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Minireview

Emerging ethnic differences in lung cancer therapy

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Although global clinical trials for lung cancer can enable the development of new agents efficiently, whether the results of clinical trials performed in one population can be fully extrapolated to another population remains questionable. A comparison of phase III trials for the same drug combinations against lung cancer in different countries shows a great diversity in haematological toxicity. One possible reason for this diversity may be that different ethnic populations may have different physiological capacities for white blood cell production and maturation. In addition, polymorphisms in the promoter and coding regions of drug-metabolising enzymes (e.g., CYP3A4 and UGT1A1) or in transporters (e.g., ABCB1) may vary among different ethnic populations. For example, epidermal growth factor receptor (EGFR) inhibitors are more effective in Asian patients than in patients of other ethnicities, a characteristic that parallels the incidence of EGFR-activating mutations. Interstitial lung disease associated with the administration of gefitinib is also more common among Japanese patients than among patients of other ethnicities. Although research into these differences has just begun, these studies suggest that possible pharmacogenomic and tumour genetic differences associated with individual responses to anticancer agents should be carefully considered when conducting global clinical trials.
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Lung cancer is the most common malignancy worldwide. Approximately 1.2 million people are diagnosed with lung cancer annually (accounting for 12.3% of all cancers); the second most common malignancy is breast cancer (10.4%), followed by colorectal cancer (9.4%). As lung cancer almost invariably has a poor prognosis, it is the largest single cause of death from cancer in the world, with a mortality of 1.1 million annually (Stewart and Kleihues, 2003). Only 15% of lung cancer patients have a disease that is confined to the lung and are candidates for surgical resection; most patients with this disease have distant metastases or pleural effusion at the time of their initial diagnosis. These patients can be treated with systemic chemotherapy, but the efficacy of currently available anticancer agents is limited and patients with advanced diseases rarely live long.

As the development of new anticancer agents and chemotherapeutic regimens is both time and money consuming, clinical trials need to be as efficient as possible. One effort in this direction has been the adoption of global clinical trials for new agents that involve trial centres on more than one continent; this strategy enables adequate sample sizes to be obtained in a relatively short-time period and eliminates the need for redundant clinical trials with similar objectives conducted in different countries. However, whether the results of clinical trials performed in one population can be fully extrapolated to other populations remains questionable because of potential differences in trial designs, study-specific criteria, patient demographics, frequency of monitoring, and population-related pharmacokinetics, pharmacodynamics and pharmacogenomics. Recently, these genetic and physiologic factors influencing cancer chemotherapy have been increasingly examined and reported.

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CLINICAL OBSERVATIONS OF TOXICITY DURING CYTOTOXIC CHEMOTHERAPY

A comparison of phase III trials for the same drug combinations against non-small cell lung cancer conducted in different countries shows a great diversity in toxicity (Sekine *et al*, 2006). Among trials studying the combination of carboplatin and paclitaxel, the dose of carboplatin was fixed in all the trials, but the dose of paclitaxel was 200 mg m^{-2} in Japanese and European trials and 225 mg m^{-2} in American trials. Grades 3–4 neutropenia was noted in 88% of the patients in the Japanese trial, 15–51% of the patients in the European trials, and 6–65% of the patients in the American trials. Meanwhile, grades 3–4 febrile neutropenia was encountered in 16% of the patients in the Japanese trial, 0–9% of the patients in the European trials, and 2–4% of the patients in the American trials (Table 1). For combinations of cisplatin and docetaxel (Table 1) and cisplatin and vinorelbine (Table 2), the incidences of grades 3–4 neutropenia and febrile neutropenia were almost the same between phase III trials performed in different areas, but the doses of docetaxel and vinorelbine in the Japanese trials were lower than those in the European and American trials. Thus, neutropenia in patients receiving a combination of platinum and antimicrotubule agents may be more severe in Japanese than in Europeans and Americans. A higher frequency of grades 3–4 neutropenia in Japanese patients than in American patients was associated with combinations of cisplatin and irinotecan (65 *vs*

Table 1 Toxicity associated with a combination of platinum and taxane

Research group	Chemotherapy dose: Platinum	Chemotherapy dose: Taxane	No. of patients	Grades 3–4 toxicity (%): NP	Grades 3–4 toxicity (%): FNP	Reference
A combination of carboplatin and paclitaxel						
Japan	6 (AUC)	200 ($mg\,m^{-2}$)	145	88	16	Ohe *et al* (2007)
Greece	6 (AUC)	200 ($mg\,m^{-2}$)	252	15	0	Kosmidis *et al* (2002)
EU	6 (AUC)	200 ($mg\,m^{-2}$)	309	51	4	Rosell *et al* (2002)
ECOG	6 (AUC)	225 ($mg\,m^{-2}$)	290	63	4	Schiller *et al* (2002)
SWOG	6 (AUC)	225 ($mg\,m^{-2}$)	206	57	2	Kelly *et al* (2001)
SWOG	6 (AUC)	225 ($mg\,m^{-2}$)	182	—	3	Gandara *et al* (2004)
USA	6 (AUC)	225 ($mg\,m^{-2}$)	190	65	—	Belani *et al* (2005)
USA	6 (AUC)	225 ($mg\,m^{-2}$)	345	6	—	Herbst *et al* (2004)
A combination of cisplatin and docetaxel						
Japan	80 ($mg\,m^{-2}$)	60 ($mg\,m^{-2}$)	151	74	2	Ohe *et al* (2007)
ECOG	75 ($mg\,m^{-2}$)	75 ($mg\,m^{-2}$)	289	69	11	Schiller *et al* (2002)
USA	75 ($mg\,m^{-2}$)	75 ($mg\,m^{-2}$)	408	75	5	Fossella *et al* (2003)

NP, neutropenia; FNP, febrile neutropenia.

Table 2 Toxicity associated with a combination of cisplatin and vinorelbine

Research group	Chemotherapy dose ($mg\,m^{-2}$): Cisplatin	Chemotherapy dose ($mg\,m^{-2}$): Vinorelbine	No. of patients	Grades 3–4 toxicity (%): NP	Grades 3–4 toxicity (%): FNP	Reference
Japan	80 (day 1)	25 (days 1, 8)	145	88	18	Ohe *et al* (2007)
Greece	80 (day 8)	30 (days 1, 8)	204	37	11	Georgoulias *et al* (2005)
France	100 (day 1)	30 (weekly)	156	83	22	Pujol *et al* (2005)
EU	120 (day 1)	30 (weekly)	206	79	4	Le Chevalier *et al* (1994)
SWOG	100 (day 1)	25 (weekly)	202	76	1	Kelly *et al* (2001)
USA	100 (day 1)	25 (weekly)	404	79	5	Fossella *et al* (2003)

NP, neutropenia; FNP, febrile neutropenia.

32%, $P<0.001$) and cisplatin and etoposide (92 *vs* 66%, $P<0.001$) for the treatment of extensive small-cell lung cancer (Lara *et al*, 2007).

How can this ethnic difference in the severity of neutropenia be explained? One possibility is that the physiological capacity of the white blood cell production and maturation may vary among different ethnic populations. An asymptomatic reduction in neutrophils (benign neutropenia) is more commonly observed in individuals of African descent than in Caucasians, and no data on this phenomenon are available for Asians (Hsieh *et al*, 2007). The mechanisms are unclear, but a lower bone marrow reserve, an intrinsic marrow difference, an abnormal cytokine response, or any combination of these factors have been suggested (Hsieh *et al*, 2007). The lower neutrophil counts were associated with higher levels of IL-8 and granulocyte colony-stimulating factor in African volunteers. Thus, these cytokines are considered to compensate for the relatively low neutrophil counts in this population (Mayr *et al*, 2007). A recent report showed that ethnicity-related low neutrophil counts were associated with neutrophil elastase (ELA2) polymorphisms (C-199A), but not with serum cytokine levels (Grann *et al*, 2007).

ETHNIC DIFFERENCES IN DRUG METABOLISING ENZYMES

An explanation for the ethnic differences in haematological toxicity may be the varying activities of drug-metabolising enzymes and transporters that are mainly associated with polymorphisms in the promoter and coding regions of these enzymes (Fujita and Sasaki, 2007). The haematological toxicity of docetaxel monotherapy was associated with the clearance of this agent in Asian patients, a phenomenon that can be largely explained by CYP3A4 activity (Yamamoto *et al*, 2000). A study conducted in the Netherlands showed that docetaxel clearance was associated with the homozygous C1236T polymorphism in the ABCB1 (p-glycoprotein) gene (ABCB1*8) but was not associated with any CYP3A4 gene polymorphisms (Bosch *et al*, 2006). In contrast, docetaxel pharmacokinetics were not associated with the percent decrease in neutrophil counts nor with any polymorphisms in the CYP3A4 and ABCB1 genes in American patients (Lewis *et al*, 2007). Another example of ethnic differences in drug-metabolising enzymes is the association between polymorphisms in genes involved in irinotecan metabolism and irinotecan-induced neutropenia. Among the patients who received irinotecan with or without another anticancer agent, grade 4 neutropenia was noted in 40–57% of the patients with UDP-glucuronosyltransferase (UGT) 1A1*28 (a polymorphism in the promoter region of the UGT1A1 gene) homozygosity, whereas neutropenia was only observed in 15% or less of the patients with wild-type alleles. This association was consistent in both Asian and Caucasian patients, although the frequency of homozygosity was about 10% in Caucasians and much lower in Asians. The UGT1A1*6 allele is another polymorphism at exon 1 that is associated with defective glucuronidating function and is found almost exclusively in Asian individuals with a frequency as high as 20% (Fujita and Sasaki, 2007). UGT1A1*6 is significantly linked to polymorphisms of UGT1A7 and UGT1A9. A haplotype including UGT1A1*6 and UGT1A7*3, noted in as many as 15% of Japanese patients, and UGT1A1*6 homozygosity, noted in 7% of Korean patients, were significantly associated with decreased glucuronosyltransferase activity for SN-38 and severe neutropenia (Han *et al*, 2006; Fujita

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et al, 2007). In 177 Japanese patients treated with irinotecan-including chemotherapy, a homozygous or double heterozygous genotype for UGT1A1*6 and UGT1A1*28 (*6/*6, *28/*28 or *6/*28) was significantly associated with severe neutropenia (Minami *et al*, 2007). In addition, patients with a homozygous C3435T polymorphism in the ABCB1 gene are four-fold more likely to develop grade 3 diarrhoea when treated with a combination of cisplatin and irinotecan (Lara *et al*, 2007).

Data on associations between polymorphisms in genes coding drug-metabolising enzymes and therapeutic efficacy remain scarce. A recent prospective study in 250 patients with metastatic colorectal cancer showed a significantly higher response rate (67 *vs* 40%) and a nonsignificant survival advantage (hazard ratio (HR): 0.81; 95% confidence interval (CI): 0.45–1.44) in patients homozygous for UGT1A1*28, compared with those with wild-type alleles; these outcomes were associated with a higher exposure to SN-38 (Toffoli *et al*, 2006). In a study of 81 NSCLC patients, those who were homozygous for UGT1A1*6 had a lower response rate (0 *vs* 50%, $P=0.038$) and a poorer MST (7.6 *vs* 17.7 months, $P=0.017$) as well as greater toxicities than the other patients (Han *et al*, 2006). The most plausible explanation for the negative effects of UGT1A1*6 on treatment outcome may be that the dose intensity or cycle number might have been reduced in patients with UGT1A1*6 because of polymorphism-associated toxicities (Fujita and Sasaki, 2007).

These pharmacogenetic analyses have been rather preliminary. Data on genotyping, pharmacokinetics, and pharmacodynamics collected from a large number of patients with different ethnic backgrounds are needed to demonstrate the cause of ethnic differences in chemotherapy-associated toxicity.

EFFICACY OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS

Epidermal growth factor receptor (EGFR), a cell membrane receptor with tyrosine kinase activity, is expressed in most patients with NSCLC and plays a role in cellular proliferation, inhibition of apoptosis, angiogenesis, metastatic potential, and chemoresistance. Small-molecule inhibitors of EGFR, such as gefitinib and erlotinib, have shown antitumor activity and have alleviated symptoms in NSCLC patients who were previously treated with standard chemotherapy. Two randomized phase II studies, IDEAL (Iressa Dose Evaluation in Advanced Lung Cancer)-1 (involving 210 patients and conducted in Europe, Australia, South Africa, and Japan) and IDEAL-2 (involving 216 patients and conducted in the USA), have evaluated the efficacy of gefitinib at a dose of either 250 mg daily or 500 mg daily in patients with advanced NSCLC in whom earlier platinum-based chemotherapy had failed. No difference in the response rates between the doses was noted, but an increased response rate was recorded for never smokers, women, and those with an adenocarcinoma histology, compared with patients who did not have these characteristics. In addition, the response rate was 28% in Japanese patients but only 9–12% in patients of other ethnicities (Fukuoka *et al*, 2003; Kris *et al*, 2003). A randomized phase III trial, ISEL (Iressa Survival Evaluation in Lung Cancer), of gefitinib *vs* a placebo in 1692 NSCLC patients who had been previously treated with one or two chemotherapeutic regimens failed to show any survival benefit of gefitinib; in the overall population, the median survival times (MSTs) in the gefitinib and placebo arms were 5.6 and 5.1 months, respectively (HR: 0.89; 95% CI: 0.78–1.03). A subgroup analysis, however, showed that the MST was longer in Asian patients receiving gefitinib than in those receiving the placebo (MST: 9.5 *vs* 5.5 months; HR: 0.66; 95% CI: 0.48–0.91). Similar results were seen for never smokers: patients receiving gefitinib survived longer than those receiving the placebo (MST: 8.9 *vs* 6.1 months; HR: 0.67, 95% CI: 0.49–0.91) (Thatcher *et al*, 2005).

A similar association between objective responses and ethnicity was observed in studies on erlotinib monotherapy for previously treated advanced NSCLC. In an American phase II trial of this agent in 57 advanced NSCLC patients with disease progression or relapse after platinum-based chemotherapy, the response rate was 12% and the MST was 8.4 months (Perez-Soler *et al*, 2004). In contrast, the combined data of two Japanese phase II trials of erlotinib in similar patient populations showed objective responses in 30 of 106 (28%) patients and an MST of 13.8 months. Among the responders, significantly higher proportions of females (50%) than males (17%) ($P=0.0009$) and of never smokers (51%) than smokers (14%) were observed ($P<0.0001$) (Tamura *et al*, 2007). A phase III trial of erlotinib or a placebo in 731 NSCLC patients previously treated with one or two chemotherapy regimens showed that the response rate in Asian patients was higher than that in patients of other ethnicities (28 *vs* 10%, $P=0.02$) (Shepherd *et al*, 2005).

These results of phases II and III trials consistently suggest that EGFR tyrosine kinase inhibitors may be more effective in Asian patients than in patients of other ethnicities.

In April 2004, the activating mutations of the EGFR gene were identified in NSCLC specimens, and cancers with these mutations were reported to be highly sensitive to gefitinib. The populations with higher responses to gefitinib (females, non-smokers and patients with an adenocarcinoma histology) also have higher incidences of EGFR mutations (Kosaka *et al*, 2004; Pao *et al*, 2004; Shigematsu *et al*, 2005). The incidence of EGFR mutations in surgically resected tissue samples is summarised in Table 3 (Kosaka *et al*, 2004; Pao *et al*, 2004; Marchetti *et al*, 2005; Qin *et al*, 2005; Shigematsu *et al*, 2005; Soung *et al*, 2005; Tokumo *et al*, 2005; Yang *et al*, 2005; Sasaki *et al*, 2006). The incidence varies from one report to another, but EGFR mutations tend to be more common among patients with an adenocarcinoma histology and among non-smokers. Among Asian patients, the average incidences of EGFR mutations were 31% overall, 47% among patients with adenocarcinoma, and 56% among non-smokers; among other ethnic populations, however, the average incidences were 7–8% overall, 13–15% among patients with adenocarcinoma, and 34–35% among non-smokers (Table 3). Thus, the percentage of responders to gefitinib or erlotinib almost paralleled the percentage of patients with EGFR mutations.

The mechanism responsible for the high frequency of EGFR mutations in Asian patients is a subject of great interest, and polymorphisms in the regulatory sequence of the EGFR gene have been vigorously investigated. The CA simple sequence repeat 1 (CA-SSR1), a highly polymorphic locus containing 14–21 CA dinucleotide repeats, is located at the 5′ end of intron 1 of the EGFR gene. Studies of CA-SSR1 repeat length and EGFR expression in breast cancer tissues have shown a constant decline in EGFR expression with increasing repeat length (Buerger *et al*, 2000, 2004). In addition, a shorter repeat length was associated with an elevated risk of lung cancer (Zhang *et al*, 2007) and poor survival in NSCLC patients (Dubey *et al*, 2006). The CA-SSR1 repeat length distribution varies according to ethnicity, with Asians tending to have longer repeats than Americans (Liu *et al*, 2003). Two single-nucleotide polymorphisms in the promoter region of the EGFR gene (−219G/T and −191C/A) were also associated with promoter activity and EGFR expression (Liu *et al*, 2005), and their polymorphic types (associated with low EGFR expression) were more common among Asians than among other ethnicities (Nomura *et al*, 2007). These observations suggest that many Asians have polymorphic types that lead to a decreased intrinsic production of EGFR protein. If a certain critical level of EGFR is required to drive the cell toward a malignant phenotype, another mechanism including activating mutations of EGFR and/or the autonomous activation of downstream signalling may be required for the development of lung cancer among Asians (Nomura *et al*, 2007).

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Table 3 Incidence of EGFR mutations in surgically resected specimens

		All cases		Adenocarcinoma		Non-smokers	
Author	Country	Total N	Mutation N (%)	Total N	Mutation N (%)	Total N	Mutation N (%)
Western areas							
Shigematsu	USA	80	11 (14)	44	11 (25)	26	7 (27)
Pao	USA	96	11 (11)	72	11 (15)	15	7 (47)
Yang	USA	219	26 (12)	164	25 (15)	34	12 (35)
Marchetti	Italy	860	39 (5)	375	39 (10)	103[a]	23 (22)
	Subtotal	1255	87 (7)	655	86 (13)	75	26 (35)
Asian areas							
Shigematsu	Japan	263	71 (27)	154	67 (44)	78	47 (60)
Kosaka	Japan	277	111 (40)	224	110 (49)	112[a]	76 (68)
Tokumo	Japan	120	38 (32)	82	37 (45)	36	25 (69)
Sasaki	Japan	95	35 (37)	71	32 (45)	36	25 (69)
Shigematsu	Taiwan	93	32 (34)	55	31 (56)	55	27 (49)
Qin	China	41	10 (24)	17	7 (41)	21	6 (29)
Soung	Korea	153	30 (20)	69	26 (38)	54	25 (46)
Shigematsu	Others	361	107 (30)	214	102 (48)	135	76 (56)
	Subtotal	1403	434 (31)	886	412 (47)	415	231 (56)
Other areas							
Shigematsu	Australia	83	6 (7)	36	5 (14)	7	4 (57)
Shigematsu	Others	158	13 (8)	75	12 (16)	31	9 (29)
	Subtotal	241	19 (8)	111	17 (15)	38	13 (34)
	Toatl	2899	540 (19)	1652	515 (31)	528	270 (51)

[a]Including only patients with adenocarcinoma histology.

INTERSTITIAL LUNG DISEASE ASSOCIATED WITH GEFITINIB AND ERLOTINIB

The frequencies of grades 3–4 common toxicities after the administration of gefitinib, including diarrhoea, skin rash, and elevated liver transaminase levels, have been similar among study populations, but the incidence of severe interstitial lung disease (ILD) associated with the administration of gefitinib differs between patients in Japan and those in other countries. In the IDEAL studies, two Japanese patients developed grades 3–4 ILD (2%), whereas no patients outside of Japan experienced ILD (Fukuoka *et al*, 2003; Kris *et al*, 2003). A retrospective study of 1976 consecutive patients treated with gefitinib at 84 institutions showed that the incidence of ILD was 3.5% and the mortality rate was 1.6%. Several risk factors for the development of gefitinib-induced ILD were identified in the Japanese population: a history of pulmonary fibrosis, a history of smoking, a poor performance status, and a male sex (Ando *et al*, 2006). A similar incidence of ILD (4.6%) was also noted in association with erlotinib chemotherapy in Japanese phase II trials (Tamura *et al*, 2007).

The association between ILD and anticancer treatment is a major topic in Japan because (1) the diagnosis of ILD can be difficult and a consensus among physicians is sometimes not reached, (2) the risk factors for ILD have not been fully established, 3) an effective treatment for ILD has not been established and the condition is often fatal, and (4) the low frequency of this complication makes it difficult to conduct pertinent clinical trials. Gefitinib-induced ILD seems to be more common among Japanese patients than among other patients, but the reasons for this ethnic difference are totally unknown.

CONCLUSION

The findings discussed here suggest that considerable variations in the toxicity and efficacy of anticancer agents may exist among patients of different ethnicities. Although research into these differences has just begun, these studies suggest that possible pharmacogenomic and tumour genetic differences associated with individual responses to anticancer agents should be carefully considered when conducting global clinical trials.

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Weekly Epoetin Beta Maintains Haemoglobin Levels and Improves Quality of Life in Patients with Non-Myeloid Malignancies Receiving Chemotherapy

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Objective: This study was aimed at investigating the effectiveness and safety of once-weekly epoetin beta for anaemic cancer patients receiving chemotherapy.
Methods: A total of 104 patients with a haemoglobin level of ≤11.0 g/dL were enrolled. Patients received a once-weekly subcutaneous dose of 36 000 IU epoetin beta for 12 weeks. If the increase in the haemoglobin level was <1.0 g/dL after 6 weeks, or a red blood cell transfusion was required between days 15 and 42, the dose of epoetin beta was increased to 54 000 IU from the subsequent week. The primary endpoint was the percentage of patients who achieved a haemoglobin increase of ≥2.0 g/dL; the haemoglobin response rate. Quality of life (QOL) was assessed using the Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire.
Results: The haemoglobin response rate was 66.3% among the 98 patients (breast cancer: $n = 25$; malignant lymphoma: $n = 21$; ovarian cancer: $n = 20$; lung cancer: $n = 15$; other cancers: $n = 17$) assessable for a haemoglobin response. Thirty-nine patients (39.8%) required a dose escalation to 54 000 IU. At the end of the study, QOL assessable patients ($n = 96$) showed a mean improvement in the FACT-An total fatigue subscale score (FSS) of 0.3 points from baseline. Patients with a haemoglobin response had a mean change in the total FSS of +3.2, compared with −3.4 for patients without a haemoglobin response. No serious adverse event of epoetin beta was observed.
Conclusions: Epoetin beta administered at an initial dose of 36 000 IU once-weekly was well tolerated, with increased haemoglobin levels and improved QOL in anaemic cancer patients receiving myelosuppressive chemotherapy.

Key words: anaemia – erythropoietin – cancer – chemotherapy – quality of life

INTRODUCTION

Anaemia is a common complication of cancer patients undergoing chemotherapy. Symptoms of anaemia, including fatigue, palpitations, dizziness and dyspnea markedly reduce patient activity, resulting in impaired quality of life (QOL). In most cases, however, physicians hesitate to prescribe red blood cell (RBC) transfusions until the haemoglobin level is <8.0 g/dL, even if the patient has symptoms related to anaemia, such as fatigue. Although the safety of blood transfusion has improved in recent years, risks still remain, such as viral infections, graft versûs host disease and haemolytic reactions.

In Europe and the United States, erythropoietin (EPO) agents have widely been used since the 1990s for the treatment of chemotherapy-induced anaemia. Although a three-times weekly dosing schedule was initially introduced (1–3), this schedule was inconvenient for outpatients. Several studies reported that once-weekly dosing of EPO increased the

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haemoglobin level and improved QOL in a manner comparable with those obtained by three-times weekly dosing (4,5).

Since EPO agents have not been approved for the treatment of chemotherapy-induced anaemia in Japan, we previously conducted a dose-finding study of weekly epoetin beta in patients with malignant lymphoma or lung cancer, resulting in a recommended weekly dose of 36 000 IU (6). In this prospective study, we investigated the haemoglobin response, the effects on QOL and the safety of once-weekly epoetin beta in anaemic patients with non-myeloid malignancies. We also investigated the effects of dose escalation to 54 000 IU in patients showing insufficient haemoglobin increase.

PATIENTS AND METHODS

Patient Eligibility

Inclusion criteria were as follows: (a) histological or cytological confirmation of non-myeloid malignancy diagnosis, (b) treatment with cyclic chemotherapy, (c) anaemia (haemoglobin level $\leq$11.0 g/dL) considered to be primarily chemotherapy-induced, (d) life expectancy of at least 4 months, (e) aged between 20 and 79 years, (f) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, (g) eligibility for the QOL questionnaire and (h) adequate hepatic and renal function.

Exclusion criteria included: (a) iron deficiency (mean corpuscular volume $<80\ \mu m^3$ or iron saturation [{Fe/(Fe+unsaturated iron-binding capacity)} $\times$ 100] $<$15.0%); (b) surgery scheduled during the study period; (c) EPO therapy within 4 weeks prior to the study; (d) documented haemorrhagic lesions; (e) pregnancy, breastfeeding or non-use of adequate birth control measures; (f) history of myocardial, pulmonary, cerebral infarction, serious drug allergy, uncontrolled hypertension, hypersensitivity to any EPO agent or any serious complication; and (g) tumor in the central nervous system.

Study Design and Treatment Schedule

This multicentre, open-label study was conducted at 14 sites in Japan.

The protocol was approved by the institutional review board of the respective hospitals, and written informed consent was obtained from all patients who participated in the study.

The initial dose of epoetin beta (Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) was 36 000 IU, and a once-weekly treatment was administered subcutaneously for 12 weeks. If the patient's haemoglobin level did not increase by $\geq$1.0 g/dL from baseline after 6 weeks of treatment, or an RBC transfusion was required between days 15 and 42, the dose of epoetin beta was increased to 54 000 IU weekly from the subsequent week. If the haemoglobin level increased to $\geq$14.0 g/dL, epoetin beta was discontinued until the haemoglobin level decreased to $\leq$12.0 g/dL, and was then restarted at two-thirds (24 000 IU or 36 000 IU) of the previous dose (36 000 IU or 54 000 IU). RBC transfusion was allowed at the discretion of the investigator during the study. An oral daily dose of 100–200 mg elemental iron was recommended if the mean corpuscular volume was $<80\ \mu m^3$ or the iron saturation was $<$15.0%.

QOL was evaluated at baseline and week 12 using the Japanese Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire (7,8), a well-validated instrument. In this study, the FACT-An total fatigue subscale, which consists of 13 fatigue related questions, was mainly analysed. The FACT-An total fatigue subscale scores (FSS) range from 0 to 52, with higher scores indicating less fatigue.

Evaluation of Efficacy and Safety

The American Society of Clinical Oncology/The American Society of Hematology guidelines (9) stipulate that the criteria for the haemopoietic effect should be an increase in haemoglobin level $\geq$1.0–2.0 g/dL in 6–8 weeks. Furthermore, there are reports (2,6), which showed that QOL is improved in patients with an increase in haemoglobin level of $\geq$2.0 g/dL.

The primary endpoint of the study was the percentage of patients achieving an increase in the haemoglobin level of $\geq$2.0 g/dL from the baseline between weeks 4 and 12, the haemoglobin response rate, excluding the data within 28 days after an RBC transfusion. The secondary endpoint was the change in FSS after 12 weeks of treatment. The percentage of patients receiving RBC transfusions between day 28 and the end of the study was also assessed. It was not expected that treatment with an EPO agent could influence transfusion requirements before day 28.

Adverse events (AEs) were assessed during the 12-week treatment period and during a 1-week observation period after the last dosing. Anti-erythropoietin antibodies were measured by the enzyme-linked immunosorbent assay and radio-immunoprecipitation (RIP) assay, and detection by either was judged as positive.

Statistical Analysis

We expected that 90 patients would need to be enrolled in the study to obtain a haemoglobin response rate of 70 $\pm$ 10% (95% confidence interval [CI]), as the primary endpoint.

Patients who received at least one dose of the study drug comprised the safety population. For efficacy analysis, the full analysis set (FAS) population was defined as eligible patients who received at least one dose of the study drug.

The changes in the haemoglobin level and FACT-An scores were calculated by subtracting each patient's baseline values from the last values. The rates of increase in haemoglobin before and after dose escalation were compared using a linear mixed-effects model. The potential factors influencing the change in FSS were examined by multiple

regression analysis. Pearson correlation coefficients were calculated to assess the association between changes in the haemoglobin level and FACT-An scores.

RESULTS

Demographics and Baseline Characteristics

A total of 104 patients were enrolled in the study between February and November 2004. Five patients discontinued the study before the first dosing for the following reasons: patient eligibility criteria violation, $n = 3$; patient denial, $n = 1$; and disease progression, $n = 1$. Thus, 99 patients were administered epoetin beta. One patient was excluded because of non-compliance with the eligibility criteria, leaving 98 patients as the FAS population. Eighty-seven patients (88.8%) completed all 12 weeks of the study. Eleven patients (11.2%) withdrew from the study. The primary reasons for withdrawal were progressive disease and AEs.

The demographics and baseline characteristics of the FAS population are listed in Table 1. Common types of cancer were breast ($n = 25$), malignant lymphoma ($n = 21$), ovarian ($n = 20$) and lung ($n = 15$). The mean age was 58.4 years (range: 23–78), and the mean body weight was 50.7 kg (range: 31.7–74.0). Most of the patients had an ECOG PS of 0 or 1 and a tumour stage of III or IV. The main chemotherapeutic agents used during the study were platinum for lung and other types of cancer, anthracycline for malignant lymphoma, taxane for breast cancer and platinum plus taxane for ovarian cancer. All patients met the criterion that they should not be iron-deficient at the time of enrollment.

Haemoglobin Response

The mean change in the haemoglobin level from baseline to the end of the study was 2.47 g/dL (standard deviation [SD]: 2.09; range: −2.8 to 6.0), as shown in Fig. 1. Figure 1 shows the mean changes in haemoglobin levels by tumour type. The pattern of changes in haemoglobin level was similar for the different tumour types. The mean increase in the haemoglobin level in patients with and without an initial EPO level of ≥100 mIU/mL were 1.76 g/dL (SD: 2.60) and 2.50 g/dL (SD: 1.85), respectively.

The haemoglobin response rates, defined as the percentage of patients achieving an increase in haemoglobin level of ≥2.0 g/dL from the baseline between weeks 4 and 12, are listed in Table 2. The overall haemoglobin response rate was 66.3% (65 of 98 patients). The median time to the haemoglobin response was 56 days from the first dosing, analysed by the Kaplan–Meier method. The percentage of patients with a haemoglobin level of ≥12.0 g/dL between weeks 4 and 12 was 59.2% (58 of 98 patients).

The percentage of patients who required dose escalation to 54 000 IU was 39.8% (39 of 98 patients). In these patients, the haemoglobin level increased after dose escalation, and the change in the haemoglobin level was 1.23 g/dL (SD: 2.19) at the end of the study. The haemoglobin response rate was 33.3% (13 of 39 patients) in patients who required dose escalation. The rate of haemoglobin increase before and after dose escalation was 0.023 g/dL/week (Weeks 0–6) and 0.266 g/dL/week (Weeks 7–12), respectively ($P = 0.0055$).

For three patients, the drug treatment was discontinued when the haemoglobin level exceeded 14.0 g/dL, and was restarted at a dose of 24 000 IU when the haemoglobin level decreased to ≤12.0 g/dL.

Quality of Life

Overall compliance in terms of the percentage of patients who completed the FACT-An was 100% at baseline and 97% (95 of 98 patients) at the end of the study. For three patients who dropped out due to progressive disease and were regarded as missing not at random, the scores at the end of the study were substituted with the minimum scores for all patients. Two patients were excluded from the evaluation of the change in the FSS because the responses to some items were missing.

The mean baseline FSS was 31.8 (SD: 11.4, $n = 98$) points. At the end of the study, the mean change from baseline was 0.3 (SD: 11.8, $n = 96$) points. The mean FSS change in the patients with progressive disease, as judged by each investigator, was −3.8 (SD: 16.7, $n = 15$) points (haemoglobin change: 2.4 g/dL). On the other hand, the mean change in patients without progressive disease was 1.9 (SD: 9.6, $n = 78$) points (haemoglobin change: 2.3 g/dL). These data indicated that progressive disease may be one of the independent variables affecting the change in FSS.

Relationship Between Haemoglobin Response and QOL Score

The results of a multiple regression analysis suggested that the change in the haemoglobin level ($P = 0.014$), the FSS at the initiation of dosing ($P < 0.0001$) and the PS at the end of the study ($P < 0.0001$) largely contributed to the change in the FSS. The correlation coefficient between the change in the FSS and the changes in the haemoglobin level was 0.280, indicating a significant correlation ($P = 0.006$, $n = 96$).

Patients who achieved an increase in the haemoglobin level of ≥2.0 g/dL experienced a 3.2-point mean change in FSS. On the other hand, patients who did not achieve an increase in haemoglobin level of ≥2.0 g/dL experienced a −3.4-point change (Fig. 2). There were no differences in the FSS at the initiation of dosing between patients with and without a change in haemoglobin level of ≥2.0 g/dL (32.0 versus 31.6). These data indicate that the change in FSS is dependent on the change in the haemoglobin level.

Concerning the relationship between the FSS at the initiation of dosing and the change in the FSS, patients with a baseline FSS of ≤36.0 reported greater improvement (mean ± SD: 1.6 ± 13.0) in the FSS at the end of the study (Table 3).

Table 1. Characteristics of the full analysis set population

	Characteristics	Total	Lung	Malignant Lymphoma	Breast	Ovarian	Other types
Sex	Male	27	11	10	0	0	6
	Female	71	4	11	25	20	11
Age (years)	Mean ± SD	58.4 ± 10.8	60.5 ± 10.5	56.5 ± 13.4	58.2 ± 9.0	54.4 ± 11.0	63.8 ± 8.0
	Range	23–78	41–78	23–74	39–77	30–75	40–76
ECOG performance status	0	48	1	9	14	13	11
	1	39	12	9	6	6	6
	2	11	2	3	5	1	0
Tumour stage	I	6	0	1	3	2	0
	II	17	1	4	7	4	1
	III	15	0	3	0	9	3
	IIIA	2	1	0	1	0	0
	IIIB	8	6	0	2	0	0
	IV	50	7	13	12	5	13
Treatment regimen	Platinum based	17	7	2	0	1	7
	Taxane based	28	5	0	19	3	1
	Anthracycline based	28	1	18	6	0	3
	Platinum, Anthracycline based	4	0	1	0	1	2
	Platinum,Taxane based	21	2	0	0	15	4
Weight (kg)	Mean ± SD	50.7 ± 8.2	53.8 ± 8.7	52.7 ± 9.9	47.9 ± 7.2	49.3 ± 6.6	50.9 ± 7.4
	Range	31.7–74.0	38.0–70.7	31.7–74.0	34.0–63.0	34.1–60.0	37.7–65.5
Haemoglobin (g/dL)	Mean ± SD	9.3 ± 1.4	9.6 ± 1.4	9.3 ± 1.4	9.4 ± 1.4	9.2 ± 1.6	9.1 ± 1.4
	Range	5.6–11.9	6.4–11.2	6.5–11.3	5.7–11.9	6.4–11.7	5.6–11.1
MCV (fL)	Mean ± SD	92.3 ± 6.5	89.0 ± 6.4	90.0 ± 5.4	91.9 ± 5.8	94.6 ± 7.5	95.7 ± 5.3
	Range	79.9–107.5	79.9–99.3	80–101	80.3–103.2	81.9–107.5	84–103.4
Reticulocyte (‰)	Mean ± SD	19.7 ± 16.4	20.8 ± 15.1	24.2 ± 24.1	18.0 ± 13.2	21.1 ± 15.6	14.1 ± 10.3
	Range	1–106	2–50	1–106	1–58	1–54	1.1–35.1
Transferrin saturation (%)	Mean ± SD	29.7 ± 22.3	22.4 ± 7.1	41.5 ± 30.6	21.9 ± 16.9	31.1 ± 24.3	30.5 ± 18.0
	Range	4.8–92.9	12.5–35.5	9.9–92.9	4.8–80.6	7.2–90.7	14.0–90.3
Serum endogenous erythropoietin (mIU/mL)	Mean ± SD	119.1 ± 310.5	64.3 ± 69.9	80.7 ± 108.0	88.4 ± 107.1	125.9 ± 144.8	252.0 ± 706.0
	Range	15.7–2970	15.7–234	17.3–399	16.7–472	23.2–578	20.4–2970
Baseline QOL FACT-An							
Anemia subscale (0–80)	Mean ± SD	50.8 ± 14.5	47.0 ± 15.9	50.6 ± 11.7	47.1 ± 13.7	53.5 ± 11.1	56.7 ± 19.5
	Range	16–80	17–74	26–67	20–71	34–75	16–80
Fatigue subscale (0–52)	Mean ± SD	31.8 ± 11.4	29.6 ± 12.9	30.3 ± 10.6	29.7 ± 10.7	33.9 ± 8.7	36.5 ± 14.1
	Range	4–52	4–52	10–43	7–50	20–48	8–52

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; QOL, quality of life; FACT-An, Functional Assessment of Cancer Therapy-Anemia; MCV, mean corpuscular volume.

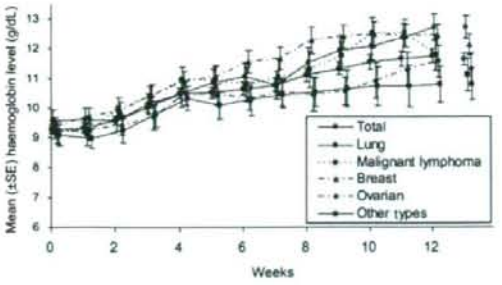

Figure 1. Change in haemoglobin level by tumor type. Mean weekly haemoglobin levels for the FAS population. Haemoglobin values within 28 days after RBC transfusion were excluded. FAS, full analysis set; RBC, red blood cell.

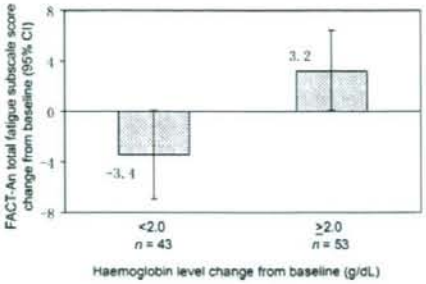

Figure 2. Changes in the FACT-An total fatigue subscale score by change in haemoglobin level. FACT-An, Functional Assessment of Cancer Therapy-Anaemia.

RBC Transfusion Requirement

The percentage of patients who received RBC transfusions between day 28 and the end of the study was only 6.1% (6 of 98 patients). The mean pretransfusion haemoglobin level at the time of the first transfusion was 6.2 g/dL (range: 5.4–7.3 g/dL). The percentage of patients whose haemoglobin level had decreased to <8.0 g/dL or who received an RBC transfusion between day 28 and the end of the study was 20.4% (20 of 98 patients).

Safety

AEs reported by at least 20% of the patients are summarised in Table 4. Death as a result of disease progression was not reported as an AE. Adverse drug reactions reported by at least 5% of patients are listed in Table 5. Among the 133 events in 48 patients (48.5%) that were considered related to the study drug, Grade III events were headache, hypertension, diarrhea, decreased serum potassium, impaired consciousness, anorexia and decreased serum phosphate. Three events (3.0%) of hypertension were reported as possibly related to epoetin beta treatment. An antihypertensive drug was administered after the onset of hypertension in one patient, who had hypertension as a comorbidity before the study. One patient (65-year-old female with malignant lymphoma) experienced a thrombovascular event, a lacunar infarction, at week 6. This event was evaluated as being unrelated to epoetin beta and was attributed to aging.

The incidence and type of AEs in patients who required dose escalation did not differ from those in patients who did not.

In two patients with ovarian and gastric cancer, anti-erythropoietin antibodies were detected only by RIP assay.

Table 2. Haemoglobin response rate by baseline haemoglobin, tumour type and dose escalation

	%	*n*
Response rate*	66.3	65/98
Response rate by baseline haemoglobin, g/dL		
<10.0	68.8	44/64
≥10.0	61.8	21/34
Response rate by tumour type		
Lung	80.0	12/15
Malignant lymphoma	66.7	14/21
Breast	76.0	19/25
Ovarian	65.0	13/20
Other types	41.2	7/17
Response rate by dose escalation		
Yes	33.3	13/39
No	88.1	52/59

*All patients, including those receiving transfusions.

Table 3. Changes in the FACT-An total fatigue subscale score by baseline FSS and final PS

Time period	Baseline		End of treatment		
	n	Mean score (SD)	*n*	Mean score (SD)	Mean change from baseline (SD)
Total	98	31.8 (11.4)	96*	31.8 (13.5)	0.3 (11.8)
Baseline FSS					
≤36.0	62	24.8 (7.9)	62	26.5 (12.0)	1.6 (13.0)
>36.0	36	43.9 (4.0)	34*	41.5 (10.3)	−2.2 (8.8)
Final PS					
0	58	35.5 (11.3)	56*	37.4 (10.3)	2.4 (10.2)
1	28	27.4 (9.1)	28	29.0 (11.5)	1.6 (12.2)
2	4	19.3 (9.4)	4	11.8 (11.4)	−7.5 (7.9)
3	3	29.7 (15.9)	3	21.0 (7.2)	−8.7 (13.8)
4	5	25.7 (7.3)	5	6.4 (7.1)	−19.3 (6.4)

*Two patients missing FSS. Collected but could not be calculated.
FSS, FACT-An total fatigue subscale score; PS, performance status.

Table 4. Frequencies of adverse events ($n = 99$)

Event	n	%	Grade*				
			I	II	III	IV	V
Neutropenia	83	83.8	3	11	24	45	0
Leukopenia	78	78.8	2	16	41	19	0
Nausea	57	57.6	38	11	8	0	0
Thrombocytopenia	55	55.6	21	9	23	2	0
Lymphopenia	52	52.5	0	18	34	0	0
Anorexia	46	46.5	22	13	10	1	0
Fatigue	39	39.4	22	14	3	0	0
Vomiting	36	36.4	18	16	2	0	0
Diarrhea	33	33.3	23	6	4	0	0
Increased lactate dehydrogenase	32	32.3	25	6	1	0	0
Peripheral neuropathy	26	26.3	21	5	0	0	0
Fever	26	26.3	17	7	2	0	0
Constipation	24	24.2	3	13	7	1	0
Increased alanine aminotransferase	24	24.2	15	6	3	0	0
Alopecia	22	22.2	7	15	0	0	0

*National cancer institutes common toxicity criteria, version 2.0.

Table 5. Frequencies of adverse drug reactions ($n = 99$)

Event	n	%	Grade*				
			I	II	III	IV	V
Increased lactate dehydrogenase	10	10.1	9	1	0	0	0
Headache	7	7.1	6	0	1	0	0
Nausea	7	7.1	5	2	0	0	0
Rash	5	5.1	3	2	0	0	0
Back pain	5	5.1	5	0	0	0	0

*National cancer institutes common toxicity criteria, version 2.0.

Neutralisation of EPO activity was detected in neither patient, and the haemoglobin level was elevated after dosing with the study drug. The investigators judged that the antibody did not cause pure red cell aplasia.

When re-examined six months after the last observation, one of these patients (ovarian cancer) was antibody negative, whereas the other (gastric cancer) could not be re-examined, having died of the underlying disease.

DISCUSSION

Several studies have been conducted to assess the effects of EPO agents in anaemic cancer patients, and increased haemoglobin levels and improvement in QOL that correlated with the increased haemoglobin level were reported (1,10).

The objectives of our study were to investigate the effects of an initial once-weekly 36 000 IU dose of epoetin beta on haemoglobin levels and QOL in patients with non-myeloid malignancy undergoing chemotherapy. The criterion for a haemoglobin response, an increase in the haemoglobin level of $\geq$2.0 g/dL, was based on a report that symptoms of anaemia assessed by the FACT-An are improved in patients with a change in the haemoglobin level of $\geq$2.0 g/dL (2,6). According to this index, the haemoglobin response rate in the present study was 66.3% (65 of 98 patients). The increases in haemoglobin levels that were observed were independent of the tumour type or the baseline haemoglobin level. None of the investigators performed a randomised comparison of a dose increase versus an unchanged dose in EPO low responders. In the present study, there was an increase in the rate of haemoglobin increase after dose escalation to 54 000 IU, and the haemoglobin response rate for patients who required a dose escalation was 33.3% (13 of 39 patients).

The secondary endpoint, the change in the FSS, showed an increase of 0.3 points; however, in patients who showed an increase in the haemoglobin level of $\geq$2.0 g/dL, the FSS was increased by 3.2 points, which was significantly higher than the -3.4-point change in patients whose haemoglobin level increased by <2.0 g/dL. A 3.2-point increase is comparable with the 3 points considered to be a clinically significant change in FSS (11). In addition, the mean change in FSS for patients with progressive diseases (PD) was -3.8 points (median: -6.5 points, range: -37 to 35 points) even though correction of anaemia was observed. In total, excluding PD cases, a 1.9- point improvement was observed.

Investigating the relationship between the FSS at the initiation of dosing and the change in the FSS showed that greater improvements in FSS were observed in patients with lower FSS. The FSS before treatment with epoetin beta was 31.8 ± 11.4 points, which is higher than the scores (FSS: 22.1–29.7 points, change in FSS: 1.6–5.2 points) in cancer patients with anaemia reported in several randomised trials (1,10,12–14). Nevertheless, the mean initial haemoglobin level (9.3 g/dL) in the present study was equal to the levels in the other trials (9.2–10.1 g/dL). Since it has been reported that the FSS after treatment with an EPO agent is aggravated in patients with an FSS exceeding 36.0 at the initiation of dosing (15), the scores were analysed after stratification at 36.0. This resulted in improved scores (1.6 ± 13.0 points) for those patients with a baseline score of $\leq$36.0, when compared with patients with a score $>$36.0 (-2.2 ± 8.8 points). The results of a multiple regression analysis of the change in the FSS demonstrated that the change in the haemoglobin level, the FSS at the initiation of dosing and the PS at the end of the study were factors that largely contributed to the change in the FSS. A positive and significant association was observed between

the degree of increase in the haemoglobin level and the degree of improvement in the FSS ($r = 0.280$, $P = 0.006$). It was comparable with the results ($r = 0.2879$, $P = 0.0002$; $r = 0.35$, $P = 0.001$ and $r = 0.2893$, $P < 0.0001$) of three other studies (1,10,16).

The RBC transfusion rate was only 6.1% (6 of 98 patients) between day 28 and the end of the study. As reported for once-weekly epoetin alfa administered to patients with various types of cancer (14), the transfusion rates between week 5 and the end of treatment were 14.5% (24 of 166 patients) for epoetin alfa and 29.3% (48 of 164 patients) for placebo. Furthermore, the mean pretransfusion haemoglobin levels for the first transfusion reported in the previous trial in the United States (7.9 and 7.8 g/dL, respectively) were higher than those (6.2 g/dL) in the present study in Japan. To evaluate the effect of EPO agents, the percentage of patients whose haemoglobin level had decreased to <8.0 g/dL or who received an RBC transfusion was considered to be a more objective index than the RBC transfusion rate in Japan, because RBC transfusion itself is prescribed at the discretion of the investigator and when the haemoglobin level is low.

Epoetin beta was well tolerated in the present study. Most of the AEs were consistent with the underlying disease or with the chemotherapy. Hypertension, which was judged to be related to epoetin beta was observed in three patients. It was alleviated either by no treatment or the administration of hypotensive agents. Lacunar infarction was also observed in one patient. A relationship to epoetin beta was ruled out, however, and this event was judged to be due to aging. Two recently published studies (17,18) targeting higher haemoglobin levels, in which survival was a primary endpoint, have raised concerns that EPO agents may have a negative impact on survival in cancer patients. A meta-analysis of 57 studies, including these two recent studies revealed an overall survival hazard ratio of 1.08 (95%CI: 0.99–1.18) and that uncertainties remain as to whether EPO agents affected survival (19). The FDA has provided new safety information on erythropoiesis-stimulating agents (ESAs), in which the target haemoglobin level is not to exceed 12 g/dL, because analyses of other studies in patients with cancer found a higher chance of serious and life-threatening adverse drug reactions or deaths with the use of ESAs (20). Although, in the present studies, there was no problem with safety when the haemoglobin level at which dosing was withheld was set at 14 g/dL, in consideration of FDA ALERTs, etc., we intend to investigate the use of lower values for target haemoglobin level and haemoglobin level at which dosing should be withheld.

In conclusion, once-weekly epoetin beta treatment increased the haemoglobin level and correspondingly improved the QOL in anaemic patients with non-myeloid malignancies receiving chemotherapy. Additionally, haemoglobin levels could be improved and controlled by once-weekly treatments at an initial dose of 36 000 IU followed by dose adjustment in the range of 24 000–54 000 IU.

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Conflict of interest statement

One of the authors, Hironobu Minami, receives honoraria from Chugai Pharmaceutical Co., Ltd. and Kirin Pharma Co., Ltd.

One of the authors, Yasuo Ohashi, consults on design and data analysis of clinical trials for Chugai Pharmaceutical Co., Ltd.

One of the authors, Nagahiro Saijo, holds stock option for Takeda Pharmaceutical Co., Ltd.

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Short Communication

Randomised phase II trial of irinotecan plus cisplatin *vs* irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

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Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m^{-2} on days 1 and 8 and cisplatin 60 mg m^{-2} on day 1 with ($n = 55$) or without ($n = 54$) etoposide 50 mg m^{-2} on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.

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Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 *vs* 9.4 months, $P = 0.002$) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003).

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The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

PATIENTS AND METHODS

Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count $\geqslant 4.0 \times 10^3\,\mu l^{-1}$, neutrophil count $\geqslant 2.0 \times 10^3\,\mu l^{-1}$, haemoglobin $\geqslant 9.5\,g\,dl^{-1}$, platelet count $\geqslant 100 \times 10^3\,\mu l^{-1}$, total serum bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, hepatic transaminases $\leqslant 100\,IU\,l^{-1}$, serum creatinine $\leqslant 1.2\,mg\,dl^{-1}$, creatinine clearance $\geqslant 60\,ml\,min^{-1}$, and $PaO_2 \geqslant 60$ torr; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

Treatment schedule

In the IP arm, cisplatin, 60 mg m^{-2}, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m^{-2}, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m^{-2}, was administered intravenously over 60 min on days 1–3. Filgrastim 50 μg m^{-2} or lenograstim 2 μg kg^{-1} was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded 10.0×10^3 μl^{-1}. Hydration (2500 ml) and a $5HT_3$ antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (http://www.umin.ac.jp/ctr/index.htm), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/faq.pdf). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months × 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2}\text{ week}^{-1}\text{)} = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3–4 toxicities were assessed by χ tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan–Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

RESULTS

Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

Table 1 Patient characteristics

	IP ($n=54$)	IPE ($n=55$)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–4%	38	43
5–9%	10	10
⩾10%	6	2

Table 2 Treatment delivery

	IP ($n=54$) No. (%)	IPE ($n=55$) No. (%)
Number of cycles delivered		
6*	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment†		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	2 (4)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

†$P=0.013$ by χ^2 test. *Protocol violation.

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patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.013$) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ($P=0.005$). Packed red blood cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ($P=0.011$). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ($P=0.16$). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.262$). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 *vs* 11%, $P=0.054$). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

Table 3 Total dose and dose intensity

	3-week regimens in this study		4-week regimen*
	IP ($n=54$) Median (range)	IPE ($n=55$) Median (range)	IPE ($n=30$) Median (range)
Total dose ($mg\,m^{-2}$)			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
Dose intensity ($mg\,m^{-2}\,week^{-1}$)			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

*From our previous study (Sekine *et al*, 2003).

Table 4 Grade 3–4 toxicities

	IP ($n=54$)			IPE ($n=55$)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopaenia	9	1	10 (19)	18	11	29 (53)*
Neutropaenia	17	11	28 (52)	24	28	52 (95)*
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopaenia	2	0	2 (4)	13	0	13 (13)†
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11)‡
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

*$P<0.001$; †$P<0.01$; and ‡$P=0.054$ by χ^2 test.

Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ($P=0.126$). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ($P=0.049$) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ($P=0.52$), respectively (Figure 1B).

DISCUSSION

This study showed that the IPE regimen in a 3-week schedule with CSF support produced a promising response rate, PFS and overall survival. Haematological toxicity in the IPE arm, however, was very severe in spite of the G-CSF support with the grade 3 febrile neutropaenia noted in 31% of patients.

In comparison between the 3-week IPE regimen in this study and the 4-week IPE regimen in the previous study, the delivery of cisplatin and etoposide was improved in the 3-week IPE regimen when compared with the 4-week IPE regimen at the cost of the irinotecan total dose. The response rate and MST were 87% and 13.7 months, respectively, in the 3-week IPE regimen and 77% and 12.9 months in the previous 4-week schedule, and toxicity profiles were comparable to each other (Sekine *et al*, 2003).

The MST of 12.4 months in the IP arm in this study was comparable to that of the previous phase III study, with an MST of 12.8 months (Noda *et al*, 2002). Thus, this study showed the reproducible excellent survival outcome of patients with ED-SCLC who were treated with the IP combination. In contrast, a recent American phase III study of the PE regimen *vs* IP regimen failed to show the superiority of the IP regimen to the PE regimen; the MST

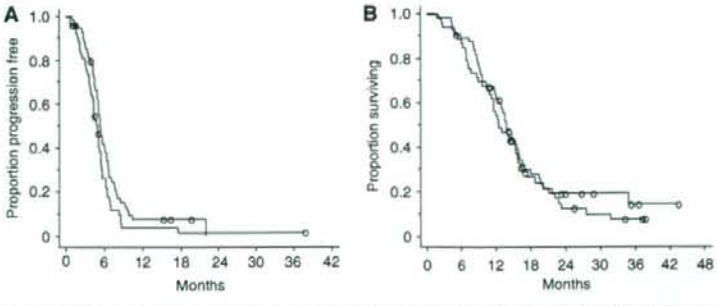

Figure 1 Progression-free survival (**A**) and overall survival (**B**). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

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for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of 60 mg m^{-2} on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of 30 mg m^{-2} on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

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Prospective Study of the Accuracy of *EGFR* Mutational Analysis by High-Resolution Melting Analysis in Small Samples Obtained from Patients with Non–Small Cell Lung Cancer

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Abstract

Purpose: Epidermal growth factor receptor (*EGFR*) mutations, especially in-frame deletions in exon 19 (DEL) and a point mutation in exon 21 (L858R), predict gefitinib sensitivity in patients with non–small cell lung cancer (NSCLC). In this study, we verified the accuracy of *EGFR* mutation analysis in small samples by high-resolution melting analysis (HRMA), which is a rapid method using PCR amplification with a dye to analyze the melting curves in NSCLC.

Experimental Design: We designed a prospective study to compare the sensitivity and specificity of HRMA and DNA sequencing with laser capture microdissection. Eligible patients with lung lesions were screened by bronchoscopy or percutaneous needle biopsy to histologically confirm the diagnosis, followed by surgical resection of the NSCLC. Small diagnostic specimens were analyzed for *EGFR* mutations by HRMA, and the surgically resected specimens were examined for mutations by HRMA and DNA sequencing.

Results: The analyses for *EGFR* mutations were conducted in 52 eligible cases of the 92 enrolled patients. *EGFR* mutations were detected in 18 (34.6%) patients. The results of HRMA from surgically resected specimens as well as DNA sequencing revealed 100% sensitivity and specificity. On the other hand, the sensitivity and specificity of HRMA from the small diagnostic specimens were 83.3% and 100%, respectively.

Conclusions: In this study, we showed that HRMA is a highly accurate method for detecting DEL and L858R mutations in patients with NSCLC, although it is necessary to consider the identification of patients with a false-negative result when the analysis is conducted using small samples.

Somatic mutations in the kinase domain of the epidermal growth factor receptor (*EGFR*) have been reported in patients with non–small cell lung cancer (NSCLC; refs. 1–3). Although many types of *EGFR* mutations have been identified, they seem to be concentrated in exons 18 to 21 of *EGFR*; ~85% to 90% of *EGFR*-mutant patients have mutations in two hotspots: a short in-frame deletion in exon 19 (DEL) and a point mutation at codon 858 in exon 21 (L858R; ref. 4). Several studies have revealed that *EGFR* mutations are strongly associated with the tumor response and clinical outcome in patients with NSCLC receiving treatment with EGFR tyrosine kinase inhibitors, such as gefitinib (Iressa, AstraZeneca; refs. 5–7). The mutational status of *EGFR*, especially the presence/absence of DEL and L858R, is a strong predictor of the sensitivity to EGFR tyrosine kinase inhibitor, and the detection of *EGFR* mutations is useful for decision-making by both patients and physicians (4, 8). Recently, a laboratory test for *EGFR* mutations has become clinically available for guiding treatment decisions.

Until now, screening for these mutations has most commonly been conducted using DNA sequencing methods. In our previous study, we used methanol-fixed, paraffin-embedded surgical specimens and performed direct sequencing and pyrosequencing with laser capture microdissection (LCM) to ensure high-quality genetic analysis of archived tissues (5, 9). However, these approaches are not useful in clinical practice for two reasons. First, although the sequencing methods require a high ratio of tumor-to-normal tissue DNA for optimal results, the diagnostic specimens obtained from cases of advanced NSCLC may contain only a small amount of tumor cells and

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