come. In contrast, plasma levels of VEGFR-2 showed a trend to decrease over the same period, whereas plasma Tie-2 levels did not seem to change (Table 6). Baseline plasma VEGF levels appeared to be lower in patients who experienced clinical benefit following vandetanib treatment: PR (median 22.3 pg/ml, $n = 6$) and SD (median 37.0 pg/ml, $n = 16$) versus PD (median 63.7 pg/ml, $n = 21$). Patients with a low (below median) baseline plasma VEGF level had a longer TTP (median, 24.1 week) than those with a high (above median) baseline VEGF level (median, 8.3 weeks) (Figure 4). No clear relationship was apparent between baseline levels of plasma Tie-2 and VEGFR-2 and tumor response.

DISCUSSION

The primary objective of this phase IIa study was to assess the ORR to three doses of vandetanib (100, 200, and 300 mg/d) in Japanese patients with advanced or recurrent NSCLC. These doses of vandetanib were selected based on the outcomes of a Japanese phase I study where it was observed that vandetanib was well tolerated up to a dose of 300 mg and objective tumor responses were observed in 4 of 9 patients with NSCLC at doses of either 200 or 300 mg.[11]

In this study, objective tumor responses were observed at all three doses of vandetanib. The ORR in the 100, 200, and 300 mg arms was 17.6% (3 of 17 patients), 5.6% (1 of 18 patients), and 16.7% (3 of 18 patients), respectively. The DCR and TTP were similar across the three dose arms. It was noted that 50% (9 of 18) of the patients in the 200 mg arm had failed two previous chemotherapy regimens, compared with 23.5% (4 of 17 patients) and 22.2% (4 of 18 patients) in the 100 and 300 mg arms, respectively. It is possible that these differences contributed to the lower ORR observed in the 200 mg arm, although the number of patients in each dose arm was too small to allow any definitive conclusions to be made.

Vandetanib was well tolerated at 100, 200, and 300 mg dose levels in this study. Overall, AEs were generally mild and manageable with symptomatic treatment, dose interruption or reduction. In addition, the AE profile was consistent with that determined during phase I evaluation in patients with advanced solid tumors[10,11] and phase II monotherapy data in NSCLC.[12] Furthermore, the AE profile was also consistent with that reported previously for agents that inhibit the VEGFR[17,18] or EGFR[4,19] signaling pathways. In general, no apparent dose dependence was noted in the incidence of the common AEs in this study except for asymptomatic QTc prolongation (24%, 56%, and 44% for the 100, 200, and 300 mg dose arms, respectively), an event that was manageable by dose interruption/reduction.

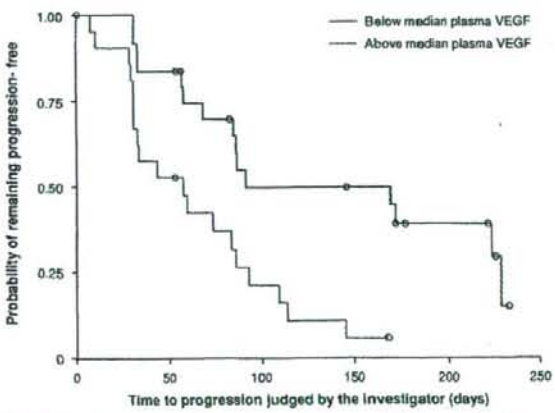

FIGURE 4. Kaplan–Meier curve of low (below median) versus high (above median) baseline plasma VEGF and time to progression.

A notable feature of this study, and the phase II program for vandetanib in NSCLC, is that patients with squamous cell histology or stable brain metastases were permitted to enter the trials. Both of these factors have been associated with an increased risk of bleeding, including severe life-threatening hemoptysis in NSCLC patients with squamous histology in a randomized phase II study of bevacizumab with carboplatin and paclitaxel.[20] These events have also been reported with other inhibitors of VEGF/VEGFR signaling, such as sunitinib and sorafenib.[17,18] Importantly, no CNS hemorrhage AEs or hemoptysis attributable to vandetanib were reported in this study.

The PK profile in this NSCLC patient population was consistent with that seen previously during Phase I evaluation in Japanese and USA/Australian patients with a range of solid tumors.[10,11]

In patients with NSCLC, specific *EGFR* mutations are associated with increased sensitivity to EGFR tyrosine kinase inhibitors,[21,22] and a better survival outcome with gefitinib has been shown to correlate with high *EGFR* gene copy number.[23] In this study, an exploratory analysis of tumor samples for amplification of *EGFR* gene copy number and somatic mutations of the *EGFR* gene revealed no clear relationship between *EGFR* mutation or gene amplification status and clinical outcome in patients receiving vandetanib. The *EGFR* mutation frequency of 4% (1 of 27 patients) is lower than that previously reported,[24,25] and further studies are needed to evaluate *EGFR* mutation status as a possible predictive marker for vandetanib therapy in advanced NSCLC.

In addition to *EGFR* mutation/amplification status, plasma profiling of cytokines and angiogenic factors may be a feasible approach for identifying blood-based prognostic and activity markers for therapies in NSCLC. Preliminary analysis of plasma concentrations of the angiogenesis markers VEGF and VEGFR-2 in the present study revealed that patients with PR or SD were more likely to have low baseline levels of VEGF than those with PD. It has been shown previously that low pretreatment levels of circulating VEGF correlated with a good response to gefitinib treatment in patients with NSCLC.[26] The significance of the relationship between these biomarkers and clinical outcome requires further investigation.

In conclusion, vandetanib monotherapy (100–300 mg/d) demonstrated antitumor activity with an acceptable safety and tolerability profile in Japanese patients with advanced NSCLC. Based only on this study, there is no com-

pelling evidence to identify the optimal dose of vandetanib monotherapy in this population of patients; further investigation of vandetanib doses in the range 100 to 300 mg is warranted in Japanese patients with advanced NSCLC. Other randomized phase II studies of vandetanib in advanced NSCLC have demonstrated improvements in progression-free survival with vandetanib 300 mg as a monotherapy versus gefitinib[12] and with the combination of vandetanib 100 mg and docetaxel.[14] Phase III evaluation of vandetanib in a broad population of patients, both as monotherapy at 300 mg (versus placebo in patients previously treated with anti-EGFR therapy [ZEPHYR]; versus erlotinib [ZEST]) and at 100 mg in combination with docetaxel (ZODIAC) or pemetrexed (ZEAL), has been initiated in global trials.

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Importance of *UDP-glucuronosyltransferase 1A1*6* for irinotecan toxicities in Japanese cancer patients

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Abstract

Recent pharmacogenetic studies on irinotecan have revealed the impact of *UDP glucuronosyltransferase (UGT) 1A1*28* on severe irinotecan toxicities. Although the clinical role of *UGT1A1*6*, which is specifically detected in East Asian patients, in irinotecan toxicities is suggested, clear evidence remains limited. To examine the impact of *6, the association of *UGT1A1* genotypes with severe irinotecan toxicities was retrospectively investigated in Japanese cancer patients. A significant *6-dependent increase in the incidence of grade 3 or 4 neutropenia was observed in 49 patients on irinotecan monotherapy ($p = 0.012$). This study further clarifies the clinical importance of *6 in irinotecan therapy in East Asians.

Keywords: UGT1A1; Pharmacogenetics; Irinotecan; SN-38

1. Introduction

Irinotecan, an anticancer prodrug, is widely applied for a broad range of carcinomas, including colorectal and lung cancers. The active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferase 1As (UGT1As) such as 1A1, 1A7, 1A9 and 1A10, to form the inactive metabolite, SN-38 glucuronide (SN-38G) [2–5]. Among the UGT

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isoforms, UGT1A1 is thought to be a predominant contributor to SN-38G formation [2,6]. The dose-limiting toxicities in irinotecan therapy are severe diarrhea and leucopenia [7], and lowered UGT activity is well correlated with severe irinotecan toxicities [8]. Since Ando et al. first reported the significant relevance of *UGT1A1*28* – a repeat polymorphism in the TATA box (−40_−39insTA) – to severe neutropenia/diarrhea [9], a number of clinical studies, primarily conducted in Caucasian patients, have shown associations between *UGT1A1*28* and lowered SN-38G formation or severe neutropenia/diarrhea [10–13]. Based on these findings, the Food and Drug Administration (FDA) of the United States approved a revision of the label for Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028), recommending "a reduction in the starting dose by at least one level of irinotecan for the *UGT1A1*28* homozygous patients". Subsequently, the clinical application of *UGT1A1*28* testing was put into practice for irinotecan therapy in the United States.

To implement personalized irinotecan therapy in Asian countries, the racial differences in *UGT1A1* polymorphisms among Caucasians, African-Americans, and Asians must be taken into consideration [14]. For East Asians, the frequency of **28* is one third of that of Caucasians or African-Americans, and another low-activity allele **6* [211G>A(G71R)], which is not detected in Caucasians or African-Americans, shows the same frequency as the **28* allele. Clinical studies in Japanese cancer patients have demonstrated that significantly low area under concentration-time curve (AUC) ratios of SN-38G to SN-38 are observed in patients having **6* and/or **28* [15–17], suggesting the necessity of typing **6* in addition to **28*. A recent report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, showed a significant association of **6* homozygotes with severe neutropenia [18]. However, data on the role of **6* in irinotecan toxicities is still limited in terms of the various irinotecan-containing regimens. In the first study by Ando et al. on Japanese cancer patients, the association of **6* with irinotecan toxicities was not evident, but a possible enhancement of **28*-related toxicities by **6* was suggested [9]. Other studies in Japanese patients showed an additive effect of **6* on the lowered UGT activity by **28* [15–17]. A significant association of the genetic marker "**6 or *28*" with severe neutropenia was also shown in our previous study, but due to a lack of **6* homozygotes in our patient population, the effect of **6* alone was not confirmed [17].

In this study, to further demonstrate the clinical importance of **6* alone, *UGT1A1* genotypes were determined using DNA extracted from paraffin-embedded specimens (non-cancerous tissues) from 75 Japanese cancer patients by the pyrosequencing method [19,20], and the associations between *UGT1A1* genotype and severe irinotecan toxicities and serum total bilirubin levels were retrospectively analyzed.

2. Materials and methods

2.1. Patients and irinotecan treatment

In a post-marketing surveillance study conducted by Daiichi Pharmaceutical Co., Ltd. (currently Daiichi Sankyo Co., Ltd., Tokyo, Japan), irinotecan was prescribed to 297 patients with various types of cancers from 1995 to 2000 at the National Cancer Center Hospital. The patients were selected through standard clinical practice according to the drug label for indications and contraindications. Methanol-fixed, paraffin-embedded archival tissue specimens, which were necessary for high-quality extraction of DNA greater than 2 kb in size [21], were available for 75 of the 297 patients and were analyzed in this study. Irinotecan was administered by intravenous 30-min infusion as a single agent or in combination chemotherapy at a dose of 60 mg/m^2 (weekly or biweekly), 100 mg/m^2 (biweekly), or 150 mg/m^2 (biweekly). Profiles of the patients in this study, including cancer type, treatment history, and regimens, are summarized in Table 1. The pre-treatment levels of serum total bilirubin were determined by a kit (VL T-BIL, Azwell Inc., Osaka, Japan) according to an enzymatic method using bilirubin oxidase [22]. Toxicities were monitored during irinotecan therapy and graded according to the Common Toxicity Criteria version 2 of the National Cancer Institute.

Because the samples in this study were residual specimens remaining after histopathological diagnosis in the hospital and not collected specifically for research purposes, the samples and their clinical information were anonymized in an unlinkable fashion according to the Ethics Guidelines for Human Genome/Gene Analysis Research by the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry of Japan. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences.

2.2. DNA extraction from paraffin-embedded tissue sections and genotyping of UGT1A1 polymorphisms

Three sections (20 μm of pathologically normal tissues around tumors) were deparaffinized twice by treat-

Table 1
Profiles of cancer patients in this study

		No. of patients
Patients genotyped (Male/female)		75 (51/24)
Age		
Mean/range (y)	50.7/34–75	
Performance Status[a]		
	0/1/2	18/48/8
Previous treatment		
Surgery[a]	+/−	71/3
Chemotherapy[b]	+/−	63/10
Radiotherapy[b]	+/−	9/64
Combination therapy and tumor type [dose of irinotecan (mg/m^2)/(w or 2w)[c]]		
Irinotecan monotherapy	Lung (60/w or 100/2w)	4
	Stomach (100/2w or 150/2w)	5
	Colon (100/2w or 150/2w)	40
With cisplatin	Lung (60/w or 100/2w)	4
	Stomach (60/2w)	11
With mitomycin C (MMC)	Stomach (150/2w)	8
	Breast (120/2w)	1
With 5-fluorouracil (5-FU)	Colon (150/2w)	2
Available data on serum bilirubin levels		37

[a] Data from one patient is lacking.
[b] Data from two patients are lacking.
[c] Weekly or biweekly.

ment with 1.5 ml of xylene at room temperature. After centrifugations, the residual pellet was then washed twice with 1.5 ml of ethanol. Finally, the pellet was dried at 37 °C for 15 min. DNA extraction was performed using a QIAamp tissue kit (QIAGEN K.K., Tokyo, Japan) according to the manufacturer's instructions with some modifications. Briefly, 540 μl of ATL lysis buffer and 60 μl of proteinase K (Qiagen) were added to each pellet, mixed thoroughly, and incubated at 56 °C for 3 h with a rotator. Any remaining tissue debris was removed by centrifugation, and the resulting supernatant was used for the extraction. Twelve microliters of RNase A (100 mg/ml) was added to the supernatant and incubated for 2 min at room temperature. Next, 600 μl of buffer AL was added and mixed thoroughly, and the mixture was incubated at 70 °C for 10 min. Six-hundred microliters of ethanol was added to the solution and mixed well, followed by extraction of DNA using a Qiagen DNA extraction column. The DNA was eluted in a final elution volume of 150 μl. The yield was determined using a NanoDrop spectrophotometer (NanoDrop Technology, Inc, Rockland, DE, USA) and the size of the extracted DNA was checked by agarose gel electrophoresis.

Genotyping of *UGT1A1*6* (211G>A, G71R), **28* (−364C>T, which is perfectly linked with −40_−39insTA in Japanese), and **60* (−3279T>G) were performed by pyrosequencing as described previously [19,20].

2.3. Association analysis and statistics

For association analysis, we focused on incidences of severe diarrhea and neutropenia (grade 3 or greater) observed during irinotecan-therapy. The incidence of severe diarrhea was very low, and the incidence of neutropenia was higher in combination therapy. Therefore, the association of neutropenia with *UGT1A1* genotypes was primarily evaluated in 49 patients with irinotecan monotherapy. As a parameter for in vivo UGT1A1 activity, serum total bilirubin levels taken at baseline from 37 patients were also used.

Statistical analysis for evaluation of the relationship between *UGT1A1* genotypes and severe neutropenia was performed using the chi-square test for trend using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA). The gene-dose effect of the genetic marker "**6 or *28*" on serum total bilirubin levels was analyzed using the Jonckheere–Terpstra (JT) test in the SAS system (version 5.0, SAS Institute, Inc., Cary, NC). The *P*-value of 0.05 (two-tailed) was set as a significant level. Multivariate logistic regression analysis on neutropenia (grade 3 or greater) was performed using JMP software (version 6.0.0, SAS Institute, Inc., Cary, NC), including variables for age, sex, body surface area, performance status, concomitant disease, history of adverse reaction, irinotecan dosage, dosing interval, and *UGT1A1* genotypes. The variables in the final model for neutropenia were chosen using the forward and backward stepwise procedure at the significance level of 0.1.

3. Results

3.1. UGT1A1 diplotypes/haplotypes

The diplotypes and haplotypes (**1, *60, *6* and**28*) of *UGT1A1* exon 1 were analyzed in 75 Japanese cancer patients (Table 1) and their frequencies were summarized (Table 2). The haplotypes were assigned according to our previous definition [15]. It should be noted that the **60* haplotype does not harbor the **28* allele (−40_−39insTA), but most of the **28* haplotype does harbor the **60* allele (−3279T>G). In this study, the **28* homozygote was not present, and the frequency of haplotype **28* (0.113) was slightly lower than that found in our previous study (0.138) [17]. In contrast, the frequency of haplotype **6* (0.213) was higher than that found in the previous study (0.167) [17].

Table 2
Frequencies of *UGT1A1* diplotypes (A) and haplotypes (B) for cancer patients in this study

		Frequency
(A) Diplotype	No. of patients (N = 75)	
*1/*1	21	0.280
*1/*60	9	0.120
*60/*60	2	0.027
*6/*1	14	0.187
*6/*60	8	0.107
*6/*6	4	0.053
*28/*1	12	0.160
*28/*60	3	0.040
*28/*6	2	0.027
*28/*28	0	0.000
(B) Haplotype[a]	No. of chromosomes (N = 150)	
*1	77	0.513
*60	24	0.160
*6	32	0.213
*28	17	0.113

[a] Haplotype definition follows the previous report [15]; *60, −3279T>G without −40_−39insTA; *6, 211G>A(G71R); *28, −40_−39insTA.

3.2. Association of UGT1A1 genotypes with serum total bilirubin levels

Serum total bilirubin levels at baseline, a parameter of in vivo UGT1A1 activity, were available from 37 patients (treated by various regimens), and we analyzed their association with *UGT1A1* genotypes (Fig. 1). The median values of total bilirubin in *60/*1, *28/*1 and *6/*1 heterozygotes were not significantly different from that of the wild type (*1/*1). Higher median values were observed for the *6 homozygotes (*6/*6) and the double heterozygotes of *6 and *28 (*6/*28) than that of the wild type (*1/*1), with increases of 1.9-fold and 2.2-fold, respectively. Since *6 and *28 are mutually independent and their reducing effects on UGT activity are equivalent [15,17], diplotypes were classified by the presence of "*6 or *28" (indicated by "+" in Fig. 1). As shown in Fig. 1, a significant "*6 or *28"-dependent increase in total bilirubin levels was observed ($p = 0.0088$, Jonckheere–Terpstra test).

3.3. Severe toxicities observed in this study

Incidences of severe diarrhea and neutropenia (grade 3 or greater) are shown in Table 3 for each irinotecan-containing regimen. Grade 3 diarrhea was observed in only 4 of the 75 subjects, and since the incidence of diarrhea was low (5.3%), an association analysis on diarrhea was not conducted. Regarding neutropenia, 26 patients experienced grade 3 or 4 neutropenia. Of these 26 patients, 90% experienced neutropenia within 2 months after starting irinotecan-therapy, and 70% within 2 weeks. Significant differences in neutropenia incidences were observed among the regimens used, and considerably high incidences were observed in the combination therapies. Accordingly, association of the *UGT1A1* genotypes with severe neutropenia was analyzed primarily in the patients who received irinotecan-monotherapy.

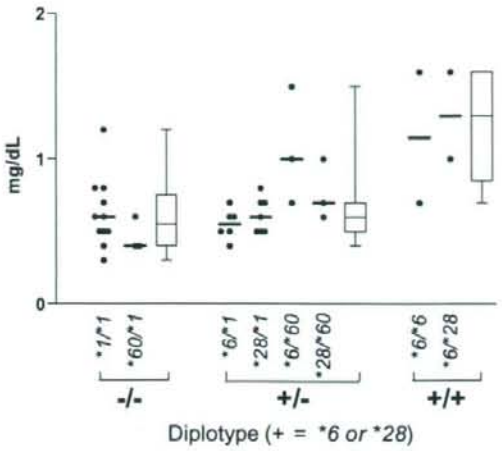

Fig. 1. Effects of *UGT1A1* genotypes on serum total bilirubin levels at baseline in Japanese cancer patients ($N = 37$). Each point represents a patient, and the median value of each diplotype is shown with a bar. All diplotypes are classified into −/−, +/−, and +/+ by the genetic marker, "*UGT1A1*6 or *28", indicated by "+", and their distributions are shown by a box representing the 25–75 percentiles with a bar at the median and lines representing the highest and lowest values. A significant "*6 or *28"-dependent increase in total bilirubin levels was observed ($p = 0.0088$, Jonckheere–Terpstra test).

Table 3
Severe toxicities observed in Japanese cancer patients

Treatment	Diarrhea[a]/total (%)	Neutropenia[b]/total (%)
Total patients	4/75 (5.3)	26/75 (34.7)
Irinotecan alone	1/49 (2.0)	6/49 (12.2)
With CDDP	2/15 (13.3)	11/15 (73.3)
With MMC	1/9 (11.1)	8/9 (88.9)
With 5-FU	0/2 (0.0)	1/2 (50.0)
P-value[c]	NS	<0.0001

[a] Grade 3.
[b] Grade 3 or 4.
[c] Chi-squre test.

3.4. Association of UGT1A1 genotypes with neutropenia

Since significant associations of *UGT1A1*6* and *28 with increased total bilirubin levels (decreased UGT-activity) were once again confirmed in this study, we assessed the clinical relevance of these haplotypes, focusing on the effect of *6 on severe neutropenia. In the 49

patients who received irinotecan monotherapy, the incidence of grade 3 or 4 neutropenia was *6-dependently increased ($p = 0.012$ in the chi-square test for trend). Namely, incidences of severe neutropenia in the *6 heterozygotes (*6/*1,*6/*60, and *6/*28) and homozygotes (*6/*6) were 2.3-fold and 15-fold higher, respectively, than that seen in the non-*6 bearing patients (*1/*1, *60/*1, *28/*1, and *28/*60) (Table 4). In this study, no *28 heterozygotes (*28/*1 and *28/*60) experienced any severe neutropenia, and there were no *28 homozygotes enrolled. Therefore, the effect of *28 could not be determined. For the *60-bearing patients without *6 or *28 (only heterozygote, *60/*1), one patient among six experienced severe neutropenia, and no significant *60-dependent increase was observed (data not shown). Although no statistically significant association of the *28 heterozygotes with severe neutropenia was confirmed in this study, the incidence of discontinuation of irinotecan monotherapy was higher in the *28-bearing patients (91%, $N = 11$) than that in the non-*28 subjects (79%, N = 38), while *60- or *6-dependent increased discontinuation rates were not found (data not shown). For the patients with cisplatin-combination therapy, a higher incidence of severe neutropenia was observed in the *6-bearing patients (*6/*1, *6/*60, and *6/*6) (100%, $N = 3$) than that in the non-*6 bearing subjects (*1/*1, *60/*1, *60/*60, and *28/*1) (66.7%, $N = 12$).

3.5. Multivariate analysis of neutropenia

In order to further clarify the clinical impact of *6 on irinotecan toxicities, multivariate logistic regression analysis on grade 3 or 4 neutropenia was conducted using variables, including *UGT1A1* genotypes and patient background factors, described in Section 2. The final model revealed a significant association of *6 with the incidence of grade 3 or 4 neutropenia at an odds ratio of 5.87 (Table 5).

4. Discussion

The clinical application of the genetic test for *UGT1A1*28* prior to irinotecan therapy has been in practice in the United States since 2005, which was based on cumulative evidence supporting the significant association of *28 with severe irinotecan toxicity [9–13]. Most of the evidence was obtained in Caucasian patients, where *28 is relatively frequent (30–40%) [14]. Although additive effects of another low activity allele, *6, which is specific for East Asians, has been also suggested [9,15–17], direct evidence in Japanese patients has remained limited. In this study, we clearly showed the significant correlation of *6 to grade 3 or 4 neutropenia in Japanese cancer patients who received irinotecan monotherapy. An increased incidence of severe neutropenia was also observed in the *6-bearing patients using cisplatin combination therapy. This finding is in accordance with a report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, which showed a significant association of *6 homozygotes with grade 4 neutropenia [18]. Since combination therapies using irinotecan may cause higher incidences of severe toxicities, the *UGT1A1* polymorphisms should be carefully considered in regimens that include irinotecan.

Since the alleles *6 and *28 are mutually independent [15] and their effects on the UGT activities were shown to be equivalent, the usefulness of the genetic marker "*6 or *28" for personalized irinotecan therapies has been suggested [17]. This was also supported in the current study, which showed a "*6 or *28"-dependent increase in serum total bilirubin levels (Fig. 1). Because of the low frequency of *28 without homozygotes among our subjects, the influence of *28 on toxicities was not clearly demonstrated, as in the case of the Korean patients where the allele frequency of *1A1*6* (23.5%) was much higher than that of *1A1*28* (7.3%) [18]. However, in the current study, the double heterozygotes of *6 and *28 (*6/*28) showed increases in serum total bilirubin levels (Fig. 1). Moreover, a higher incidence of severe neutropenia in the *6/*28 patients was observed, although the patient number was small ($N = 2$) (Table 4). This finding also indi-

Table 4
Association of *UGT1A1* genotypes with severe neutropenia (grade 3 or 4) in irinotecan monotherapy

Diplotype[b]	Neutropenia[a]/total (%)	Effect of *6 (%)	
−/−	1/20 (5.0)	non-*6/non-*6	(3.4)
*28/−	0/9 (0.0)		
*6/−	3/16 (18.8)	*6/non-*6	(22.2)
*6/*28	1/2 (50.0)		
*6/*6	1/2 (50.0)	*6/*6	(50.0)
P-value[c]		0.012	

[a] Grade 3 or 4.
[b] "−" represents "*1 or *60".
[c] Chi-square test for trend.

Table 5
Multivariate logistic regression analysis of severe neutropenia (grade 3 or 4) in irinotecan monotherapy

Variable	Coefficient	SE	*P*-value	Odds ratio	(95% Confidence limit)
*UGT1A1*6*	1.77	0.809	0.0289	5.87	(1.37–39.6)

$R^2 = 0.157$, Intercept = 3.15, $N = 49$.

cates the importance of "*6 or *28" in severe neutropenia, and in fact, a gene-dose effect of "*6 or *28" ($p = 0.04$ in the chi-square test for trend) and its significant contribution in multivariate analysis ($p = 0.0326$) were also confirmed (data not shown).

For the *60 haplotype (-3279T>G without -40_-39insTA), no association of *60 with severe neutropenia was observed in this study, which coincides with reports of other studies on Japanese cancer patients [17,23]. As for the *27 allele [686C>A(P229Q)], it was linked with the *28 allele and the haplotype was defined as the *28 subtype, *28c [15]. One *28c-heterozygous patient with irinotecan monotherapy showed no severe neutropenia, suggesting a small contribution of the *27 allele (data not shown).

In this study, the association between *UGT1A1* genotypes and antitumor activity was difficult to evaluate because of the small number of subjects stratified into each tumor type. Further clinical studies are needed to establish methods for selection of the appropriate regimen or dosage based on the *UGT1A1* genotypes, where a balance between toxicity and antitumor effect should be considered.

In conclusion, this study demonstrated the significant association of *UGT1A1*6* with severe irinotecan-mediated neutropenia. The current data also supported the usefulness of the genetic marker "*6 or *28" for personalized irinotecan therapy in Japanese, and likely East Asian, patients.

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ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

Section Editor: Mark J. Ratain, MD

Population Differences in the Use of EGFR-targeted Agents

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H&O What do we currently know about epidermal growth factor receptor (EGFR)-targeted therapies and population differences?

NS EGFR-targeted therapies can largely be divided into 2 categories: EGFR small molecule tyrosine kinase inhibitors (TKIs) and antibodies.

First of all, we know that the response rates for EGFR TKIs such as gefitinib (Iressa, Astrazeneca) and erlotinib (Tarceva, Osi Phamaceuticals) are significantly higher in Asians, females, adenocarcinomas, and non-smokers. Survival rates are also better than in the total population. As for the toxicity profiles, incidences of pulmonary toxicities are higher in males, smokers, and squamous cell carcinomas. Within the Asian population, we currently know that the frequency of interstitial pneumonia is significantly higher in Japanese patients than in the Chinese and Korean population.

Secondly, there are the antibodies such as cetuximab (Erbitux, Imclone). This year at the American Society of Clinical Oncology meeting, the results from an interesting FLEX study—a randomized, multicenter, phase III investigation that compared cetuximab in combination with cisplatin/vinorelbine versus cisplatin/vinorelbine alone in advanced non-small cell lung cancer patients—were presented. There were very few data pertaining to the Asian population, but when the researchers divided data of the Caucasians and the Asians, the results seemed to be better in Caucasians. In the Asian population, there was no difference in survival rates; I think this is because the majority of Asian people receive small-molecule EGFR TKIs after antibody treatment, a factor that may confuse the survival results.

H&O How are these differences explained by EGFR and K-Ras mutation rates in certain populations?

NS About 30–40% of Asians are said to have an EGFR mutation. In Caucasians, the reported mutation rate is less than 10%. This corresponds with study results that show the same type of difference—a higher response and survival rates in the Asian population to EGFR inhibitor therapy—between the 2 populations. We currently do not know much about the mutation rates in other populations such as blacks, hispanics, etc., although it is said that in the hispanic population, the mutation rate seems to be very low.

In the European Society of Medical Oncology (ESMO) meeting this September, Professor Tony Mok from the Chinese University of Hong Kong presented results from the IRESSA Pan-Asia Study (IPASS), which clearly showed that EGFR mutation is related to response and survival.

The IPASS study, of which I was one of the co-workers, was an open label, randomized, parallel-group trial that tested gefitinib versus carboplatin/paclitaxel (carbo/paclitaxel) as first line treatment in a selected population of patients from Asia. It included 1,217 Asian people whose tumors were of adenocarcinoma histology, who had not

received prior chemotherapy, and who were non smokers or light smokers. Japanese people were about 20% of the participants; Chinese were about 30%; the rest were from other Asian countries. The aim of the trial was to demonstrate that gefitinib was non inferior to carbo/paclitaxel doublet chemotherapy.

Subjects were randomized (about 600 subjects in each arm) to gefitinib or carbo/paclitaxel (ie, standard chemotherapy). The primary endpoint was progression-free survival (PFS).

Results showed that the gefitinib group had superior PFS and higher tumor response compared with intravenous carbo/paclitaxel chemotherapy in the overall population. However, although the PFS in the gefitinib group was significantly better, we noticed that the 2 curves for gefitinib and carbo/paclitaxel crossed at 5–6 months. Interestingly, during the first 5–6 months, the carbo/paclitaxel group was doing better, but after that point, the gefitinib group showed better PFS. These were 2 very strange curves. Statistically, when we analyzed the differences using the Cox proportional hazard model, there was a significant difference between the 2 groups, overall favoring gefitinib. However, there is really no consensus as to whether crossed curves can be analyzed by the Cox proportional hazard model.

Also noteworthy was that among the 1,217 patients, about one-third were anaylized by biomarkers such as EGFR mutation, EGFR amplification, and EGFR expression. We found that in patients with EGFR mutation, gefitinib did significantly better than carbo/paclitaxel. However, in patients with the wild-type EGFR, the PFS of the carbo/paclitaxel group was significantly better than that of the gefitinib group. This was a very interesting observation.

As you know, patients who have an EGFR mutation do not have a K-Ras mutation, and vice versa. One might therefore speculate that, in a sense, K-Ras mutation is inversely associated with the efficacy of EGFR-targeted therapy, but the truth is that there is not enough data in lung cancer. In colon cancer, if the EGFR is mutated, anti-EGFR antibodies such as cetuximab are not effective. In lung cancer, we do not have much data mainly because K-Ras mutation rate is not very high.

H&O Have there been studies investigating the differences within the Asian population (ie, Japanese, Korean, Chinese, etc.)?

NS This is a difficult question because we have very few data. We do know that the mutation rates of the Japanese and Koreans are nearly the same—around 30–40%. At present, we do not have sufficient data on the mutation rates of the Chinese and other Asian countries, so we have not been able to make a complete comparison yet.

H&O What technology is there to detect EGFR mutation, and how reasonable is it to use it to predict EGFR TKI efficacy?

NS Some claim that other biomarkers such as EGFR amplification and fluorescence in situ hybridization (FISH) could also be indicators; but in my mind, they are not very reliable. I believe that EGFR mutation is the most reliable predictor we currently know. And reliability here depends on the number of samples; we need to get enough samples to analyze. How we detect mutation is a separate issue—a technical problem. I think that if we use copy numbers of the EGFR for amplification parameters, it would be reasonably reliable because it is very quantitatvie.

The problem with FISH results is that they contain 2 elements. FISH positive includes EGFR amplification and high polysomy. However, EGFR amplification is closely correlated to mutation whereas high polysomy does not show any correlation.

When studies include both, the end analysis may be very complicated. This is the case with the majority of the data from the University of Colorado Cancer Center or from Dr. Federico Cappuzzo at the Istituto Clinico Humanitas IRCCS in Italy, who sees FISH technology to be the best method for patient selection when the main endpoint is survival. But I think the mix of 2 different kinds of FISH data is very difficult for us to interpret. Even in the IPASS trial, analysis of survival based on FISH positivity showed a similar tendency but the analysis based on EGFR mutation was much more clear.

I also think that clinical factors such as nonsmoking, females, adenocarcinomas, etc. are related to these EGFR mutations. So at this point, I believe that EGFR mutation is most highly predictive. If patients have the mutation, nearly 80% of them will respond.

H&O Should EGFR TKIs be included in the initial therapy for patients with EGFR mutation?

NS This is a crucial question. And as was evident from my results at the ESMO meeting this year, we can conclude that for patients with EGFR mutation, the first choice of therapy could be gefitinib. For patients without EGFR mutation, chemotherapy should be chosen as the first choice of therapy. But, the IPASS data are for PFS and not overal survival (OS). We still need to wait for OS data, and it will take some time.

But I think the important thing is to focus on the primary endpoint of a clinical trial. If the primary endpoint is OS, it is rather easy for us to interpret the results. If

the primary endpoint is PFS or time to treatment failure (TTF), it is rather difficult to make hard conclusions. PFS and TTF are not that accurate, making them softer endpoints, which do not directly relate to patient benefit.

H&O What sort of studies do you think are necessary to investigate this topic further? Are there any ongoing that are noteworthy?

NS Right now, there are talks of 2 randomized Japanese trials: one by researchers at Tohoku University and the other by the West Japan Oncology Group (WJOG). The study designs are very similar; both are testing gefitinib versus platinum doublet in EGFR-mutated patients. The Tohoku group is testing carbo/paclitaxel, whereas the WJOG group is testing cisplatin plus docetaxel, for their chemotherapy arm. The primary endpoint is PFS. Both trials are currently accruing patients.

However, the IPASS data has heavily influenced these clinical trials because they have already shown that PFS in EGFR-mutated patients is significantly better in the gefitinib group than in the chemotherapy group. So the question whether to continue these 2 randomized trials has become an ethical one, and still remains unanswered.

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Molecularly Targeted Therapy for Lung Cancer: Recent Topics

Many clinical trials of molecular target drugs have been done against advanced lung cancer, however, majority did not meet the primary endpoint. Positive studies of EGFR-TKI such as BR21 and Interest used unselected populations of non-small cell lung cancer. It was quite difficult to explain why they were positive. In the present review, the difficulties of clinical trial design in molecular target drugs were discussed based on the differences of the magnitude of antitumor activity and the target tumor cell population between cytotoxic drugs and molecular target therapy. **(J Lung Cancer 2008;7(1):1 - 8)**

Key Words: Lung cancer, Molecular target therapy, EGFR-TKI, Clinical trial

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The therapeutic efficacy of cytocidal anticancer drugs for lung cancer has reached a plateau(1 ~ 4), and it is extremely important to develop of new therapeutic agents. However, the majority of clinical trials of molecularly targeted drugs for lung cancer have yielded negative data, and the only drugs currently approved anywhere in the world are the EGFR-TKIs such as gefitinib and erlotinib and the anti-VEGF antibody, bevacizumab. Historically, matrix metalloprotease inhibitors(5), PKC α inhibitors, Ras kinase inhibitors(6), bexarotene, trastuzumab(7), etc.(8), have all been assessed with the prolongation of survival by simultaneous or consecutive use with cytocidal anticancer agents, but only negative data have been obtained (Table 1).

Table 1. Molecular Target-based Therapy in Lung Cancer

Specific target-based drugs	Combination	Results
Gefitinib (EGFR)	Y	Negative
	N	Negative, vs placebo
	N	Negative in Japanese, vs DTX
		Positive in Global, vs DTX
Erlotinib (EGFR)	Y	Negative
	N	Positive, vs placebo
Cetuximab (EGFR)	Y	Positive
Lonafarnib (ras)	Y	Negative
Bexarotene (RXR)	Y	Negative
Affinitac (PKC α)	Y	Negative
Sorafenib (Raf, VEGF etc)	Y	Negative
Trastuzumab	Y	Negative
Cetuximab	Y	Negative
Environment specific target-based drugs	**Combination**	**Results**
MMPI (Marimastat, Prinomastat)	Y	Negative
Bevacizumab	Y	Positive

N: No, Y: Yes

EGFR-TKIs

EGFR-TKIs are molecularly targeted drugs that selectively modify molecular biological abnormalities of tumor cells themselves(9 ~ 12). The amazing antitumor effect of EGFR-TKIs in cases in which platinum-taxane therapy failed attracted interest(13 ~ 16), but it was difficult to demonstrate that they contributed to any survival benefit(17 ~ 20). Erlotinib is used as second-line and third-line chemotherapy in cases of platinum-

taxane failure, and it has shown a survival benefit in comparison with placebo in unselected non-small cell cancer(21). By contrast, it was impossible to show any overall survival benefit of gefitinib in a group of similar cases that were almost the same although the results were marginal(22,23), and while significant prolongation of survival time was observed in Asians (no Japanese were included) by post-study stratification, no difference in survival time at all from the placebo control group was observed in Caucasians. Moreover, four trials of standard chemotherapy (carboplatin+paclitaxel, gemcitabine+cisplatin) ±EGFR-TKI all yielded negative data(17~20), and in a comparative study with gefitinib as intensification chemotherapy for stage III non-small cell cancer the survival time of the gefitinib group was instead significantly poorer than in the control group(24). Adjuvant studies using EGFR-TKIs in resected cases was started in Japan and North America but case entry was poor, and it was stopped before completion(25).

Two comparative studies of docetaxel versus the EGFR-TKI gefitinib in cases in which platinum-taxane was ineffective yielded different results. Even though the response rate to gefitinib by the Japanese patients was higher than in the Western population, it was impossible to demonstrate non-inferiority versus docetaxel in the V15-32 study conducted in Japan(26). By contrast, non-inferiority was demonstrated in the Interest study conducted in a large number of cases in Western countries(27).

The majority of the results of these studies were not what the investigators expected (Table 2), and numerous questions have arisen.

1) In placebo-controlled studies in cases in which platinum-taxane therapy was ineffective, the ISEL study (gefitinib) was negative(22), whereas BR-21 (erlotinib) was positive(21). The efficacy of gefitinib was marginal, but no difference at all was observed in the Western subjects. Differences in dosage were stated as the reason, but that is not a satisfactory explanation.

2) Does not the fact that Intact I & II (gefitinib)(17,18) and Talent(19) & Tribute (erlotinib)(20) were all negative studies conflict with the evidence in BR-21 study. There is the explanation based on their effects on the cell cycle that anti-cancer drugs and EGFR-TKIs act antagonistically when administered simultaneously.

3) Non-inferiority versus docetaxel was demonstrated in the Interest study (gefitinib) even though the ISEL study (gefitinib) was negative. By contrast, although Japanese patients, who have a high response rate to EGFR-TKIs, were used as the study subjects of the V15-32 study (gefitinib), the docetaxel control group tended to have better survival at each time point of 10-12 months after the beginning of treatment.

Table 2. RCTs (Randomized Clinical Trials) of Erlotinib & Gefitinib

	Early	Stage III	Advanced	
Erlotinib	RADIANT (n=945, vs. placebo, *on going*)		First line TALENT (n=1172, CDDP/GEM± Erlotinib, *negative*) TRIBUTE (n=1059, CBDCA/PTX± Erlotinib, *negative*) SATURN (n=850, CT x 4 → vs. placebo, *on going*)	Relapsed BR.21 (n=731, vs. placebo, *positive*) TITAN (n=648, vs DTX, *on going*)
Gefitinib	BR.19 (n=1242, vs. placebo, *terminated*) Japanese trial (n=670, vs. placebo, *terminated*)	SWOG0023 (n=840, CRT→DTX→gefitinib, *terminated*)	First line INTACT1 (n=1093, CDDP/GEM± Gefitinib, *negative*) INTACT2 (n=1037, CBDCA/PTX± Gefitinib, *negative*)	Relapsed ISEL (n=1692, vs. placebo, *negative*) V15-32 (n=484, vs. DTX, *negative*) INTEREST (n=1466, vs. DTX, *positive*)

4) In the SWOG S0023, which evaluated differences according to whether gefitinib was used after radiochemotherapy, survival time was significantly shorter in the gefitinib group(24). Reason. Although considerable patient selection was involved, it was a randomized controlled trial.

5) Do the results of the Interest and BR-21 studies suggest that the efficacy of gefitinib and erlotinib is equivalent?(21,27) Is it legitimate to speculate and argue whether there are differences in efficacy based on the results of clinical studies with completely different study designs.

These questions suggest that the basic assumptions underlying clinical trial results of anticancer drugs can not be applied to molecularly targeted therapy.

Against this background the following are conceivable.

1) The response rates of Western people and Asian people to EGFR-TKIs are different, and the reason for the difference is a difference in EGFR mutation rate(28～44).

2) At present it is unknown whether EGFR mutations are a predictor of the therapeutic efficacy of EGFR-TKIs or even a predictor of the therapeutic efficacy of cytotoxic anticancer drugs(26).

3) EGFR-TKIs display a potent antitumor effect in cells that possess the target, but have no effect at all on cells that do not possess it. By contrast, because cytotoxic anticancer drugs exert an antitumor effect against whole tumor mass (Fig. 1), the effect that they have on survival time is different from that of molecularly targeted drugs even if the response rates are equivalent according to the RECIST criteria (Fig. 2). The concept of "long NC" does not apply to molecularly targeted drugs such as EGFR-TKIs. Actually, in the V15-32 study the response rate to gefitinib was approximately twofold compared

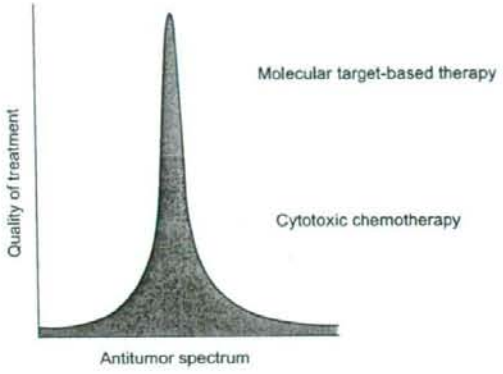


Fig. 1. Improvement of treatment quality.

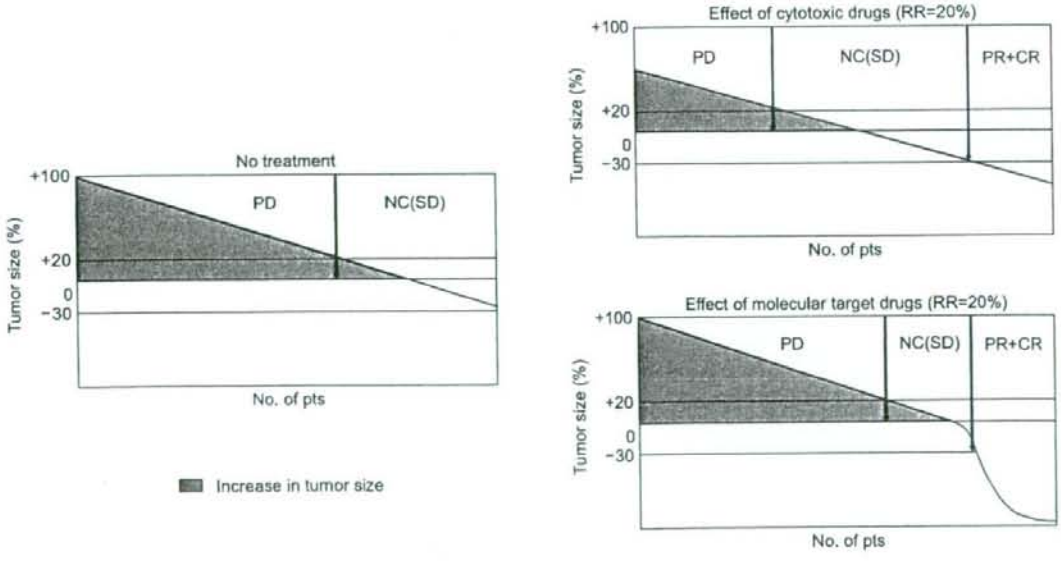


Fig. 2. Difference in the effect of cytotoxic drugs and molecular target drugs (waterfall plots).

with docetaxel(26), but non-inferiority could not be demonstrated, and survival time at each time point assessed in the gefitinib group was slightly poorer than in the docetaxel group at each time point during early phase after the beginning of treatment (Table 3, Fig. 3, 4). Waterfall plots are being used often recently. We can show the differences in efficacy between anticancer drugs and molecularly targeted drugs in figures (Fig. 2).

The basis of molecularly targeted therapy is that it should be used to treat patients who harbor the target. The problem lies in the degree of sensitivity and specificity of the biomarkers that are capable of detecting the molecular target. The molecular target of EGFR-TKIs is a mutated EGFR, and while a response rate of approximately 80% can be achieved when mutations are present, a response of 10% is obtained even when there are no mutations(28~32). Moreover, it is not easy to obtain samples that are sufficient to detect mutations. Attempts are being made to devise a method of detection that uses blood, etc., as the specimen, but the results have not been satisfactory. Changes in surrogate tissue seem merely to reflect germ line variation, and their meaning is different from that of assessments that use tumor tissue and reflect both germ line variation including SNPs and somatic mutation. Attempts have also been made to predict therapeutic efficacy on the basis of gene expression(40), protein expression(41), etc., in addition to mutations, but no reliable results have been reported.

Table 3. Overall Survival (ITT)

	Gefitinib	RR	Docetaxel	RR
No. of Pts	245	22.5%	244	12.8%
No. of events	156		150	
One year survival (%)	48%		54%	

Hazard ratio=1.12 (0.89~1.40) p=0.330. Non-inferiority could not be demonstrated.

Anti-EGFR Antibody

There have been few results of research on the effect of EGFR antibodies (cetuximab, panitumab, matuzumab) on lung cancer. The antibodies recognize epitopes on the cell surface and have been found to exert their antitumor activity by blocking signal transduction pathway or by antibody-dependent cell-mediated cytotoxicity (ADCC). The mechanism by which they block signal transduction systems has not been elucidated. According to the results of in vitro studies, the majority of the antitumor activity of the antibodies appears to be attributable

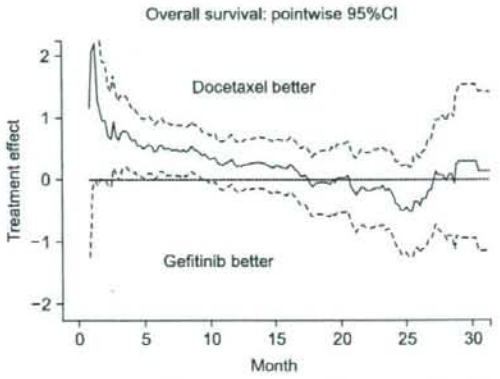
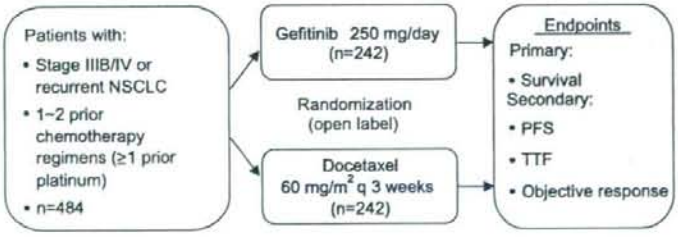


Fig. 4. Treatment effect at each time point. Analysed by Prof. Masahiro Takeuchi, Kitasato University, Division of Biostatistics & Division of Pharmaceutical Medicine. Courtesy of Prof. M. Takeuchi, Kitasato University.



- Stratified for histology, gender, PS, study site
- Non-inferiority design: Upper limit of hazard ratio<1.25

Fig. 3. Trial V15-32: Phase III trial of gefitinib vs. docetaxel in 2nd/3rd line NSCLC.

to ADCC. In a study comparing CDDP+vinorelbine±cetuximab, Gatzemeier and Rosell obtained an improvement in response rate and prolongation of progression-free time in comparison with anticancer drug therapy alone(45), and Kelley et al. conducted a study comparing simultaneous and consecutive treatment with cetuximab in combination with CBDCA+ paclitaxel and obtained better treatment results in the simultaneous administration group(46). Assessment of improvement in the results of treatment by applying EGFR antibodies to the treatment of other stages of lung cancer seems necessary in the future(47,48).

Anti-VEGF Antibody (Bevacizumab)

Anti-VEGF antibody is intended to improve treatment results by selectively modifying the molecular biological properties of the host that constitutes the tumor environment(49). When negative data for matrix metalloproteases persisted, it was concluded that "target-less molecularly targeted agents" that act on the tumor environment in this way do not contribute to improving the results of treatment. However, the remarkable improvement in results of treatment with IFL+bevacizumab for colorectal cancer(50) and reproducible results with FOLFOX4+ bevacizumab(51) suggested that even drugs that acted on the tumor environment could produce a significant survival benefit and improvement in cure rate. The ECOG reported positive data for PTL+CBDCA±bevacizumab(52,53) in previously untreated advanced non-small cell cancer, but despite strict patient selection that accepted only non-squamous cell carcinoma patients as subjects, a mere 2-month survival benefit and a significantly high rate of adverse effects, such as bleeding, were observed. The enormous cost of treatment was seen as another problem. The AVAIL study, which was primarily conducted in Europe, compared gemcitabine+CDDP±bevacizumab, and prolongation of progression-free time was observed in the bevacizumab group(54), but, unfortunately, there was no prolongation of overall survival time. Moreover, in the 7.5 mg/kg dosage group of the ECOG phase II trial, the results of treatment were poor. It is unknown whether these inconsistencies were simply attributable to differences in the prognostic factors of the patients entered in the study or were based on the chemotherapy regimen that was used. Research on biomarkers that might predict the efficacy of target-less molecularly targeted drugs or be correlated with their efficacy has been lagging. Bevacizumab has already begun to be used in Japan in combination with FOLFOX4 to treat colorectal cancer. Training of clinical oncologists who sufficiently understand the emergency management of thrombosis and bleeding is needed.

Multiple-target Molecularly Targeted Drugs ("Dirty" Targeted Drugs)

A great number of anticancer drugs that act on a variety of targets have been developed, and clinical trials have been conducted in lung cancer. From the standpoint of the process of drug development, the fact that a drug that selectively modifies a certain target has been developed does not necessarily mean that it will act on that target alone. Thus, viewed from the opposite vantage point, developing drugs that are designed to modify many targets just from the beginning may also serve as a strategy. Since signal transduction systems are constructed of complex networks, attempting to impede tumor growth by simultaneously inhibiting several of their pathways is one possible approach. However, as the number of targets increases, proof of principle studies become more difficult. In addition, it will be necessary to consider the choice between using dirty targeted drugs that have many targets or using combinations of targeted drugs that have different targets. Moreover, even being called "dirty" seems unavoidable, because many investigators themselves have not sorted out what the targets are in the clinical trials of Sorafenib(55), Sunitinib(56), Vandetanib(57), etc.(58), which are currently being tested. Every time results of clinical studies are obtained, there is a feeling that they are going to cause a headache. Selection of a population that possesses the target would seem essential for clinical studies of molecularly targeted drugs. On the other hand, because there are no targets for molecularly targeted drugs that are cancer-environment-specific, patient selection is not performed. Because the "dirty" targeted drugs that are currently being used are equipped with both functions, it is claimed that a combined effect can be achieved, but there is also a possibility that we are doing a biologically fatal contradiction.

Clinical Studies and Biomarkers

When molecularly targeted drugs were introduced, there was

a widespread theory that "because the efficacy of molecularly targeted drugs is exhibited in the form of a cytostatic effect instead of a cytotoxic effect, it is impossible to evaluate them by ordinary clinical trial methodology". However, the hypothesis has been demonstrated to be false. 1) despite being targeted therapy, effective compounds cause tumor shrinkage, 2) matrix metalloproteases and other drugs that act on the tumor environment have yielded negative data in phase 3 studies every single time, and 3) drug-specific adverse effects associated with increases in dose are observed with drugs other than antibodies, it now appears possible to evaluate molecularly targeted drugs by conventional clinical studies. Facts that have subsequently become clear include that 1) targeted drugs are effective only in cells that possess the target and are completely ineffective in cells that do not, 2) drugs that act downstream of signal transduction have poor selectivity, and it is difficult to demonstrate efficacy, and 3) drugs that act on specific molecular biological characteristics of the cancer environment in a certain sense do not have a target. Thus, when a specific molecular biological target is present on the cancer cells themselves, it seems ideal to select subjects who have the target and use it to treat them. Success has been achieved with Herceptin in breast cancer by using that strategy, and it is not difficult to plan clinical trials of Rituxan for lymphomas, Gleevec for CML, etc., because all of the cancer cells retain the original target. Patient selection for EGFR-TKIs seems to be the most strategic task, and the establishment of validated biomarkers with high sensitivity and excellent selectivity also seems to be an important task. V15-32 research has shown that it is impossible to predict survival curves in clinical studies that include whole patients without selection. By contrast, because drugs that act on the cancer environment, as represented by Avastin, do not have a target, all types of cancers are candidates for treatment. The exception is patients who develop severe toxicity. This category of drugs basically cannot be expected to be effective when used alone. They are used in combination, and cancer chemotherapy intensifying effects, etc., have been shown. Because these drugs can be expected to be effective to a certain degree in all patients without selection and they ultimately seem to intensify the efficacy of anticancer drugs, it seems possible to make comparisons by means of survival curves and proportional hazard models of treatment with cytocidal anticancer drugs.

CONCLUSION

Effect of Molecularly targeted therapy of lung cancer is less clear-cut than for other diseases. Despite EGFR-TKIs displaying a remarkable antitumor effect in taxane-platinum-resistant cases, it can be pointed out that it has been impossible to demonstrate any prolongation of survival time and that there are far too few segmented cases, especially in Western countries, in order to perform patient selection based on EGFR mutations.

Comparative studies in patients selected according to their clinical characteristics and whether they have EGFR mutations are currently being conducted, and it will be very interesting to see what kind of results they yield. Avastin seems likely to be approved in Japan, but caution is required in regard to toxicity. What kind of results will be obtained when "dirty" targeted drugs are subjected to clinical studies without patient selection is unknown territory.

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