Immunotherapy Project
for Patients with Lung Cancer

「Basic Research」

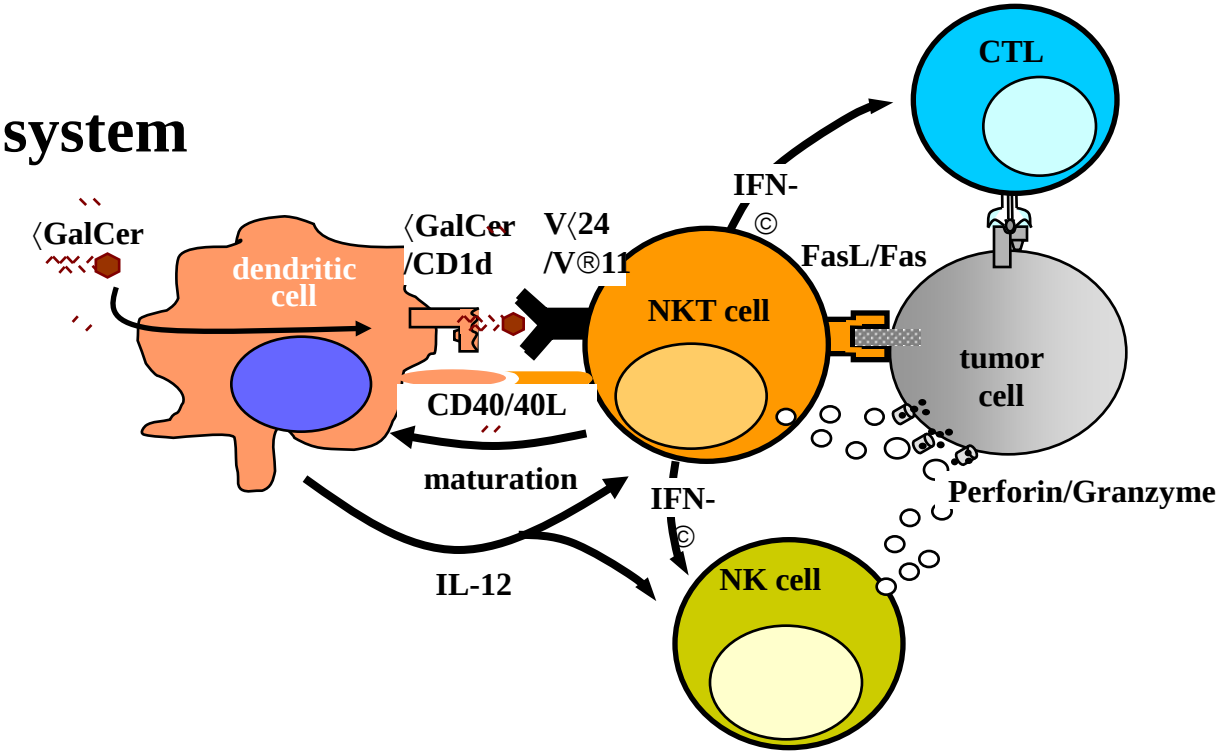
Invariant Natural Killer T cells (iNKT cells)

— Characteristics —

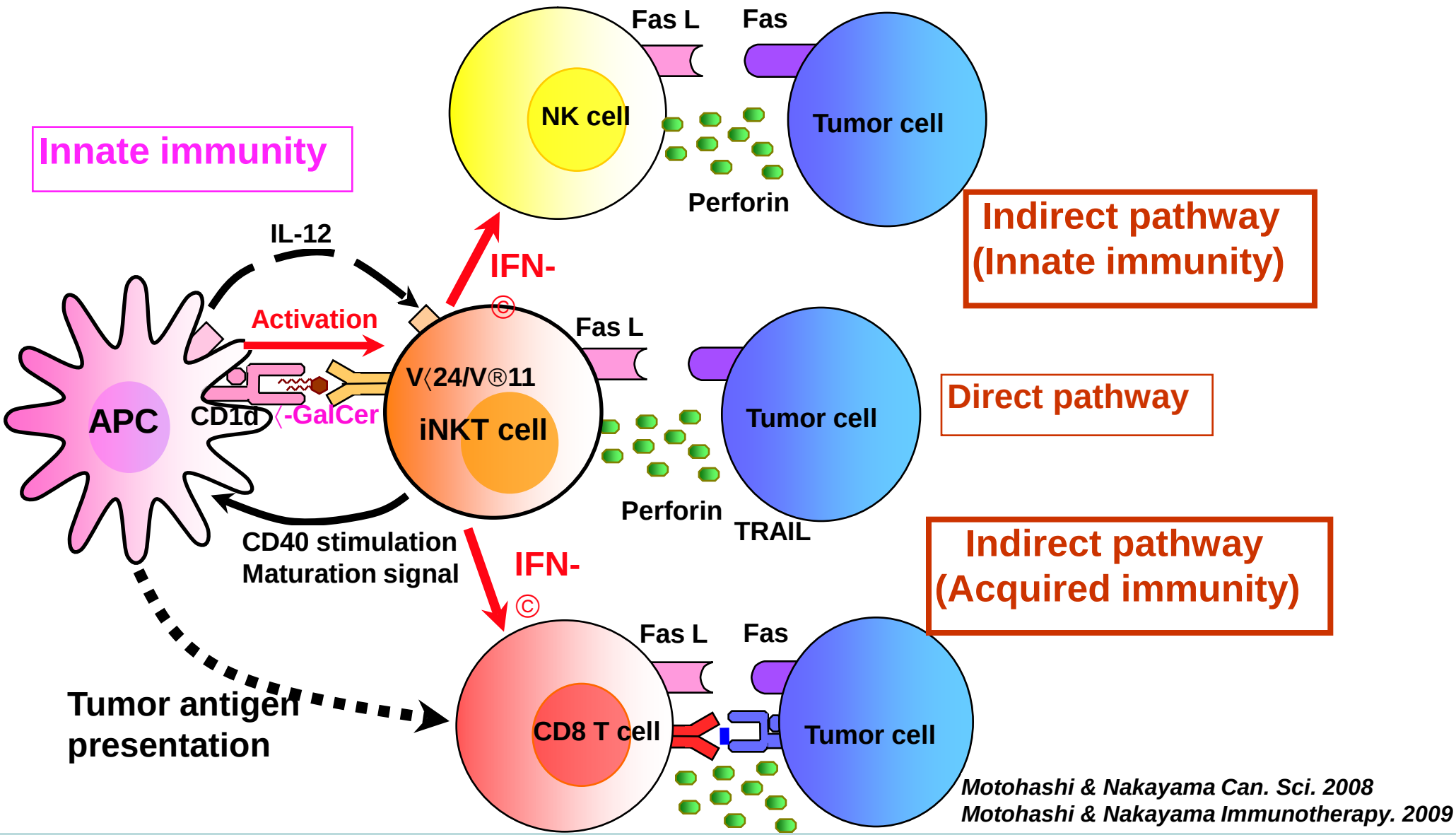
1. Possess both T cell receptor and NK receptor
2. Express invariant antigen receptor (human:V⟨24, mice:V⟨14)
3. Recognize glycolipid antigens (⟨-Galactosylceramide or microbial pathogen-derived glycolipids), presented by MHC class Ib molecule, CD1d

— Functions —

1. Regulation of immune system
2. Anti-tumor Immunity
3. Allergy defense
4. Autoimmune disease
5. Transplantation



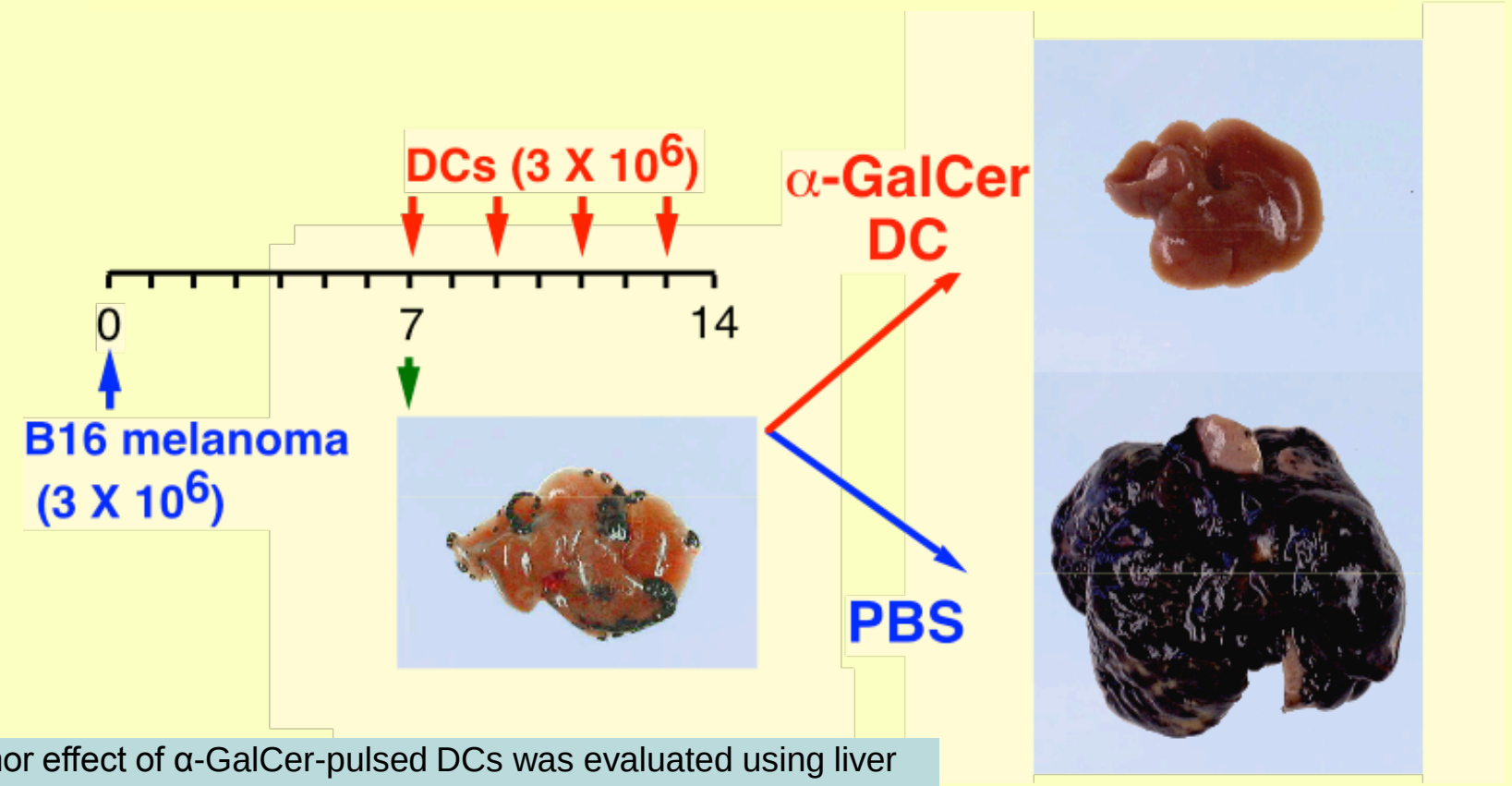
Anti-tumor immune responses induced by ⟨GalCer-activated human iNKT cells



Motohashi & Nakayama Can. Sci. 2008
Motohashi & Nakayama Immunotherapy. 2009

The α- GalCer/CD1d complex activates NKT cells to upregulate CD40L and cytotoxic molecule expressions. CD40L on NKT cells stimulates CD40 on DC, leading to their activation to produce IL-12. IL-12 from DC activates NKT cells to produce IFN-γ which in turn stimulates NK cells and CD8 CTLs mediating antitumor cytotoxicity. α-GalCer-activated NKT cells also induce maturation of DC, which contributes to the upregulation of Th1 responses.

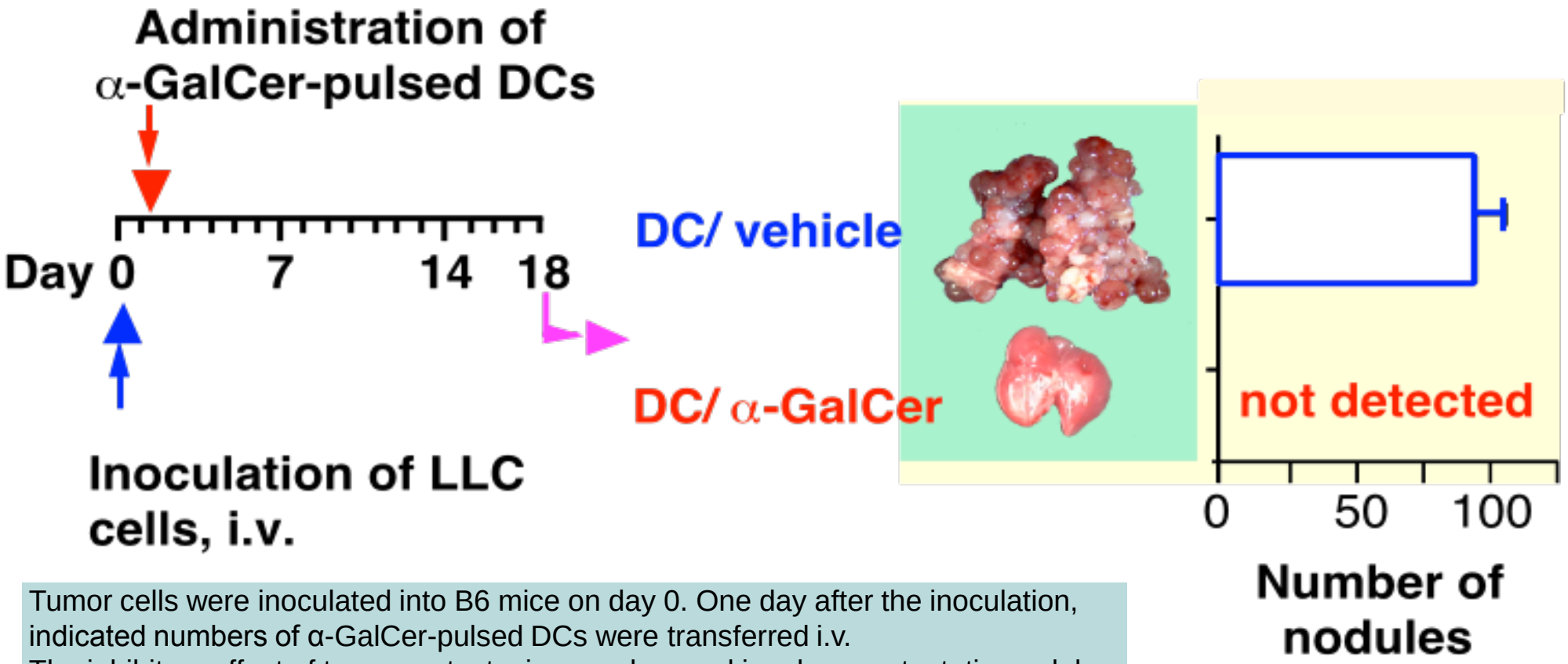
Regression of established metastatic tumors by ⟨-GalCer- pulsed DCs.



The antitumor effect of α-GalCer-pulsed DCs was evaluated using liver metastasis of B16 melanoma. Effects of i.v. administration of α-GalCer-pulsed DCs on established B16 melanoma foci were assessed. The transfer of pulsed DCs started on day 7, day 9, or day 11. The same numbers of pulsed DCs were injected every other day until day 13. All mice were sacrificed on day 14.

Toura et al, J. Immunol. 1999

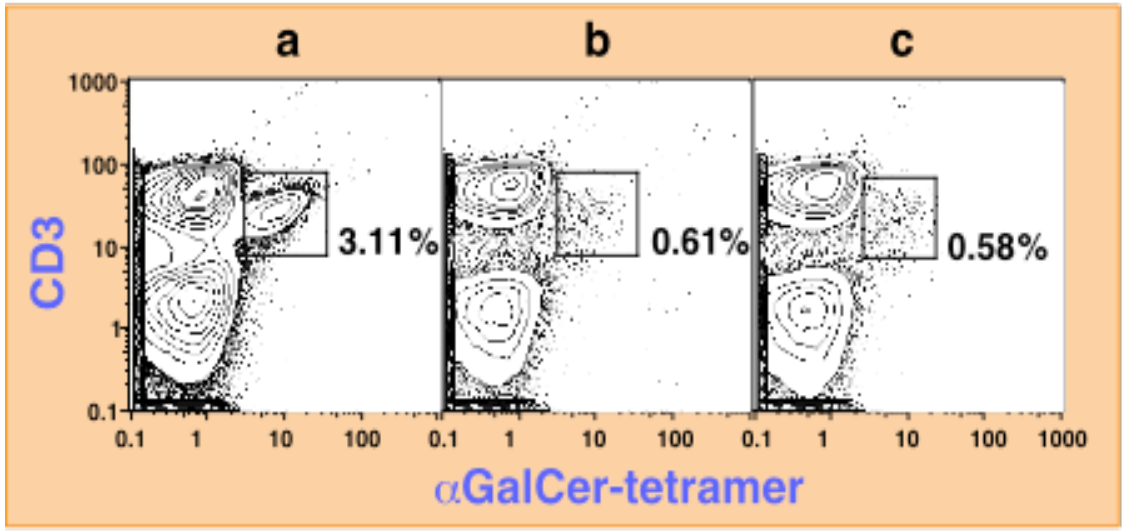
Inhibition of tumor metastasis by α-GalCer-pulsed DCs



Tumor cells were inoculated into B6 mice on day 0. One day after the inoculation, indicated numbers of α-GalCer-pulsed DCs were transferred i.v.
The inhibitory effect of tumor metastasis was observed in a lung metastatic model using LLC cells. A single i.v. injection of α-GalCer-pulsed DCs (3 x 106), but not vehicle-pulsed DCs, induced a complete inhibition of lung metastasis of LLC.

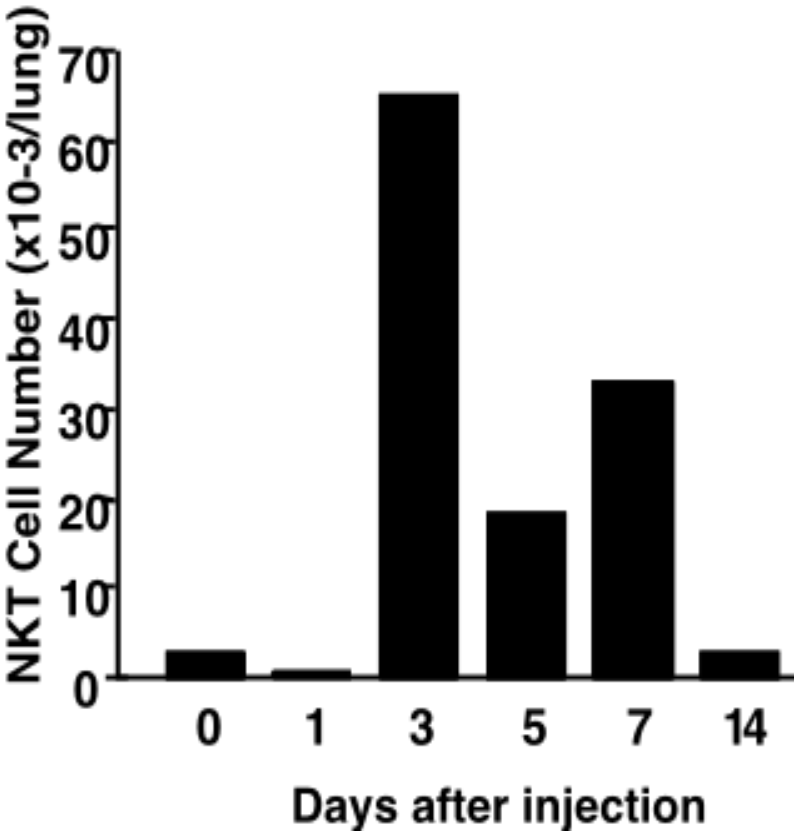
Toura et al, J. Immunol. 1999

Vα14NKT cells are expanded in the lung after αGalCer-pulsed DC administration



Treatment

a: αGalCer-pulsed DC ($3x10^6$)
b: vehicle-pulsed DC ($3x10^6$)
c: no treatment
40 hours after i. v. inj.



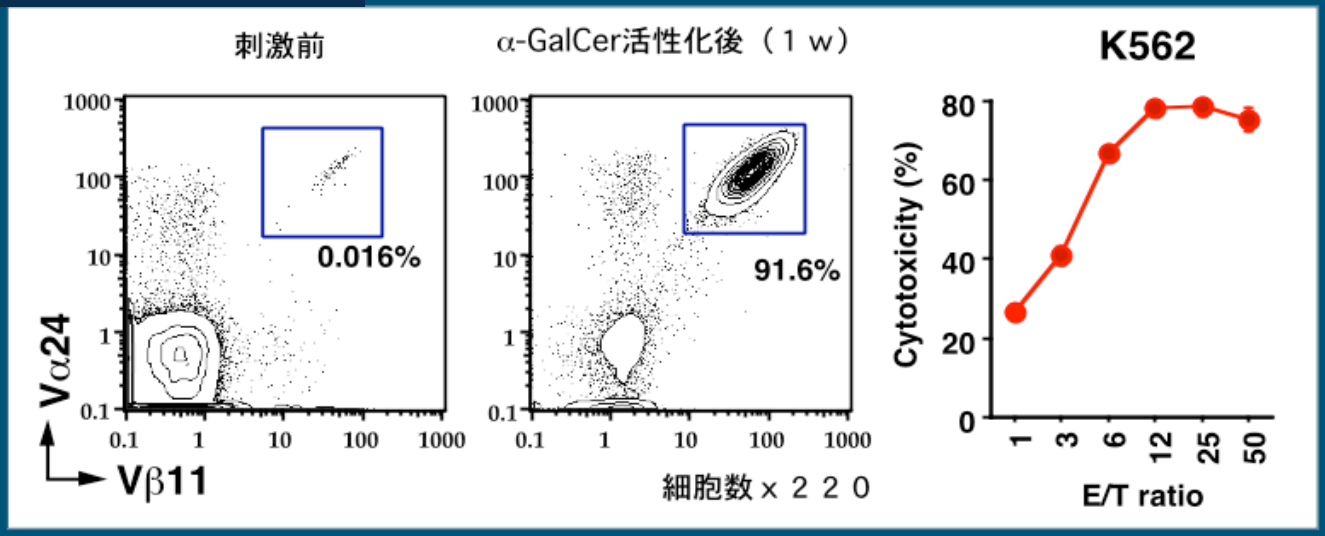
Numbers of V⟨14 NKT cells in the lung were monitored after ⟨-GalCer-pulsed DC administration. Levels of V⟨14 NKT cells were assessed by flow cytometry with an ⟨-GalCer/CD1d tetramer that detected V⟨14 NKT cells even with activated status. As shown in the chart on the left, the absolute numbers of V⟨14 NKT cells increased 3 days after ⟨-GalCer-pulsed DC injection, and increased levels were sustained for at least 1 week (right). These results suggest that endogenous V⟨14 NKT cells expanded due to treatment with ⟨-GalCer-pulsed DCs and accumulated in the lung.

Motohashi et al, Int. J. Cancer. 2002

Regression of established metastatic tumors by ⟨-GalCer- pulsed DCs.

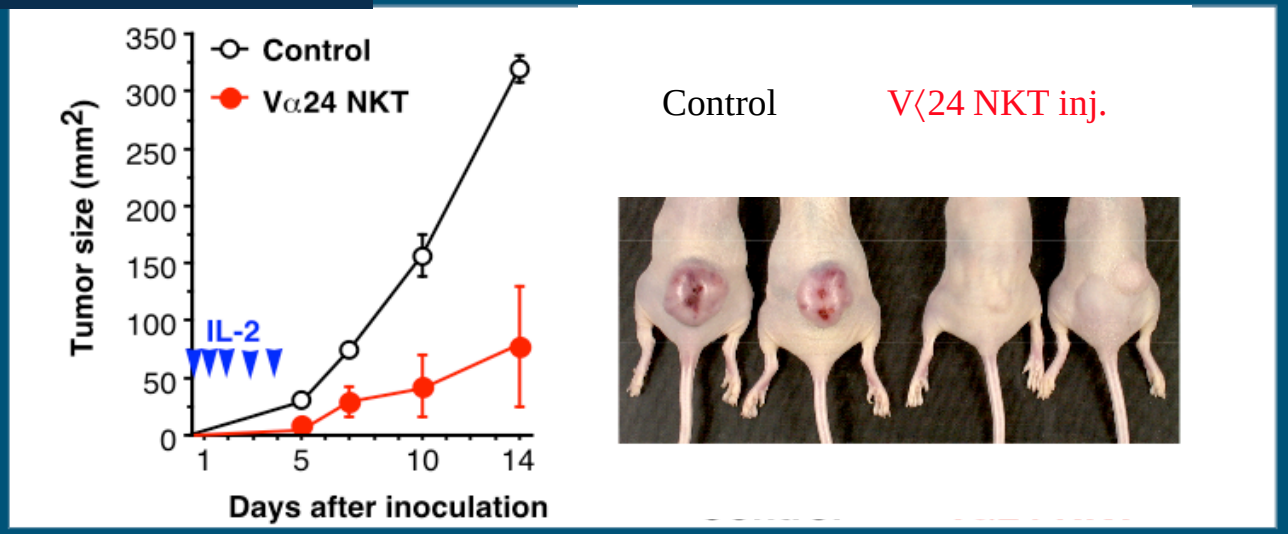
in vitro

(Kawano et al. Can. Res. 59:5102, 1999)



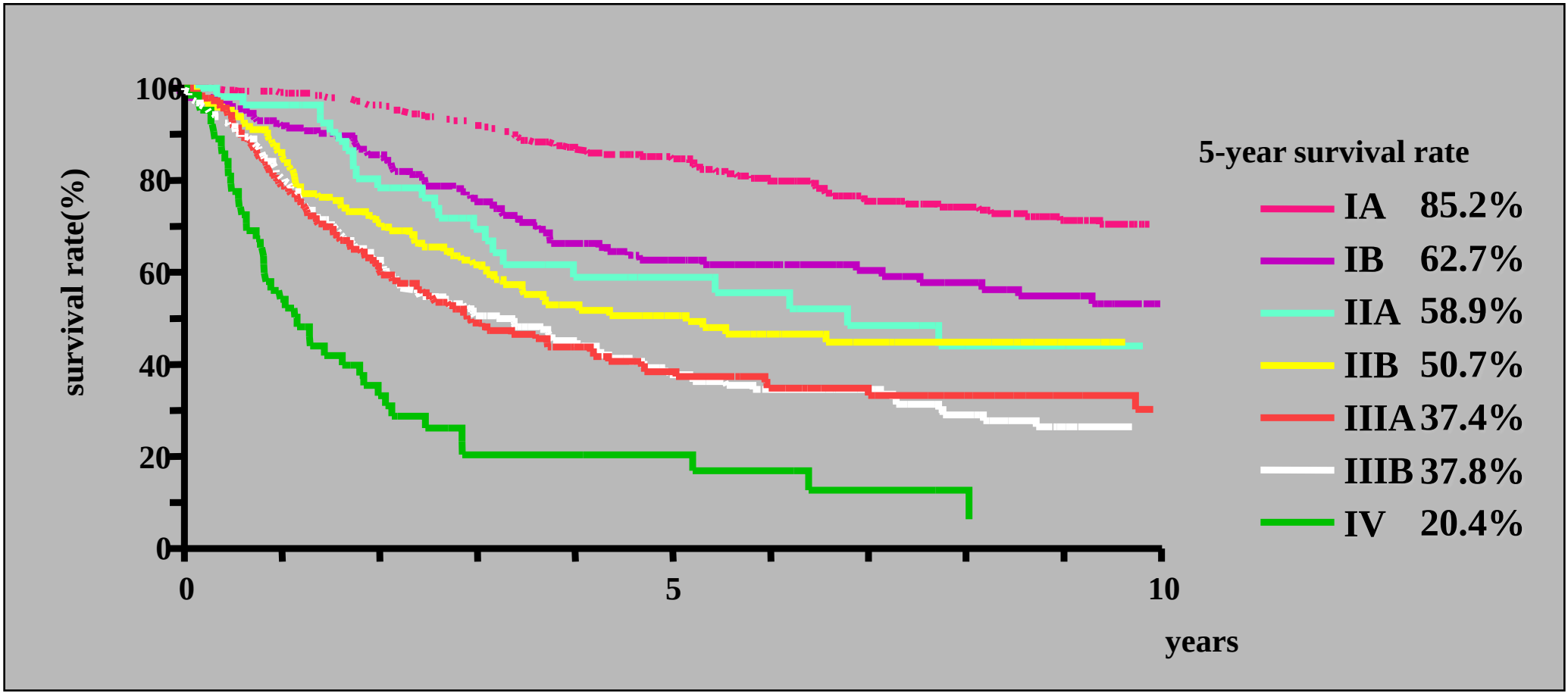
In vitro: Human V⟨24 NKT cells purified from umbilical cord blood were cultured with ⟨-GalCer and IL-2 for 7 days, and their cytotoxic activity against tumor cells was assessed. As shown in the upper chart, >91% of the cultured cells were found to express the invariant V⟨24/V®11 NKT cell antigen receptor. These cells displayed a potent cytotoxic activity against K562 (upper, right).

in vivo



In vivo: To further investigate the function of ⟨-GalCer-activated V⟨24 NKT cells, we carried out a Winn's assay. As shown in the lower chart, the tumor growth was dramatically inhibited if T.Tn tumor cells were inoculated together with activated V⟨24 NKT cells, suggesting cytotoxic activity of ⟨-GalCer-activated V⟨24 NKT cells *in vivo*.

Overall survival curve of primary lung cancer after surgery (1984 - 2004, Chiba University)



The survival curves for earlier stages show better survival than advanced stages, underscoring the importance of early detection for lung cancer. In addtion, we need effective adjuvant therapy after surgery to improve the survival rate of advanced stages.

Metastasis site after curative operation of lung cancer

Autopsy results

Brain: 18%

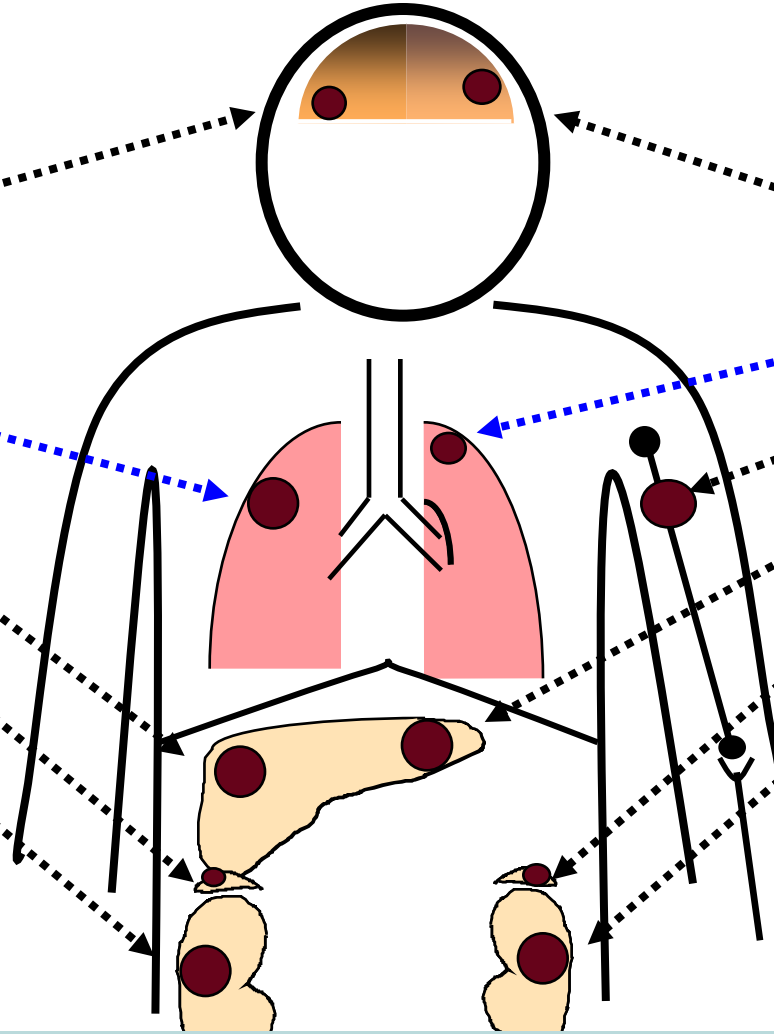
Lung: 52%

Liver: 36%

Adrenal gland: 33%

Kidney: 18%

Matthews et al.



First relapse organs during follow up

Brain: 19%

Lung: 49%

Bone: 17%

Liver: 7%

Adrenal gland: 2%

Kidney: 1%

Chiba Univ.

In patients with non small cell lung cancer, it is well known that there is a high incidence of micrometastasis in distant organs at the time of resection, even though the surgery is considered complete. In the left panel, Matthews and coworkers reported the autopsy results of patients whose lung cancer was curatively resected, but who died within 30 postoperative days. Matthews found a high incidence of micrometastasis in the lung, liver, adrenal glands and so on. We investigated the first relapse organs in patients with curative resection during the postoperative follow-up, showing a high recurrence rate in the lungs and brains similar to the autopsy results. From our research, it is clear that suppression of intrapulmonary metastasis is crucial to improve survival.

Profiles of patients with primary lung cancer and healthy volunteers

	Lung cancer	Volunteer
Total	60	20
Age +SD (mean)	51~81+7.8 (67.6)	51~85+10.7 (65.6)
Gender		
Male	43	10
Female	17	10
Histology		
Ad*	40	
Sq**	18	
Large***	2	
Clinical Stage		
Stage IA&IB	26	
Stage IIA&IIB	6	
Stage IIIA&IIIB	16	
Stage IV	3	
Recurrence	9	

The study population was comprised of 60 patients (mean age 67.6, range 51–81, years; 43 males and 17 females) and 20 healthy volunteers (mean age 65.6, range 51–85 years; 10 males and 10 females). Primary lung cancer patients were histologically diagnosed and classified into 4 clinical stages according to the UICC system. 20 patients received no treatment, including surgery, radiation therapy or chemotherapy, before collecting blood samples. Healthy volunteers had no past or present history of malignant disease and received no corticosteroid hormones.

Motohashi et al, Int. J. Cancer, 2002

Decreased NKT cells in patients with primary lung cancer

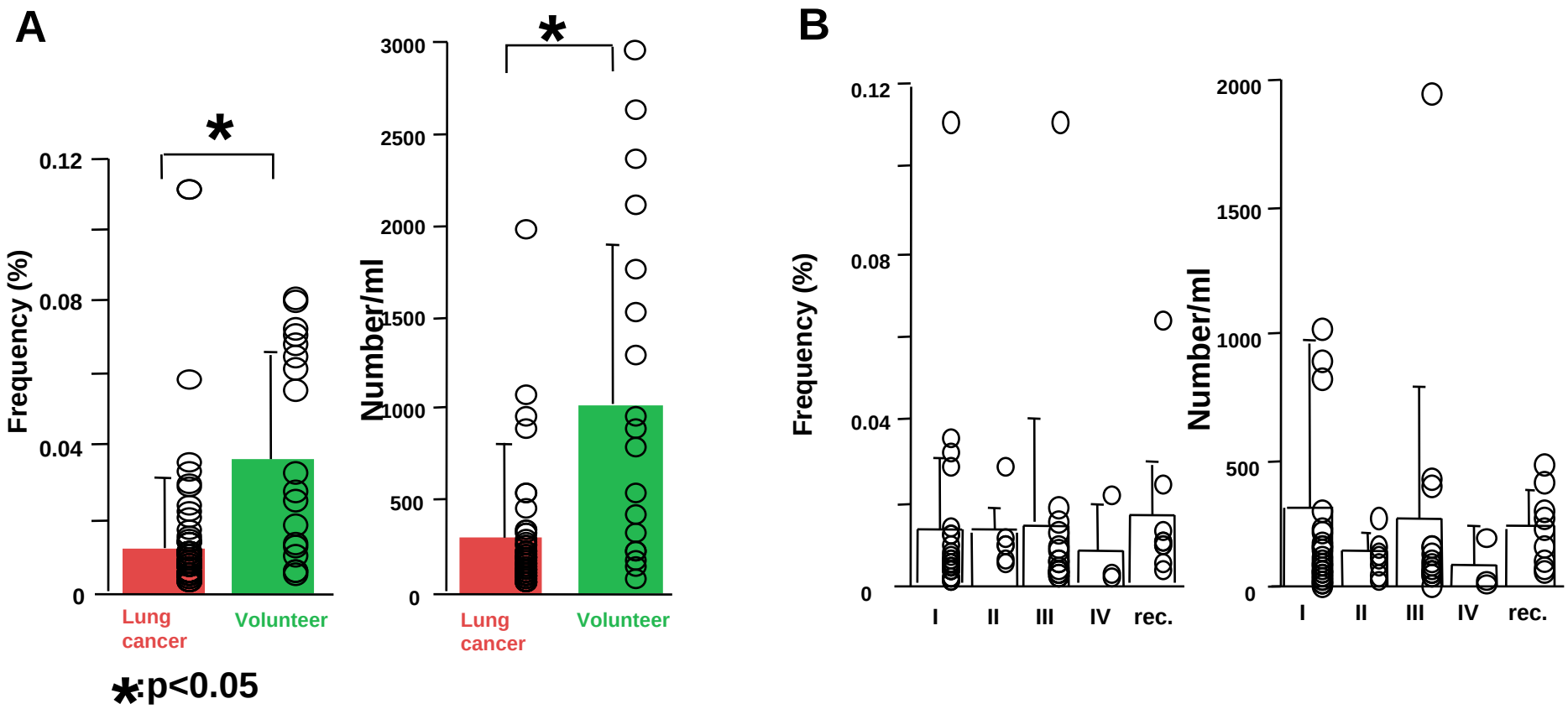


Figure A shows the mean value of percentages with distribution of V⟨24 NKT cells (V⟨24+V®11+) per total lymphocytes (left) and mean absolute numbers with distribution of V⟨24 NKT cells in 1 ml of peripheral blood (right). Numbers of peripheral blood V24⟨ NKT cells were significantly lower in cancer patients compared to healthy volunteers (p <0.0001).
Figure B shows frequencies and absolute numbers of V⟨24 NKT cells in patients of a certain clinical stage. No significant correlation between clinical stage and the number of V⟨24 NKT cells was revealed.

Motohashi et al, Int. J. Cancer. 2002

Frequency of IFN© producing V⟨24 NKT cells in primary lung cancer patients

PBMC

↓

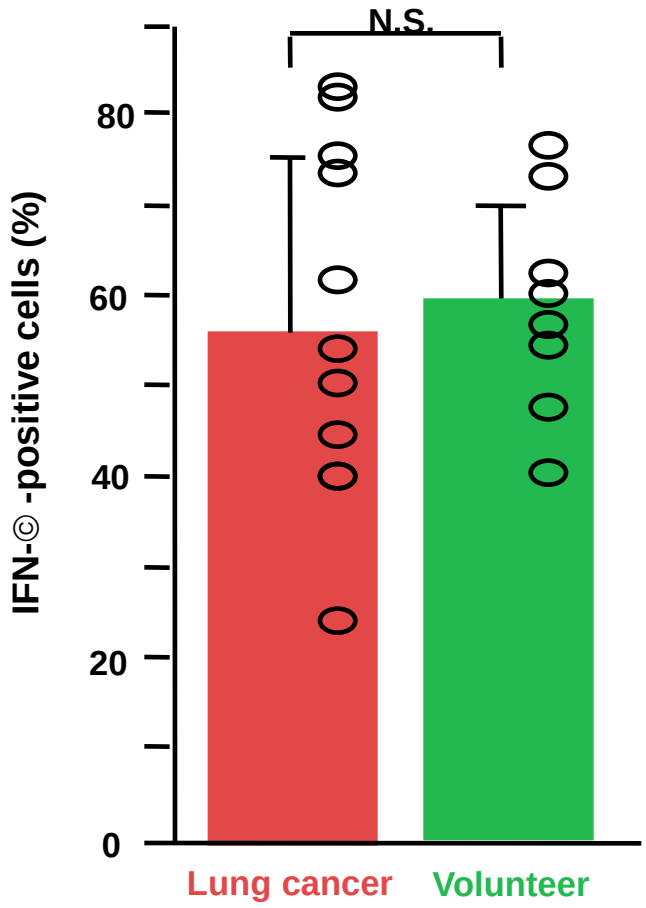
Stimulation with anti-CD3 (14 hours)

↓

Single cell sorting of V⟨24V®11 NKT cell

↓

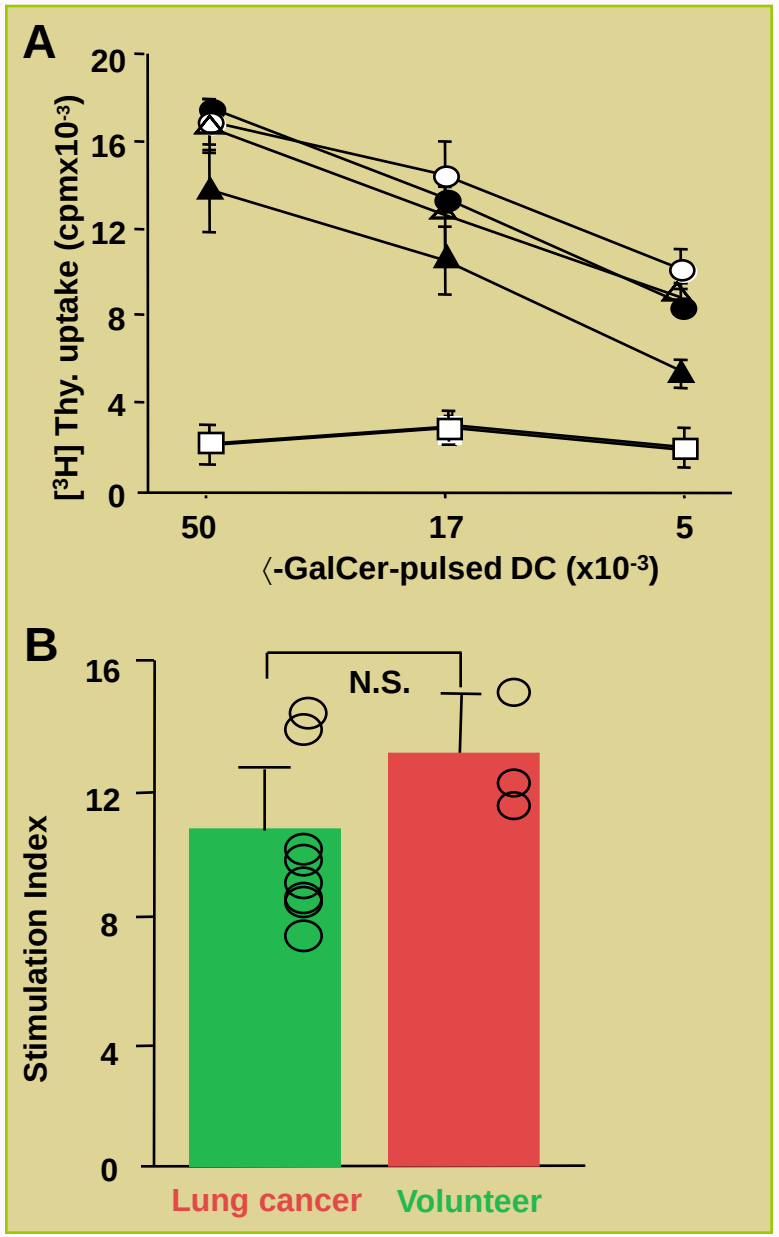
RT-PCR IFN©/V⟨24-C⟨



We carried out single-cell sorting and single-cell RT-PCR to evaluate the function of V⟨24 NKT cells in patient PBMCs. No significant difference between patients and healthy volunteers was observed (p=0.695). These results suggest that the circulating V⟨24 NKT cells in tumor-bearing patients were functionally normal in terms of IFN-© production.

Motohashi et al, Int. J. Cancer. 2002

Normal dendritic cell function of lung cancer patients

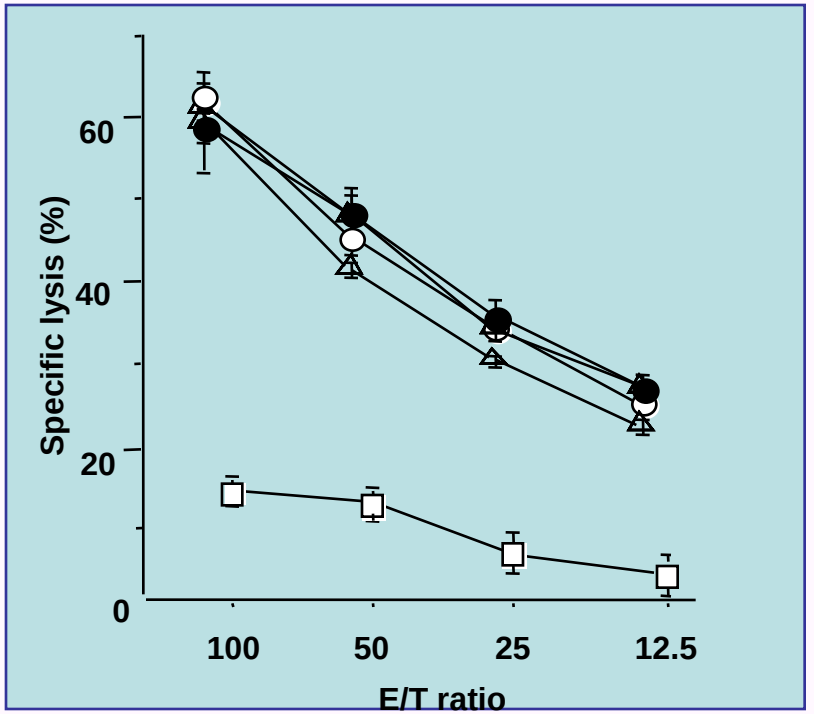


DC source

○: Patient 1
●: Patient 2
▲: Patient 3
△: healthy volunteer
□: no Ag-pulsed

responder

mouse V⟨14NKT



Assessment of DC function in lung cancer patients.

The ability of DCs to present ⟨-GalCer (Fig A, B) and the levels of cytotoxicity induced by ⟨-GalCer-pulsed DCs (Right Fig.) also showed no difference between patient and volunteer DCs.

Motohashi et al, Int. J. Cancer. 2002

Levels of NKT cells in human vs. mouse lungs

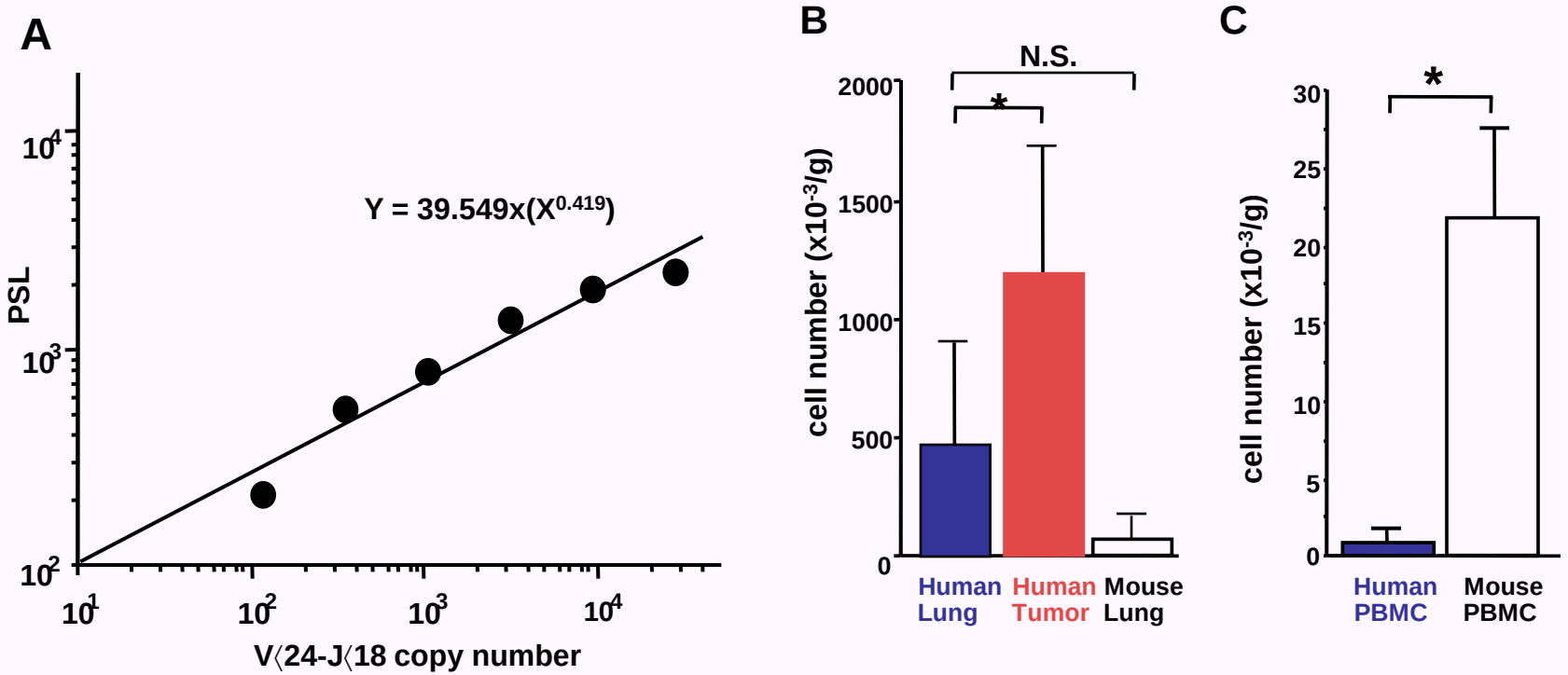
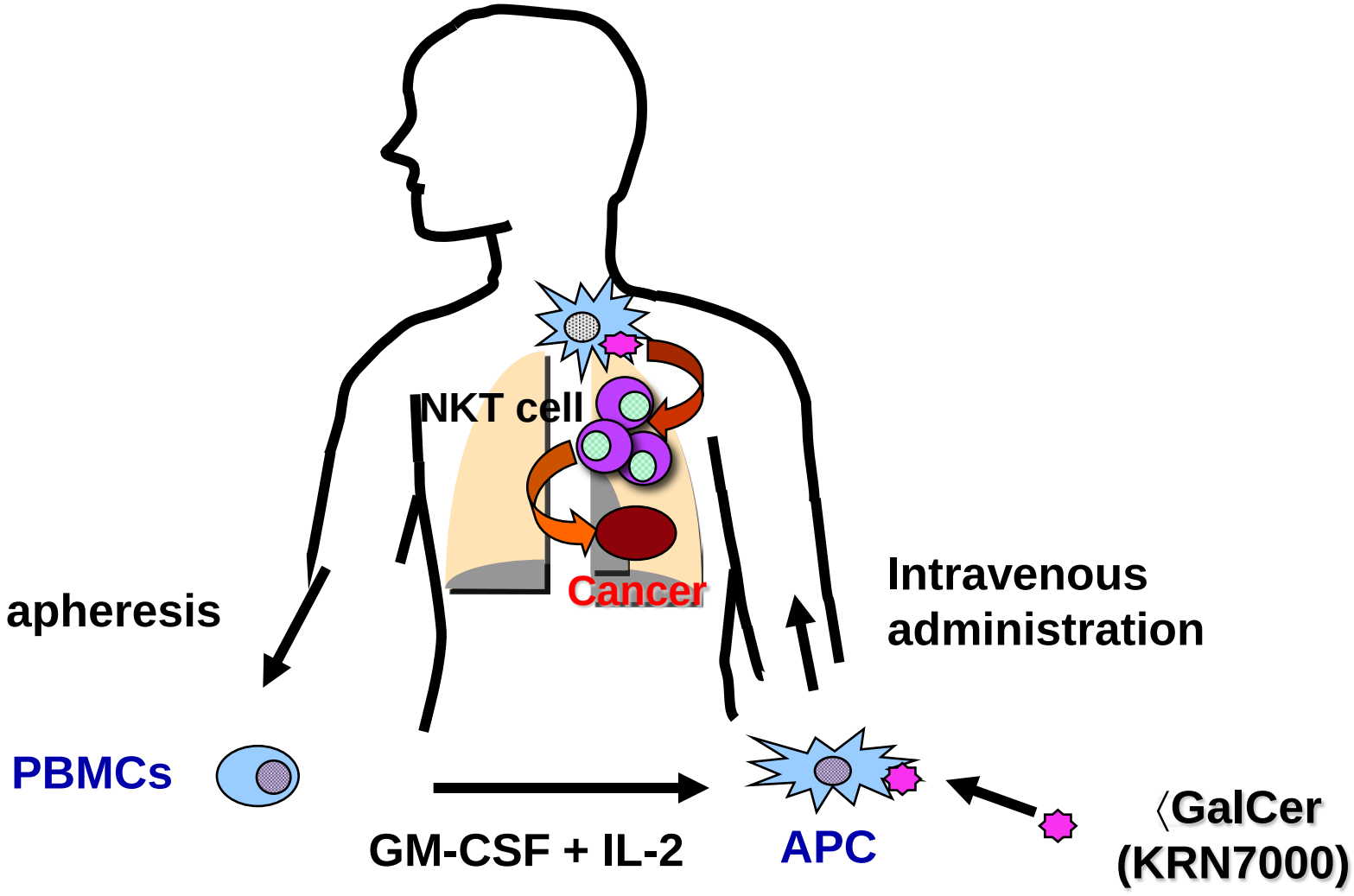


Figure A: A standardization curve of quantitative PCR was obtained with titrated amounts of standard DNA.
Figure B: Noncancerous regions of the lung obtained from patients contained about 5 x 10^5 copy numbers of V〈24-J〈Q sequence per gram. The tumor tissue itself contained increased amounts of V〈24 NKT cells at concentrations about 2.5 times that in noncancerous lung tissue.
Figure C: Concentrations of NKT cells in the mouse's peripheral blood were more than 10 times higher than in young adult human V〈24 NKT cells

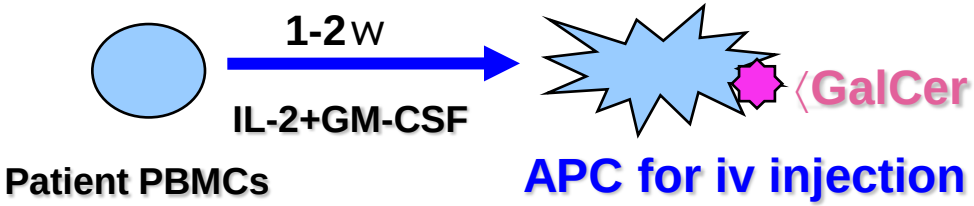
Motohashi et al, Int. J. Cancer. 2002

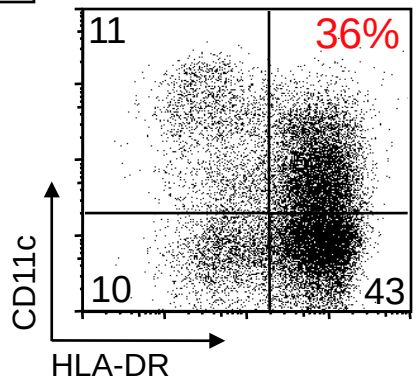
iNKT cell-based immunotherapy for lung cancer



We demonstrated that IL-2/GM-CSF-cultured PBMCs are superior to monocyte-derived dendritic cells cultured with GM-CSF and IL-4 in their ability to expand V□24 invariant NKT cells.

〈GalCer-pulsed IL-2/GMCSF cultured PBMCs



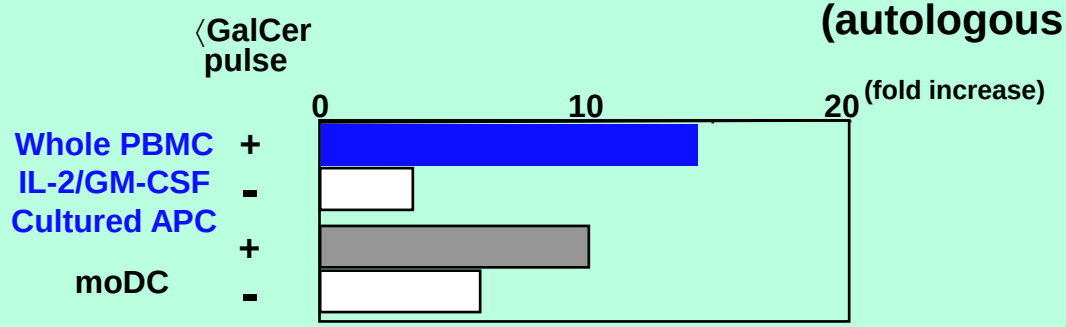


DC: HLA-DR^{+}CD11c^{+} （10−40%）

1. Large numbers of functional APCs

Apheresis: ~2x10^{9} PBMCs --> Culture ->1x10^{9}/m^{2} iv

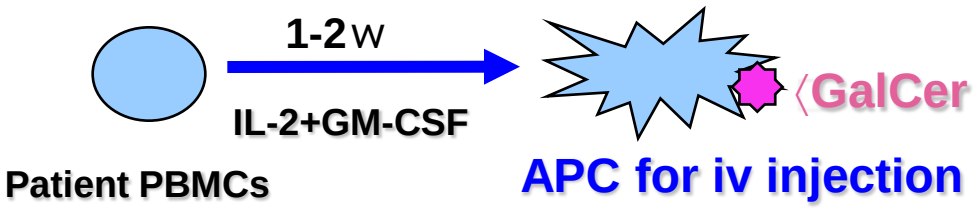
Levels of NKT cell proliferation stimulated with APCs (autologous NKT)

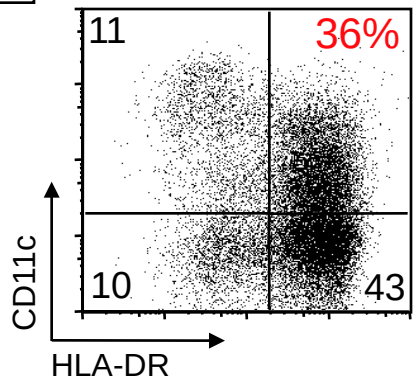


We demonstrated that IL-2/GM-CSF-cultured PBMCs were superior to monocyte-derived dendritic cells cultured with GM-CSF and IL-4 in their ability to expand V□24 invariant NKT cells.

Ishikawa et al. Int.J.Can. 2006

〈GalCer-pulsed IL-2/GMCSF cultured PBMCs

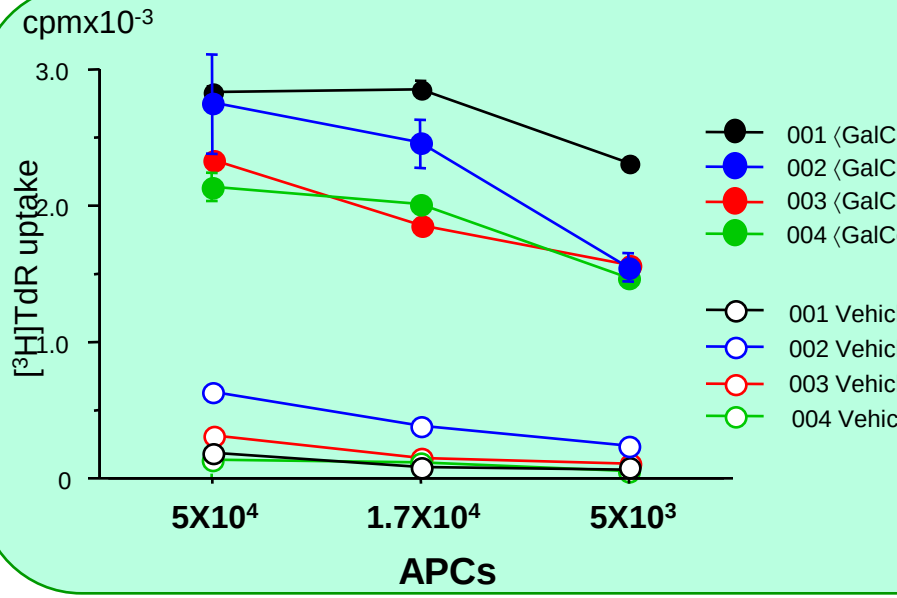


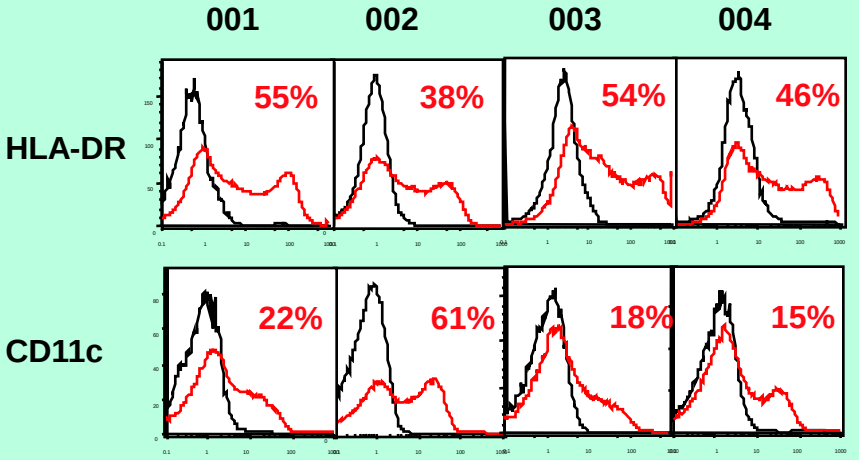


DC: HLA-DR$^+$CD11c$^+$ (10−40%)

1. Large numbers of functional APCs

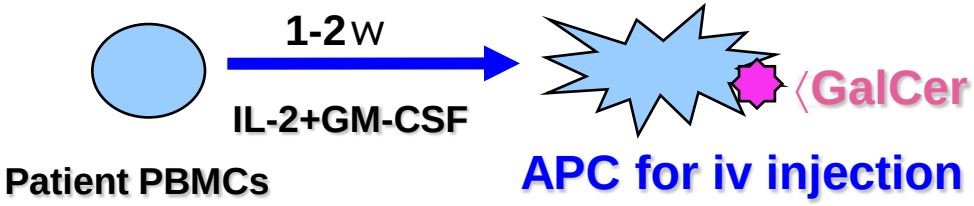
2. Small variation in function of 〈GalCer presentation

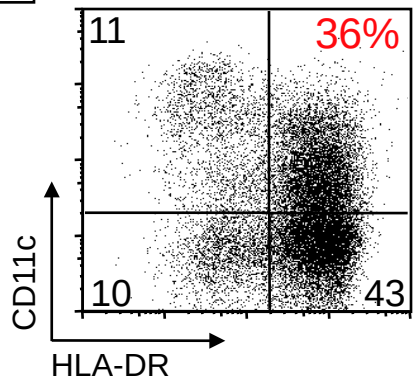




Cell proliferation was assessed by 3H-thymidine uptake.
The results clearly show that human APCs from patients proliferated in response to □-GalCer.

⟨GalCer-pulsed IL-2/GMCSF cultured PBMCs

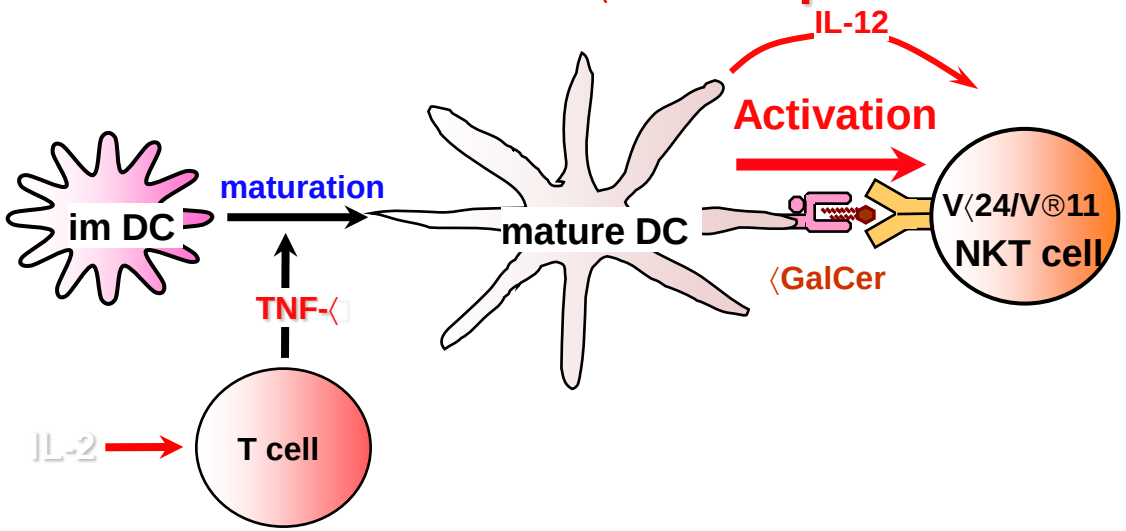




DC: HLA-DR⁺CD11c⁺ (10−40%)

1. Large numbers of functional APCs

2. Small variation in function of ⟨GalCer presentation

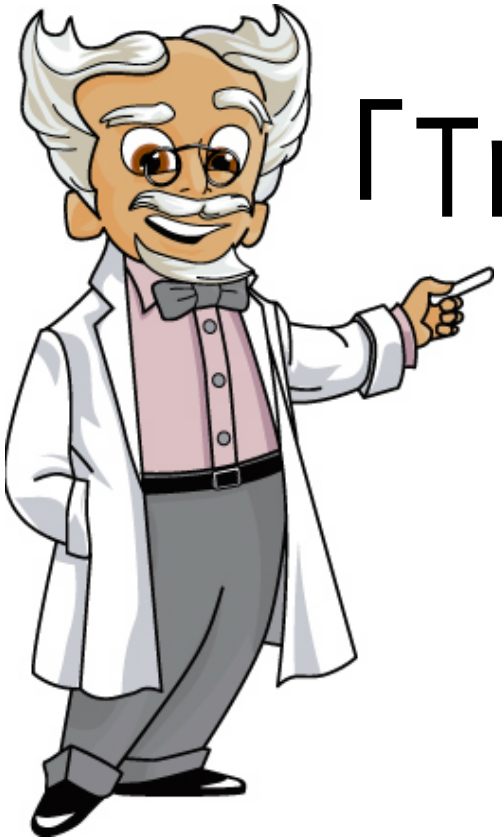


In our established APC culture system, all PBMCs were cultured with granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-2. T cells present in the PBMC culture were stimulated with IL-2 and secreted a substantial amount of tumor necrosis factor (TNF)-α, which converted immature DCs into mature DCs in vitro without any additive maturation agents.

Motohashi et al. Cancer sci. 2008

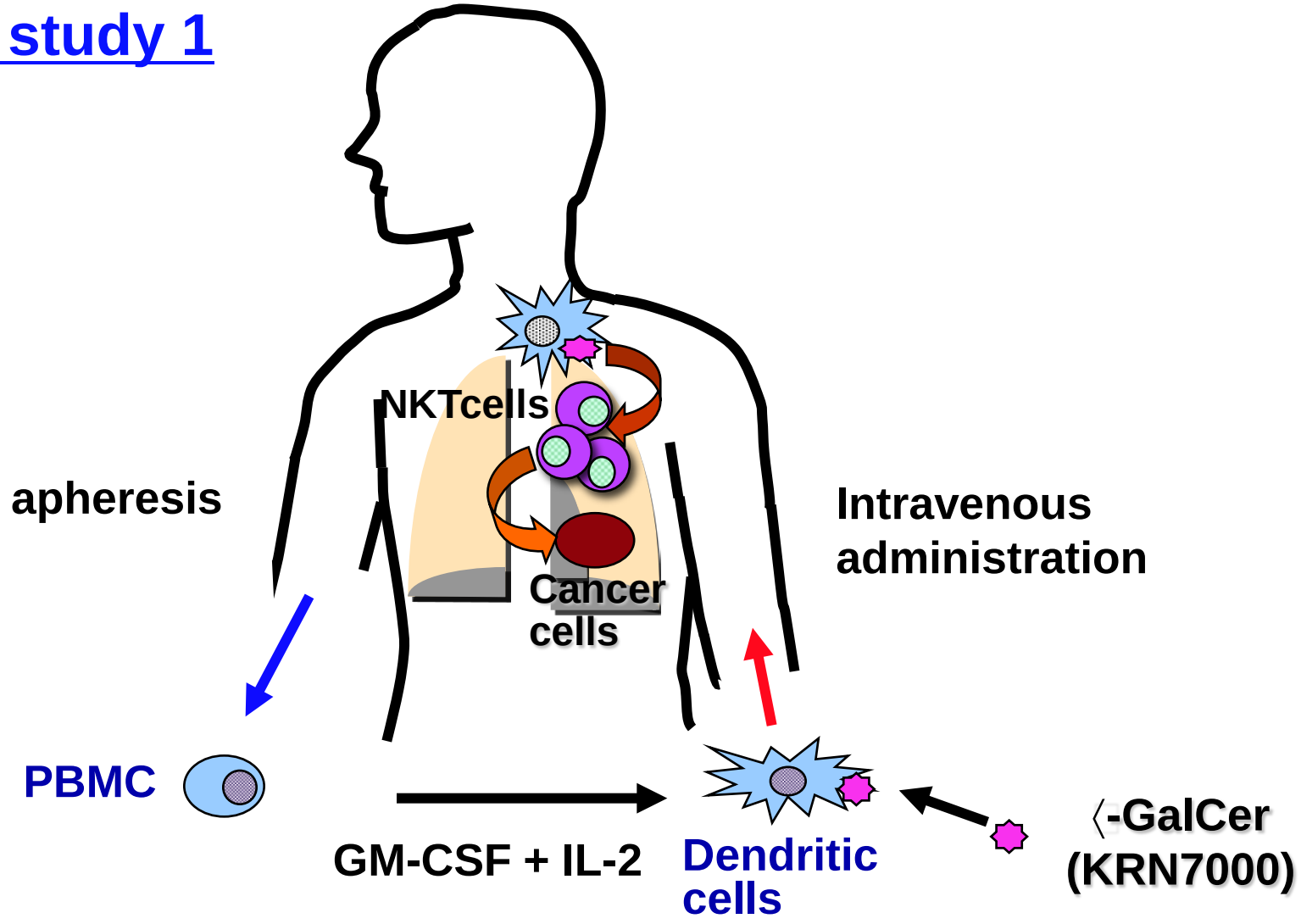
Immunotherapy Project

for Patients with Lung Cancer



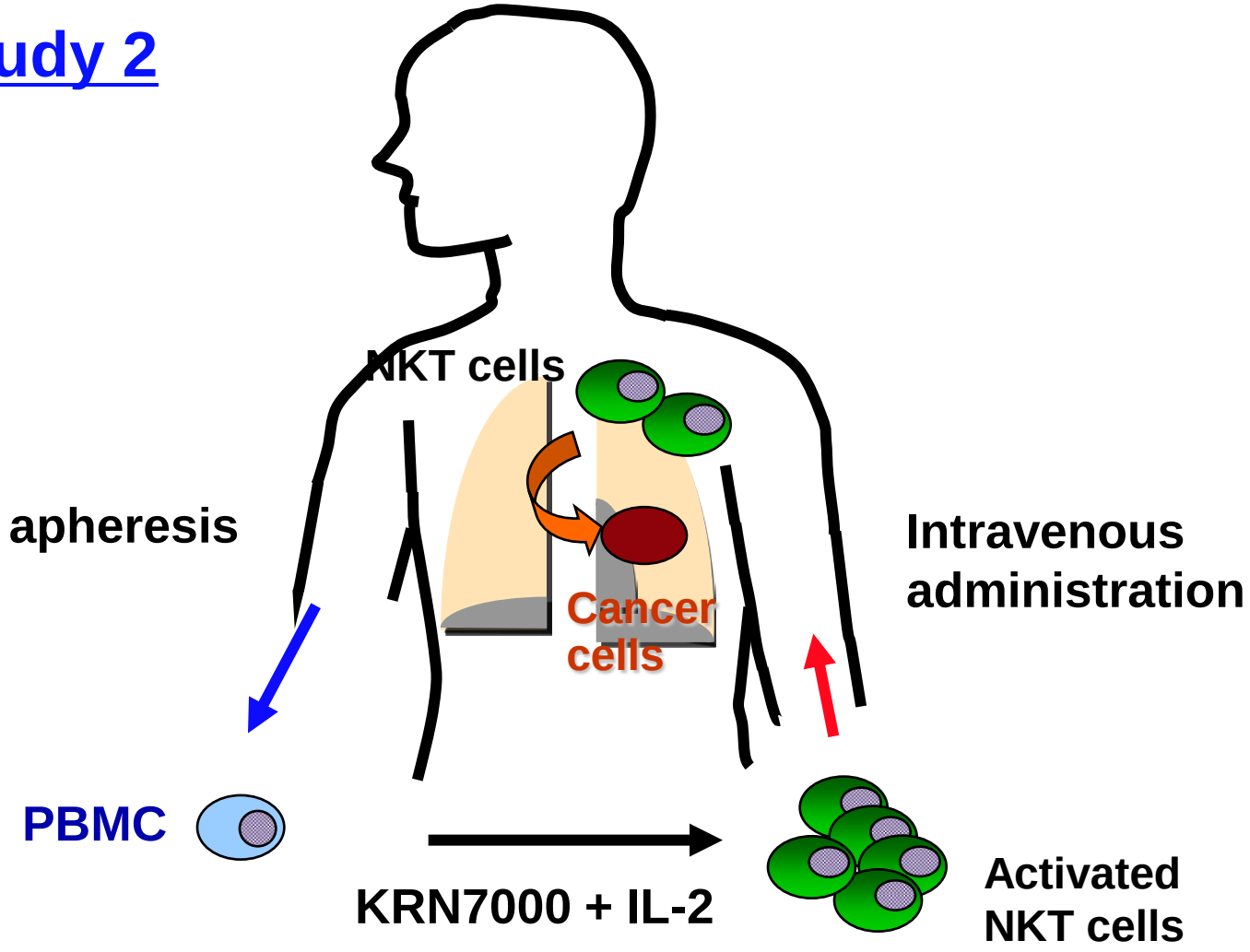
「Translational Research」

Clinical study 1



From 2001 to 2008, we performed phase I and phase I-II clinical studies on patients with lung cancer at Chiba University.
The results indicate that this cell therapy was safe, and we clearly demonstrated both NKT-specific immune responses and a correlation between the increased IFN-© production and prolonged median survival time.

Clinical study 2

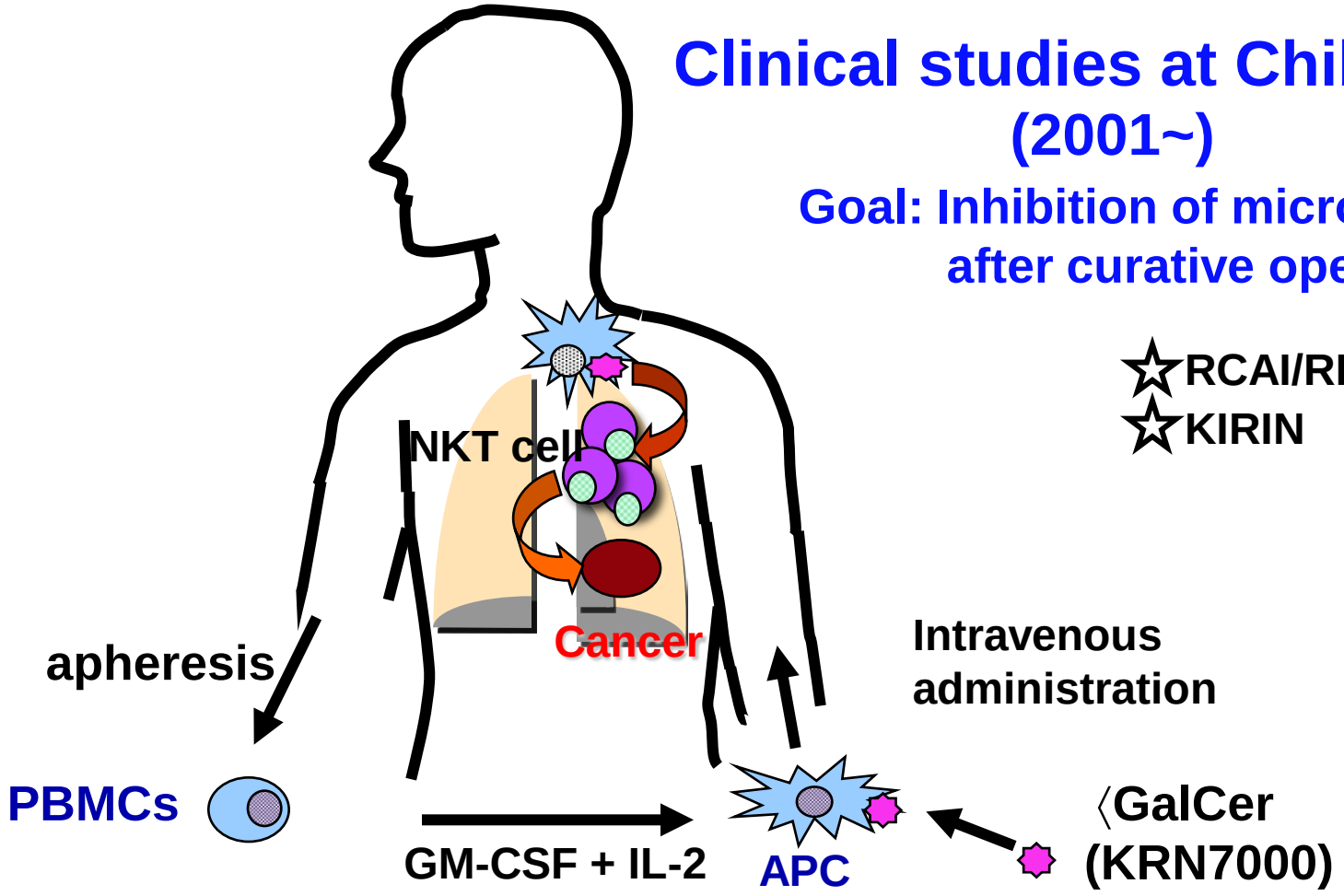


Our clinical trial with activated Vα24 NKT cell administration, which began in 2003, was well tolerated and was carried out safely with only minor adverse reactions even in patients in which the disease was quite advanced.

Clinical studies at Chiba Univ. (2001~)

Goal: Inhibition of micrometastasis after curative operation

☆RCAI/RIKEN
☆KIRIN



- Phase I study (2001-2003)
- Phase I-II study (2004-2007)
- Phase I study (2009-)

Ishikawa, et al. Clin. Cancer Res. 2005
Motohashi, et al. J Immunol. 2009
Transbronchial injection

PBMCs → IL-2+〈GalCer →

- Phase I study (2003-2006) *Motohashi, et al. Clin Can. Res. 2006*

To apply the potent anti-tumor activity of invariant NKT cells to lung cancer treatment, we started to perform a phase I clinical study in 2001.

Murine and human NKT cells are activated by a specific glycolipid Ag, α-galactosylceramide (αGalCer).

[We thank Kirin Brewery for providing clinical grade αGalCer (KRN7000) for these experiments.]

Immunomonitoring and clinical outcome

MST: 18.6 months

Case	Baseline NKT	FACS Max	ELISPOT Max	Anti-tumor effect	TTP (days)	Follow-up (months)	Outcome
5	0.004%	2.6 (d56)	19.5 (d56)	SD	252	36.6	dead
4	0.057%	6.2 (d14)	15.3 (d56)	PD	71	19.4	dead
2	0.014%	11.9 (d14)	10.8 (d56)	SD	>84	32.2	dead
13	0.009%	21.1 (d21)	5.6 (d35)	PD	63	27.6	alive
25	0.041%	0.7 (d14)	5.3 (d35)	PD	77	18.9	alive
8	0.015%	0.9 (d14)	4.9 (d21)	PD	63	16.4	dead
18	2.000%	3.2 (d14)	3.3 (d14)	PD	77	21.6	alive
10	0.240%	0.5 (d49)	2.8 (d35)	SD	260	32.7	alive
19	2.500%	1.8 (d14)	2.1 (d49)	PD	61	14.5	dead
3	0.023%	0.9 (d21)	2.0 (d56)	SD	153	18.6	dead
12	0.200%	1.0 (d49)	1.7 (d49)	SD	208	25.0	dead
17	0.039%	3.6 (d21)	1.4 (d21)	PD	84	5.1	dead
22	0.023%	1.3 (d21)	1.4 (d14)	PD	72	17.4	dead
24	0.013%	1.9 (d84)	1.3 (d42)	PD	35	13.0	dead
16	0.015%	1.0 (d14)	1.1 (d28)	PD	70	3.6	dead
23	0.026%	1.7 (d21)	1.1 (d42)	PD	72	9.6	dead
1	0.017%	1.3 (d21)	1.0 (d21)	PD	71	7.5	dead

A

B

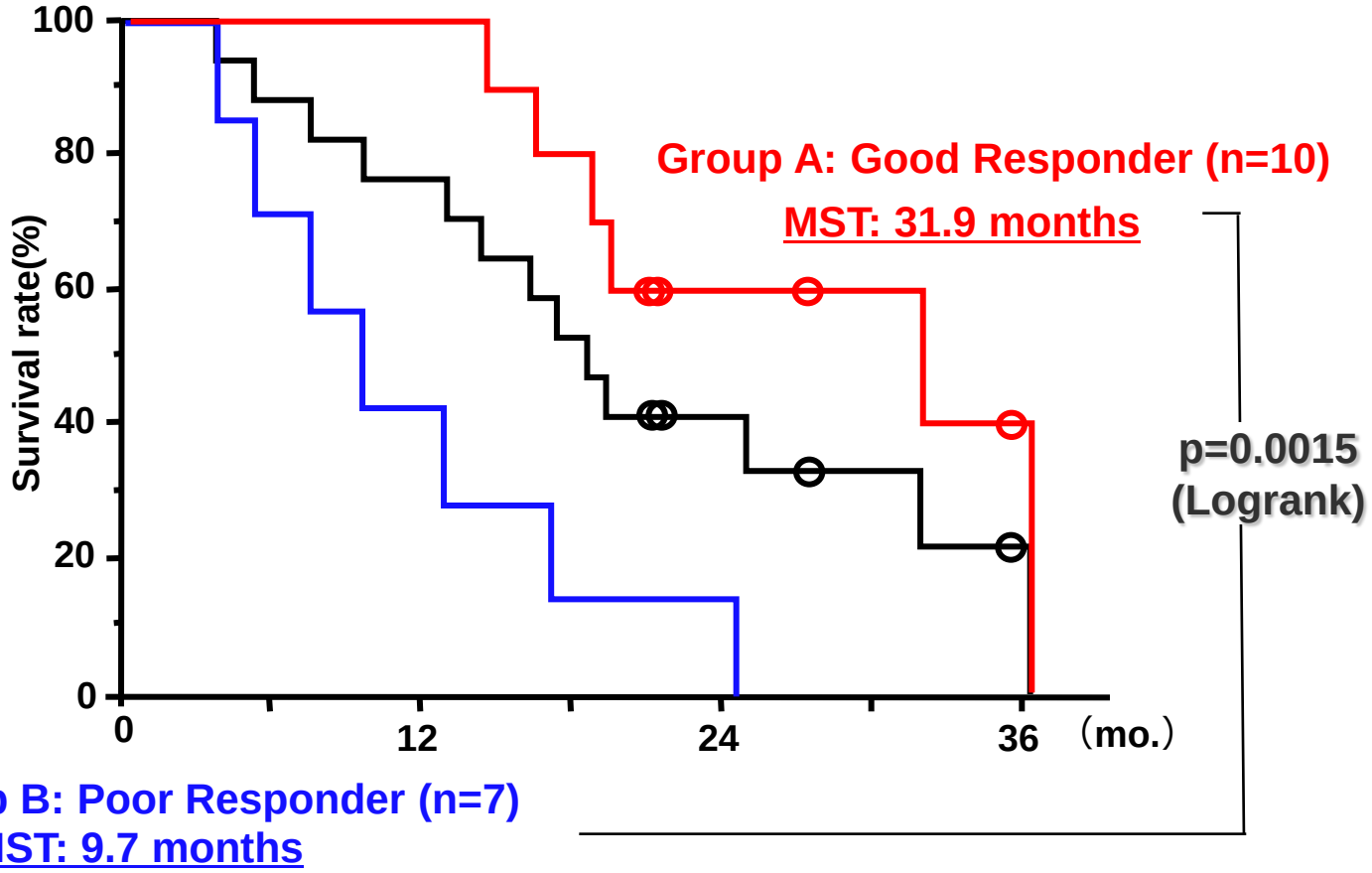
CR: complete response (no tumor) PR: partial response (~80%) SD: stable disease (80-120%) PD: progressive disease (120%~)

Increased IFN-© production correlates with better overall survival

All cases (n=17)
MST: 18.6 months
2-year survival rate: 35.4%

Group A: Good responder, IFN© spots > 2 fold
Group B: Poor responder, IFN© spots < 2 fold

Historical control
MST: 4.6 months
(c-stage; IIIB, IV, standard chemotherapy)
J. Clin. Oncol. 18:2095, 2000



MST:
median survival time

The increased IFN-γ-producing cells that resulted from αGalCer stimulation in PBMCs were significantly associated with prolonged MST. These results are encouraging and warrant further evaluation.

Motohashi, et al. J Immunol. 2009

Summary

1. ⟨GalCer-pulsed DCs administration was well tolerated and could induce NKT cell-specific immune responses.
2. Follow-up data indicated that at least 10 patients who displayed high IFN-©-producing properties after ⟨GalCer-pulsed DC treatment achieved prolonged overall survival times.
3. The augmentation of IFN-© production, which might be related to this extended survival time, was detected after treatment. We are now investigating a novel biomarker that can predict IFN-© responsiveness.

Trans-bronchial injection of ⟨GalCer-pulsed APCs in patients with advanced or recurrent non-small cell lung cancer (Phase I study)

Purpose: To activate the iNKT cells in the tumor microenvirnment more efficiently, we designed a trans-bronchial injection of ⟨GalCer-pulsed APCs using bronchoscopy.

1) Primary endpoint

- Investigate the safety profile

  (CTCAE v3.0, Common Terminology Criteria for Adverse Events)

2) Secondary endpoints

- Measure NKT cell-specific immune responses

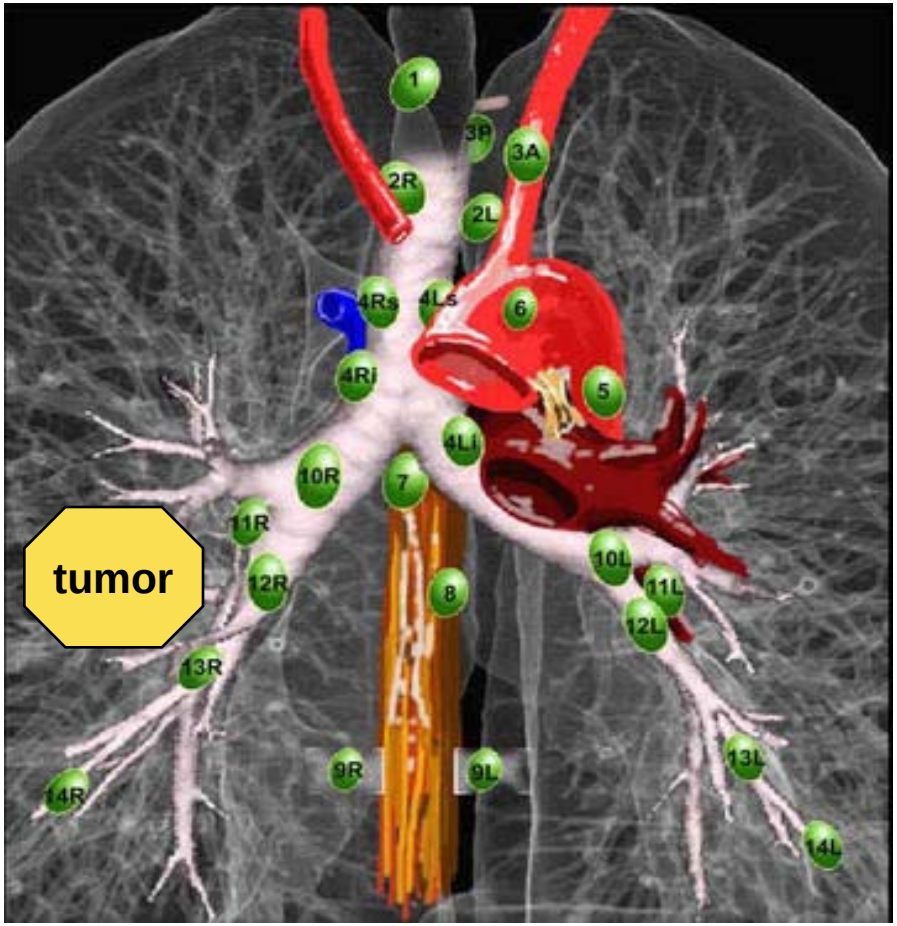
  PBMCs: FACS Analysis, ELISPOT Assay

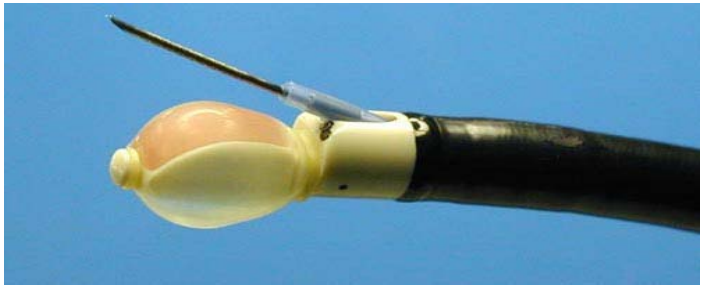
  Biopsy specimens: Real-time PCR

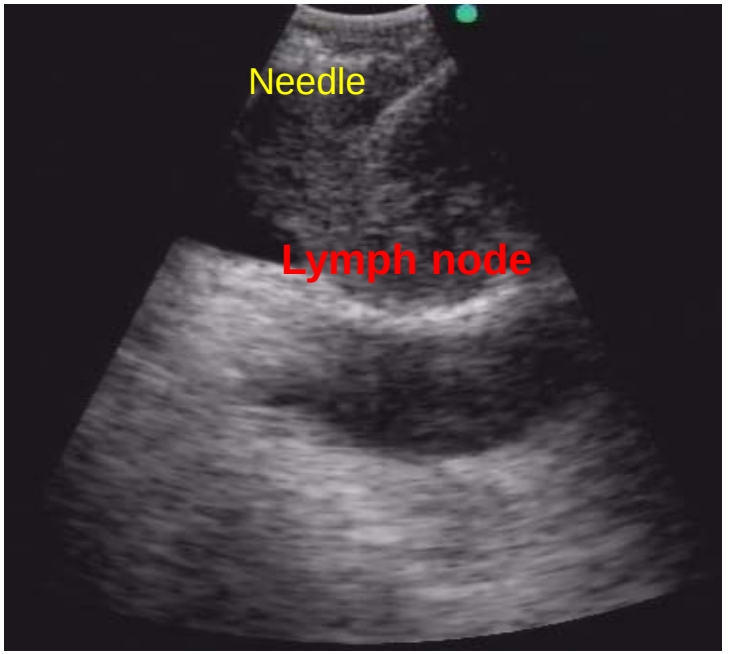
- Evaluate anti-tumor activity

  (RECIST, Response Evaluation Criteria in Solid Tumor)

Endobronchial Ultrasonography: EBUS







The image on the left shows the mapping of draining lymph nodes from lung cancer. Because it is important to evaluate lymph node metastasis from lung cancer before surgery, our department has developed a new modality: endobronchial ultrasound-guided transbronchial needle aspiration, or EBUS-TBNA. This technique is basically bronchoscopy using an ultrasonic transducer and a EBUS scan to show the lymph nodes. Using this technique, we can visualize and puncture mediastinal and hilar lymph nodes under direct EBUS guidance with minimal complications. This procedure enables injection of αGalCer-pulsed APCs into the draining lymph nodes.

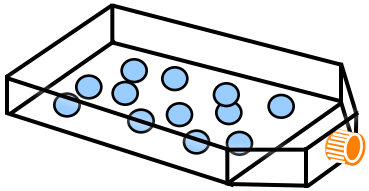
Preparation of Antigen Presenting Cells

[day 0]

Leukapheresis

Cell Processing Center

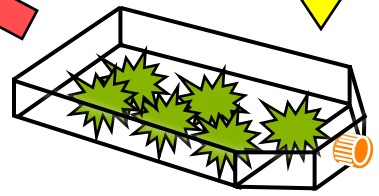
Collected whole PBMCs



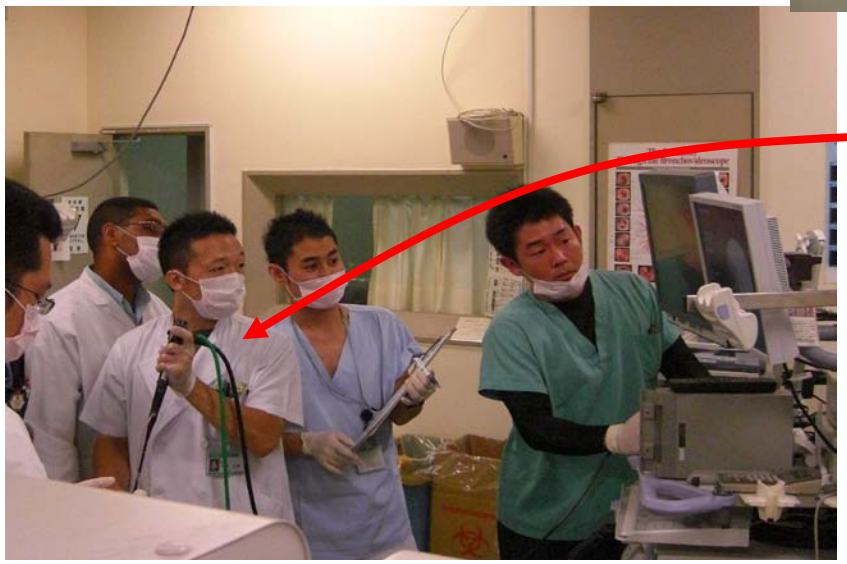
cultured with
IL-2 and *GM-CSF*
for 7 or 14 days

〈GalCer

〈GalCer-pulsed
IL-2/GM-CSF-cultured PBMCs



[day 6 or 13]



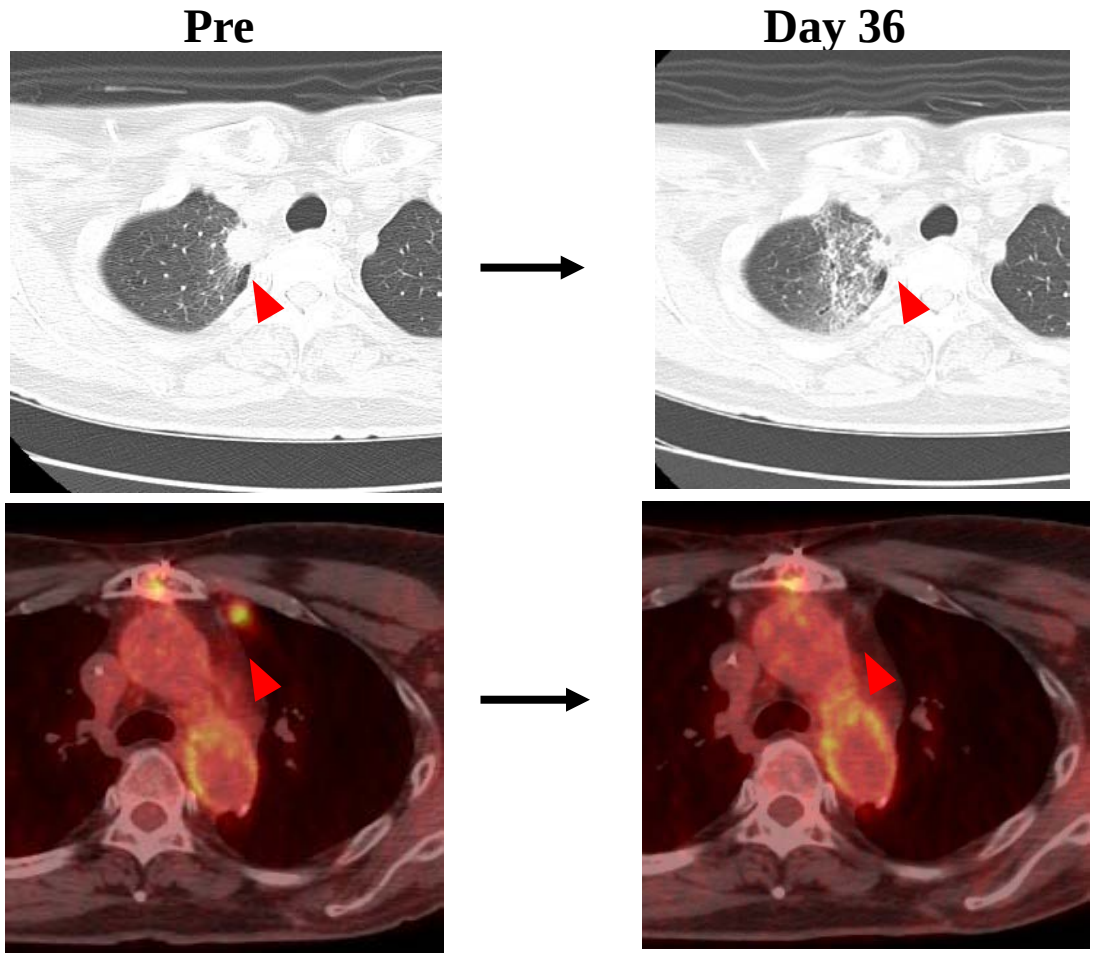
Trans-bronchial injection [day 7 or 14]

All procedures were done according to Good Manufacturing Practice standards. Eligible patients underwent peripheral blood leukapheresis and PBMCs were collected. Thereafter, whole PBMCs were cultured with IL-2 and GM-CSF. The cultured cells were pulsed with αGalCer on the day before administration. On days 9 and 16, the patients received the cultured cells via bronchoscopy.

Anti-tumor response (intra-tumoral injection case)

Case IT001: 56 y.o. male

Rt. lung cancer (Adenocarcinoma, c-stage Ⅳ)

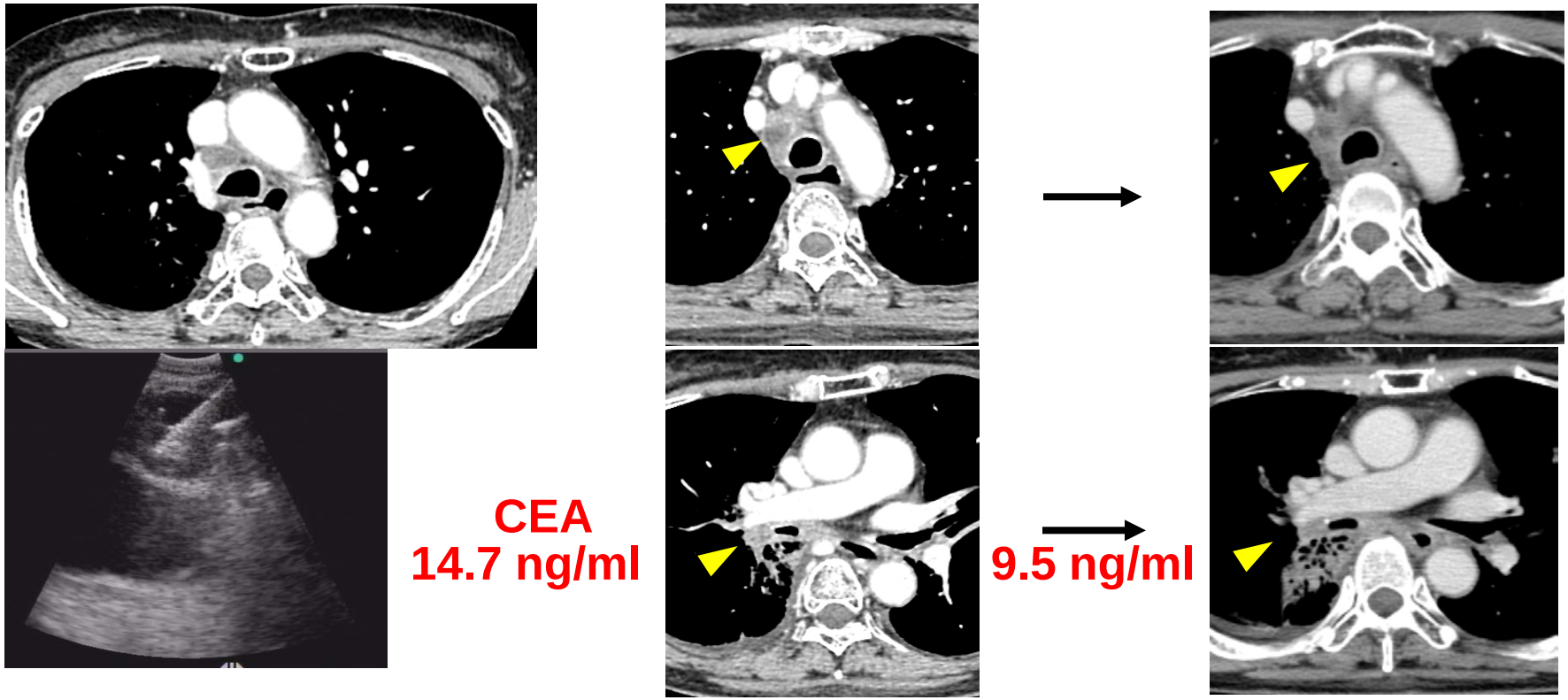


This shows a case of intratumoral injection. A 56 year-old man was diagnosed with right lung cancer, adenocarcinoma, c-stage Ⅳ. He received chemotherapy and radiation therapy, and αGaCer-pulsed APCs were injected into the primary tumor of the right lung via bronchoscopy. A chest CT revealed reduction of the tumor and a FDG-PET showed the accumulation of the para-sternal lymph node was diminished after intratumoral injection of □GalCer-pulsed APCs.

Anti-tumor response (intra-nodal injection case)

Case IN003: 61 y.o. female

Rt. lung cancer (Adenocarcinoma, c-stage ⅢB)



A 61 year-old woman was diagnosed with right lung cancer, adenocarcinoma, c-stage ⅢB. She received chemotherapy and radiation therapy, but had a recurrent of mediastinal lymph nodes. A chest CT showed mediastinal lymphadenopathy and tumor of the right hilum. Then, αGaCer-pulsed APCs were injected into the mediastinal lymph node. The left figure shows the medeastinal lymph node with a fine needle inserted. 100 million cells of αGaCer-pulsed APCs in a 2 ml suspension were injected. A chest CT revealed a slight reduction of the mediastinal lymph node and hilar tumor after this intranodal injection. The tumor marker CEA decreased from 14.7 to 9.5.

Summary

- **We performed trans-bronchial intra-nodal and intra-tumoral injection of ⟨GalCer-pulsed APCs in 11 patients (total injections: 22 times). This procedure proved to be safe and well-tolerated.**
- **Trans-bronchial injection of αGalCer-pulsed APCs can lead to systemic NKT cell-specific immune responses.**
- **With greater numbers of treatments, we will assess the correlation between immune responses and antitumor effects.**

Reference

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