Review Article

γδ T cell therapy for the treatment of non-small cell lung cancer

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Abstract: γδ T cells are attractive effector cells for cancer immunotherapy as they can secrete cytokines abundantly and exert potent cytotoxicity against a wide range of cancer cells. They comprise 1-5% of peripheral blood T cells, the majority expressing the Vγ9Vδ2 T cell receptor that recognizes phosphoantigens. Direct *in vivo* activation of Vγ9Vδ2 T cells in cancer patients as well as adoptive transfer of *ex vivo* expanded Vγ9Vδ2 T cells has been investigated in several clinical trials. We previously established a large-scale *in vitro* expansion method for Vγ9Vδ2 T cells using zoledronate and interleukin-2 (IL-2). We found that Vγ9Vδ2 T cells from patients with advanced cancer as well as from healthy donors underwent extensive proliferation under these conditions. Such cultured Vγ9Vδ2 T cells retained cytokine secretion capacity and mediated cytotoxicity against a variety of cancer cell lines. Recently, we conducted a phase I clinical study to evaluate safety and potential anti-tumor effects of re-infusing *ex vivo* expanded γδ T cells in patients with advanced or recurrent non-small-cell lung cancer (NSCLC) refractory to or intolerant of current conventional treatments. There were no severe adverse events related to the therapy. All patients remained alive during the study period with a median survival of 589 days and median progression-free survival of 126 days. Six patients had stable disease (SD), whereas the remaining six evaluable patients experienced progressive disease (PD) four weeks after the sixth transfer. We conclude that adoptive transfer of zoledronate-expanded γδ T cells is safe and feasible in patients with NSCLC, refractory to other treatments.

Keywords: Non-small-cell lung cancer (NSCLC); cell therapy; γδ T cells; adoptive transfer

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide; more than one million people die every year (1). Non-small-cell lung cancer (NSCLC) accounts for approximately 85% of all cases and most patients with NSCLC are diagnosed at an advanced stage and have a poor prognosis, with a 5-year survival rate of <5%. Conventional treatment for this disease consists of surgery, radiotherapy, chemotherapy, and multimodality therapies. The patient's cancer staging, histology, and tolerance including performance status and comorbidities used to determine the indication for the treatment. Recently, treatment decisions for NSCLC are driven by their tumour genotype or phenotype, such as mutations in epidermal growth factor receptor (*EGFR*) and the fusion oncogene *EML4-ALK* (2). Bevacizumab, a monoclonal antibody that binds to vascular endothelial growth factor-A, erlotinib and gefitinib, small molecule tyrosine kinase inhibitors (TKIs) that inhibit EGFR, and crizotinib, a TKI that inhibits EML4-ALK are widely used for the treatment. So-called "immune checkpoint blockade" T-cell modulating agents, such as antibodies against cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed death 1 (PD-1) and PD-L1, are currently being investigated. Despite the introduction of these new treatment modalities, outcomes remain poor, requiring for new treatment approaches. Active immunotherapy such as adoptive T cell-transfer represents one promising approach for lung cancer therapy (3). Growing body of evidence suggests that γδ T cells are

Table 1 Lymphocytes for immunotherapy

	αβ T cell	γδ T cell	NKT cell
PBMC (%)	65-75	1-5	<1
Distribution	Blood, lymphoid organ	Blood, epithelium, lymphoid organ	Blood, bone marrow, liver, lung
Cell surface molecules	αβ TCR	γδ TCR	Invariant TCR (Vα24, Vβ11)
	CD3	CD3	CD3
	CD4/CD8	$CD4^-CD8^-$/$CD8\alpha\alpha^+$	CD4/CD8
		NKG2D	NK receptors
Antigen	MHC/peptide complex	IPP, ApppI, MICA/B	CD1d/glycolipid
MHC restriction	Yes	No	No
TCR diversity	Very diverse	Relatively restricted, expression variance dictated by tissue localization	Restricted
Cytotoxicity	Yes	Yes	Yes
Function	Adaptive immunity	Immune regulation, surveillance, homeostasis	Immune regulation

Abbreviations: TCR, T cell receptor; MICA/B, MHC class I-related molecules A and B; IPP, isopentenyl pyrophosphate; PMBC, peripheral blood mononuclear cell.

attractive candidates for anticancer immunotherapy. This review discusses recent advances in basic γδ T cell research and data from clinical trials on the use of γδ T cells in the treatment of lung cancers.

γδ T cell

While most human peripheral blood T lymphocytes express αβ T cell receptor (TCR) (αβ T cell), 1-5% of peripheral blood T cells express γδ TCR (γδ T cell) (4) and <1% express invariant TCR (Vα24, Vβ11) (NKT cell). Major differences between these three lymphocytes are summarized in *Table 1*. γδ T cells like αβ T cell are derived from bone marrow derived precursor cells; αβ/γδ lineage commitment occurs during thymocyte development. The transcriptome analysis of γδ T cells demonstrated that the gene signature of γδ T cells is a hybrid of those from αβ T and NK cells, with more 'NK cell' genes than αβ T cells have and more 'T cell' genes than NK cells (5).

γδ T cells fulfill a role of rapid lymphoid stress-surveillance system to stress-induced tissue perturbation (*Figure 1A*). The lymphoid stress-surveillance response is initiated by many forms of surface expression of stress-induced ligands for γδ TCR or NKG2D and provides an immediate source of cytokines, chemokines, and other factors that can substantially affect adaptive immunity (6). There are two main subsets of γδ T cells in humans: one expressing the TCR variable regions Vγ9 and Vδ2, represents the majority of peripheral-blood γδ T lymphocytes; the second subset of Vδ1 T cells is resident mainly within epithelia, where these cells serve as a first line of defense against infections or malignancies (7). During the intrathymic development, γδ T cell precursors express transcription factors, such as PLZF1, T-bet, Eomes, RORγt and SOX13. Distinct expression of transcription factors in γδ T cell subsets that bear particular TCR V regions determine the acquisition of discrete sets of homing receptors for particular peripheral tissues and effector functions with restricted expression patterns for cytokines (*Figure 1B*) (8).

For lymphocytes to be suitable for stress surveillance, they express various receptors and recognize a spectrum of molecules signifying cellular dysregulation (*Figure 2*) (6,9). Historically, γδ T cells were shown to display a unique reactivity to mycobacteria rather than major histocompatibility complex (MHC) class I/peptide complex (10). It is now well known that low molecular weight phosphoantigens, such as isopentenyl pyrophosphate (IPP), are the ligands for Vγ9Vδ2 TCR (11). IPP is an intermediate metabolite of mevalonate/cholesterol pathway in mammalian cells. Though microbial IPP level may not reach the minimum required for γδ T cell activation and thus do not explain Vγ9Vδ2 T cell responses to infection, an intermediate of the alternative, nonmevalonate pathway

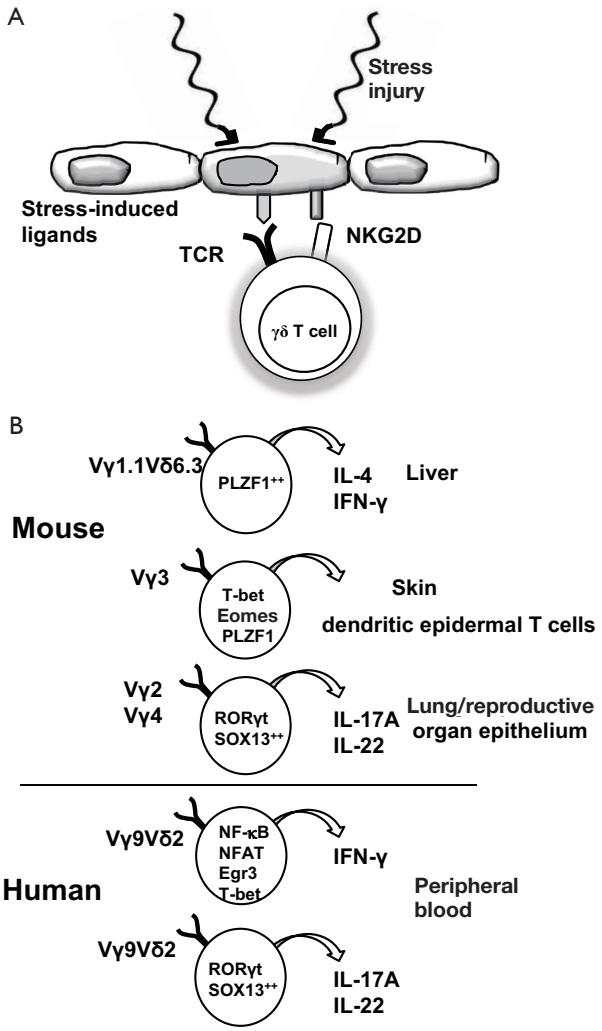

Figure 1 Lymphoid stress surveillance (A) and Tissue resident γδ T cells (B). (A) Injury or stress that upregulate surface expression of ligands for γδ TCR or for NKG2D initiate the lymphoid stress-surveillance response; (B) The intrathymic developmental programming determines the tissue distribution of γδ T cells. The emerging γδ thymocyte subsets with distinct transcriptional modules bear particular TCR V regions, which control the acquisition of discrete sets of HRs and effector functions. Abbreviations: TCR, T cell receptor; HR, homing receptor.

of isoprenoid biosynthesis, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP), has 10,000 times more effective bioactivity to Vγ9Vδ2 T cells than IPP (12). Stress-induced MHC class I-related molecules A and B (MICA/B) as well as UL16-binding proteins (ULBP) 1-4 and Retinoic acid early transcript 1 (RAET1) are recognized by NKG2D and unique products of common pathogens are recognized by TLRs. The cells can then display several types of function appropriate to different types of stress, directed against microbial infection or tumor cells. As immediate effector cells, γδ T cells secrete cytokines (IFN-γ, IL-17, IL-5, IL-13, IL-10, IL-4, LT-β) and chemokines (MIP-1α/β, RANTES) and exert cytotoxicity and antibody dependent cellular cytotoxicity (ADCC) upon pleiotropic antigen recognition. Therefore, γδ T cells are implicated in the first line of the defense against pathogens and have anti-tumor activity against cancers. Especially, robust cytotoxicity and IFN-γ and TNF-α secretion contribute to anti-tumor immunity.

Tumor cell recognition by Vγ9Vδ2 T cells

Tumor surveillance is a key component of lymphoid stress surveillance; Transformation-induced changes are efficiently recognized by γδ T cells that may result in the enhance tumor immunogenicity. It has been reported that mutant *p53*, which is present in more than half of all human cancers, can significantly upregulate mevalonate pathway activity in cancer cells, which contributes to the γδ T cell recognition of tumor cells (13). Therefore, IPP and its isomer dimethylallyl pyrophosphate (DMAPP) accumulate in the tumor cells and are recognized by γδ TCR (14). Aminobisphosphonates, which are used to treat osteoporosis and metastatic bone disease of malignant tumors such as multiple myeloma, breast and prostate cancer, inhibit the farensyl pyrophospate (FPP) synthase, that mediates the conversion of IPP and DMAPP to FPP, leading to intracellular accumulation of upstream metabolites, including IPP and DMAPP (*Figure 2*) (9). Therefore, bisphosphonate sensitize tumor cells for the efficient recognition by γδ T cells. Recently, another metabolite, triphosphoric acid 1-adenosin-5'-yl ester 3-(3-methylbu-3-enyl) ester (ApppI) that is synthesized from IPP and ATP is considered as a natural activator of Vγ9Vδ2 T cells (15). Of note, ApppI is readily detectable in tumor cells such as Daudi cells without aminobisphosphonate treatment and may thus represent a natural ligand of γδ T cells. Moreover, Vγ9Vδ2 TCRs interact with F1-ATPase expressed at the tumor cell surface; MICA and MICB as well as ULBP 1-4 expressed by different types of epithelial tumor cells are recognized by γδ T cells through NKG2D receptors in a MHC unrestricted manner (16). Thus, γδ T cells can directly recognize molecules that are expressed on cancer cells without need of antigen processing

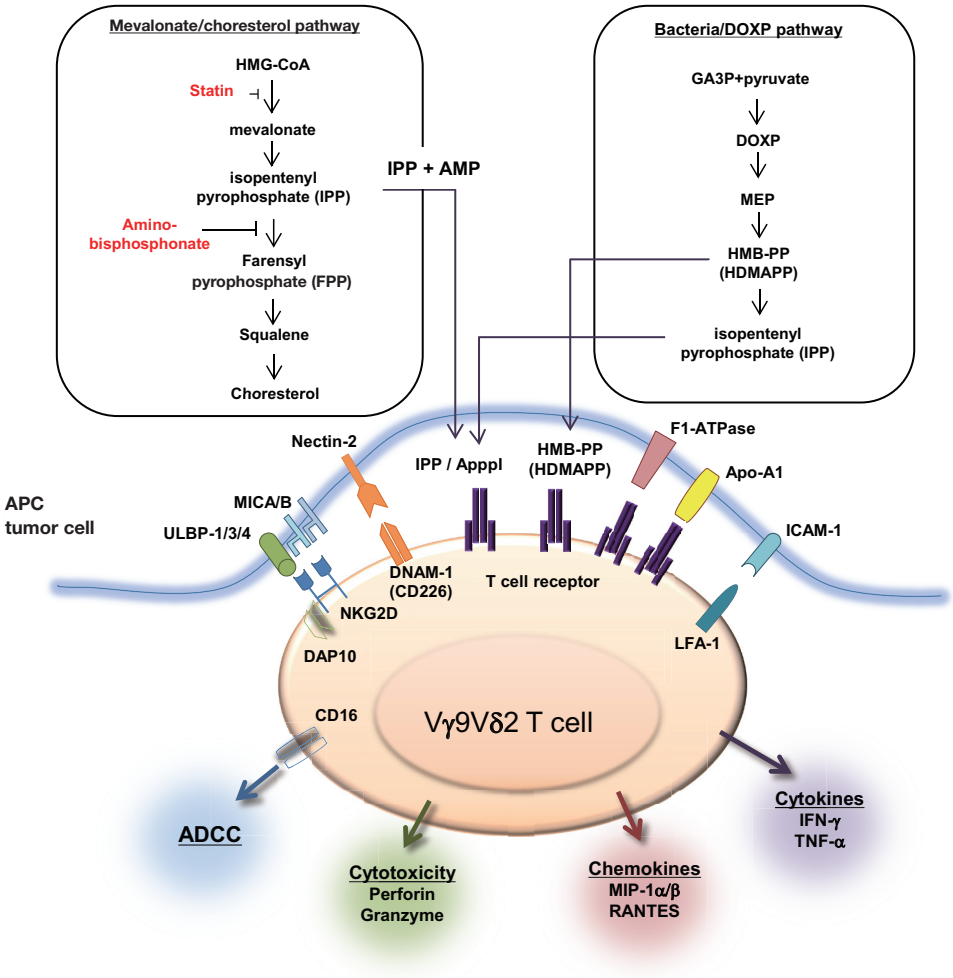

Figure 2 Tumor cell ligands recognized by human γδ T cells. Left panel, IPP is an intermediate metabolite produced through the mevalonate/cholesterol production pathway in mammalian cells. Pharmacological agents that can block upstream (statins) or downstream (aminobisphosphonate) this pathway lead to decreased or increased intracellular IPP levels, respectively. Endogenous IPP accumulation is observed in diverse tumor cells; IPP metabolites can be converted into ApppI, which could then be presented at the cell surface with much higher affinity to γδ TCR than IPP. Right panel, in pathogen-infected cells (e.g., mycobacterial infection), bacterial HMB-PP, or HDMAPP produced through the DOXP pathway could be presented. Abbreviations: IPP, isopentenyl pyrophosphate; TCR, T cell receptor; HMB-PP, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; HDMAPP, 4-hydroxy-3-dimethylallyl pyrophosphate.

and presentation, suggesting that they may exert anti-tumor effects even on target cells with reduced or absent expression of MHC class I molecules. These results imply the potential application of γδ T cell-mediated immunotherapy for the treatment of advanced cancers.

Immunotherapy with *in vivo* activation of γδ T cells

As shown in *Figure 3*, two strategies could be developed to apply the anti-tumor activity of γδ T cells to cancer immunotherapy: *in vivo* administration of compounds that activate γδ T cells or adoptive transfer of *ex vivo* expanded γδ T cells (17). Fever observed in patients under bisphosphonate treatment make us aware that bisphosphonate activated γδ T cells in peripheral blood mononuclear cells (PBMCs). Kunzmann *et al.* reported four of ten patients given pamidronate for increased bone resorption had a substantial increase in the percentage of γδ T cells in their PBMCs (18). Since then, immunotherapy seeking to exploit γδ T cells to destroy malignant cells

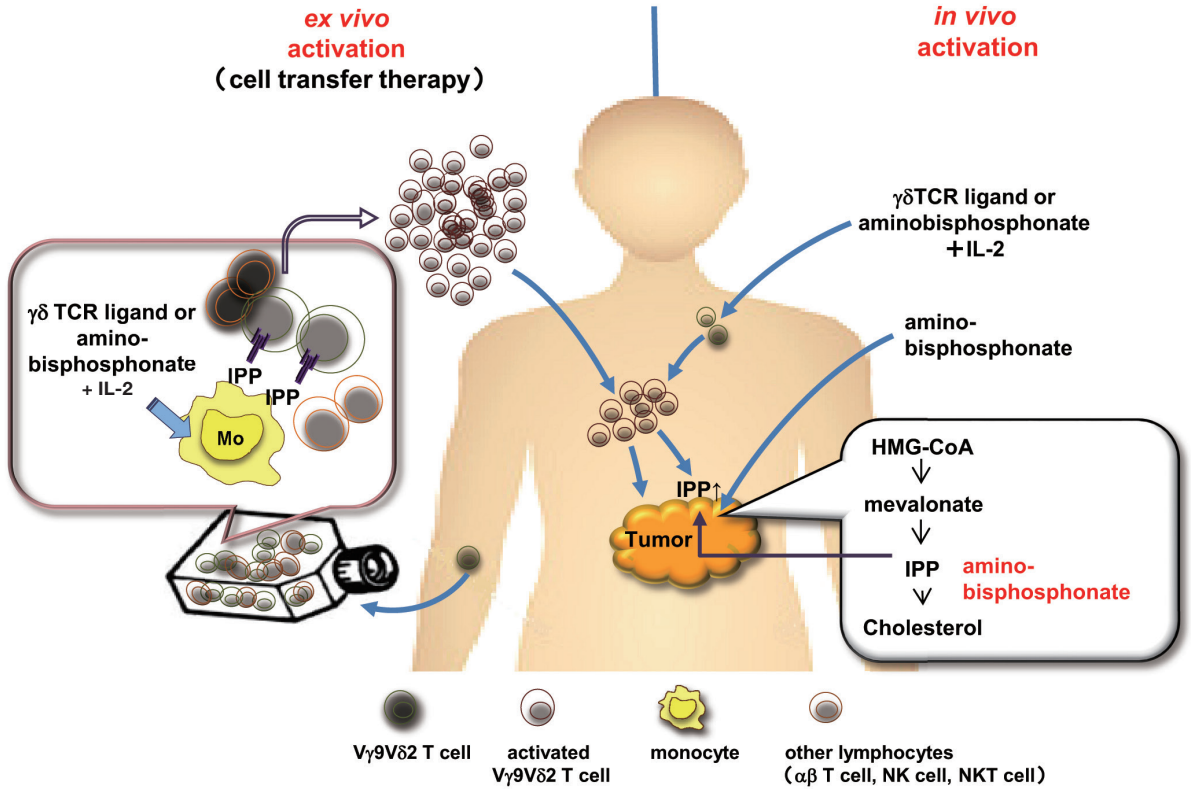

Figure 3 Strategies for γδ T cell based immunotherapy. Left panel, the adoptive cell transfer of *in vitro* expanded γδ T cells. Right panel, the *in vivo* activation of γδ T cells by phosphoantigens (e.g., BrHPP) or aminobisphosphonates and low-dose IL-2. The concomitant injection of aminobisphosphonate leads to intracellular accumulation of IPP/ApppI in tumor cells by blocking the mevalonate pathway, resulting in the sensitization of tumor cells to γδ T cells. Abbreviations: BrHPP, phosphorylated bromohydrin; IL-2, interleukin-2; IPP, isopentenyl pyrophosphate.

was developed by administering aminobisphosphonate and interleukin-2 (IL-2), to activate and expand γδ T cells *in vivo*. A pilot study on the intravenous infusion of low-dose IL-2 in combination with pamidronate in patients with relapsed and/or refractory low-grade non-Hodgkin lymphoma or multiple myeloma demonstrated that activation and proliferation of γδ T cells *in vivo* was observed in five patients (55%) and partial responses were seen in three of the nine for which expansion of γδ T cells was observed *in vitro* (19). Dieli *et al.* treated hormone-refractory prostate cancer with either zoledronate in combination with IL-2 (n=9) or zoledronate alone (n=9) (20). Neither group of patients experienced any severe adverse events. The response rate was 67% in the first group and 22% in the second group, with actual responses dependent on the expansion, number, and phenotype of γδ T cells. While these aminobisphosphonates indirectly activate γδ T lymphocytes as a consequence of the inhibition of FPP (a key enzyme of the mevalonate pathway) that leads to intracellular accumulation of endogenous phosphoantigens, direct activation of γδ T cells by synthetic stimulators have also been described. In phase I trial, synthetic stimulators, phosphorylated bromohydrin (BrHPP) that mimics the biological properties of natural phosphoantigens, was administered to the patients with IL-2 (21). While BrHPP administration induces a potent γδ T cell expansion in patients, anti-tumor activity was not clear. One of the disadvantage of *in vivo* activation of γδ T cells is that the proliferative response is transient, probably because repeated injection of BrHPP and IL-2 induced activation induced cell death of Vγ9Vδ2 T cell and an exhaustion of the response (22).

Immunotherapy with *ex vivo* activated γδ T cells

Adoptive transfer of γδ T cells following *ex vivo* expansion

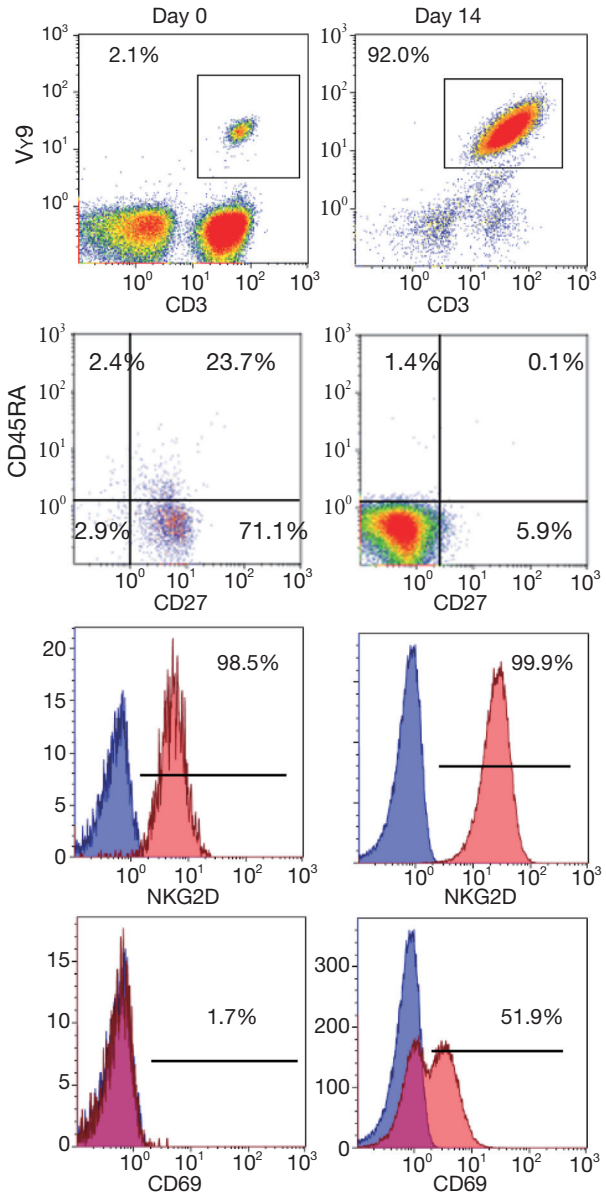

Figure 4 Typical phenotype of zoledronate-expanded γδ T cells by flow cytometric analysis. Fresh peripheral blood mononuclear cells or cells cultured for 14 days were stained with anti-CD3 and TCRVγ9 mAb. The $CD3^+Vγ9^+$ population was further analyzed with anti-CD27, CD45RA, NKG2D or CD69 mAbs.

by using IL-2 and phosphoantigen or aminobisphosphonate could represent an alternative to their *in vivo* activation. Efficient Vγ9Vδ2 T cell expansion can be obtained by co-culturing PBMCs with γδ TCR ligands, such as 2-methyl-3-butenyl-1-pyrophosphate (2M3B1-PP) and BrHPP. More than 1×10^9 *ex vivo* expanded γδ T cells were administered to the patients with metastatic renal cell carcinoma, multiple myeloma, non-small cell lung cancer (23-28). Adoptive transfer of γδ T cells was well tolerated and objective clinical responses in some of the patients were reported. The injection of zoledronate might precede the infusion of expanded γδ T cells in patients with cancer. By blocking the mevalonate pathway, zoledronate leads to intracellular accumulation of IPP/ApppI in tumor cells that are then recognized by Vγ9Vδ2 T cells (*Figure 3*).

We have established a large-scale *in vitro* expansion method for Vγ9Vδ2 T cells using zoledronate and IL-2 (29,30). PBMC were stimulated with 5 μM zoledronate and IL-2. Fourteen days after *in vitro* stimulation, *ex vivo* expanded γδ T cells were harvested and scrutinized for their sterility and purity. As shown in *Figure 4*, the percentage of $CD3^+TCRVγ9^+$ T cells in PBMC in this example was 2.1% on day 0. The dominant populations were $CD27^+CD45RA^+$ naive or $CD27^+CD45RA^-$ central memory phenotypes. When γδ T cells were efficiently stimulated, the frequency of γδ T cells increased to more than 92.0% of the cultured cells in successful γδ T cell cultures on day 14. The cultured γδ T cells upregulated NKG2D and CD69 expression and displayed $CD27^-CD45RA^-$ effector memory phenotype. We found that Vγ9Vδ2 T cells from patients with advanced cancer as well as from healthy donors underwent extensive proliferation under these conditions. Such cultured Vγ9Vδ2 T cells retained cytokine secretion capacity and mediated cytotoxicity against a variety of cancer cell lines, including lung cancer cell lines. As shown in *Figure 5*, γδ T cells attached to the tumor cells, resulting in collapse of the tumor cell membranes and apoptosis.

γδ T cells and lung cancer

There are several reports that γδ T cells can recognize and kill lung cancer cells. By analysis of tumor infiltrating lymphocytes (TIL), γδ T cells were detected at human lung cancer; both $Vδ1^+$ and $Vδ2^+$ TILs killed the N592 lung cancer cell line (31). It has been reported that Vγ9Vδ2 T cells stimulated by synthetic phosphoantigen BrHPP lysed tumor cell lines, including a primary NSCLC cell line (lung-ca 459) (32). We examined whether zoledronate-activated Vγ9Vδ2 T cells displayed cytotoxic activity against a lung cancer cell lines with different histology, mutated genes, and sensitivity to chemotherapeutic drugs, using a panel of lung cancer cell lines described in *Table 2*. Zoledronate-activated γδ T cells recognized and killed these lung cancer cell lines irrelevant to their histology,

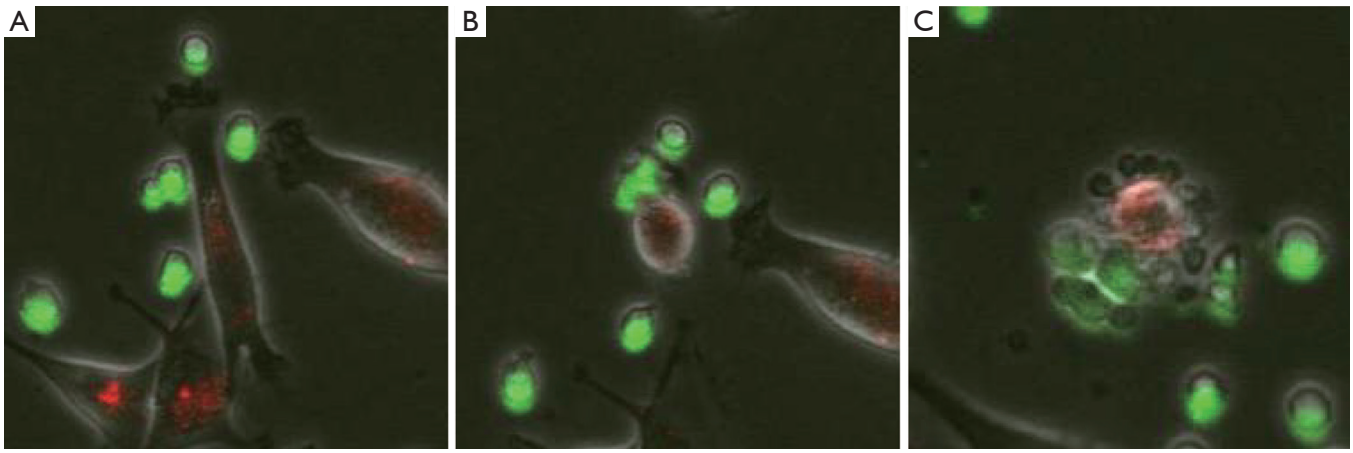

Figure 5 γδ T cell cytotoxicity. γδ T cells (green staining with CFSE) recognized and killed the lung cancer cell line, NCI H460 (red staining with PKH-26), by direct contact. In tumor cells attacked by the γδ T cells, collapse of the cell membranes led to apoptosis. It took approximately 2 h to progress from A to C.

Table 2 Lung cancer cell lines

Cell line	NCI-H1299	A549	NCI-H460	NCI-H358
Histology	NSCLC	Ad	La	BAC
Mutation				
EGFR	wt	wt	wt	wt
KRAS	wt	mut	mut	mut
p53	del	wt	wt	del
Sensitivity to drug IC50 (μM)				
CDDP	16.60	33.90	0.800	21.50
Paclitaxel	0.02	0.03	0.008	0.05
5FU	0.20	1.90	0.600	4.70
Gemcitabine	0.04	0.01	0.008	>0.10
Gefitinib	29.60	2.90	14.100	5.40
Surface marker				
HLA	++	+	++	++
CD54	+		–	+
CD166	+	+	++	++
MICA	++	+	++	–
Sensitivity to γδ T cell	+	+	+++	++

Abbreviations: NSCLC, non-small-cell lung cancer; Ad, adenocarcinoma; La, large cell carcinoma; BAC, bronchioloalveolar carcinoma; wt, wild type; mut, mutation; del, deletion.

mutated genes, and sensitivity to chemotherapeutic drugs. These results support the idea that γδ T cells can be used in immunotherapy for lung cancer.

γδ T cell therapy for the treatment of NSCLC

We conducted a phase I clinical study to evaluate safety and potential antitumor effects of re-infusing *ex vivo* expanded γδ T cells in patients with recurrent or advanced NSCLC (33,34). The research protocol was approved by the Ethics Committee of the University of Tokyo Hospital, and it was registered at the University Hospital Medical Information Network Clinical Trials Registry (Unique trial number: C000000336) on March 1, 2006. Written informed consent was obtained from each patient before they entered the study. The study was performed in accordance with the Declaration of Helsinki. Patients aged ≥20 years with advanced or recurrent NSCLC refractory to or intolerant of current conventional treatments were eligible for the study. Fifteen patients underwent adoptive immunotherapy with these γδ T cells. Patient's PBMCs were stimulated with zoledronate (5 μM) and IL-2 (1,000 IU/mL) for 14 days. Harvested cells, mostly γδ T cells, were given intravenously every two weeks without additional IL-2, a total of six times. If there were some clinical benefit, the treatment was repeated until the disease progressed. Though adverse events were observed in five patients, such as elevated liver enzymes, Flu-like symptoms, bacterial pneumonia, radiation pneumonitis, tumor pain, dyspnea, and weight loss, there were no severe adverse events related to the therapy (*Table 3*).

The results of phase I study of γδ T cell therapy was summarized in *Table 4*. The number of intravenous γδ T cell infusions ranged from 3 to 12. Twelve patients completed a

Table 3 Adverse events

Adverse events	CTCAE grade			
	1	2	3	4
Flu-like symptoms	2			
Bacterial pneumonia			1	
Radiation pneumonitis			1	
Dyspnea		1		
Weight loss	1			
Tumor pain		1		
Increased GGT			1	
Increased AST	1			
Increased ALT		1		

Table 4 Clinical results of adoptive γδ T cell transfer therapy for the treatment of NSCLC

Patient ID	No. γδT infusions	Plasma IFN-γ	Plasma MICA	Clinical response	PFS (d)	OS (d)
1	6	–	+	PD	62	640
2	6	–	–	SD	188	1,505
3	6	+	+	PD	105	295
4	12	+	–	SD	126	738
5	6	+	–	SD	285	861
6	3	–	+	Withdrawn (pneumonitis)	276	360
7	6	+	–	SD	244	244
8	4	–	–	Withdrawn (bacterial pneumonia)	200	937
9	4	–	–	PD	34	589
10	12	+	–	SD	139	965
11	6	–	–	Excluded (prostate cancer)		
12	8	–	+	PD	100	567
13	6	–	–	SD	237	616
14	6	+	–	PD	120	269
15	6	+	–	PD	72	202
				Median	126	589

Abbreviations: NSCLC, non-small-cell lung cancer; MICA, MHC class I-related molecules A; PFS, progression-free survival; OS, overall survival; PD, progressive disease; SD, stable disease.

course of six injections, three of whom received additional infusions. All patients remained alive during the study period with a median survival of 589 days and median progression-free survival (PFS) of 126 days. Maruyama *et al*. reported that median PFS was two months with gefitinib (250 mg/d) or docetaxel (60 mg/m^2) in patients with advanced/metastatic NSCLC who had failed one or two chemotherapy regimens (35). Considering that the study population of ours was also quite similar to that study, the clinical responses achieved here are promising, though the number of study was small. According to the Response Evaluation Criteria In Solid Tumors, six patients had stable disease (SD), whereas the remaining six evaluable patients experienced progressive disease (PD) four weeks after the sixth transfer. We conclude that adoptive transfer of zoledronate-expanded γδ T cells is safe and feasible in patients with NSCLC, refractory to other treatment.

The number of peripheral γδ T cells gradually increased with increasing numbers of infusion. However, the increases of γδ T cells in PBMC after transfer therapy had no association with their clinical responses. It remains to be elucidated whether transferred γδ T cells actually infiltrated in the tumor. Though the association failed to achieve statistical significance, plasma IFN-γ elevation is a potential indicator of better prognosis in the small patient group reported here (*Table 4*). In contrast, soluble MICA in patients' plasma was associated with poor prognosis; even though the presence of MICA in the plasma did not impair the *ex vivo* expansion of γδ T cells under our culture conditions. It has been reported that MICA is expressed by many cancers including primary lung cancers, and its recognition by NKG2D contributes to immunosurveillance against cancer. However, tumor cells shed MICA molecules into the serum to escape from recognition by immune cells. Soluble MICA downregulates NKG2D expression by CD8 T cells, NK cells, and γδ T cells (36). We are currently conducting phase II study to examine the efficacy and safety of adoptive γδ T cell therapy for the treatment of NSCLC. We will examine whether these molecules, IFN-γ and soluble MICA, can be applied as biomarkers; detection of IFN-γ or soluble MICA might be a marker for better prognosis or resistance to γδ T cell therapy, respectively, helping to determine in advance which patients would be likely to benefit from this treatment.

Future directions

Recent advances in tumor immunology have identified crucial roles of immune suppressive cells and immune checkpoint systems in inhibiting anti-tumor immune responses in cancer patients. Immune suppressive cells, such as regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC), increase in cancer-bearing hosts and suppress anti-cancer immune responses. Despite general concerns that chemotherapy would inhibit the efficacy of immunotherapy because of bone marrow suppression and immunosuppressive effects, anticancer therapies can enhance anti-tumor immune response by depleting immunosuppressive cells (37). Because persistent presentation of tumor antigens makes T cell tolerant or unresponsive to the antigens, the physical removal of tumor burden by surgery can potentiate an immune response. Monoclonal antibodies that block CTLA-4, PD-1 and PD-L1 molecules inhibited tumour-induced immune suppression, thereby allowing T cells to continue to survive, proliferate, infiltrate into the tumor site, produce cytokines and promoting tumour rejection (38). These results suggest that there is great potential for surgery, chemotherapies and molecularly targeted agents, to work synergistically with γδ T cell based immunotherapy, making combinatorial strategies a key area of future clinical research. The clinical efficacy of γδ T cell transfer therapy should be evaluated further in prospective clinical trials, and combinations of this newly emerging therapy with established treatments are expected to improve the survival of lung cancer patients in the future.

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