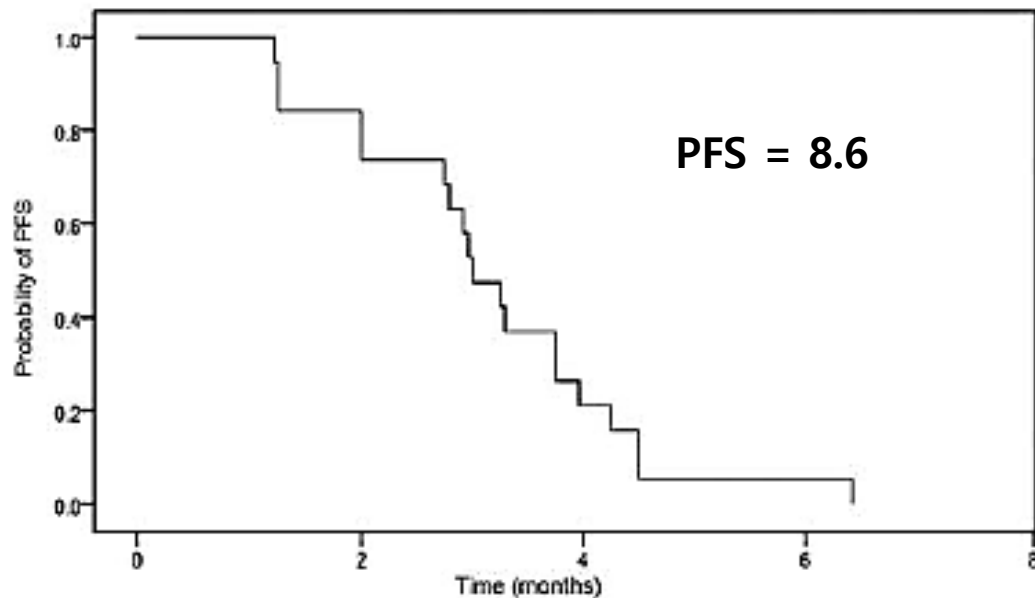
# Clinical Trial of NK Cell

## Lung cancer

### A Trial of Autologous Ex vivo-expanded NK Cell-enriched Lymphocytes with Docetaxel in Patients with Advanced Non-small Cell Lung Cancer as Second- or Third-line Treatment: Phase IIa Study

Anticancer Res, 2013

#### The Kaplan Meier estimated progression-free survival



#### Tumor response to docetaxel and NK cell-enriched lymphocytes

Patient's reaction after cancer treatment	Number of Patients (% Total 19)
Complete Reaction (CR)	0 (0)
Partial Reaction (PR)	2 (10.5)
Stable Disease (SD)	12 (63.2)
Progressive disease (PD)	5 (26.3)

Docetaxel -- anticancer drug and NK cells (at least  $2.0 \times 10^9$ ) were administered on the same period (2~6 doses) as the second and third line treatment to non-small-cell lung cancer patients whom conventional anti-cancer method has failed to cure. Compared to the high fatality level of the patients whom the first line treatment had failed to cure, result showed higher effectiveness such as higher number of patients with partial reaction with few side-effects, and **increased progress-free survival level by 3 times.**



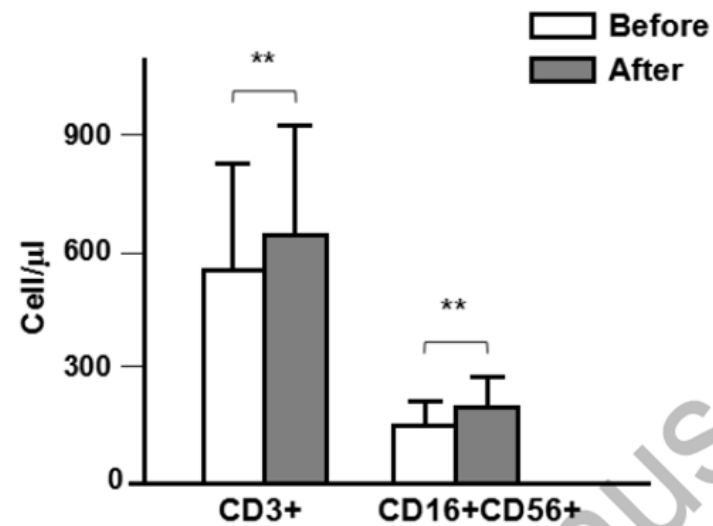
# Clinical Trial of NK Cell

## Liver Cancer

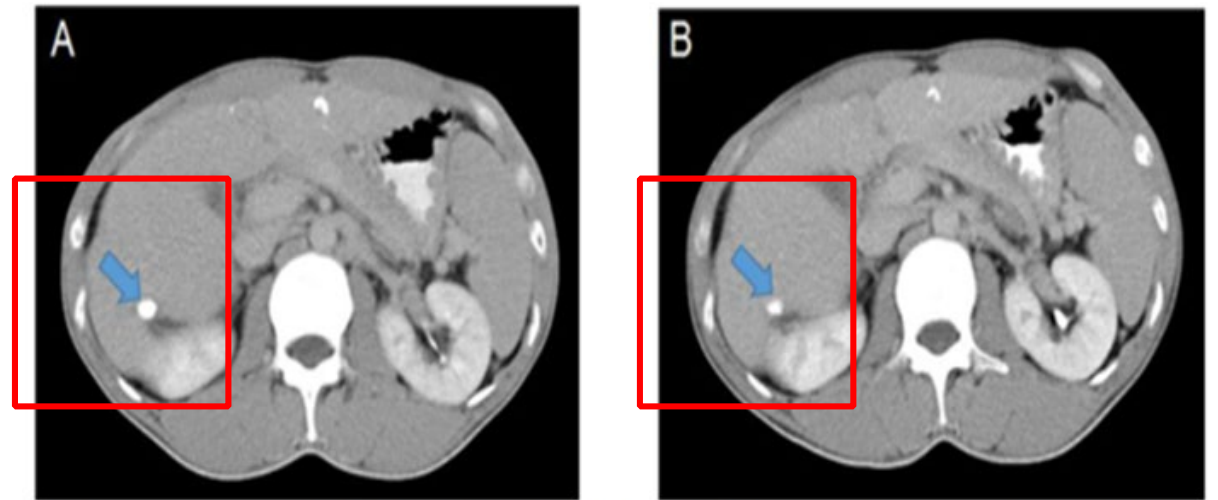
### Effect of NK cell immunotherapy on immune function in patients with hepatic carcinoma : A preliminary clinical study

Cancer Biology & Therapy, 2017

#### Changes in T cells and NK cells in peripheral blood before and after NK Cell Treatment



#### CT scan before NK cell treatment (left) and after 3 month (right) of 38 years old relapsing patient who went into stable state after NK cell treatment



After performing NK immune cell treatment on total 16 patients with phase 2-4 metastatic and relapsing liver cancer, 18.8% showed partial reaction, 50.0% showed stable disease and 31.2% showed progressive disease reaction after 3 months. There had been no side-effects and non-reaction survival period was 7.5 months. **Patients who received NK cell treatment for more than 4 times had longer non-reaction survival period than the patients with received less than 4 times**



# Clinical Trial of NK Cell

## Esophageal cancer

### Role of NKG2D ligand on NK cell immunotherapy in advanced esophageal cancer patients after operation

Chinese J of Clin Oncol, 2013

	Time to progression (month)	Overall survival (month)
Chemotherapy only	5.00	11.00 (9.38-12.62)
MICA- group : Chemo + NK	5.80	10.4 (8.77-12.02)
MICA+ group : Chemo + NK	9.90	16.9 (11.67-22.12)

NK cell treatment was given to the patients simultaneously with chemotherapy who have esophageal cancer cells that express MICA . The side-effects of anti-cancer drugs in patients – Leukopenia and peripheral neurotoxicity – were eased after the NK cell treatment. **The tumor progression and overall survival showed significant increase (P<0.05)**

Autologous NK cell treatment was given to the progressive esophageal cancer patients simultaneously with chemotherapy. It had no side-effect and enhanced immunity to increase survival and tumor progression on patients with cancer cells that express MICA – ligand recognized by NK cell's activation receptor, NKG2D.



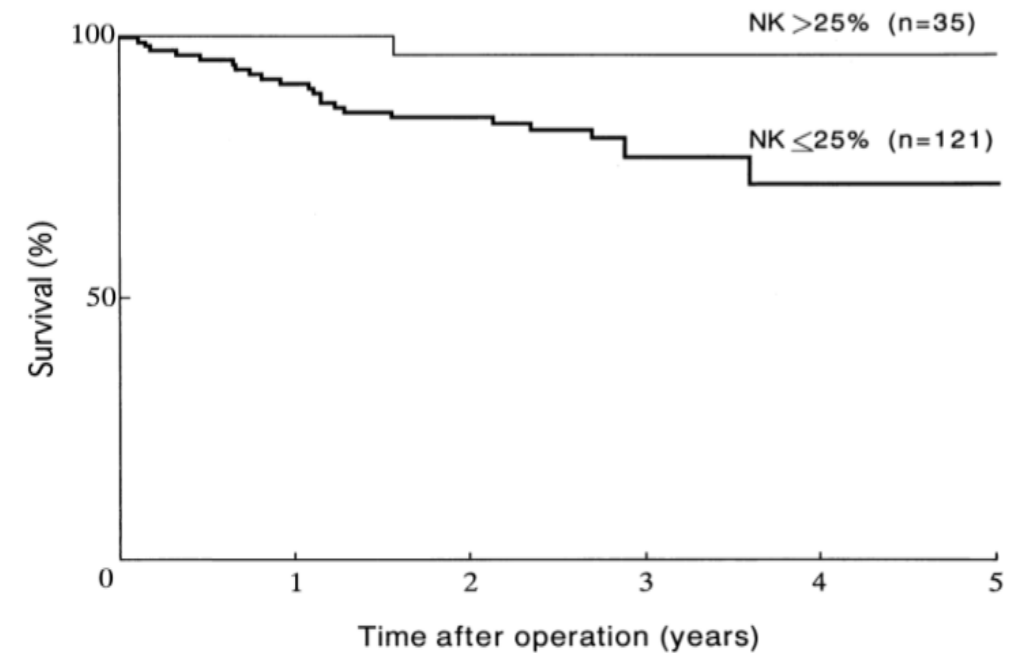
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## Gastric Cancer

### Prognostic Significance of Natural Killer Cell Activity in Patients With Gastric Carcinoma: A Multivariate Analysis

THE AMERICAN JOURNAL OF GASTROENTEROLOGY, 2001

1. Patients with activated NK cells had 5-year survival rate of 94.6% (Activation>25%), which was higher than patients with lower NK cell activation (Activation  $\leq$  25%).
2. NK cell activation showed relations to the tumor size and it's progression.
3. Measurement of NK cell activation can be suitable to use for prognosis and follow-up clinical management of the patients.



**“Gastric cancer patients with higher NK cell activation rate, had higher 5-year survival rate than patients with lower activation.”**

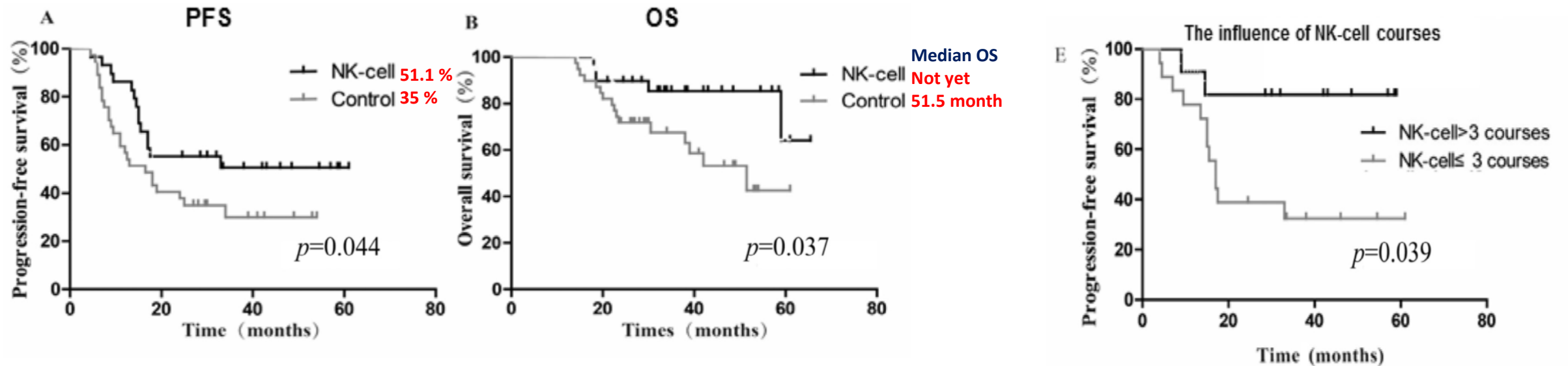


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## Colorectal cancer

Adoptive transfer of natural killer cells in combination with chemotherapy improves outcomes of patients with locally advanced colon carcinoma

Cytotherapy, 2018



<2010-2016 cohort study>

NK cell treatment was performed simultaneously with chemotherapy on colorectal cancer patients with local progression . It prevented relapse that accompanies side-effects and lead to a longer survival. It was especially effective for undifferentiated carcinoma and no considerable side-effect had been reported.



# Clinical Trial of NK Cell

## Colorectal cancer

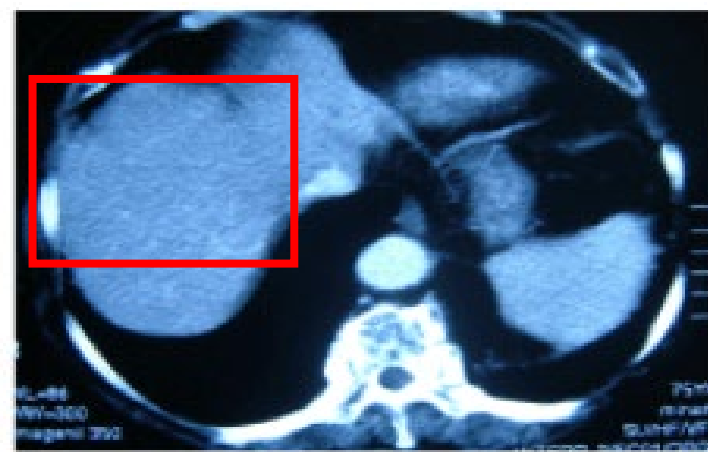
**After the surgeries for colorectal cancer and lung metastasis, the third metastasis was detected in the liver, but could not be treated with surgery**

<http://www.nco-clinic.jp>

1. Cancer was spread to patient's lung and liver on 5 months and 14 months respectively after the surgery for colorectal cancer. Resection surgery followed
2. There were also lymph node metastasis. Four types of anti-cancer drugs were used as a supplement.
3. Multiple metastases were found in the liver. Chemotherapy was used but stopped due to the side effects.
4. After switching the anti-cancer drug to TS-1, highly activated NK cell treatment was initiated.
5. After five doses of highly active NK cells, CT images confirmed that most liver metastases disappeared and lymph node metastases became smaller (Photo 1)



Before treatment



After treatment



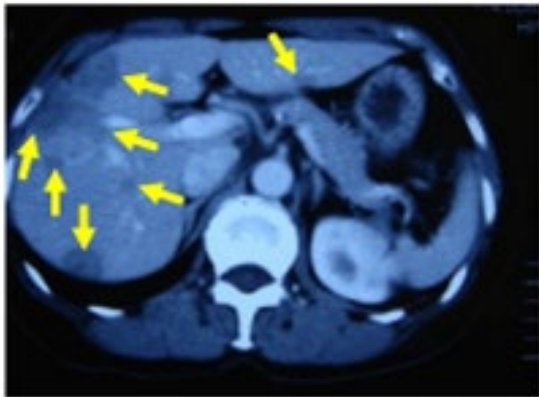
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## Breast cancer

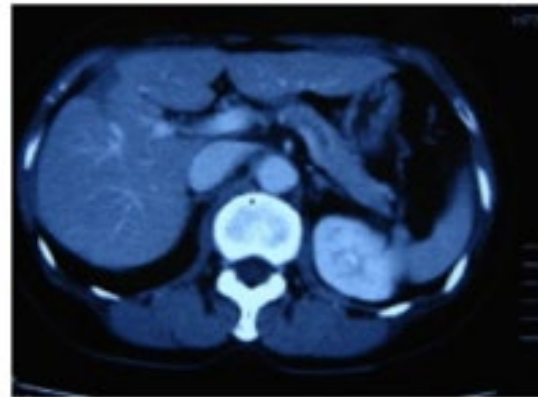
After total mastectomy, local recurrence of breast cancer and multiple hepatic metastases were observed

<http://www.nco-clinic.jp>

Photo 1 Local recurrence, multiple hepatic metastases

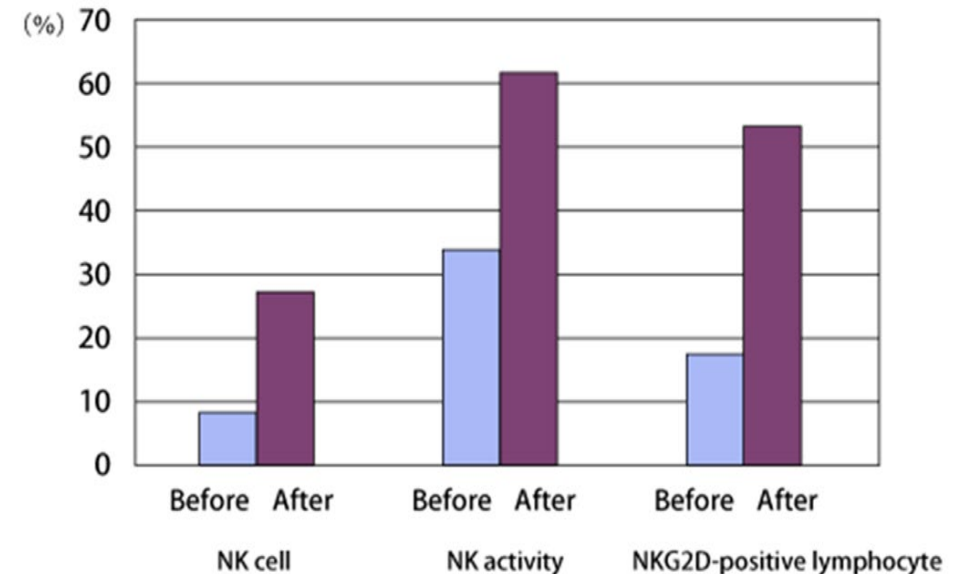


Before treatment



After 5 months of treatment

Figure Change in lymphocytes in the blood before and after the treatment



Anti-cancer drugs and NK cell treatment were administered simultaneously to the patient who had multiple metastasis. After 5 months, metastasis had disappeared. After 10 doses, NK cell rate has increased by 3 times from 8.7% before treatment to 27.6% after treatment. Activation of NK cells was doubled as well. **Combined with anti cancer drugs, NK cell treatment was very effective.**



# Clinical Trial of NK Cell

## Ovarian Cancer

### Natural Killer Cell Activity and Progression-Free Survival in Ovarian Cancer

Gynecol Obstet Invest, 1993

NK cell treatment was performed to 17 patients with progressive epithelial ovarian carcinoma. Disease progression was measured during the follow-up period.

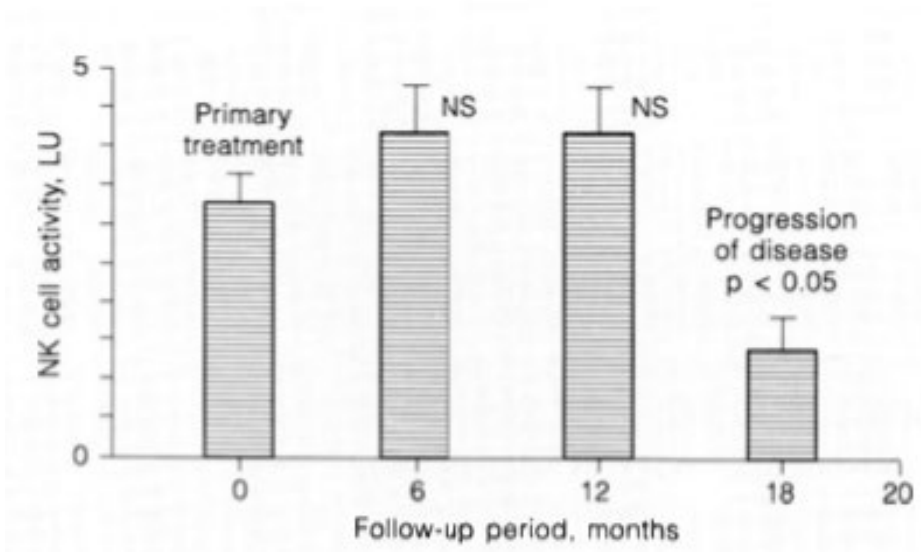


Fig 1. Decrease of NK cell activity at the time of disease progression

**Table 1.** NK cell activity of PBLs (at the time of primary treatment) in patients who had disease progression and in patients with disease-free survival

Disease progression	Number of cases	NK activity, LU (mean $\pm$ SD)	p
Yes	8	3.31 $\pm$ 0.7	< 0.05
No	9	7.18 $\pm$ 1.34	

At the time of surgical treatment, Average NK cell activity was considerably lower in patients with relapse or disease progression.

**“NK cell activation was considerably lower in patients with disease progression at the time of relapse”**

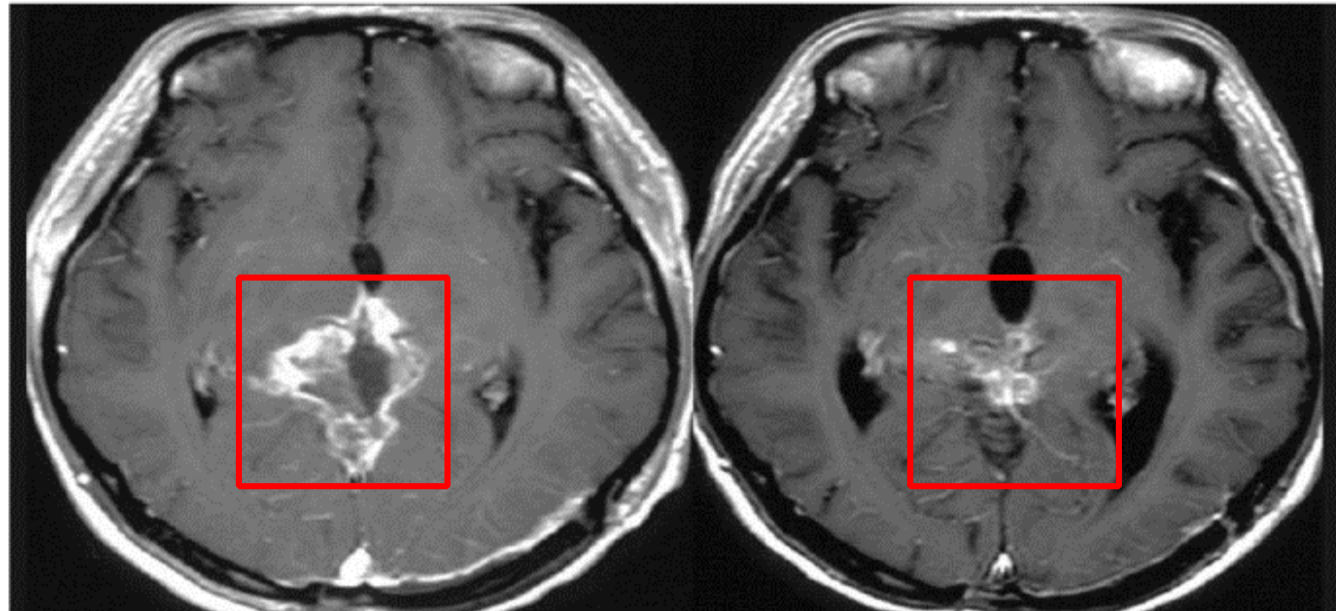


# Clinical Trial of NK Cell

## Brain tumor

### Autologous Natural Killer Cell Therapy for Human Recurrent Malignant Glioma

Anticancer research, 2004



Autologous NK cells were administered to the brain tumor patients with relapse (1<sup>st</sup> dose: 5.1 billion cells, 2<sup>nd</sup> dose: 4.6 billion cells). M R image before the treatment (left) and after the treatment (right). **Autologous NK cell treatment is effective for decreasing tumor size of the relapsing glioma.**



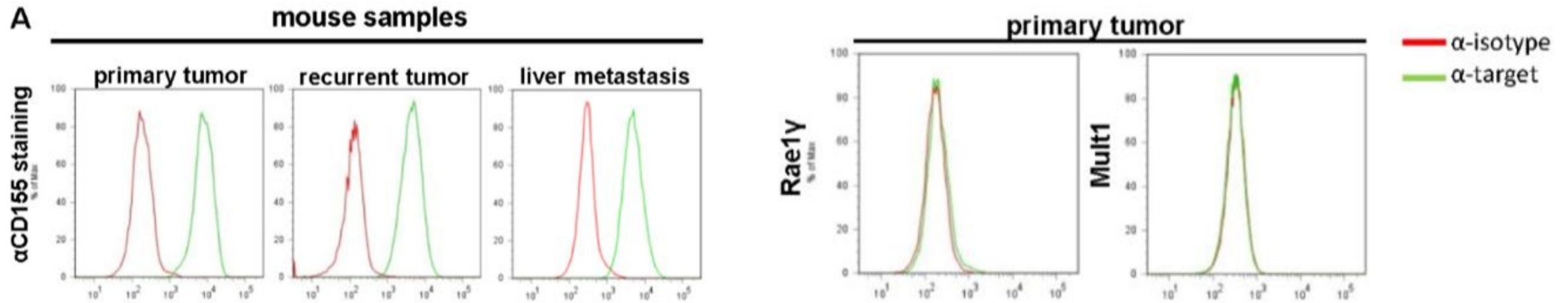
# Clinical Trial of NK Cell

## Pancreatic cancer – *in vivo*

**Perioperative, spatiotemporally coordinated activation of T and NK cells prevents recurrence of pancreatic cancer**

American Association for Cancer Research, 2017

### Coordinated perioperative immunotherapy for resectable PDAC



After investigation of NK cell ligand in a tumor, Rae1r and Multi1 was not expressed. However, tumor cells that was generated from relapsing cancer, liver metastasis and primary tumor material, had high expression rate of CD155. CD155 is one of the activation receptors, DNAM-1 ligands. As DNAM-1 increases, More CD155 will be recognized therefore more destroying ability.

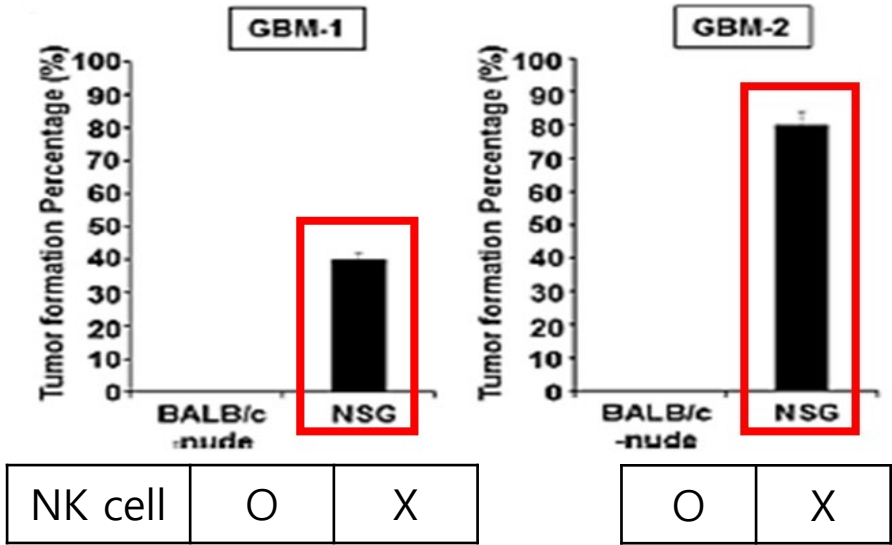
**“Mass expression of CD155 ligand on the surface of the pancreatic cancer cell is recognized by NK cell’s activation receptor, DNAM-1 to destroy it”**



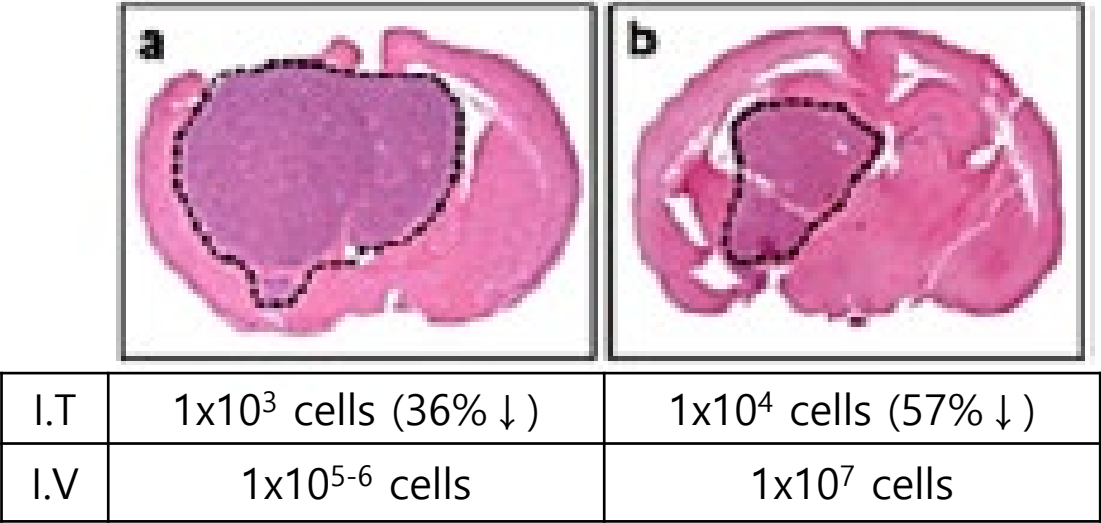
## Brain tumor - *in vivo*

Natural killer (NK) cells inhibit systemic metastasis of glioblastoma cells and have therapeutic effects against glioblastomas in the brain

BMC Cancer,2015



After administering brain tumor in lung metastasis test, the mice with NK cells did not show metastasis on lungs, therefore NK cells plays important role in preventing metastasis of the tumor.



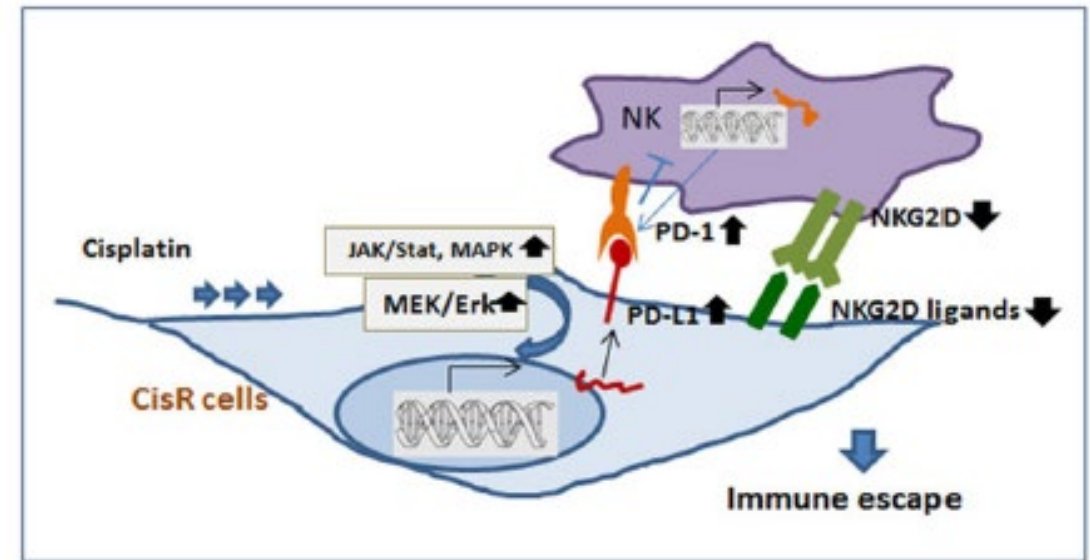
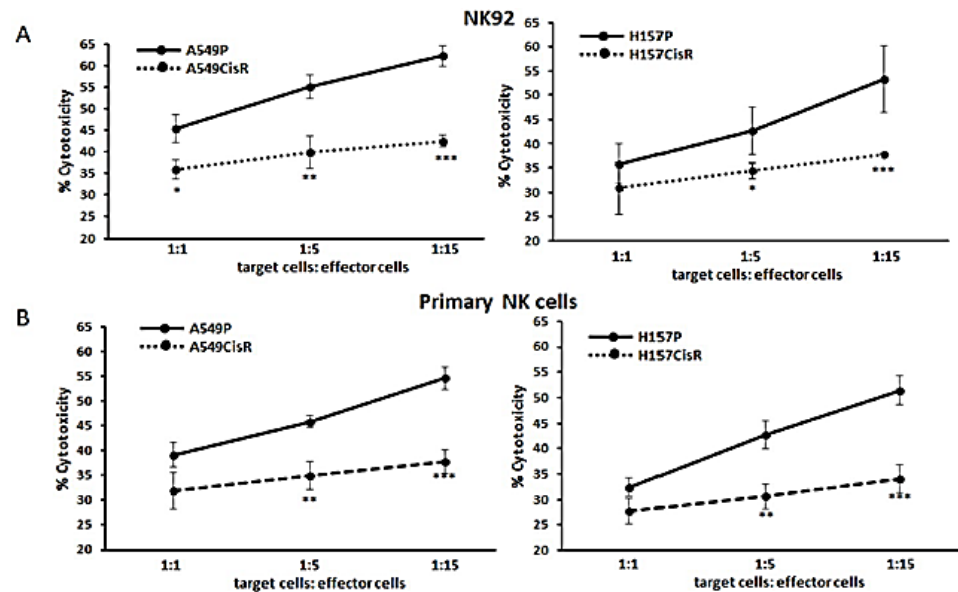
When 10 million NK cells were administered to the blood every week for three times, they penetrated to the disease area to decrease the size of the tumor by 57%, which shows an anti-tumor effect.

“NK cell plays vital role in preventing metastasis of tumor and decreasing the size of the tumor”

## Lung cancer - *in vitro*

### A Enhancing NK cell-mediated cytotoxicity to cisplatin-resistant lung cancer cells via MEK/Erk signaling inhibition

Nature Scientific Reports, 2017



Cell lines of non-small-cell lung cancer, A549CisR and H157CisR were put in to anti-cancer drug -- Cisplatin, of which they have tolerance to, together with NK 92(nk cell line), and NK cells separated from the blood. A high anti-cancer effect was also shown not only in lung cancer cell lines but also in cell lines that has Cisplatin tolerance. The cell lines that has tolerance to Cisplatin showed high expression of PD-1 (immune deflecting) antigens. By adding PD-1 or PD-L1 antigen, NK cell showed much higher cytotoxicity. This shows MEK/Erk signaling system has a important role in this effect.



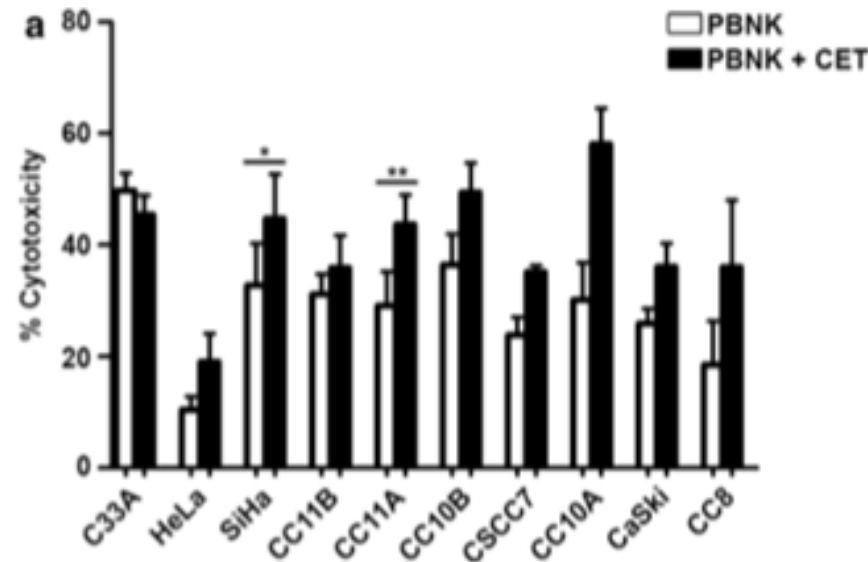
# Clinical Trial of NK Cell

## Uterine cancer - *in vitro*

### High-efficiency lysis of cervical cancer by allogeneic NK cells derived from umbilical cord progenitors is independent of HLA status

Cancer Immunol Immunother, 2017

Cervical cancer  
cell lines



In 10 different cell lines of the cervical cancer that represent HPV(Human papillomavirus), research has been conducted on therapies using PBNK alone and combined usage of PBNK and CET. Combined treatment using PBNK and CET showed possibility for better effectiveness.

CET : Cetuximab (inhibits EGFR) – Anti cancer drug  
PBNK : Peripheral blood natural killer cells

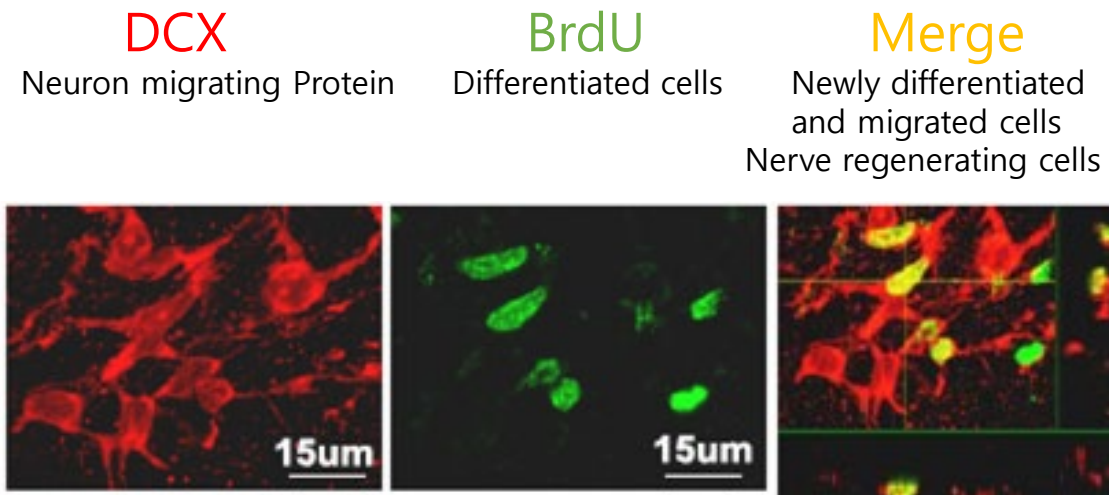


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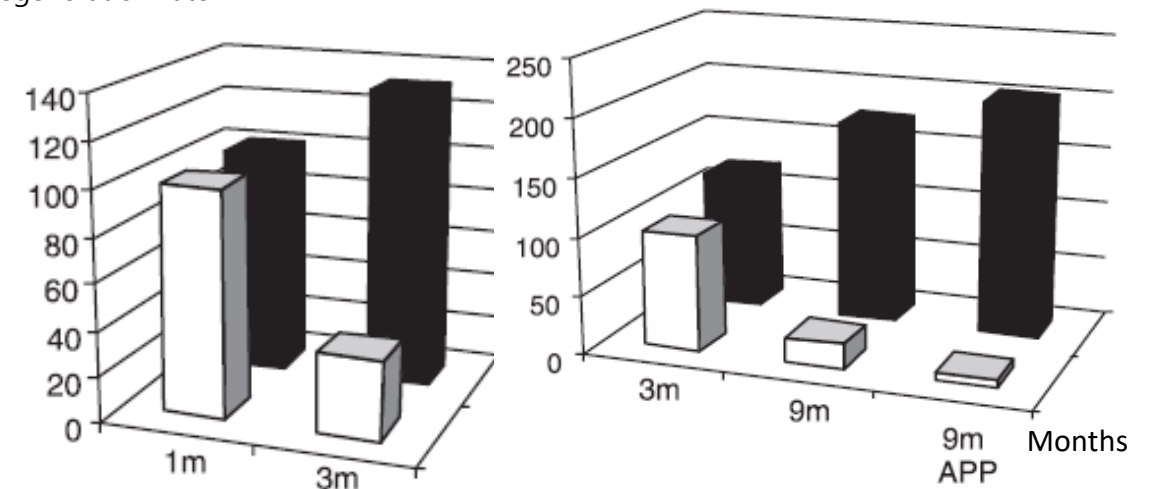
## Alzheimer's disease - *in vivo*

### IFN-gamma enhances neurogenesis in wild-type mice and in a mouse model of Alzheimer's disease

FASEB J, 2008



Nerve regeneration rate



White column: Normal Mouse  
Black column: Mouse with IFN- $\gamma$  expressing DN A implemented.

IFN- $\gamma$  expressing DNA implemented mouse had increasing nerve regeneration rate as it gets older. It had maximum effect among the mice that had Alzheimer's disease inducing DNA. **Massive IFN- $\gamma$  produced by NK cells can help on nerve regeneration in Alzheimer's disease patients.**



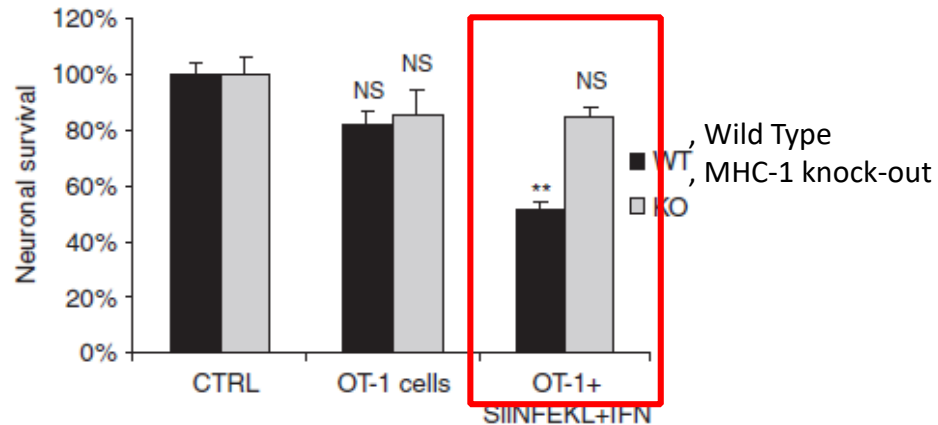
# Clinical Trial of NK Cell

## Parkinson disease- *in vivo*, *in vitro*

### MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration

Nat Commun, 2014

MHC-1 induction in dopamine neuron of midbrain using Oxidative stimuli (L-DOPA)



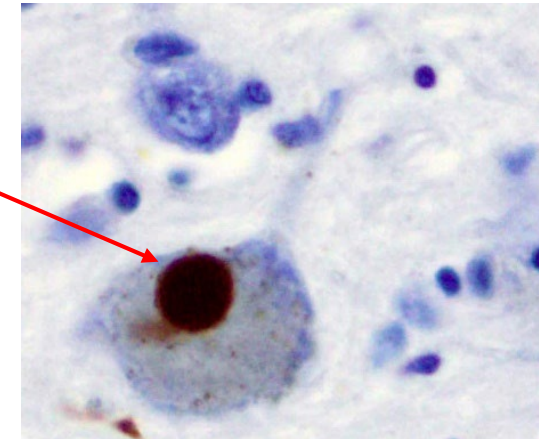
When inducing MHC-I antigen among the nerve cells, it is experimentally supported that T-cells destroy neurons, which shows a possibility that **Parkinson disease might be caused by auto immune disorder.**

### T cells from patients with Parkinson's disease recognize $\alpha$ -synuclein peptides

Nature, 2017

$\alpha$ -synuclein's Parkinson's disease induction is a result of the autoimmune reaction

Lewy body  $\alpha$ -synuclein



Parkinson's disease is induced when  $\alpha$ -synuclein, the protein that helps a nerve transfer between brain cells, cumulate within dopamine nerve cells. An autoimmune reaction can be induced by T-cells that are activated by  $\alpha$ -synuclein recognizing a neuron as a pathogen to attack.

**“NK cells produces IFN- $\gamma$  which controls auto-immune reaction.  
NK cell injection has a potential to regulate the autoimmune reaction”**



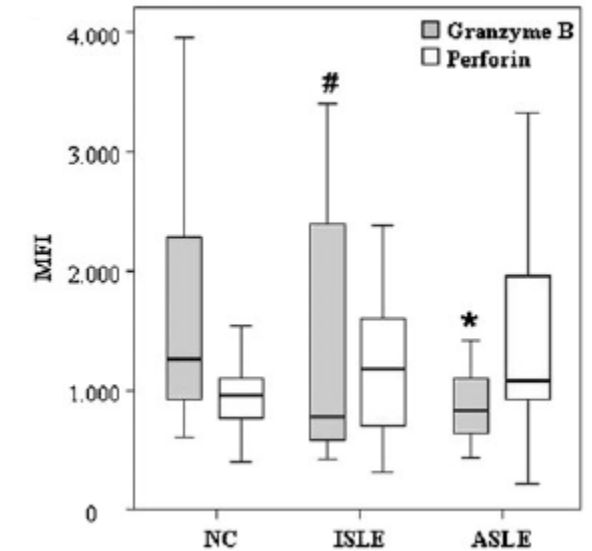
# Clinical Trial of NK Cell

## Auto-immune disorder : systemic lupus erythematosus (Lupus) – Clinical Data

### NK cells dysfunction in systemic lupus erythematosus: relation to disease activity.

Clin Rheumatol. 2012

		Normal Control	Inactive SLE	Active SLE
WBC	Cells/ul	7,273±1,737	7,077±2,405	6,294±1,989*
NK cell	%	3.3±1.8	1.8±1.0	1.4±1.2*
	Cells/ul	240.0±150.0	120.0±80.0*	90.0±60.0*
CD56 <sup>bright</sup>	%NK	6.2±3.6	9.3±8.1	7.6±6.0
	Cells/ul	14.0±7.0	9.0±6.0*	7.0±6.0*
CD56 <sup>dim</sup>	%NK	93.8±3.6	90.7±8.0	92.4±6.1
	Cells/ul	220.0±150.0	110.0±70.0*	80.0±60.0*



\*, p<0.05

Number and proportions of NK cells in peripheral blood is especially lower in active lupus patients. Also, expression of Granzyme B is lower in lupus patients compared to the normal control group.

**“Injection of the large number of NK cells are effective for Lupus patients”**



## Auto-immune disorder : Rheumatoid arthritis and Sjögren syndrome – Clinical Data

**A significantly impaired natural killer cell activity due to a low activity on a per-cell basis in rheumatoid arthritis**

Mod Rheumatol, 2009

	Number of N K cells (/ul)	Proportion of NK cells (%)	NK cell activation according to E:T ratio (% lysis)			NK cell activation (Cell lysis unit)	
			50:1	20:1	10:1	LU	LU/NK cell
Rheumatoid Arthr itis	267 ± 371*	13.3 ± 9.5*	21.6 ± 14.1**	11.9 ± 8.2**	6.9 ± 4.8**	26.0 ± 18.5**	0.21 ± 0.16*
Sjögren syndrome	133 ± 82**	9.1 ± 4.3**	29.8 ± 13.1**	19.4 ± 22.9**	8.5 ± 5.6**	39.8 ± 39.1**	0.37 ± 0.34
Normal Control	298 ± 196	15.5 ± 6.8	52.6 ± 19.5	30.0 ± 16.7	16.7 ± 10.5	106.5 ± 110.5	0.34 ± 0.22

Number of NK cells, its proportions and cytotoxicity activation are lower in rheumatoid arthritis and Sjögren syndrome patients. Also, NKG2D, which is important for NK cell's immediate immune response, and CD244, which is important for signaling NK cells, are significantly lower in rheumatoid and Sjögren syndrome patients. CD 16, which is important in NK cell's activation is significantly lower in rheumatoid arthritis patients compared to the normal control group.

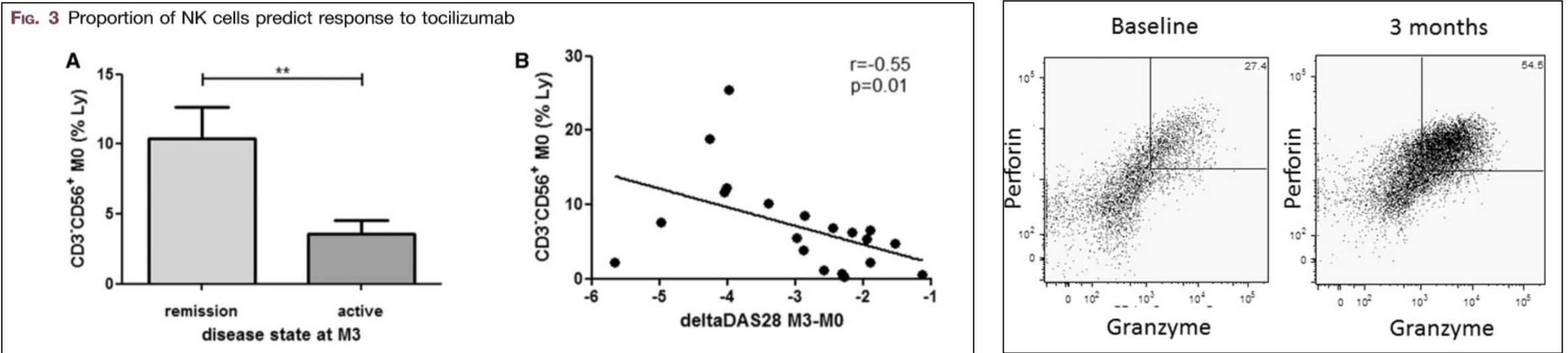
**NK cell with activation receptors like NKG2D is effective in treating the patients with autoimmune disorder when used as a supplementary treatment**



## Autoimmune disorder : Rheumatoid arthritis – Clinical Data

**High levels of natural killer cells are associated with response to tocilizumab in patients with severe rheumatoid arthritis**

Rheumatology , 2015



- If NK cell proportion is lower in blood, DAS28, the inflammation measurement of rheumatoid arthritis is higher. This shows NK cell's role in protection against rheumatoid arthritis
- Groups with rheumatoid arthritis eased through Tocilizumab showed increased NK cell rate in peripheral blood.
- Also NK cells perforin content was increased after treating RA patients with Tocilizumab.

**“High number of NK cell and activation is effective in treating rheumatoid arthritis”**