Original Article

Optimizing the timing of 3.6 mg Pegfilgrastim Administration for Dose-Dense Chemotherapy in Japanese Patients with Breast Cancer

Daisuke Takabatake[a]*, Yukiko Kajiwara[b] Shoichiro Ohtani[c], Yoko Suzuki[b],
Mari Yamamoto[d], Shinichiro Kubo[d], Masahiko Ikeda[d], Mina Takahashi[e],
Fumikata Hara[f], Kenjiro Aogi[e], Shozo Ohsumi[e], Yutaka Ogasawara[g],
Yoshitaka Nishiyama[h], Hajime Hikino[i], Kinya Matsuoka[j], Tadahiko Shien[b],
Naruto Taira[b], and Hiroyoshi Doihara[b]

[a]Kochi Health Science Center, Kochi 781-0915, Japan, [b]Okayama University Hospital, Okayama 700-8558, Japan,
[c]Hiroshima Citizens Hospital, Hiroshima 730-8518, Japan, [d]Fukuyama Citizens Hospital, Fukuyama, Hiroshima 721-8511, Japan,
[e]Shikoku Cancer Center, Matsuyama 791-0280, Japan, [f]Cancer Institute Hospital, Tokyo 135-8550, Japan,
[g]Kagawa Prefectural Center Hospital, Takamatsu 760-8557, Japan, [h]Okayama Saiseikai General Hospital, Okayama 700-8511, Japan,
[i]Matsue Red Cross General Hospital, Matsue 690-8506, Japan, [j]Ehime Prefectural Central Hospital, Matsuyama 790-0024, Japan

Perioperative dose-dense chemotherapy (DDCT) with pegfilgrastim (Peg) prophylaxis is a standard treatment for high-risk breast cancer. We explored the optimal timing of administration of 3.6 mg Peg, the dose approved in Japan. In the phase II feasibility study of DDCT (adriamycin + cyclophosphamide or epirubicin + cyclophosphamide followed by paclitaxel) for breast cancer, we investigated the feasibility, safety, neutrophil transition, and optimal timing of Peg treatment by administering Peg at days 2, 3, and 4 post-chemotherapy (P2, P3, and P4 groups, respectively). Among the 52 women enrolled, 13 were aged > 60 years. The anthracycline sequence was administered to P2 (n = 33), P3 (n = 5), and P4 (n = 14) patients, and the taxane sequence to P2 (n = 38) and P3 (n = 6) patients. Both sequences showed no interaction between Peg administration timing and treatment discontinuation, treatment delay, or dose reduction. However, the relative dose intensity (RDI) was significantly different among the groups. The neutrophil count transition differed significantly among the groups receiving the anthracycline sequence. However, the neutrophil count remained in the appropriate range for both sequences in the P2 group. The timing of Peg administration did not substantially affect the feasibility or safety of DDCT. Postoperative day 2 might be the optimal timing for DDCT.

Key words: dose-dense chemotherapy, breast cancer, pegfilgrastim

P erioperative dose-dense chemotherapy (DDCT), which is currently the standard choice for patients at a high risk of breast cancer, involves the escalation of dose intensity by shortening the interval between consecutive administrations of drugs [1]. This treatment strategy has been made possible by the prophylactic administration of granulocyte colony-stimulating factor (G-CSF). Pegfilgrastim (Peg) is a long-acting PEGylated form of the recombinant human G-CSF analog filgrastim that is prophylactically administered during each cycle in chemotherapy regimens with 14-day intervals. Although it is generally recommended that Peg is administered 24 h after chemotherapy in DDCT, the optimal timing is uncertain.

Peg treatment sometimes causes early leukocytosis, and there are a few case reports of severe adverse effects

such as splenic rupture [2]. Several studies have compared post-chemotherapy day 2 administration versus day 4, or 24 h versus 72 h versus 96 h [3,4]. The results of these studies were inconsistent. Moreover, in Japan, the approved dose of 3.6 mg is different from the 6 mg dose used in western countries, and the optimal timing of administration has not been estimated for the 3.6 mg dose.

We conducted a multicenter phase II study to estimate the efficacy and feasibility of sequential DDCT consisting of anthracycline followed by taxane for Japanese patients with breast cancer [5]. Patients at each participating institution were arbitrarily assigned to receive Peg administration at day 2 (24 h), day 3 (48 h) or day 4 (72 h). In the present *post-hoc* analysis, to identify the optimal timing of 3.6 mg Peg administration in DDCT for Japanese patients with breast cancer, we investigated the effect of timing (post-chemotherapy day 2, 3 or 4) on the feasibility and safety of Peg treatment, and we evaluated the transition of the neutrophil count.

Patients and Methods

Study design and eligibility.   We previously conducted a prospective, multicenter, open-label phase II study to evaluate the feasibility of DDCT for Japanese women with breast cancer; the study design and eligibility have been reported [5]. Briefly, the eligibility criteria were: age 20-70 years, Eastern Cooperative Oncology Group performance status ≤ 2, left ventricle ejection fraction $\geq 60\%$, and operable human epidermal growth factor receptor 2 (HER2)-negative breast cancer with axillary lymph node metastasis. The study protocol was registered at the University Hospital Medical Information Network (UMIN 000022097) and was approved by the institutional review board of each participating institution. All patients provided written informed consent before enrollment.

Treatment procedure.   The patients received preoperative full-sequence DDCT as four cycles of adriamycin + cyclophosphamide (AC) or epirubicin + cyclophosphamide (EC) at 2-week intervals, followed by four cycles of paclitaxel (PTX) at 2-week interval. The AC regimen consisted of 60 mg/m^2 adriamycin and 600 mg/m^2 cyclophosphamide. The EC regimen consisted of 90 mg/m^2 epirubicin and 600 mg/m^2 cyclophosphamide. The dose of PTX was 175 mg/m^2.

Prophylactic G-CSF (3.6 mg Peg) was administered subcutaneously on days 2-4 of each cycle. Adverse effects were evaluated using the Common Terminology Criteria for Adverse Events ver. 4.0.

The patients' white blood cell and neutrophil counts were monitored on days 1 and 8 of the first chemotherapy cycle for each agent, and at each administration. The eligibility criteria for each drug were as follows: neutrophil count $\geq 1,500$, platelet count $\geq 10 \times 10^5$, hemoglobin level ≥ 9.1 g/dl, and non-hematologic adverse event $\leq$ grade 2. The protocol-off criteria were as follows: (1) > 14-day delay in the administration of each drug due to any adverse effects, (2) any non-hematologic adverse event > grade 4, (3) a patient wanted to discontinue study treatment, or (4) the attending physician determined that it would be difficult for the patient to continue the study treatment.

Study outcomes.   The end points of our previous feasibility study were the rates of pathological complete response (pCR), febrile neutropenia, treatment completion, and toxicities, and the relative dose intensity (RDI) [5]. In contrast, the main purpose of the present *post-hoc* analysis was to determine the optimal timing of the administration of Peg at the dose of 3.6 mg in Japanese patients.

We categorized the patients into three administration groups, P2, P3, and P4, which received Peg on days 2 (24 h), 3 (48 h) and 4 (72 h) after chemotherapy, respectively. The timing of Peg administration was arbitrary among the institutions. The transition of the neutrophil count was recorded for each patient, and the mean transition values were determined for the P2, P3, and P4 administration groups, and for the age groups of ≥ 60 years and < 60 years. We then estimated the interaction between the timing of Peg administration and the discontinuation, delay, or dose reduction of the treatment drug. "Delay" was defined as a delay in drug administration for > 7 days. We also determined the RDI in each group, the incidence of grade 3-4 neutropenia, and the incidence of febrile neutropenia. The RDI was defined as follows: RDI (%) = dose intensity (DI) by actual dose / DI by scheduled dose × 100, where DI is the total actual dose (mg/m^2)/week.

Statistical analysis.   Anthracycline and taxane were evaluated separately to determine whether the timing of Peg administration affected the transition of the neutrophil count, by a multivariate analysis of variance (MANOVA). The frequency of protocol changes

because of treatment discontinuation or delay and the incidence of grade 3-4 hematologic adverse events were compared among the groups using Fisher's exact test and Pearson's chi-square test. We used an analysis of variance (ANOVA) to compare the average RDI among the groups. All statistical analyses were performed using JMP software (ver. 10, SAS Institute, Cary, NC, USA).

Results

Between April 2016 and January 2017, 53 eligible women were enrolled in the study at nine institutions; one patient was excluded after a postoperative pathological evaluation revealed that her cancer was HER2-positive. The patients' characteristics are summarized in Table 1. The median patient age was 52 (range 33-69) years, and 13 patients (25.0%) were ≥60 years old. The anthracycline and taxane sequences were evaluated separately. In the P2, P3, and P4 groups, 33 (63.5%), 5 (9.6%), and 14 (26.9%) patients received the anthracycline sequence, respectively. In the P2, P3, and P4 groups, 38 (86.4%), 6 (13.6%), and 0 (0%) patients received the taxane sequence, respectively.

The results of our analyses are summarized in Table 2. Treatment was discontinued in 10 (19.2%) patients because of adverse events. More specifically, during the anthracycline sequenc, 4 (12.1%), 1 (20%), and 3 (21.4%) patients dropped out of the study in the P2, P3, and P4 groups, respectively, and 5 (15.2%) patients in the P2 group received a delayed administration because of adverse events. A dose reduction was required for 3 patients (9.1%) in the P2 group and 1 patient (8.3%) in the P4 group.

During the taxane sequence, 2 (5.3%) patients in the P2 group dropped out of the study, whereas 5 (13.9%)

patients in the P2 group and 1 (16.7%) in the P3 group received delayed treatment. Grade 3-4 neutropenia and febrile neutropenia were observed in 38.5% and 11.5% of the patients, respectively. In particular, most of the patients who experienced febrile neutropenia dropped out of the study, but the treatment discontinuation rate was not significantly different among the groups (Table 2).

Among the patients who completed the treatment, severe adverse events were rare. During the anthracycline sequence, the RDI values were 98.1%, 100%, and 85.9% in the P2, P3, and P4 groups, respectively, and the RDI of the P4 group was significantly lower compared to those of the other groups ($p = 0.043$). Among patients receiving the taxane sequence, the RDI was 95.2% and 98.2% in the P2 and P3 groups, respectively ($p = 0.178$).

Figure 1A,B shows the transition of the average neutrophil count in the different groups for the anthracycline and taxane sequences, respectively. The statistical analysis revealed that the timing of the Peg administration significantly affected the neutrophil transition
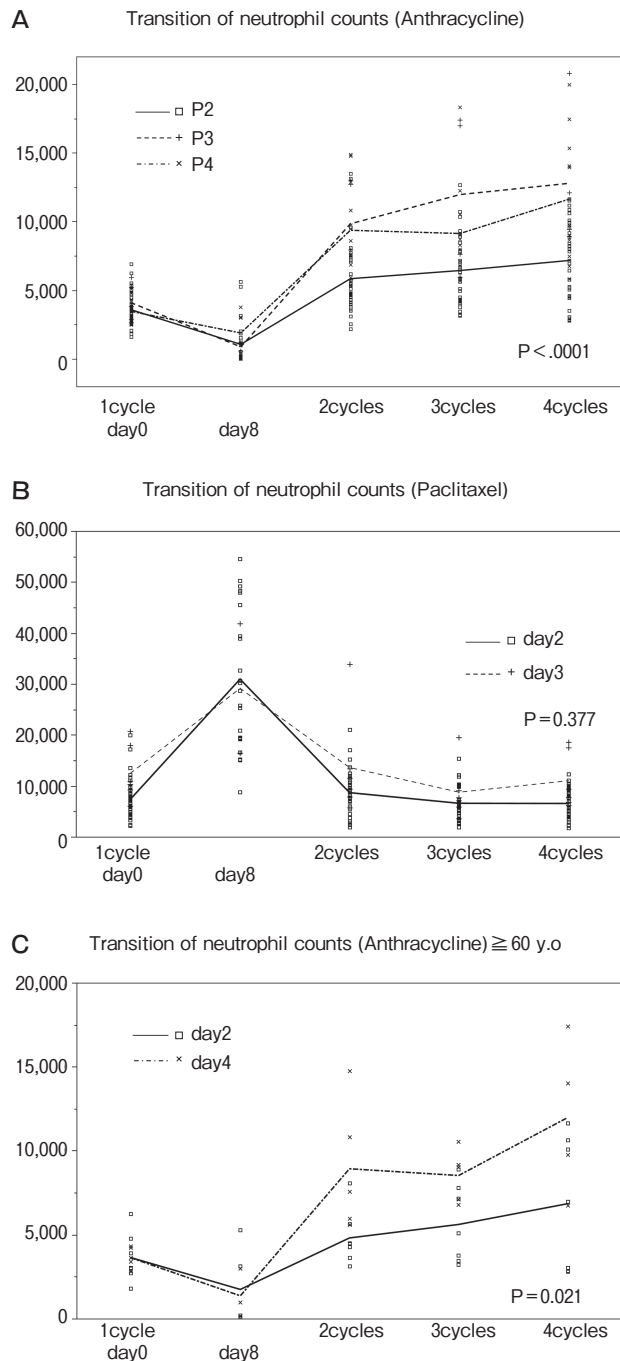
Table 1   Patient characteristics

	n (%)	
age (median)	52 (33−69)	
≧ 60	13 (25.0%)	
< 60	39 (75.0%)	
Peg administration	anthracycline	PTX
day 2 (P2)	33 (63.5%)	38 (86.4%)
day 3 (P3)	5 (9.6%)	6 (13.6%)
day 4 (P4)	14 (26.9%)	0 (0%)

Peg, pegfilgrastim; PTX, paclitaxel.

Table 2   Summary of results

Discontinuation of Treatment

Peg administration	anthracycline	PTX
P2	12.1% (4/33)	5.3% (2/38)
P3	20% (1/5)	0% (0/6)
P4	21.4% (3/14)	–
total	15.4% (8/52)	4.5% (2/44)
p-value	0.689	1.0

Delay of Treatment

Peg administration	anthracycline	PTX
P2	15.2% (5/33)	13.9% (5/36)
P3	0% (0/5)	16.7% (1/6)
P4	0% (0/12)	–
total	10.0% (5/50)	14.3% (6/42)
p-value	0.239	1.0

Dose Reduction

Peg administration	anthracycline	PTX
P2	9.1% (3/33)	0% (0/36)
P3	0% (0/5)	0% (0/6)
P4	8.3% (1/12)	–
total	8.0% (4/50)	0% (0/42)
p-value	0.783	–

P2, P3, and P4: day 2, 3, and 4 of Peg administration, respectively. Peg, pegfilgrastim; PTX, paclitaxel.

A

Transition of neutrophil counts (Anthracycline)

B

Transition of neutrophil counts (Paclitaxel)

C

Transition of neutrophil counts (Anthracycline) ≧ 60 y.o

Fig. 1　　**A**, Transition of the average neutrophil count at the start of each cycle in the anthracycline sequence. Here and in panels B and C, *the squares and solid lines* indicate the P2 group; *the pluses and dashed lines* indicate the P3 group, and the *chain lines and x* indicate the P4 group; **B**, Transition of the average neutrophil count at the start of each cycle in the taxane sequence; **C**, Transition of the average neutrophil count at the start of each cycle in the anthracycline sequence in patients aged >60 years.

with anthracycline treatment but not with taxane treatment. In addition, the neutrophil count was maintained in the appropriate range in the P2 group for both sequences. These results were reproduced in the patients who were ≥60 years old in the anthracycline sequence (Fig. 1C). In the taxane sequence, all of the patients aged ≥60 years were in the P2 group.

When we analyzed the neutrophil transition by age group, there was no significant difference between the patients ≥60 and those <60 years old (data not shown). However, it should be noted that the patients who had dropped out were excluded from these analyses.

The results revealed that there was no interaction between the timing of Peg administration and the discontinuation, delay, or dose reduction events. However, during administration of the anthracycline sequence, the RDI of the P4 group was significantly lower than that of the other groups.

Discussion

We investigated the effect of the timing of 3.6 mg Peg administration in DDCT, and our findings revealed that the timing had no effect on the feasibility or safety in Japanese patients with breast cancer. However, the transition of the neutrophil count and the RDI were both significantly different among the three administration groups during the anthracycline sequence.

Although it is recommended that Peg is administered 24 h after chemotherapy, the timing widely used in Japan is 24-72 h [6,7], and the optimal timing of DDCT has not been determined. Several studies in western countries have investigated the timing of Peg administration. A randomized phase III GIM2 study, which focused on leukocytosis, evaluated which of 24, 72, or 96 h was the safest time point for Peg administration [3], and concluded that Peg administration at 72 h was the safest. This timing was adopted in the GIM2 study. In the GBG33 study, the efficacy of Peg administration on day 2 versus day 4 was evaluated with respect to the incidence of grade 4 leucopenia in DDCT for patients with breast cancer [4]. The results failed to demonstrate that Peg was more effective if administered on day 4 than on day 2. Thus, in western countries too, the timing of Peg administration has not been defined and remains controversial.

However, all of the previous studies evaluated Peg administered at the dose of 6 mg, the approved dose in

western countries, and the chemotherapy regimens were not sorted. To the best of our knowledge, there has been no evaluation of 3.6 mg Peg in DDCT, which is the dose approved in Japan. The U.S. Oncology Group performed a dose-finding study that compared Peg 30, 60, and 100 µg/kg [8], and it reported that the safety profiles were similar among the three doses; however, the pharmacokinetics of Peg were dependent on the dose and neutrophil count. The subsequent randomized phase III study of a single 6 mg fixed dose led to its approval in western countries [9].

A dose-finding study performed in patients with breast cancer in Japan [10] compared 3.6 mg Peg with 6 mg Peg administered on day 2 in the docetaxel+adriamycin+cyclophosphamide (TAC) regimen. The neutrophil transition and the rate of febrile neutropenia were not significantly different between the two doses. The results of these previous western studies may thus be adaptable to the 3.6 mg dose in Japan, but the neutrophil counts were slightly different on day 14 between 3.6 mg and 6 mg Peg [10]. The interval of DDCT is 14 days, which differs from the 21-day schedule for the TAC regimen. A further evaluation of DDCT using the 3.6 mg Peg dose might be required.

In the GIM2 study, early leukocytosis was evaluated as a safety indicator. In real-world clinical settings, the neutrophil count is not routinely examined on day 2 or 8, and the 3.6 mg dose may cause milder leukocytosis than the 6 mg dose. Consequently, the rates of completion, dose reduction, and delay or the patient age was the defined evaluation target in our analyses.

The results of our analyses demonstrated that the neutrophil transition was significantly different only during the anthracycline sequence (not during the taxane sequence) among the three Peg administration groups. Although the reason for this result is not clear, a self-regulating neutrophil-mediated clearance mechanism has been reported in which the neutrophil nadir rapidly declined as neutrophils started to recover [8]. This was probably because of the differences in the nadir point between regimens. In the present patient cohort, at the start of the new chemotherapy cycles 2, 3, and 4, the neutrophil counts of the P3 and P4 groups were higher than that of the P2 group during the anthracycline sequence. In addition, the taxane sequence of the P3 group showed a higher neutrophil count compared to that of the P2 group.

Several cases of splenic rapture or capillary leak syndrome and acute myeloid leukemia have been reported to be caused by Peg administration [2,11,12]. Early leukocytosis is considered a risk factor. Accordingly, at the start of a new cycle, it is desirable for the leucocyte count to be within the normal range as much as possible.

We also speculate that the low RDI of the P4 group might have been due to the high treatment discontinuation rate in this group. It is better to avoid day 4 Peg administration. We observed that the patient's age did not affect the neutrophil transition at any timing of Peg administration (data not shown). However, a few patients were ≥60 years old, and our previous feasibility study revealed that the completion rate was significantly lower in older patients compared to younger patients [5]. Only patients who completed the study treatment were included in the present analyses, and the results based on age were therefore not conclusive.

There were some major limitations to this study. Although the patients were recruited prospectively, the present analysis was not preplanned, and thus the number of patients in each group was small, and the patients were not randomly assigned. Randomized studies are needed to draw more concrete conclusions.

In summary, our preliminary finding indicated that the timing of 3.6 mg Peg administration did not greatly affect the efficacy or the safety of DDCT; however, the RDI was lower with Peg day 4 administration. Peg administration on days 2 and 3 was available in the anthracycline sequence at the convenience of each institution. In addition, administration on both days 2 and 3 was allowed in the taxane sequence. However, the neutrophil count was maintained in the appropriate range with day 2 administration.

In conclusion, our findings indicate that in both the anthracycline and taxane sequences, 3.6 mg Peg administration on day 2 might be the optimal timing for DDCT in Japanese patients with breast cancer.

References

1. Norton L: Theoretical concepts and the emerging role of taxanes in adjuvant therapy. Oncologist (2001) 6 Suppl 3: 30–35.

2. Watring NJ, Wagner TW and Stark JJ: Spontaneous splenic rupture secondary to pegfilgrastim to prevent neutropenia in a patient with non-small-cell lung carcinoma. Am J Emerg Med (2007) 25: 247–248.

3. Lambertini M, Bruzzi P, Poggio F, Pastorino S, Gardin G, Clavarezza M, Bighin C, Pronzato P and Del Mastro L: Pegfilgrastim administration after 24 or 72 or 96 h to allow dose-dense anthracycline- and taxane-based chemotherapy in breast cancer patients: a single-center experience within the GIM2 randomized phase III trial. Support Care Cancer (2016) 24: 1285–1294.

4. Loibl S, Mueller V, von Minckwitz G, Conrad B, Koehne CH, Kremers S, Forstbauer H, Linder M, Nekljudova V and Moebus V: Comparison of pegfilgrastim on day 2 vs. day 4 as primary prophylaxis of intense dose-dense chemotherapy in patients with node-positive primary breast cancer within the prospective, multi-center GAIN study: (GBG 33). Support Care Cancer (2011) 19: 1789–1795.

5. Takabatake D, Kajiwara Y, Ohtani S, Itano Y, Yamamoto M, Kubo S, Ikeda M, Takahashi M, Hara F, Aogi K, Ohsumi S, Ogasawara Y, Nishiyama Y, Hikino H, Matsuoka K, Takahashi Y, Shien T, Taira N and Doihara H: The efficacy and feasibility of dose-dense sequential chemotherapy for Japanese patients with breast cancer. Breast Cancer (2018) 25: 717–722.

6. Morita S, Kikumori T, Tsunoda N, Inaishi T, Adachi Y, Ota A, Shibata M, Matsuoka A, Nakanishi K, Takeuchi D, Mizutani T, Shimokata T, Hayashi H, Maeda O and Ando Y: Feasibility of dose-dense epirubicin and cyclophosphamide with subcutaneous pegfilgrastim 3.6 mg support: a single-center prospective study in Japan. Int J Clin Oncol (2017).

7. Mizuno Y, Fuchikami H, Takeda N, Iwai M and Sato K: Efficacy of reduced dose of pegfilgrastim in Japanese breast cancer patients receiving dose-dense doxorubicin and cyclophosphamide therapy. Jpn J Clin Oncol (2017) 47: 12–17.

8. Holmes FA, Jones SE, O'Shaughnessy J, Vukelja S, George T, Savin M, Richards D, Glaspy J, Meza L, Cohen G, Dhami M, Budman DR, Hackett J, Brassard M, Yang BB and Liang BC: Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. Ann Oncol (2002) 13: 903–909.

9. Green MD, Koelbl H, Baselga J, Galid A, Guillem V, Gascon P, Siena S, Lalisang RI, Samonigg H, Clemens MR, Zani V, Liang BC, Renwick J, Piccart MJ and International Pegfilgrastim 749 Study G: A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol (2003) 14: 29–35.

10. Masuda N, Tokuda Y, Nakamura S, Shimazaki R, Ito Y and Tamura K: Dose response of pegfilgrastim in Japanese breast cancer patients receiving six cycles of docetaxel, doxorubicin, and cyclophosphamide therapy: a randomized controlled trial. Support Care Cancer (2015) 23: 2891–2898.

11. Hershman D, Neugut AI, Jacobson JS, Wang J, Tsai WY, McBride R, Bennett CL and Grann VR: Acute myeloid leukemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy. J Natl Cancer Inst (2007) 99: 196–205.

12. Arshad M, Seiter K, Bilaniuk J, Qureshi A, Patil A, Ramaswamy G and Liu D: Side effects related to cancer treatment: CASE 2. Splenic rupture following pegfilgrastim. J Clin Oncol (2005) 23: 8533–8534.