Table 2 Subject characteristics by initial dose cohort

Variable	10 mg on MWF (n=3)	15 mg on MWF (n=4)	20 mg on MWF (n=6)	Total (n=13)
Sex (n)				
Male	2	3	3	8
Female	1	1	3	5
Age (years)				
Median	62.0	61.0	61.5	62.0
Range	53–77	28–71	49–67	28–77
Weight (kg)				
Median	58.2	63.05	55.85	58.2
Range	56.0–63.9	52.9–69.5	45.4–82.0	45.4–82.0
Height (cm)				
Median	162.0	164.5	161.5	163.0
Range	156–171	143–176	147–175	143–176
Platelets (10^9/L)				
Median	376	185.5	257.5	252
Range	305–609	167–215	178–301	167–609
AST (U/L)				
Median	25	23.5	19	20
Range	14–72	16–31	16–25	14–72
ALT (U/L)				
Median	12	22	14.5	15
Range	11–59	12–37	7–21	7–59
Bilirubin (μmol/L)				
Median	6.84	12.825	11.115	10.26
Range	6.84–10.26	5.13–18.81	6.84–13.68	5.13–18.81
Creatinine (μmol/L)				
Median	53.04	65.86	66.74	62.76
Range	53.04–68.07	33.59–81.33	32.71–95.47	32.71–95.47
QT (ms)				
Median	345	360	410	390
Range	337–346	334–390	395–444	334–444
QTcF (ms)				
Median	394	394	413	403
Range	377–397	376–416	383–432	376–432
Primary site, histology/cytology [n (%)]				
Lung, adenocarcinoma	0 (0.0)	1 (25.0)	2 (33.3)	3 (23.1)
Rectum, adenocarcinoma	0 (0.0)	1 (25.0)	1 (16.7)	2 (15.4)
Non-Hodgkin lymphoma, CTCL	2 (66.7)	0 (0.0)	0 (0.0)	2 (15.4)
Colon, adenocarcinoma	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Esophagus, squamous cell carcinoma	0 (0.0)	1 (25.0)	0 (0.0)	1 (7.7)
Small intestine, leiomyosarcoma	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Larynx, squamous cell carcinoma	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Pleura, mesothelioma	1 (33.3)	0 (0.0)	0 (0.0)	1 (7.7)
Thymus, squamous cell carcinoma	0 (0.0)	1 (25.0)	0 (0.0)	1 (7.7)
WHO performance status [n (%)]				
0	2 (66.7)	1 (25.0)	4 (66.7)	7 (53.8)
1	1 (33.3)	3 (75.0)	2 (33.3)	6 (46.2)
2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

ALT alanine transaminase, *AST* aspartate aminotransferase, *CTCL* cutaneous T-cell lymphoma, *MWF* Monday, Wednesday, and Friday, *WHO* World Health Organization

Table 3 Duration of exposure and duration of treatment by initial dose cohort

	10 mg MWF (n=3)	15 mg MWF (n=4)	20 mg MWF (n=6)	Total (n=13)
Duration of exposure[a] (days)				
Mean ± SD	72.7±29.14	52.8±34.56	106.0±85.92	81.9±64.12
Median	82.0	51.0	71.5	75.0
Range	40–96	22–87	26–253	22–253
Duration of treatment[b] (days)				
Mean ± SD	32.0±12.49	18.5±9.33	43.2±36.69	33.0±27.03
Median	36.0	18.0	27.5	26.0
Range	18–42	10–28	12–109	10–109

[a] Duration of exposure was defined as the time from the last known date the study drug was taken minus the time that the study drug was started + 1 (interruption periods were included). [b] Duration of treatment was defined as the total number of days that the study drug was taken (interruption periods were not included)

Safety and tolerability

All patients who received at least one dose of panobinostat experienced more than one AE. AEs occurring in at least 20% of the safety population, regardless of whether they were related to the study drug, are shown in Table 4. Grade 3 or 4 AEs occurred in 8 patients. Three grade 4 events were reported in 2 patients: thrombocytopenia for each patient, and decreased hemoglobin concentration.

The most frequently reported AEs, regardless of whether they were related to the study drug, were diarrhea and nausea (10 patients each, 76.9%), but most of the episodes were mild to moderate in degree. Thrombocytopenia was reported in 12 of 13 patients (92.3%); the exact MedDRA

Table 4 Adverse events (AEs), regardless of whether they were related to the study drug, by preferred terms, occurring in at least 20% of the population and in the initial dose cohort

Preferred terms	All grades				Grade 3/4			
	10 mg (n=3)	15 mg (n=4)	20 mg (n=6)	All (n=13)	10 mg (n=3)	15 mg (n=4)	20 mg (n=6)	All (n=13)
Patients with AEs, n (%)	3 (100.0)	4 (100.0)	6 (100.0)	13 (100.0)	1 (33.3)	2 (50.0)	5 (83.3)	8 (61.5)
Thrombocytopenia[a]	2 (66.7)	4 (100.0)	6 (100.0)	12 (92.3)	0 (0.0)	2 (50.0)	3 (50.0)	5 (38.5)
Hemoglobin decreased	0 (0.0)	0 (0.0)	3 (50.0)	3 (23.1)	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Diarrhea	3 (100.0)	3 (75.0)	4 (66.7)	10 (76.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea	3 (100.0)	3 (75.0)	4 (66.7)	10 (76.9)	1 (33.3)	0 (0.0)	0 (0.0)	1 (7.7)
Vomiting	2 (66.7)	2 (50.0)	4 (66.7)	8 (61.5)	1 (33.3)	0 (0.0)	0 (0.0)	1 (7.7)
Fatigue	0 (0.0)	1 (25.0)	4 (66.7)	5 (38.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pyrexia	2 (66.7)	0 (0.0)	3 (50.0)	5 (38.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Weight decreased	2 (66.7)	2 (50.0)	1 (16.7)	5 (38.5)	0 (0.0)	1 (25.0)	0 (0.0)	1 (7.7)
Blood albumin decreased	0 (0.0)	0 (0.0)	3 (50.0)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Blood thyroid-stimulating hormone increased	0 (0.0)	1 (25.0)	2 (33.3)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C-reactive protein increased	1 (33.3)	0 (0.0)	2 (33.3)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anorexia	2 (66.7)	1 (25.0)	4 (66.7)	7 (53.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Back pain	0 (0.0)	1 (25.0)	2 (33.3)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pain in extremity	1 (33.3)	0 (0.0)	2 (33.3)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dizziness	1 (33.3)	2 (50.0)	1 (16.7)	4 (30.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dysgeusia	2 (66.7)	0 (0.0)	2 (33.3)	4 (30.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cough	0 (0.0)	2 (50.0)	1 (16.7)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pruritus	1 (33.3)	0 (0.0)	2 (33.3)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[a] Thrombocytopenia including the MedDRA terms "thrombocytopenia" and "platelet count decreased"

(Medical Dictionary for Regulatory Activities) terms used were "thrombocytopenia" (7/13) and "platelet count decreased" (5/13). Thus, thrombocytopenia was the most frequently reported AE in this trial. Of the eight patients in whom grade 3/4 AEs occurred, five (62.5%) experienced thrombocytopenia. Although these patients required an interruption of the study drug, platelet counts recovered rapidly to grade 1 or less within 7 days in most cases.

The incidence of fatigue increased with increasing doses (0 of 3 patients at the 10-mg dose, 1 of 4 patients at the 15-mg dose, and 4 of 6 patients at the 20-mg dose), and this trend was the same as that observed in previous studies in a non-Japanese population [10].

Newly occurring or worsening abnormal electrocardiographic findings in the initial dose cohort are provided in Table 5. Absolute QT/QTcF prolongation was not observed in any of the patients. QT prolongation >60 ms was recorded in one patient in the 10-mg dose group, and QTcF prolongation >60 ms was recorded in another patient. Neither of these patients had any relevant symptoms. T wave abnormalities on the electrocardiogram, which were reported as AEs, were suspected to be related to the study drug in one patient (20-mg dose group).

Pharmacokinetics

Pharmacokinetic data were available for 13 patients. Plasma panobinostat concentration profiles on days 1 and 15 are shown in Fig. 1. After oral administration, panobinostat was rapidly absorbed, and the t_{max} was 1–2 h. The mean elimination $t_{1/2}$ of panobinostat ranged from 9 to 14 h on day 1 and from 17 to 18 h on day 15, respectively (Table 6). The plasma concentration of panobinostat at 48 h was below the lower limit of quantification on day 1 in most patients.

Pharmacodynamics

In five patients (three with colorectal cancer, one with non-small cell lung cancer, and one with esophageal cancer), the percentage of HBF increased over time during the study period. In the remaining patients, no suggestive trend in HBF was observed, and the differences in HBF from day 1 to the end of the study were ≤0.2%. No relation between panobinostat administration and HBF was observed.

Antitumor activity

Thirteen patients were evaluable for response. Tumor types and responses are shown in Table 7. Seven of 13 patients had stable disease. No complete responses or partial responses were observed. Two patients in the 15-mg cohort had progressive disease. Of 11 patients with solid tumors, 5 (1 in the 10-mg cohort and 4 in the 20-mg cohort) had stable disease. Two patients in the 20-mg cohort with stable disease had progression-free survival of 164 and 253 days, respectively. The two CTCL patients (both in the 10-mg cohort) had stable disease. The best PGA and CA responses indicated stable disease.

Discussion

This study showed that a dosing schedule of 20 mg oral panobinostat once daily for three noncontiguous days (MWF) weekly was well-tolerated in Japanese patients with advanced solid tumors or CTCL. No DLT was observed in patients in any cohort, and the MTD was not reached in the study. Panobinostat may be tolerable at higher doses; however, this possibility should be explored in future studies.

Table 5 Abnormal electrocardiographic findings by initial dose cohort

	10 mg on MWF (n=3)	15 mg on MWF (n=4)	20 mg on MWF (n=6)	Total (n=13)
Maximum absolute QT/QTcF [n (%)]				
QT >500 ms (CTCAE grade 3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
QT >480 ms and ≤500 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
QTcF >500 ms (CTCAE grade 3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
QTcF >480 ms and ≤500 ms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Maximum QT/QTcF increase from baseline [n (%)]				
>30 ms and ≤60 ms in QT	1 (33.3)	0 (0.0)	1 (16.7)	2 (15.4)
>60 ms in QT (CTCAE grade 2)	1 (33.3)	0 (0.0)	0 (0.0)	1 (7.7)
>30 ms and ≤60 ms in QTcF	1 (33.3)	0 (0.0)	0 (0.0)	1 (7.7)
>60 ms in QTcF (CTCAE grade 2)	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)

CTCAE Common Terminology Criteria for Adverse Events, *MWF* Monday, Wednesday, and Friday

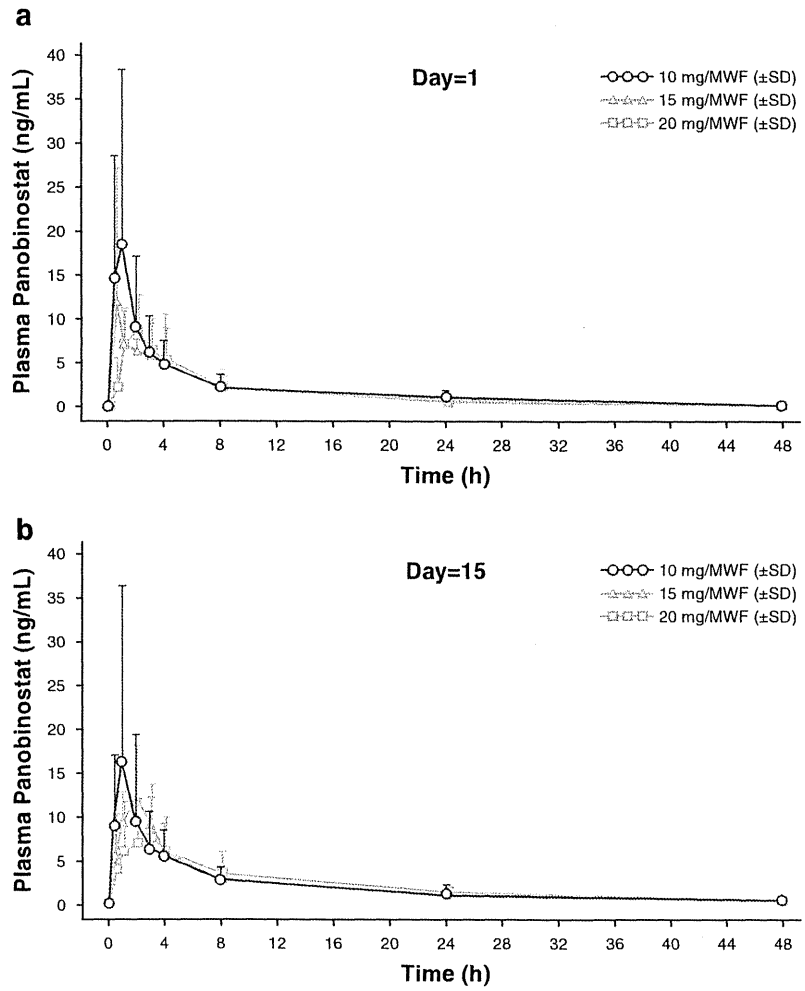

Fig. 1 **a** Panobinostat plasma concentrations on day 1 by initial dose cohort (mean + SD). Panobinostat was administered orally once daily on Monday, Wednesday, and Friday of each week. Pharmacokinetic data of panobinostat were obtained from 3, 4 and 6 patients in 10 mg, 15 mg and 20 mg cohorts, respectively. **b** Panobinostat plasma concentrations on day 15

The most frequently reported grade 3 or 4 AE was thrombocytopenia, but all such events were transient and resolved after the study drug was interrupted. No hemorrhage-related AEs were reported. Thrombocytopenia is a commonly reported AE and is a classic side effect of DACIs [11]. Recently, it was reported that DACIs inhibit GATA-1 gene expression in megakaryocytes by decreasing the transactivation function of GATA-1 itself. GATA-1 is a prototypic erythro-megakaryocytic transcription factor that plays an essential role in the differentiation of megakaryocytes and erythrocytes [12]. This GATA-1 reducing activity of DACIs may lead to a delay in megakaryocyte maturation and may cause thrombocytopenia. This proposed mechanism of action is supported by the in vivo result that administration of a potent HDACI (FR235225) to rats for 7 days resulted in a decrease in the peripheral platelet count and an increase in splenic megakaryocytes in a dose-dependent manner [13]. The rapid recovery of platelet counts seen in our study suggests that the mechanism of DACI-induced thrombocytopenia might be different from that of the typical cytotoxic agent–induced myelosuppression.

Because of the possible interaction of DACIs with the HERG K^+ channel, cardiac toxicity is a safety concern of DACIs [14, 15]. However, no serious cardiac toxicity was reported in our study. QTcF prolongation of 30 to 60 ms and of ≥60 ms was observed in 7.7% of the study population in our study, which was not greater than the results obtained in an integrated analysis of oral panobinostat in Western patients: QTcF changes of 30 to 60 ms and of ≥60 ms in 79 patients (27.1%) and 11 patients (3.8%), respectively, out of a total of 291 patients who received 20 mg panobinostat weekly on MWF [16].

The average exposure (C_{max} and AUC) did not increase with increasing dose, which may have been due to the large interindividual variability (coefficient of variation: 20–90% for C_{max} and AUC) and the limited number of pharmacokinetic profiles. Therefore, we were unable to draw any conclusions regarding the dose proportionality, or the lack thereof, of panobinostat pharmacokinetics in the Japanese

Table 6 Pharmacokinetic parameters by initial dose cohort

Time and pharmacokinetic parameter[a]	10 mg on MWF (n=3)	15 mg on MWF (n=4)	20 mg on MWF (n=6)
Day 1			
t_{max} (h)[b]	1.0 (0.5–2.0)	1.2 (0.5–4.0)	1.5 (0.5–3.0)
C_{max} (ng/mL)	20.5±18.9	16.6±11.4	10.8±3.0
$AUC_{0\text{-}24\ h}$ (h · ng/mL)	91.2 (36.5, 146)[c]	67.4±30.6	66.5±28.7
$AUC_{0\text{-}inf}$ (h · ng/mL)	129 (44.6, 214)[c]	79.0±44.8	91.3±43.5[d]
$t_{1/2}$ (h)	15.8 (9.27, 22.3)[c]	9.2±3.9	12.8±5.1
Vz/F (L)	2249 (1500, 2998)[c]	2633±894	3878±2061[d]
CL/F (L/h)	135 (46.7, 224)[c]	230±101	263 ±144[d]
Day 15			
t_{max} (h)[b]	1.0 (0.5–4.0)	1.5 (0.4–2.0)	2.0 (0.5–8.0)
C_{max} (ng/mL)	19.4 ±18.3	14.4±4.3	11.6±6.1
$AUC_{0\text{-}24}$ (h · ng/mL)	89.1±60.0	88.5±25.7	87.9±40.8
$AUC_{0\text{-}inf}$ (h · ng/mL)	177 (107, 247)[c]	133±35.1[d]	153±57.5[e]
$t_{1/2}$ (h)	18.4±6.3	17.8±5.5	18.4±5.0[f]
Vz/F (L)	2094 (1334, 2854)[c]	2548±540[d]	3598±1086[e]
CL/F (L/h)	67.0 (40.4, 93.6)[c]	118±27.2[d]	150±68.6[e]

[a] Values are means ± SDs, unless otherwise noted. [b] Values are medians (ranges). [c] n=2; values are mean (individual values). [d] n=3. [e] n=4. [f] n=5. AUC, area under the curve; CL/F, apparent clearance; Vz/F, volume of distribution during the terminal phase

subjects. Additional research is necessary to address this issue properly. When plasma concentrations of panobinostat after oral administration were compared between Japanese and non-Japanese subjects in a previous study [10], the average concentration appeared to be somewhat lower in the Japanese subjects. However, the range of individual values between the two populations largely overlapped. This apparent difference may have been attributable to the large interindividual variability and limited number of patients; therefore, these data did not conclusively indicate an ethnic difference in the pharmacokinetic profile of panobinostat. A meta-analysis including other ongoing studies will enable us to clarify the cause of the large interindividual variability, including potential ethnic factors.

HBF is the predominant hemoglobin in the fetus, but it is gradually replaced by adult hemoglobin after birth. Experi-

Table 7 Tumor response to panobinostat

Tumor type	Dose level (mg/day)	Prior medication use		PFS (days)	Best response
		No. of regimens	Antineoplastic drugs used		
CTCL	10	2	Predonine, cyclosporine	–	Stable disease
CTCL	10	4	Etoposide, INF-γ, nidran	–	Stable disease
Mesothelioma	10	4	Cisplatin, gemcitabine, irinotecan	78	Stable disease
Esophagus	15	3	Fluorouracil, cisplatin, nedplatin, docetaxel	85	Unknown
CRC	15	5	Irinotecan, fluorouracil, doxorubicin, mitomycin c, cisplatin, oxaliplatine, s-1	78	Unknown
Thymus	15	1	Paclitaxel, carboplatin	24	Progressive disease
NSCLC	15	5	Paclitaxel, carboplatin, gefitinib, gemcitabine, irinotecan	23	Progressive disease
Larynx	20	5	Cisplatin, fluorouracil, carboplatin, paclitaxel, s-1, docetaxel	≥25	Unknown
CRC	20	3	Oxaliplatine, s-1, fluorouracil, irinotecan	51	Unknown
CRC	20	3	Tegafur uracil, irinotecan, s-1, fluorouracil, oxaliplatine	79	Stable disease
Leiomyosarcoma	20	1	Imatinib	164	Stable disease
NSCLC	20	6	Vinorelbine, cisplatin, gefitinib, s-1, gemcitabine, docetaxel	71	Stable disease
NSCLC	20	2	Gemcitabine, cisplatin, docetaxel	253	Stable disease

CRC colorectal cancer, *CTCL* cutaneous T-cell lymphoma, *INF-γ* interferon gamma, *NSCLC* non-small cell lung cancer, *PFS* progression-free survival

mental studies have shown that DACIs can induce the re-expression of HBF [17]. We measured HBF to evaluate whether it could be used as a pharmacodynamic biomarker of DACIs; however, no apparent relation was observed between panobinostat administration and HBF. In our study, an absolute increase in HBF over time was observed in all three colorectal cancer patients. This finding supports recent evidence of HBF-containing red blood cells within colorectal tumor tissues, which suggests that the colonic microenvironment may stimulate extramedullary fetal-type hematopoiesis [18].

Unfortunately, despite promising preclinical evidence, little clinical activity was observed in this trial. No objective responses were observed, although one patient with leiomyosarcoma and one with non-small cell lung cancer achieved progression-free survival of >5 months. However, encouraging activity at higher doses was recently reported. Panobinostat induced clinical responses in CTCL patients who received doses of 20 or 30 mg on MWF, although in the trial a dose of 30 mg on MWF was considered excessively toxic [7] Additionally, clinical responses have been observed in heavily pretreated patients with Hodgkin lymphoma who received panobinostat at doses ≥30 mg on MWF weekly or ≥45 mg on MWF every other week [19].

Panobinostat should be explored at higher doses than evaluated in this trial. Based on the patient population evaluated in this trial (i.e. advanced solid tumors or CTCL), and emerging global clinical data at that time the decision was made to stop dose escalation at 20 mg. Of note, in a preliminary report of a trial in Western patients (conducted in Australia, Germany, and the United States) with solid tumors or Non-Hodgkin lymphoma receiving oral panobinostat on a MWF schedule, dose-limiting toxicities of grade 3 diarrhea and grade 4 thrombocytopenia were observed at 30 mg and grade 3 fatigue at 20 mg [8]. However, the results obtained here suggest that single-agent treatment with panobinostat at 20 mg on MWF might be suboptimal and that greater clinical benefit might be observed at higher doses.

Panobinostat is likely to have greater therapeutic potential when administered in combination with other therapeutic agents. Recently, panobinostat in combination with bortezomib showed antitumor activity against relapsed or refractory multiple myeloma in a phase Ib trial. Clinical efficacy was observed in 18 (14 partial or better responses and 4 minor responses) of 28 evaluable patients (64%). Responses were also seen in patients refractory to prior bortezomib treatment, which suggests synergy of the combination [20]. Furthermore, from a theoretical standpoint, many anticancer agents have the potential to synergize with the epigenetic regulation mediated by panobinostat; e.g., drugs that have an overlapping mechanism of action or drugs that affect the same target through a complementary mechanism of action, which needs to be confirmed in future clinical trials.

In conclusion, a dose of 20 mg of panobinostat administered orally on MWF has been confirmed to be safe and tolerable for patients with advanced solid tumors or cutaneous T-cell lymphoma, although further studies should be conducted to establish the MTD. Given the promising data concerning the efficacy of panobinostat in Western patients with Hodgkin lymphoma and multiple myeloma, studies in Japanese patients with hematologic tumors should also be undertaken.

References

1. Bolden JE, Peart MJ, Johnstone RW (2006) Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 5:769–784. doi:10.1038/nrd2133
2. Prince HM, Bishton MJ, Harrison SJ (2009) Clinical studies of histone deacetylase inhibitors. Clin Cancer Res 15:3958–3969. doi:10.1158/1078-0432.CCR-08-2785
3. Atadja P (2009) Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. Cancer Lett 280:233–241. doi:10.1016/j.canlet.2009.02.019
4. Qian DZ, Kato Y, Shabbeer S, Wei Y, Verheul HM, Salumbides B, Sanni T, Atadja P, Pili R (2006) Targeting tumor angiogenesis with histone deacetylase inhibitors: the hydroxamic acid derivative LBH589. Clin Cancer Res 12:634–642. doi:10.1158/1078-0432.CCR-05-1132
5. O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B, Curly T, Moskowitz C, Portlock C, Horwitz S, Zelenetz AD, Frankel S, Richon V, Marks P, Kelly WK (2006) Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. J Clin Oncol 24:166–173. doi:10.1200/JCO.2005.01.9679
6. Shao W, Growney J, Feng Y, Wang P, Yan-Neale Y, O'Connor G, Kwon P, Yao Y, Fawell S, Atadja P (2008) Potent anticancer activity of a pan-deacetylase inhibitor panobinostat (LBH589) as a single agent in in vitro and in vivo tumor models. AACR Meeting Abstracts 2008:735
7. Ellis L, Pan Y, Smyth GK, George DJ, McCormack C, Williams-Truax R, Mita M, Beck J, Burris H, Ryan G, Atadja P, Butterfoss D, Dugan M, Culver K, Johnstone RW, Prince HM (2008) Histone deacetylase inhibitor panobinostat induces clinical responses with associated alterations in gene expression profiles in cutaneous T-cell lymphoma. Clin Cancer Res 14:4500–4510. doi:10.1158/1078-0432.CCR-07-4262
8. Prince HM, George D, Patnaik A, Mita M, Dugan M, Butterfoss D, Masson E, Culver KW, Burris HA III, Beck J (2007) Phase I study of oral LBH589, a novel deacetylase (DAC) inhibitor in advanced solid tumors and non-hodgkin's lymphoma. ASCO Meet Abstr 25:3500
9. Duvic M, Hymes K, Heald P, Breneman D, Martin AG, Myskowski P, Crowley C, Yocum RC, Bexarotene Worldwide Study Group (2001) Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II-III trial results. J Clin Oncol 19:2456–2471
10. Woo MM, Culver K, Li W, Liu A, Scott J, Parker K, Jalaluddin M, Laird G, Cooper MR, Schran HF (2008) Panobinostat (LBH589) pharmacokinetics (PK): implication for clinical safety and efficacy. Ann Oncol 19:487P

11. Lane AA, Chabner BA (2009) Histone deacetylase inhibitors in cancer therapy. J Clin Oncol 27:5459–5468. doi:10.1200/JCO.2009.22.1291
12. Chang AN, Cantor AB, Fujiwara Y, Lodish MB, Droho S, Crispino JD, Orkin SH (2002) GATA-factor dependence of the multitype zinc-finger protein FOG-1 for its essential role in megakaryopoiesis. Proc Natl Acad Sci USA 99:9237–9242. doi:10.1073/pnas.142302099
13. Matsuoka H, Unami A, Fujimura T, Noto T, Takata Y, Yoshizawa K, Mori H, Aramori I, Mutoh S (2007) Mechanisms of HDAC inhibitor-induced thrombocytopenia. Eur J Pharmacol 571:88–96. doi:10.1016/j.ejphar.2007.06.015
14. Bates SE, Rosing DR, Fojo T, Piekarz RL (2006) Challenges of evaluating the cardiac effects of anticancer agents. Clin Cancer Res 12:3871–3874. doi:10.1158/1078-0432.CCR-06-1017
15. Strevel EL, Ing DJ, Siu LL (2007) Molecularly targeted oncology therapeutics and prolongation of the QT interval. J Clin Oncol 25:3362–3371. doi:10.1200/JCO.2006.09.6925
16. Weber HA, Tai F, Paul S, Schindler J, Woo MM, Spence S, Marlowe J, Lin R (2009) QT interval measurements in patients with hematologic malignancies and solid tumors treated with oral panobinostat (LBH589). ASH Annu Meet Abstr 114:3781
17. Witt O, Monkemeyer S, Ronndahl G, Erdlenbruch B, Reinhardt D, Kanbach K, Pekrun A (2003) Induction of fetal hemoglobin expression by the histone deacetylase inhibitor apicidin. Blood 101:2001–2007. doi:10.1182/blood-2002-08-2617
18. Wolk M, Martin JE, Reinus C (2006) Development of fetal haemoglobin-blood cells (F cells) within colorectal tumour tissues. J Clin Pathol 59:598–602. doi:10.1136/jcp.2005.029934
19. Dickinson M, Ritchie D, DeAngelo DJ, Spencer A, Ottmann OG, Fischer T, Bhalla KN, Liu A, Parker K, Scott JW, Bishton M, Prince HM (2009) Preliminary evidence of disease response to the pan deacetylase inhibitor panobinostat (LBH589) in refractory Hodgkin Lymphoma. Br J Haematol 147:97–101. doi:10.1111/j.1365-2141.2009.07837.x
20. San-Miguel JF, Sezer O, Siegel D, Guenther A, Mateos M, Prosser I, Cavo M, Blade J, Boccadoro M, Bengoudifa BR, Klebsattel M, Bourquelot PM, Anderson KC (2009) A Phase IB, multi-center, open-label dose-escalation study of oral panobinostat (LBH589) and I.V. Bortezomib in patients with relapsed multiple myeloma. ASH Annu Meet Abstr 114:3852

INTERNAL MEDICINE

□ CASE REPORT □

Development of Cushing's Syndrome During Effective Chemotherapy for Small Cell Lung Cancer

Koichi Suyama, Yoichi Naito, Kiyotaka Yoh, Seiji Niho, Koichi Goto, Hironobu Ohmatsu, Yutaka Nishiwaki and Yuichiro Ohe

Abstract

Paraneoplastic Cushing's syndrome caused by ectopic adrenocorticotropin (ACTH) production has been reported. However, most cases of this syndrome are diagnosed before first-line chemotherapy or at the time of disease recurrence. Here, we present a 53-year-old man who gradually developed the symptoms of Cushing's syndrome during effective chemotherapy for small cell lung cancer. His symptoms were controlled using mitotane, but his primary cancer progressed and he died 5 months after the start of chemotherapy. This very rare case of Cushing's syndrome associated with small cell lung cancer during effective chemotherapy is presented here.

Key words: Cushing's syndrome, ACTH, lung cancer, chemotherapy

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Introduction

Paraneoplastic Cushing's syndrome caused by ectopic adrenocorticotropin (ACTH) production has been reported. However, most cases of this syndrome are diagnosed before first-line chemotherapy or at the time of disease recurrence. A few reports have described the gradual emergence of the symptoms of Cushing's syndrome during effective treatment for lung cancer. Identifying the symptoms of Cushing's syndrome at an early stage is important from the perspective of early diagnosis. Here, we present a case of paraneoplastic Cushing's syndrome that emerged gradually during effective chemotherapy for small cell lung cancer.

Case Report

A 53-year-old man presented with a cough, sputum, and dyspnea lasting for about two months. A plain chest radiograph at another hospital showed an abnormal shadow in a hilum of the left lung. A bronchoscopy revealed a small cell lung cancer (SCLC). He was referred to our hospital for treatment.

The patient had smoked 30 cigarettes a day for 32 years. Computed tomography (CT) of the chest revealed a mass in the left hilum of the lung and mediastinal lymph node swelling (Fig. 1A). No tumors other than those in the left thorax and no enlarged lymph nodes except those were found. His laboratory findings, including the serum potassium level, were almost normal. Regarding serum tumor markers, squamous cell carcinoma-related antigen and carcinoma-related antigen were not detected, but the serum neuron-specific enolase (NSE) level was 32.1 ng/mL (normal, <16.3 ng/mL) and the serum Pro-GRP level was 473 pg/mL (normal, <46 pg/mL). The clinical stage was T2N3M0, indicating limited SCLC.

At the time of hospitalization, the physical findings were not characteristic of a Cushingoid appearance. Because of the large radiation field, chemotherapy using cisplatin and etoposide was first performed (Fig. 2). After two cycles of chemotherapy, a tumor reduction was confirmed using CT (Fig. 1B). Thereafter, the patient began to complain of chest pain. We consulted a cardiologist, and three stenosed lesions were discovered in his coronary arteries. Percutaneus transluminal coronary angioplasty was performed for one lesion. Thereafter, we reinitiated chemotherapy after changing cisplatin to carboplatin to reduce the cardiac burden. Prior to the fourth round of chemotherapy, he developed hypoka-

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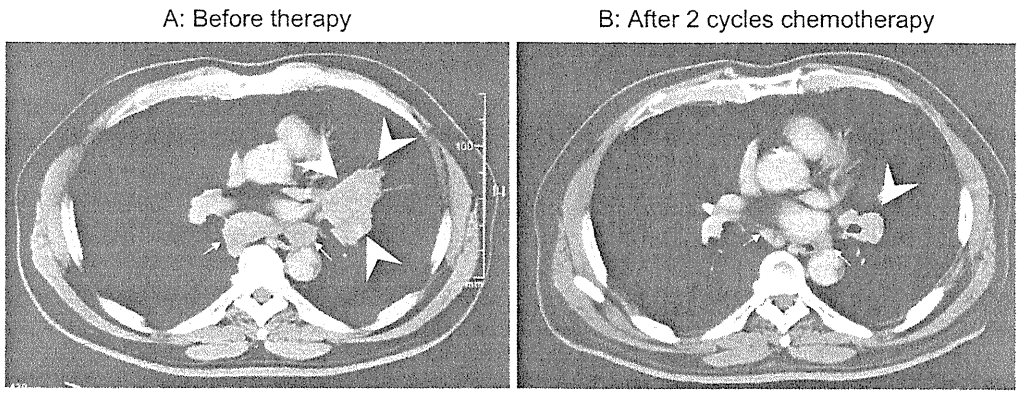
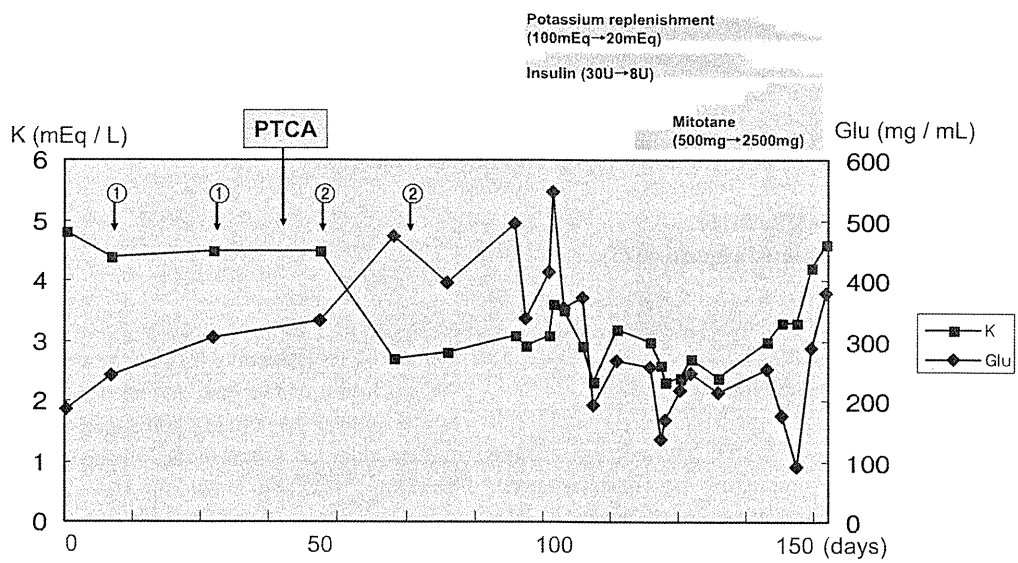


Figure 1. **A: Computed tomography (CT) image obtained before chemotherapy. A chest CT revealed a mass in the left hilum of the lung (arrowhead) and mediastinal lymph node swelling (arrow). B: CT image obtained after 2 cycles of chemotherapy. The main tumor and swollen lymph node show signs of reduction.**



①: CDDP+ETP ②: CBDCA+ETP
PTCA: Percutaneous transluminal coronary angioplasty

Figure 2. **Clinical course.**

lemia. His blood glucose level also gradually began to increase. Because these symptoms were not severe, we continued the chemotherapy. The serum tumor marker kept decreasing (NSE: max 50.3 ng/mL→21.5 ng/mL, Pro-GRP: max 1172 pg/mL→695 pg/mL) during the chemotherapy period. After completing 4 cycles of chemotherapy, he had developed severe hypokalemia, diabetes, hypertension and a depressive state (Fig. 2). He also exhibited centripetal obesity and a buffalo hump. We started the administration of potassium and insulin. However, no response to treatment was observed. The NSE and pro-GRP levels, which had been declining, began to rise. We speculated that these findings were consistent with Cushing's syndrome. The plasma ACTH concentration was 481.0 pg/mL (normal range, 7.2-63.3 pg/mL) and the plasma cortisol concentration was 144.0 μg/dL (normal range, 4.0-18.3 μg/dL). The serum ACTH concentration failed to be suppressed after treatment with 1 mg of dexamethasone overnight. It also was not suppressed after metyrapone loading. Magnetic resonance imaging (MRI) did not reveal a pituitary mass. These results suggested that the patient's Cushing's syndrome was caused by ectopic ACTH production associated with the SCLC.

The patient was treated with mitotane (500 mg/day). We gradually increased the amount of mitotane, reaching a final dosage of 2500 mg/day. After the start of mitotane treatment, his hypokalemia and hyperglycemia gradually improved. The amount of required potassium and insulin also decreased (Fig. 2). The plasma ACTH and cortisol concentration also decreased (ACTH: 481 pg/mL→329.0 pg/mL, cortisol: 144.0 μg/dL→89.0 μg/dL). However, his primary

lung cancer was progressing. Second-line chemotherapy could not be started because of the patient's uncontrollable symptoms, poor performance status, and the refusal of the patient to undergo chemotherapy. He died 5 months after the start of the initial chemotherapy.

Discussion

A previous retrospective study demonstrated that the incidence of paraneoplastic Cushing's syndrome is 5% or less among all SCLC patients (1). In the recent literature, the incidence of SCLC associated with paraneoplastic syndrome has seemed to decrease (2). A possible explanation for this trend might be the recent improvements in diagnosis, chemotherapy, and radiotherapy. However, SCLC patients who develop paraneoplastic syndrome still have a poor prognosis because of various complications. Reportedly, 43% of SCLC patients with ectopic ACTH production experienced severe infections that contributed significantly to their eventual deaths (1). Another study reported a high rate of fatal infections (about 28%) and nonfatal infections in Cushing's syndrome (3). The cause of such infections might be hypercortisolism. The early diagnosis of Cushing's syndrome is very important for improving patient survival.

As shown in the case presentation, the chemotherapy was considered to have been effective. However, the patient gradually developed the symptoms of Cushing's syndrome. Most cases of Cushing's syndrome reportedly develop at the time of the initial presentation or the relapse of SCLC (1). Patients who develop Cushing's syndrome often have a poor outcome because of chemoresistance. The worsening of Cushing's syndrome during effective chemotherapy is thought to be very rare. One possible reason for the poor outcome in the present case is that chemoresistant cell clones might have produced the ACTH. In other words, cell clones that survived the chemotherapy might have begun to proliferate rapidly after chemotherapy. Thus, "the chemoresistant cancer cell clones that produced ACTH" might have contributed to the poor outcome of the present patient with SCLC who developed ectopic ACTH syndrome. Vanhees et al reported a case of syndrome of inappropriate antidiuretic hormone (SIADH) associated with effective chemotherapy in SCLC (4). They hypothesized that the release of ADH from the malignant cells during the early tumor breakdown from chemotherapy resulted in SIADH. As well as this hypothesis, the present case might have had the possibility of developing Cushing's syndrome from the release of ACTH from malignant cells in the period of rapid cell necrosis due to effective chemotherapy.

Once Cushing's syndrome is suspected, a differential diagnosis must be made by performing an overnight dexamethasone test and metyrapone test. If no ectopic ACTH production is present, the serum ACTH level should be greatly suppressed after the administration of 1 mg dexamethasone and should increase after the administration of metyrapone. If pituitary Cushing's disease is present, the serum ACTH level should also increase after the administration of metyrapone. The ACTH level in the present patient did not respond to the administration of dexamethasone and metyrapone. These results indicated that the patient had ectopic ACTH production; in this manner, a final diagnosis of Cushing's syndrome as a result of SCLC was confirmed. He was treated with mitotane to counteract the ectopic ACTH production. Mitotane, or o,p'DDD, can block the adrenocortical steroid synthesis by inhibition of cholesterol side-chain cleavage and 11β-hydroxylase. This inhibition affects extra-adrenal cortisol disposition by inducing its hepatic clearance, reducing hormone production, and ameliorating the symptoms of hormone excess (5). A recent study from a single center showed the ideal therapeutic control of the ectopic ACTH secretion syndrome by using mitotane (6). In that study, 20 of the 23 patients showed clinical improvement of Cushing's syndrome manifestations. The present patient's symptoms arising from Cushing's syndrome began to improve by using mitotane, but his SCLC also began to progress and could not be stopped, mainly because treatment of the cancer itself could not be resumed.

We could not perform an immunohistochemical study for ACTH using primary or metastatic tumor specimens for the diagnosis of ectopic ACTH secretion. ACTH produced from neoplasms is said to have a different structure than that of wild-type ACTH, and conventional immunohistochemical staining using a polyclonal anti-ACTH antibody may not be useful in tumor cells (7). The predominant form of ACTH in tumor extracts is reportedly a large ACTH molecule that cannot be detected using the usual immunohistochemical staining (8).

In summary, we have described a rare case of Cushing's syndrome that progressed even during effective chemotherapy for SCLC. The clinical symptoms of Cushing's syndrome must be kept in mind when treating patients with lung cancer, since early detection and appropriate treatment can overcome the otherwise poor prognosis.

The authors state that they have no Conflict of Interest (COI).

References

1. Shepherd FA, Laskey J, Evans WK, Goss PE, Johansen E, Khamsi F. Cushing's syndrome associated with ectopic corticotropin production and small-cell lung cancer. J Clin Oncol **10**: 21-27, 1992.
2. Noorlander I, Elte JW, Manintveld OC, et al. A case of recurrent non-small-cell lung carcinoma and paraneoplastic Cushing's syndrome. Lung Cancer **51**: 251-255, 2006.
3. Delisle L, Boyer MJ, Warr D, et al. Ectopic corticotropin syndrome and small-cell carcinoma of the lung. Clinical features, outcome, and complications. Arch Intern Med **153**: 746-752, 1993.
4. Vanhees SL, Paridaens R, Vansteenkiste JF. Syndrome of inappropriate antidiuretic hormone associated with chemotherapy-induced tumor lysis in small-cell lung cancer: Case report and literature review. Ann Oncol **11**: 1061-1065, 2000.
5. Veytsman I, Nieman L, Fojo T. Management of endocrine manifestations and the use of mitotane as a chemotherapeutic agent for

adrenocortical carcinoma. J Clin Oncol **27**: 4619-4629, 2009.

6. Donadille B, Groussin L, Waintrop C, et al. Management of Cushing's syndrome due to ectopic adrenocorticotropin secretion with 1,ortho-1, para'-dichloro-diphenyl-dichloro-ethane: findings in 23 patients from a single center. J Clin Endocrinol Metab **95**: 537-544, 2010.
7. Singer W, Kovacs K, Ryan N, Horvath E. Ectopic ACTH syndrome: clinicopathological correlations. J Clin Pathol **31**: 591-598, 1978.
8. Gewirtz G, Yalow RS. Ectopic ACTH production in carcinoma of the lung. J Clin Invest **53**: 1022-1032, 1974.

Clinical Outcome of Small Cell Lung Cancer with Pericardial Effusion but without Distant Metastasis

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Background: Pericardial effusion is defined as M1a in the Union Internationale Contre le Cancer seventh tumor, node, metastasis edition for lung cancer. The clinical course of small cell lung cancer (SCLC) with pericardial effusion but without distant metastasis (M1a) has not been adequately investigated.

Methods: The medical records of patients with SCLC treated at the National Cancer Center Hospital East between July 1992 and December 2007 were reviewed. During this period, 766 patients were newly diagnosed as having SCLC. Thirty-three of the 416 patients with limited disease (LD) SCLC (8%) had pericardial effusion. Seventy-nine patients with LD-SCLC (19%) had ipsilateral pleural effusion or dissemination. Of these, 16 patients had both pericardial and ipsilateral pleural effusion. We divided the 96 M1a patients into two subgroups: group A ($n = 33$) included patients with pericardial effusion, and group B ($n = 63$) included patients with ipsilateral pleural effusion or disseminated pleural nodules but without pericardial effusion.

Results: The median survival time among the patients with LD-M1a was 13.4 months (95% confidence interval: 10.7–16.6 months), and the 1-, 2-, 3-, and 5-year survival rates were 56%, 18%, 9%, and 8%, respectively. The survival of the patients with LD-M1a was intermediate between those of the patients with LD-M0 and patients with extensive disease M1b ($p < 0.0001$). The overall survival period was not statistically different between groups A and B ($p = 0.5182$). Nineteen patients in group A received chemoradiotherapy, but only two patients survived for more than 2 years (2- and 5-year survival rate: 11% both). Twenty-six patients in group B received chemoradiotherapy, and four patients survived for more than 5 years (5-year survival rate: 18%).

Conclusions: Long-term survival was achieved among patients with SCLC with pericardial effusion but without distant metastasis who successfully underwent chemoradiotherapy, although 5-year survival rate in these patients was relatively lower than in patients with SCLC with ipsilateral pleural effusion but without pericardial effusion or distant metastasis.

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Key Words: Small cell lung cancer, Limited disease, Pericardial effusion.

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Lung cancer is the leading cause of cancer-related deaths worldwide. Small cell lung cancer (SCLC) accounts for approximately 15% of all forms of lung cancer. Compared with non-SCLC, SCLC grows rapidly, quickly disseminates to the regional lymph nodes and distant sites, and is sensitive to chemotherapy with a response rate of 70 to 80%. The Veterans Administration Lung Study Group proposed a clinical two-stage system for SCLC that distinguishes limited disease (LD) and extensive disease (ED). LD is defined as being limited to one hemithorax, including mediastinal, contralateral hilar, and ipsilateral supraclavicular lymph nodes, whereas ED represents tumor spread beyond these regions.[1] The current standard care for LD-SCLC is a combination of chemotherapy and thoracic radiotherapy (TRT). Conversely, ED-SCLC is treated with chemotherapy alone. The original definition of LD was a tumor volume that could be encompassed by a reasonable radiotherapy plan. According to the International Association for the Study of Lung Cancer (IASLC)'s consensus report, however, the classification of LD-SCLC includes bilateral hilar or supraclavicular nodal involvement and ipsilateral pleural effusion, regardless of whether the cytological findings are positive or negative.[2] Pericardial effusion has not been defined precisely.

In 2007, the IASLC proposed a new tumor, node, metastasis (TNM) classification for lung cancer,[3–6] and the Union Internationale Contre le Cancer (UICC) seventh TNM edition has been available since 2009. According to the UICC seventh TNM edition, malignant pleural or pericardial effusion and tumor with pleural nodules are defined as M1a, leading to stage IV. An analysis of 12,620 patients with SCLC in the IASLC database demonstrated that patients who have ipsilateral pleural effusion without extrathoracic metastases (M1a) have a survival that is intermediate between stages I and III without effusion and stage IV. Nevertheless, no information regarding the presence of pericardial effusion is available in the IASLC database.[7]

Our previous retrospective analysis also demonstrated that the survival of patients with LD-SCLC with ipsilateral pleural effusion was intermediate between those of patients with LD without ipsilateral pleural effusion and patients with

ED, and long-term survival was achieved by patients with LD-SCLC who successfully underwent definitive TRT after their ipsilateral pleural effusion had disappeared after induction chemotherapy.[8] In this retrospective study, we investigated the clinical course and overall survival among patients with LD-SCLC with pericardial effusion, compared with those among patients with ED-SCLC or LD-SCLC with or without ipsilateral pleural effusion.

PATIENTS AND METHODS

In this study, LD-SCLC was defined as disease limited to one hemithorax, including mediastinal, contralateral hilar, and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion; ED-SCLC was defined as tumor spread beyond these manifestations.

We retrospectively reviewed the medical records of patients with lung cancer treated at the National Cancer Center Hospital East between July 1992 and December 2007.

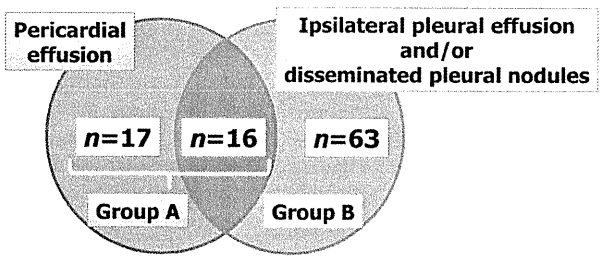

FIGURE 1. Patients with small cell lung cancer with M1a. Group A included patients with pericardial effusion, and group B included patients with ipsilateral pleural effusion or disseminated pleural nodules, but without pericardial effusion.

During this period, 766 patients were newly diagnosed as having SCLC. Four hundred sixteen patients were diagnosed as having LD-SCLC and 350 were diagnosed as having ED-SCLC using conventional staging procedures, including a medical history and physical examination, chest radiography, computed tomography (CT) scan of the chest, CT scan or ultrasound of the abdomen, bone scan, and CT scan or magnetic resonance imaging of the brain. Thirty-three of the 416 patients with LD-SCLC (8%, 95% confidence interval [CI]: 6–11%) had pericardial effusion and were included in this study. Seventy-nine of the 416 patients with LD-SCLC (19%, 95% CI: 15–23%) had ipsilateral pleural effusion or dissemination. Four patients had a disseminated mass without pleural effusion detected using CT scan. Sixteen patients with LD-SCLC had both pericardial and ipsilateral pleural effusion. Therefore, 63 patients with LD-SCLC had ipsilateral pleural effusion or dissemination without pericardial effusion. We divided the 96 M1a patients into two subgroups: group A included patients with pericardial effusion, and group B included patients without pericardial effusion. Group B patients had ipsilateral pleural effusion or disseminated pleural nodules (Figure 1).

The overall survival time was defined as the interval between the start of treatment and death or the final follow-up visit. The median overall survival time was estimated using the Kaplan-Meier analysis method.[9] Survival data were compared among the groups using a log-rank test. This study was approved by an institutional review board.

RESULTS

The patient characteristics are listed in Table 1. Eighty-three percent of the patients were male, and 81% had a performance status of 0 or 1. Fifty-four percent of the patients

TABLE 1. Patient Characteristics

	ED-SCLC (M1b)	LD-SCLC with Pericardial Effusion (M1a) (Group A)	LD-SCLC with Ipsilateral Pleural Effusion but without Pericardial Effusion (M1a) (Group B)	LD-SCLC (M0)
No. of patients	350	33	63	320
Sex				
Male	291	29	50	262
Female	59	4	13	58
Age (yr)				
Median	66	67	68	66
Range	28–85	37–82	46–83	22–87
Performance status				
0	22	0	4	108
1	224	25	47	190
2	63	6	9	15
3–4	41	2	3	7
Treatment delivered				
Chemotherapy	316	14	36	50
Chemoradiotherapy	25	19	26	224
Surgery + chemotherapy	0	0	0	33
Surgery alone	0	0	0	10
Best supportive care	9	0	1	3

LD, limited disease; SCLC, small cell lung cancer; ED, extensive disease.

TABLE 2. Timing of Thoracic Radiotherapy in Patients with M1a Small Cell Lung Cancer

Timing of Thoracic Radiotherapy	LD-SCLC with Pericardial Effusion (M1a) (Group A, n = 19)	LD-SCLC with Ipsilateral Pleural Effusion but without Pericardial Effusion (M1a) (Group B, n = 26)
Concurrently with the first course of chemotherapy	0	3
Concurrently with the second course of chemotherapy	0	4
Concurrently with the third course of chemotherapy	8	5
Concurrently with the fourth course of chemotherapy	4	0
Sequentially after chemotherapy	7	14

LD, limited disease; SCLC, small cell lung cancer.

received chemotherapy, and 38% received chemoradiotherapy. Six percent of the patients underwent surgical resection with or without adjuvant chemotherapy. Among the 96 patients with LD-M1a, all but one patient received chemotherapy (n = 50) or chemoradiotherapy (n = 45). Three patients underwent TRT (twice daily, 45 Gy in total) concurrently with the first course of chemotherapy. Four, 13, and four patients underwent TRT (once daily, 50 Gy in total) concurrently with the second, third, and fourth courses of chemotherapy, respectively. Twenty-one patients underwent TRT (once daily, 50 Gy in total) sequentially after chemotherapy. Among the group A patients, 12 patients underwent TRT concurrently with the third or fourth course of chemotherapy, and seven patients underwent TRT sequentially after chemotherapy. TRT was conducted if the pericardial effusion disappeared after induction chemotherapy. Among the group B patients, 12 patients underwent TRT concurrently with chemotherapy, and 14 patients underwent TRT sequentially (Table 2). Thirteen patients received prophylactic cranial irradiation of 25 Gy (seven patients in group A and six patients in group B).

Figure 2 shows the survival of all 766 patients with SCLC belonging to category M. The survival of patients with LD-M1a was intermediate between those of patients with LD-M0 and ED-M1b ($p < 0.0001$). Six hundred eighty-two patients have died. The median follow-up time was 65.8 months, ranging from 3.2 to 160.1 months. The median survival time among the patients with LD-M1a was 13.4 months (95% CI: 10.7–16.6 months), and the 1-, 2-, 3-, and 5-year survival rates were 56%, 18%, 9%, and 8%, respectively.

Survival analyses for the subgroup of patients with LD-M1a (n = 96) are shown in Figures 3, 4 and Table 3. Overall survival was not statistically different between groups A and B (p = 0.5182). All 14 patients who received chemotherapy in group A died within 3 years. One patient in group B who received chemotherapy as an initial treatment survived for more than 5 years, but this patient received chemoradiotherapy as a second-line treatment after a local

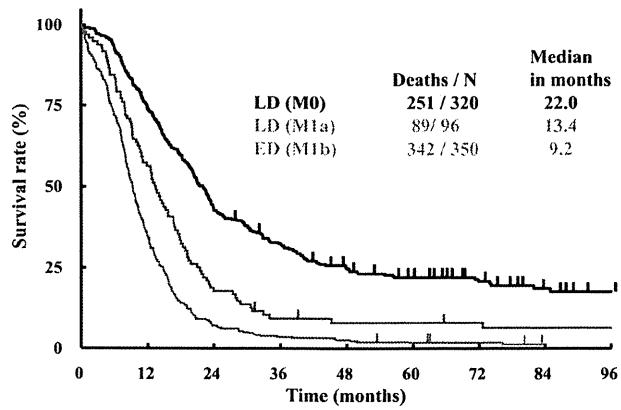

FIGURE 2. Overall survival among all 766 patients with M-category small cell lung cancer. LD, limited disease; ED, extensive disease.

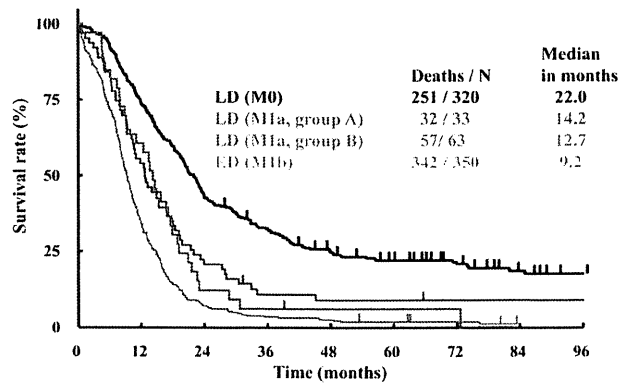

FIGURE 3. Overall survival among patients with M-category small cell lung cancer, subgroups A and B. LD, limited disease; ED, extensive disease.

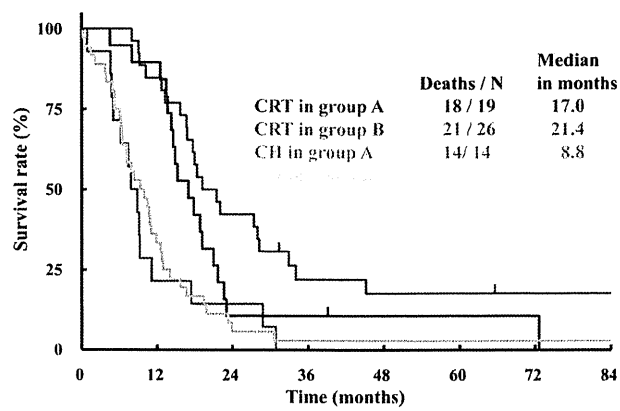

FIGURE 4. Overall survival among M1a patients with small cell lung cancer according to subgroups A, B, and initial treatment delivered. CRT, chemoradiotherapy; CH, chemotherapy.

TABLE 3. Survival Data

Subgroup	No. of Patients	Median Survival Time (mo) (95% CI)	1-yr Survival Rate (%)	2-yr Survival Rate (%)	3-yr Survival Rate (%)	5-yr Survival Rate (%)
ED (M1b)	350	9.2 (8.5–10.0)	34	7	3	2
LD (M0)	320	22.0 (20.0–23.5)	74	43	33	22
LD with pericardial effusion (group A)	33	14.2 (9.1–17.5)	61	12	6	6
Receiving CRT	19	17.0 (13.6–21.0)	89	11	11	11
Receiving Chemotherapy	14	8.8 (4.7–11.1)	21	14	0	0
LD with ipsilateral pleural effusion but without pericardial effusion (group B)	63	12.7 (10.2–16.7)	54	21	11	9
Receiving CRT	26	21.4 (16.7–28.2)	85	42	22	18
Receiving chemotherapy	36	9.3 (6.3–11.8)	33	6	3	3

CI, confidence interval; ED, extensive disease; LD, limited disease; CRT, chemoradiotherapy.

TABLE 4. Six Patients with M1a Small Cell Lung Cancer who Survived for More Than 5 yr

Age (yr)	Sex	Group	Initial Treatment	Survival Time (mo)	State
64	M	A	Chemoradiotherapy	72.6	Dead
70	F	B	Chemoradiotherapy	146.5	Alive
53	M	B	Chemotherapy[a]	140.4	Alive
73	F	B	Chemoradiotherapy	138.0	Alive
72	M	B	Chemoradiotherapy	117.0	Alive
68	M	B	Chemoradiotherapy	65.5	Alive

[a] This patient received chemoradiotherapy as a second-line treatment after a local recurrence. Therefore, all six patients received chemoradiotherapy and achieved long-term survival for more than 5 yr.

M, male; F, female.

recurrence. Four of the 26 patients who received chemoradiotherapy in group B survived for more than 5 years (Table 4). Conversely, only 2 of the 19 patients who received chemoradiotherapy in group A survived for more than 2 years. One patient developed a local recurrence at 4 years and 10 months after the initiation of first-line chemoradiotherapy and died of lung cancer 14 months later. The remaining patient also developed a local recurrence at 2 years and 9 months after the initiation of first-line chemoradiotherapy and received second-line chemotherapy. This patient was still alive at the time of the data cutoff.

DISCUSSION

This retrospective analysis demonstrated that the survival of patients with SCLC and ipsilateral pleural or pericardial effusion (M1a) was intermediate between those of M0 and M1b patients. It is suitable that patients with ipsilateral pleural effusion or pericardial effusion belong to M1a category in the UICC seventh TNM edition. No statistically significant difference in the overall survival between M1a patients with pericardial effusion (group A) and those with ipsilateral pleural effusion but without pericardial effusion (group B) was observed. Among the patients who successfully underwent chemoradiotherapy, the patients in group B had 2-, 3-, and 5-year survival rates of 42%, 22%, and 18%, respectively, whereas the patients in group A had a 2-year survival rate of only 11%. Our previous retrospective analyses demonstrated that the median survival time of patients with cytologically positive and cytologically negative pleural effusion were 9.3 and 12.7 months, respectively. Furthermore, all 11 patients with cytologically positive pleural effusion died within 3 years.[8] Long-term survival for more than 5 years was achieved only by patients with cytologically negative pleural effusion. We speculate that an inflammatory process, such as atelectasis, causes ipsilateral pleural effusion in some patients. Conversely, most pericardial effusion is believed to be malignant. Therefore, long-term survival was seldom achieved by patients with pericardial effusion, even if they received chemoradiotherapy.

Recently, the applicability of the UICC seventh TNM edition for SCLC was investigated using the California Cancer Registry database. This database included 108 and 1518 M1a patients with pericardial effusion and pleural dissemination, respectively. No significant difference in overall survival was observed among patients with pleural or pericardial effusion (median survival time: 7 versus 7 months, 2-year survival rate: 16.7% versus 9.7%, respectively).[10] These data were comparable with our results. Nevertheless, no information regarding the treatment performed for the M1a patients was included in the previous article.

Our retrospective analysis has several limitations. First, the number of M1a patients with pericardial effusion was only 33, because only 8% of the patients with LD-SCLC exhibited pericardial effusion. Second, we did not conduct a cytological examination of the pericardial effusion. Pericardial puncture or drainage is usually performed in patients with cardiac tamponade. None of the patients in group A had cardiac tamponade; therefore, a pericardial puncture was technically difficult. Third, examination period was more than 15 years, from 1992 to 2007. Irinotecan, active for SCLC, has been commonly used from 2000 in Japan. Patients in this study were treated with a potential range of different chemotherapeutic agents during the period, which was not controlled.

Only 2 of 19 patients (11%) who received chemoradiotherapy in group A survived for more than 3 years. Con-

versely, all 14 patients who did not receive chemoradiotherapy in group A died within 3 years. TRT probably improves local control and achieves long-term survival in some patients. Definitive TRT is recommended in M1a patients with SCLC, if ipsilateral pleural or pericardial effusion has disappeared after induction chemotherapy.

In conclusion, the survival of patients with SCLC and ipsilateral pleural or pericardial effusion (M1a) is intermediate between those of M0 and M1b patients. No statistically significant difference in the overall survival of M1a patients with pericardial effusion and those with ipsilateral pleural effusion but without pericardial effusion was observed. Long-term survival was achieved among M1a patients with pericardial effusion who successfully underwent chemoradiotherapy, although 5-year survival rate in these patients was relatively lower than in M1a patients with ipsilateral pleural effusion but without pericardial effusion.

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REFERENCES

1. Zelen M. Keynote address on biostatistics and data retrieval. *Cancer Chemother Rep 3* 1973;4:31–42.
2. Stahel RA, Ginsberg R, Havemann K, et al. Staging and prognostic factors in small cell lung cancer: a consensus report. *Lung Cancer* 1989;5:119–126.
3. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol* 2007;2:706–714.
4. Postmus PE, Brambilla E, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for revision of the M descriptors in the forthcoming (seventh) edition of the TNM classification of lung cancer. *J Thorac Oncol* 2007;2:686–693.
5. Rami-Porta R, Ball D, Crowley J, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. *J Thorac Oncol* 2007;2:593–602.
6. Rusch VW, Crowley J, Giroux DJ, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. *J Thorac Oncol* 2007;2:603–612.
7. Shepherd FA, Crowley J, Van Houtte P, et al. The International Association for the Study of Lung Cancer lung cancer staging project: proposals regarding the clinical staging of small cell lung cancer in the forthcoming (seventh) edition of the tumor, node, metastasis classification for lung cancer. *J Thorac Oncol* 2007;2:1067–1077.
8. Niho S, Kubota K, Yoh K, et al. Clinical outcome of chemoradiation therapy in patients with limited-disease small cell lung cancer with ipsilateral pleural effusion. *J Thorac Oncol* 2008;3:723–727.
9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–481.
10. Ignatius Ou SH, Zell JA. The applicability of the proposed IASLC staging revisions to small cell lung cancer (SCLC) with comparison to the current UICC 6th TNM Edition. *J Thorac Oncol* 2009;4:300–310.

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Clinical Cancer Research

Phase I Dose-Escalation Study and Biomarker Analysis of E7080 in Patients with Advanced Solid Tumors

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Abstract

Purpose: E7080, an oral multitargeted receptor tyrosine kinase inhibitor, has antiangiogenic and antitumor activity. This Phase I study investigated maximum tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetics (PK), pharmacodynamics (PD), and efficacy in patients with advanced solid tumors.

Experimental Design: In this sequential, dose-escalation, open-label study E7080 was administered orally twice daily in a 2-week-on/1-week-off cycle. Plasma angiogenic proteins, circulating endothelial cells (CEC) and circulating progenitor cells (CEP) were measured for biomarker analysis.

Results: Twenty-seven patients (median age 53 years, performance status 0/1) were enrolled. E7080 was escalated from 0.5 to 1, 2, 4, 6, 9, 13, 16, and 20 mg bid by conventional 3-patient cohorts. During cycle 1, no grade 3/4 toxicity was observed up to 13 mg bid. DLTs included grade 3 AST/ALT increase in 1 patient at 16 mg bid and grade 3 platelet count decrease in 2 patients at 20 mg bid. The MTD of 13 mg bid was determined. After repeated doses, C_{max} and area under the plasma concentration–time curve increased in a dose-dependent manner. After 14 days' treatment, c-kit(+) CEPs and CECs significantly decreased in cycle 1, but c-kit(−) CEPs and CECs did not. Change from baseline in c-kit(+) CEC ratio in cycle 1 and baseline SDF1α, c-kit(+) CEPs and c-kit(+) CEP ratio significantly correlated with the E7080 therapeutic effect.

Conclusion: E7080 has manageable toxicity up to 13 mg bid when administered in a 2-week-on/1-week-off cycle and shows preliminary activity for durable disease control. Biomarker analysis suggested antiangiogenic activity correlated with antitumor activity in patients with a wide range of solid tumors. *Clin Cancer Res; 17(8); 2528–37. ©2011 AACR.*

Introduction

Angiogenesis, the development and proliferation of a vascular network, is fundamental to both initial tumor growth and progression to metastatic disease. VEGF is a key factor to drive tumor angiogenesis (1), and platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) also play an important role. PDGF receptor (PDGFR) tyrosine kinases are expressed on the surface of pericytes and smooth muscle cells, and both induce proliferation and contribute to vascular maturation (2, 3). FGF receptor (FGFR) tyrosine kinases expressed on the surfaces of endothelial cells (EC) and smooth muscle cells, promote signals for cell proliferation and survival, as well as the development and stabilization of blood vessels (4, 5). Upon inhibition of tumor VEGF, PDGF, and FGF may also be upregulated to induce and maintain angiogenic activity (6, 7).

The tyrosine kinase receptors for these angiogenic factors, along with their associated signaling pathways, represent putative targets for pharmacotherapeutic intervention in cancer patients. Several molecules have been developed specifically to target tyrosine kinase receptors. Multitargeted tyrosine kinase inhibitors exhibited notable antitumor effect and showed acceptable tolerability profiles (8–10). However, differences in target kinase selectivity and potency may influence individual efficacy and toxicity profiles.

E7080, an oral multitargeted receptor tyrosine kinase inhibitor with antiangiogenic and antitumor activity,

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

This article presents original material from a Phase I study of E7080. Findings from this study have been presented at the 44th Annual Meeting American Society of Clinical Oncology, May 30 to June 3, 2008, and the 13th Workshop of the Japanese Society for Molecular Target Therapy of Cancer, June 25, 2009.

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Translational Relevance

Tyrosine kinase receptors for angiogenic factors along with their associated signaling pathways represent recognized targets for pharmacotherapeutic intervention. E7080 is an oral multitargeted receptor tyrosine kinase inhibitor that has antiangiogenic and antitumor activity, and strongly inhibits a wide range of tyrosine kinases. This Phase I dose-escalation study determined the maximum tolerated dose, dose-limiting toxicities, pharmacokinetics, pharmacodynamics, and preliminary efficacy of E7080. The correlation of certain biomarkers with antitumor activity was also evaluated. E7080 showed a manageable toxicity at 13 mg or less bid doses (only 3 DLTs at ≥16 mg bid) and preliminary activity for durable disease control. Biomarker analysis of circulating endothelial and progenitor cells, suggested an antiangiogenic activity, which correlated with antitumor activity in patients with a wide range of advanced solid tumors.

strongly inhibits a wide range of tyrosine kinases, including VEGFR-1 (Flt1), VEGFR-2 (KDR), and VEGFR-3 (Flt4), FGFR-1, PDGFRβ, and c-kit (11). E7080 decreased VEGFR-2 phosphorylation in both ECs [half maximal inhibitory concentration (IC_{50}) 0.83 nmol/L] and cell-free assays (IC_{50} 4 nmol/L; refs. 11, 12). In addition, E7080 has been shown to inhibit the growth of vascular EC and the formation of vascular-like tube structures in culture cells, and suppress tumor progression in murine models with various tumor types (11–13). Inhibition of xenograft tumor growth by E7080 was observed at doses as low as 1.0 and 10.0 mg/kg, suggesting greater efficacy of this agent compared to preapproved VEGFR2 inhibitors, including sorafenib and sunitinib (13–15).

A phase I dose-escalation study was conducted to investigate the safety, pharmacokinetics (PK), pharmacodynamics (PD; via biomarker analysis), and preliminary efficacy of E7080 in patients with advanced solid tumors. In addition, the correlation of certain biomarkers with antitumor activity was evaluated.

Patients and Methods

Study design

This single-center, open-label, sequential dose-escalation study of E7080 (ClinicalTrials.gov identifier NCT00280397; study identification number E7080-J081-103) was conducted at the National Cancer Center Hospital (Tokyo, Japan) between January 24, 2006 and September 8, 2008. All patients provided written, informed consent and study approval was obtained from the Institutional Review Board at the National Cancer Center Hospital. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. As stipulated by Japanese guidelines, the initial starting dose of E7080 was set at the human equivalent (based on body surface area) of one third of the toxic low dose obtained in 4-week animal toxicity studies. These studies established the toxic low dose as 0.1 mg/kg, at which testicular toxicity was observed in dogs. The human equivalent dose is calculated as 3.2 mg, thus 1.0 mg was set as the initial dose for E7080 in this study.

The primary objective of the study was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of oral E7080 administered twice daily in a 2-week-on/1-week-off cycle in patients with advanced solid tumors. Secondary objectives included the assessment of PK, safety and tolerability, as well as determining a recommended dose for Phase II trials, and describing any observed tumor responses. Exploratory objectives included the characterization of PD markers of antitumor activity.

Eligibility criteria

Patients aged 20 to 75 years with histologically or cytologically confirmed advanced solid tumors that were resistant to standard therapy, or for which no standard therapy exists, and with Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, a life expectancy of 3 or more months, and adequate organ function were eligible. Postmenopausal women with amenorrhea for 12 or more months, or women of childbearing potential who were not pregnant, were eligible for inclusion in the study. All females and fertile male patients had to use adequate contraceptive methods during the study.

Patients were excluded if they had received previous anticancer treatments (including surgery or radiotherapy) or anticoagulant therapy (blood transfusions, blood agents, and hematopoietic factors) for at least 4 weeks prior to study entry or had incompletely recovered from prior therapy-related toxicity, except alopecia (evidence of grade ≥2 toxicity). Additional exclusion criteria included: brain metastases (symptomatic or requiring treatment); abnormal bone marrow, liver, or renal function [hemoglobin <9.0 g/dL, neutrophil count <1,500/μL, platelet count <100,000/μL, serum bilirubin >1.5 mg/dL, aspartate aminotransferase (AST) >100 IU/L, alanine aminotransferase (ALT) >100 IU/L, serum creatinine >1.5 mg/dL, or creatinine clearance <50 mL/min, measured by the Cockcroft–Gault method (16)], history of drug or alcohol abuse; infection with human immunodeficiency virus, hepatitis B or C; history of ischemic heart disease or clinically significant cardiac disorder within 6 months prior to study start; prolongation of the QT interval corrected using Fridericia's formula (QTcF) at screening (QTcF: >450 milliseconds for males and >470 milliseconds for females) or arrhythmia requiring treatment; history of cerebral infarction, hemorrhagic or thrombotic disease; evidence or history of malabsorption syndrome, surgery involving gastrointestinal anastomoses 4 or less weeks prior to enrollment or were recovering from surgery within 3 weeks of enrollment. Other exclusions included patients with duplicate resting mean systolic blood pressure ≥160 mmHg and diastolic blood pressure

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≥90 mmHg measurements or evidence of proteinuria at screening, those taking antiplatelet/anticoagulant therapy at screening and throughout the study, and patients receiving any other investigational drug within 4 weeks prior to study entry. Prophylactic administration of drugs including antiemetics, antihypertensives, and antidiarrheal agents was prohibited during Cycles 0 and 1. Concomitant use of cytochrome P450 (CYP3A4) inhibitors or inducers was prohibited throughout the study, due to potential interactions with or effects on metabolism of E7080.

Study treatment

Eligible patients were sequentially enrolled on escalating doses of oral E7080 using a standard 3 + 3 design. Dosing was scheduled to begin at 0.5 mg bid. In cycle 0 (7-day cycle), patients received a single oral dose of E7080 on day 1 for PK analysis and received no drug on days 2 to 7. In cycle 1 (21-day cycle), which immediately proceeded cycle 0, patients received E7080 bid on days 1 to 14. All patients were hospitalized for E7080 administration and evaluation during the full 28 days of cycles 0 and 1, thereafter the study was continued on an outpatient basis.

After tolerability was confirmed in cycles 0 and 1, the dose was doubled if a hematologic toxicity (≤grade 1 including anemia or lymphocytopenia) or nonhematologic toxicity (excluding alopecia and hypertension) in Cycle 1 was observed. When grade 2 toxicity occurred in 1 or more patient, the dose was escalated by 50% or less and, if grade 3 toxicity occurred, the dose was escalated by 33.3% or less thereafter.

Before dose escalation, all 3 patients in each cohort were required to complete cycle 1 of treatment. If no patients experienced a DLT at the first dose level, then the dose was escalated for the next 3 patients. If 1 of these experienced DLT, then 3 more patients were accrued at the same dose level. If none of these additional patients experienced a DLT, then the dose was escalated for the subsequent 3 patients. Dose escalation was terminated when 2 or more patients experienced a DLT at a given dose level. No intrapatient dose escalations were allowed. The presence of DLTs was assessed during cycles 0 and 1. From cycle 2 onwards, patients remained on study treatment at the same dose level as cycle 1 until tumor progression, unacceptable toxicity, or withdrawal due to other reasons.

Dose delays and reductions. To allow a patient to recover from any toxicities, a treatment cycle could be delayed for 14 or less days. Any patient who experienced a DLT that resolved sufficiently to allow continued treatment was eligible for treatment at a reduced dose level (≤75% and ≤50% of the previous dose for the first and second dose reductions, respectively). A maximum of up to 2 dose reductions was permitted.

Safety assessments

DLTs and MTD. DLTs were defined as grade 3 or more platelet count decrease, grade 4 neutropenia, any grade 3 or more nonhematologic toxicity (with exceptions of grade 4 hypertension not controlled by any antihypertensive drugs and grade ≥3 vomiting and diarrhea not controlled by antiemetic or antidiarrheal drugs), and failure to administer more than 75% of the planned doses of E7080 during the same cycle due to toxicity.

The MTD was defined as the highest dose at which no DLT was experienced by the first 3 patients in that cohort, or the dose at which a DLT was experienced by no more than 1 of 6 patients evaluable for toxicity.

Laboratory assessments and adverse events

Safety assessments scheduled for screening, throughout the study, and on study discontinuation included medical history, ECOG performance status, physical examination, vital signs, laboratory tests (hematology, blood biochemistry, and blood coagulation), urinalysis, electrocardiogram, and pregnancy testing. Adverse events (AE), including DLTs, were assessed throughout the study according to the Common Terminology Criteria for AEs (CTCAE Version 3.0; ref. 17).

Pharmacokinetics

In cycle 0, patients received a single oral dose of E7080 for PK analysis. Blood samples were collected at predose on day 1 and at 1, 3, 5, 6, 8, 12, 24, 48, 96, and 168 hours following administration. In cycle 1, patients received E7080 twice daily on days 1 to 14 of a 21-day cycle, except day 14 when E7080 was administered only once in the morning for PK analysis. Blood samples were collected from each patient before the first dose on days 1, 5, 8, 11, and 14 and at 1, 3, 5, 6, 8, 12, 24, 48, 96, and 168 hours after administration on day 14. Urine samples were collected 0 to 12 hours (the time equivalent to the interval between doses) after administration on day 14 in cycle 1. Plasma and urine E7080 concentrations were determined using liquid chromatography with tandem-mass spectrometry (Sumitomo Chemical Co. Ltd.).

Antitumor activity

Best overall tumor response and disease progression were measured using the Response Evaluation Criteria in Solid Tumors (RECIST; ref. 18). Tumor assessments were evaluated at screening, in cycles 2 and 3, and in every 2 cycles thereafter.

PD and baseline biomarkers

Blood samples for PD analysis were collected from each patient at predose of day 1 and 15 in cycle 1. Circulating endothelial cells (CEC) and circulating progenitor cells (CEP), which reflect active vascular turnover and angiogenesis (19, 20) were collected and measured within 24 hours of blood collection by fluorescence activated cell sorting (FACS). Briefly, peripheral blood mononuclear cells were incubated for 30 minutes at 4°C with fluorescein isothiocyanate (FITC)-conjugated anti-human CD34, FITC-conjugated anti-human CD45, phyroerythrin-conjugated anti-human CD117 (c-kit), and with FITC-conjugated anti-human CD133. Cells were then washed with phosphate-buffered saline and fixed in 4% paraformaldehyde,

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prior to FACS analysis, performed by SRL MediSearch Inc. using a FACScan cytometer and CellQuest software (Becton Dickinson).

To quantify CEC and CEP, the number of CD34-positive and CD45-negative cells was isolated, and CD133-negative cells and CD133-positive cells were determined as CEC and CEP, respectively. In addition, CEC and CEP were divided into c-kit positive [c-kit(+)] and negative [c-kit(−)] subpopulations. C-kit(+) ratio (%) was calculated as [c-kit(+) CEC or CEP]/[total CEC or CEP].

Plasma samples were collected before the first dose and stored at −80°C until assayed. Samples were analyzed in triplicate for baseline levels of angiogenic proteins and cytokines using a BioPlex (Bio-Rad Laboratories, Inc) assay (Mitsubishi Chemical Medience Corp.; ref. 21). Soluble VEGFR-1 (sVEGFR-1) and soluble VEGFR-2 (sVEGFR2) were measured by enzyme-linked immunosorbent assay (22).

Correlations of biomarker levels with the therapeutic effect of E7080 were investigated. Therapeutic effect was defined as the treatment duration from the first E7080 dose to discontinuation due to progressive disease or toxicity.

Statistical analysis

All patients who received at least 1 E7080 dose and had evaluable data were included in the safety, efficacy, PK, and PD analyses. PK analysis of plasma E7080 concentration-versus-time data were analyzed using WinNonlin Version 5.2 software. Noncompartmental analysis was performed to determine PK parameters of E7080. PD analysis was performed using Spearman's rank correlation coefficient for correlation analysis and Wilcoxon signed rank test to determine change from pretreatment.

Results

Patient characteristics

Twenty-seven evaluable patients received E7080. Demographic and baseline characteristics of these patients are shown in Table 1. Patients with a wide range of solid tumors were enrolled, with colon cancer being the most frequent (33.3%). The majority of patients (81.4%) had received 2 or more prior chemotherapy regimens.

Study treatment

Of the 27 patients who received E7080, 26 patients completed at least cycle 1, and 10 patients continued treatment for ≥6 cycles. One patient who received 6 mg bid did not complete cycle 0 due to a postrenal failure AE (not a DLT) and was excluded from the efficacy and PD populations. Across all dose groups, the main reason for study withdrawal in patients who completed at least cycle 1 was progressive disease (20/26 patients). Other reasons for withdrawal were AEs ($n = 2$), start of treatment in next cycle delayed ≥15 days ($n = 2$), withdrawal of consent ($n = 1$), and investigator decision ($n = 1$).

Table 1. Patient characteristics (treated patients, $N = 27$)

Characteristic	
Mean age, y (range)	50.7 (26–70)
Gender, *n* (%)	
Male	10 (37.0)
Female	17 (63.0)
ECOG performance status, *n* (%)	
0	10 (37.0)
1	17 (63.0)
2	0 (0)
Mean time since initial diagnosis, months (range)	46.04 (9.7–120.1)
Site of primary lesion, *n* (%)	
Colon cancer	9 (33.3)
Sarcoma	7 (25.9)
Non–small cell lung cancer	5 (18.5)
Other	6 (22.2)
Histologic/cytologic diagnosis, *n* (%)	
Adenocarcinoma	15 (55.6)
Squamous cell carcinoma	3 (11.1)
Bone or soft-tissue carcinoma	7 (25.9)
Other	2 (7.4)
Prior treatment history, *n* (%)	
Surgery	25 (92.6)
Radiotherapy	6 (22.2)
Chemotherapy	26 (96.3)
Number of prior chemotherapy regimens, *n* (%)	
0	1 (3.7)
1	4 (14.8)
2	3 (11.1)
3	9 (33.3)
≥4	10 (37.0)

Safety

DLTs and MTD. No DLTs were observed during cycle 0 and 1 of the dose escalation at 0.5, 1, 2, 4, 6, 9, and 13 mg bid dose levels. DLTs were reported in 2 patients at 20 mg bid, both of whom experienced grade 3 platelet count decrease. Consequently, 3 patients were accrued at the 16 mg bid dose, 1 of whom developed DLT (increased grade 3 AST and ALT). Of the other 2 patients in the 16 mg bid group, 1 developed grade 2 platelet count decrease in cycle 1 and grade 2 fatigue in cycle 2, while the other patient experienced grade 3 fatigue, grade 3 proteinuria, and grade 2 edema in cycle 2. No additional patients were treated at the 16 mg bid dose level as it was judged to be an intolerable dose. Based on the DLTs observed, the MTD was defined as 13 mg bid for this dosing schedule.

Adverse events

The most frequently reported AEs (≥50% of patients) were: hematuria (74.1%), fatigue (70.4%), hypertension