Table 1 Levels of evidence

| | |
|---|---|
| I | Evidence obtained from meta-analysis of multiple, well-designed, controlled studies. Randomized trials with low false-positive and low false-negative errors (high power) |
| II | Evidence obtained from at least one well-designed experimental study. Randomized trials with high false-positive and/or low false-negative errors (low power) |
| III | Evidence obtained from well-designed, quasi-experimental studies such as non-randomized, controlled single-arm, pre-post, cohort, time, or matched case-control series |
| IV | Evidence from well-designed, non-experimental studies such as comparative and correlational descriptive and case studies |
| V | Evidence from case reports and clinical examples |

Table 2 Case-series studies on small cell lung cancer in the elderly

| Authors (year) | Age | Number of patients (%) | Limited disease (%) | PS 0–1 (%) | Comorbidity (%) | Optimal treatment (%)[a] | TRD (%) | MST (month) |
|---|---|---|---|---|---|---|---|---|
| Nou (1996)[8] | <70 | 235 (68) | 50 | NA | NA | NA | 7 | 11 |
| | ⩽70 | 110 (32) | 48 | NA | NA | NA | 8 | 7 |
| Dajczman et al. (1996)[9] | <70 | 231 (74) | 40 | 80 | 56 | 44 | 5 | 9 |
| | ⩽70 | 81 (26) | 43 | 52 | 75 | 23 | 5 | 6 |
| Tebbutt et al. (1997)[10] | <70 | 102 (67) | 46 | 60 | NA | 83 | NA | No difference |
| | ⩽70 | 51 (33) | 49 | 55 | 63 | 47 | 4 | No difference |
| Jara et al. (1999)[11] | <70 | 59 (62) | 42 | 71 | 58 | 59 | NA | 8 |
| | ⩽70 | 36 (38) | 36 | 69 | 78 | 39 | NA | 5 |

MST, median survival time; NA, not available; PS, performance status; TRD, treatment-related death.
[a]Optimal treatment was defined as four or more treatment cycles, relative total dose of 85% or higher, or no definition described.

cycles, relative total does of 85% or higher, or no definition available, was delivered to 23–47% of the elderly patients compared with 44–83% of the younger patients. The incidence of treatment-related death and patient survival, however, did not differ between the two age groups.

## Chemotherapy for elderly patients in good general condition

Among elderly lung cancer patients, 10–30% are in good general condition without comorbidity,[9–13] and the standard chemotherapy for the general population, including cyclophosphamide, doxorubicin and vincristine (CAV), cisplatin and etoposide (PE), and CAV alternating with PE regimens, can be given to this population (Evidence level, IV). Subgroup analyses of phase II and phase III trials of SCLC by age showed that myelosuppression and doxorubicin-induced cardiotoxicity were severer in the elderly patients than in the younger patients, and that their incidence of treatment-related death tended to be higher. About 80% of elderly patients, however, received optimal treatment, and their survival was comparable to that of younger patients (Table 3).[14–16] Thus, the standard chemotherapy should be tried in these patients, although a reduction in treatment cycles and chemotherapy dose, or prolongation of treatment intervals may be needed more often than in younger patients.

## Chemotherapy for unselected elderly patients

The standard chemotherapy for younger patients is not indicated for 70–90% of elderly patients because of poor performance status or the presence of complications. Oral etoposide and teniposide has been tried in these patients, but randomized trials showed that it was more toxic and had no survival benefit over the standard chemotherapy (Table 4).[17,18] A randomized trial of two-drug

**Table 3** Subgroup analyses of phase III trials of small cell lung cancer by age

| Authors (year) | Treatment | Age | Number of patients | Limited disease (%) | PS 0–1 (%) | Optimal treatment (%)[a] | Grade 3–4 toxicity (%) | TRD (%) | MST (month) |
|---|---|---|---|---|---|---|---|---|---|
| Paccagnella et al. (1996)[14] | CAV–PE (±TRT) | <70 | 254 | 58 | ND | RDI 78 | NA | 3 | 12 |
| | | ⩽70 | 32 | 56 | ND | RDI 67 | NA | 9 | 12 |
| Siu et al. (1996)[15] | CAV–PE (±TRT) | <70 | 520 | 100 | 88 | 92 | Neutropenia[b] (60)<br>Thrombocytopenia (10)<br>Cardiac (0.2) | 2 | 15 |
| | | ⩽70 | 88 | 100 | 84 | 82 | Neutropenia[b](64)<br>Thrombocytopenia[15]<br>Cardiac (3) | 5 | 13 |
| Yuen et al. (2000)[16] | PE+TRT | <70 | 331 | 100 | 96 | 90 | Neutropenia[b](58)<br>Thrombocytopenia (21)<br>Infection (6) | 1 | 22 |
| | | ⩽70 | 50 | 100 | 90 | 78 | Neutropenia[b](82)<br>Thrombocytopenia (36)<br>Infection (10) | 10 | 14 |

CAV, cyclophosphamide, doxorubicin and vincristine; MST, median survival time; NA, not available; ND, no difference; PE, cisplatin and etoposide; PS, performance status; RDI, relative dose intensity; TRD, treatment-related death; TRT, thoracic radiotherapy.

[a] Optimal treatment was defined as four or more treatment cycles.

[b] Grade 4 only.

Table 4 Phase III studies comparing standard and low intensive chemotherapy in elderly or poor risk patients with small cell lung cancer

| Authors (year) | Chemotherapy regimen | Number of patients | Age ⩾ 70 (%) | PS ⩾ 2 (%) | RR (%) | Grade 3–4 toxicty (%) | TRD (%) | MST (month) |
|---|---|---|---|---|---|---|---|---|
| Girling (1996)[17] | Oral E (50 mg) bid days 1–10 | 171 | Median 67 | 100 | 61 | Neutropenia[a](14), Infection (4) | 14 | 4.3[b] |
| | Standard EV or CAV | 168 | Median 68 | 100 | 73 | Neutropenia[a] (12), Infection (7) | 10 | 6.1[b] |
| Souhami et al. (1997)[18] | Oral E (100 mg) bid days 1–5 | 75 | 52 | 48 | 33 | Neutropenia (3), Infection (5) | 2 | 4.8[b] |
| | Standard CAV/PE | 80 | 44 | 56 | 46 | Neutropenia (3), Infection (6) | 1 | 5.9[b] |
| MRC (1996)[19] | EV | 156 | 25 | 54 | 55 | Leukopenia[a] (4)[b], Stomatitis[c] (34)[b] | 1 | 4.6 |
| | EVMC | 154 | 27 | 52 | 54 | Leukopenia[a] (16)[b], Stomatitis[c] (54)[b] | 7 | 4.7 |
| James et al. (1996)[20] | Half dose CAV/PE, q11 days | 78 | Median 63 | 63 | 59 | Leukopenia (23)[b], Infection (5) | 0 | 6.4 |
| | Standard CAV/PE, q3w | 89 | Median 63 | 67 | 45 | Leukopenia (7)[b], Infection (5) | 1 | 5.8 |
| Earl et al. (1991)[21] | Planned CEV | 155 | Median 65 | 31 | NA | NA | NA | 8.2 |
| | Required CEV | 145 | Median 66 | 35 | NA | NA | NA | 6.8 |

CAV, cyclophosphamide, doxorubicin and vincristine; CEV, cyclophosphamide, etoposide and vincristine; E, etoposide; EV, etoposide and vincristine; EVMC, etoposide, vincristine, methotrexate and cyclophosphamide; MST, median survival time; NA, not available; PE, cisplatin and etoposide; PS, performance status; RR, response rate; TRD, treatment-related death.

[a] Including grade 2–4 toxicity.

[b] Statistically significant.

[c] Including grade 1–4 toxicity.

versus four-drug combinations showed severer toxicity in the four-drug arm with no improvement in survival.[19] A regimen of cisplatin and etoposide (PE) alternating with cyclophosphamide, doxorubicin, and vincristine (CAV) every 10–11 days at half the standard dose failed to reduce toxicity or improve survival compared with the standard PE alternating CAV regimen in a randomized trial.[20] Another randomized trial of cyclophosphamide, etoposide, and vincristine (CEV) given as needed to palliate symptoms, versus CEV given at fixed 3- to 4-week treatment intervals showed that patients randomized to receive chemotherapy as needed had a median interval between cycles of 5 weeks and received only 50% as much total chemotherapy as the patients randomized to the fixed schedule. Although the median survival times were equivalent between both arms, better symptomatic control was achieved with the fixed interval treatment.[21] Thus, these less intensive treatments than the standard treatment are not less toxic or useful for palliation.

The combination of carboplatin and etoposide has been one of the most frequently evaluated regimens in elderly patients with SCLC, and has yielded a response rate of 70–90% and a median survival of 8–10 months for ED and 12–15 months for LD with acceptable toxicity in phase II trials (Table 5).[22,23,25] Modification of the carboplatin dose based on creatinine clearance levels can be especially useful in elderly patients, because many of them have impaired renal function. As a result, this two-drug combination periodically repeated every 3- to 4-weeks has become standard treatment in this patient population (Evidence level, II).

## Treatment of elderly patients with limited disease who are in good general condition

A retrospective review of 1208 patients (including 398 SCLC patients, 107 patients more than 70 years of age, 114 patients with PS 2 or higher, and 352 patients with body weight loss greater than 5%) in six EORTC clinical trials (including three for NSCLC, one for SCLC, and two for esophageal cancer) showed that age did not influence the frequency or severity of acute and delayed toxicity of thoracic radiotherapy.[27] Retrospective subset analysis of patients with limited SCLC who were treated with concurrent chemoradiotherapy in phase III trials showed that 80% of the patients 70 years of age or older completed the planned treatment, although hematological toxicity was severer in the elderly group than the younger group (Table 3).[15,16] Only patients with good general condition were included in these trials; 90% had PS 0–1 and 82% had less than 5% body weight loss in the one study,[16] and 84% had PS 0–1 in the other.[15] Thus, the standard chemoradiotherapy can be given to elderly patients in good general condition with PS 0–1, normal organ function and no comorbidity (Evidence level, IV).

## Treatment for unselected elderly patients with limited disease

There are three phase II trials of concurrent chemoradiotherapy in this patient population. Although the chemotherapy cycles in these trials were reduced compared with the standard 4–6 cycles, the 5-year survival rates reached to 13–25% with manageable toxicity (Table 6).[28–30] Thus, a combination of full-dose thoracic radiotherapy and two cycles of chemotherapy may be the optimal treatment in unselected elderly patients with limited disease (Evidence level, III).

## Discussion

It has been thought to be difficult to establish standard treatments for elderly patients with SCLC, because they form a heterogeneous population in terms of general condition and treatment outcome varies from report to report. However, by classifying studies on the treatment of this population into three types and characterizing subjects included in the studies, relatively consistent results were obtained. To select the optimal treatment for elderly patients, two groups needed to be considered separately: elderly patients in good general condition and all others. The former can be treated with the same strategy as younger patients with minor modifications, if any.

Among elderly patients, 30–50% have PS 2 or higher, and 60–80% have complications in major organs including the kidney, heart, and lung.[6,9–11] They have been treated with oral etoposide or combination chemotherapy at decreased doses or longer intervals. These less intensive treatments than the standard treatment, however, were not less toxic or useful for palliation in the elderly with decreased activity. By contrast, two-drug combination chemotherapy, including a combination of etoposide and carboplatin, produced response rates (RRs) and median survival times (MSTs) comparable to those of younger patients with

Table 5 Phase II trials for elderly or poor risk patients with small cell lung cancer

| Authors (year) | Chemotherapy regimen ($mg/m^2$) | Number of patients | Age $\geqslant 70$ (%) | PS $\geqslant 2$ (%) | RR (%) | Grade 3–4 toxicty (%) | TRD (%) | MST (month) |
|---|---|---|---|---|---|---|---|---|
| Evans et al. (1995)[22] | Oral E (100 mg) days 1–7 Carbo (150) day 1 | 47 | Median 69 | 30 | 71 | Neutropenia (84) Thrombocytopenia (21) Stomatitis (2) | 18 | LD 14 ED 11 |
| Matsui et al. (1998)[23] | Oral E (40) days 1–14 Carbo[a] day 1 | 38 | 100 | 34 | 81 | Neutropenia (53) Thrombocytopenia (53) Infection (8) | 5 | LD 15 ED 9 |
| Westeel et al. (1998)[24] | P (30) A (40) V (1) day 1 E (100) days 1, 3, 5 | 41 | 100 | 66 | 88 | Infection (6) Emesis (9) | 0 | ED 11 |
| Okamoto et al. (1999)[25] | E (100) days 1–3 Carbo[a] day 1 | 36 | 100 | 25 | 75 | Neutropenia (86) Thrombocytopenia (50) Infection (5) | 3 | LD 12 ED 10 |
| Samantas et al. (1999)[26] | Oral E (100 mg) days 1–12 Carbo (80) weekly | 60 | Median 66 | 59 | 32 | Neutropenia (6) Thrombocytopenia (2) Infection (3) | 3 | 5.5 |

Carbo, carboplatin; E, etoposide; ED, extensive disease; LD, limited disease; MST, median survival time; PAVE, cisplatin, doxorubicin, vincristine and etoposide; PS, performance status; RR, response rate; TRD, treatment-related death.

[a] Dose adjusted for creatinine clearance.

Table 6 Phase II trials of chemoradiotherapy for elderly or poor risk patients with limited small cell lung cancer

| Authors (year) | Chemotherapy radiotherapy (Gy/fraction) | Number of patients | Age ⩾ 70 (%) | PS ⩾ 2 (%) | RR (%) | Grade 3–4 toxicty (%) | TRD (%) | MST (month) | 5-YS (%) |
|---|---|---|---|---|---|---|---|---|---|
| Westeel et al. (1998)[28] | PAVE × 3, PE × 1 20/5, 30/10, 40/15 | 25 | Median 72 | 28 | 92 | Thrombocytopenia[a](9) Infection (18) Esophagitis[a](9) | 3 | 16 | 24 |
| Murray et al. (1998)[29] | CAV × 1, PE × 1 20/5, 30/10 | 55 | 67 | 45 | 89 | Infection(4) | 5 | 13 | 18 |
| Jeremic et al. (1998)[30] | Carbo + oral E × 2 45/30 (twice daily) | 72 | 100 | 17 | 75 | Leukopenia (8) Thrombocytopenia (12) Infection (3) Esophagitis (3) | NA | 15 | 13 |

CAV, cyclophosphamide, doxorubicin and vincristine; Carbo, carboplatin; E, etoposide; MST, median survival time; NA, not available; PAVE, cisplatin, doxorubicin, vincristine and etoposide; PE, cisplatin and etoposide; PS, performance status; RR, response rate; TRD, treatment-related death; 5-YS, five-year survival rate.

[a] Grade 4 only.

acceptable toxicity in elderly patients. Carboplatin is especially useful for the elderly, because it requires only minimum hydration, its non-hematological toxicity is mild, and the dose can be adjusted according to patient's creatinine clearance. Japanese Clinical Oncology Group (JCOG) evaluated toxicity and efficacy of this method in a phase II study (JCOG9409), and showed that grade 4 neutropenia and thrombocytopenia were noted in 44% and 12% of patients, respectively, and that CR and PR were obtained in 6% and 69%, respectively.[25] We started a large phase III trial in 1997, comparing etoposide (80 mg/m$^2$ days 1–3) and carboplatin (AUC = 5) with etoposide (the same dose) and cisplatin (25 mg/m$^2$ days 1–3) in elderly patients with SCLC (JCOG 9702). Up to the present, more than 200 patients were registered in this study.

A recent phase III trial showed that a combination of cisplatin and irinotecan was superior to a combination of cisplatin and etoposide in patients with extensive SCLC, but only patients 70 years of age or younger were included in this study.[31] In addition, there is no clinical trial of irinotecan in elderly patients with SCLC. Another anticancer agent promising in the treatment of SCLC is amrubicin, which yielded a response rate of 79% and median survival time of 11 months in patients with extensive SCLC.[32] Further studies are necessary to evaluate these new agents in the treatment of elderly patients with SCLC.

The chemoradiotherapy used in younger patients may be too intensive for most elderly patients with limited SCLC. One approach that avoids excessive toxicity is to reduce the dose of the chemotherapy or radiotherapy. A recent meta-analysis of chemotherapy alone versus chemotherapy plus radiotherapy in patients with limited SCLC demonstrated survival benefit of radiotherapy added to chemotherapy in patients less than 70 years of age, but the benefit disappeared in the older patients.[33] This finding indicates that the standard treatment in this setting might be chemotherapy alone. The currently available phase II studies of treatment of limited SCLC in the elderly, however, showed that two cycles of chemotherapy plus full-dose radiotherapy produced long-term survivors with acceptable toxicity.[28–30] Thus, which modality should be modified remains controversial, but reduced cycles of chemotherapy combined with full-dose radiotherapy appears to be the treatment of choice at present.

The criteria for the classification of elderly patients into two groups in this review were based on PS, function of major organs, and comorbidity. However, they may be inadequate to evaluate this heterogeneous elderly population. In future clinical trials, it will be important to evaluate the influence of cancer treatment on the functional status of the elderly. A comprehensive geriatric assessment designed to improve the health care of elderly people consists mainly of instruments for evaluating activities of daily living, physical function, cognitive function, and emotional status.[34, 35] It has been used as a diagnostic tool to screen for problems and to determine the needs of the geriatric population for in-home assistance, home-health service, or hospital care, but it may be also useful for our purpose.

In conclusion, although the evidence levels based on clinical trials currently available are low, it is possible to select the optimal treatment for elderly patients with SCLC by dividing them into patients in good and poor general condition.

## References


1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer* 1993;54:594–606.
2. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. *CA Cancer J Clin* 2000;50:7–33.
3. Murren J, Glatstein E, Pass HI. Small cell lung cancer. In: DeVita Jr VT, Hellman S, Rosenberg SA, editors. *Cancer: principles and practice of oncology*. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 983–1018.
4. Stephens RJ, Johnson DH. Treatment and outcomes for elderly patients with small cell lung cancer. *Drugs Aging* 2000;17:229–47.
5. Shepherd FA, Bezjak A. Treatment of small cell lung cancer in the elderly patients. In: Pass HI, Mitchell JB, Johnson DH, Turrisi AT, Minna JD, editors. *Lung cancer: principles and practice*. 2nd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 1081–91.
6. Sekine I, Fukuda H, Kunitoh H, Saijo N. Cancer chemotherapy in the elderly. *Jpn J Clin Oncol* 1998;28:463–73.
7. American Society of Clinical Oncology. Clinical practice guidelines for the treatment of unresectable non-small-cell lung cancer. *J Clin Oncol* 1997;15:2996–3018.
8. Nou E. Full chemotherapy in elderly patients with small cell bronchial carcinoma. *Acta Oncol* 1996;35:399–406.
9. Dajczman E, Fu LY, Small D, Wolkove N, Kreisman H. Treatment of small cell lung carcinoma in the elderly. *Cancer* 1996;77:2032–8.
10. Tebbutt NC, Snyder RD, Burns WI. An analysis of the outcomes of treatment of small cell lung cancer in the elderly. *Aust N Z J Med* 1997;27:160–4.
11. Jara C, Gomez-Aldaravi JL, Tirado R, et al. Small-cell lung cancer in the elderly – is age of patient a relevant factor? *Acta Oncol* 1999;38:781–6.
12. Oshita F, Kurata T, Kasai T, et al. Prospective evaluation of the feasibility of cisplatin-based chemotherapy for elderly lung cancer patients with normal organ functions. *Jpn J Cancer Res* 1995;86:1198–202.
13. Goodwin JS, Hunt WC, Humble CG, Key CR, Samet JM. Cancer treatment protocols. Who gets chosen? *Arch Intern Med* 1988;148:2258–60.

14. Paccagnella A, Favaretto A, Cipiani A, et al. Treatment of small cell lung cancer in elderly patients. *Proc Am Soc Clin Oncol* 1996;15:387 (abstract).
15. Siu LL, Shepherd FA, Murray N, et al. Influence of age on the treatment of limited-stage small-cell lung cancer. *J Clin Oncol* 1996;14:821–8.
16. Yuen AR, Zou G, Turrisi AT, et al. Similar outcome of elderly patients in intergroup trial 0096: cisplatin, etoposide, and thoracic radiotherapy administered once or twice daily in limited stage small cell lung carcinoma. *Cancer* 2000;89: 1953–60.
17. Girling DJ. Comparison of oral etoposide and standard intravenous multidrug chemotherapy for small-cell lung cancer: a stopped multicentre randomised trial. Medical Research Council Lung Cancer Working Party. *Lancet* 1996; 348:563–6.
18. Souhami RL, Spiro SG, Rudd RM, et al. Five-day oral etoposide treatment for advanced small-cell lung cancer: randomized comparison with intravenous chemotherapy. *J Natl Cancer Inst* 1997;89:577–80.
19. Medical Research Council Lung Cancer Working Party. Randomised trial of four-drug vs less intensive two-drug chemotherapy in the palliative treatment of patients with small-cell lung cancer (SCLC) and poor prognosis. Medical Research Council Lung Cancer Working Party. *Br J Cancer* 1996;73:406–13.
20. James LE, Gower NH, Rudd RM, et al. A randomised trial of low-dose/high-frequency chemotherapy as palliative treatment of poor-prognosis small-cell lung cancer: a Cancer Research Campaign trial. *Br J Cancer* 1996;73: 1563–8.
21. Earl HM, Rudd RM, Spiro SG, et al. A randomised trial of planned versus as required chemotherapy in small cell lung cancer: a Cancer Research Campaign trial. *Br J Cancer* 1991; 64:566–72.
22. Evans WK, Radwi A, Tomiak E, et al. Oral etoposide and carboplatin. Effective therapy for elderly patients with small cell lung cancer. *Am J Clin Oncol* 1995;18:149–55.
23. Matsui K, Masuda N, Fukuoka M, et al. Phase II trial of carboplatin plus oral etoposide for elderly patients with small-cell lung cancer. *Br J Cancer* 1998;77:1961–5.
24. Westeel V, Murray N, Gelmon K, et al. New combination of the old drugs for elderly patients with small-cell lung cancer: a phase II study of the PAVE regimen. *J Clin Oncol* 1998;16:1940–7.
25. Okamoto H, Watanabe K, Nishiwaki Y, et al. Phase II study of area under the plasma-concentration-versus-time curve-based carboplatin plus standard-dose intravenous etoposide in elderly patients with small-cell lung cancer. *J Clin Oncol* 1999;17:3540–5.
26. Samantas E, Skarlos DV, Pectasides D, et al. Combination chemotherapy with low doses of weekly carboplatin and oral etoposide in poor risk small cell lung cancer. *Lung Cancer* 1999;23:159–68.
27. Pignon T, Gregor A, Schaake Koning C, et al. Age has no impact on acute and late toxicity of curative thoracic radiotherapy. *Radiother Oncol* 1998;46: 239–48.
28. Westeel V, Murray N, Gelmon K, et al. New combination of the old drugs for elderly patients with small-cell lung cancer: a phase II study of the PAVE regimen. *J Clin Oncol* 1998;16:1940–7.
29. Murray N, Grafton C, Shah A, et al. Abbreviated treatment for elderly, infirm, or noncompliant patients with limited-stage small-cell lung cancer. *J Clin Oncol* 1998;16:3323–8.
30. Jeremic B, Shibamoto Y, Acimovic L, Milisavljevic S. Carboplatin, etoposide, and accelerated hyperfractionated radiotherapy for elderly patients with limited small cell lung carcinoma: a phase II study. *Cancer* 1998;82:836–41.
31. Noda K, Nishiwaki Y, Kawahara M, et al. Japan Clinical Oncology Group. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 2002;346:85–91.
32. Yana T, Negoro S, Yokota M, Fukkuoka M. Phase II study of amrubicin (SM-5887), a 9-amino-anthracycline, in previously untreated patients with extensive stage small-cell lung cancer: a West Japan Lung Cancer Group Trial. *Proc Am Soc Clin Oncol* 1998;17:450a.
33. Pignon JP, Arriagada R, Ihde DC, et al. A meta-analysis of thoracic radiotherapy for small-cell lung cancer. *N Engl J Med* 1992;327:1618–24.
34. Applegate WB, Blass JP, Williams TF. Instruments for the functional assessment of older patients. *N Engl J Med* 1990; 322:1207–14.
35. Stuch AE, Siu AL, Wieland GD, Rubenstein LZ. Comprehensive geriatric assessment: a meta-analysis of controlled trials. *Lancet* 1993;342:1032–6.

*MUTATION IN BRIEF*

# Haplotypes of *CYP3A4* and Their Close Linkage With *CYP3A5* Haplotypes in a Japanese Population

**Hiromi Fukushima-Uesaka[1], Yoshiro Saito[1]*, Hidemi Watanabe[1], Kisho Shiseki[1], Mayumi Saeki[1], Takahiro Nakamura[1], Kouichi Kurose[1], Kimie Sai[1], Kazuo Komamura[2,3], Kazuyuki Ueno[4], Shiro Kamakura[2], Masafumi Kitakaze[2], Sotaro Hanai[3], Toshiharu Nakajima[5], Kenji Matsumoto[5], Hirohisa Saito[5], Yu-ichi Goto[6], Hideo Kimura[6], Masaaki Katoh[7], Kenji Sugai[7], Narihiro Minami[6,7], Kuniaki Shirao[8], Tomohide Tamura[8], Noboru Yamamoto[8], Hironobu Minami[9], Atsushi Ohtsu[10], Teruhiko Yoshida[11], Nagahiro Saijo[8], Yutaka Kitamura[12,13], Naoyuki Kamatani[12], Shogo Ozawa[1], and Jun-ichi Sawada[1]**

*[1]Project Team for Pharmacogenetics, National Institute of Health Sciences, Tokyo, Japan; [2]Division of Cardiology, [3]Research Institute, [4]Department of Pharmacy, National Cardiovascular Center, Osaka, Japan; [5]Department of Allergy and Immunology, National Research Institute for Child Health and Development, National Center for Child Health and Development, Tokyo, Japan; [6]National Institute of Neuroscience, [7]National Center Hospital for Mental, Nervous and Muscular Disorders, National Center of Neurology and Psychiatry, Tokyo, Japan; [8]Division of Internal Medicine, National Cancer Center Hospital, [11]Genetics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan; [9]Division of Oncology/Hematology, [10]Division of GI Oncology/Digestive Endoscopy, National Cancer Center Hospital East, Chiba, Japan; [12]Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan; [13]Mitsubishi Research Institute, Inc., Tokyo, Japan*

*Correspondence to: Yoshiro Saito, Project Team for Pharmacogenetics, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan; Tel.: +81-3-3700-9453; Fax: +81-3-5717-3832; E-mail: yoshiro@nihs.go.jp

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**In order to identify single nucleotide polymorphisms (SNPs) and haplotype frequencies of *CYP3A4* in a Japanese population, the distal enhancer and proximal promoter regions, all exons, and the surrounding introns were sequenced from genomic DNA of 416 Japanese subjects. We found 24 SNPs, including 17 novel ones: two in the distal enhancer, four in the proximal promoter, one in the 5'-untranslated region (UTR), seven in the introns, and three in the 3'-UTR. The most common SNP was c.1026+12G>A (IVS10+12G>A), with a 0.249 frequency. Four non-synonymous SNPs, c.554C>G (p.T185S, *CYP3A4*16*), c.830_831insA (p.E277fsX8, **6*), c.878T>C (p.L293P, **18*), and c.1088 C>T (p.T363M, **11*) were found with frequencies of 0.014, 0.001, 0.028, and 0.002, respectively. No SNP was found in the known nuclear transcriptional factor-binding sites in the enhancer and promoter regions. Using these 24 SNPs, 16 haplotypes were unambiguously identified, and nine haplotypes were inferred by aid of an expectation-maximization-based program. In addition, using data**

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**from 186 subjects enabled a close linkage to be found between *CYP3A4* and *CYP3A5* SNPs, especially among the SNPs at c.1026+12 in *CYP3A4* and c.219-237 (IVS3-237, a key SNP site for *CYP3A5*3*), c.865+77 (IVS9+77) and c.1523 in *CYP3A5*. This result suggested that *CYP3A4* and *CYP3A5* are within the same gene block. Haplotype analysis between *CYP3A4* and *CYP3A5* revealed several major haplotype combinations in the *CYP3A4-CYP3A5* block. Our findings provide fundamental and useful information for genotyping *CYP3A4* (and *CYP3A5*) in the Japanese, and probably Asian populations.** © 2003 Wiley-Liss, Inc.

KEY WORDS: CYP3A4; CYP3A5; SNP; haplotype; Japanese

## INTRODUCTION

The human cytochrome P450 (CYP) 3A subfamily has been estimated to be involved in the metabolism of half of the prescription drugs (Wrighton et al., 1996; Thummel and Wilkinson, 1998; Guengerich, 1999). The *CYP3A43* (MIM# 606534), *CYP3A4* (MIM# 124010), *CYP3A7* (MIM# 605340), and *CYP3A5* (MIM# 605325) genes consist of a cluster spanning 231 kb on chromosome 7 (Finta and Zaphiropoulos, 2000; Gellner et al., 2001). Among the family members, CYP3A4 is a predominant form in the adult human liver. CYP3A4 induction is mediated by pregnane/steroid X receptor (PXR), constitutive androstane receptor (CAR) and the vitamin D receptor by its binding to the distal xenobiotic-responsive enhancer module (XREM), especially to the distal nuclear receptor-binding element-1 (dNR1, imperfect DR-3 motif, -7733 to -7719 from the transcriptional start site) and dNR3 (imperfect DR-3 motif, -7290 to -7270 from the transcriptional start site), and to the proximal promoter region, especially to the proximal PXR response element (prPXRE, ER-6 motif, -169 to -152 from the transcriptional start site) (Goodwin et al., 1999, 2002; Drocourt et al., 2002). Recently, it has been reported that hepatic nuclear factor-4a (HNF-4a) also increases the activity of basal and a PXR/CAR-mediated reporter gene with the *CYP3A4* enhancer/promoter by its binding to the region immediately upstream of the dNR1 site in XREM (-7785 to -7772 from the transcriptional start site) (Tirona et al., 2003).

Up to 40-fold interindividual variations in CYP3A4 expression levels have been observed in the human liver. Furthermore, there is a 10-fold variation in the metabolism of CYP3A4 substrates *in vivo* (Thummel and Wilkinson, 1998; Guengerich, 1999). These interindividual differences are likely to be associated with efficacy and adverse effects of drugs. Thus, it is clinically important to predict CYP3A4 activity in the liver or other tissues, such as the intestine.

It has been suggested that approximately 85% of the interindividual variability in hepatic CYP3A4 activity is due to genetic factors (Ozdemir et al., 2000). Thus, several researches have focused on the identification of *CYP3A4* genetic variants (Lamba et al., 2002a). To date, 25 *CYP3A4* alleles (haplotypes), including 6 subtypes, have been publicized on the Human Cytochrome P450 Allele Nomenclature Committee homepage (www.imm.ki.se/CYPalleles). As for Caucasian populations, sequence-based genotyping was performed for 213 (Eiselt et al., 2001) and 53 (Lamba et al., 2002b) DNA samples. With Asian populations, however, only a PCR-SSCP analysis was performed with DNA samples from 102 Chinese subjects. In this report, 3 polymorphisms in the exons, including a frame-shift variant (*CYP3A4*6*), were identified by subsequent sequencing of variant samples found by PCR-SSCP (Hsieh et al., 2001). In other reports, only a small number of samples (24 or 30) were sequenced (Dai et al., 2001; Lamba et al., 2002b). Thus, there has been no comprehensive sequence analysis of *CYP3A4* for Asian populations, including the Japanese. Furthermore, there has been no report on *CYP3A4* haplotype analysis for any population. Increasingly, association studies have shown that haplotypes, linked combinations of SNPs, have the advantage of giving more precise detection of the phenotype-genotype link than do the individual SNPs (Judson et al., 2000). Therefore, in order to identify SNPs and haplotypes in the Japanese, we sequenced the distal enhancer region (-7989 to -7114 from the translational start codon, corresponding to -7886 to -7011 from the transcriptional start site), the proximal promoter region (up to 913 basepairs upstream of the translational start codon, corresponding to up to -810 from the transcriptional start site), all the exons, and the surrounding intronic regions of *CYP3A4* for 416 Japanese individuals. Then, linkage disequilibrium analysis was performed for the *CYP3A4* and *CYP3A5* genes together, using the data from 186 identical subjects described in the previous report (Saeki et al., 2003). Strong linkage was found between the SNPs in these two genes. Therefore, we further inferred haplotype combinations of the region covering *CYP3A4* and *CYP3A5*.

## METHODS

### Human genomic DNA samples

All 416 Japanese subjects were either patients with arrhythmia who were administered anti-arrhythmic drugs, cancer patients who were administered irinotecan or paclitaxel, patients with epilepsy who were administered anti-epileptic drugs, or patients with allergic diseases (atopic dermatitis and/or asthma) who were administered steroidal drugs. Genomic DNA was extracted directly from blood leukocytes (343 samples) or from lymphocytes immortalized with the Epstein-Barr virus (73 samples). This study was approved by the ethical review boards of the National Cardiovascular Center, the National Cancer Center, the National Center of Neurology and Psychiatry, the National Center for Child Health and Development, and the National Institute of Health Sciences. Written informed consent was obtained from all subjects.

### Polymerase chain reaction (PCR) conditions and DNA sequencing

First, the entire *CYP3A4* gene (GenBank Accession # AF280107.1) was amplified in 3 amplicons: the distal enhancer region to exon 2, the proximal promoter region to exon 7, and exons 5 to 13. The primer sequences can be obtained by a request to the corresponding author. Genomic DNA (150 ng) was amplified using 1.25 units of *Z*-Taq (Takara Shuzo, Tokyo, Japan) with 0.2 μM primers. The first-round PCR was 30 cycles of 5 sec at 98°C, 5 sec at 55°C, and 190 sec at 72°C. Next, the promoter region and each exon were amplified by *Ex*-Taq (0.625 units) (Takara Shuzo) with the appropriate sets of *CYP3A4*-specific primers (0.5 μM). Second-round PCR consisted of 5 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 1 min at 55°C, and 2 min at 72°C, and then a final extension for 5 min at 72°C. The PCR products were treated with the PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH) and directly sequenced on both strands using the ABI BigDye Terminator Cycle Sequencing Kit (version 3, Applied Biosystems, Foster City, CA). The primers for the second round PCR were also used for sequencing, except for the distal enhancer region and exons 1, 2, 4, 11, and 12. Excess dye was removed with a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 or 3730 DNA Analyzer (Applied Biosystems). Conditions for PCR and *CYP3A5* (GenBank Accession # NG_000004.2) sequencing were described previously (Saeki et al., 2003). The amplification and sequencing of *CYP3A7* (GenBank Accession # AF280107.1) promoter region were performed according to Kuehl et al. (2001). All the SNPs were confirmed by repeating the PCR on genomic DNA and sequencing the newly generated PCR products.

### Haplotype analysis

Some of the haplotypes were unambiguous from subjects with homozygous SNPs at all sites or a heterozygous SNP at only one site, and will be publicized on the Human Cytochrome P450 Allele Nomenclature Committee homepage. Separately, the diplotype configurations (a combination of haplotypes) were inferred by LDSUPPORT software, which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies (Kitamura et al., 2002). Linkage analysis was performed by SNPAlyze software (Dynacom Co., Yokohama, Japan). Nucleotide diversity ($\pi$) was calculated using DnaSP software (Rozas and Rozas, 1999).

## RESULTS

The distal enhancer region, proximal promoter region, all exons, and surrounding intronic regions of *CYP3A4* for 416 Japanese subjects were sequenced. Twenty-four SNPs, including 17 novel ones [2 were in the distal enhancer region, 4 in the proximal promoter region, 1 in the 5'-untranslated region (UTR), 7 in the introns and 3 in the 3'-UTR] were detected (see Table 1). All of the allelic frequencies were in the Hardy-Weinberg equilibrium ($p$>0.387 or higher). Since we did not find apparent differences in SNP frequencies among the subjects with the different disease types (data not shown), the data for all subjects were analyzed as one group. The most common SNP was c.1026+12G>A (IVS10+12G>A) with a 0.249 frequency.

Table 1 Diplotype configulation of *CYP3A4*

| Exon/Intron | | Enhancer | Enhancer | Promoter | Promoter | Promoter | Promoter | 5'-UTR | Intron 1 | Intron 3 | Intron 5 | Intron 6 | Exon 7 | Intron 7 | Intron 7 | Intron 7 | Exon 9 | Intron 9 | Intron 9 | Exon 10 | Intron 10 | Exon 11 | 3'-UTR | 3'-UTR | 3'-UTR | Number of subjects | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position on AF280107.1 | | g.54133 | g.54436 | g.61371 | g.61382 | g.61407 | g.61981 | g.61971 | g.65993 | g.68113 | g.76236 | g.77509 | g.77639 | g.77763 | g.77845 | g.78311 | g.79697_79698 | g.79754 | g.79851_79852 | g.82106 | g.82266 | g.83903 | g.88049 | g.88649 | g.88242 | | |
| cDNA change[1] | | c.-7904 C>A | c.-7601 delA | c.-666 G>A | c.-655 A>G | c.-630 A>G | c.-156 C>A | c.-66 C>G | c.72-41 delA | c.218+22 A>G | c.433-17 T>G | c.522-18 A>C | c.554 C>G | c.670+8 G>A | c.670+90 T>C | c.671-40 A>G | c.830_831 insA | c.865+22 G>A | c.865+119_120 delAT | c.878 T>C | c.1028+12 G>A | c.1088 C>T | c.1505 T>G | c.1505 T>C | c.1778 C>A | | |
| Amino acid change | | | | | | | | | | | | | p.T185S | | | | p.E277fsX8 | | | p.I293P | | p.T363M | | | | | |
| Reference | | Novel | Novel | Novel | Novel | Novel | Novel | Novel | Novel | Novel | Dai et al. (2001) | Novel | *16 | Novel | Novel | Novel | *6 | Novel | Dai et al. (2001) | *18 | Dai et al. (2001) | *11 | Novel | Novel | Novel | | |
| Diplotype[2] | *1A/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 226 | 381 |
| | *1G/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 107 | |
| | *1K/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 3 | |
| | *1H/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1J/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1M/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1N/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1P/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1Q/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1R/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1S/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1T/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1H/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 5 | |
| | *1n/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1c/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1w/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1V/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1y/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1z/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1ea/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *1G/*1G | | | | | | | | | | | | | | | | | | | | [redacted] | | | | | 21 | |
| | *1H/*1G | | | | | | | | | | | | | | | | | | | | | | | | | 3 | |
| | *1K/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 38 (amino acid change) |
| | *1Bc/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| | *16B/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 9 | |
| | *1BB/*1A | | | | | | | | | | | | | | | | | | | | | | | | | 17 | |
| | *16B/*1G | | | | | | | | | | | | | | | | | | | | [redacted] | | | | | 1 | |
| | *1Bc/*1G | | | | | | | | | | | | | | | | | | | | [redacted] | | | | | 1 | |
| | *1BB/*1G | | | | | | | | | | | | | | | | | | | | [redacted] | | | | | 3 | |
| | *16B/*1BB | | | | | | | | | | | | | | | | | | | | [redacted] | | | | | 1 | |
| | *1BB/*1BB | | | | | | | | | | | | | | | | | | | [redacted] | [redacted] | | | | | 1 | |
| SNP frequency | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.004 | 0.001 | 0.001 | 0.014 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.028 | 0.249 | 0.002 | 0.001 | 0.001 | 0.010 | 416 | |

| | | Frequency |
|---|---|---|
| Haplotype[2] | *1A | 0.734 |
| | *1G | 0.189 |
| | *1H | 0.010 |
| | *1J | 0.005 |
| | *1K | 0.001 |
| | *1L | 0.001 |
| | *1M | 0.001 |
| | *1N | 0.001 |
| | *1P | 0.001 |
| | *1Q | 0.001 |
| | *1R | 0.001 |
| | *1S | 0.001 |
| | *1T | 0.001 |
| | *1n | 0.001 |
| | *1c | 0.001 |
| | *1w | 0.001 |
| | *1v | 0.001 |
| | *1y | 0.001 |
| | *1z | 0.001 |
| | *1ea | 0.001 |
| | *6 | 0.001 |
| | *1Bc | 0.001 |
| | *16B | 0.014 |
| | *1BB | 0.028 |

[1] Position on the cDNA sequence (A of the translational start codon is 1, based on GenBank Accession # AF280107.1) or position from the end of the nearest exon.

[2] White cell, wild-type; gray cell, heterozygous alteration; black cell, homozygous alteration. The unambiguously assigend haplotypes approved by Human Cytochrome P450 Allele Nomenclature Committee are described by using capital alphabetical letters.

**Table 2: Haplotype Combinations of CYP3A4 and CYP3A5**

| Haplotype combination (CYP3A4-CYP3A5) | Frequency |
|---|---|
| *1A-*3A | .696 |
| *1G-*1E | .120 |
| *18B-*1E | .027 |
| *1A-*3C | .022 |
| *1A-*3F# | .022 |
| *1G-*3A | .019 |
| *1A-*1E | .019 |
| *16B-*1E | .016 |
| *1G-*1f | .014 |
| *1G-*1g | .005 |
| *1H-*1g | .005 |
| *11c-*1E | .003 |
| *1A-*1h | .003 |
| *1A-*3H# | .003 |
| *1A-*3i | .003 |
| *1A-*3J# | .003 |
| *1aa-*1E | .003 |
| *1G-*3C | .003 |
| *1G-*3G# | .003 |
| *1H-*1E | .003 |
| *1M-*3F# | .003 |
| *1S-*3C | .003 |
| *1v-*1i | .003 |
| *1w-*3A | .003 |

#The haplotypes *CYP3A5*3F*, **3G*, **3H* and **3J*, published in the Human Cytochrome P450 Allele Nomenclature Committee homepage, were formally described as **3d*, **3e*, **3f* and **3h* in our previous paper (Saeki et al., 2003).

As for the SNPs identified in the exons, 4 reported non-synonymous SNPs were detected: c.554C>G (p.T185S, *CYP3A4*16*), c.830_831insA (p.E277fsX8, resulting in an early stop codon TGA at 285, **6*), c.878T>C (p.L293P, **18*), and c.1088 C>T (p.T363M, **11*) with frequencies of 0.014, 0.001, 0.028, and 0.002, respectively (Table 1). *CYP3A4*16* or **18* was always detected together with the SNP, c.1026+12G>A.

Two novel SNPs were found in the distal enhancer regions. The positions are 16 bases upstream of the HNF-4a binding motif and 221 bases downstream of dNR-1. Also, four novel SNPs were detected in the proximal promoter regions, but these were at least 100 bases from prPXRE. The functional influence of the non-coding SNPs, located in the 5'-UTR, introns, and 3'-UTR, is currently unknown. The calculated nucleotide diversity ($\pi$) using all samples was 0.00008.

Using the detected SNPs in *CYP3A4*, haplotype analysis was then performed. Some haplotypes were first unambiguously assigned by homozygous SNPs at all sites (**1G* and **18B*) or a heterozygous SNP at only one site (**1H-*1T* and **16B*). They are described in capital alphabetical letters in Table 1 (These haplotypes will be publicized on the Human Cytochrome P450 Allele Nomenclature Committee homepage). Separately, we estimated the diplotype configuration (a combination of haplotypes) for each subject by LDSUPPORT software. The diplotype configurations of all the subjects had a probability (certainty) of >99.99%. The additionally inferred haplotypes were seven **1* subtypes (**1u-*1aa*) and two **11* subtypes (**11b and *11c*) (Table 1). The most frequent haplotype was **1A* (frequency: 0.734), followed by **1G* (0.189), **18B* (0.028), **16B* (0.014), and **1H* (0.010). The frequencies of the other haplotypes were less than 0.01.

In addition to *CYP3A4* haplotypes, we previously identified *CYP3A5* haplotypes, another *CYP3A* family gene with a polymorphic expression pattern (Saeki et al., 2003). Using the data from 186 subjects (also included in this study), linkage disequilibrium analysis was performed with the SNPs of *CYP3A4* and *CYP3A5* simultaneously.

The most frequent SNP in *CYP3A4*, c.1026+12G>A, showed a strong linkage with c.219-237A>G (IVS3-237A>G) inversely (namely G>A; *CYP3A5*3*, Kuehl et al., 2001) [$\rho^2$ = 0.722 and Chi$^2$ = 271.0 ($p$<0.0001)], c.865+77G>T [$\rho^2$ = 0.638 and Chi$^2$ = 241.0 ($p$<0.0001)] and c.1523C>T inversely [$\rho^2$ = 0.606 and Chi$^2$ = 221.3 ($p$<0.0001)] in *CYP3A5*. In other words, c.1026+12G (wild type) in *CYP3A4* is linked to c.219-237G and c.1523T (**3A*) in *CYP3A5*. These three SNPs in *CYP3A5* also showed a weak linkage with the SNP at c.554 (**16*) or c.878 (**18*) in *CYP3A4*. These results suggested that *CYP3A4* and *CYP3A5* are within the same gene block.

Then, we further inferred combinations of *CYP3A4* and *CYP3A5* haplotypes utilizing LDSUPPORT software (Table 2). Using the data with the probability (certainty) over 0.98 from 184 subjects, we calculated the frequencies of haplotype combinations in the *CYP3A4-3A5* block (Table 2). The most frequent combination (*CYP3A4-CYP3A5*) was **1A-*3A* (frequency: 0.696), followed by **1G-*1E* (0.120), **18B-*1E* (0.027), **1A-*3C* (0.022), and **1A-*3F* (0.022). The frequencies of the other haplotypes were less than 0.02.

CYP3A7 is known as a fetal form of CYP3A, but has been reported to be expressed in 14 out of 15 Japanese adult subjects (Tateishi et al., 1999). We also searched for *CYP3A7*1B and *1C*, which were shown to be the main polymorphisms responsible for *CYP3A7* expression in Caucasian adult livers (Burk et al., 2002), but could not detect these polymorphisms in 268 samples (data not shown). Instead, we detected an SNP c.–425G>C (A of the translational start site for *CYP3A7* is numbered +1, rs3823647 in the dbSNP database) in this area with a 0.011 frequency, and found a perfect linkage between this SNP and *CYP3A5*1f* SNPs (c.166-102C>T and c.1253+177C>T) [$\rho^2$ = 1.00 and Chi$^2$ = 340 ($p$<0.0001)] using data from 170 samples (Saeki et al., 2003).

## DISCUSSION

Here, we report the screening of *CYP3A4* SNPs in a Japanese population. Overall, we detected 17 novel and 7 known SNPs, including four non-synonymous ones (Table 1). *CYP3A4*11* (p.T363M) and **16* (p.T185S) have reduced *in vitro* catalytic activities against testosterone with lowered protein expression levels (Eiselt et al., 2001; Murayama et al., 2002). *CYP3A4*6* (p.E277fsX8) was found in a patient with a lowered urinary 6ß-hydroxycortisol to free cortisol ratio, suggesting decreased CYP3A4 activity (Hsieh et al., 2001). On the other hand, *CYP3A4*18* (p.L293P) induced unchanged or rather increased activity to testosterone and chlorpyrifos *in vitro* (Dai et al., 2001; Murayama et al., 2002). The subjects with these non-synonymous SNPs (total frequency: 0.045) may have an altered CYP3A4 activity.

Large ethnic differences in *CYP3A4* SNPs have been reported, such as *CYP3A4*1B* (c.-392A>G), which has been detected in 9% of Caucasians, 53% of Africans, and no Asians (Walker et al., 1998). As for SNPs located in the exons, p.M445T (**3*) and p.F189S (**17*) were only found in Caucasians, p.R162Q (**15*) was in Africans, and p.D174H (**10*) were in both Caucasians and Africans (Dai et al., 2001; Lamba et al., 2002b). Of the SNPs detected in this study, **6* was previously found in the Chinese, **16* in Mexicans and the Japanese, and **18* in the Chinese (Hsieh et al., 2001; Dai et al., 2001; Lamba et al., 2002b). These ethnic differences of the SNPs also imply haplotype differences. We found 25 haplotypes, including 16 unambiguous ones. However, no detailed haplotypes have been reported in other ethnic populations. Comparable studies should be done in the future.

In the following order, *CYP3A43*, *CYP3A4*, *CYP3A7*, and *CYP3A5*, are in a gene cluster spanning 231 kb on chromosome 7. This study also showed that a close linkage between *CYP3A4* and *CYP3A5* SNPs, especially among the SNPs at c.1026+12 in *CYP3A4*, and c.219-237 (a key SNP site for *CYP3A5*3*), c.865+77 and c.1523 in *CYP3A5*. c.1026+12 in *CYP3A4* is approximately 91 kb from c.219-237 in *CYP3A5* and 116 kb from c.1523 in *CYP3A5*. c.219-237A>G in *CYP3A5* induces aberrant splicing, resulting in defective activity (Kuehl et al., 2001).

Since CYP3A4 and CYP3A5 largely metabolize the same substrates, it is worth analyzing the haplotype combinations (Table 2). Major combinations (*CYP3A4-3A5*) were **1A-*3A* and **1G-*1E*. Our previous study showed that *CYP3A5*3* was the predominant defective allele in a Japanese population (Saeki et al., 2003). According to the obtained haplotype combinations, the *CYP3A4* haplotypes containing the c.1026+12G allele (such as **1A*) are linked to *CYP3A5*3* with a 97% probability. Inversely, 88% of the *CYP3A4* haplotypes with c.1026+12A (such as **1G*) are linked to *CYP3A5*1*. Thus, these results suggested that genotyping at the IVS10+12 position in *CYP3A4* can predict if the subject has *CYP3A5*3* in a Japanese population. In addition, the activity-decreasing haplotype *CYP3A4*16B* perfectly linked with *CYP3A5*1E*, but not **3*, suggesting that the resulting expression of CYP3A5 can compensate for decreased CYP3A4 activity. Recently, the importance of

haplotype analysis has been shown in phenotype-genotype association studies as well as candidate gene discovery (Judson et al., 2000). Our data also demonstrate the usefulness of haplotype analysis for the prediction of total CYP3A activity. The haplotype combination analysis should include *CYP3A7* haplotypes in the future.

In conclusion, we assigned *CYP3A4* haplotypes and showed its close linkage with *CYP3A5* haplotypes. The assigned haplotypes provide fundamental and useful information for genotyping *CYP3A4* and *CYP3A5* in the Japanese, and probably the Asian populations.

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## REFERENCES

Burk O, Tegude H, Koch I, Hustert E, Wolbold R, Glaeser H, Klein K, Fromm MF, Nuessler AK, Neuhaus P, Zanger UM, Eichelbaum M, Wojnowski L. 2002. Molecular mechanisms of polymorphic *CYP3A7* expression in adult human liver and intestine. J Biol Chem 277:24280-24288.

Dai D, Tang J, Rose R, Hodgson E, Bienstock RL, Mohrenweiser HW, Goldstein JA. 2001. Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. J Pharmacol Exp Ther 299:825-831.

Drocourt L, Ourlin JC, Pascussi JM, Maurel P, Vilarem MJ. 2002. Expression of *CYP3A4*, *CYP2B6*, and *CYP2C9* is regulated by the vitamin D receptor pathway in primary human hepatocytes. J Biol Chem 277:25125-25132.

Eiselt R, Domanski TL, Zibat A, Mueller R, Presecan-Siedel E, Hustert E, Zanger UM, Brockmoller J, Klenk H, Meyer UA, Khan KK, He Y, Halpert JR, Wojnowski L. 2001. Identification and functional characterization of eight CYP3A4 protein variants. Pharmacogenetics 11:447-458.

Finta C, Zaphiropoulos PG. 2000. The human cytochrome P450 3A locus. Gene evolution by capture of downstream exons. Gene 260:13-23.

Gellner K, Eiselt R, Hustert E, Arnold H, Koch I, Haberl M, Deglmann CJ, Burk O, Buntefuss D, Escher S, Bishop C, Koebe HG, Brinkmann U, Klenk HP, Kleine K, Meyer UA, Wojnowski L. 2001. Genomic organization of the human *CYP3A* locus: identification of a new, inducible *CYP3A* gene. Pharmacogenetics 11:111-121.

Goodwin B, Hodgson E, Liddle C. 1999. The orphan human pregnane X receptor mediates the transcriptional activation of *CYP3A4* by rifampicin through a distal enhancer module. Mol Pharmacol 56:1329-1339.

Goodwin B, Hodgson E, D'Costa DJ, Robertson GR, Liddle C. 2002. Transcriptional regulation of the human *CYP3A4* gene by the constitutive androstane receptor. Mol Pharmacol 62:359-365.

Guengerich FP. 1999 Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol 39:1-17.

Hsieh KP, Lin YY, Cheng CL, Lai ML, Lin MS, Siest JP, Huang JD. 2001. Novel mutations of CYP3A4 in Chinese. Drug Metab Dispos 29:268-273.

Judson R, Stephens JC, Windemuth A. 2000. The predictive power of haplotypes in clinical response. Pharmacogenomics, 1: 15-26.

Kitamura Y, Moriguchi M, Kaneko H, Morisaki H, Morisaki T, Toyama K, Kamatani N. 2002. Determination of probability distribution of diplotype configulation (diplotype distribution) for each subject from genotypic data using the EM algorithm. Ann Hum Genet 66:183-193.

Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. 2001. Sequence diversity in *CYP3A* promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27:383-391.

Lamba JK, Lin YS, Schuetz EG, Thummel KE. 2002a Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 54:1271-1294.

Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, Schuetz EG. 2002b. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. Pharmacogenetics 12:121-132.

Murayama N, Nakamura T, Saeki M, Soyama A, Saito Y, Sai K, Ishida S, Nakajima O, Itoda M, Ohno Y, Ozawa S, Sawada J. 2002. *CYP3A4* gene polymorphisms influence testosterone 6a-hydroxylation. Drug Metabol Pharmacokin 17:150-156.

Ozdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, Kashuba AD. 2000. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics 10:373-388.

Rozas J, Rozas R. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174-175.

Saeki M, Saito Y, Nakamura T, Murayama N, Kim SR, Ozawa S, Komamura K, Ueno K, Kamakura S, Nakajima T, Saito H, Kitamura Y, Kamatani N, Sawada J. 2003. Single nucleotide polymorphisms (SNPs) and haplotype frequencies of *CYP3A5* in a Japanese population. Hum Mut 21:653 (Mutation in Brief #618).

Tateishi T, Watanabe M, Moriya H, Yamaguchi S, Sato T, Kobayashi S. 1999. No ethnic difference between Caucasian and Japanese hepatic samples in the expression frequency of CYP3A5 and CYP3A7 proteins. Biochem Pharmacol. 57:935-939.

Tirona RG, Lee W, Leake BF, Lan LB, Cline CB, Lamba V, Parviz F, Duncan SA, Inoue Y, Gonzalez FJ, Schuetz EG, Kim RB 2003. The orphan nuclear receptor HNF4a determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. Nat Med 9:220-224.

Thummel KE, Wilkinson GR. 1998. In vitro and in vivo drug interactions involving human CYP3A. Annu Rev Pharmacol Toxicol 38:389-430.

Walker AH, Jaffe JM, Gunasegaram S, Cummings SA, Huang CS, Chern HD, Olopade OI, Weber BL, Rebbeck TR. 1998, Characterization of an allelic variant in the nifedipine-specific element of CYP3A4: ethnic distribution and implications for prostate cancer risk. Hum Mutat 12:289 (Mutation in Brief #191).

Wrighton SA, VandenBranden M, Ring BJ. 1996. The human drug metabolizing cytochromes P450. J Pharmacokinet Biopharm 24:461-473.

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**Short Communication**

# Phase I study of cisplatin analogue nedaplatin (254-S) and paclitaxel in patients with unresectable squamous cell carcinoma

**I Sekine*[,1], H Nokihara[1], A Horiike[1], N Yamamoto[1], H Kunitoh[1], Y Ohe[1], T Tamura[1], T Kodama[1] and N Saijo[1]**

[1]*Divisions of Internal Medicine and Thoracic Oncology, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan*

The recommended phase II dose of paclitaxel 180 mg m$^{-2}$ given as a 3-h infusion followed by nedaplatin 100 mg m$^{-2}$ in a 1-h infusion every 3–4 weeks was determined in 52 chemo-naive patients with unresectable squamous cell carcinoma (SCC), with a promising response rate for lung SCC of 55%.
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Squamous cell carcinoma (SCC) arises from the epithelial tissue of many different organs. Although localised diseases can be treated using surgical resection or curative radiotherapy, advanced SCC continues to have a poor prognosis and the standard treatment has not been established (DeVita *et al*, 2001). Cisplatin-based chemotherapy has been used for the treatment of advanced SCC, regardless of the site of tumour origin (DeVita *et al*, 2001).

Nedaplatin (cis-diammine-glycolate-*O,O'*-platinum II, 254-S) is a second-generation platinum derivative that has an antitumour activity comparable to that of cisplatin (Kobayashi *et al*, 1991) but is less toxic to the kidney (Kameyama *et al*, 1990), as seen in preclinical experiments. Nedaplatin produced promising response rates in phase II trials for the treatment of SCC arising from the head and neck (Inuyama *et al*, 1992), lung (Yamamoto *et al*, 2000), oesophagus (Taguchi *et al*, 1992), and uterine cervix (Noda *et al*, 1992). Paclitaxel is another promising drug for the treatment of advanced SCC, as shown by the favourable response rates obtained in phase II trials for head and neck (Forastiere *et al*, 1998), non-small-cell lung (Sekine *et al*, 1996), oesophageal (Ajani *et al*, 1994), and cervical (McGuire *et al*, 1996) cancers.

A combination of nedaplatin and paclitaxel is a promising chemotherapeutic regimen because a significant synergistic effect was obtained for this combination in a preclinical mice tumour model (Yamada *et al*, 2001), and the combination of platinum compounds and paclitaxel is one of many standard regimens (Schiller *et al*, 2002). The objectives of this phase I trial were (1) to evaluate the toxicity of the regimen and to determine the maximum tolerated dose (MTD) and recommended phase II dose (RPTD) of nedaplatin and paclitaxel, and (2) to observe the antitumour effects of this regimen on SCC arising in various organs.

## PATIENTS AND METHODS

### Patient selection

The eligibility criteria for enrolment in the trial were as follows: histologically or cytologically proven SCC; unresectable disease; measurable disease; no previous chemotherapy; age between 20 and 75 years; performance status of 0 or 1 (Oken *et al*, 1982); adequate bone marrow function (white blood cell (WBC) count $\geqslant 4.0 \times 10^9 l^{-1}$, neutrophil count $\geqslant 2.0 \times 10^9 l^{-1}$, haemoglobin $\geqslant 10.0$ g dl$^{-1}$ and platelet count $\geqslant 100 \times 10^9 l^{-1}$), liver function (total bilirubin $\leqslant 1.5$ mg dl$^{-1}$ and transaminase $\leqslant 100$ IU l$^{-1}$), and renal function (serum creatinine $\leqslant 1.5$ mg dl$^{-1}$ and creatinine clearance $\geqslant 60$ ml min$^{-1}$); and a $PaO_2$ $\geqslant 60$ Torr. Patients were excluded from the trial for any of the following reasons: uncontrolled malignant pleural or pericardial effusion; a concomitant serious illness contraindicating chemotherapy; pregnancy; or breast-feeding. All patients gave their written informed consent.

### Treatment schedule

The levels and respective doses of paclitaxel (mg m$^{-2}$) and nedaplatin (mg m$^{-2}$) are shown in Table 1. Paclitaxel diluted in 500 ml of 5% glucose was administered as a 3-h intravenous infusion with premedication as previously described (Sekine *et al*, 1996). Normal saline (500 ml) and granisetron (40 $\mu$g kg$^{-1}$) in 100 ml of normal saline were given intravenously, followed by nedaplatin diluted in 250 ml of normal saline administered in a 1-h intravenous infusion. This treatment was repeated every 3–4 weeks.

### Toxicity assessment and treatment modification

Complete blood cell counts and differential counts, routine chemistry determinations, and a chest X-ray were performed at least once a week throughout the course of treatment. If grade 4 neutropenia was noted, the neutrophil count was repeated 4 days later to determine whether the grade 4 neutropenia had lasted for 5 days or longer. Acute toxicity was graded according to the NCI Common Toxicity Criteria, version 2.0, issued in 1998 (JCOG, 1998). Subsequent cycles of chemotherapy were delayed if any of the following toxicities were noted on day 1: WBC count $\leqslant 3.0 \times 10^9 l^{-1}$, neutrophil count $\leqslant 1.5 \times 10^9 l^{-1}$, platelet count $\leqslant 100 \times 10^9 l^{-1}$, serum creatinine level $\geqslant 1.6$ mg dl$^{-1}$, grade 2 elevated hepatic transaminase level or total serum bilirubin, fever $\geqslant 38$°C, or a performance status $\geqslant 2$.

*Correspondence: Dr I Sekine; E-mail: isekine@ncc.go.jp
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**Table 1** Dose level and number of patients accrued

| Level | Paclitaxel ($mg\,m^{-2}$) | Nedaplatin ($mg\,m^{-2}$) | No. of patients: Accrued | Evaluable for DLT[a] | Developing DLT[a] |
|---|---|---|---|---|---|
| 1 | 135 | 60 | 6 | 6 | 2 |
| 2 | 150 | 60 | 3 | 3 | 0 |
| 3 | 150 | 80 | 3 | 3 | 0 |
| 4 | 180 | 80 | 7 | 6 | 1 |
| 5 | 180 | 100 | 12 | 12 | 4 |
| 6 | 210 | 100 | 21 | 19 | 8 |

[a]Dose-limiting toxicity.

The treatment was terminated if the above-mentioned toxicity did not disappear in 3 weeks. If grade 4 leukopenia, grade 4 neutropenia for 5 days or longer, grade 3–4 febrile neutropenia, or grade 3–4 neutropenia with infection was noted, $50\,mg\,m^{-2}$ of granulocyte colony-stimulating factor (G-CSF) was given subcutaneously, and the doses of paclitaxel and nedaplatin were reduced by 25% in subsequent chemotherapy cycles.

### Dose-limiting toxicity, MTD, and RPTD

The dose-limiting toxicity (DLT) was defined as grade 4 neutropenia lasting 5 days or longer, grade 3–4 febrile neutropenia, grade 3–4 neutropenia with infection, grade 4 leukopenia, a platelet count $<20\times10^{9}\,l^{-1}$, and grade 3 or greater nonhaematological toxicity other than nausea and vomiting. Doses were escalated according to the frequency of DLT evaluated during the first cycle of chemotherapy. Three patients were initially enrolled at each dose level. If none of the patients experienced DLT, the next cohort of patients was treated at the next higher dose level. If one of the three patients experienced DLT, then three additional patients were enrolled at the same dose level, bringing the total to six patients for that dose level. If two or fewer patients experienced DLT, the next cohort of patients was treated at the next higher dose level. If three or more of the six patients experienced DLT, that level was considered to be the MTD. If two or all the initial three patients experienced DLT, that level was considered to be the MTD. The recommended dose for phase II trials was defined as the dose preceding the MTD. Six to 15 additional patients were enrolled at the RPTD to confirm that the frequency of DLT was less than one-third.

### Response evaluation

The objective tumour response was evaluated according to the WHO criteria issued in 1979 (WHO, 1979).

### Study design, data management, and statistical considerations

The protocol and consent form were approved by the Institutional Review Board of the National Cancer Center, Tokyo Japan. Data management, periodic monitoring, and the final analysis were performed by the Study Coordinator. A patient accrual period of 24 months and a follow-up period of 12 months were planned. The overall survival time was estimated using the Kaplan–Meier method (Armitage and Berry, 1994). Survival time was measured from the date of study registration until the date of death from any cause.

## RESULTS

### Patient characteristics

Between August 1999 and December 2002, 53 patients were registered in the study. One patient at level 5 developed a bone fracture prior to treatment and did not receive chemotherapy. This patient was excluded from all the analyses. Of the remaining 52 patients (42 males and 10 females) with a median age of 62 years (range 49–75), 42 (81%) patients had lung SCC, followed by thymic SCC in five patients and head and neck SCC in four patients. Of the 52 patients, 24 and 24 had metastatic and locally advanced diseases, respectively.

### Treatment delivery, toxicity, MTD, and RPTD

Treatment delivery was summarised in Table 2. Severe toxicity was mainly manifested as leucopenia, neutropenia, and associated infection, but the frequency of these symptoms did not differ between dose levels (Table 3). Grade 3 anaemia and thrombocytopenia were only noted in one patient (5%) each; both these patients had been treated at dose level 6. No grade 3–4 nausea, neuropathy, or myalgia was noted. A grade 3–4 elevation in creatinine, grade 3–4 hyponatremia, appetite loss, and diarrhoea were only observed at level 6. One patient treated at level 6

**Table 2** Treatment delivery

| | No. of patients (%): Levels 1–4 ($n=19$) | Level 5 ($n=12$) | Level 6 ($n=21$) |
|---|---|---|---|
| *Chemotherapy cycles* | | | |
| 5 | 1 (5) | 0 (0) | 0 (0) |
| 4 | 7 (37) | 4 (33) | 5 (24) |
| 3 | 2 (11) | 2 (17) | 3 (14) |
| 2 | 5 (26) | 4 (33) | 8 (38) |
| 1 | 4 (21) | 2 (17) | 5 (24) |
| Median | 3 | 3 | 2 |
| *Dose reduction in subsequent cycles* | | | |
| None | 12 (63) | 9 (75) | 12 (50) |
| Required | 3 (16) | 1 (8) | 4 (19) |
| Not administered | 4 (21) | 2 (17) | 5 (24) |

**Table 3** Toxicity in all courses

| | Levels 1–4 ($n=19$) | | | Level 5 ($n=12$) | | | Level 6 ($n=21$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 3–4 (%) | 3 | 4 | 3–4 (%) | 3 | 4 | 3–4 (%) |
| Leukopenia | 6 | 0 | (32) | 5 | 0 | (42) | 6 | 1 | (33) |
| Neutropenia | 3 | 10 | (68) | 2 | 9 | (92) | 3 | 12 | (71) |
| Anaemia | 0 | 0 | (0) | 0 | 0 | (0) | 1 | 0 | (5) |
| Thrombocytopenia | 0 | 0 | (0) | 0 | 0 | (0) | 1 | 0 | (5) |
| AST | 0 | 0 | (0) | 0 | 0 | (0) | 1 | 0 | (5) |
| ALT | 0 | 0 | (0) | 1 | 0 | (8) | 0 | 1 | (5) |
| Creatinine | 0 | 0 | (0) | 0 | 0 | (0) | 0 | 1 | (5) |
| Hyponatremia | 0 | 0 | (0) | 0 | 0 | (0) | 2 | 1 | (14) |
| Infection | 4 | 0 | (21) | 4 | 0 | (33) | 6 | 0 | (29) |
| Appetite loss | 0 | 0 | (0) | 0 | 0 | (0) | 1 | 0 | (5) |
| Dianthoea | 0 | 0 | (0) | 0 | 0 | (0) | 2 | 0 | (10) |
| Constipation | 0 | 0 | (0) | 0 | 0 | (0) | 0 | 1 | (5) |
| Arrhythmia | 2 | 0 | (11) | 0 | 0 | (0) | 0 | 0 | (0) |
| Lung toxicity | 0 | 0 | (0) | 0 | 0 | (0) | 2 | 0 | (10) |

developed grade 2 leukopenia, fever, watery diarrhoea, and grade 4 ileus, but recovered in 5 days. Two patients at level 6 developed grade 3 interstitial pneumonitis, but quickly recovered with oxygen therapy alone in one patient and with oxygen and steroid therapy in the other patient. No treatment-related deaths occurred in the study.

In all, 19 DLTs were noted in 15 patients. Of the 1nine DLTs, 13 were neutropenic fever or documented infection and six were nonhaematological. At level 6, only two of the first six patients developed DLT; therefore, 15 additional patients were entered at this level to confirm the frequency of DLT. Two patients were excluded from the DLT analysis because G-CSF was administered before the duration of grade 4 neutropenia had been determined (protocol violation). Of the remaining 13 patients, six developed DLT. Thus, eight (42%) of the 19 patients evaluated for DLT developed DLT at level 6; this dose level was therefore determined to be the MTD. An additional six patients were registered at level 5, and four (33%) of the 12 patients at level 5 developed DLT; this level was determined to be the RPTD.

### Objective responses and survival

Of the 42 patients with lung SCC, two CRs and 21 PRs were noted, and the overall response rate (95% confidence interval) was 55% (39–70%). No difference in the response rates for levels 1–4 and levels 5–6 were observed. One PR was noted in a patient with thymic SCC, and one PR was noted in a patient with head and neck SCC. The overall survival time (95% confidence interval) in all patients ($n = 52$) was 11.1 (6.4–15.8) months.

## DISCUSSION

This study showed that the combination of nedaplatin and paclitaxel was feasible with acceptable toxicity, and that the RPTD of nedaplatin was 100 mg m$^{-2}$ over 1 hour, which is the full dose of this agent, while that of paclitaxel was 180 mg m$^{-2}$ over 3 h. These doses are comparable to doses for practical use and those determined by previous phase I trials of cisplatin or carboplatin in combination with paclitaxel, where 180–225 mg m$^{-2}$ of paclitaxel was given with the full dose of platinum-agent (Akiyama *et al*, 2001; Kurata *et al*, 2001). The toxicity profile in the present study was similar to that of the carboplatin and paclitaxel combination (Akiyama *et al*, 2001).

The primary objectives of phase I trials are to evaluate toxicity and to establish a recommended drug dose for a given administration schedule; an additional goal of these trials is to look for evidence of the drug's antitumour activity. Objective tumour responses to newly investigated drugs are a promising clue for determining specific tumour types for subsequent phase II trials; therefore, patients with various tumours are usually registered in phase I trials (Sekine *et al*, 2002). In cases where some information on the antitumour activity of a drug is available, patients can be selected so that the chance of a response is maximised. This study was a histology-oriented phase I trial, and objective tumour responses were observed in about half of the patients.

The combination of nedaplatin and paclitaxel is particularly promising for the treatment of patients with lung SCC, as shown by the high response rate of 55%. Adenocarcinoma, large-cell carcinoma, adenosquamous carcinoma, and SCC of the lung have been grouped together as non-small-cell lung cancer because treatment response and prognosis are similar for these histologies. A recent cDNA microarray analysis of non-small-cell lung cancer tissue, however, showed that the gene expression profiles of SCC and adenocarcinoma are different (Kikuchi *et al*, 2003), and these differences may lead to different responses to anticancer agents, including nedaplatin. Thus, optimal chemotherapy regimens for the treatment of non-small-cell lung cancer should be established according to each tumour's histology. The numbers of patients with head and neck SCC and patients with thymic SCC were too small to comment on the antitumour effects of this regimen.

In conclusion, the combination of nedaplatin and paclitaxel is a feasible treatment, and the RPTD is paclitaxel 180 mg m$^{-2}$ given as a 3-h infusion followed by nedaplatin 100 mg m$^{-2}$ in a 1-h infusion every 3–4 weeks. This regimen was highly effective for the treatment of untreated lung SCC.

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## REFERENCES

Ajani JA, Ilson DH, Daugherty K, Pazdur R, Lynch PM, Kelsen DP (1994) Activity of taxol in patients with squamous cell carcinoma and adenocarcinoma of the esophagus. *J Natl Cancer Inst* 86: 1086–1091

Akiyama Y, Ohe Y, Tamura T, Sawada M, Inoue A, Kusaba H, Yamamoto N, Sekine I, Kunitoh H, Kodama T, Saijo N (2001) A dose escalation study of paclitaxel and carboplatin in untreated Japanese patients with advanced non-small cell lung cancer. *Jpn J Clin Oncol* 31: 482–487

Armitage P, Berry G (1994) Survival analysis. In *Statistical Methods in Medical Research*, Armitage P, Berry G (eds) 3rd edn, pp 469–492. Oxford: Blackwell Scientific Publications

DeVita Jr VT, Hellman S, Rosenberg SA (2001) *Cancer: Principles & Practice of Oncology*, 6th edn Philadelphia: Lippincott Williams & Wilkins

Forastiere AA, Shank D, Neuberg D, Taylor IV SG, DeConti RC, Adams G (1998) Final report of a phase II evaluation of paclitaxel in patients with advanced squamous cell carcinoma of the head and neck: an Eastern Cooperative Oncology Group trial (PA390). *Cancer* 82: 2270–2274

Inuyama Y, Miyake H, Horiuchi M, Hayasaki K, Komiyama S, Ota K (1992) A late phase II clinical study of *cis*-diammine glycolato platinum, 254-S, for head and neck cancers. *Gan To Kagaku Ryoho* 19: 871–877 (Japanese)

JCOG Administrational Committee (1998) National Cancer Institute – Common Toxicity Criteria (NCI-CTC Version 2.0, Jan 30, 1998). Japanese edition by JCOG. Available at http://www.med.gunma-u.ac.jp/event/CTCJ_v20.pdf

Kameyama Y, Okazaki N, Nakagawa M, Koshida H, Nakamura M, Gemba M (1990) Nephrotoxicity of a new platinum compound, 254-S, evaluated with rat kidney cortical slices. *Toxicol Lett* 52: 15–24

Kikuchi T, Daigo Y, Katagiri T, Tsunoda T, Okada K, Kakiuchi S, Zembutsu H, Furukawa Y, Kawamura M, Kobayashi K, Imai K, Nakamura Y (2003) Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. *Oncogene* 22: 2192–2205

Kobayashi H, Takemura Y, Miyachi H, Ogawa T (1991) Antitumor activities of new platinum compounds, DWA2114R, NK121 and 254-S, against human leukemia cells sensitive or resistant to cisplatin. *Invest New Drugs* 9: 313–319

Kurata T, Tamura T, Shinkai T, Ohe Y, Kunitoh H, Kodama T, Kakinuma R, Matsumoto T, Kubota K, Omatsu H, Nishiwaki Y, Saijo N (2001) Phase I and pharmacological study of paclitaxel given over 3 h with cisplatin for advanced non-small cell lung cancer. *Jpn J Clin Oncol* 31: 93–99

McGuire WP, Blessing JA, Moore D, Lentz SS, Photopulos G (1996) Paclitaxel has moderate activity in squamous cervix cancer. A Gynecologic Oncology Group study. *J Clin Oncol* 14: 792–795

Noda K, Ikeda M, Yakushiji M, Nishimura H, Terashima Y, Sasaki H, Hata T, Kuramoto H, Tanaka K, Takahashi T, Hirabayashi K, Yamabe TH, Hatae M (1992) A phase II clinical study of *cis*-diammine glycolato

platinum, 254-S, for cervical cancer of the uterus. *Gan To Kagaku Ryoho* 19: 885–892 (Japanese)

Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 5: 649–655

Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH, The Eastern Cooperative Oncology Group (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 346: 92–98

Sekine I, Nishiwaki Y, Watanabe K, Yoneda S, Saijo N, Kikuchi K, Kawakami Y, Abe S, Fujita A, Shida A, Nukiwa T, Mori K, Saito R, Yoneda S, Kabe J, Hayashi I, Niitani K, Noda K, Kurita Y (1996) Phase II study of 3-h infusion of paclitaxel in previously untreated non-small cell lung cancer. *Clin Cancer Res* 2: 941–945

Sekine I, Yamamoto N, Kunitoh H, Ohe Y, Tamura T, Kodama T, Saijo N (2002) Relationship between objective responses in phase I trials and potential efficacy of non-specific cytotoxic investigational new drugs. *Ann Oncol* 13: 1300–1306

Taguchi T, Wakui A, Nabeya K, Kurihara M, Isono K, Kakegawa T, Ota K (1992) A phase II clinical study of *cis*-diammine glycolato platinum, 254-S, for gastrointestinal cancers. 254-S Gastrointestinal Cancer Study Group. *Gan To Kagaku Ryoho* 19: 483–488 (Japanese)

Yamada H, Uchida N, Maekawa R, Yoshioka T (2001) Sequence-dependent antitumor efficacy of combination chemotherapy with nedaplatin, a newly developed platinum, and paclitaxel. *Cancer Lett* 172: 17–25

Yamamoto N, Tamura T, Kurata T (2000) Phase I and pharmacokinetic (PK) study of (Glycolate-0, 0′)-diammine platinum (II) (Nedaplatin: 254-S) in elderly patients with non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 19: 203a (abstr 792)

WHO (1979) *Handbook for Reporting Results of Cancer Treatment*, WHO Offset Publication No. 48 Geneva: World Health Organization