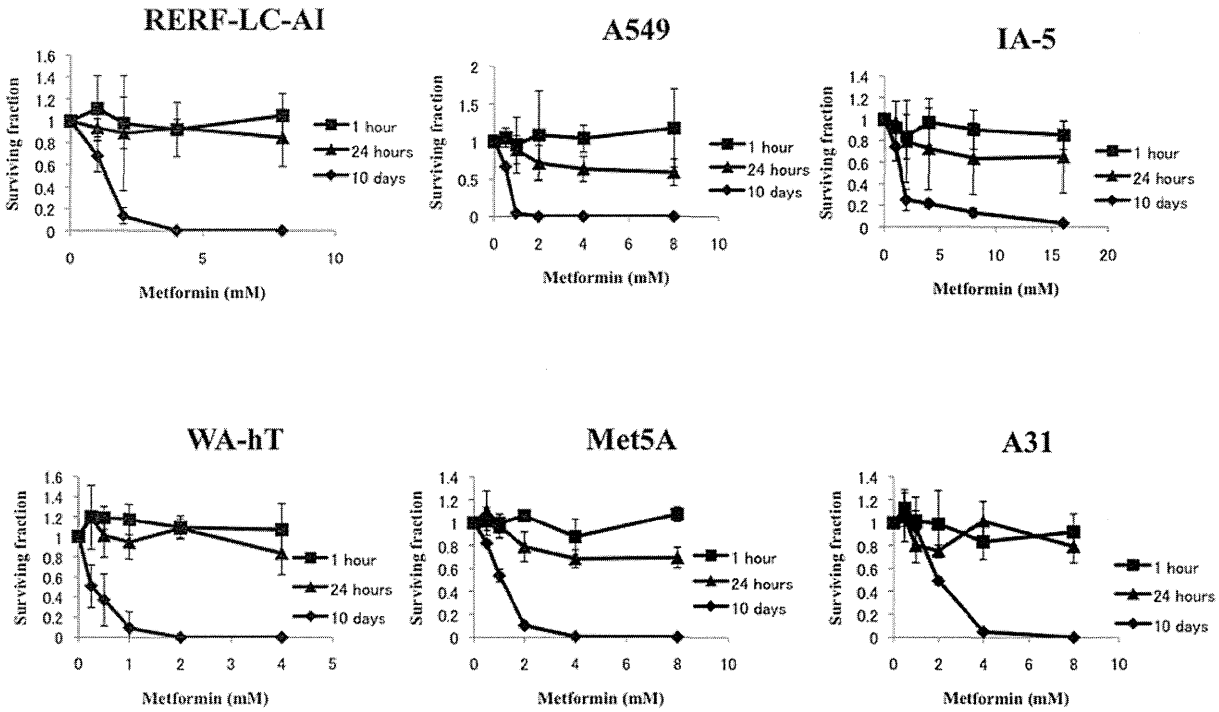

Figure 1. Survival curves drawn based on the results of the clonogenic assay. The cells were exposed to various concentrations of metformin for 1 or 24 h before being trypsinized and plated for colony formation in complete medium. Otherwise, the cells were trypsinized and plated for colony formation by further culture for 10 days in metformin-containing complete medium. RERF-LC-AI, A549, IA-5, and WA-hT represent squamous, adeno-, large cell, and small cell lung carcinoma cell lines, respectively. Met5A and A31 represent non-transformed human mesothelial and mouse fibroblast cell lines, respectively. Each experiment was conducted in triplicate and repeated 3 times. The mean value of each triplicate represents the value of each experiment, while the mean ± SD of the 3 experimental results were calculated. The dot and bar represent mean and SD at each point.

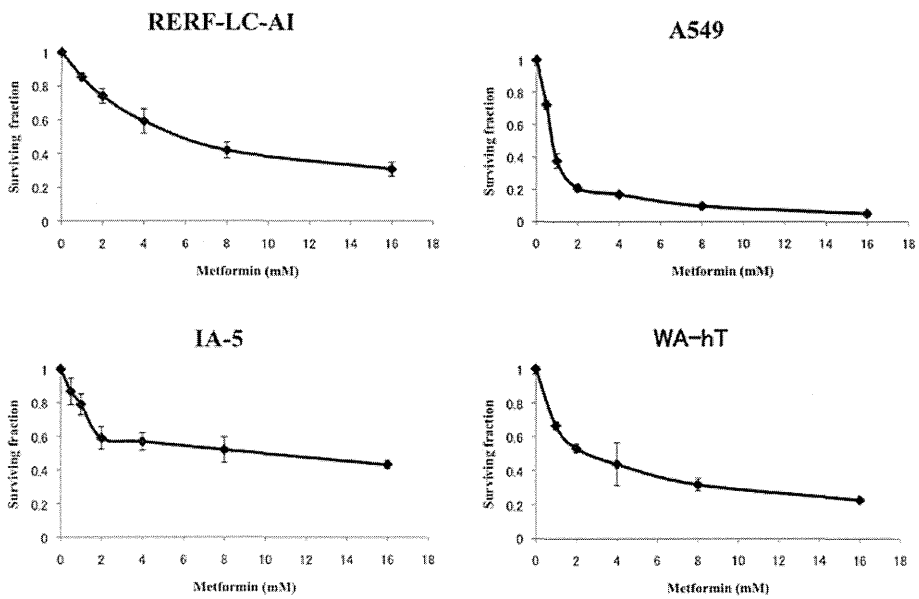

Figure 2. Survival curves drawn based on the results of the cell survival assay. The cells were plated at a concentration of 10^5 cells/plate with complete medium containing metformin at various concentrations, and were further cultured for 4 days with metformin until the surviving cells were counted. Each experiment was conducted in triplicate and repeated 3 times. The mean value of each triplicate represents the value of each experiment, and the mean ± SD of the 3 experimental results were calculated. The dot and bar represent mean and SD at each point.

(Fig. 4A). Apoptosis assessed by determining the activities of caspases 3, 8 and 9 revealed results similar to that of Hoechst staining (Fig. 4B).

Cell cycle distribution. In cell cycle analysis, the effects of metformin at IC_{30} and IC_{70} were compared to those of cisplatin at IC_{70} and at a higher concentration in each cell line. Although

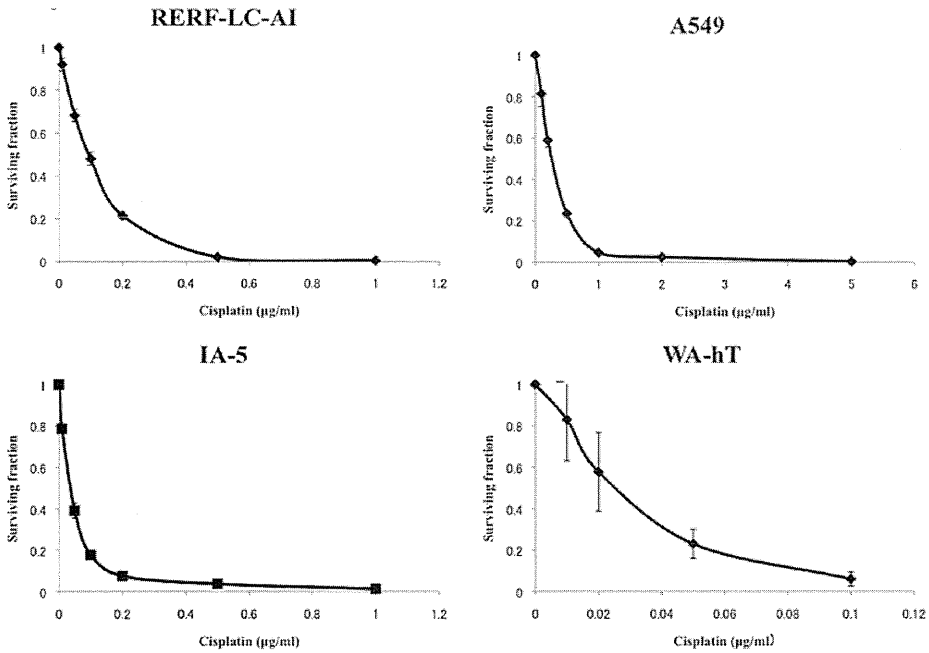

Figure 3. Survival curves drawn based on the results of the cell survival assay. The cells were treated with various concentrations of cisplatin instead of metformin. Other procedures were conducted as mentioned in Fig 2

there was no statistically significant difference except for the case of RERF-LC-AI, metformin induced G0/G1 phase accumulation in all 4 cell lines, whereas cisplatin at IC_{70} caused significant G2/M phase accumulation in RERF-LC-AI and WA-hT, and G0/G1 phase accumulation in A549 cells. On the other hand, higher concentrations of cisplatin caused significant G2/M phase accumulation in all 4 cell lines (Fig. 5).

Interaction of metformin and cisplatin. In this experiment, cisplatin at IC_{50} and IC_{90} in each cell line was combined with metformin. The inhibitory effects of metformin on cell proliferation were slightly suppressed with cisplatin at IC_{50} in all cells, except A549 cells. A higher dose (IC_{90}) of cisplatin almost completely countervailed or even reversed the effects of metformin in all cell lines except for A549, where a modest, but significant, sub-additive effect was observed (Fig. 6).

Discussion

Metformin inhibited clonogenicity and cell proliferation in all 4 cell lines in a similar manner. On the other hand, WA-hT cells showed significantly higher sensitivity to cisplatin compared to the other cell lines. Concerning clonogenicity, the inhibitory effect of metformin was not specific to cancer cells because non-transformed mouse fibroblast and human mesothelial cell lines were also inhibited. Contrary to molecular targeted agents which specifically kill cancer cells harboring their specific targets, classical cytotoxic agents kill cells similarly even between cancer and non-transformed cells *in vitro* but preferably kill the former over the latter *in vivo* with differential effects to the cells according to different dividing capabilities. Therefore, it is speculated that metformin does not attack cancer-specific target molecules. In addition, the clonogenic assay disclosed that it is necessary to expose the cells to metformin for long periods of time to exert the inhibitory effects. As the surviving fraction reached a plateau in the range of 0.1-0.3 in the various cell lines with increasing doses of metformin (Fig. 2), the concentrations of IC_{30} and IC_{70} were chosen for further elucidating the mechanism of action.

Metformin did not enhance apoptosis at relatively low concentrations, as assessed by Hoechst staining and caspase activities in all cell lines except for WA-hT. Although the differences were not statistically significant except in RERF-LC-AI, metformin at IC_{30} and IC_{70} in each cell line tended to cause G0/G1 phase accumulation. Specifically, metformin exerted cytotoxicity by G0/G1 arrest in RERF-LC-AI, A549, and IA-5 and by both G0/G1 arrest and apoptosis in WA-hT cells. Although metformin may induce a different effect at higher concentrations, such high concentrations would not be clinically relevant. More specifically, the mean peak plasma concentration (C_{max}) in 5 Japanese diabetes patients was reportedly 0.85 ± 0.19 μg/ml (5.1±1.1x10^{-3} mM) when 250 mg of metformin was orally administered (21). Since the maximal single dose of 750 mg metformin is prescribed 3 times a day, an estimation of the C_{max} would be 15x10^{-3} mM. Although the dose range employed in the present research is approximately 100-fold higher than the plasma concentration that is achieved by conventional clinical application, the present results still seem clinically relevant for several reasons; a) chronic administration of metformin for months or even years in the clinical setting would be possible and the long-term exposure may augment its effects as demonstrated in Fig. 1; b) metformin accumulates in tissues at much higher concentrations than in the blood (22); and c) a dose-finding study for cancer treatment might determine the maximal tolerated dose of metformin at a much higher level than currently used for diabetic patients. Moreover, further elucidating the antiproliferative action of

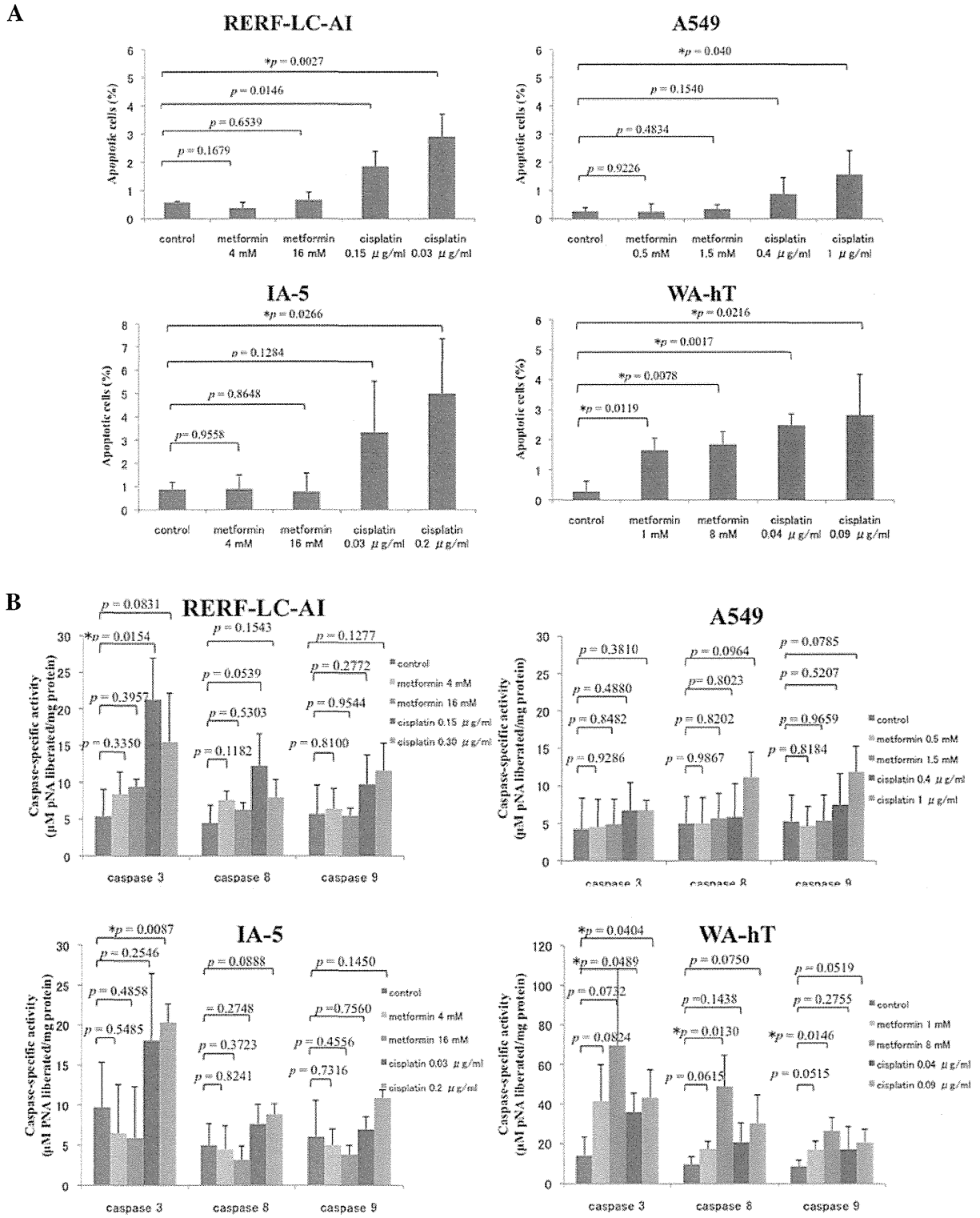

Figure 4. Apoptotic induction by treatment with metformin or cisplatin assessed by (A) Hoechst staining and (B) by caspase activities. The concentrations of metformin were IC_{30} and IC_{70} for each cell line. The concentrations of cisplatin were IC_{70} and higher for each cell line. Differences from the control (no exposure to agents) were compared using the Student's t-test, and the resulting p-values are presented. *p<0.05 (two-tailed). Each experiment was repeated 3 times, and the mean ± SD values of the 3 experimental results are presented.

metformin may lead to the discovery of crucial target molecules for more effective new agents.

There is an increasing number of reports on the antineoplastic effects of metformin highlighting controversy in relation to its apoptotic induction and cell cycle alteration. Contrary to some studies reporting enhanced apoptosis in triple-negative breast cancer (6) and pancreatic cancer cells (10), others failed to observe apoptosis in non-triple-negative

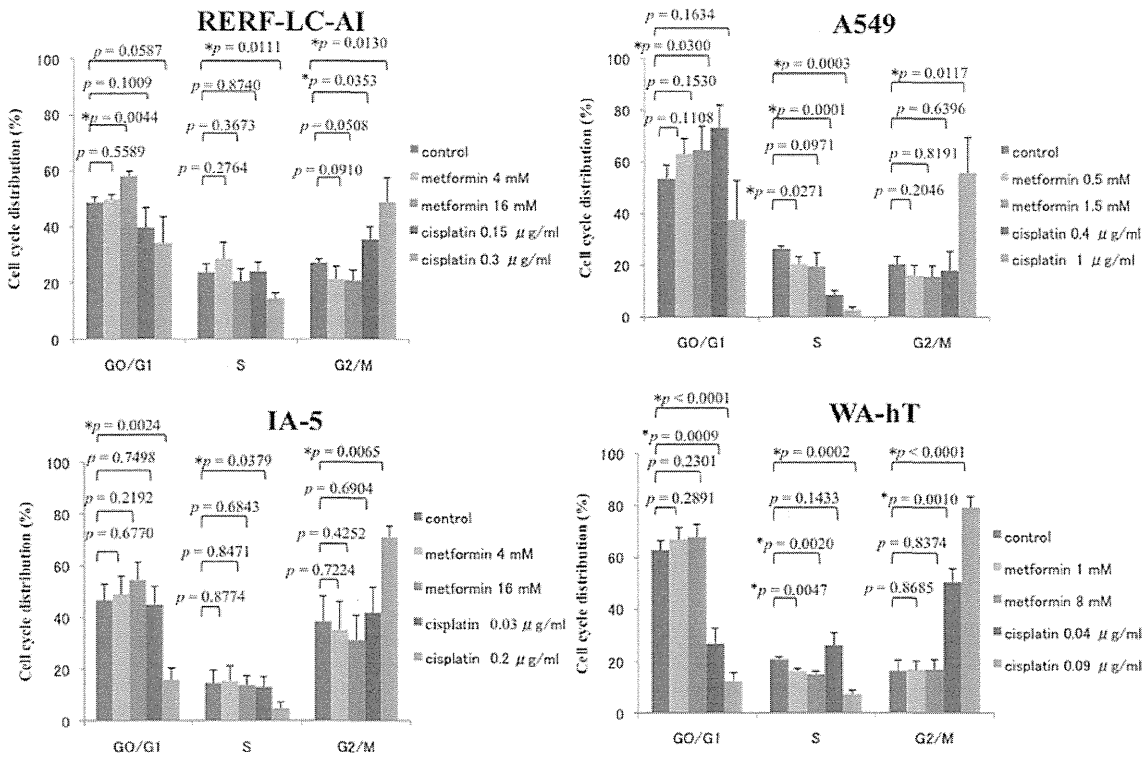

Figure 5. Alteration of cell cycle distribution by metformin or cisplatin assessed using the propidium iodide single-color method with a flow cytometer. Similarly to apoptosis analysis, the concentrations of metformin were IC_{30} and IC_{70} for each cell line, while the concentrations of cisplatin were IC_{70} and higher for each cell line. Differences from the control (no exposure to agents) were compared using Student's t-test and the resulting p-values are presented. *p<0.05 (two-tailed). Each experiment was repeated 3 times, and the mean ± SD values of the 3 experimental results are presented.

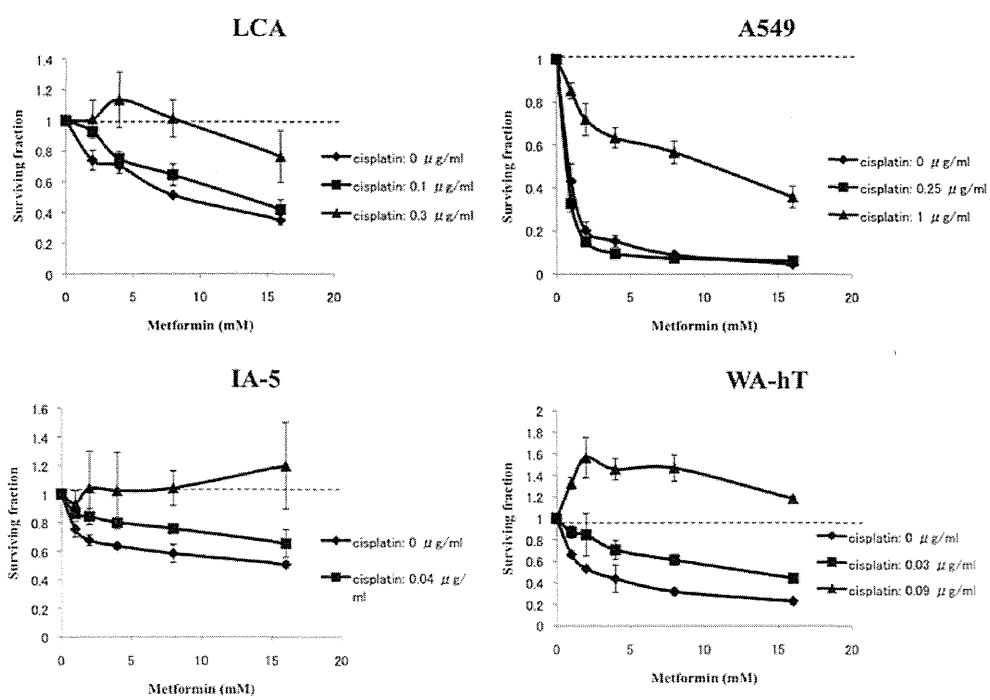

Figure 6. Interaction between metformin and cisplatin on cell proliferation inhibition. The cell survival assay was conducted using various concentrations of metformin as described in Fig. 2 except that the cells were exposed to 3 different concentrations of cisplatin, its IC_{50} and IC_{90} for each cell line and 0, together with metformin. In each curve with a defined cisplatin concentration, a fraction against the value without metformin was plotted at each metformin concentration point. Therefore, the curves located above the curve without cisplatin represent the antagonistic effects between the 2 agents. In particular, curves exceeding the line at fraction 1 (dotted lines) represent inverse effects, i.e., cell proliferation-enhancing effects by metformin treatment. Each experiment was conducted in triplicate and repeated 3 times. The mean value of each triplicate represents the value in each experiment, and the mean ± SD of the 3 experimental results were calculated. The dot and bar represent mean and SD at each point.

breast cancer (5) and a prostatic cancer cell line (9). Notably, cell cycle accumulation at the S phase was observed in a study on triple-negative breast cancer (6) and a pancreatic cancer cell line (10) that accompanied apoptosis enhancement, whereas an arrest at the G1 check point was observed in the non-triple-negative breast cancer (5) and the prostatic cancer cell line (9), and this arrest was not accompanied by apoptosis enhancement. In addition, G1 arrest was observed in an ovarian cancer cell line (12). The present results also differed among the cell lines, each of them representing a different histological type of human lung cancer. Metformin did not exert apoptosis induction but showed a tendency toward G0/G1 arrest in RERF-LC-AI, A549, and IA-5 cell lines, similarly to the results with non-triple-negative breast cancer (5) and prostatic cancer cell lines (9). However, WA-hT simultaneously underwent apoptosis and slight G0/G1 arrest. In view of the diversified results even among the 4 cell lines of a single disease, it would not be inappropriate to conclude that metformin affects different cancers or cancer cell lines differently.

The results of the combined effects of metformin and cisplatin are noteworthy. Administration of cisplatin at IC_{50} along with metformin decreased the sensitivity to metformin in all 4 cell lines. Moreover, the combined use of metformin and high-dose cisplatin enhanced metformin-induced growth in all cell lines, excluding A549, because the cell survival curves of the other 3 cell lines exceeded the lines of fraction 1. Controversy exists in the literature with respect to the interaction with cisplatin. According to the research conducted by Gotlieb *et al* (11) and Rattan *et al* (13), metformin with cisplatin synergistically killed ovarian cancer cells. In contrast, Janjetovic *et al* (18) reported an antagonistic interaction between metformin and cisplatin in human glioma, rat glioma, human neuroblastoma, mouse fibrosarcoma, and human leukemia cell lines, possibly via an AMP kinase-independent upregulation of the Akt survival pathway. However, they also found augmented cisplatin sensitivity by metformin in a mouse melanoma cell line. Harhaji-Trajkovic *et al* (19) reported the antagonistic action of metformin on cisplatin. These findings again suggest that metformin affects different types of cancer, differently. On the other hand, the interaction between metformin and cisplatin in the present study was unexceptionally antagonistic in all 4 cell lines.

In conclusion, metformin inhibited the proliferation of various histological types of human lung cancer cell lines, possibly by varied mechanisms including apoptosis induction and G0/G1 arrest according to the cell line. Metformin and cisplatin were antagonistic in all 4 investigated cell lines. Taking account of its limited clinical adverse effects, metformin may have the potential for use in cancer therapy with adequate consideration of drug-drug interaction.

Acknowledgements

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References

1. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR and Morris AD: Metformin and reduced risk of cancer in diabetic patients. BMJ 330: 1304-1305, 2005.
2. Bowker SL, Majumdar SR, Veugelers P and Johnson JA: Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 29: 254-258, 2006.
3. Jiralerspong S, Palla SL, Giordano SH, *et al*: Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 27: 3297-3302, 2009.
4. Mazzone PJ, Rai HS, Beukemann M, Xu M, Abdallah R and Sasidhar M: The effect of metformin and thiazolidinedione use on lung cancer. Chest 138: 882A, 2010.
5. Alimova IN, Liu B, Fan Z, *et al*: Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. Cell Cycle 8: 909-915, 2009.
6. Liu B, Fan Z, Edgerton SM, *et al*: Metformin induces unique biological and molecular responses in triple negative breast cancer cells. Cell Cycle 8: 2031-2040, 2009.
7. Zakikhani M, Dowling R, Fantus IG, Sonenberg N and Pollak M: Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 66: 10269-10273, 2006.
8. Rocha GZ, Dias MM, Ropelle ER, *et al*: Metformin amplifies chemotherapy-induced AMPK activation and antitumoral growth. Clin Cancer Res 17: 3993-4005, 2011.
9. Ben Sahra I, Laurent K, Loubat A, *et al*: The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene 27: 3576-3586, 2008.
10. Wang LW, Li ZS, Zou DW, Jin ZD, Gao J and Xu GM: Metformin induces apoptosis of pancreatic cancer cells. World J Gastroenterol 14: 7192-7198, 2008.
11. Gotlieb WH, Saumet J, Beauchamp MC, *et al*: In vitro metformin anti-neoplastic activity in epithelial ovarian cancer. Gynecol Oncol 110: 246-250, 2008.
12. Rattan R, Giri S, Hartmann LC and Shridhar V: Metformin attenuates ovarian cancer cell growth in an AMP-kinase dispensable manner. J Cell Mol Med 15: 166-178, 2011.
13. Rattan R, Graham RP, Maguire JL, Giri S and Shridhar V: Metformin suppresses ovarian cancer growth and metastasis with enhancement of cisplatin cytotoxicity in vivo. Neoplasia 13: 483-491, 2011.
14. Pollak M: Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 8: 915-928, 2008.
15. Goodwin PJ, Ligibel JA and Stambolic V: Metformin in breast cancer: time for action. J Clin Oncol 27: 3271-3273, 2009.
16. Algire C, Zakikhani M, Blouin MJ, Shuai JH and Pollak M: Metformin attenuates the stimulatory effect of a high-energy diet on in vivo LLC1 carcinoma growth. Endocr Relat Cancer 15: 833-839, 2008.
17. Iliopoulos D, Hirsch HA and Struhl K: Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Research 71: 3196-3201, 2011.
18. Janjetovic K, Vucicevic L, Misirkic M, *et al*: Metformin reduces cisplatin-mediated apoptotic death of cancer cells through AMPK-independent activation of Akt. Eur J Pharmacol 651: 41-50, 2011.
19. Harhaji-Trajkovic L, Vilimanovich U, Kravic-Stevovic T, Bumbasirevic V and Trajkovic V: AMPK-mediated autophagy inhibits apoptosis in cisplatin-treated tumour cells. J Cell Mol Med 13: 3644-3654, 2009.
20. Sugimoto T, Takiguchi Y, Kurosu K, *et al*: Growth factor-mediated interaction between tumor cells and stromal fibroblasts in an experimental model of human small-cell lung cancer. Oncol Rep 14: 823-830, 2005.
21. Iwase M, Nohara S, Sasaki N, *et al*: Pharmacokinetics study of low dose of metformin clinically used in Japan. J Japan Diab Soc 47: 197-201, 2004.
22. Wilcock C and Bailey CJ: Accumulation of metformin by tissues of the normal and diabetic mouse. Xenobiotica 24: 49-57, 1994.

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ORIGINAL REPORT

Carboplatin- or Cisplatin-Based Chemotherapy in First-Line Treatment of Small-Cell Lung Cancer: The COCIS Meta-Analysis of Individual Patient Data

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose
Since treatment efficacy of cisplatin- or carboplatin-based chemotherapy in the first-line treatment of small-cell lung cancer (SCLC) remains contentious, a meta-analysis of individual patient data was performed to compare the two treatments.

Patients and Methods
A systematic review identified randomized trials comparing cisplatin with carboplatin in the first-line treatment of SCLC. Individual patient data were obtained from coordinating centers of all eligible trials. The primary end point was overall survival (OS). All statistical analyses were stratified by trial. Secondary end points were progression-free survival (PFS), objective response rate (ORR), and treatment toxicity. OS and PFS curves were compared by using the log-rank test. ORR was compared by using the Mantel-Haenszel test.

Results
Four eligible trials with 663 patients (328 assigned to cisplatin and 335 to carboplatin) were included in the analysis. Median OS was 9.6 months for cisplatin and 9.4 months for carboplatin (hazard ratio [HR], 1.08; 95% CI, 0.92 to 1.27; $P = .37$). There was no evidence of treatment difference between the cisplatin and carboplatin arms according to sex, stage, performance status, or age. Median PFS was 5.5 and 5.3 months for cisplatin and carboplatin, respectively (HR, 1.10; 95% CI, 0.94 to 1.29; $P = .25$). ORR was 67.1% and 66.0%, respectively (relative risk, 0.98; 95% CI, 0.84 to 1.16; $P = .83$). Toxicity profile was significantly different for each of the arms: hematologic toxicity was higher with carboplatin, and nonhematologic toxicity was higher with cisplatin.

Conclusion
Our meta-analysis of individual patient data suggests no differences in efficacy between cisplatin and carboplatin in the first-line treatment of SCLC, but there are differences in the toxicity profile.

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INTRODUCTION

Small-cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers. At presentation, approximately 70% of patients are diagnosed as having extensive disease and the remaining patients are diagnosed as having limited disease.[1]

The main international guidelines recommend platinum-based chemotherapy as the standard of care for first-line therapy of SCLC.[2-4] However, whether cisplatin or carboplatin are equally effective in the treatment of SCLC is still contentious. These two platinum compounds have different toxicity profiles. Cisplatin is associated with more GI adverse effects, neurotoxicity, and renal function impairment, and its administration requires a prolonged hydration,[5,6] but carboplatin is associated with more myelosuppression.

Although the mechanisms of action are similar, it is unclear whether carboplatin and cisplatin have the same clinical efficacy. For some tumors such as ovarian cancer, randomized studies[7,8] supported the use of carboplatin instead of cisplatin; for other tumors, such as germ cell and head and neck tumors, cisplatin is superior to carboplatin.[9] Several meta-analyses have addressed the issue of cisplatin-based versus carboplatin-based chemotherapy in the first-line treatment of advanced non–small-cell lung cancer. Cisplatin-based regimens resulted in slightly superior outcomes compared

with carboplatin-based chemotherapy in terms of objective response rate (ORR) and, in certain subgroups, prolonged overall survival (OS), without being associated with a significant increase in toxic effects.[10,11]

With the aim of comparing the efficacy of cisplatin versus carboplatin in the first-line treatment of SCLC, we conducted a meta-analysis of individual patient data (COCIS; Carboplatin- or Cisplatin-Based Treatment for SCLC) on patients enrolled onto randomized trials comparing the effectiveness of these two compounds.

PATIENTS AND METHODS

Identification of Eligible Trials

A literature search was performed in December 2008 and was updated in June 2009 to identify all published and unpublished randomized trials comparing cisplatin- and carboplatin-containing chemotherapy as first-line treatment of patients with SCLC.[12] The search was performed by using PubMed, EMBASE, MEDLINE, and the Cochrane Database. Proceedings of the main international meetings (American Society of Clinical Oncology, European Society for Medical Oncology, European Cancer Conference, and World Conference on Lung Cancer) were searched from 2005 onward. The following key words were used: "small cell lung carcinoma," "carboplatin," "cisplatin," and "randomized trial."

Data Collection and Study Quality

Individual patient data were requested for all patients within each of the four identified trials. A list of the types of data collected is available in Appendix Table A1 (online only). Before performing the analyses, data from each study were carefully checked and verified for coherence with the original publications: database quality was good for all of the eligible studies.

Statistical Methods

All of the analyses planned and prespecified in the meta-analysis protocol were performed according to the intention-to-treat principle. All the analyses were stratified by trial, and all tests were two-sided.

The primary end point was OS, defined as the time between date of random assignment and date of death or last date of follow-up for censored patients. OS curves were estimated by using the Kaplan-Meier technique and were compared by using the stratified log-rank test. Median follow-up was calculated according to the inverted Kaplan-Meier technique.[13]

Because the meta-analysis was based on individual patient data, heterogeneity of treatment effect on OS among trials was assessed by the likelihood ratio of two trial-stratified models, one with trial-specific treatment estimates and one with overall treatment estimates.[14] Under the null hypothesis of no heterogeneity, this statistic follows approximately a χ^2 distribution on J − 1 degrees of freedom (where J is the total number of

Table 1. Characteristics of the Four Randomized Trials Included in the Meta-Analysis

Variable	Joss et al[21]	Skarlos et al[22]	Okamoto et al[23]	Lee et al[24]
Treatment schedule				
Cisplatin arm	Cisplatin 30 mg/m^2 days 1–3 + doxorubicin 40 mg/m^2 day 1 + etoposide 100 mg/m^2 days 1-3 Followed (usually after 17-21 days) by cyclophosphamide 1,000 mg/m^2 day 1 + methotrexate 20 mg/m^2 days 14, 17 + vincristine 1.4 mg/m^2 day 1 + lomustine 40 mg/m^2 day 1	Cisplatin 50 mg/m^2 days 1-2 + etoposide 100 mg/m^2 days 1-3 every 3 weeks up to six cycles	Cisplatin 25 mg/m^2 days 1-3 + etoposide 80 mg/m^2 days 1-3, every 3-4 weeks up to four cycles	Cisplatin 60 mg/m^2 day 1 + etoposide 120 mg/m^2 day 1; 100 mg/m^2 twice a day orally days 2-3 every 3 weeks up to six cycles
Carboplatin arm	Carboplatin 80 mg/m^2 day 1 + teniposide 80 mg/m^2 day 1 once per week	Carboplatin 300 mg/m^2 day 1 + etoposide 100 mg/m^2 days 1-3 every 3 weeks up to six cycles	Carboplatin AUC 5 day 1 + etoposide 80 mg/m^2 days 1-3 every 3-4 weeks up to four cycles	Carboplatin AUC 5 day 1 + gemcitabine 1,200 mg/m^2 days 1 and 8 every 3 weeks up to six cycles
Radiotherapy	N/A	LD: OR → Chest RT (concurrent with third cycle) and PCI ED: CR → Chest RT (concurrent with third cycle) and PCI	N/A	LD: OR → Chest RT CR also PCI
Primary end point	N/S	N/S	Overall survival	Overall survival
Planned sample size	N/S	N/S	220	241
Actual sample size	59	143	220	241
Start of accrual	September 1989	September 1987	September 1998	January 1999
End of accrual	September 1991	November 1991	January 2004	October 2001
Median follow-up, months[13]	N/A (all patients dead)	26.3	58.9	24.0
No. of deaths recorded	59 (100%)	111 (78%)	203 (92%)	216 (90%)
Eligibility criteria				
Age limitations, years	N/S	< 75	≥ 70 (PS 0-2) < 70 (PS 3)	Both < 70 and ≥ 70
PS	0-3	0-2	0-2 (≥ 70 years) 3 (< 70 years)	0-2 (ED) ≥ 2 (LD)
Stage	ED	ED LD	ED	ED (PS 0-2) Poor prognosis LD (PS ≥ 2 and/or increased ALP)

Abbreviations: ALP, alkaline phosphatase; AUC, area under curve; CR, complete response; ED, extensive disease; LD, limited disease; N/A, not applicable; N/S, not specified; OR, objective response; PCI, prophylactic cranial irradiation; PS, performance status; RT, radiotherapy.

trials). Findings of the meta-analysis are depicted in classic Forest plots, with point estimates and 95% CIs for each trial and for the studies overall; diamond size is proportional to study size.

Further exploratory analyses were performed in the subgroups and were based on the main baseline patients' characteristics of sex, age (younger than 70 years *v* 70 or older), stage (limited *v* extensive), and Eastern Cooperative Oncology Group (ECOG) performance status (0 to 1 *v* 2 to 3) to describe possible heterogeneity of treatment effect. An interaction test was also performed.

Secondary end points were progression-free survival (PFS), ORR, and treatment toxicity. PFS was defined as the time between date of random assignment and date of progression, or date of death for patients without progression, or last date of follow-up for censored patients. PFS analyses were similar to those for OS. ORRs were compared by using the stratified Mantel-Haenszel χ^2 test for combining two-by-two tables, and the Breslow-Day test was used to detect differences in treatment effect among the trials.[14] For ORR, patients achieving a complete response or partial response were considered as responders, and all others were considered as nonresponders.

Toxicity variables were dichotomized as (1) any grade (grade 1 to 5) versus no toxicity and (2) severe (grade 3 to 5) versus no/mild toxicity (grade 0 to 2). Toxicity rates were compared by using the stratified exact tests; Zelen's exact test was used to detect differences in toxicity effects among the trials,[15] and the pooled odds ratio with 95% CI was estimated by means of the exact method.

Statistical analyses were performed by using S-PLUS (S-PLUS 6.0 Professional, release 1; Insightful Corporation, Seattle, WA) and SAS 9.2 (SAS Institute, Cary, NC); the graphs were generated by using SigmaPlot 8.0 for Windows (SPSS, Chicago, IL) and R 2.13 (R Foundation for Statistical Computing, Vienna, Austria) software packages. Exact tests were performed by using StatXact 7 (Cytel Software, Cambridge, MA).

Table 2. Baseline Characteristics of the Patients by Treatment Group

Characteristic	Cisplatin-Based (n = 328) No.	%	Carboplatin-Based (n = 335) No.	%	All Patients (N = 663) No.	%
Clinical trial						
Okamoto et al[23]	110	33.5	110	32.8	220	33.2
Lee et al[24]	120	36.6	121	36.1	241	36.3
Skarlos et al[22]	71	21.6	72	21.5	143	21.6
Joss et al[21]	27	8.2	32	9.6	59	8.9
Age, years						
Median	67		66		67	
Range	27-85		36-86		27-86	
< 70	192	58.5	194	57.9	386	58.2
> 70	136	41.5	141	42.1	277	41.8
Sex						
Male	255	77.7	261	77.9	516	77.8
Female	73	22.3	74	22.1	147	22.2
Stage						
Limited disease	107	32.6	103	30.7	210	31.7
Extended disease	221	67.4	232	69.3	453	68.3
ECOG performance status						
0	37	11.3	42	12.5	79	11.9
1	204	62.2	193	57.6	397	59.9
2	66	20.1	77	23.0	143	21.6
3	21	6.4	23	6.9	44	6.6

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

RESULTS

Characteristics of the Trials

Of the nine publications evaluated at the initial stage, five were excluded for the following reasons: two because of data included in another article[16,17]; one because it was not a randomized trial[18]; one because it was a randomized phase II noncomparative trial[19]; and one because of a preplanned, systematic cross-over[20] (Appendix Fig A1, online only). The remaining four trials were eligible with a total of 663 patients: one trial was conducted in Switzerland,[21] one in Greece,[22] one in Japan,[23] and one in the United Kingdom.[24] The results of all four trials have already been published in full-length articles.

Thanks to the efforts of the principal investigators and data centers, individual patient data were available for all four eligible trials. Main characteristics of the four trials are described in Table 1. All four trials compared carboplatin- versus cisplatin-based doublets with the exception of the Joss et al trial,[21] in which a carboplatin doublet (considered the experimental arm) was compared with an alternating cisplatin-based schedule that included seven different drugs (considered the standard arm).

Patient Characteristics and Treatment Outcomes

Of the 663 eligible patients, 328 patients (49.5%) were assigned to cisplatin and 335 (50.5%) to carboplatin. Baseline characteristics of

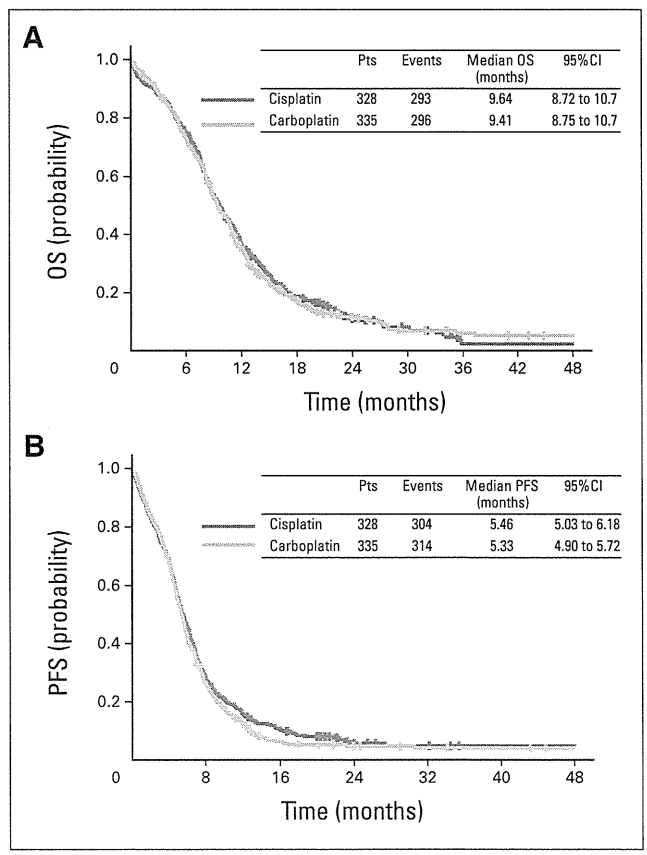

Fig 1. (A) Overall survival (OS) and (B) progression-free survival (PFS) curves by treatment arm. Pts, patients.

the 663 patients are described in Table 2. Median age was 67 years (range, 27 to 86 years). Most of the patients were males (78%) and had a good performance status (0 or 1 in 72%). Two trials[21,23] were limited to extensive disease, and the UK trial[24] allowed the inclusion of patients with limited disease who had a poor prognosis defined by poor performance status and/or high levels of alkaline phosphatase. The Greek trial[22] was the only trial that allowed the inclusion of patients with limited disease independent of their prognosis.

In the two trials[22,24] that enrolled patients with limited disease, thoracic radiotherapy was administered to 123 patients (32.1%), with similar proportions in the two treatment arms (34.2% in the cisplatin arm and 30.1% in the carboplatin arm). Information about prophylactic cranial irradiation was available in the same two trials[22,24]: prophylactic cranial irradiation was administered to 23.0% of patients, again with similar proportions in the two treatment groups (23.3% in the cisplatin arm and 22.8% in the carboplatin arm).

Median follow-up according to the Schemper and Smith method[13] was 31.9 months (29.4 months in the cisplatin arm and 31.9 months in the carboplatin arm). OS curves for patients according to treatment arms are shown in Figure 1A. Overall, 589 deaths were recorded (89%), with median OS of 9.6 months in the cisplatin arm and 9.4 months in the carboplatin arm. The corresponding hazard ratio (HR) was 1.08 (95% CI, 0.92 to 1.27; $P = .37$ with the log-rank test stratified by trial). The 6-month survival rate was 75.3% and 72.7% and the 1-year survival rate was 36.2% and 35.0% for cisplatin and carboplatin, respectively. As shown in Figure 2A, there was evidence of heterogeneity among the four trials ($P = .062$; $I^2 = 59\%$) with the Swiss trial reporting high HR values. A sensitivity analysis was performed excluding the Swiss trial,[21] and the heterogeneity disappeared ($P = .801$; $I^2 = 0\%$). With the exclusion of that trial, the HR was 1.01 (95% CI, 0.85 to 1.19; $P = .94$). Survival analysis by subgroups is shown in Figure 3A; there was no evidence of significant heterogeneity among subgroups of treatment effect around the overall effect.

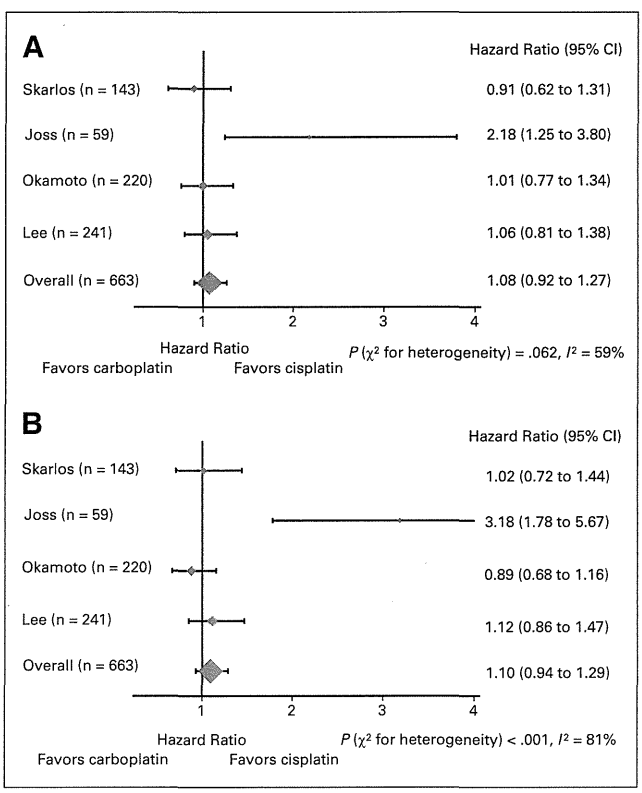

Fig 2. Forest plot of (A) overall survival and (B) progression-free survival by trial.

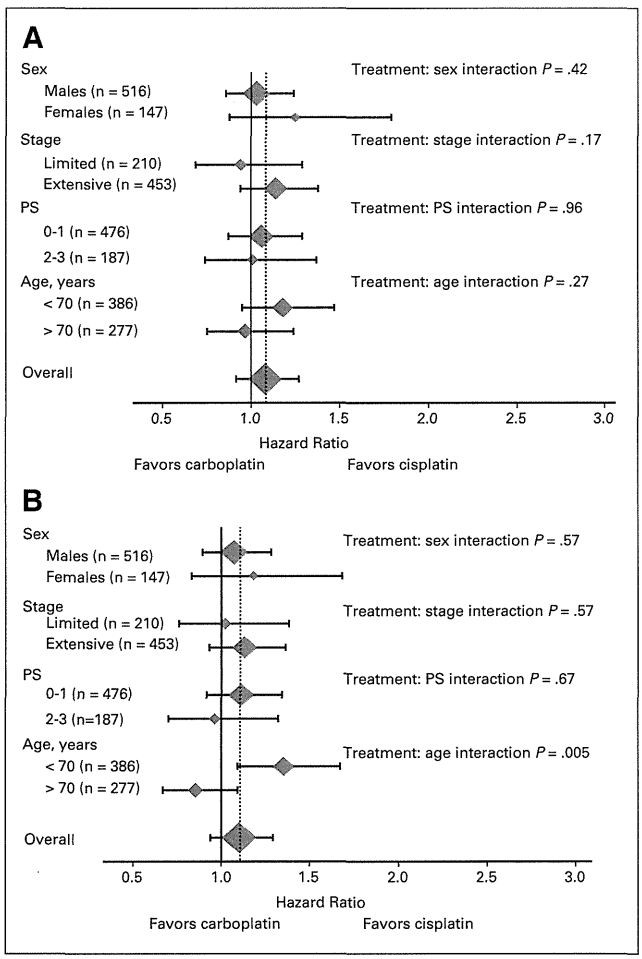

Fig 3. Forest plot of (A) overall survival and (B) progression-free survival by patients' subgroups. PS, performance status.

PFS curves for patients according to assigned treatment are shown in Figure 1B. Overall, 618 progressions were recorded (93%), with median PFS equal to 5.5 and 5.3 months for cisplatin and carboplatin, respectively. The corresponding HR was 1.10 (95% CI, 0.94 to 1.29; $P = .25$ with a log-rank test stratified by trial). The 6-month PFS was 45.4% and 40.8% and the 1-year PFS was 16% and 12.2% for patients assigned to cisplatin and carboplatin, respectively. A Forest plot of treatment effect on PFS is shown in Figure 2B; there was statistically significant heterogeneity ($P < .001$; $I^2 = 81\%$) with the Swiss trial reporting high HR values. A sensitivity analysis was performed excluding the Swiss trial,[21] and heterogeneity disappeared ($P = .477$; $I^2 = 0\%$). With the exclusion of that trial, the HR was 1 (95% CI, 0.85 to 1.19; $P = .95$). PFS analysis by subgroups is shown in Figure 3B; there was no evidence of heterogeneity among subgroups of treatment effect

Table 3. Toxicity

Toxicity	Patients With Toxicity Information	Any Grade						Severe Toxicity (grade ≥ 3)					
		Cisplatin (%)	Carboplatin (%)	Exact OR	95% CI	P*	P† for Homogeneity	Cisplatin (%)	Carboplatin (%)	Exact OR	95% CI	P*	P† for Homogeneity
Leucopenia	655	74	77	1.22	0.81 to 1.88	.357	< .001	34	34	0.96	0.67 to 1.37	.863	< .001
Neutropenia	458	86	90	1.53	0.81 to 2.92	.177	.397	64	73	1.74	1.07 to 2.83	.021	.999
Anemia	512	84	89	1.72	0.99 to 3.03	.049	.046	16	25	1.73	1.12 to 2.89	.011	< .001
Platelets	512	39	71	3.36	2.83 to 6.34	< .001	< .001	14	42	3.78	2.86 to 7.19	< .001	< .001
Nausea/vomiting	655	72	63	0.66	0.47 to 0.93	.013	.012	6	3	0.49	0.21 to 1.11	.066	.999
Stomatitis	655	25	21	0.78	0.52 to 1.17	.239	.065	1	< 1	0.24	0.01 to 3.32	.320	.999
Diarrhea	458	19	22	1.23	0.76 to 2.00	.415	.999	2	2	0.99	0.18 to 5.40	.999	.999
Constipation	239	39	51	1.58	0.92 to 2.73	.091	.999	3	5	1.51	0.35 to 7.48	.749	.999
Neurotoxicity	416	19	7	0.29	0.14 to 0.58	< .001	.243	1	< 1	0.35	0.01 to 7.27	.569	.999
Renal toxicity	415	25	10	0.34	0.19 to 0.61	< .001	.787	1.5	5	0.28	0.01 to 3.78	.351	.540
Toxic deaths	655	—	—	—		—	—	1.9	1.5	0.80	0.19 to 3.18	.769	.101

Abbreviation: OR, odds ratio.
*Exact text stratified by trial.
†Exact test for homogeneity of odds ratios.

around the overall effect, with the exception of a significant interaction with age, favoring cisplatin-based treatment in younger patients and carboplatin-based treatment in older patients.

ORR was 67.1% (220 of 328; exact 95% CI, 61.8% to 71.9%) with cisplatin and 66.0% (221 of 335; exact 95% CI, 60.7% to 70.8%) with carboplatin ($P = .83$ stratified by clinical trial). Relative risk of ORR was 0.98 (95% CI, 0.84 to 1.16). The test for heterogeneity was significant ($P = .035$; $I^2 = 65\%$). In this case, heterogeneity also disappeared after excluding the Swiss trial[21] ($P = .611$, $I^2 = 0\%$).

In the Japanese trial,[23] one patient assigned to the cisplatin arm was not eligible for toxicity analysis: no chemotherapy was administered because delirium occurred after registration. In the UK trial,[24] one patient in each arm did not start treatment, and neither patient was eligible for toxicity analysis. Finally, the data center of the Swiss trial[21] was not able to retrieve the toxicity information used for original publication. However, all the information that was available was used for this analysis. Overall, 655 of the 663 patients were included in the toxicity analysis, although information was not available for all adverse effects (Table 3). Carboplatin-containing chemotherapy is associated with more myelosuppression, with a significantly higher incidence of severe neutropenia, anemia, and thrombocytopenia. Patients treated with cisplatin had significantly more nausea/vomiting, neurotoxicity, and renal toxicity. Heterogeneity among studies was found for some adverse effects, probably due to the different drugs and doses used.

DISCUSSION

The COCIS meta-analysis of individual patient data shows that carboplatin-based regimens appear to be equally effective in terms of OS, PFS, and ORR compared with cisplatin-based combinations for the first-line therapy of SCLC, differing only in their toxicity profiles. Because of the small sample sizes of SCLC trials comparing carboplatin- with cisplatin-based chemotherapy, the COCIS meta-analysis allowed us to overcome the problem of reduced statistical power. The upper CI of the HR for OS (1.27) is higher than the margin usually considered acceptable for defining noninferiority. However, after excluding the Swiss trial,[21] the upper CI becomes 1.19, so we can rule out that risk of death with carboplatin is more than 20% worse than with cisplatin. These data support the increased use of carboplatin instead of cisplatin as part of standard treatment for SCLC.

A potential limitation of the COCIS meta-analysis is the difference in treatment schedules among the trials, especially considering that our results for all outcomes considered are burdened by a statistically significant heterogeneity. Sensitivity analysis suggested that the primary source of this heterogeneity was the Swiss study,[21] the only one that showed statistically significant superiority of cisplatin, which is different from the results of all the other trials. When this study was excluded from the analysis, the test for heterogeneity did not reach statistical significance. In the Swiss study, however, a great disparity is apparent between the treatment arms. Patients randomly assigned to the cisplatin arm received an alternating schedule of seven different drugs versus patients randomly assigned to carboplatin plus teniposide, which appeared substantially weaker. However, the overall results of the COCIS meta-analysis were not substantially affected by this trial, because it randomly assigned 59 patients, representing only 8.9% of all patients included in our meta-analysis. Of the remaining trials, two[22,23] compared platinum-based doublets that differed only for the platinum compound (carboplatin plus etoposide *v* cisplatin plus etoposide), although in one study,[24] the treatment arms also differed in the platinum companion (gemcitabine *v* etoposide). We recognize that these differences may contribute to the clinical heterogeneity of the meta-analysis. However, clinical heterogeneity may improve the generalizability of the observed results. In other words, the consistently similar efficacy between treatments in the three trials comparing cisplatin- and carboplatin-based doublets and the absence of statistical heterogeneity in the analysis excluding the Swiss trial represent relevant evidence for the choice of a platinum compound in clinical practice.

Another bias could be the role of thoracic radiotherapy in the group of patients with limited disease. However, the accrual of these patients was well balanced in both treatment groups. In the two trials[22,24] enrolling patients with limited disease, thoracic radiotherapy

was administered to a similar proportion of patients in the two treatment groups.

Another possible bias of this meta-analysis is related to the different doses of cisplatin and carboplatin used in the eligible trials. Cisplatin dose ranged from 60 to 100 mg/m^2 given in one dose or fractionated in 2 to 3 days, and carboplatin dose was based on either body surface area (at 80 or 300 mg/m^2) or on area under the curve 5. The carboplatin dose (80 mg/m^2) used in the Swiss trial was low and may explain the inferior outcome of the carboplatin arm. The cisplatin dose investigated in the UK trial (60 mg/m^2) was at the inferior limit of the activity dose to allow the enrollment of patients with poor prognosis. However, to date, no evidence exists of a dose-response effect associated with platinum agents within the range of the doses used in these studies, except for the low carboplatin dose used in the Swiss trial. Therefore, it is unlikely that these minor differences in platinum doses affected our findings.

As expected from literature and from clinical experience with the two drugs, the range of toxicity of the two platinum agents was different. Carboplatin-based regimens were associated with more cases of grade 3 to 4 hematologic toxicities. To date, the availability of granulocyte colony-stimulating factors and erythropoietins could also improve the control of corresponding hematologic toxicities.[25,26] Cisplatin-based therapies were associated with more nonhematologic toxicities of any grade. Considering that all eligible trials started accrual during the 1980s and 1990s, it is likely that with the introduction of newer and more effective antiemetic agents,[27] the incidence of nausea and vomiting associated with intermediate- to high-dose cisplatin can be further ameliorated. Grade less than 3 neurotoxicity and renal toxicity were statistically worse in cisplatin-based chemotherapy. Despite low or moderate intensity in the majority of patients, this toxicity could affect the quality of life of many patients.

We did not address the end point of health-related quality of life because only two trials[21,24] included this evaluation. Moreover, the tools used were different. Overall, in those two trials, there was no significant difference in quality of life at the different assessment points that could be attributed to treatment.

Before collecting data from individual studies for the COCIS meta-analysis, we performed a meta-analysis based on literature data.[28] Individual patient data permit us to draw more definite conclusions than in the previous analysis for the reasons given by Piedbois and Buyse.[29] In fact, the general results are substantially similar but, in contrast to meta-analysis based on abstracted data, the individual patient data approach of the COCIS meta-analysis allows the investigator to evaluate the reliability of the randomization methods, check the trial data, repeat the original or perform other analyses, and update the patients' outcomes. Furthermore, availability of individual data allowed subgroup analysis with exploratory intent. No evidence of significant differences in OS between cisplatin and carboplatin according to sex, stage, performance status, or age were apparent. Unfortunately, caution is needed to use this information for managing patients with limited disease because the majority of patients with limited disease included in this meta-analysis had bulky disease or poor prognosis. In other words, only a small group of patients had limited disease, and we think that no definite conclusions should be drawn in this subgroup of patients.

In our opinion, the question of which platinum compound to use is a relevant clinical issue, particularly in patients with SCLC who have a poor prognosis. This is the first and only individual patient data meta-analysis in which we collected all the available trials that addressed this issue and all of them have been published as full-length articles. On the basis of our results, the choice of the platinum compound for first-line treatment of patients with SCLC in clinical practice should take into account the expected toxicity profile, age, the patient's organ function, and the patient's comorbidities.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

1. Govindan R, Page N, Morgensztern D, et al: Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: Analysis of the Surveillance, Epidemiologic, and End Results database. J Clin Oncol 24:4539-4544, 2006
2. Simon GR, Turrisi A, American College of Chest Physicians: Management of small cell lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 132:324S-339S, 2007 (suppl 3)
3. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Small cell lung cancer—Version v2.2012. http://www.nccn.org
4. Stahel R, Thatcher N, Früh M, et al: 1st ESMO Consensus Conference in lung cancer: Lugano 2010—Small-cell lung cancer. Ann Oncol 22:1973-1980, 2011
5. Johnson SW, O'Dwyer PJ: Pharmacology of cancer chemotherapy: Cisplatin and its analogues, in DeVita VT Jr, Hellman S, Rosenberg SA (eds): Cancer: Principles and Practice of Oncology (ed 7). Philadelphia, PA, Lippincott Williams & Wilkins, 2005 pp 344-358
6. Go RS, Adjei AA: Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. J Clin Oncol 17:409-422, 1999
7. du Bois A, Lück HJ, Meier W, et al: A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst 95:1320-1329, 2003
8. Ozols RF, Bundy BN, Greer BE, et al: Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: A Gynecologic

Oncology Group study. J Clin Oncol 21:3194-3200, 2003

9. Lokich J, Anderson N: Carboplatin versus cisplatin in solid tumors: An analysis of the literature. Ann Oncol 9:13-21, 1998

10. Ardizzoni A, Boni L, Tiseo M, et al: Cisplatin-versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: An individual patient data meta-analysis. J Natl Cancer Inst 99:847-857, 2007

11. Hotta K, Matsuo K, Ueoka H, et al: Meta-analysis of randomized clinical trials comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer. J Clin Oncol 22:3852-3859, 2004

12. Pignon JP, Hill C: Meta-analyses of randomised clinical trials in oncology. Lancet Oncol 2:475-482, 2001

13. Schemper M, Smith TL: A note on quantifying follow-up in studies of failure time. Control Clin Trials 17:343-346, 1996

14. Smith CT, Williamson PR, Marson AG: Investigating heterogeneity in an individual patient data meta-analysis of time to event outcomes. Stat Med 24:1307-1319, 2005

15. Reis IM, Hirji KF, Afifi AA: Exact and asymptotic tests for homogeneity in several 2 x 2 tables. Stat Med 18:893-906, 1999

16. Kosmidis PA, Samantas E, Fountzilas G, et al: Cisplatin/etoposide versus carboplatin/etoposide chemotherapy and irradiation in small cell lung cancer: A randomized phase III study—Hellenic Cooperative Oncology Group for Lung Cancer Trials. Semin Oncol 21:23-30, 1994

17. Steele JP: Gemcitabine/carboplatin versus cisplatin/etoposide for patients with poor-prognosis small cell lung cancer: A phase III randomized trial with quality-of-life evaluation. Semin Oncol 28:15-18, 2001

18. Kumagai M, Kutsuzawa T, Fuyuno G, et al: Comparison of carboplatin/etoposide/vincristine with cisplatin/etoposide as combination chemotherapy for small cell lung cancer. Tokaj J Exp Clin Med 20:209-214, 1995

19. Socinski MA, Weissman C, Hart LL, et al: Randomized phase II trial of pemetrexed combined with either cisplatin or carboplatin in untreated extensive-stage small-cell lung cancer. J Clin Oncol 24:4840-4847, 2006

20. Monnet I, Chariot P, Quoix E, et al: Extensive small-cell lung cancer: A randomized comparison of two chemotherapy programs with early crossover in instance of failure—Association pour le Traitement des Tumeurs Intra-Thoraciques (ATTIT). Ann Oncol 3:813-817, 1992

21. Joss RA, Alberto P, Hürny C, et al: Quality versus quantity of life in the treatment of patients with advanced small-cell lung cancer? A randomized phase III comparison of weekly carboplatin and teniposide versus cisplatin, adriamycin, etoposide alternating with cyclophosphamide, methotrexate, vincristine and lomustine: Swiss Group for Clinical Cancer Research (SAKK). Ann Oncol 6:41-48, 1995

22. Skarlos DV, Samantas E, Kosmidis P, et al: Randomized comparison of etoposide-cisplatin vs. etoposide-carboplatin and irradiation in small-cell lung cancer: A Hellenic Co-operative Oncology Group study. Ann Oncol 5:601-607, 1994

23. Okamoto H, Watanabe K, Kunikane H, et al: Randomised phase III trial of carboplatin plus etoposide vs split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small-cell lung cancer: JCOG 9702. Br J Cancer 97:162-169, 2007

24. Lee SM, James LE, Qian W, et al: Comparison of gemcitabine and carboplatin versus cisplatin and etoposide for patients with poor-prognosis small cell lung cancer. Thorax 64:75-80, 2009

25. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Myeloid growth factors—Version v1.2011. http://www.nccn.org

26. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Cancer- and chemotherapy-induced anemia—Version v2.2012. http://www.nccn.org

27. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Antiemesis—Version v1.2012. http://www.nccn.org

28. Rossi A, Di Maio M, Martelli O, et al: Cisplatin versus carboplatin-based regimens in the treatment of small cell lung cancer (SCLC): Meta-analysis of published randomized clinical trials (RCTs). J Thorac Oncol 4:S397-S398, 2009 (suppl 1; abstr D6.3)

29. Piedbois P, Buyse M: Meta-analyses based on abstracted data: A step in the right direction, but only a first step. J Clin Oncol 22:3839-3841, 2004

Takeda et al. BMC Cancer 2012, 12:327
http://www.biomedcentral.com/1471-2407/12/327

BMC Cancer

STUDY PROTOCOL Open Access

Impact of treatment with bevacizumab beyond disease progression: a randomized phase II study of docetaxel with or without bevacizumab after platinum-based chemotherapy plus bevacizumab in patients with advanced nonsquamous non–small cell lung cancer (WJOG 5910L)

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Abstract

Background: Bevacizumab, a humanized antibody to vascular endothelial growth factor (VEGF), shows clinical activity against human cancer, with its addition to standard chemotherapy having been found to improve outcome in patients with advanced nonsquamous non–small cell lung cancer (NSCLC). However, there have been no evidence-based studies to support the continued use of bevacizumab beyond disease progression in such patients treated with the drug in first-line therapy. We have now designed a randomized phase II trial to examine the clinical benefit and safety of continued bevacizumab treatment in patients with advanced nonsquamous NSCLC whose disease has progressed after first-line treatment with bevacizumab plus a platinum-based doublet.

Methods/Design: WJOG 5910L was designed as a multicenter, open-label, randomized, phase II trial by the West Japan Oncology Group of docetaxel (arm A) versus docetaxel plus bevacizumab (arm B) in patients with recurrent or metatstatic nonsquamous NSCLC whose disease has progressed after first-line treatment with bevacizumab plus a platinum-based doublet. Patients in arm A will receive docetaxel at 60 mg/m^2 and those in arm B will receive docetaxel at 60 mg/m^2 plus bevacizumab at 15 mg/kg, with each drug administered on day 1 every 21 days until progression or unacceptable toxicity. The primary endpoint of the study is progression-free survival, with secondary endpoints including response rate, overall survival, and safety, for patients treated in either arm.

Trial registration: UMIN (University Hospital Medical Information Network in Japan) 000004715

Keywords: Bevacizumab, Beyond disease progression, Non-small cell lung cancer

Background

Lung cancer is the most common cause of cancer-related death worldwide, with non–small cell lung cancer (NSCLC) accounting for ~75% of all lung cancer cases [1]. Platinum-based chemotherapy regimens are the standard first-line treatment for individuals with advanced NSCLC, but the efficacy of such regimens has reached a plateau [2]. Both experimental and clinical studies have identified many molecules that contribute to the various biological behaviors of malignant tumors including NSCLC.

Vascular endothelial growth factor (VEGF), an endothelial cell–specific mitogen, is the major regulator of angiogenesis in normal and malignant tissue [3,4]. Increased expression of VEGF has been detected in most types of tumor in humans, including NSCLC, and, in many instances, it is associated with increased risk of recurrence, metastasis, or death [5-8]. Preclinical studies

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have shown that a murine monoclonal antibody specific for VEGF inhibits the growth of human tumor xenografts when administered alone or together with chemotherapy [9-11]. A humanized variant of this antibody, bevacizumab, has shown clinical activity against human cancer, with its addition to standard chemotherapy having been found to improve outcome in the treatment of individuals with metastatic colorectal cancer (mCRC) [12].

Randomized phase III trials have evaluated the addition of bevacizumab to cytotoxic chemotherapy in chemonaïve patients with advanced NSCLC. ECOG 4599 was the first of these trials and established the combination of bevacizumab and cytotoxic chemotherapy as a new standard of care for eligible patients [13]. This trial randomized 878 patients with NSCLC of stage IIIb or IV to chemotherapy alone (carboplatin and paclitaxel) or the same chemotherapy regimen with bevacizumab. Individuals with squamous cell carcinoma, brain metastases, or hemoptysis and those receiving anticoagulation therapy were excluded. Median overall survival (OS) increased from 10.3 to 12.3 months as a result of the addition of bevacizumab (hazard ratio [HR], 0.79; $P < 0.001$), and median progression-free survival (PFS) increased from 4.5 to 6.2 months (HR, 0.66; $P < 0.001$). Toxicities that showed statistically significant but minimal increases in frequency associated with the addition of bevacizumab included hypertension (6.0 versus 0.7%), hemoptysis (1.9 versus 0.2%), and epistaxis (0.7 versus 0.2%) of grade ≥3 as well as neutropenia of grade 4 (25.5 versus 16.8%) and febrile neutropenia (5.2 versus 2.0%). The AVAiL trial randomized patients to receive cisplatin and gemcitabine alone or together with bevacizumab at a dose of either 7.5 or 15 mg/kg [14]. Although OS was originally selected as the primary endpoint, this was changed to PFS during accrual. Like ECOG 4599, the exclusion criteria of AVAiL were broad: a squamous tumor histology, mixed adenosquamous histology if predominantly squamous, hemoptysis greater than one-half teaspoon of bright red blood per event, tumor-invading or abutting major blood vessels, brain metastases or spinal cord compression, uncontrolled hypertension, thrombotic or hemorrhagic disorders in the prior 6 months, and therapeutic anticoagulation within 10 days of the first dose. The median PFS was improved in both the 7.5 mg/kg group (6.7 months; HR, 0.75; $P = 0.003$) and 15 mg/kg group (6.5 months; HR, 0.82; $P = 0.03$) as compared with the chemotherapy-alone arm (6.1 months). OS was not significantly improved by the addition of bevacizumab in this study.

On the basis of these results, bevacizumab plus cytotoxic chemotherapy has become a standard of care for first-line therapy in a subgroup of patients with advanced NSCLC. However, there are no evidence-based studies to support the use of bevacizumab beyond disease progression in NSCLC patients receiving the antibody as first-line therapy. Preclinical studies have shown that VEGF is expressed throughout the life cycle of a tumor [15,16], and that VEGF inhibition results in marked antitumor effects when the inhibitor is administered throughout tumor development. Rapid tumor revascularization has also been shown to occur after removal of anti-VEGF therapy, suggesting that vascular regrowth may be a normal physiological response to the removal of VEGF inhibition [17,18]. Sustained VEGF inhibition has thus been shown to achieve and maintain tumor regression. Insight into the effect of treatment with bevacizumab beyond disease progression has been provided by the non-randomized, prospective bevacizumab treatment registry known as the BRiTE Study for mCRC [19]. In this prospective study, the impact of treatment with bevacizumab beyond first progression was examined. A total of 1445 patients manifested disease progression and received either further treatment with bevacizumab ($n = 642$, 44%), treatment other than bevacizumab ($n = 531$, 37%), or no treatment ($n = 253$, 18%) after progression. Despite having a patient population more representative of general CRC patients, the group that continued bevacizumab beyond first tumor progression showed a median OS of 31.8 months ($n = 642$), compared with a median OS of 19.9 months for those patients who received treatment other than bevacizumab ($n = 531$), a value similar to that reported in previous randomized studies. On the basis of these findings, the prospective European AIO 0504 trial was undertaken and is currently under way for examination of the effect of the addition of bevacizumab to fluoropyrimidine-based chemotherapy in patients with mCRC who show disease progression while receiving first-line standard chemotherapy plus bevacizumab.

With this background, the current randomized trial (University Hospital Medical Information Network in Japan [UMIN] 000004715) was designed to evaluate whether the addition of bevacizumab to docetaxel alone (the standard second-line treatment for NSCLC) might improve PFS when administered as second-line treatment in NSCLC patients who have progressed after first-line treatment with bevacizumab plus a platinum-based doublet.

Methods/Design

Study design

This open-label, randomized, phase II study of the West Japan Oncology Group (WJOG 5910L) was designed to evaluate the addition of bevacizumab to standard therapy with docetaxel. The primary endpoint of the study is PFS, with secondary endpoints including response rate, OS, and safety, for patients treated with either docetaxel alone or docetaxel plus

bevacizumab. The study has been approved by the institutional ethics committee of each participating institution.

Eligibility criteria

Study entry is limited to patients aged ≥20 years with histologically or cytologically confirmed non-squamous NSCLC that is either recurrent or metastatic, with documented disease progression after first-line treatment with bevacizumab plus platinum-based doublet chemotherapy, and with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. A patient who has received pre- or postoperative chemotherapy is eligible if the last administration of the prior adjuvant regimen occurred at least 12 months before the onset of platinum-based chemotherapy plus bevacizumab. A patient with a history of treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (gefitinib or erlotinib) before platinum-based chemotherapy plus bevacizumab is also eligible if he or she harbors an *EGFR* mutation. Adequate bone marrow, renal, and liver function is required. A lesion not previously irradiated that is measurable according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 is required for evaluation of response. All patients must sign informed consent forms approved by the relevant institutional review board.

Exclusion criteria include: prior treatment with docetaxel; active or recent history of hemoptysis (at least one-half teaspoon of bright red blood per event); central nervous system metastases; active thrombosis or embolism; serious infection; serious uncontrolled medical conditions including heart disease, diabetes, or hypertension; uncontrolled effusion (pleural, peritoneal, or pericardial effusion requiring drainage for symptom management); major surgery within 4 weeks prior to registration; minor surgical procedures, radiation therapy, or transfusion within the previous 14 days; evidence of interstitial pneumonitis or pulmonary fibrosis on the baseline chest x-ray; pregnancy or lactation; and a history of cancer within the previous 5 years.

Patient registration

After eligibility criteria are confirmed and informed consent obtained, eligible patients are registered and the planned treatment is initiated by investigators. The accrual began in February 2011 and is to continue for 2 years.

Treatment plan

In this multicenter phase II trial, patients are randomly assigned on a 1:1 basis to docetaxel or docetaxel plus bevacizumab (Figure 1). The study focuses on the outcome of a second bevacizumab-based line of treatment, seeking clinical predictors that might help identify patients likely to benefit from bevacizumab therapy beyond progression (Figure 2). Stratification factors were thus chosen on the basis of the hypotheses that patients who show progressive disease during first-line therapy are unlikely to benefit from second-line therapy and that bevacizumab as a maintenance therapy after treatment with bevacizumab plus a platinum-based doublet until disease progression may augment the efficacy of chemotherapy in second-line treatment. Random assignment was stratified by (i) baseline ECOG performance status (0 or 1 versus 2), (ii) history of treatment with EGFR tyrosine kinase inhibitors (gefitinib or erlotinib versus neither), (iii) duration of treatment with bevacizumab plus a platinum-based doublet (<6 versus ≥6 weeks), and (iv) time to disease progression after the last bevacizumab administration of the first-line treatment (<3 versus ≥3 weeks). Patients treated in arm A will receive docetaxel (60 mg/m^2) and those in arm B will receive docetaxel (60 mg/m^2) plus bevacizumab (15 mg/kg) on day 1 every 21 days until progression or unacceptable toxicity. Patients in arm B are allowed to receive docetaxel alone as a result of bevacizumab-related toxicity, but they are not allowed to receive bevacizumab alone. A computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain, CT scans of the chest and abdomen, bone scan or positron emission tomography (PET) scan, and an electrocardiogram are required before onset of the study treatment. Patients undergo tumor assessment at baseline, every 4 weeks during the first 12 weeks, and every 6 weeks thereafter. Adverse events are recorded

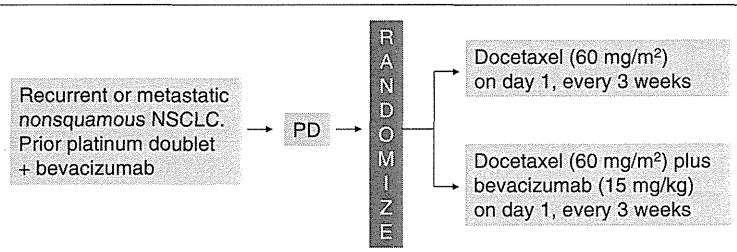

Figure 1 WJOG 5910L treatment design. PD, progressive disease.

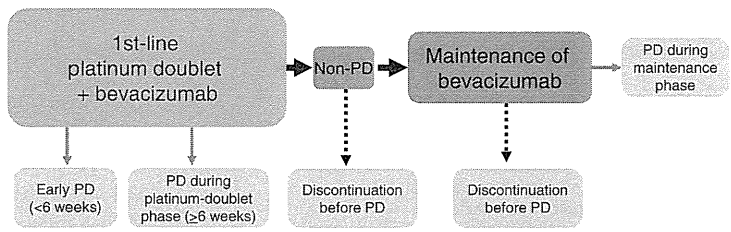

Figure 2 Disease course patterns for first-line chemotherapy. Red arrows indicate discontinuation of first-line treatment because of PD; broken black arrows indicate discontinuation before PD.

based on the National Cancer Institute Common Toxicity Criteria (version 4.0).

Statistical considerations

The primary and secondary efficacy endpoint analysis will be performed with the intent-to-treat population. The emphasis of the efficacy analysis will be on estimating the size of the difference in treatment effect between arm A and arm B. The sample size was calculated on the basis of hypothesis testing in terms of PFS, which was defined as the time from registration until disease progression or death, whichever occurs earlier. Patients who have not experienced progression or death by the end of follow-up for the study will be censored on the date of the last tumor assessment. The trial is based on a randomized phase II screening design as described previously [20]. A previous randomized phase II study evaluated the efficacy of chemotherapy with or without bevacizumab in patients with advanced nonsquamous NSCLC who were treated with first-line chemotherapy without bevacizumab. The primary endpoint of PFS tended to be longer with chemotherapy plus bevacizumab than for chemotherapy alone (HR, 0.66; 95% confidence interval, 0.38 to 1.16) [21]. Accordingly, the present trial is designed as a one-sided test to detect a ≥30% reduction in the HR associated with PFS favoring the experimental arm with a type I error (alpha) rate of 0.20. The median PFS for docetaxel alone is estimated to be 2.0 months based on the results of previous phase III trials. According to these parameters, a total of 90 or more events (total from both arms combined) is required to achieve a statistical power of >80%. Taking into account patients who prove to be ineligible or who are lost to follow-up, the sample size is planned as 100 patients.

Conclusion

The WJOG 5910L trial is designed to examine the clinical benefit and safety of continued bevacizumab treatment beyond first disease progression in patients with advanced nonsquamous NSCLC whose disease has progressed after first-line treatment with bevacizumab plus a platinum-based doublet. The information obtained by the study may prompt early completion of the planned patient accrual.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

M.T. and I.O. developed the study concept and initiated the project. K.N. and Y.N. coordinated the study concept and protocol design. T.Y. is responsible for statistical analysis. All authors have read and approved the final manuscript.

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References

1. Hoffman PC, Mauer AM, Vokes EE: **Lung cancer.** *Lancet* 2000, **355**:479–485.
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH: **Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer.** *N Engl J Med* 2002, **346**:92–98.
3. Folkman J: **What is the evidence that tumors are angiogenesis dependent?** *J Natl Cancer Inst* 1990, **82**:4–6.
4. Ferrara N: **The role of vascular endothelial growth factor in pathological angiogenesis.** *Breast Cancer Res Treat* 1995, **36**:127–137.
5. Mattern J, Koomagi R, Volm M: **Association of vascular endothelial growth factor expression with intratumoral microvessel density and tumour cell proliferation in human epidermoid lung carcinoma.** *Br J Cancer* 1996, **73**:931–934.
6. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF: **Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract.** *Cancer Res* 1993, **53**:4727–4735.
7. Brown LF, Berse B, Jackman RW, Tognazzi K, Guidi AJ, Dvorak HF, Senger DR, Connolly JL, Schnitt SJ: **Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer.** *Hum Pathol* 1995, **26**:86–91.
8. Seto T, Higashiyama M, Funai H, Imamura F, Uematsu K, Seki N, Eguchi K, Yamanaka T, Ichinose Y: **Prognostic value of expression of vascular**

endothelial growth factor and its flt-1 and KDR receptors in stage I non-small-cell lung cancer. *Lung Cancer* 2006, **53**:91–96.
9. Ferrara N, Gerber HP, LeCouter J: **The biology of VEGF and its receptors.** *Nat Med* 2003, **9**:669–676.
10. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N: **Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo.** *Nature* 1993, **362**:841–844.
11. Borgstrom P, Gold DP, Hillan KJ, Ferrara N: **Importance of VEGF for breast cancer angiogenesis in vivo: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin.** *Anticancer Res* 1999, **19**:4203–4214.
12. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F: **Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer.** *N Engl J Med* 2004, **350**:2335–2342.
13. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH: **Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer.** *N Engl J Med* 2006, **355**:2542–2550.
14. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leighl N, Mezger J, Archer V, Moore N, Manegold C: **Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil.** *J Clin Oncol* 2009, **27**:1227–1234.
15. Fontanini G, Vignati S, Boldrini L, Chine S, Silvestri V, Lucchi M, Mussi A, Angeletti CA, Bevilacqua G: **Vascular endothelial growth factor is associated with neovascularization and influences progression of non-small cell lung carcinoma.** *Clin Cancer Res* 1997, **3**:861–865.
16. Hanrahan V, Currie MJ, Gunningham SP, Morrin HR, Scott PA, Robinson BA, Fox SB: **The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression.** *J Pathol* 2003, **200**:183–194.
17. Mancuso MR, Davis R, Norberg SM, O'Brien S, Sennino B, Nakahara T, Yao VJ, Inai T, Brooks P, Freimark B, Shalinsky DR, Hu-Lowe DD, McDonald DM: **Rapid vascular regrowth in tumors after reversal of VEGF inhibition.** *J Clin Invest* 2006, **116**:2610–2621.
18. Vosseler S, Mirancea N, Bohlen P, Mueller MM, Fusenig NE: **Angiogenesis inhibition by vascular endothelial growth factor receptor-2 blockade reduces stromal matrix metalloproteinase expression, normalizes stromal tissue, and reverts epithelial tumor phenotype in surface heterotransplants.** *Cancer Res* 2005, **65**:1294–1305.
19. Grothey A, Sugrue MM, Purdie DM, Dong W, Sargent D, Hedrick E, Kozloff M: **Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRiTE).** *J Clin Oncol* 2008, **26**:5326–5334.
20. Rubinstein LV, Korn EL, Freidlin B, Hunsberger S, Ivy SP, Smith MA: **Design issues of randomized phase II trials and a proposal for phase II screening trials.** *J Clin Oncol* 2005, **23**:7199–7206.
21. Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi PD, Hart L, Melnyk O, Ramies D, Lin M, Sandler A: **Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small-cell lung cancer.** *J Clin Oncol* 2007, **25**:4743–4750.

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ORIGINAL ARTICLE

Comparison of chemotherapeutic efficacy between LCNEC diagnosed using large specimens and possible LCNEC diagnosed using small biopsy specimens

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Abstract
Background It is often difficult to diagnose large cell neuroendocrine carcinomas (LCNEC) of the lung using small biopsy specimens. Some recent studies attempted to diagnose LCNEC using biopsy specimens; in 2011, the International Association for the Study of Lung Cancer pathological panels suggested possible LCNEC as a diagnosis for LCNEC by using biopsy specimens. Here, we compared the chemotherapeutic efficacy in possible LCNEC and LCNEC diagnosed using surgically resected specimens.
Methods We retrospectively reviewed patients who received platinum-based chemotherapy as first-line chemotherapy at our institution during September 2002–September 2011. Further, we compared the clinical characteristics, chemotherapeutic responses, and survival outcomes of patients diagnosed as having "LCNEC definite" with those diagnosed as having "possible LCNEC."
Results We selected 34 patients of whom 10 were diagnosed with LCNEC using surgically resected specimens and 24 patients with possible LCNEC were diagnosed using small biopsy specimens. In both groups, almost all patients were men and were smokers. Small-cell carcinoma-based chemotherapy, such as platinum plus irinotecan or platinum plus etoposide, was used for treating 60 % LCNEC patients (6/10) and 67 % possible LCNEC patients. In the LCNEC and possible LCNEC groups, respectively, the response rate was 70 and 54 % ($p = 0.39$), median progression-free survival was 2.9 and 4.4 months ($p = 0.20$), and median survival time was 12.8 and 9.1 months ($p = 0.50$).
Conclusion No statistically significant differences were found in chemotherapeutic responses and survival outcomes between the 2 groups, which suggests that chemotherapeutic efficacy is similar in both possible LCNEC and LCNEC.

Keywords LCNEC · Possible LCNEC · Small cell carcinoma · Chemotherapy · Biopsy

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Introduction

In the 2004 edition of the World Health Organization (WHO) classification, large cell neuroendocrine carcinoma (LCNEC) of the lung was defined using detailed criteria for each subtype of neuroendocrine tumor; LCNEC was subcategorized as a variant of large cell carcinoma. The histological findings of LCNEC are large tumor cells with a low nuclear/cytoplasm ratio, prominent nucleoli, a high

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mitotic rate (11 or more mitotic figures in 10 high-power fields), a high degree of necrosis, and neuroendocrine (NE) morphologic features, such as rosette formation, organoid nesting, and palisading. Immunohistochemical positive staining for at least 1 NE marker, such as neural cell adhesion molecule (NCAM), chromogranin A, and synaptophysin, is also required [1].

LCNEC is a rare tumor accounting for approximately 3 % of all resected pulmonary malignancies [2–4]. Most previous reports have found that LCNEC predicted poorer survival than expected for stage-matched non-small-cell lung carcinoma (NSCLC) [2–4]. The malignant behavior and poor prognosis of LCNEC have been reported to be similar to those of small-cell lung carcinoma (SCLC) [5, 6]. However, these reports were limited to surgically resected specimens, because it is difficult to fully meet the histological criteria required to diagnose LCNEC using small biopsy specimens. One of the serious problems with LCNEC is that there are few studies evaluating the clinical features and prognosis of advanced cases, since diagnosis of advanced LCNEC using a small specimen is often difficult. There is no established therapeutic strategy for LCNEC, particularly for advanced cases.

Recently, Igawa et al. [7] attempted to diagnose advanced LCNEC using biopsy specimens, and reported that the pathological findings of LCNEC on biopsy specimens were defined NSCLC with some NE morphology and 1 or more positive NE markers with a high Ki-67/MIB 1 labeling index. Shimada et al. [8] also reported similar results. In 2011, Travis and colleagues suggested use of the term "possible LCNEC" for NSCLC with NE morphology and positive NE markers (NCAM, chromogranin A, and/or synaptophysin), excluding definite adenocarcinoma and squamous cell carcinoma, in a small biopsy specimen [9]. To evaluate the diagnosis of possible LCNEC, we compared the efficacy of chemotherapy in LCNEC and possible LCNEC in this study.

Patients and methods

Patients

From September 2002 to September 2011, we selected patients consecutively whose pathological diagnoses were LCNEC or possible LCNEC who received platinum-based chemotherapy as first-line chemotherapy from patient records at Shizuoka Cancer Center. We excluded patients who received concurrent chemo-radiotherapy. LCNEC and possible LCNEC were diagnosed using either primary or metastatic lesions. The sampling method was not defined, i.e., whether it was by biopsy or surgery. LCNEC was diagnosed according to the 2004 WHO criteria, using samples obtained by surgically resection. The diagnosis of possible LCNEC was made when LCNEC was highly suspected, but it was difficult to fulfill the conventional WHO criteria. All cases had confirmed positivity of 1 or more immunohistochemical NE markers (NCAM, chromogranin A, and synaptophysin) and showed a high MIB 1 labeling index (more than 40 %).

Evaluation

Chemotherapeutic response was accessed according to the Response Evaluation Criteria in Solid Tumors (RECIST): Revised RECIST guideline (version 1.1) [10]. To define disease progression or relapse, patients were evaluated by physical examination, chest radiography, and computed tomography (CT) of the chest and abdomen. In some patients, we used positron emission tomography (PET)–CT, magnetic resonance imaging (MRI), or bone scintigraphy to detect the extent of disease progression. Their clinical disease staging was reassessed according to the latest Union for International Cancer Control (UICC) staging criteria (7th edition) [11].

Progression-free survival (PFS) was scored as an event of documented disease recurrence or death measured from the start of first-line chemotherapy to the date of an event or the last follow-up. Overall survival (OS) was measured from the start of first-line chemotherapy to the date of death or the last follow-up.

Statistical analysis

All categorical variables and objective response rates were analyzed using the chi-squared test or Fisher's exact test, as appropriate. Distributions of PFS and OS were estimated using the Kaplan–Meier method, and the LCNEC and possible LCNEC groups were compared using the log-rank test. All p values were 2 sided, and values <0.05 were considered statistically significant. All analyses were performed using JMP 9 software (SAS Institute, Cary, NC, USA). This study was approved by the institutional review board.

Results

A total of 34 patients were eligible for this retrospective study, including 10 LCNEC patients diagnosed using surgically resected specimens. The resection sites for diagnosis of LCNEC were the lung ($n = 6$), brain metastasis ($n = 3$), and bone metastasis ($n = 1$). All 24 possible LCNEC patients were diagnosed using small biopsy specimens, and the biopsy sites were transbronchial biopsy ($n = 18$), CT-guided needle biopsy ($n = 4$), surgical