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Intrapleural hypotonic cisplatin treatment for malignant pleural effusion in 80 patients with non-small-cell lung cancer: a multi-institutional phase II trial

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To assess the effect and toxicity of hypotonic cisplatin treatment (HPT) consisting of the intrapleural administration of cisplatin in distilled water for malignant pleural effusion in patients with non-small-cell lung cancer (NSCLC). Non-small-cell lung cancer patients with cytologically proven and previously untreated malignant pleural effusion were enrolled into this study. Firstly, the lung was fully re-expanded by a tube thoracostomy, and then 25 mg cisplatin in 500 ml of distilled water was instilled through a chest tube and then the tube was clamped. After 1 h, the tube was declamped and allowed to drain. The chest tube was removed when the pleural effusion volume decreased to 200 ml or less per day. A complete response (CR) was considered to occur when the pleural effusion disappeared. A partial response (PR) was determined to occur when the volume of pleural effusion remained under $\frac{1}{4}$ of hemithorax. The response at 4 weeks was evaluated by an extramural review. Out of 84 patients enrolled from February 1998 to August 2002, 80 patients were eligible and analysed in the present study. The toxicity of HPT was acceptable. Neither a haematological toxicity of any grade nor grade 4 nonhaematological toxicity was observed. Grade 3 nonhaematological toxicities were observed, including nausea (4%), vomiting (3%), pyothorax (1%) and dyspnoea (1%). The median time of drainage from HTP was 4 days. Twenty-seven (34%) and 39 (49%) patients achieved CR and PR, respectively, for an overall response rate of 83% (95% confidence interval, 74–91%). The median duration of the response was 206 days. The median survival time of all patients was 239 days. Hypotonic cisplatin treatment for malignant pleural effusion of NSCLC is therefore considered to be feasible and effective. A phase III study of HPT is thus warranted.

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Malignant pleural effusion is considered to be the first clinical manifestation of malignancy as well as the first sign of recurrent cancer. Among the various kinds of malignancies, lung cancer is the leading cause of malignant effusion (Chernow and Sahn, 1977; Shan, 1987). The standard treatment for a patient with a symptomatic malignant pleural effusion due to non-small-cell lung cancer (NSCLC), whose life expectancy is not too short, is considered to be a tube thoracotomy with subsequent pleurodesis (Ruckdeschel *et al*, 1991; Walker-Renard *et al*, 1994).

Ichinose *et al* (1997) reported that intraoperative intrapleural treatment using hypotonic cisplatin solution (cisplatin solution diluted by distilled water) effectively controlled malignant pleural effusion and/or pleural dissemination found at thoracotomy in NSCLC patients. According to their experimental data, hypotonic cisplatin solution demonstrated a significantly greater antitumour activity than either isotonic cisplatin or distilled water alone (Ichinose *et al*, 1993).

Ushijima *et al* (1997) applied this hypotonic cisplatin treatment (HPT) consisting of the intrapleural administration of cisplatin in distilled water after tube thoracostomy to treat malignant pleural effusion due to NSCLC and gastric cancer, and reported successful results in several patients. A multi-institutional phase II trial was thus conducted to assess the effect and toxicity of HPT for malignant pleural effusion due to NSCLC.

PATIENTS AND METHODS

Patient eligibility

The patients were eligible for this phase II trial if they had cytologically proven and previously untreated malignant pleural

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effusion of NSCLC, which had either not yet been treated or had been treated 4 weeks or more before enrolment. All participants were required to be under 80 and 80 year of age, with a leucocyte count of $\leqslant 4000\,\mu l^{-1}$, a platelet count of $>100\,000\,\mu l^{-1}$, a serum bilirubin level $\leqslant 1.5\,mg\,dl^{-1}$, a normal creatinine level and serum glutamic oxaloacetic transaminase/glutamic pyruvic transaminase levels of no more than twice the upper limit of normal. The patients were also required to have a sufficient re-expansion of the lung after chest tube drainage and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1 or 2 after drainage. The extent of re-expansion was re-assessed by an extramural review committee. Systemic chemotherapy or radiotherapy to the lung, mediastinum and pleura was not given for 4 weeks after HPT. This trial was approved by the institutional review boards of each participating institution, and written informed consent was obtained from all patients.

Treatment methods

The lung was fully expanded by a thoracostomy using a chest tube with a double lumen, and then the patients were enrolled into the trial via facsimile by the administration office of the Kyushu Yamaguchi Thoracic Oncology Group in the National Kyushu Cancer Center. First, premedications, intramuscular injection of 15 mg of pentazocine and intrapleural administration of 10 ml of 1% lidocaine were performed. Thereafter, 25 mg of cisplatin in 500 ml of distilled water was instilled through a chest tube. The hypotonic cisplatin solution was prepared as follows: 50 ml cisplatin solution containing 25 mg cisplatin was injected into the bottle containing distilled water of 500 ml. The chest tube was clamped for 1 h. The patients were then asked to change position (supine and bilateral decubital) from time to time during the treatment regimen, and then the tube was declamped and allowed to drain under a negative pressure of 10–15 cm H_2O. When the drainage effusion was less than 200 ml a day, the chest tube was removed. Any patient whose drainage effusion continued for over 2 weeks was withdrawn from the trial and was also judged to be a nonresponder.

Evaluations

Toxicities were evaluated according to ECOG common toxicities criteria (Oken *et al*, 1982).

The response to HPT was evaluated based on the findings of posteroanterior and lateral chest radiographs 4 weeks after HPT. The response criteria used were as follows: a complete response (CR) when no pleural effusion was observed; a partial response (PR) when pleural effusion was observed, but the level of effusion was less than 25% of the long axis of the hemithorax; and no response (NR) when effusion was larger than that defined by PR. A chest radiograph in responding patients was taken at least every month in order to monitor the condition of the controlled pleural effusion. The response and duration of response were determined by an extramural review committee.

A progression of effusion was defined as when pleural effusion of more than 25% of the long axis of the hemithorax was observed or tube drainage was needed. The effusion-progression-free survival time was defined as the time from the enrolment until the progression of effusion or death without a progression of effusion. The overall survival was defined as the time from enrolment until death from any cause.

Statistical analysis

The primary end point of the study was the overall response rate including CR and PR. Based on the assumption that a response rate of higher than 75% would warrant further investigation of this treatment, and that a rate below 60% would make such an investigation unnecessary, a total sample size of 62 patients was required with an alpha error of 0.05 and a beta error of 0.20, using the mini-max two-stage sequential design by Simon. The first stage of the study required 30 patients, and if at least 18 responses were observed, then a second stage required 32 patients would be conducted. Since ineligible patients would be included, the accrual of at least 70 patients was thus planned.

RESULTS

Patient characteristics

Eighty-four patients were enrolled into this trial from February 1998 to August 2002. However, four patients were later judged to be ineligible: two patients had malignant pleural effusion due to either parotid gland cancer or uterus cancer, one patient had bilateral malignant effusion and one patient had a poor PS who had no chest X-ray film in the standing position. As a result, 80 patients were eligible and thus were analysed in the present study. As shown in Table 1, they included 40 men and 40 women with a median age of 67 years. Most patients had an ECOG PS of 0 or 1 and a histology of adenocarcinoma. In addition, 73% of the patients had received no prior therapy. Although the extent of a re-expansion of the lung after tube thoracotomy was judged to be equivocal in four patients at an extramural review committee, those patients were included in the analysis.

Adverse events

Neither the haematological toxicity of any grade nor of grade 4 nonhaematological toxicity was observed in all 80 eligible patients (Table 2). Grade 3 nonhaematological toxicities were observed, including nausea (4%), vomiting (3%), dyspnoea (1%) and pyothorax (1%).

Table 1 Patient characteristics

Enrolled patients	84
Eligible patients	80
Gender	
Male	40 (50%)
Female	40 (50%)
Age median (range)	67 (35–89)
ECOG performance status	
0	24 (30%)
1	47 (59%)
2	9 (11%)
Stage	
IIIB	35 (44%)
IV	45 (56%)
Histological type	
Adenocarcinoma	77 (96%)
Squamous cell carcinoma	3 (4%)
Prior therapy	
None	58 (73%)
Surgical resection only	7 (9%)
Chemotherapy or radiotherapy	10 (13%)
Surgical resection plus chemotherapy or radiotherapy	5 (6%)
Re-expansion of lung	
Sufficient	76 (95%)
Equivocal	4 (5%)

Response

Among the 80 patients including one patient whose response was not evaluable due to complications of pyothorax, 27 patients (34%) had CR and 39 patients (49%) achieved PR with an overall response rate of 83% (95% confidence interval 74–91%). There were also 13 patients with NR. Two of these 13 patients were withdrawn from the trial since their effusion could not be controlled within 2 weeks after HPT. The median duration of drainage after HPT in the responding patients was 2 days ranging from 1 to 22 days. The median time of response was 206 days in 66 responding patients ranging 36–949 days.

Survival

The median follow-up period was 1045 days (range, 424–2061 days). The median effusion-progression-free survival time and 1-year effusion-progression-free survival rate of all 80 patients were 173 days and 31.8% (95% confidence interval, 22–42%). The median survival time and the 1-year survival rate of all 80 patients were 239 days and 39% (95% confidence interval, 28–49%) as shown in Figure 1.

DISCUSSION

The agents administered intrapleurally for the management of malignant pleural effusion were classified as either non-anticancer or anticancer drugs. The non-anticancer drug had a sclerosing agent that produces pleurodesis. The most frequently used agent is talc in the United States (Hausheer and Yarbro, 1985), tetracycline or doxycycline (Putnam *et al*, 1999) in the United Kingdom and OK432, which is prepared from a substrain of *Streptococcus pyrogenes* A3, in Japan (Saka *et al*, 1994). In a majority of cases, talc is administered by pleural pourdage through thoracoscopy using either local anaesthesia or general anaesthesia by the surgical team. Although the response rate to thoracoscopic talc insertion is reported to be over 90%, the requirement of thoracoscopy leads to some limitations in the use of talc. In addition, the frequency of severe chest pain induced by treatment with talc pleurodesis was reported to be 17% (Hartman *et al*, 1993). On the other hand, no such pain was observed in the 80 patients who received this intrapleural HPT for malignant effusion due to NSCLC. The intrapleural administration of the tetracycline or doxycycline was reported to be effective for the control of malignant effusion. However, it has recently become difficult to use tetracycline or doxycycline since these have been withdrawn from the market in 1991 and 1997, respectively. The administration of OK432 in saline solution is easily performed through a chest tube and it is reported to control over 70% of malignant pleural effusion cases. However, its usage is still limited to only Japan.

Although anticancer drugs administered intrapleurally for the management of malignant pleural effusions are expected to have both cytotoxic and sclerosing effects, the mechanism of action for controlling effusion remains unclear. The results of etoposide (Holoye *et al*, 1990), fluorouracil (Suhrland and Weisberger, 1965), mitomycin-C (Luh *et al*, 1992) or doxorubicin (Desai and Figueredo, 1979) investigated in various trials have not proven to be sufficiently attractive and these agents are therefore not presently in use. In a randomised trial comparing intrapleural administration of bleomycin with intrapleural tetracycline, bleomycin has been proven to be superior to tetracycline for the management of malignant pleural effusion (Moffett and Ruckdeschel, 1992). However, bleomycin has not been used extensively due to its high expense. The Lung Cancer Study Group performed a phase II trial of the intrapleural administration of combination chemotherapy using cisplatin and cytarabine in 46 patients, of whom about half had NSCLC (Rusch *et al*, 1991). Tohda *et al* (1999) also performed a similar phase II trial using cisplatin plus etoposide in 70 NSCLC patients. The overall response rate was reported to be 49% in the former trial and 46% in the later trial. As the criteria of the response and proportions of the disease in the subjects vary between the different trials, an accurate comparison of the results is difficult. However, the response rate reported in

Table 2 Nonhaematological toxicities

Grade	1	2	3	4
Nausea	21	11	3 (4%)	0
Vomiting	7	7	2 (3%)	0
Fever	11	0	0	0
Dyspnoea	5	2	1 (1%)	0
Chest pain	13	5	0	0
Infection	0	0	1 (1%)	0

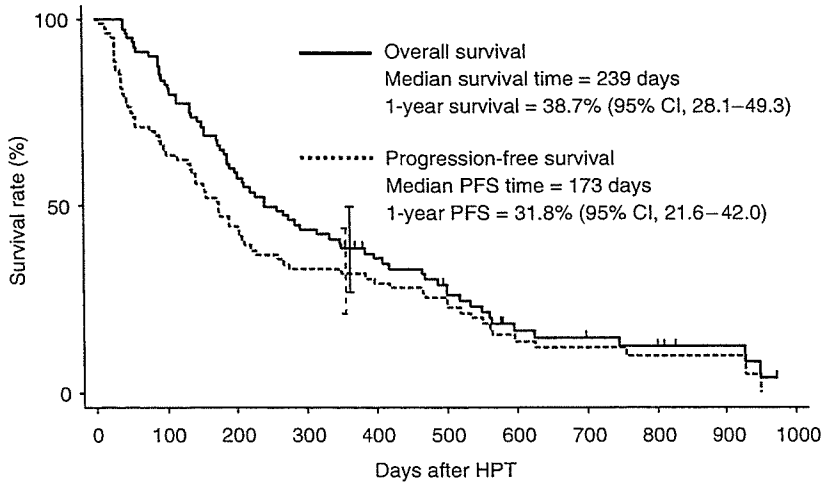

Figure 1 Overall survival and progression-free survival. Each tick mark and bar represents a patient who is alive and the 95% confidence interval of the survival rate, respectively.

those intrapleural cisplatin-based chemotherapy trials seems to be, on the whole, inferior to that in the trials using a non-anticancer agent.

In the present phase II trial of the intrapleural administration of hypotonic cisplatin solution in 80 patients with NSCLC, the overall response rate was 83%. The criteria of the response in this trial were similar to those of the cisplatin-based intrapleural chemotherapy trials mentioned above. The main difference between the previously reported intrapleural cisplatin-based chemotherapy and our HPT is the use of isotonic saline in the former and distilled water in the latter for a dilution of cisplatin, which itself is an isotonic solution. Ichinose *et al* (1993) found that hypotonic cisplatin solution whose cisplatin concentration and osmolarity ranged between 5 and 50 $\mu g\,ml^{-1}$ and between 2.8 and 28 $mOsm\,l^{-1}$, respectively, had a significantly stronger antitumour activity than either isotonic cisplatin or distilled water alone in an *in vitro* experiment of short-time exposure ranging from 0.5 to 10 min. In a prospective study in patients whose malignant pleural effusion and/or pleural dissemination were found at thoracotomy, intraoperative intrapleural HPT for 15 min before the closure of thorax prolonged the control of the pleural disease (Ichinose *et al*, 1997). In addition, a randomised phase III trial demonstrated the intraoperative intrapleural HPT in resected NSCLC patients with a positive intrapleural lavage cytology finding to significantly decrease the occurrence of malignant effusion and/or pleural dissemination after operation (Ichinose *et al*, 2002). The cisplatin concentration (50 $\mu g\,ml^{-1}$) and hypotonicity (28 $mOsm\,l^{-1}$) were the same as those in the present trial. The mechanism by which the HPT shows an antitumour effect is considered to be as follows: (1) distilled water itself has a direct cytotoxicity (Ichinose *et al*, 1993), (2) tumour cells exposed to hypotonic cisplatin increase their cellular cisplatin level since the cells become swollen by the hypotonic solution (Ichinose *et al*, 1993) and (3) chloroaqua and diaqua, formed by the hydrolysis of cisplatin in distilled water, are also believed to be active antitumour agents (Rosenberg, 1978; Sherman and Lippard, 1987).

The incidence of the toxicities seen in this study was significantly lower than that in the cisplatin-based chemotherapy trials. Grade 3 non-haematological toxicities were observed, including nausea (4%), vomiting (3%), dyspnoea (1%) and infection (1%) in the present trial, while 90% of the patients had gastrointestinal toxicities including grade 3 and 49 and 9% of the patients experienced haematologic toxicities of grades 1 plus 2 and that of grade 3, respectively, in the trial of the Lung Cancer Study Group. Tohda *et al* (1999) reported a gastrointestinal toxicity of grade 3 in 27% of the patients. The main reasons for a different incidence of toxicities between the two trials and our trial are considered to be due to the differences in intrapleural exposure time of the agent and the administered dosage between trials: (1) the clamping time after the intrapleural administration was only 1 h in our trial compared to either 4 or 72 h in the previous trials and (2) the dose of cisplatin administered was 25 $mg\,body^{-1}$ once or twice in our trial, while it was 80 and 100 $mg\,m^{-2}$ in the previous trials. Regardless of the administration of cisplatin with lower dosage as well as the shorter treatment time, the response rate of the HPT, nevertheless, seems to be superior to that of the previously reported chemotherapy trials.

In addition, the overall survival and effusion-progression-free survival curves are closely similar as shown in Figure 1. This observation indicates that malignant pleural effusion was controlled by HPT in most patients.

Although HPT is considered to be a feasible and active treatment for malignant effusion due to NSCLC, further trials are called for. The Japan Clinical Oncology Group (JCOG) conducted a randomised phase II study for malignant pleural effusion due to NSCLC, by comparing bleomycin, OK-432 and cisplatin plus etoposide. We therefore intend to conduct a phase III study to compare HPT with the best arm of the JCOG trial.

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REVIEW ARTICLE

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Methodological aspects of current problems in target-based anticancer drug development

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Abstract Differently from the conventional antineoplastic agents, target-based drugs are designed a priori, based on our knowledge of various physiological molecules that has been obtained by the development of molecular biology. This "Copernican revolution" in drug development may imply a paradigm shift in this field. However, contrary to the initial expectations, many drugs developed by this approach are now faced with difficulties, mainly because of the fundamental and theoretical limits of this approach. All of the physiological functions are not always known in all target molecules. In low-molecular-weight drugs, i.e., "inhibitors," targets disperse, due to the structural similarities in physiological molecules. This double-faced "out-of-focusing" causes many problems in various steps of drug development, drug design, clinical trials, and administration to patients. Many drugs are now being abandoned because of unexpectedly lower response rates or unforeseeable adverse effects, and the variety of the drugs exhibits a kaleidoscopic appearance. The double-faced "out-of-focusing" derives from the methodological limits in molecular biology, i.e., elementalism, and limits in our techniques for drug development. To overcome these currently inevitable limits, it appears essential to elucidate the specific changes in target molecules that chiefly promote tumor growth and, consequently, strongly predict response to the administered drugs. Precise and efficient detection of responder populations is the key to the development and establishment of target-based anticancer therapies.

Key words Target-based anticancer agents · Imatinib · Gefitinib · Trastuzumab

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Introduction – from discovery to design: a "Copernican revolution" in drug development?

In the past decade, a new approach for cancer treatment has emerged. In contrast to conventional drug development, this new approach, now widely referred to as "target-based" therapies, employs drugs that have been designed to work on a single molecule functioning in the body. Thus far, drugs have been discovered by the screening and chemical modification of naturally occurring compounds, according to biological, i.e., phenomenological, activities. Target-based drugs, on the other hand, are designed a priori, based on the knowledge of each physiological molecule that has been obtained by the development of molecular biology. This dramatic change in methodology may imply a "Copernican revolution" in drug development and a paradigm shift in this field. However, contrary to the initial expectations, many drugs that have been developed by this approach are now confronted with difficulties. Although target-based drugs are defined as those designed to target a single molecule in cells, they are, in fact, developed by the screening and chemical modification of known inhibitors or newly synthesized compounds, as are classical drugs. The exceptions are recombinant protein drugs and antibody drugs. The latter, in particular, have become possible and available because of the target-based approach. Low-molecular-weight drugs, i.e., "inhibitors", predominate in this field, and a limited number of antibody drugs are now available or being developed. Although some recombinant protein drugs have been developed, none are now regarded as promising. This discrepancy between the ideal and the real in the techniques used for drug development underlies the currently inevitable limits of the target-based approach.

Furthermore, our knowledge obtained by molecular biology and biochemistry is not always complete. Using only these theoretically "elementalistic" methodologies, it appears difficult to fully understand cells or organisms, which may form "complex" systems. This elementalistic tendency in the methodologies underlying target-based drug development also leads to its fundamental and theoretical limits. In this article, we discuss the methodological aspects of current problems in target-based anticancer therapies.

Current status of target-based anticancer drug development

In Table 1, we have summarized studies of target-based anticancer drugs reported at the American Society for Clinical Oncology (ASCO) annual meetings from 2002 to 2005, and several intriguing tendencies can be seen in this field. First, growth factors and their receptors predominate as the target molecules. Particularly, higher priorities appear to be given to inhibitors of tyrosine kinase receptors, except for the first tyrosine kinase inhibitor, imatinib, which counteracts the disease-specific fusion gene product including the nuclear tyrosine kinase, c-ABL. Second, low-molecular-weight compounds referred to as inhibitors are predominant. There are no recombinant protein drugs in the Table, with the exception of angiostatin and endostatin, which were developed, but have now been abandoned. Only two antibody drugs, bevacizumab and cetuximab, are consistently being studied, although there are some antibody drugs that are now regarded as established target-based anticancer agents. However, more importantly, the most remarkable tendency in this field is that drugs have a rapid turnover, with the life span of many drugs being short. In Table 1, there are only four drugs that have been consistently developed during this 4-year period. Although the cost of development of each target-based drug is vast, many drugs are being abandoned for various reasons, mainly the unexpectedly lower response rates and unforeseeable adverse effects. For the more efficient and effective development of target-based anticancer agents, it appears to be important to discuss carefully these negative aspects and their causes in the development of this category of drugs. We discuss these problems below.

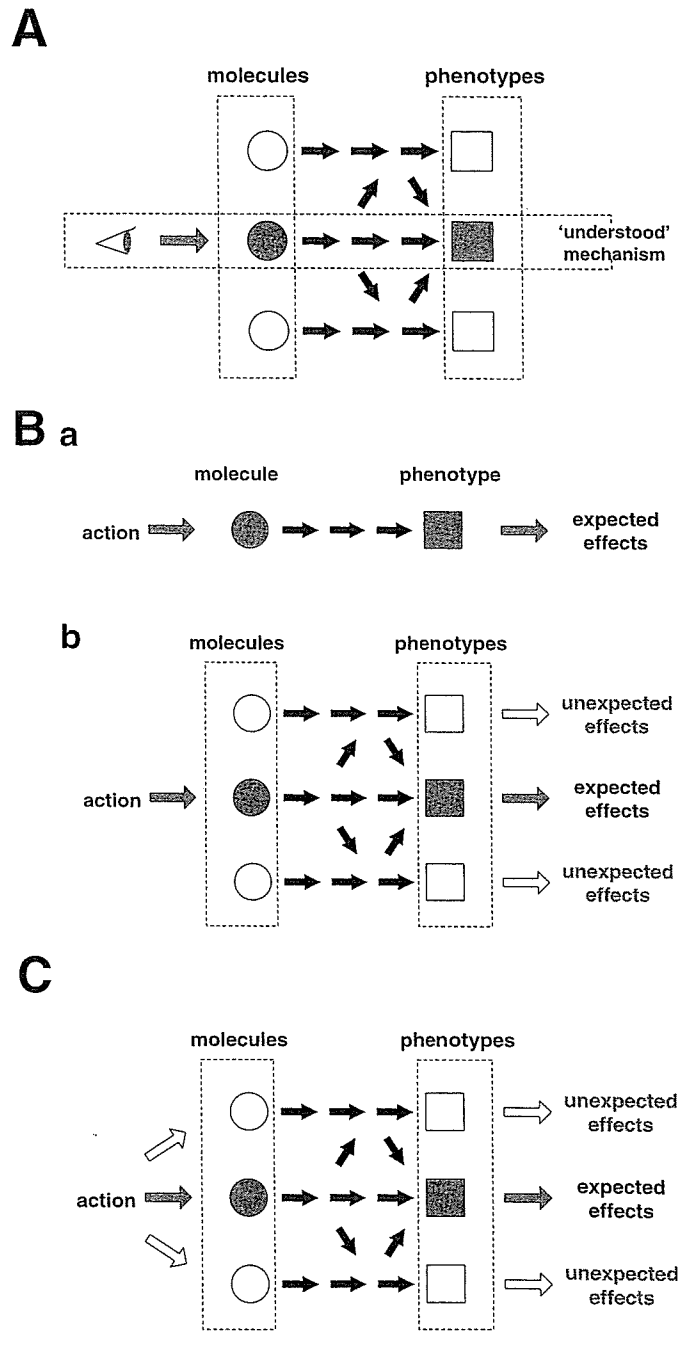

Fig. 1A–C. Schematic representations of the relationships among drugs, target molecules, phenotypes, and effects. **A** Our viewpoint; **B** recombinant protein/antibody drugs; **C** low-molecular-weight drugs

Problem I: mechanisms of action and drug design

Target-based anticancer agents can be classified into two categories: (a) recombinant proteins/antibodies and (b) low-molecular-weight compounds. In drugs in the former category, mechanisms of action appear simple and unequivocal (Fig. 1B*a*). However, these agents test our real and net knowledge of the physiological functions of the molecules in question. Angiostatin and endostatin are examples of proteins that were regarded as candidates in this category. Development of these recombinant protein drugs unexpectedly faced a deadlock, since the initial experimental results could not be reproduced in several independent experimental systems. Apart from the lack of reproducibility associated with technical problems, this symbolic example indicates the fundamental and theoretical limits of target-based drug development. In fact, all of the physiological functions are not always known in all the molecules regarded as a targets (Fig. 1A). As another example, some matrix metalloprotease (MMP) inhibitors were regarded as promising candidates as neo-

vascularization inhibitors, although they had been developed as inhibitors of invasive tumor growth. This example also reflects the fundamental and theoretical limits of target-based approaches.

It is evident that molecular biology and biochemistry largely underlie target-based drug development, because this approach address a single molecule that functions in the body of an organism. However, as is widely recognized, molecular biology and biochemistry are theoretically elementalistic methodologies. Cells are "complex" systems in which the relationship between a part and the whole is not simple, as it is in a clock or a car. From these molecular biology and biochemistry approaches only, it is sometimes difficult to understand the real relationship between a part and the whole, i.e., the functions of physiological molecules and cellular phenotypes or phenomena in the whole body of an organism. Nevertheless, molecular biology and biochemistry are essential for molecule-based drug design. Attention must be paid to the "understood" functions of physiological molecules. Before designing target-based drugs, it appears to be important to verify objectively all of our knowledge of the physiological functions of the molecule in question. Thus, target-based approaches involve fundamental and theoretical limits.

In drugs classified as low-molecular-weight compounds, this problem becomes more critical (Fig. 1C). As mentioned above, many anti-receptor tyrosine kinase inhibitors are now being developed. However, this category, tyrosine kinase is comprised of vast numbers of diverse molecules. In fact, receptor tyrosine kinases can be classified into four subtypes, according to structural similarity, and the structures of the functional domains exhibiting tyrosine kinase activity are very similar in each subtype.[1] It is now known that an agent which was initially designed to target a single tyrosine kinase molecule can exhibit its inhibitory effect on other tyrosine kinase molecules, due to structural similarity in the kinase domains. A typical example is imatinib (STI571; Gleevec). Imatinib was originally designed to inhibit tyrosine kinase activity in the products of the *BCR-ABL* fusion gene that is a hallmark of chronic myeloblastic leukemia (CML).[2] The tyrosine kinase activity in BCR-ABL fusion proteins is derived from the unique tyrosine kinase protein which functions in nuclei, c-ABL. Because there is a similarity between the kinase domain structure of c-ABL and those of other tyrosine kinases, such as platelet-derived growth factor receptors (PDGFR) and c-KIT, it has been demonstrated that imatinib also inhibits the kinase activity in these other receptor tyrosine kinases.[3] Indeed, imatinib has been tested to see whether it exhibits growth inhibitory effects on gastrointestinal stromal tumors (GIST) that overexpress c-KIT tyrosine kinase,[4] and it is now the first-choice drug for the treatment of this neoplastic disease.

Similarly, SU5416[5] and SU6668[6] were initially developed as inhibitors of vascular endothelial growth factor receptor (VEGFR), Flt-1 (VEGFR-1), and Flk-1 (VEGFR-2). These drugs have also been proven to inhibit other tyrosine kinase activities, including PDGFR, fibroblast growth factor receptor (FGFR), and c-KIT.[7] Thus, in the category of low-molecular-weight compounds referred to as "inhibitors," molecular targets inevitably disperse, due to the structural similarities in the functional domains between the initially targeted molecules and related proteins. In CML, in which tumor growth depends exclusively on the activity of a single, unique, i.e., disease-specific, molecule, BCR-ABL fusion protein, this problem does not emerge. However, this case is exceptional. The *BCR-ABL* fusion gene is found in leukemia cells in almost 100% of CML patients. On the other hand, in almost all other malignancies, the contribution of a targeted molecule to tumor growth is not always exclusive, and is sometimes marginal, and the extent of contribution varies widely between individuals. This problem leads directly to other problems concerning, for eaxmple, clinical trials, criteria for administration, and adverse effects, which are discussed below.

Problem II: clinical trials

In clinical trials in general, inflation of the false-negative rate, in other words, decrease in the statistical power, influences the results greatly, particularly when the fraction of the drug-sensitive population in a trial is small compared to the whole patient population.[8,9] The sample size, i.e., the number of patients, in clinical trials is generally determined based on the expected difference of treatment effects between the experimental and control arms, with given α and β levels. According to calculation by the logrank test, when one requires 80% statistical power to detect an improvement in 1-year survival from 10% to 20% with a two-sided 0.05 level of significance in a placebo-controlled parallel group trial, 127 patients need to be randomized to each treatment arm. This estimation is implicitly based on the assumption that the drug should be uniformly effective in all patients. However, if the drug has an effect only in a limited fraction of the whole patient population ("responder fraction") and the rest of the population has no benefit from the treatment, the statistical power decreases dramatically. Figure 2 shows the relationship between the responder fraction and the statistical power. In the case that the responder fraction is 30%, the false-negative rate reaches 50%, which implies that studies are judged as negative with a one-in-two probability, despite the fact that the treatment itself has a clinical benefit. Needless to say, in order to judge this case to be positive with a higher probability, larger sample sizes are required. For positive results, the sample size is inversely proportional to the responder fraction.

When the dependence of tumor growth on a target molecule is not exclusive and, consequently, the sensitivity to a drug targeting the molecule varies depending on the individual, the responder fraction in the patient population is limited, and studies trying to demonstrate a clinical benefit of the treatment face difficulties. This problem may partly explain the rapid turnover and the short life spans of target-based anticancer drugs.

Table 1. Clinical trials on molecular target-based drugs reported at ASCO meetings 2002–2005

Code name	INN	Commercial name	Targeted Molecule	2002	2003	2004	2005
1. Growth factor antibody							
anti-VEGF	Bevacizumab	Avastin	VEGF Ab	CR,R(I-II)	CR(cIII), B(cIII), Pa(cII), Pr(cII), Lu(cII)	Hp(sIII), Pa(cII), R(cII), B(cI), CR(cII)	B(cIII, cII), Lu(cII/III, cII), CR(cIII, cII), G(cII), Hp(sII,cII), Pa(cI), R(cII), Ov(cII,sII)
VEGF Trap			VEGF1,2 Ab		–(sI)		
2. Growth factor receptor antibody							
IMC-1C11			VEGFR2 Ab	CR(I)			
anti-VEGFR1		Angiozyme	VEGFR1 Ab	–(I)			
ABX-ECF			EGFR Ab	R(I-II)	CR(sII)		
EMD72000	Matuzmab		EGFR Ab	ST(I)	–(sI)	CR(sI), HN(sI), Pa(cI)	
IMC-C225	Cetuximab	Erbitux	EGFR Ab	HN, Lu, CR(II-III)	–(sI/II), CR(cII), Lu(cII)	HN(cIII), Lu(cII), CR(cII), CR(cIII)	CR(cIII,cII),Lu(sII)
rhuMAb2C4	Pertuzumab		Her2 Ab		–(sI)		
3. Growth factor inhibitor							
ABT-526			(VEGF,bFGF,IL-8,HGF) Ihb			My(cII)	
VEGF Trap			VEGF Ihb			–(sI)	
VEGF-antisense	Veglin		VEGF Ihb			–(sI), Hm(sI)	
ABT-510			(VEGF,bFGF,IL-8,HGF) Ihb			–(cI)	S(sII),R(sII)
4. Tyrosine Kinase Receptor Inhibitor							
PKI166/CGP75166			TK(EGFR,erbB2) Ihb	–(I)			
SU6668			TK(VEGFR) Ihb	–(I)			
CP-547632			TK(VEGFR) Ihb	–(I)		Lu(cI)	
STI571	Imatinib	Gleevec	TK(BCR-ABL) Ihb	CML,GIST(I-III)	R(sII), Pr(sII)		
ZD1839	Gefitinib	Iressa	TK(EGFR) Ihb	Lu,R,HN,CR(I-II)	B(sII), R(sII)		
SU5416	Semxnanib		TK(VEGFR) Ihb	AML,Lu,R,Pr(I-II)	MI(sII)		
OSI-774	Erlotinib	Tarceva	TK(EGFR) Ihb	–,Lu(I-III)	B(sII/cI),CR(sII/cI),Lu(sII/cI)	Lu(cIII), Lu(sII), Hp(sII), R(sII), CR(cII), B(cI), Pa(cI), GI(sII)	Pa(cIII), Pa(cII), Hp(sII), Lu(sII), B(sII), –(cI)
CI-1033	Canertinib		TK(pan-erbB) Ihb	–(I)	–(sI)	Ov(sII), –(sI)	
PTK787/ZK222584	Vatalanib		TK(VEGFR1,2,3) Ihb	GI,–(I)	GI(sI),CR(sI,cI),AML(sI, cI)	CR(cII)	CR(cIII,cI,cI/II), Hp(sI), Ov(cI)
ZD6474			TK(VEGFR,EGFR) Ihb	–(I)	–(sI)	Lu(cII)	Lu(cII)
EKB-569			TK(EGFR,Her2) Ihb		Pa(cI)		
CEP-701			TK(FLT3) Ihb		AML(sII)		
BAY43-9006	Sorafenib		TK(Raf Kinase,VEGFR,PDGFR) Ihb		–(sI, cI)	R(sII), Pa(cI/II), –(cI)	R(sIII,sII), HN(sII), Lu(cI), MI(cI,sI/II), –(sI)
GW572016	Lapatinib		TK(ErbB1,ErbB2) Ihb		–(Is)	B(cII), –(sI), –(cI)	B(sII,cI), Lu(sII), –(cI)
CP-724714			TK(EebB2)			B(sI)	
AZD2171			TK(VEGFR) Ihb			–(sI)	–(sI),Pr(sI)
GW786034			TK(VEGFR1,2,3) Ihb			–(sI)	–(sI)
AMG706			TK(VEGFR1,2,3,PDGFR,KIT,RET) Ihb				–(sI)
AEE788			TK(EGFR,Her2,VEGFR) Ihb				–(sI),GI(sI)
CHIR-258			TK(VEGFR,FLT3,EGFR,KIT) Ihb				–(sI)
BIBF1120			TK(VEGFR,PDGFR,KIT,FGFR) Ihb				–(sI)
BMS-354825	Dasatinib		TK(SRC,BCR-ABL,PDGFR,KIT) Ihb				–(sI),GIST(sI)

Table 1. Continued

Code name	INN	Commercial name	Targeted Molecule	2002	2003	2004	2005
5. Inhibitors of downstream signal transduction molecules							
R115777		Zarnestra	Farnesyl transferase Ihb	CR,Pa,Lu,B,Pr(II-III)			
SCH66336	Lonafarnib	Sarasar	Farnesyl transferase Ihb	–(I)			
LY317615			Protein Kinase Cb Ihb	–(I)			
CI-1040			MEK Ihb	–(I)	Lu(sII), B(sII), CR(sII), Pa(sII)		–(sI)
CCI-779	Temsirolimus		mTOR Ihb		R(cI)	R(cI), B(cII)	Lu(cII), B(cII)
RAD001	Everolimus		mTOR Ihb			GIST(cI/II)	–(sI)
AP23573			mTOR Ihb			–(sI)	S(sII), Hm(sII)
PD0325901			MEK Ihb				–(sI/II)
CP-751821			IGF-1R Ihb				My(sI)
6. Inhibitors of cell cycle regulatory proteins							
HMR1275	Alvocidib		CDK Ihb	Lu,CR, –(I)	Lu(cI)		
BMS-387032			CDK2/Cyclin E Ihb		–(sI)		
7. Inhibitors of proteasome							
PS-341	Bortezomib	Velcade	Proteasome Ihb	My, –(I-II)	Lu(sII),B(cI),Hm(cI),Ov(cI)	My(cIII), CR(cII), Hp(sI/II), Lu(cII), Lu(sII), Lu(cI), B(sII), Pr(cI/II)	
8. Inhibitors of chromatin regulator molecules							
CI-994			Histon deacetylase Ihb	Lu,Pa, –(I-II)	Lu(cII)		
MS-275			Histon deacetylase Ihb		–(sI)		
9. Inhibitors of Secretory Proteins etc							
BB-2516	Marimastat		MMP Ihb	CR(II)			
AG3340	Prinomastat		MMP Ihb	Lu,Es(II-III)			
CP-471358			MMP Ihb	–(I)			
rh-angiostatin			HmRcmb angiostatin	–(I)			
rh-endostatin			HmRcmb endostatin	–(I)			
10. Others							
TLK286			Glutathione-S-transferase P1 Ihb		Lu(sII),Ov(sII)		
Celecoxib	Celecoxib	Celebrex	COX-2 Ihb		B(sII-III),Ut(sII),Lu(cII)		
Rituximab	Rituximab	Rituxan	CD20 Ab			Ly(sIII)	

cI,cII,cIII, phase I, II, III trials using combination treatment regimen; sI,sII,sIII, phase I, II, III trials using single treatment regimen; B, breast cancer; CR, colorectal cancer; Es, esophagus cancer; Gl, glioma; Hm, hematological malignancies; HN, head & neck cancer; Hp, hepatic cancer; Lu, lung cancer; Ly,lynphoma; Ml, melanoma; My, myeloma; Ov, ovarian cancer; Pa, pancreatic cnacer; Pr, prostate cancer; R, renal cancer; S, sarcoma; Ut, uterine; –, unspecified; Ab, antibody; Ihb, inhibitor; TK, tyrosine kinase; CDK, cycline dependent kinase; MMP, matrix metalloproteinase; HmRcmb, human recombinant

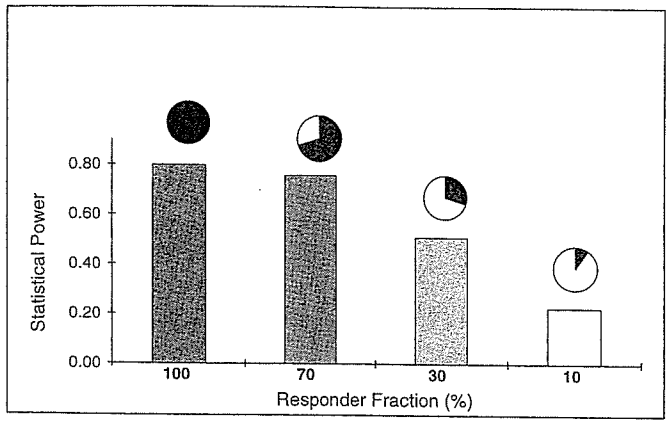

Fig. 2. Relationship between the responder fraction and statistical power: a hypothetical calculation. The 1-year survival rates for the experimental and control arms are assumed to be 20% and 10%, respectively. Exponential distributions are assumed for both treatment arms. Two-year accrual and 1-year follow-up periods are assumed

Problem III: criteria for administration

The problems concerning criteria for drug administration are essentially the same as those discussed above. In the section above, "Problem I: mechanisms of action and drug design," it has been made clear that the significance of target molecules in the pathological state in question, i.e., tumor growth, should be fully understood and established. It is now widely accepted that genomic instability underlies tumorigenesis in various neoplasms. Genomic instability comprises the "mutator phenotype," in which mutation rates in the genome are markedly elevated and mutation occurs in various genes, and the "chromosomal instability," which causes diverse abnormalities in chromosomal number and structure. These structural alterations of the genome frequently lead to the deregulated expression of various genes. Therefore, particularly in cancer, found genetic changes, either in the structure or in the expression status, do not necessarily imply that tumor growth depends on these changes. As discussed above, the fusion gene derived from an abnormal chromosome translocation, *BCR-ABL*, is found in almost 100% of CML patients, and the growth of CML cells is entirely dependent on this chimera gene. Indeed, the regulated expression of BCR-ABL causes leukemia in a model system using transgenic animals.[10] Thus, the rationale for the administration of a tyrosine kinase inhibitor, imatinib, to patients with CML is unquestionable. Indeed, it is known that imatinib treatment of CML shows high response rates. On the other hand, when imatinib is administered to patients with gastrointestinal stromal tumors (GISTs) that express a growth factor tyrosine kinase receptor, c-KIT, are the circumstances the same?

Almost all GISTs express c-KIT. The problem is that c-*kit* mutations in regions including exon 11, which code the transmembrane domain, are reported in GIST.[11] Inheritance of these mutations is known to cause a familial predisposition to GIST.[12] These c-*kit* mutations may alter the tyrosine kinase activity in c-KIT proteins. In sporadic cases of GIST, the frequency of these c-*kit* mutations is reported to be lower than 70%.[7] It may be important to discriminate GISTs depending on abnormally elevated tyrosine kinase activity due to c-*kit* mutations from GISTs that simply express wild-type c-KIT molecules. In fact, it has been reported that clinical outcomes in GIST patients differ widely depending on the mutation status of the c-*kit* gene,[13] which strongly suggests that tumor growth in GISTs with c-*kit* mutations, particularly mutations in exon 11, is highly dependent on elevated tyrosine kinase activity in mutated c-KIT molecules. However, at present, the rationale for the administration of imatinib to patients with GIST is based on the immunohistochemical confirmation of simple c-KIT expression in tumor cells. Interestingly, it was reported at the ASCO 2003 meeting that other neoplasms expressing c-KIT did not respond to imatinib treatment.

There is a similar problem in target-based therapies for non-small-cell lung cancer (NSCLC). Gefitinib (ZD1839; Iressa;) and erlotinib (OSI-774; Tarceva) inhibit the tyrosine kinase activity in epidermal growth factor receptor 1 (EGFR1), which is frequently expressed in various cancers, including NSCLC. These target-based drugs were initially intended to be used for all patients with tumors expressing EGFR. However, it was reported that tumors with *EGFR1* mutations, particularly a 15-bp inframe deletion in exon 19, were more sensitive to gefitinib than those without the mutations.[14,15] However, Hirsch and colleagues[16] reported that *EGFR1* amplification (to be precise, multiplicity in the copy number due to chromosome 7 polysomy or aneuploidy) was more closely related to gefitinib/erlotinib sensitivity. Although several comparative studies have been done,[16–19] there is still a controversy (Table 2). At present, there seems to be a consensus that tumors harboring *EGFR1* mutations are relatively more sensitive to gefitinib/erlotinib, and that tumors with these mutations frequently carry *EGFR1* amplification. The problem is that the tyrosine kinase activity in mutant EGFR1 has not been biochemically determined. Mutant EGFR1 may be less active, and cancer cells may try to compensate for insufficient tyrosine kinase activity to promote cell growth with an increase in the gene copy number, particularly when cells have chromosomal instability. Gefitinib/erlotinib may be more effective in such tumor cells. This may be one possible explanation for the linkage. Needless to say, some tumors may have only one copy of the mutated *EGFR1* gene, and other tumors may carry several copies of wild-type *EGFR1* as a simple reflection of aneuploidy, because they are not dependent on its tyrosine kinase activity. The most important information is whether or not the tumor growth depends on EGFR1 tyrosine kinase activities. The rationales for the administration of these target-based drugs should be based on this information. In anti-EGFR therapies in NSCLC, clinical testing to determine the *EGFR1* gene structures may be regarded as routine in the near future.

There is a similar, but more serious problem with trastuzumab (Herceptin). Trastuzumab is the first humanized monoclonal antibody drug that has been developed as

Table 2. EGFR status and clinical outcomes in patients treated with EGFR tyrosine kinase inhibitors

Authors (year)	Gene copy number			Gene mutation			Protein expression			Reference
	Method	Correlation with response	Correlation with survival	Method/axon	Correlation with response	Correlation with survival	Antibody	Correlation with response	Correlation with survival	
Cappuzzo (2005)	FISH	Yes	Yes	Sequencing /18,19,21	Yes	No	Clone 31G7 monoclonal Ab	Yes	Yes	16
Tsao (2005)	FISH	Yes	Yes	Sequencing /18–21	Yes	No	OakoEGFR PharmOx kite	No	Yes	18
Takano (2005)	Quantitative real-time PCR	Yes	No	Sequencing /18–21	Yes	Yes	Not done	–	–	19
Bell (2005)	Quantitative real-time PCR	No	No	Sequencing /18–21	Yes	No	Not done	–	–	17

FISH, fluorescent in situ hybridization; PCR, polymerase chain reaction; EGFR, epidermal growth factor receptor

a target-based anticancer agent. It reacts with an EGFR family member, ErbB-2/HER2/NEU receptor tyrosine kinase. As with imatinib therapy for GIST, the rationales for the administration of trastuzumab are currently based on the immunohistochemical grading of the HER2/NEU expression level in tumor cells. Efforts have been made to achieve accuracy and reproducibility in the immunohistochemical assays for HER2/NEU expression. However, it is known that response rates are not different between patients with tumors that have different grades of HER2/NEU expression.[20] As in the case of *EGFR1* in NSCLC, the existence of c-*erbB-2*/c-*neu* gene amplification makes this problem more complicated. It has been reported that response rates for trastuzumab treatment are relatively higher in tumors with c-*erbB-2*/c-*neu* gene amplification than in those without this gene amplification.[21] On the other hand, Baselga et al.[20] reported that response rates and time to progression were not different between HER2/NEU-overexpressing tumors and tunors with c-*erbB-2*/c-*neu* gene amplification. Fluorescent in situ hybridization (FISH) performed on tissue specimens is currently used to detect c-*erbB-2*/c-*neu* gene amplification. Interestingly, it is known that tumors with c-*erbB-2*/c-*neu* gene amplification, confirmed by FISH, do not necessarily overexpress HER2/NEU. Apart from technical problems in performing tissue FISH, these observations suggest that the relationship between the expression level of a given gene and changes in its structure, either mutation or amplification, is not simple as has been expected.

What does tumor growth depend on? The answer to this question is the only touchstone with which we can rationalize the administration of target-based anti-cancer drugs. Some tumors may be dependent on target molecules, but others may not. Some genetic changes may reflect the dependence of tumor growth on the elevated activity of target molecules, but others may not. Comprehensive and thorough studies to elucidate the complex relationships among the structures, expression levels, and functions of the gene in question are required. Once these relationships are elucidated, it will be possible to establish a system for accurate and, consequently, efficient clinical testing to support target-based anticancer therapies. However, there seems to be easiness in our approaches to clinical testing for target-based therapies. Needless to say, cost-effectiveness must be also considered. DNA sequencing, which has recently become markedly easier than it used to be, is still expensive and not available at all medical facilities, while immunohistochemistry is relatively inexpensive and widely available. However, once the specific genetic changes on which tumor growth highly depends are found, it may be possible to develop a new technique that is specialized for detecting those changes and, is, consequently, efficient and inexpensive. The development of commercialized custom testing may also improve cost-effectiveness in clinical testing for target-based anticancer therapies.

Problem IV: adverse effects

As stated earlier, all of the physiological functions are not always known in all the molecules regarded as targets. In the category of low-molecular-weight compounds, "inhibitors," molecular targets are disperse, due to the structural similarities in the functional domains between the initially targeted molecules and related proteins. This double-faced out-of-focusing sometimes causes unexpected adverse effects of target-based anticancer agents, markedly delaying clinical trial steps and sometimes leading to the abandonment of development of the agent. This problem may partly explain the kaleidoscopic changes in the variety of anticancer drugs in development, as discussed above (see Table 1). A symbolic example is an MMP inhibitor, marimastat, which was initially developed as an inhibitor of invasive tumor growth. The MMP family regulates physiological cell traffic in fibrous tissue matrix, particularly in inflammation, immune response, bone/cartilage regeneration, and vascularization, as well as regulating pathological processes such as tumor invasion and metastasis. As a result, severe arthralgia and bone pain were frequently observed in patients who received marimastat.[22,23] This unforeseeable (but, in a sense, foreseeable) adverse effect finally led to suspension of the development of this drug. In the case of gefitinib, its crucial side effect, pulmonary fibrosis, raised a social problem, and finally led to the suspension of governmental approval of this drug in the United States.

Problem V: verification processes

When a new therapeutic approach has been introduced, it appears to be important to examine the currently available clinical results retrospectively, in order to establish its significance. The first-generation target-based anticancer drugs, i.e., imatinib, gefitinib, and trastuzumab, are now regarded as established. As shown by the data in Table 1, it is clear that combined therapies using these drugs and conventional antineoplastic agents are now frequently being tested. However, with these first-generation drugs, the overall response rates are not necessarily as high as initially expected. Comprehensive and thorough studies of the gene structure and the expression status, using clinically obtained materials, e.g., tumor tissue specimens, may elucidate specific changes in target molecules which chiefly promote tumor growth and, consequently, strongly predict response to the administered drugs. In fact, such studies have already been carried out in NSCLC patients treated with gefitinib/erlotinib.[16–19] However, the conclusions of the studies differ widely (Table 2). Needless to say, careful processing of the statistical data is essential. In addition, it is also essential to elucidate the qualitative (not biological but biochemical, i.e., enzymological) differences among mutated EGFR1 proteins and wild-type ones, which have not, thus far, been addressed. The confusion in this field may be partly due to a lack of this information. Such basic studies are also required to be carried out in parallel with the re-examination of the clinical data. Similar problems have also been raised for imatinib, particularly for GIST, and trastuzumab.

All of the methodological problems discussed above converge on the following two problems: (1) information about the target molecules is never complete (Fig. 1A), and (2) drugs never target only the target molecules (Fig. 1B,C). The former problem derives from the methodological limits of molecular biology and biochemistry, i.e., elementalism, and the latter from the methodological limits in our techniques for drug development. These limits are currently inevitable. However, elaborative and careful verification of the clinically obtained data, if supported by precise and sensitive analyses, may provide us with important information concerning criteria for drug administration and, consequently, improve the efficacy of treatment. Precise and efficient detection of responder populations is the key to the development and establishment of target-based anticancer therapies.

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Initial Experience With Video-Assisted Thoracoscopic Surgery for Pulmonary Metastasis in the Prone Position

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Abstract: We performed video-assisted thoracoscopic surgery for pulmonary metastasis with the patient in the prone position because the use of the full lateral decubitus position was not possible owing to a deformity of the upper extremity, which existed because of a previous operation for osteosarcoma. In cases where the lateral decubitus position cannot be used, the prone position is both safe and effective for treating dorsal lesions of the lung by means of video-assisted thoracoscopic surgery.

Key Words: video-assisted thoracoscopic surgery, pulmonary metastasis, osteosarcoma, prone position

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Video-assisted thoracoscopic surgery (VATS) is now routinely used as a minimally invasive approach for the treatment of patients with intrathoracic disease. The patients are usually placed in the full lateral decubitus position to undergo VATS. In the present case, however, the patient could not be placed in the full lateral decubitus position owing to a deformity in his upper extremity as a result of a previous operation for osteosarcoma. We therefore selected the prone position to treat a metastatic lesion in the dorsal portion of the left lower lobe.

CASE

A 19-year-old man underwent a large resection for osteosarcoma of the right upper extremity. Postoperatively, the patient received adjuvant chemotherapy consisting of methotrexate, doxorubicin, and cisplatin. Two years after the initial operation, a computed tomography scan revealed a dorsal lesion in the left S^1 segment as shown in Figure 1. The right full lateral decubitus position is generally needed to treat left intrathoracic lesions by the VATS procedure. However, the patient could not be placed in that position owing to a deformity of the right upper limb. Under general anesthesia, the patient was placed in the prone position as shown in Figure 2A. Ventilation was commenced with a double-lumen endotracheal tube to allow for 1-lung ventilation and a collapse of the ipsilateral lung. The left pleural cavity was investigated under a thoracoscope inserted in the seventh intercostal space on the mid-axillary line. Two additional ports were then approached under direct vision using 12 mm trocars through the intercostal space at the left mid-scapular line of the back position as shown in Figure 2B. In the dorsal portion of the left lower lobe, the metastatic lesion was found. The lesion was resected using the Endo GIA Universal Roticulator (Tyco Healthcare Group, Norwalk, CT). The specimen was retrieved with an Endopouch Retriever (Ethicon Endo-Surgery, Inc., Cincinnati, OH). A histologic examination revealed the proliferation of spindle cells in fascicles with osteoidlike structures, which were typical of osteosarcoma (Fig. 3). The patient's postoperative recovery was uneventful. The patient was discharged on the seventh postoperative day. The patient provided his written, informed consent according to institutional guidelines before preparing this case report.

COMMENT

This is the first report of a pulmonary metastasectomy through VATS in the prone position. VATS is now routinely used as a minimally invasive approach for the treatment of patients with intrathoracic disease. Patients are usually placed in the lateral decubitus position for most such procedures. There have been some previous reports describing the treatment of spinal deformities,[2] mediastinal neurogenic tumors,[3] and transdiaphragmatic adrenalectomy[4] using VATS with the patient in the prone

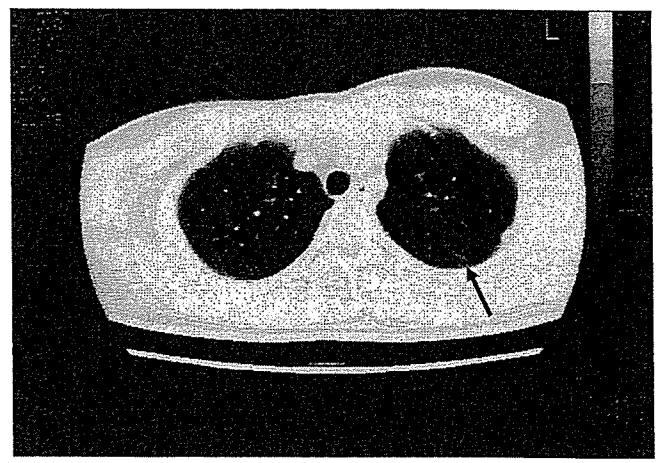

FIGURE 1. Computed tomography showing the presence of a posterior nodule in the lower lobe of the left lung (arrow).

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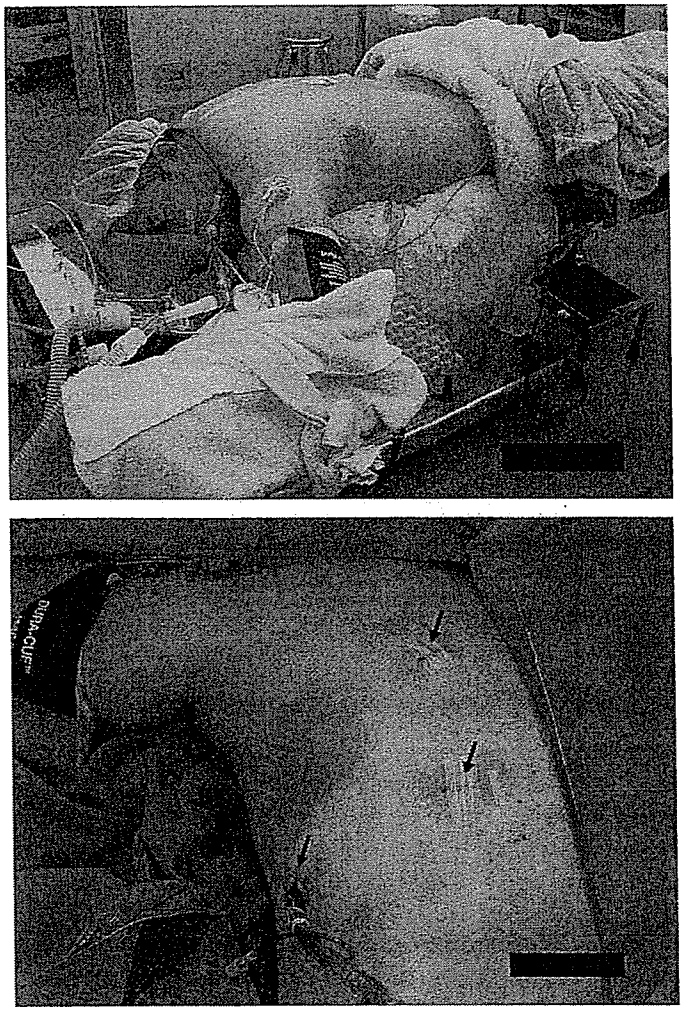

FIGURE 2. A, An intraoperative photograph of the patient set-up. B, Port sites of the back position.

position. To the best of our knowledge, there have been no reports describing the treatment of intrapulmonary lesions through VATS with the patient in the prone position. We previously reported a single-stage thoracoscopic procedure for bilateral spontaneous pneumothorax in the supine position.[5] In the supine position, however, it is difficult to treat an intrapulmonary lesion in the back. Prone positioning during general anesthesia more strongly affects the cardiopulmonary function than when patients are in the supine position. According to a hemodynamic evaluation, the inferior vena caval compression decreased the venous return and increased the intrathoracic pressure while also decreasing the left

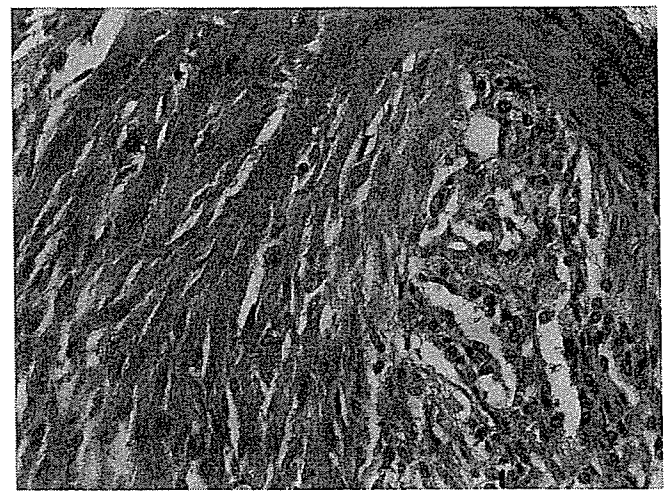

FIGURE 3. Osteosarcoma metastasis to the lung composed of a proliferation of spindle cells in the fascicles with osteoidlike structures. Hematoxylin-eosin, ×120.

ventricular compliance.[6] However, the prone position has been reported to only minimally affect respiratory system compliance while improving the functional residual capacity and oxygenation.[1] In this case, no severe hemodynamic changes were recognized after careful observations during 1-lung ventilation. In conclusion, when the full lateral decubitus position cannot be used, the prone position is both a safe and effective alternative for VATS when treating dorsal lesions of the lung.

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Prognostic value of expression of vascular endothelial growth factor and its flt-1 and KDR receptors in stage I non-small-cell lung cancer

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KEYWORDS
Non-small cell lung cancer;
Vascular endothelial growth factor;
Fms-like tyrosine kinase receptor-1;
Kinase insert domain-containing receptor;
Prognosis

Summary
Background: Angiogenesis plays an important role in tumorigenesis and has attracted interest as a potential target in cancer treatment.
Patients and methods: We examined the prognostic value of the expression of vascular endothelial growth factor (VEGF) and of the VEGF receptors (VEGFRs) fms-like tyrosine kinase receptor-1 (flt-1) and kinase insert domain-containing receptor (KDR) in non-small-cell lung cancer (NSCLC). Sixty patients with surgical stage I NSCLC who had not undergone induction therapy or adjuvant therapy were selected from among 170 patients with NSCLC who had undergone surgery from January to December 1995. Specimens obtained at surgical resection were subjected to immunohistological staining, and the relationship between postoperative outcome and the expression of VEGF and its receptors was investigated. All patients included in the analysis had been followed up for 5 years or longer or until death.
Results: Patients with tumors expressing VEGF or KDR tended to have poorer outcomes, and VEGF expression and KDR expression were positively correlated. In contrast, flt-1 expression was not correlated with VEGF expression or outcome. Outcomes were poor in patients with tumors positive for both VEGF and VEGFRs. Multivariate analysis identified expression of both flt-1 and KDR and VEGF and KDR as possible independent prognostic factors.

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Conclusions: Our results suggest that expression of VEGF and VEGFR are associated with a poor prognosis via autocrine and paracrine growth stimulation of cancer cells. Moreover, tumors expressing both flt-1 and KDR may have greater malignant potential and are associated with a poor prognosis.

1. Introduction

Tumor angiogenesis is essential for cancer growth and metastasis [1,2]. The correlations of tumor vessel density with metastasis [3–5] and survival time [6,7] have been examined, and tumor vessel density has been shown to affect prognosis [8]. Recently, a variety of molecules involved in tumor angiogenesis have been identified, and such issues as the relationships between angiogenesis factors and tumor vessel density have been examined [9]. Vascular endothelial growth factor (VEGF) is one of the most important angiogenesis factors [10], and its expression within tumors is suggested to affect prognosis through angiogenesis [11,12]. A monoclonal antibody targeting VEGF has shown promise against pulmonary adenocarcinoma [13] and is now being evaluated in a phase III study. VEGF binds to VEGF receptors (VEGFRs) on endothelial cells and stromal fibroblasts and plays a key role in angiogenesis. The receptor fms-like tyrosine kinase receptor-1 (flt-1 or VEGFR-1) has a high affinity for VEGF but low angiogenic activity and is thought to be a VEGF-neutralizing receptor; in contrast, kinase insert domain-containing receptor (KDR) is believed to enhance the angiogenic effects of VEGF [14]. These VEGFRs are also expressed on cancer cells [15,16], but their biological significance is unclear. To clarify the prognostic significance of VEGF and VEGFRs, in the present study we examined survival and the expression of VEGF and VEGFRs by tumor cells in patients with non-small-cell lung cancer (NSCLC).

2. Subjects and methods

2.1. Patients

Immunohistological staining was performed in December 1996 of tumor specimens from 170 consecutive patients who had undergone resection for NSCLC from January to December 1995 at the Osaka Medical Center for Cancer and Cardiovascular Diseases and the Kumamoto Regional Medical Center. Reviewers of histologic features were blinded to the patients' clinical characteristics. Of the 170 patients with tumors for which immunohistological staining was performed, those with postoperative stage I NSCLC who had not undergone induction or postoperative adjuvant chemotherapy and had been followed up for 5 years postoperatively were included in the analysis. Clinical outcomes were assessed in December 2003.

2.2. Immunohistochemical staining method

Formalin-fixed, paraffin-embedded, 3 μm-thick sections were obtained from each of the 170 specimens of primary lesions. All specimens were stained with hematoxylin and eosin for histologic diagnosis. Immunohistochemical staining was performed with the avidin–biotin-peroxidase complex method. Sections were briefly immersed in citrate buffer (0.01 mol/l citric acid: pH 6.0) and were incubated for four 5 min intervals at 100 °C in a microwave oven for antigen retrieval. The sections were then incubated with an anti-flt-1 antibody (1:200, rabbit polyclonal; Santa Cruz Biochemical, Santa Cruz, CA) and an anti-KDR antibody (1:200, rabbit monoclonal; Santa Cruz Biochemical). VEGF immunohistochemial staining methods were performed without microwave pretreatment with an anti-human VEGF antibody (1:100, polyclonal; Santa Cruz Biochemical, Santa Cruz, CA). After overnight incubation at 4 °C with the primary antibody, slides were washed in phosphate-buffered saline and then exposed to the secondary antibody, a biotinylated anti-rabbit antibody (1:200; Jackson Laboratories, West Grove, PA) for 30 min at room temperature. The slides were then washed in phosphate-buffered saline, and incubated with the avidin–biotin-peroxidase complex (1:200; Dako, Glostrup, Denmark) for 30 min at room temperature. The chromogenic substrate of peroxidase was a solution of 0.05% 3,3′-diaminobenzidine tetrahydrochloride/0.03% H_2O_2/10 mmol/l imidazole in 0.05 mol/l Tris buffer. The assay conditions were established with human umbilical cord vessels as positive controls. Normal rabbit IgG for the monoclonal or polyclonal antibody, at the same concentration as the primary antibody, was used as the negative control.

2.3. Immunohistochemial evaluation

The slides were independently reviewed by two of the authors (T.S. and M.H.) who were blinded to the clinicopathological data. Intense staining was scored as 2, weak as 1, and negative as 0. Cells with a staining intensity of 1 or 2 were regarded as positive cells. The percentage of positive cells was calculated by counting more than 1000 cancer cells in randomly chosen high-power fields (10 × 40). Protein expression was considered positive when immunostaining was seen in at least 10% of cancer cells. If the discrepancies existed between the reviewers, a consensus judgment was reached through discussion.

2.4. Statistical analysis

Association between factors, such as correlation of gene expression positivity, was examined by the chi-square test. Overall survival is defined for NSCLC recurrence patients as the time from surgery until death from any cause, while death of no recurrence patients or survival more than 5 years is considered to be censored. We firstly estimated survival curves by the Kaplan–Meier method conducted univariate logrank test. Then, we selected final prognostic factors by

the backward stepwise Cox regression method from ones exhibiting logrank p-value <0.10, considered to show their possible significance. We adopted the 10% significance level for the variable selection of the backward stepwise process. All data were analyzed using SAS version 9 (SAS Institute Inc., Cary, NC).

3. Results

Of the 170 patients from whom resected specimens were subjected to immunohistological staining, 62 had pathological stage II or III NSCLC and 108 had stage I NSCLC. Thirty-four of the 108 patients with stage I NSCLC had received induction or adjuvant treatment or both, and of the remaining 74 patients, 14 were lost to follow-up after less than 5 years. Thus, 60 patients were included in the analysis. Thirty-two patients were male and 28 were female, median age was 69 years (age range, 44–87 years), and the T factor was T1 in 43 patients and T2 in 17 patients.

3.1. VEGF and VEGFR expression

Both VEGF and VEGFRs appeared on tumor microvessels. In cancer cells, VEGF was expressed in the cytoplasm, and VEGFRs were expressed on the cell membrane and in the cytoplasm near the cell membrane. The overall positivity rates for flt-1, KDR, and VEGF were 32%, 48%, and 30%, respectively (Table 1). No differences were seen in the rates of expression according to histologic type, differentiation grade, or T factor. The expression of flt-1 was not correlated with either KDR expression or VEGF expression. However, KDR expression was positively correlated with VEGF expression ($p=0.003$).

3.2. VEGF and VEGFR expression and survival

Postoperative survival time in patients with stage I NSCLC was examined on the basis of expression by tumors of VEGF, flt-1, and KDR. Kaplan–Meier survival curves and the logrank test showed no difference in survival between patients with VEGF-positive tumors and those with VEGF-negative tumors ($p=0.09$; Fig. 1). Survival time did not differ between patients with flt-1-positive tumors and those with flt-1-negative tumors ($p=0.10$; Fig. 2a). In contrast, survival was shorter in patients with KDR-positive tumors than in patients with KDR-negative tumors ($p=0.05$; Fig. 2b). Survival time was shorter in patients with tumors positive for both flt-1 and VEGF than in patients with tumors negative for both flt-1 and VEGF or in patients with tumors positive for either flt-1 or VEGF ($p=0.05$; Fig. 3a). Similar results were obtained for KDR ($p=0.04$; Fig. 3b). Survival time was significantly shorter in patients with tumors positive for both flt-1 and KDR than in patients with tumors negative for both flt-1 and KDR or in patients with tumors positive for either flt-1 or KDR ($p=0.002$; Fig. 4).

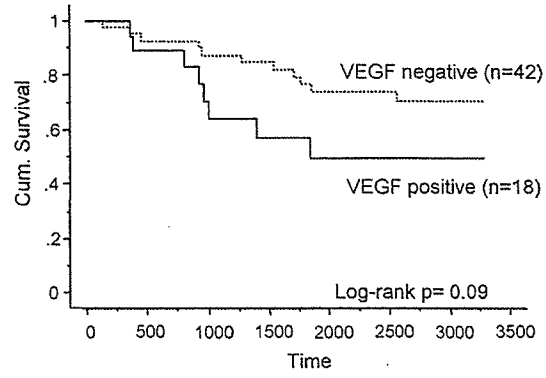

Fig. 1 Postoperative survival curves according to VEGF expression. There was no statistical significantly difference in survival between patients with VEGF-positive tumors (5-year survival rate: 48%) and those with VEGF-negative tumors (5-year survival rate: 74%).

3.3. Multivariate analysis

After univariate logrank test, we screed five factors with cut-off of $p=0.10$: VEGF expression, KDR expression, VEGF and VEGFRs co-expression, positive for both KDR and flt-1 (Table 2). Then the backward stepwise Cox regression analysis finally selected two factors predicting shorter survival, tumors positive for both KDR and flt-1 and tumors

Table 1 Relationship between expression rate of VEGF and VEGFRs (flt-1 and KDR) and clinicopathological factors

	Number *n* (%)	VEGF *n* (%)	flt-1	KDR *n* (%)
All patients	60	18 (30)	19 (32)	29 (48)
Histological type				
Adenocarcinoma	53	14 (26)	27 (25)	25 (47)
Squamous cell carcinoma	6	2 (33)	6 (100)	1 (17)
Differentiation				
Well-differentiated	31	5 (16)	7 (23)	15 (48)
Moderately differentiated	22	8 (36)	9 (41)	10 (45)
Poorly and un-differentiated	7	7 (100)	5 (75)	5 (75)
T factor				
T1	43	11 (26)	9 (21)	19 (44)
T2	17	7 (41)	10 (59)	10 (59)

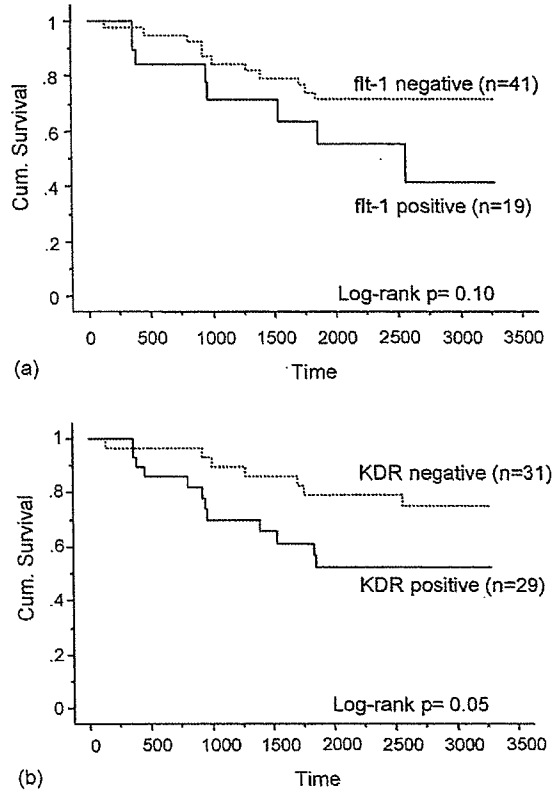

Fig. 2 Postoperative survival curves according to VEGFR expression. (a) flt-1: Survival time did not differ between patients with flt-1-positive tumors (5-year survival rate: 64%) and those with flt-1-negative tumors (5-year survival rate: 72%). (b) KDR: Survival was shorter in patients with KDR-positive tumors (5-year survival rate: 52%) than in patients with KDR-negative tumors (5-year survival rate: 80%).

positive for both KDR and VEGF with 0.10 significance level (Table 3).

4. Discussion

The cytokine VEGF discovered by Folkman is an important vascular growth factor [10]. Both flt-1 and KDR are receptors for VEGF, which is involved in vascular growth, but they are present mainly in endothelial cells and stromal cells [14]. In the present study, we attempted to clarify the prognostic value of VEGF and VEGFR expression in NSCLC cells. We limited the analysis to patients with postoperative stage I disease who had not undergone perioperative therapy, because other prognostic factors in these patients are relatively uniform.

Previous studies have shown that VEGF acts via flt-1 or KDR to promote tumor angiogenesis, which stimulates tumor growth and metastasis. These studies have clarified the role of VEGF in tumor vessel formation [17] and have suggested an association between VEGF expression and the malignant potential of tumor cells. Although both flt-1 and KDR can be expressed in tumor cells, the relationship between their expression in tumor cells and prognosis remains unclear. In vitro experiments have shown that the addition of VEGF

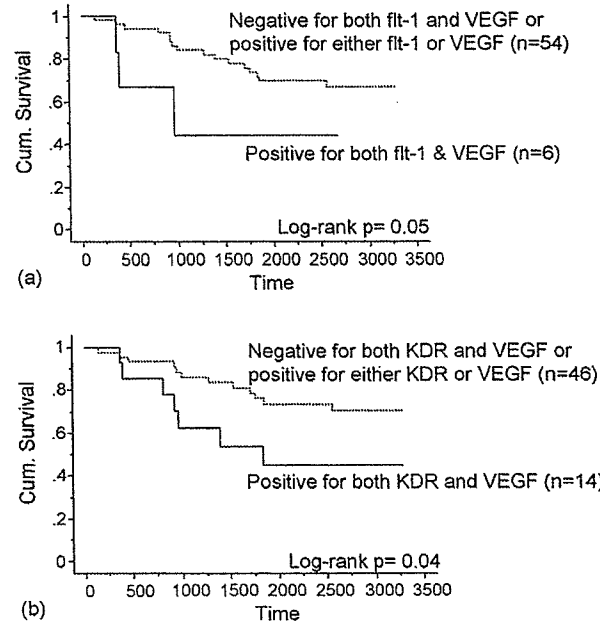

Fig. 3 Postoperative survival curves according to co-expression of VEGF and VEGFR. (a) flt-1: Survival time was shorter in patients with tumors positive for both flt-1 and VEGF (5-year survival rate: 44%) than in patients with tumors negative for both flt-1 and VEGF or in patients with tumors positive for either flt-1 or VEGF (5-year survival rate: 72%). (b) KDR: Survival time was shorter in patients with tumors positive for both KDR and VEGF (5-year survival rate: 44%) than in patients with tumors negative for both flt-1 and VEGF or in patients with tumors positive for either KDR or VEGF (5-year survival rate: 76%).

increases the growth of cells expressing KDR [18]; this finding suggests the possibility of both autocrine and paracrine growth in VEGF-producing cells [19,20]. Conversely, tyrosine phosphorylation of flt-1 by VEGF is weak despite the high affinity of flt-1 for VEGF, suggesting that flt-1 neutralizes the effects of VEGF. The rate of flt-1 expression in tumor cells is low, and the biological characteristics of tumors expressing flt-1 are unknown [21].

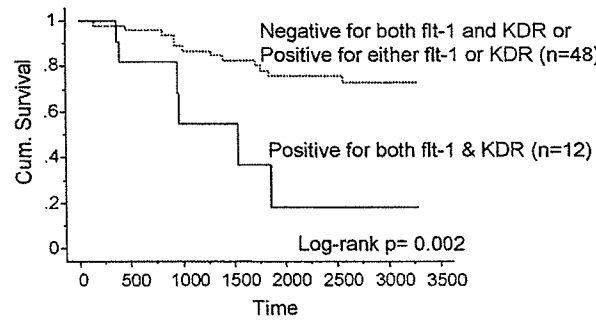

Fig. 4 Postoperative survival curves according to co-expression of flt-1 and KDR. Survival time was significantly shorter in patients with tumors positive for both flt-1 and KDR (5-year survival rate: 20%) than in patients with tumors negative for both flt-1 and KDR or in patients with tumors positive for either flt-1 or KDR (5-year survival rate: 76%).