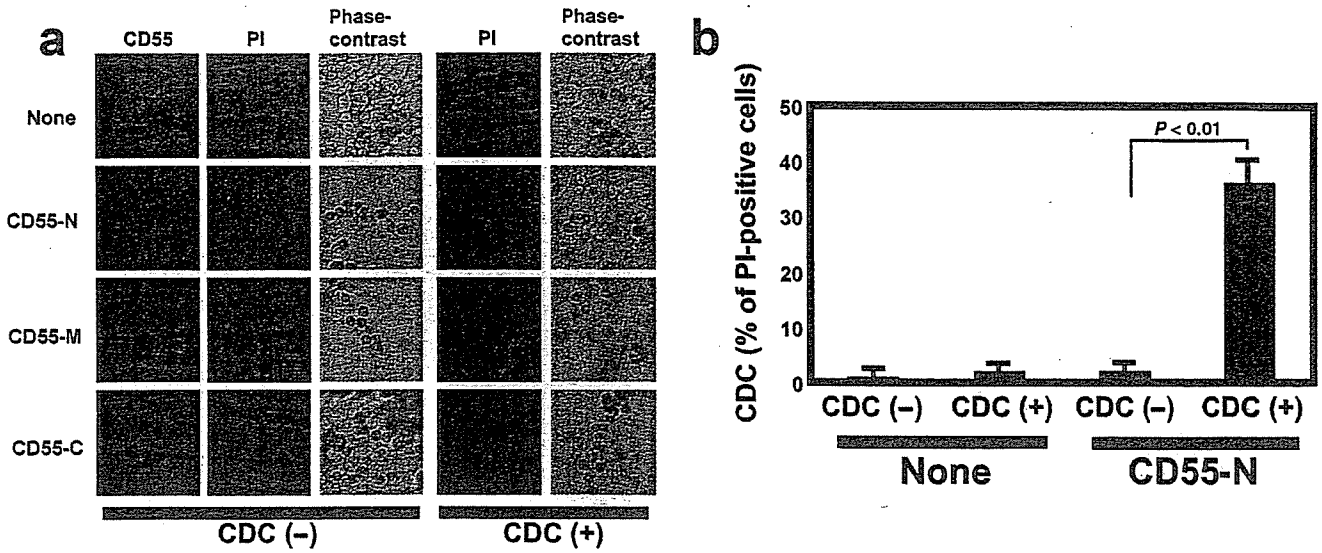

Fig. 4. Blockade of endogenous CD55 on breast cancer cells by small interfering RNA (siRNA). (a,b) SK-BR3 cells were transfected with siRNA against three parts of CD55, namely CD55-N, CD55-M and CD55-C, for 72 h. After transfection, the cells were stained with the anti-CD55 antibody and DAPI, and then the complement-dependent cytotoxicity (CDC) assay with trastuzumab was carried out with or without adding fresh human AB serum (a, left and right panels). (b) The percentage of propidium iodide-positive cells was calculated by counting 100 cells. Data are the mean ± SD (error bars) from experiments with triplicate samples. All statistical tests were two-sided Student's *t*-tests.

Because the breast cancer cell line SK-BR3 expresses Her2/neu and CD55 on its cell surface, siRNAs against three parts of CD55 (CD55-N for 1–380 nucleotides; CD55-M for 381–817 nucleotides; and CD55-C for 821–1146 nucleotides) were designed and introduced into SK-BR3 cells (Fig. 4). To detect dying cells, PI staining was used for the CDC assay with trastuzumab, and then the percentage of PI-positive cells was evaluated under laser scanning confocal microscopy. Most SK-BR3 cells expressed CD55 molecules without transfection of siRNA against CD55 (Fig. 4a, left). In contrast, expression of CD55 on SK-BR3 cells transfected with CD55-N disappeared 72 h after transfection, or became much weaker than without transfection of siRNA against CD55 (Fig. 4a, right). SK-BR3 cells transfected with CD55-M or CD55-C did not reveal knock down of CD55 expression to the level seen with CD55-N (Fig. 4a). Only 3.0 ± 1.0% of SK-BR3 cells without transfection of siRNA (mock transfection) against CD55 became PI-positive by CDC with trastuzumab, whereas 36.0 ± 6.0% of cells were PI-positive by CDC with trastuzumab after the transfection of siRNA (Fig. 4b). This suggested that siRNA against nucleotides 1–380 of CD55 (i.e. CD55-N) was effective for decreasing CD55 expression and sensitivity to CDC on adherent cells such as SK-BR3.

Blockade of CD55 expression by siRNA overcomes resistance to CDC in fresh lymphoma cells

To investigate the effect of siRNA against CD55 on fresh lymphoma cells, lymphoma cells were isolated from the lymph nodes of five patients with recurrent lymphomas and transfected with siRNA against CD55 (Fig. 5). As shown in Fig. 5a, lymphoma cells from all five cases with recurrent lymphoma strongly expressed CD55 molecules under laser scanning confocal microscopy. When fresh lymphoma cells were transfected with CD55-N for 24 h, but not CD55-M and CD55-C, CD55 expression on fresh lymphoma cells was significantly knocked down under laser scanning confocal microscopy, compared with the control (Fig. 5a, left columns). The percentage of PI-positive cells showed no significant differences among transfections with and without CD55-N, CD55-M and CD55-C before the CDC assay (Fig. 5b). The percentage of PI-positive cells in the transfection with CD55-N significantly increased from 7.1 ± 2.8% to 67.9 ± 8.1%. This indicates that the siRNA against CD55 (CD55-N) could efficiently knock down the expression of CD55 on SK-BR3 and freshly isolated lymphoma cells from recurrent lymphomas, and that it could induce cell death in SK-BR3 and freshly isolated lymphoma cells from recurrent lymphomas by CDC. This suggests that the degree of CD55 expression can determine resistance to CDC with antibody therapy, and that the therapies, which target CD55 molecules such as siRNA and its monoclonal antibody, would be helpful in antibody therapy for bulky disease.

Discussion

Treatment of malignancies has been largely based on chemotherapy and radiotherapy. Although improvement in response rates and survival has been obtained with these therapies over the years, a significant proportion of patients do not respond to treatment, or they relapse. Moreover, conventional cytotoxic therapy is often associated with significant morbidity. Recently, molecular targeting therapy has been developed[22] and monoclonal antibodies against CD20 and HER2/neu have been used for molecular targeting therapy.[1–3] Also, in recent therapies for malignancies, monoclonal antibodies have emerged as important therapeutic agents.

In the preset study, we have shown a negative correlation between the size of extirpated lymph nodes and susceptibility to CDC with rituximab, but the level of CD20 expression did

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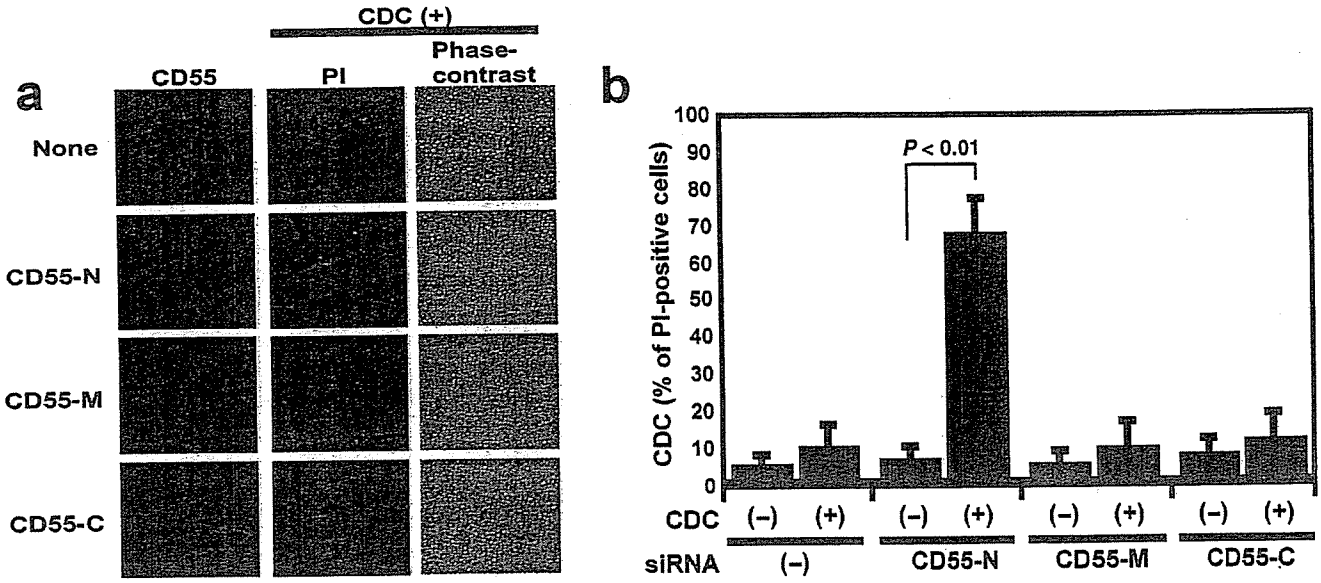

Fig. 5. Blockade of CD55 on primary lymphoma cells by small interfering RNA (siRNA). (a,b) Lymphoma cells from the lymph nodes of five patients with chemotherapy refractory and resistant lymphoma were transfected with siRNA against three parts of CD55, namely CD55-N, CD55-M and CD55-C, for 24 h. (a) After transfection, the cells were stained with anti-CD55 antibody and propidium iodide (PI), and then the complement-dependent cytotoxicity (CDC) assay with rituximab was carried out with or without adding fresh human AB serum. (b) The percentage of PI-positive cells was calculated by counting 100 cells. Data are the mean ± SD (error bars) from experiments with triplicate samples. All statistical tests were two-sided Student's *t*-tests.

not correlate with the size of the lymph node or susceptibility to CDC with rituximab. To date, no other studies have analyzed the relationship between size of lymph node and susceptibility to CDC with rituximab. It has been shown previously that CDC is directly correlated with CD20 expression.[11,23] In contrast, Manches *et al.*[24] have reported in detail that there is no direct correlation between lysis and expression of CD20 in global lymphoma such as FL, mantle cell lymphoma (MCL), small lymphocytic lymphoma (SLL), diffuse large B cell lymphoma (DLCL), and non-tumor B cells, as we showed in the current study. They also suggested that other regulators such as C-reactive protein (CRP) might play important roles in this complement system.

Although antibody therapy is a good tool, resistance sometimes occurs due to unknown mechanisms.[8,25] Patients with bulky mass, especially more than 7 cm of lymphoma mass, often show resistance to rituximab and are not curable.[26] We have demonstrated that CDC activity negatively correlates with the size of extirpated lymph nodes, and that the formula's intercept is 7.447 cm. This suggests that CDC is ineffective to tumors greater than 7.447 cm in size, and that our observation is consistent with the report of Coiffier *et al.*[26] Additionally, CD55 expression significantly correlates with the size of extirpated lymph nodes, suggesting that CD55 expression may play an important role in CDC resistance with antibody therapy. High densities of Daudi and Raji cells, associated with bulky mass, also became resistant to CDC with rituximab, and expression of CD55 increased during cell culture (Terui *et al.*, unpublished data). The relationship between cell density and size of tumors, resistance to CDC and CD55 expression are the same in not only extirpated lymph nodes from patients but also in experimental cell lines. Although previous reports have discussed whether CD55 can be an indicator of prognosis, no one has reported the relationship between cell density and tumor size, resistance to CDC and CD55 expression. Low or high CD55 expression has been reported in CLL cells.[11] However, some researchers have reported that *in vitro* susceptibility to rituximab-induced CDC could not be predicted by the levels of CD55 protein in CLL cells, nor *in vivo* in FL and CLL patients.[12,13] On the other hand, Golay *et al.*[27] have reported that relative levels of CD55 and CD59 may become useful markers to predict clinical responses. Overexpression of CD55 on some tumor cell lines and in colorectal carcinomas has been shown to be an indicator of poor prognosis. This result is consistent with the present study, as we found that CD55 expression in bulky disease may be a useful indicator of this prognosis. Recently, Madjd *et al.*[28] reported that loss of CD55 is related to poor prognosis in breast cancer. High expression of CD55 was significantly associated with low-grade lymph node negativity and with good prognosis. Survival analysis showed that CD55 overexpression was associated with a more favorable outcome. On the other hand, loss of CD55 is associated with poor survival. They established a novel anti-CD55 antibody for use in immunohistochemistry. Although they classified weak to strong intensity of CD55, it is possible that the antibody recognized the non-glycosylated SCR3 domain of CD55 molecule, but not the glycosylated CD55 molecule. The authors pointed out that loss of CD55 is associated with poor prognosis, but not with monoclonal antibody resistance. In the present study, we demonstrated that blockage of CD55 overcomes resistance to antibody therapy and that CDC plays an important role in tumor attack in antibody therapy. As the mechanism that we refer to is different from their study, it may depend on the type of cancer investigated.

Malignant progression has been reported to be associated with tumor hypoxia, and the inside of the bulky mass showed low oxygen partial pressure (PO_2) (<10 mmHg).[29] Because hypoxia induces COX-2 expression and prostaglandin E_2 (PGE_2) production in not only human vascular endothelial cells[30] but also tumor cells,[31,32] PGE_2 may be produced more in bulky tumors with hypoxia. Recently, it has been reported that PGE_2 upregulates expression of the complement inhibitor CD55 in colorectal cancer.[33] This suggests that bulky mass of lymphoma and other cancers may express CD55 to high levels via PGE_2 production.

It has been reported that the protective activity of rituximab or the 1F5 antibody is completely abolished in syngeneic knockout animals lacking C1q, the first component of the classical complement pathway C ($C1qa^{-/-}$).[34] This indicates that complement activation is fundamental for rituximab therapeutic activity *in vivo*. As CDC is more rapidly and efficiently triggered by monoclonal antibodies in cells with higher expression of their target molecules, we focused on how sensitivity to CDC can be recovered in the resistance to monoclonal antibody therapy. In antibody therapy, blockage of CD55 may be useful for recovery of sensitivity to CDC. It has been reported that anti-CD55 and anti-CD59 antibodies can enhance CDC sensitivity with rituximab, and that CD55 and CD59 may become useful markers to predict the clinical response.[24] Although they did not mention the therapy against resistance to antibody therapy using anti-CD55 and anti-CD59 antibodies,[24] there are three ways to block the function of CD55: (i) blocking the antibody against CD55; (ii) siRNA[35] for CD55; and (iii) small molecules as CD55 inhibitors. We have demonstrated that siRNA for CD55 successfully inhibited functional CD55 protein, and that CDC activity was enhanced in the CD55-knock down breast cancer cell line SK-BR3 and in clinical samples from lymphoma patients. In particular, siRNA is a better tool for blocking CD55, as siRNA can inhibit not only expression of CD55 but also the function of CD55. Nagajothi *et al.* also showed genetic and biochemical methods to decrease CD55 expression and other GPI-anchored proteins.[36] This suggests that a decline in CD55 levels could be enough to make the tumor sensitive to CDC with rituximab and trastuzumab.

In conclusion, we have shown that CD55 blockade by siRNA enhances rituximab-mediated cytotoxicity. This observation gives us a novel strategy to suppress bulky disease-related resistance to monoclonal antibody treatment.

Acknowledgments

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Review Article

Gene Therapy for Breast Cancer. – Review of Clinical Gene Therapy Trials for Breast Cancer and *MDR1* Gene Therapy Trial in Cancer Institute Hospital

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Gene therapy for advanced breast cancer is anticipated to be a useful therapeutic approach. Strategies in ongoing clinical protocols can be divided into four groups: (1) suppression of oncogenes or transfer of tumor-suppressor genes; (2) enhancement of immunological response; (3) transfer of suicide genes; (4) protection of bone marrow using drug resistance genes. We have started a clinical study of multidrug resistance (*MDR1*) gene therapy. Advanced breast cancer patients received high dose chemotherapy and autologous peripheral blood stem cell transplantation (PBSCT) with *MDR1*-transduced hematopoietic cells, and then were treated with docetaxel. Two patients have been treated so far, and *in vivo* enrichment of *MDR1*-transduced cells with docetaxel treatment has been seen. Both patients are in complete remission and had no apparent adverse effects from the *MDR1* gene transfer.

Breast Cancer 13:8-15, 2006.

Key words: Breast cancer, Gene therapy, *MDR1*, Adenoviral vector, Retroviral vector

The cure rate of advanced or recurring breast cancer is under 5%, so the usual goal of treatment is prolongation of survival or improvement of quality of life (QOL), not cure[1]. Endocrine therapy for hormone-receptor-positive patients, chemotherapy, radiation therapy, bisphosphonates for bone diseases, and trastuzumab for HER2-overexpressed patients, have all been shown to be effective for advanced breast cancer, but none has been shown to increase the cure rate.

Gene therapy for advanced breast cancer is expected to be a useful therapeutic approach. Strategies in ongoing clinical protocols can be divided into four groups: (1) suppression of oncogenes or transfer of tumor-suppressor genes; (2) enhancement of immunological response; (3) transfer of suicide genes; (4) protection of bone marrow using drug resistance genes (Table 1)[2,3]. There are three major methods for gene transfer: (1) transduction of naked DNA such as lipofection (transient expression); (2) transduction of adenoviral vector or vaccinia virus vector (transient expression); (3) transduction of retroviral vector (stable expression). In this paper, ongoing clinical trials of gene therapy for breast cancer are reviewed, and a clinical trial of multiple drug resistance 1 (*MDR1*) gene therapy at our institution is described.

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Present Status of Clinical Trials of Gene Therapy for Breast Cancer

Suppression of Oncogene Expression or Transfer of Tumor-Suppressor Gene

The carcinogenic process requires an accumulation of multiple gene mutations or abnormalities of gene expression. Common gene abnormalities in breast cancer include p53 gene mutation, ErbB2/HER2 gene amplification, c-myc gene amplification, and cyclin D1 gene amplification[4]. Several clinical trials aim to improve those gene abnormalities by local or systemic gene transfer.

A) Transfer of the normal p53 gene: Mutations of the p53 gene are the most frequently found gene abnormalities among various malignancies, including breast cancer[5]. Tumor cells with mutated p53 genes show defects of cell-cycle regulation,

Table 1. Clinical Studies of Gene Therapy for Breast Cancer

Strategy	Gene	vector	Investigator
1 suppression of oncogene or transfer of tumor suppressor gene	p53	adenovirus	von Mehren Cristofanilli Baynes
	E1A	lipofection	Hortobagyi
	antisense (c-fos, c-myc)	retrovirus	Holt
	MDA-7	adenovirus	Bucholz
2-A transfer of cytokine gene	IL-2	lipofection adenovirus	Lyerly Stewart
	IL-12	retrovirus	Park
	GM-CSF	adenovirus	Suzuki
	TNF + NeoR	retrovirus	Rosenberg
2-B transfer of costimulatory molecule gene	B7.1 (CD80)	lipofection adenovirus	Urba Schuchter
2-C transfer of antigen gene	MUC1	vaccinia virus	Kufe
	HER-2	naked DNA	Patel
	MUC1 + CD80	vaccinia virus	Eder
	MUC1 + IL-2	vaccinia virus	Velu
3 transfer of suicide gene	HSV-TK	retrovirus	Favrot
	Cytosine deaminase	lipofection	Lemoine
	CYP 2B6	retrovirus	Harris
4 transfer of drug resistance gene	MDR1	retrovirus	Stewart Cowan Deisseroth Hesdorffer O'Shaughnessy Takahashi

according to http://www.wiley.co.uk/genetherapy/clinical

and transfer of normal p53 genes causes cell-cycle arrest or apoptosis. Clinical studies of p53 gene therapy using adenoviral vectors (Advexin, Introgen *et al.*) for various tumor types, including breast cancer, are ongoing. Von Mehren and Cristofanilli have begun clinical studies of a combination of local injection of p53-adenoviral vector into skin metastatic lesions or locally advanced breast cancer and systemic chemotherapy. Baynes has initiated a clinical study of high dose chemotherapy associated with transplantation of autologous peripheral blood stem cells (PBSC) that have been purged *ex vivo* by p53-adrnovirus infection. Baynes's group has shown that p53 gene transfer has no effect on normal PBSC.

B) Suppression of the ErbB2/HER2 gene: The ErbB2/HER2 gene encodes an 185 kD protein and is a member of the epidermal growth-factor receptor family. This gene is amplified in 20-30% of breast cancer patients, and correlates with a poor prognosis and resistance to hormone therapy[4]. Monoclonal humanized murine antibody to ErbB2/HER2 protein (trastuzumab/Herceptin™) is effective in advanced, ErbB2/HER2-overexpressing breast cancer patients[6]. The adenovirus type 2 or type 5 E1A gene inhibits expression of the ErbB2/HER2 gene, and E1A gene transfer into ErbB2/HER2-overexpressed tumors causes tumor reduction and enhances sensitivity to chemotherapy *in vitro* and *in vivo*[7]. At MD Anderson Cancer Center, patients with breast cancer or ovarian cancer overexpressing ErbB2/HER2 were treated with gene therapy using a local injection of E1A gene-liposome into skin lesions or pleural/peritoneal effusion[8]. There was no serious adverse effect other than fever or pain at the injection sites. In

six cases in which tumor cells in body fluids could be analyzed, reduction of ErbB2/HER2 expression and a decrease in tumor cells were shown. E1A gene transfer also reduced tumor growth of non-HER2-overexpressing cells, and E1A gene transfer to tumor tissues of breast cancer or head and neck cancer by lipofection showed minor response in HER2-negative tumors[9].

C) Suppression of c-myc and c-fos gene: Arteaga and Holt made a retroviral vector which overexpresses antisense mRNA to c-myc and c-fos genes under the control of mammary tumor virus (MMTV) promoter. Transfer of this vector into a breast cancer cell line suppressed tumor formation in animal models[10]. They have started a clinical trial of gene therapy for malignant effusion or meningitis in breast cancer patients who have failed standard therapy. Effusions will be drained and replaced with a solution of the vector, then periodically drained to follow the disease and assess gene transfer[11].

D) Transfer of melanoma differentiation associated protein 7 (MDA-7): MDA-7 is a novel tumor suppressor gene, and its transfer into tumor cells causes growth suppression and apoptosis. However, MDA-7 gene transfer into normal cell lines does not[12]. A clinical trial of gene therapy that injects MDA-7- adenoviral vector (Ad-mda7, ISGN 241) into tumor cells has started (Buchholz). There was no serious adverse effect in a phase I study, and a combination phase I/II study with irradiation has begun.

Augmentation of Immunological Response to Cancer Cells

Breast cancer cells have long been supposed to have low antigenecity and to be resistant to immune therapy. So far, reports of nonspecific immune therapies such as BCG have shown that those therapies are not effective for breast cancer[13]. But since the 1990s, many breast cancer-associated antigens have been reported, and various clinical studies of specific immune therapy for breast cancer, such as vaccination therapy targeted to ErbB2/HER2, are ongoing[14, 15]. Immune therapy by gene transfer includes: 1) transfer of cytokine genes that enhance immune response, 2) transfer of co-stimulatory molecule genes, and 3) transfer of antigen molecule genes.

A) Transfer of cytokine genes

i) Interleukin-2 (IL-2): Injection of IL-2 gene-adenoviral vector into tumor tissues[16], or subcutaneous injection of inactivated tumor cells that were transduced *ex vivo* by IL-2 gene lipofection (Lyerly) may cause a systemic immune reaction in tumor cells. In a phase I/II study, Stewart *et al.*[17] treated 23 cases with breast cancer or malignant melanoma by injection of 10^7-10^{10}pfu adenovirus-IL-2 into subcutaneous tumors. There was no side effect other than local inflammation of injection sites, and reduction in diameter of subcutaneous tumors was reported in 24% of patients, but there was no PR.

ii) Interleukin-12 (IL-12): Retroviral transfer of IL-12 gene into skin fibroblasts of patients *ex vivo*, then injection of the fibroblasts into tumor tissues may activate a tumor-specific immune response. In a phase I study, nine cases with advanced neoplasm including breast cancer were treated by Kang *et al.* Reduction of tumor at injection sites was shown in four cases, and reduction of tumor at remote sites was shown in one melanoma case. There was no side effect other than slight pain at the injection sites[18].

iii) Granulocyte-macrophage colony stimulating factor (GM-CSF): Retroviral transfer of GM-CSF gene into tumor cells and injection of those cells into subcutaneous tissue may activate systemic immune reaction to tumor cells (Suzuki). The same gene therapy for renal cell cancer has been done in Japan.

iv) Tumor necrosis factor (TNF): Retroviral transfer of TNF gene and Neo gene into tumor cells *ex vivo* and subcutaneous injection of tumor cells may activate systemic immune response to tumor cells[19].

B) Transfer of co-stimulatory molecule gene: Transfer of T cell co-stimulatory molecule CD80 (B7.1) gene into tumor cells by lipofection and injection of those tumor cells into subcutaneous tissue (Urba), or direct injection of CD80-adenoviral vector into tumor tissue (Schuchter) may activate T cell growth and immune response.

C) Transfer of antigen gene: Clinical studies of MUC1(CA15-3) gene transfer by vaccinia virus into tumor cells and injection of tumor cells into subcutaneous tissue (Kufe), simultaneous transfer of MUC1 and CD80 gene (Eder), or HER2 gene transfer (Patel), have been ongoing. Scholl *et al.* repeatedly administered vaccinia virus containing MUC1 and IL-2 genes (TG1031) intramuscularly to patients with metastatic breast cancer. In 31 patients, two patients (6%) had PR and 15 patients had SD[20].

Suicide Gene Therapy

Transfer of drug-activating enzyme gene into tumor cells and treatment with a prodrug form of chemotherapeutic agents causes a high concentration of the activated drug in the tumor tissue and apoptosis of tumor cells. Not only transduced cells, but also circumferential cells are reported to die with this gene therapy (bystander effect).

A clinical trial of retroviral herpes simplex virus thymidine kinase (HSV-TK) gene transfer into breast cancer tumor tissues and treatment with gancylovir is ongoing (Favrot).

A phase I study of injection of HER2 promoter-driven cytosine deaminase (CD) gene plasmid into metastatic skin lesions of breast cancer and treatment with prodrug (fluorocytosine) has been reported. Fluorocytosine is transformed into 5FU by the CD gene. Expression of the CD gene in HER2-positive tumor cells has been shown in 9/11 cases at day 2 and 3/10 cases at day 7. Tumor reduction was shown in 4 of 12 cases[21].

Retroviral P450 2B6 (CYP2B6) gene transfer into metastatic cutaneous tissues and oral cyclophosphamide therapy causes efficient conversion of prodrug cyclophosphamide into active metabolite phosphoramide mustard in the tumor tissues. In a phase I study, nine breast cancer and three melanoma patients were treated with CYP2B6 vector (MetXia-P450). One breast cancer patient had a PR and four (33%) had stable diseases (SD) $\geqq$ 3 months[22].

Bone Marrow Protection by Drug-Resistance Gene

Breast cancer is sensitive to chemotherapy. Response rates of advanced breast cancer for most combination chemotherapy are between 40% and 70% (complete response (CR) rate 10-30%), but duration of response is 7-10 months for PR, and 9-18 months for CR. High dose chemotherapy with autologous blood stem cell transplantation for advanced breast cancer has shown high complete response rates (up to 50%), and 10-15% patients have enjoyed durable remission[23, 24]. However, most patients will relapse after transplantation. Randomized studies comparing high dose chemotherapy and conventional chemotherapy showed that median survival times appear to be no better than those achieved with conventional chemotherapy, so far[25]. Probably high dose chemotherapy cannot completely eradicate residual disease, and insufficient bone marrow function after the reconstitution is a major problem in post-transplantation chemotherapy. One approach to overcome the current situation would be the transplantation of the drug-resistant gene-transduced hematopoietic stem cells so that normal bone-marrow cells will be protected from the toxic effect of anticancer drugs.

A multidrug resistance 1 (*MDR1*) gene was cloned from cancer cell lines resistant to various anticancer drugs[26]. The *MDR1* gene product (P-glycoprotein, P-gp) is a 170 kD glycoprotein consisting of two trans-membranous domains and two ATP-binding domains. P-gp ATP-dependently excretes various drugs such as doxorubicin, vinka-alkaloids, or taxanes from cytoplasm to extra-cellular fluid. *Ex vivo* transfer of *MDR1* genes into hematopoietic stem cells and transplantation might make post-transplant chemotherapy feasible. Chemotherapeutic drugs such as docetaxel and paclitaxel, which have good clinical activity in the treatment of breast cancer and are efficiently effluxed by P-gp, might be the best choice for this strategy. Using a retroviral vector, Sorrentino *et al.*[27] transplanted *MDR1*-transduced bone marrow into irradiated mice and then treated them with paclitaxel. Paclitaxel treatment increased *MDR1*-transduced leukocytes in peripheral blood (*in vivo* amplification), and *MDR1*-transduced mice showed reduced bone marrow suppression by paclitaxel (bone marrow protection). Then, several groups have undertaken clinical studies of *MDR1* gene therapy for advanced breast cancer or other neoplasms[28-30].

A group at MD Anderson Cancer Center first reported the results of clinical trials[28]. They performed retroviral gene transfer without using cytokines, and in suspension or with autologous stromal cells. *In vitro* transduction efficiency was 2.8% with the solution method and 5.6% with the stromal method, detected by *in situ* PCR. But three to four weeks after transplantation, direct PCR assay of peripheral blood leukocytes in patients showed positive results in 0/10 with the solution method, and 5/8 with the stromal method. These data show insufficient transduction efficiency without using cytokines. NCI also reported the results of a clinical trial of retroviral *MDR1* gene therapy[30]. They transferred *MDR1* genes into bone marrow mononuclear cells or peripheral blood stem cells stimulated by IL-3, IL-6, and SCF. *Ex vivo* transduction efficiency was 0.2-0.5%. They treated transplanted patients with paclitaxel, but

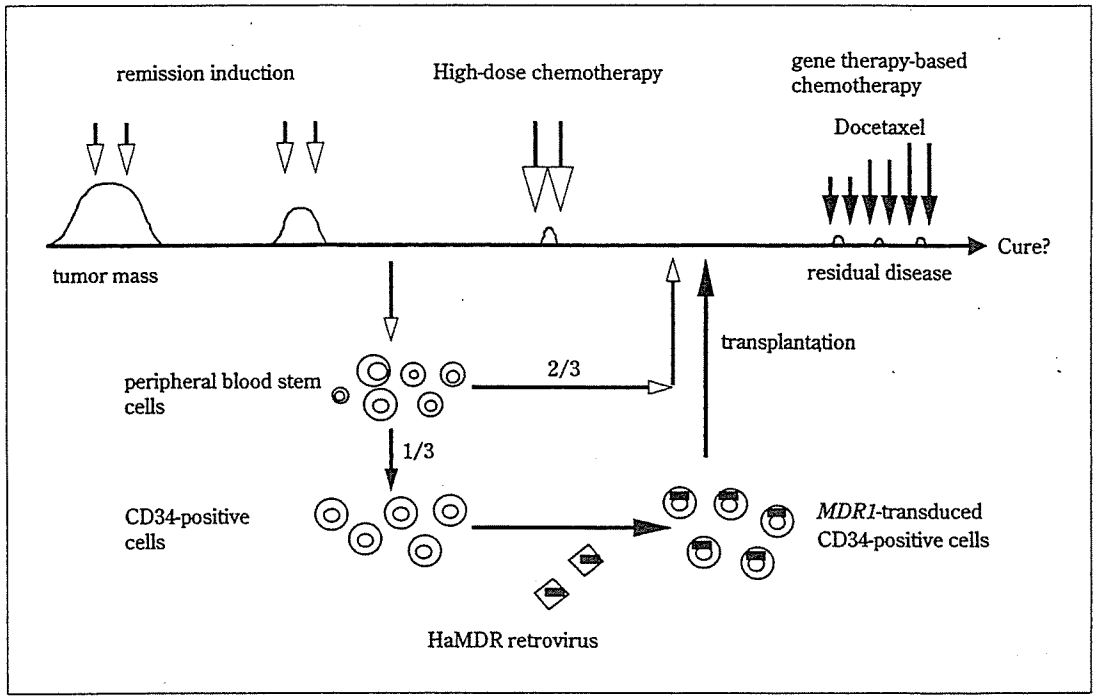

Fig 1. Schema of *MDR1* gene therapy for advanced breast cancer patients in Cancer Institute Hospital.

they could not show any enrichment of *MDR1*-transduced white blood cells by PCR. A group at Columbia University also transferred *MDR1* genes into bone marrow mononuclear cells or peripheral blood stem cells stimulated by IL-3, IL-6, and stem cell factor (SCF). They showed that 20-70% of BFU-E or CFU-GM colonies from transferred CD34-positive cells were positive for *MDR1* by PCR. BM from patients 3-12 weeks after transplantation showed *MDR1*-positivity by PCR in 2/5 patients. They also analyzed P-gp expression in bone marrow cells using flow cytometry, but they could not show any expression. Clinical studies of *MDR1* gene therapy are now ongoing at several institutions (Stewart, Cowan, Disseroth, Hesdorffer, O'Shaughnessy).

MDR1 Gene Therapy in Cancer Institute Hospital

Our group also started *MDR1* gene therapy for breast cancer. This study was approved by the Ministry of Health and the Ministry of Education and Science on February 24, 2000. The outline of the protocol is shown in Fig 1. We selected histologically confirmed, metastatic breast cancer patients who achieved good PR or CR to a precedent conventional dose chemotherapy regimen (using anthracycline and/or taxane). We used a HaMDR vector in which wild type *MDR1* cDNA (Kyoto University) had been inserted into pHa vector (NCI) derived from Harvey mice sarcoma virus (HaMSV). Peripheral blood stem cells (PBSC) were harvested by cyclophosphamide and G-CSF. CD34-positive cells were selected from about one third of PBSC, and HaMDR was transferred into those cells stimulated by SCF, thrombopoietin, IL-6, Flt-3 ligand, and soluble IL-6 receptor. Transduced PBSC were checked for safety (presence of replication-competent retrovirus, etc.) and then frozen. Patients were treated with high-dose cyclophosphamide, thiotepa, and carboplatin. Then unprocessed and *MDR1* gene-transduced PBSC were transplanted together. After bone marrow was reconstituted and patient status was normalized, patients were treated with 50% of standard dose docetaxel, then with increased doses up to 100% if grade 4 neutropenia was not recorded. Gene transfer efficiency and P-gp expression were checked with PCR and flowcytometry analysis, using peripheral leukocytes and bone marrow cells.

So far, two patients have finished high-dose chemotherapy, PBSC transplantation with *MDR1* gene transfer, and then docetaxel chemotherapy (Table 2). Peripheral blood P-gp-positive leuko-

Table 2. Case 1 of *MDR1* Gene Therapy in Cancer Insitute Hospital

October-00	Informed consent, approval by Insitutinal Review Board
November-00	PBSC harvest and *MDR1* gene transfer #1
February-01	PBSC harvest and *MDR1* gene transfer #2
April-01	High dose chemotherapy and transplantation of *MDR1*-transuced PBSC
June-01	Start of docetaxel chemotherapy
October-01	CR after 5 cycles of docetaxel
February-02	Final docetaxel therapy (#10)
March-05	No sign of relapse/leukemia

cytes increased to 5% after transplantation but decreased gradually. During docetaxel chemotherapy after transplantation, *in vivo* expansion of the *MDR1*-transduced cells (up to 10%) was obser ved. Comparison of two patients suggests the presence of a bone-marrow protection effect by *MDR1* expression during docetaxel chemotherapy, but this is not clear. No serious side effect was observed, and the patients have been in complete remission for 3 years.

Retroviral gene therapy causes random insertion of exogenous genes into genome DNA of target cells, so it may cause carcinogenesis by activation of oncogene or inactivation of tumor suppressor gene. At the end of 2002, occurrence of T cell leukemia in two patients after gene therapy for X-linked severe combined immune deficiency (X-SCID) was reported. A genetic defect in the γC gene, which is a common domain of multiple interleukin receptors (IL-2R, IL-4R, IL-7R, *et al.*), causes severe defects of T cell and natural killer cells as well as severe immune deficiency in X-SCID patients. Retroviral γC gene transfer using autologous CD34-positive hematopoietic cells in X-SCID patients restored immune system in 9 of 11 patients[31]. But T cell leukemia occurred in three patients (one more patient in January 2005) of those 9. In the leukemic cells, retroviral vector was inserted in the LMO2 gene, which causes T cell leukemia[32]. Then the FDA recommended suspension of all clinical trials of retroviral gene therapy for hematopoietic stem cells. We also suspended *MDR1* gene therapy for the third patient in January 2003. After thorough investigation of retroviral gene therapy trials for hematopoietic stem cells all over the world, no leukemia event has been found in clinical gene therapy trials, other than the French X-SCID trial (American Society for Gene Therapy Annual Meeting, 2003). Screening of the Mouse Retroviral Cancer Gene database showed that retroviral insertion into γC and LMO2 gene was found in two cases each, and insertion into both genes were found in one case. This fact suggests that both genes are oncogenes, and that the two genes can collaborate[33]. In X-SCID gene therapy, a double hit with retroviral activation of LMO2 gene and exogenous activated γC gene might be necessary for leukemogenesis. If so, retroviral gene therapy with non-oncogenic genes might have a low risk of cancer[34].

Thereafter, gene therapy using retroviral vector resumed, and retroviral gene transfer into hematopoietic cells of adenosine deaminase deficiency patients was begun in Japan at the end of 2003. We also resumed our *MDR1* gene therapy after changing the protocol (informed consent with regard to the adverse effects and more thorough investigation of patients' peripheral blood), and started high-dose chemotherapy and transplantation of PBSC with *MDR1* gene transfer to the third patient in July 2004.

We also started investigation of insertion sites of HaMDR vector in the first two patients. A clonality study of leukocytes from case 1 showed eight long-lived clones of *MDR1*-tranduced hematopoietic stem cells. No sign of expansion of any clones has been observed.

To summarize the data of our own and other institutions' clinical studies of retroviral *MDR1* gene therapy, first, there has been no serious side effect, including secondary neoplasm, but thorough investigations including retroviral insertion sites are necessary. Second, maintenance of *MDR1*-transduced hematopoietic cells for more than one year was confirmed. Third, the *MDR1*-transduced cells were selectively enriched *in vivo* by chemotherapy. Whether *MDR1* gene therapy can protect bone marrow from chemotherapy is not yet certain. We have almost finished proof-of-concept stage for the gene therapy, and we should be able to show clinical benefits compared with conventional therapy.

The techniques and knowledge of gene therapy are still limited, so we must proceed with caution, and we must inform patients of both the risks and benefits of the therapy.

Acknowledgements

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■原　著■

転移・再発乳癌におけるvinorelbineの有用性と認容性の検討

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原著

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転移・再発乳癌におけるvinorelbineの有用性と認容性の検討

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Efficacy and Safety of Vinorelbine in Women with Metastatic Breast Cancer：Tokudome N*1, Ito Y*1, Takahashi S*1, Suga S*1, Sugihara T*1, Ohsako T*1, Morizono H*1, Senuma K*1, Miura H*1, Watanabe C*1 and Hatake K*1 (*1Department of medical oncology, Cancer Institute Hospital)

We evaluated the efficacy and safety of vinorelbine in women with metastatic breast cancer.

[Patients and Methods] 58 patients who had metastatic breast cancer were started vinorelbine between May, 2005 to January, 2006. The dose of vinorelbine was 25mg/m^2, and it was administrated intravenously at day1 and 8 of a 3 week cycle.

[Results] 46.6%, 81.0%, 86.2% of patients were pretreated with anthracycline, taxane and capecitabine, respectively. The response rate was 10.3%, and time to treatment failure was 91 days. The major toxicity was superficial phlebitis (60.3%) and grade 3 or 4 neutropenia (29.3%). These events were clinically tolerable.

[Conclusion] Vinorelbine demonstrated reasonable activity in heavily pretreated patients. Early introduction to metastatic breast cancer patients is recommended for better efficacy.

Key words：Vinorelbine, Metastatic breast cancer

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はじめに

Vinorelbineは転移・再発乳癌において，とくにanthracycline系薬剤あるいはtaxane系薬剤の既治療例に対する標準的な治療レジメンとして認められつつある．とくに，近年術前・術後化学療法としてこれらの2剤を使用する症例が増え，そのような症例が再発をきたした場合には第一選択の薬剤となり得る．

Vinorelbineは転移・再発乳癌に対して日本では2005年5月に承認された．当院でのvinorelbine単剤投与につき，その成績を後ろ向きに検討した．

1. 対象と方法

2005年5月30日～2006年1月31日までに当院でvinorelbine単剤投与を行った58症例．全例に外来通院での治療が行われた．

Vinorelbineは25mg/m^{2}をDay 1，8に投与し，21日を1サイクルとして繰り返し，病勢の進行がみられるまで，あるいは原病による全身状態の悪化や副作用により中止するまで継続した．

評価項目は奏効率，治療継続期間（time to treatment failure；TTF），毒性とした．有効性については乳癌取扱い規約第15版に基づいて，完全奏効（Complete Response；CR），部分奏効（Partial Response；PR），安定（Stable Disease；SD），進行（Progressive Disease；PD）を判定し[1]，毒性はNational Cancer Center Institute Common Terminology Criteria for Adverse Events v3.0（CTCAE）に基づいて判定した．

*1 癌研究会有明病院化学療法科

表1　患者背景

		V単剤群
症例数		58
年齢	中央値	53.7 (29～79)
	< 50	18 (31.0%)
	≧ 50	40 (69.0%)
進行・再発の別	進行乳癌	6 (10.3%)
	再発乳癌	52 (89.7%)
転移部位	軟部組織	25 (43.1%)
	骨	37 (63.8%)
	内臓	49 (84.5%)
病巣部位数(臓器)	1	8 (13.8%)
	2	16 (27.6%)
	3	12 (20.7%)
	4	12 (20.7%)
	≧ 5	11 (19.0%)

表2　患者背景

		V単剤群	
再発に対する化療レジメン数	0	1 (1.7%)	
	1	7 (12.1%)	
	2	9 (15.5%)	
	3	17 (29.3%)	70.7%
	4	12 (20.7%)	
	≧ 5	12 (20.7%)	
再発に対するanthracycline治療歴	あり	27 (46.6%)	
	なし	21 (36.2%)	
再発に対するtaxane治療歴	あり	47 (81.0%)	
	paclitaxelのみ	12 (20.7%)	
	docetaxelのみ	25 (43.1%)	
	両剤使用	14 (24.1%)	
再発に対するcapecitabine治療歴	あり	50 (86.2%)	
	なし	8 (13.8%)	

2. 結　果

1) 患者背景

患者背景を表1, 2に示した.

58症例の平均年齢は53.7歳で, 50歳以上が40例と, 全体の69%を占めた. とくに60歳以上の症例は14例(24.1%)であった. trastuzumabによる心機能障害のためにtrastuzumabの投与が不可能であった1例を除き全例がHER2陰性であった.

転移部位の分類は軟部組織転移（局所の皮膚, 胸壁, 所属リンパ節など), 骨転移, 内臓転移(肝, 肺, 胸膜, 卵巣, 脳, 消化管など)に分類したが, 84.5%の症例に内臓転移が存在した. また35例（60.3%）が3臓器以上に転移を有するなど, 多臓器に転移を有する症例が多くみられた.

さらに, vinorelbineをサルベージ療法としている症例が大半を占めたことを反映して, 表2に示すように前治療歴が濃厚な症例が多く, vinorelbine前に3以上のレジメン（術前・術後化学療法, ホルモン療法を含まない）が投与されている症例は41例（70.7%）を占めた. このうち, 再発・転移に対してanthracyclineを含むレジメンが行われた症例は27例（46.6%）あったが, 再発に対してanthracyclineを含むレジメンを用いていない21症例のうち17例（81.0%）は術前あるいは術後で用いていた. また, 再発・転移に対してpaclitaxelあるいはdocetaxelのいずれかのtaxaneを用いた症例は47例（81.0%）あったが, 用いていなかった11例のうち7例（63.6%）は術前・術後での使用歴があった. とくに前治療にpaclitaxel, docetaxelの両方を用いていた症例は14例（24.1%）であった. さらに, capecitabineを用いた症例は50例（86.2%）と, ほとんどの症例ではcapecitabineの使用歴があった.

2) 腫瘍効果

総合効果判定は, CRが0例, PRが6例であり, 奏効率(CR+PR)は10.3%であった. とくに, SDを含めたclinical benefit（CR+PR+SD）は55.2%であった(表3).

前治療の数別の奏効率を表3に示した. 3レジメン以上の前治療歴をもつ41症例でも奏効率は12.2%となり, clinical benefitは53.7%の症例で認められた.

また, 前治療としてanthracyclineを用いた症例での奏効率は10.8%,paclitaxel,docetaxelいずれかのtaxaneの前治療歴のある症例では10.6%であった. とくに, paclitaxel・docetaxel両剤を用いた症例でも7.1%の奏効率が得られた. さらに, anthracycline,taxane両方の前治療歴がある症例は33例(56.9%)であったが, その奏効率は12.1%であった. 前治療にcapecitabineを用いた症例の奏効率は12.0%で, とくにanthracycline,taxane, capecitabineの前治療歴のある30例(51.7%)の奏効率は13.3%であった. このように, 濃厚な前治療歴がある症例でも一定の奏効率が認められた.

これらの症例の平均観察期間は233.8日

表3　腫瘍効果 (n=58)

		CR	PR	SD	PD	NE	奏効率	
総合効果判定		0	6	26	24	2	10.3%	
再発に対する化療レジメン数	0	0	0	0	1	0	0.0%	
	1	0	1	2	3	0	16.7%	
	2	0	0	7	2	1	0.0%	
	3	0	2	7	8	0	11.8%	12.2%
	4	0	3	4	4	1	25.0%	
	≧5	0	0	6	6	0	0.0%	
anthracycline治療歴	あり	0	4	17	16	0	10.8%	
	なし	0	2	9	8	2	9.5%	
taxane治療歴	あり	0	5	22	18	2	10.6%	
	paclitaxelのみ	0	2	10	9	1	9.0%	
	docetaxelのみ	0	4	15	15	1	11.4%	
	両剤使用	0	1	7	6	0	7.1%	
capecitabine治療歴	あり	0	6	23	20	1	12.0%	
	なし	0	0	4	4	0	0.0%	

(19～406日) である．図1に示すようにTTFのKaplan-Meier曲線の中央値は91.0日，約3カ月であった．

3) 毒性

毒性の主なものを表4に示した．最も高率に発現したのは骨髄抑制であり，とくに好中球減少は75.9%の症例に認められたが，そのうちGrade 3 (好中球数<1000/mm³) 以上は29.3%で出現した．発熱性好中球減少症は1例に認められたのみであった．悪心・嘔吐などの消化器毒性は比較的少なく，悪心は全体の25.9%で認めたが，Grade3以上は3.4%の症例に認めたのみであった．Vinorelbineに特徴的な有害事象として末梢神経障害や表在性静脈炎がある．それぞれの発現率は19.0%，60.3%であったが，重症例は認められなかった．静脈炎に対しては8例 (13.8%) の症例でステロイド (dexamethasone 8 mg) の前投薬を行い，5例 (8.6%) に鎖骨下静脈へのポート挿入を行った．

3. 考　察

転移・再発乳癌においては，1st lineあるいは2nd lineとしてanthracyclineやtaxaneが用いられてきた．このためvinorelbineはcapecitabineとともに3rd line以降として位置付けられる薬剤であったが，近年術前あるいは術後化学療法にanthracyclineやtaxaneを用いることが一般的になったため，今後再発をきたす症例に対してはcapecitabineあるいはvinorelbineが1st lineになると考えられる．

これまでの報告では，vinorelbineは転移・再発乳癌における1st lineとして奏効率35～50%と報告されている[2~5]．さらに，投与量はそれぞれの試験によって20～35mg/m²/週とばらつきがあるものの，2nd～3rd line としてはanthracycline，taxane後であっても奏効率は16～36%と良好であり，約12～18週の無増悪期間 (Time To Progression；TTP) あるいはTTFが報告されている[5~9]．とくに，Toiらが報告したanthracycline，taxaneの前治療歴がある50症例での成績では，今回と同様の投与量・投与方法で奏効率20%，clinical benefitは58%と良好な成績が得られ，TTPも115.0日という結果であった[9]．

以上の結果を踏まえると，今回の症例は3レジメン以上の前治療歴がある症例が70.7%を占め，さらに多臓器転移を有する症例が大部分であったこともあり，奏効率としては10.3%と比較的低めではあったが，clinical benefitは55.2%と，病勢の進行をある程度抑制することは可能であった．

Vinorelbineはtaxaneと同じくtubulinに作用する薬剤ではあるが，これらの結果から部分交差耐性しか持たないものと考えられる．また，毒性が他の薬剤に比較して少なく，単剤でも1st lineでanthracycline,taxaneと遜色ない奏効率が得られ

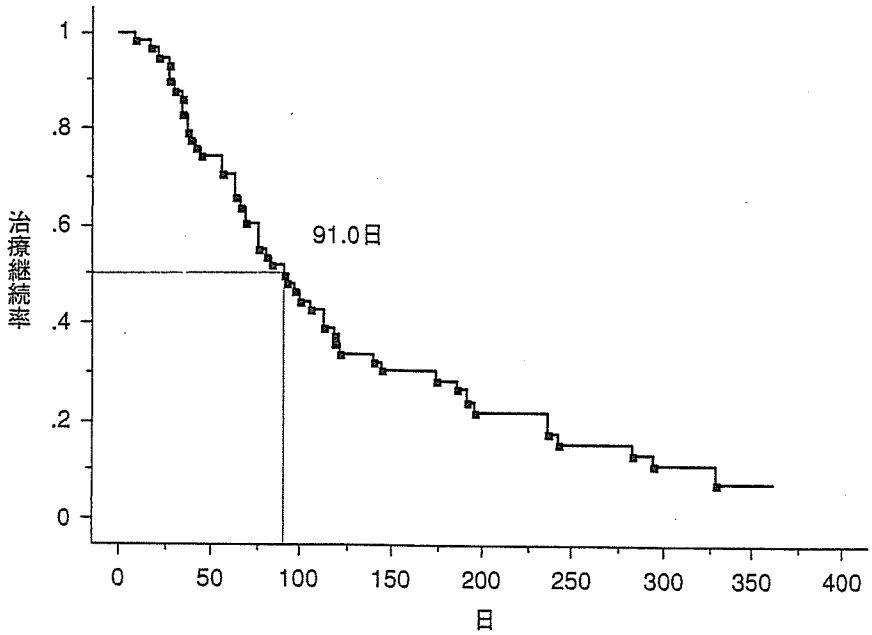

図1　Time to treatment failure, Kaplan－Meier (n＝58)

表4　副作用 (n＝58)

	Grade 1	2	3	4	発現率 (%)	G3以上の率 (%)
血色素量低下	15	6	2	0	39.7	3.4
白血球数減少	**14**	**24**	**5**	**0**	**74.1**	**8.6**
好中球数減少	**13**	**14**	**14**	**3**	**75.9**	**29.3**
発熱性好中球減少症	0	0	1	0	2.4	2.4
血小板減少	3	0	0	0	7.1	0.0
GOT上昇	22	4	1	0	46.6	1.7
GPT上昇	18	3	0	0	36.2	0.0
疲労	13	2	0	0	25.9	0.0
悪心	15	6	2	0	39.7	3.4
嘔吐	2	0	1	0	7.1	2.4
表在性静脈炎	**0**	**35**	**0**	**0**	**60.3**	**0.0**
知覚性神経障害	9	2	0	0	19.0	0.0
脱毛	2	0	0	0	3.4	0.0
発熱	13	2	0	0	25.9	0.0

ること，またとくにtrastuzumabとの併用によって68～78%という高い奏効率が得られるという点を考慮すれば，高い奏効率や長期の奏効期間を得るためには，より早い時期での使用を検討する必要がある[10~11]．

また，有害事象の評価の結果，数字の上では認容性は良好と考えられるが，実地臨床では表在性静脈炎（疼痛，皮膚の発赤，びらん形成）が問題となる症例が非常に多い．乳癌術後の症例では点滴が健側上肢に限定されること，また今回の検討では前治療歴が多い症例がほとんどであったこともその要因である．それに対して，投与前のステロイド点滴や中心静脈へのポート挿入といった対策を積極的に講じていく必要がある．

まとめ

1）vinorelbineは前治療歴が濃厚な症例に対して高い奏効率(CR＋PR)を得るのは難しいが，比較的高いclinical benefit (CR＋PR＋SD) を得ることができる．

2）高い奏効率や長期の奏効期間を得るためには，より早期での使用を検討する必要がある．

3）高頻度にみられる骨髄抑制への対応は比較的容易だが，表在性静脈炎の対策が必要な症例が多い．

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