***Conclusions:*** Plx-inv CD204%$^{high}$ was associated with shortened OS and DFS and early recurrence in the peritoneal cavity and locoregional space. The prognostic value of plx-inv CD204%$^{high}$ was also applicable to patients who received adjuvant chemotherapy. High accumulation of M2 macrophages at plx-inv represents an important predictor of poor prognosis.



## 1. Introduction

Pancreatic cancer is an aggressive malignancy with a high incidence of recurrence and low rates of survival, even when curative resection is achieved [1,2]. However, the mechanisms underlying this intractability have yet to be elucidated. Neural invasion has been accepted as an important prognostic factor for invasive ductal carcinoma (IDC) of the pancreas [3–7]. Patients with severe neural invasion are categorised as unresectable cases [8] and experience pain, cachexia, peritoneal dissemination and poor prognosis [9–11].

*In vivo* and *in vitro* models have been established to shed light on the mechanisms underlying neural invasion [9,12–15]. In our previous study [12], highly expressed genes in nerve tissues of the mouse model using Capan-1, a human pancreatic cancer cell line, included macrophage-related genes such as lysozyme [16], macrophage-expressed gene 1 glycoprotein [16] and early growth response 1 [17]. In other experimental studies, the paracrine regulation of neurotrophins was associated with the recruitment of macrophages in neural invasion and the migration of tumour cells [14,15]. Accumulation of macrophages at sites of neural invasion is considered to support tumour cell proliferation and is presumably related to poor prognosis.

Macrophages that have infiltrated into cancer stroma are termed tumour-associated macrophages (TAMs) and promote tumour progression and metastasis [18]. Increased density of TAMs is associated with poor prognosis in cancers of the thyroid, prostate, stomach, bile duct and pancreas [19–23]. TAMs express an M2-skewed phenotype, which is activated in chronic inflammation, scavenge debris and promote angiogenesis and tissue remodelling [18]. M2 macrophages show high expression of scavenger receptor (SR)-A (CD204). High accumulation of CD204-positive cells at the periphery of pancreatic IDC was correlated with shorter overall survival (OS) and disease-free survival (DFS) in our previous study [23]. However, to the best of our knowledge, the clinical impact of M2 macrophages in neural invasion sites has not been elucidated in any kind of malignancies. The aim of the present study was to investigate the prognostic value of M2 macrophages at neural invasion in patients with pancreatic IDC who underwent curative pancreaticoduodenectomy.

## 2. Methods

### 2.1. Patients

A total of 177 patients underwent curative (R0) pancreaticoduodenectomy and were histologically diagnosed with pancreatic IDC at our institution between September 1992 and June 2011. Seven patients were excluded due to surgical mortality ($n = 3$), incomplete follow-up data ($n = 2$) and poor-quality surgical specimens ($n = 2$). The remaining 170 patients were included in this investigation. The median patient age at the time of surgery was 65 years [range, 34–84 years], and 63 (37.1%) were women. Sixty patients received postoperative adjuvant chemotherapy, consisting of gemcitabine in 40 patients (66.7%), S-1 (an oral fluoropyrimidine) in 10 (16.7%), gemcitabine plus S-1 in 6 (10.0%) and 5-fluorouracil plus cisplatin in 4 (6.7%). Inclusion criteria for adjuvant chemotherapy basically conformed to the criteria of the nationwide Japanese randomised phase III trial [24]. Neoadjuvant therapy was performed in four patients. Lymphadenectomy was performed according to the Japanese General Rules for the Study of Pancreatic Cancer [25]. All patients signed an institutional review board-approved informed consent form.

### 2.2. Evaluation of clinicopathological features

Each resected specimen was fixed in 10% formalin at room temperature, and the size and gross appearance of the tumour were recorded [3]. The entire tumour was cut at intervals of 0.5–0.7 cm, and the specimens were routinely processed and embedded in paraffin. Serial sections (3-μm thick) of each tumour were cut, and one section was stained with haematoxylin and eosin (HE). Histopathological findings were examined according to the definitions of the Japan Pancreas Society [25]. The following clinicopathological factors were investigated to assess their prognostic value: age; sex; Eastern Cooperative Oncology Group performance status (ECOG PS); presence of adjuvant chemotherapy; serum level of carcinoembryonic antigen (CEA); serum level of carbohydrate antigen (CA)19-9; tumour differentiation; tumour size; serosal invasion; retroperitoneal invasion; portal vein invasion; lymphatic invasion (ly); vessel invasion (v); intrapancreatic neural invasion (ne); lymph node involvement and extrapancreatic nerve plexus invasion

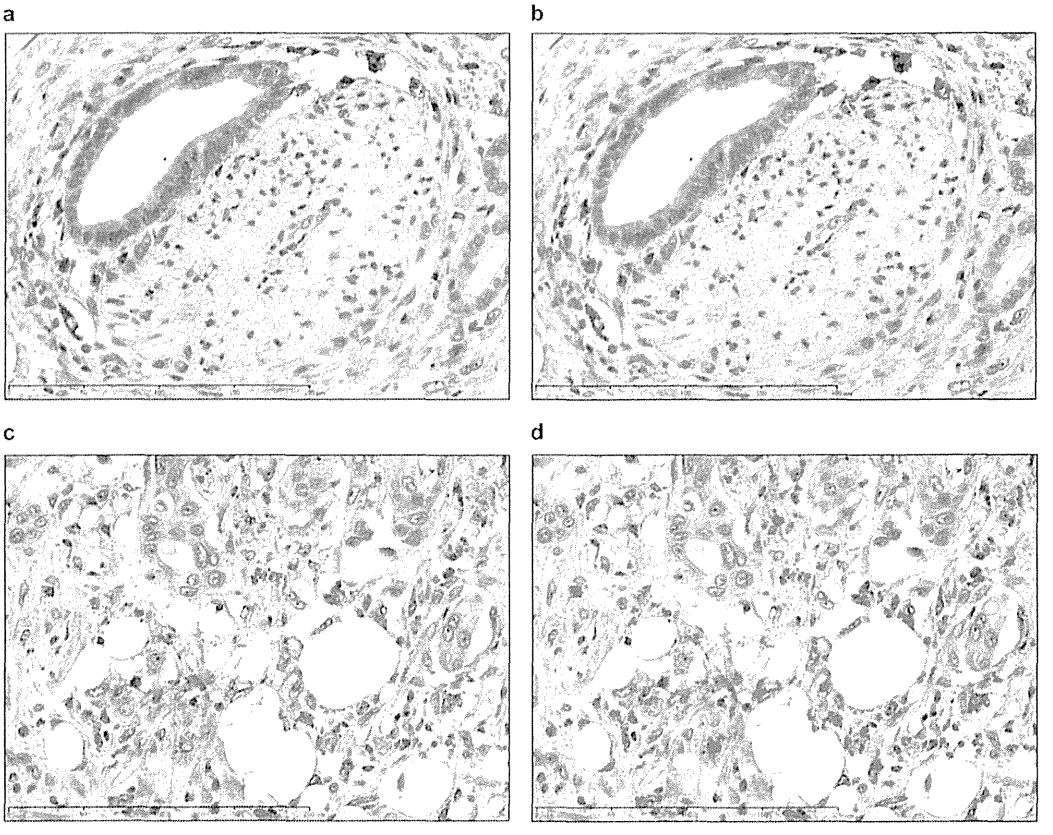


Fig. 1. (a) CD204-positive cells at an extrapancreatic nerve plexus invasion (plx-inv) (magnification, ×400). (b) Red areas represent CD204-positive cells, and the percentage area of CD204-positive cells was calculated as (area of CD204-positive cells/measured area) × 100 using the automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany). (c) CD204-positive cells at the tumour periphery (magnification, ×400). (d) CD204-positive cells are expressed as red areas.

(plx-inv). Ly, v and ne were classified into four groups based on the most extensively involved area observed under low-power magnification (×100): no invasion of cancer cells; slight invasion of a few cancer cells (1–3 points); moderate invasion (4–8 points) and severe invasion (>8 points). Pathological stage was evaluated according to the 7th edition of the International Union Against Cancer (UICC) classification (IA/IB/IIA versus IIB/III/IV) [26]. Cut-off values for continuous variables were determined from median values for all patients.

### 2.3. Definition of the tumour periphery and plx-inv

HE-stained sections at the maximal diameter of the tumour were evaluated at a magnification of ×40, and the margin of the tumour was marked on each slide. The periphery of the primary tumour was defined as fields that included cancer cells and adjacent non-cancerous cells at a magnification of ×100 [23]. As described in our previous study [3], plx-inv was defined as invasion of tumour cells inside the perineurium, apart from both the pancreatic capsule and main tumour, and was evaluated at a magnification of ×400 in all sections. Plx-inv distance was defined as the distance from the plx-inv to the main tumour. The cut-off for plx-inv distance was set at 2500 μm, and the prognostic value was evaluated [3].

### 2.4. Immunohistochemical staining and evaluation

Mouse anti-human CD204 antibody (Scavenger Receptor class A-E5, 1:400 in blocking buffer; Transgenic, Kumamoto, Japan) was used for immunohistochemical staining [23]. The percentage area of CD204-positive cells (CD204%) was calculated as (area of CD204-positive cells/measured area) × 100 using the automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany) [23]. The mean CD204% for three hot spots at the tumour periphery and plx-inv was calculated in each patient. Median CD204% for all patients with plx-inv was used to determine CD204%$^{high}$ as equal to or above the median. Prognostic analyses for CD204%$^{high}$ at the periphery and plx-inv were performed.

### 2.5. Assessment of recurrence

Contrast-enhanced computed tomography or magnetic resonance imaging was performed every 3 months after surgery. Sites of recurrence were categorised as

Table 1
Prognostic analyses for overall survival and disease-free survival in patients with invasive ductal carcinoma of the pancreas ($n = 170$).

| Parameter | $n$ | % | Overall survival | | | Disease-free survival | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | P | HR | 95% CI | P |
| *(a) Univariate analysis* | | | | | | | | |
| Age ⩾ 65 | 80 | 47.1 | 1.077 | 0.775–1.498 | 0.657 | 1.114 | 0.808–1.535 | 0.510 |
| Sex, male | 107 | 62.9 | 0.876 | 0.625–1.227 | 0.442 | 0.960 | 0.689–1.339 | 0.811 |
| ECOG PS ⩾ 1 | 28 | 16.5 | 1.975 | 1.266–3.081 | 0.003* | 1.403 | 0.909–2.166 | 0.126 |
| Absence of adjuvant chemotherapy | 110 | 64.7 | 1.715 | 1.186–2.481 | 0.004* | 1.501 | 1.061–2.123 | 0.022* |
| CEA ⩾ 3.4 ng/ml | 88 | 51.8 | 1.419 | 1.019–1.975 | 0.038* | 1.584 | 1.147–2.186 | 0.005* |
| CA19-9 ⩾ 111.5 U/ml | 85 | 50.0 | 0.904 | 0.648–1.260 | 0.550 | 1.026 | 0.743–1.417 | 0.877 |
| Tumour differentiation, moderate/poor | 126 | 74.1 | 1.248 | 0.857–1.817 | 0.248 | 1.514 | 1.042–2.199 | 0.030* |
| Tumour size ⩾ 3.0 cm | 81 | 47.6 | 1.615 | 1.160–2.248 | 0.005* | 1.596 | 1.156–2.203 | 0.004* |
| Serosal invasion (+) | 46 | 27.1 | 0.865 | 0.591–1.266 | 0.457 | 1.164 | 0.811–1.671 | 0.411 |
| Retroperitoneal invasion (+) | 145 | 85.3 | 1.174 | 0.724–1.904 | 0.516 | 1.060 | 0.668–1.682 | 0.805 |
| Portal vein invasion (+) | 40 | 23.5 | 1.479 | 1.014–2.156 | 0.042* | 1.186 | 0.818–1.722 | 0.368 |
| Ly, moderate to severe | 46 | 27.1 | 1.634 | 1.130–2.365 | 0.009* | 1.620 | 1.135–2.312 | 0.008* |
| V, moderate to severe | 103 | 60.6 | 1.779 | 1.254–2.524 | 0.001* | 1.636 | 1.168–2.292 | 0.004* |
| Ne, moderate to severe | 106 | 62.4 | 1.812 | 1.270–2.583 | 0.001* | 1.637 | 1.164–2.302 | 0.005* |
| Lymph node involvement (+) | 141 | 82.9 | 1.505 | 0.968–2.341 | 0.069 | 1.554 | 1.002–2.409 | 0.049* |
| Pathological stage IIB/III/IV | 143 | 84.1 | 1.414 | 0.903–2.214 | 0.130 | 1.463 | 0.937–2.284 | 0.094 |
| Peripheral CD204%$^{high}$ | 85 | 50.0 | 1.777 | 1.272–2.484 | 0.001* | 1.570 | 1.135–2.172 | 0.006* |
| Plx-inv (+) | 91 | 53.5 | 1.612 | 1.147–2.264 | 0.006* | 1.785 | 1.280–2.489 | 0.001* |
| Plx-inv distance ⩾ 2500 μm | 56 | 32.9 | 1.949 | 1.368–2.777 | <0.001* | 2.274 | 1.597–3.238 | <0.001* |
| Plx-inv CD204%$^{high}$ | 48 | 28.2 | 1.779 | 1.247–2.539 | 0.001* | 1.904 | 1.341–2.705 | <0.001* |
| *(b) Multivariate analysis* | | | | | | | | |
| Absence of adjuvant chemotherapy | 110 | 64.7 | 1.741 | 1.143–2.651 | 0.010* | 1.559 | 1.042–2.332 | 0.031* |
| CEA ⩾ 3.4 ng/ml | 88 | 51.8 | 1.437 | 1.011–2.041 | 0.043* | 1.602 | 1.139–2.253 | 0.007* |
| Tumour size ⩾ 3.0 cm | 81 | 47.6 | 1.610 | 1.147–2.262 | 0.006* | 1.616 | 1.160–2.250 | 0.005* |
| Ly, moderate to severe | 46 | 27.1 | 1.254 | 0.839–1.876 | 0.270 | 1.151 | 0.775–1.709 | 0.487 |
| V, moderate to severe | 103 | 60.6 | 1.505 | 1.010–2.242 | 0.045* | 1.291 | 0.879–1.897 | 0.192 |
| Peripheral CD204%$^{high}$ | 85 | 50.0 | 2.167 | 1.522–3.086 | <0.001* | 1.831 | 1.297–2.583 | 0.001* |
| Plx-inv CD204%$^{high}$ | 48 | 28.2 | 2.008 | 1.362–2.962 | <0.001* | 2.046 | 1.400–2.991 | <0.001* |

* $P < 0.05$. Prognostic analyses were carried out using Cox regression model. HR, hazard ratio; 95% CI, 95% confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; Ly, lymphatic invasion; V, vessel invasion; Ne, intrapancreatic neural invasion, Peripheral CD204%$^{high}$, percentage of CD204-positive cells area at the periphery ⩾ 3.34; Plx-inv, extrapancreatic nerve plexus invasion; Plx-inv CD204%$^{high}$, percentage of CD204-positive cells area at plx-inv ⩾ 0.57.

liver metastasis, peritoneal dissemination, locoregional recurrence and distant lymph node metastasis. Peritoneal dissemination was defined as marked peritoneal nodules, increased ascites or malignant ascites as confirmed by cytology. Locoregional recurrence was defined as tumour in a dissected space or metastasis in regional lymph nodes according to the 7th edition of the UICC classification [26]. Distant lymph node metastasis was defined as marked lymph node swelling apart from the regional space.

### 2.6. Statistical analysis

Uni- and multivariate analyses for OS, DFS and time to each type of recurrence were performed using a Cox regression model. Factors showing values of $P < 0.05$ for both OS and DFS in univariate analyses were included in multivariate analyses. Pearson's correlation coefficient $r$ was used to evaluate the correlation among covariates. The observation period was until March 2013, and the median duration was 17.6 months [95% confidence interval (CI), 14.5–20.6]. OS was defined as the time from surgery to death or the date censored at last follow-up. DFS was calculated as the time from surgery to tumour relapse or death or the date censored at last follow-up. Survival curves were drawn using the Kaplan–Meier method, and the differences between patient groups were analysed by log-rank test. $P$-values were two-sided, with the significance level at $P < 0.05$. Statistical analyses were performed using SPSS version 19.0 software (SPSS, Chicago, IL).

## 3. Results

### 3.1. Distribution of CD204%

CD204 accumulation at the primary tumour was measured in all 170 patients, and median CD204% at the tumour periphery was 3.34% [range, 0.16–14.04%]. Plx-inv was observed in 91 patients (53.5%). CD204-positive cells and the measured area at plx-inv are shown in Fig. 1a and b, and CD204-positive cells and the measured area at the tumour periphery are shown in Fig. 1c and d. Median CD204% at plx-inv was 0.57% [range,

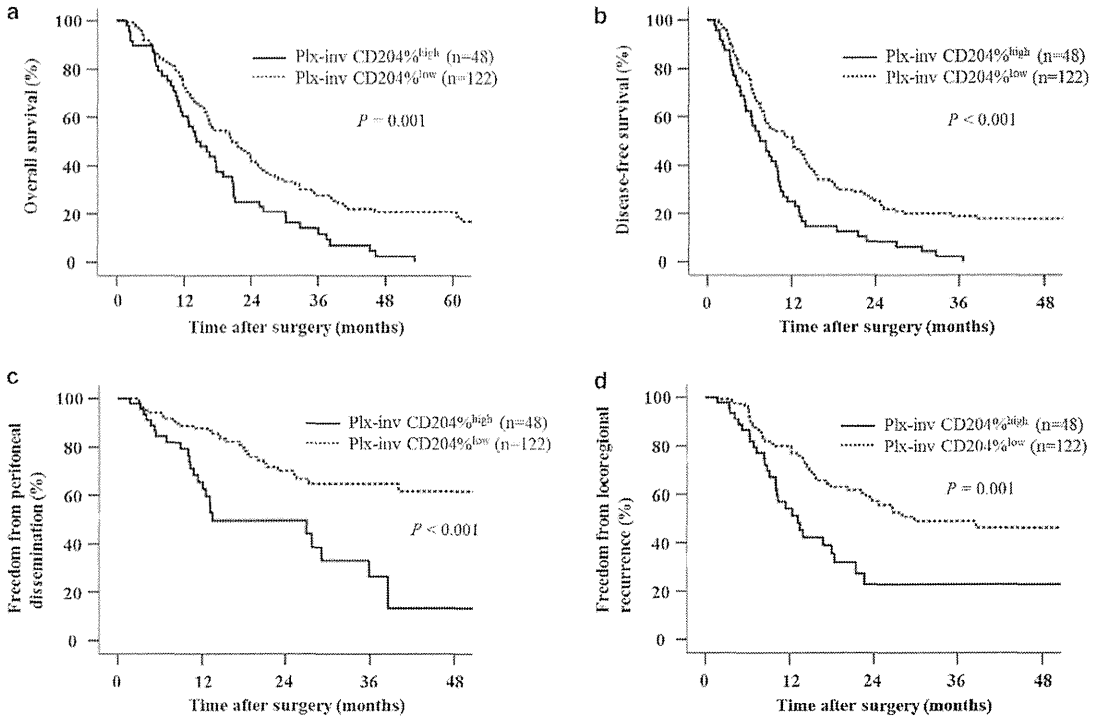


Fig. 2. (a) Kaplan–Meier curve for overall survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%). (b) Kaplan–Meier curve for disease-free survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%). (c) Kaplan–Meier curve for peritoneal dissemination-free survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%). (d) Kaplan–Meier curve for locoregional recurrence-free survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%).

0.00–7.76%]. Forty-eight patients with CD204% at plx-inv $\geqslant$0.57% were categorised as plx-inv CD204%$^{high}$. There were 43 patients with CD204% at plx-inv <0.57% and 79 patients without plx-inv, who were categorised as plx-inv CD204%$^{low}$.

### 3.2. Prognostic analyses of clinicopathological factors

The median OS and DFS were 17.8 months [95% CI, 14.7–20.9] and 9.8 months [95% CI, 7.9–11.6], respectively. Univariate analysis identified absence of adjuvant chemotherapy, CEA $\geqslant$ 3.4 ng/ml, tumour size $\geqslant$ 3.0 cm, moderate to severe ly, v and ne, peripheral CD204%$^{high}$, plx-inv, plx-inv distance $\geqslant$ 2500 μm and plx-inv CD204%$^{high}$ as candidates for correlation with both shorter OS and shorter DFS ($P < 0.05$) (Table 1a). Strong correlations were observed between plx-inv CD204%$^{high}$ and the following covariates: moderate to severe ne, $r = 0.299$, $P < 0.001$; plx-inv, $r = 0.584$, $P < 0.001$; and plx-inv distance $\geqslant$ 2500 μm, $r = 0.534$, $P < 0.001$. Therefore, these covariates were excluded from the multivariate analysis. Multivariate analysis revealed absence of adjuvant chemotherapy (hazard ratio [HR], 1.741; $P = 0.010$), CEA $\geqslant$ 3.4 ng/ml (HR, 1.437; $P = 0.043$), tumour size $\geqslant$ 3.0 cm (HR, 1.610; $P = 0.006$), moderate to severe v (HR, 1.505; $P = 0.045$), peripheral CD204%$^{high}$ (HR, 2.167; $P < 0.001$), and plx-inv CD204%$^{high}$ (HR, 2.008; $P < 0.001$) as independent risk factors for shorter OS (Table 1b). In terms of DFS, absence of adjuvant chemotherapy (HR, 1.559; $P = 0.031$), CEA $\geqslant$ 3.4 ng/ml (HR, 1.602; $P = 0.007$), tumour size $\geqslant$ 3.0 cm (HR, 1.616; $P = 0.005$), peripheral CD204%$^{high}$ (HR, 1.831; $P = 0.001$) and plx-inv CD204%$^{high}$ (HR, 2.046; $P < 0.001$) represented independent risk factors for shorter DFS (Table 1b). OS and DFS curves according to the level of plx-inv CD204% are shown in Fig. 2a and b.

### 3.3. Time to relapse according to site of recurrence

Median times to tumour relapse were 7.3 months [95% CI, 5.5–9.1] for liver metastasis (71 patients, 41.8%), 12.1 months [9.2–15.0] for peritoneal dissemination (57 patients, 33.5%), 10.0 months [7.1–13.0] for locoregional recurrence (76 patients, 44.7%) and 8.8 months [4.0–13.6] for distant lymph node recurrence (46 patients, 27.1%). Multivariate analyses showed that absence of adjuvant chemotherapy (HR, 1.924; $P = 0.030$) and moderate to severe ly (HR, 2.634; $P < 0.001$) correlated with early relapse to liver metastasis (Table 2). Peripheral CD204%$^{high}$ was a predictor of peritoneal dissemination (HR, 1.815; $P = 0.031$)

(Table 2). Plx-inv CD204%$^{high}$ was independently associated with peritoneal dissemination (HR, 2.886; $P < 0.001$) and locoregional recurrence (HR, 2.483; $P < 0.001$) (Table 2 and Fig. 2c and d).

### 3.4. Prognostic analyses stratified by presence of adjuvant chemotherapy

Adjuvant chemotherapy represented an independent prognostic factor for OS and DFS as a definitive therapeutic modality (Table 1, Fig. 3a and b). Multivariate analyses to test prognostic factors with adjuvant chemotherapy were re-examined and revealed that only plx-inv CD204%$^{high}$ was associated with both shorter OS (HR, 2.624; $P = 0.011$) and shorter DFS (HR, 2.257; $P = 0.038$) in patients with plx-inv who underwent postoperative adjuvant chemotherapy (Table 3).

## 4. Discussion

The present study demonstrated that the accumulation of CD204-positive cells, representing M2 macrophages, at plx-inv of pancreatic IDC was an independent predictor of shorter OS and DFS in patients who underwent curative pancreaticoduodenectomy for pancreatic IDC. The prognostic impact of plx-inv CD204%$^{high}$ was maintained in patients who received adjuvant chemotherapy. Infiltration of M2 macrophages at plx-inv of pancreatic IDC was revealed as a key factor to explain the aggressiveness of pancreatic IDC for the first time in this study.

Peritoneal dissemination has long been considered a poor prognostic factor for patients with pancreatic IDC [27–29]. Patients with plx-inv CD204%$^{high}$ showed early relapse to the peritoneal cavity in this study. The interaction between M2 macrophages and tumour cells at plx-inv was suggested to play a crucial role in peritoneal recurrence, which led to poor survival. From the perspective of surgical anatomy, nerve fibres of the plexus pancreaticus capitalis might provide a convenient pathway for infiltrating tumour cells. As recent experimental study showed that macrophages around nerves were recruited in response to cytokine secreted by invading tumour cells and increased migration of tumour cells [15], M2 macrophages might promote the invasiveness of tumour cells at plx-inv, leading tumour cells to disperse into the peritoneal space and resulting in peritoneal dissemination. This speculation warrants further studies to observe the distribution of M2 macrophages in metastatic sites of pancreatic IDC and to test the role of M2 macrophages in metastatic tumour models.

Immunophysiologically, neural injury leads to the accumulation of macrophages in the peripheral nerve system, although few macrophages exist in intact nerves [30]. Ceyhan et al. reported that neuritis was caused by the invasion of malignant tumour cells into the pancreas

Table 2
Multivariate analysis for early relapse according to the sites of recurrence in patients with invasive ductal carcinoma of the pancreas ($n = 170$).

| Parameter | Liver metastasis ($n = 71$) | | | | | Peritoneal dissemination ($n = 57$) | | | | | Locoregional recurrence ($n = 76$) | | | | | Distant lymph node metastasis (n = 46) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | HR | 95% CI | *P* | *n* | % | HR | 95% CI | *P* | *n* | % | HR | 95% CI | *P* | *n* | % | HR | 95% CI | *P* |
| Absence of adjuvant chemotherapy | 50 | 70.4 | 1.924 | 1.065–3.476 | 0.030* | 34 | 59.6 | 1.107 | 0.602–2.036 | 0.743 | 52 | 68.4 | 1.734 | 0.995–3.022 | 0.052 | 32 | 69.6 | 1.660 | 0.794–3.471 | 0.178 |
| CEA ⩾ 3.4 ng/ml | 40 | 56.3 | 1.347 | 0.819–2.215 | 0.241 | 23 | 40.4 | 0.934 | 0.531–1.640 | 0.811 | 36 | 47.4 | 1.238 | 0.773–1.985 | 0.374 | 26 | 56.5 | 1.516 | 0.810–2.839 | 0.193 |
| Tumour size ⩾ 3.0 cm | 38 | 53.5 | 1.492 | 0.925–2.405 | 0.101 | 22 | 38.6 | 0.968 | 0.558–1.681 | 0.909 | 34 | 44.7 | 1.114 | 0.698–1.780 | 0.650 | 23 | 50.0 | 1.327 | 0.732–2.407 | 0.351 |
| Ly, moderate to severe | 28 | 39.4 | 2.634 | 1.574–4.408 | <0.001* | 14 | 24.6 | 0.839 | 0.436–1.617 | 0.601 | 21 | 27.6 | 0.878 | 0.501–1.539 | 0.649 | 17 | 37.0 | 1.909 | 0.993–3.671 | 0.053 |
| V, moderate to severe | 46 | 64.8 | 1.146 | 0.660–1.988 | 0.629 | 30 | 52.6 | 1.126 | 0.618–2.052 | 0.698 | 47 | 61.8 | 1.323 | 0.774–2.261 | 0.306 | 29 | 63.0 | 1.133 | 0.560–2.292 | 0.729 |
| Peripheral CD204%$^{high}$ | 37 | 52.1 | 1.517 | 0.931–2.472 | 0.095 | 30 | 52.6 | 1.815 | 1.055–3.124 | 0.031* | 36 | 47.4 | 1.501 | 0.933–2.417 | 0.094 | 21 | 45.7 | 1.305 | 0.706–2.412 | 0.396 |
| Plx-inv CD204%$^{high}$ | 18 | 25.4 | 0.916 | 0.518–1.619 | 0.763 | 24 | 42.1 | 2.886 | 1.615–5.159 | <0.001* | 28 | 36.8 | 2.483 | 1.485–4.151 | <0.001* | 15 | 32.6 | 1.564 | 0.795–3.073 | 0.195 |

* $P < 0.05$. Multivariate analysis was carried out using Cox regression hazard model. HR, hazard ratio; 95% CI, 95% confidence interval; CEA, carcinoembryonic antigen; Ly, lymphatic invasion; V, vessel invasion; Peripheral CD204%$^{high}$, percentage of CD204-positive cells area at the periphery ⩾ 3.34; Plx-inv CD204%$^{high}$, percentage of CD204-positive cells area at extrapancreatic nerve plexus invasion ⩾ 0.57.

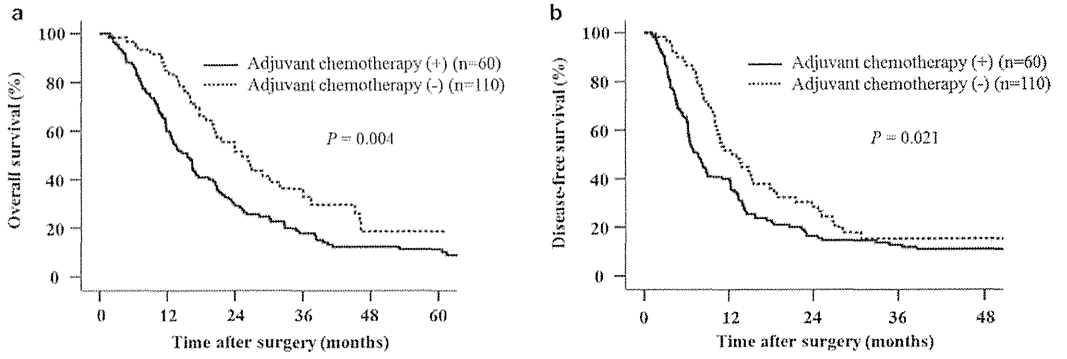


Fig. 3. (a) Kaplan–Meier curve for overall survival stratified by the presence of adjuvant chemotherapy. (b) Kaplan–Meier curve for disease-free survival stratified by the presence of adjuvant chemotherapy.

Table 3
Multivariate analysis for overall survival and disease-free survival in patients who received adjuvant chemotherapy ($n = 60$).

| Parameter | $n$ | % | Overall survival | | | Disease-free survival | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | $P$ | HR | 95% CI | $P$ |
| CEA ⩾ 3.4 ng/ml | 23 | 38.3 | 1.514 | 0.704–3.257 | 0.289 | 1.883 | 0.969–3.658 | 0.062 |
| Tumour size ⩾ 3.0 cm | 26 | 43.3 | 1.283 | 0.663–2.484 | 0.460 | 1.179 | 0.641–2.170 | 0.596 |
| Ly, moderate to severe | 21 | 35.0 | 1.775 | 0.833–3.782 | 0.137 | 1.475 | 0.667–3.263 | 0.337 |
| V, moderate to severe | 19 | 31.7 | 2.178 | 1.059–4.479 | 0.034* | 1.476 | 0.769–2.833 | 0.242 |
| Peripheral CD204%$^{high}$ | 32 | 53.3 | 1.206 | 0.601–2.420 | 0.598 | 0.890 | 0.472–1.679 | 0.719 |
| Plx-inv CD204%$^{high}$ | 20 | 33.3 | 2.624 | 1.242–5.544 | 0.011* | 2.257 | 1.045–4.879 | 0.038* |

* $P < 0.05$. Multivariate analysis was carried out using Cox regression model. HR, hazard ratio; 95% CI, 95% confidence interval; CEA, carcinoembryonic antigen; Ly, lymphatic invasion; V, vessel invasion; Peripheral CD204%$^{high}$, percentage of CD204-positive cells area at the periphery ⩾ 3.34; Plx-inv CD204%$^{high}$, percentage of CD204-positive cells area at extrapancreatic nerve plexus invasion ⩾ 0.57.

[31]. In our previous experimental study [12], neural invasion over a long distance could lead to severe neural damage. Additionally, the present study showed strong positive correlations among ne, plx-inv, long plx-inv distance and plx-inv CD204%$^{high}$. Taken together with the paracrine regulation between macrophages and tumour cells at plx-inv [14,15], severe neural invasion of tumour cells appears to recruit M2 macrophages due to neural damage. Moreover, the neural system was suggested as an expedient structure for interaction between tumour cells and M2 macrophages that promotes pancreatic cancer cell proliferation.

Adjuvant chemotherapy after complete resection of pancreatic IDC has been established as the definitive standard of care within the last decade [24,32,33]. In the present study, plx-inv CD204%$^{high}$ was the only independent prognostic factor for poor OS and DFS in the group of patients with adjuvant chemotherapy. According to recent reports, immunoregulatory cytokines such as interleukin-6 and prostaglandin E2, which are present in the tumour microenvironment, are associated with chemoresistance and tumour-induced differentiation of tumour-promoting M2 macrophages [34,35]. Additional therapy to suppress M2 macrophages might thus prove effective, particularly against cases with plx-inv and high accumulation of M2 macrophages. Depletion of macrophages by zoledronic acid has been reported to enhance the effects of sorafenib in an *in vivo* model of metastatic liver cancer [36]. A phase II randomised controlled study of tasquinimod (oral quinolone-3-carboxamide) for metastatic castrate-resistant prostate cancer patients prolonged progression-free survival and confirmed the pharmacological efficacy of this agent for inhibiting S100A9 [37], which is a protein expressed in inflammatory cells that induces the maturation of macrophages [38]. Therefore, anti-M2 macrophage therapy may have potential as an innovative treatment for pancreatic IDC.

Limitations of this study include the retrospective manner of the investigation. Adjuvant chemotherapy was performed in 60 patients and was an independent factor predictive of OS and DFS, but the indication was influenced by time trends, and some degree of selection bias might have been present. Although OS and DFS for our patient cohort were comparable with the other previous studies [24,32,33], further investigation in patients with standardised adjuvant chemotherapy is needed. Moreover, since only resectable pancreatic cancer was studied, it is unknown whether the results can be extrapolated to the much higher numbers of unresectable cases.

In conclusion, pancreatic cancer patients with high accumulation of CD204-positive cells at plx-inv who underwent curative resection showed a high incidence

of recurrence in the form of peritoneal dissemination and locoregional recurrence and shorter OS and DFS. The impact of CD204-positive cells at plx-inv on OS and DFS was maintained in the setting of adjuvant chemotherapy. Increased infiltration of M2 macrophages at plx-inv may represent an important finding for detecting patients with aggressive IDC of the pancreas.

## Conflict of interest statement

None declared.

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# Safety, Tolerability, Pharmacokinetics and Antitumor Activity of Ganitumab, an Investigational Fully Human Monoclonal Antibody to Insulin-like Growth Factor Type 1 Receptor, Combined with Gemcitabine as First-line Therapy in Patients with Metastatic Pancreatic Cancer: A Phase 1b Study

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**Objective:** Previous Phase 1 studies have shown the acceptable safety profile of ganitumab—a fully human monoclonal antibody to insulin-like growth factor Type 1 receptor—in patients with advanced solid tumors. However, ganitumab 20 mg/kg in combination with gemcitabine had not been administered to patients with metastatic pancreatic cancer. To evaluate the safety, tolerability, pharmacokinetics and antitumor activity of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m$^2$ as first-line therapy in patients with metastatic pancreatic cancer, we conducted a Phase 1b study.

**Methods:** Eligible patients were adults with previously untreated metastatic adenocarcinoma of the pancreas. Patients received gemcitabine 1000 mg/m$^2$ on Days 1, 8 and 15 plus ganitumab 20 mg/kg on Days 1 and 15 of each 28-day cycle. Gemcitabine was administered intravenously over 30–60 min. Ganitumab was administered intravenously over 60 min after completing gemcitabine infusion.

**Results:** Six patients were enrolled and received the study treatment. All patients had thrombocytopenia and leukopenia. Other most common adverse events were neutropenia and nausea. One patient had a dose-limiting toxicity defined as Grade 3 neutropenia with fever. Exposure to ganitumab 20 mg/kg was not affected by the administration of gemcitabine. No apparent pharmacokinetic drug–drug interaction was observed. No anti-ganitumab antibodies were detected. Five patients had a measurable tumor region at baseline. Of these, four patients had a best response of stable disease.

**Conclusions:** Ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m$^2$ was tolerable and showed an acceptable safety profile in patients with untreated metastatic pancreatic cancer.

*Key words: clinical trial Phase 1 – ganitumab – gemcitabine – pancreatic neoplasms – receptor, insulin-like growth factor type 1*

## INTRODUCTION

The insulin-like growth factor (IGF) system—the circulating ligands (insulin, IGF-1 and IGF-2), multiple receptors and binding proteins—plays a major role in cancer cell proliferation (1–3). In this system, IGF-1 acts as the primary regulator of growth, whereas IGF-2 has metabolic and mitogenic effects (4). Furthermore, a recent review has shown that the IGF Type 1 receptor (IGF-1R) plays a role in maintaining the malignant phenotype and disruption of IGF-1R activation leads to inhibited growth and motility of cancer cells (3). Thus, this family of growth factors, especially the IGF-1R, may present an excellent target for new therapeutic agents for anticancer treatment (5,6).

Ganitumab (previously known as AMG 479) is a fully human monoclonal antibody directed to IGF-1R. As a single agent, it inhibited the interaction of IGF-1R with IGF-1 and IGF-2 without cross-reacting to insulin receptor in IGF-1R-expressing pancreatic carcinoma cell lines (7). In addition, the combination of ganitumab with gemcitabine resulted in additive inhibitory activity both *in vitro* and *in vivo* (7). These results indicate that ganitumab is a clinical candidate for the treatment of patients with pancreatic cancer (PC).

Previous Phase 1 studies have shown that ganitumab can be administered safely to patients with advanced solid tumors at doses up to 20 mg/kg intravenously every 2 weeks (8,9). In a randomized Phase 2 study, ganitumab 12 mg/kg combined with gemcitabine 1000 mg/m$^2$ has shown evidence of activity with improved 6-month overall survival rates compared with gemcitabine alone in patients with metastatic PC (mPC) (10).

However, it is uncertain whether a higher dose level of ganitumab is needed to treat patients with mPC. A recent analysis using the data of the randomized Phase 2 study assessed the effect of ganitumab exposure on survival, and its results revealed that the progression-free survival and overall survival were longer in the high-exposure group than in the low-exposure group (11). According to this finding, a pharmacokinetic (PK) analysis was performed to determine a sufficient dose level, and the results showed that >90% of patients with mPC would reach high exposures when administered ganitumab 20 mg/kg (11).

Considering that ganitumab 20 mg/kg in combination with gemcitabine has not been administered in patients with mPC, we conducted a Phase 1b study to evaluate the safety, tolerability, PKs and antitumor activity of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m$^2$ as first-line therapy in this population.

## PATIENTS AND METHODS

### Study Design and Ethical Considerations

This Phase 1b, open-label study was conducted from August 2010 to February 2011 at three institutions in Japan. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Its protocol was reviewed and approved by the institutional review board of the participating institutions. All patients provided their written informed consent.

### Patient Population

Patients aged at least 20 years were eligible for the study if they had histologically or cytologically confirmed metastatic adenocarcinoma of the pancreas; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; and adequate hematologic, renal and hepatic functions. Adequate functions were defined as follows: hemoglobin ≥9 g/dl; absolute neutrophil count $\geq 1.5 \times 10^9$/l; platelet count $\geq 100 \times 10^9$/l; activated partial thromboplastin time ≤1.3 × the upper limit of normal (ULN) and international normalized ratio (INR) ≤1.5 (for patients who did not receive anticoagulation therapy); creatinine clearance >60 ml/min; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 × ULN (≤5 × ULN for patients with liver metastases); total bilirubin ≤1.5 × ULN; and fasting blood glucose level ≤160 mg/dl.

Patients were excluded if they had received or were receiving any treatment for PC. Other exclusion criteria included the following: islet cell carcinoma, acinar cell carcinoma, non-adenocarcinoma, or adenocarcinoma originated from biliary tree or cystadenocarcinoma; a history of central nervous system metastases; internal or external biliary drain; a history of other malignancies; and myocardial infarction or uncontrolled cardiovascular disease including acute coronary syndrome or congestive heart failure within 6 months before enrollment. Pregnant women, breastfeeding women or patients who did not use adequate contraceptive precautions despite having a partner were also excluded.

### Study Treatment

Initially, six patients received the study treatment (i.e. gemcitabine plus ganitumab), and three additional patients were to be enrolled if additional data for the safety or PK analysis were needed. Patients received gemcitabine 1000 mg/m$^2$ on Days 1, 8 and 15 as well as ganitumab 20 mg/kg on Days 1 and 15 of each 28-day cycle. Gemcitabine was administered intravenously over 30–60 min. Ganitumab was administered intravenously over 60 (±10) min after the completion of gemcitabine infusion. The infusion rate of ganitumab was slowed down (up to 120 min infusion) if patients could not tolerate the first infusion.

The dose of gemcitabine was reduced to Level 1 (750 mg/m$^2$) or Level 2 (563 mg/m$^2$) if patients had treatment-related neutropenia, thrombocytopenia or Grade 3 or greater non-hematologic toxicities that required dose reduction. The dose of ganitumab was reduced by 50% if patients had treatment-related Grade 3 or greater thrombocytopenia without Grade 2 or greater bleeding; febrile neutropenia; Grade 4 neutropenia; or Grade 3 neutropenia lasting 8 days or more. Antiemetic premedication for prophylaxis of nausea/vomiting associated with gemcitabine was allowed if necessary. Premedication with antihistamines,

corticosteroids or both was also allowed if patients had an infusion reaction. Patients continued the study treatment until the disease progression if they wished to receive it and had no unacceptable toxicities.

### Outcome Measures

Medical history was collected within 14 days before enrollment. Patients were hospitalized at least 5 days from Day 1 of treatment. Adverse events were monitored throughout the study and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Dose-limiting toxicity (DLT) was defined as any Grade 3 or greater toxicity that related to ganitumab during the first 28 days. DLTs did not include lymphopenia and infusion reaction. Fatigue, nausea, diarrhea, vomiting, leukopenia, neutropenia, febrile neutropenia, thrombocytopenia, hemoglobin decrease, increased AST or ALT, hyperglycemia and pulmonary embolism were included in DLTs if they met any of the following criteria: Grade 3 or greater neutropenia with fever (body temperature $>38.5^{\circ}C$); Grade 4 leukopenia or neutropenia lasting 8 days or more; Grade 4 thrombocytopenia lasting 8 days or more; Grade 3 or greater thrombocytopenia (for patients who were receiving anticoagulation therapy); Grade 3 or greater thrombocytopenia accompanied by Grade 2 or greater bleeding; Grade 3 or greater thrombocytopenia requiring platelet transfusion; Grade 4 hemoglobin decrease; Grade 3 fatigue lasting 8 days or more; Grade 4 fatigue; Grade 3 or greater nausea, diarrhea or vomiting despite maximum supportive care; AST or ALT $>8 \times$ ULN; AST or ALT $>5 \times$ ULN and $\leq 8 \times$ ULN lasting 15 days or more (for patients with baseline values $\leq 2.5 \times$ ULN); AST or ALT $>2 \times$ baseline value and $\leq 8 \times$ ULN lasting 15 days or more (for patients with baseline values $>2.5 \times$ ULN and $\leq 4 \times$ ULN); AST or ALT $>3 \times$ ULN accompanied by total bilirubin $>2 \times$ ULN or INR $>1.5$; any pulmonary embolism that required full-dose anticoagulation therapy (except for deep vein thrombosis); or Grade 4 hyperglycemia with ketoacidosis or hyperosmolar non-ketotic coma.

Blood pressure, pulse rate, body temperature and body weight were measured on Days 1, 8 and 15 of each treatment cycle. ECOG performance status was assessed on Day 1 of each cycle. Electrocardiograms were recorded before starting gemcitabine infusion and after completing ganitumab infusion on Days 1 and 15 of Cycle 1, Day 15 of Cycle 2 and Day 15 of every 3 cycles thereafter. Laboratory tests were performed periodically throughout the study.

Serum samples for PK analysis of ganitumab were collected before starting gemcitabine infusion, within 5 min before completing ganitumab infusion, and 3 and 24 h after completing ganitumab infusion on Day 1 of Cycle 1; and before starting gemcitabine infusion on Days 8 and 15 of Cycle 1. Serum concentration of ganitumab was determined by using a validated double anti-idiotypic antibody sandwich immunoassay (8).

Plasma samples for PK analysis of gemcitabine were collected before starting gemcitabine infusion, within 5 min before completing gemcitabine infusion, and at 15, 30 and 90 min as well as 24 h (Day 1 only) after completing gemcitabine infusion on Days 1 and 8 of Cycle 1. Plasma concentration of gemcitabine was determined by using a validated method developed by Covance Bioanalytical Services, LLC. (Indianapolis, IN, USA).

Furthermore, serum samples for assessment of anti-ganitumab antibodies were collected pre-dose of gemcitabine on Day 1 of Cycles 1, 2 and 3, and every 2 cycles thereafter. Anti-ganitumab binding antibodies were detected by using a validated bridging immunoassay. Samples positive for anti-ganitumab binding antibodies were to be evaluated additionally for potential neutralizing capabilities in a cell-based assay.

Tumor response was evaluated at screening and every 8 weeks after starting the treatment by using computed tomography or magnetic resonance imaging and was classified according to the response evaluation criteria in solid tumors (12).

### Statistical Considerations

All data were summarized descriptively. The PK parameters of ganitumab and gemcitabine were estimated by using non-compartmental methods with Phoenix WinNonlin software Version 6.1 (Pharsight Corporation, Mountain View, CA, USA). Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as the mean or the median combined with the standard deviation (SD) or the range. All data were analyzed by using SAS® System Version 9.1.3 (SAS Institute, Cary, NC, USA).

## RESULTS

### Patient Disposition, Demographics and Baseline Characteristics

A total of six patients were enrolled into the study. All patients received at least one dose of ganitumab and gemcitabine and were included in the safety and PK analyses. Of these, one patient had no measurable tumor region at baseline. This patient was excluded from the efficacy analysis. At the time of data analysis, all patients discontinued the study treatment: three patients because of disease progression, two because of adverse events (Grade 2 sudden hearing loss and Grade 1 interstitial pneumonia) and one according to the protocol (Grade 4 neutropenia that did not resolve within the prespecified period). The mean number of treatment cycles was 3 (range, 2–5). The mean relative dose intensity (=[total dose received/total dose expected per initial dose] $\times$ 100) was 91% (range, 57–100%) for ganitumab and 90% (range, 68–100%) for gemcitabine.

Table 1 shows the demographic and baseline characteristics of the study patients. The median age was 62 (range, 43–69) years. Three patients (50%) had ECOG performance status of zero. All patients had Stage IV PC. No patients received prior radiotherapy or other medication for PC.

**Table 1.** Demographic and baseline characteristics of the study patients

| | Number of patients ($n = 6$) |
|---|---|
| Median age, years (range) | 62.0 (43–69) |
| Sex, *n* (%) | |
| Male | 5 (83.3) |
| Female | 1 (16.7) |
| Median weight, kg (range) | 58.05 (49.0–75.4) |
| ECOG performance status, *n* (%) | |
| 0 | 3 (50.0) |
| 1 | 3 (50.0) |
| Medical and surgical history, *n* (%) | |
| Yes | 6 (100.0) |
| Disease stage, *n* (%) | |
| IV | 6 (100.0) |
| Prior radiotherapy, *n* (%) | |
| No | 6 (100.0) |
| Prior other medication for cancer, *n* (%) | |
| No | 6 (100.0) |

ECOG, Eastern Cooperative Oncology Group.

### Safety

Table 2 summarizes the common adverse events. All patients had thrombocytopenia and leukopenia. Other most common adverse events were neutropenia and nausea. Most adverse events were mild to moderate in severity. One patient had a DLT defined as Grade 3 neutropenia with fever. This patient experienced pyrexia (38.9°C) on Day 3 followed by Grade 3 neutropenia on Day 4.

Serious adverse events were reported in two patients: Grade 2 constipation in one; and Grade 3 decreased appetite and Grade 3 nausea in one. Of these, decreased appetite and nausea were considered to be related to ganitumab and gemcitabine by the investigator. The patient who had treatment-related serious adverse events was hospitalized and recovered with medication.

Three patients discontinued the study treatment owing to adverse events mentioned above. These events were considered to be related to ganitumab. Of these, neutropenia and sudden hearing loss resolved with treatment discontinuation and standard medication (prednisolone, adenosine triphosphate disodium hydrate and mecobalamin for sudden hearing loss; and filgrastim for neutropenia). Interstitial pneumonia did not resolve during the study.

One patient had Grade 2 hyperglycemia. This patient had a history of diabetes, and the blood glucose level was high (7.3 mmol/l) at screening. Hyperglycemia did not resolve during the study despite the medication, and the event was considered to be related to ganitumab and gemcitabine.

All patients were tested for anti-ganitumab antibodies and no one was positive for anti-ganitumab binding antibodies. No neutralizing antibodies were detected.

**Table 2.** Adverse events occurring in at least two patients or categorized into Grade 3 or 4

| Preferred term | Number of patients by adverse event grade ($n = 6$) | | | | Percentage of Grade 3/4 events |
|---|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 | |
| Hematologic | | | | | |
| Thrombocytopenia | 0 | 4 | 2 | 0 | 33 |
| Leukopenia | 1 | 4 | 1 | 0 | 17 |
| Neutropenia | 0 | 1 | 2 | 2 | 67 |
| Lymphopenia | 0 | 3 | 1 | 0 | 17 |
| Non-hematologic | | | | | |
| Nausea | 3 | 1 | 1 | 0 | 17 |
| Constipation | 1 | 3 | 0 | 0 | 0 |
| Decreased appetite | 1 | 1 | 1 | 0 | 17 |
| Vomiting | 2 | 1 | 0 | 0 | 0 |
| Weight decreased | 1 | 2 | 0 | 0 | 0 |
| Angiopathy | 2 | 0 | 0 | 0 | 0 |
| Cancer pain | 1 | 1 | 0 | 0 | 0 |
| Fatigue | 1 | 1 | 0 | 0 | 0 |
| Infusion-related reaction | 0 | 2 | 0 | 0 | 0 |
| Pyrexia | 2 | 0 | 0 | 0 | 0 |
| Rash | 2 | 0 | 0 | 0 | 0 |
| Laboratory changes of interest | | | | | |
| ALT increased | 3 | 1 | 0 | 0 | 0 |
| AST increased | 3 | 0 | 0 | 0 | 0 |
| Hemoglobin decreased | 1 | 3 | 0 | 0 | 0 |
| Blood sodium decreased | 0 | 0 | 1 | 0 | 17 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

## PHARMACOKINETICS

Figure 1 shows the individual values of area under the serum concentration–time curve (AUC) of ganitumab in this study and previous studies. The distribution of AUC values after the first infusion of ganitumab 20 mg/kg in this study was similar to that in the Phase 1 study in Japanese patients with advanced solid tumors (9). Furthermore, individual AUC values in this study were higher than any value after the first infusion of ganitumab 12 mg/kg in the Phase 2 study in patients with mPC (10).

Figure 2 shows the individual values of dose-normalized AUC and maximum observed concentration ($C_{max}$) of gemcitabine on Days 1 and 8. Both of the individual AUC and $C_{max}$ fluctuated and did not show meaningful changes between before (i.e. Day 1) and after (i.e. Day 8) administration of ganitumab. The mean (SD) $C_{max}$ of gemcitabine was 12 990 (3727) ng/ml on Day 1 and 13 380 (6239) ng/ml on Day 8. The mean (SD) $AUC_{0-last}$ of gemcitabine was 7740 (2173) and 6957 (3260) h·ng/ml, respectively.

### Antitumor Activity

In the analysis of tumor response, four patients (80%) had a best response of stable disease and one had progressive disease. The mean percent change of maximum tumor reduction from baseline was 6.6% (SD, 28.9%). The median time to progression was 58.0 (range, 37–113) days. Three patients had a time to progression longer than 100 days (113, 113 and 106 days).

## DISCUSSION

This is the first study which evaluated the tolerability of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m$^2$, and the results show that this regimen was tolerable for patients with previously untreated mPC. Although three of six patients discontinued the study treatment owing to adverse events, these adverse events were generally manageable with treatment discontinuation and standard therapy. One event, interstitial pneumonia, did not resolve during the study, but its severity was mild.

The safety profile of this regimen was consistent with those in the previous studies. In our study, the most common adverse events were thrombocytopenia, leukopenia, neutropenia and nausea. These events were frequently reported in the previous single-agent studies of ganitumab (8,9). In these studies, patients with advanced solid tumors refractory to standard treatment received up to 20 mg/kg of ganitumab every 2 weeks, and the most common toxicities included fatigue and thrombocytopenia (8), as well as neutropenia and leukopenia (9). Neutropenia and thrombocytopenia were also frequently reported in the patients who received ganitumab 12 mg/kg in combination with gemcitabine 1000 mg/m$^2$ (10). Furthermore, leukopenia and neutropenia are the most common severe toxicities of gemcitabine (13). These results suggest that the safety profile of ganitumab does not differ whether it is administered as monotherapy or in combination with gemcitabine, even though its dose is increased to 20 mg/kg. They also suggest that ganitumab and gemcitabine may be combined without synergistic increase of toxicity.

In our study, Grade 2 hyperglycemia was reported in one patient. Although this patient had a history of diabetes, hyperglycemia was noted in 5 of 50 patients without diabetes in the previous single-agent study (8). Ganitumab did not bind to the insulin receptor in non-clinical experiments (7), but hyperglycemia is one of the major toxicities of IGF-1R inhibitors and mild increases in blood glucose levels occur in ~25% of patients treated with anti-IGF-1R antibodies (14). Thus, careful monitoring for hyperglycemia is considered to be necessary. It should also be noted that sudden hearing loss occurred in one patient. A previous study in patients with

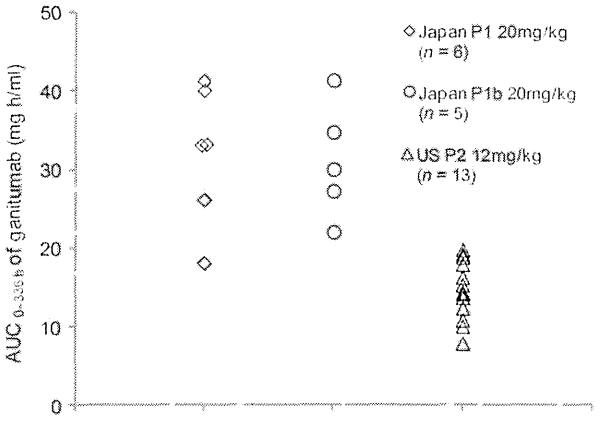


**Figure 1.** Individual AUC values of ganitumab at the first infusion. $AUC_{0-336\ h}$, the area under the concentration–time curve from time 0–336 h. Phase 1 study in Japan includes patients with non-pancreatic cancer who received ganitumab alone. Phase 1b study in Japan and Phase 2 study in the USA include patients with pancreatic cancer who received ganitumab after gemcitabine infusion. In the Japanese Phase 1b study, one patient was excluded from the pharmacokinetic analysis, because the serum concentration data were not available.

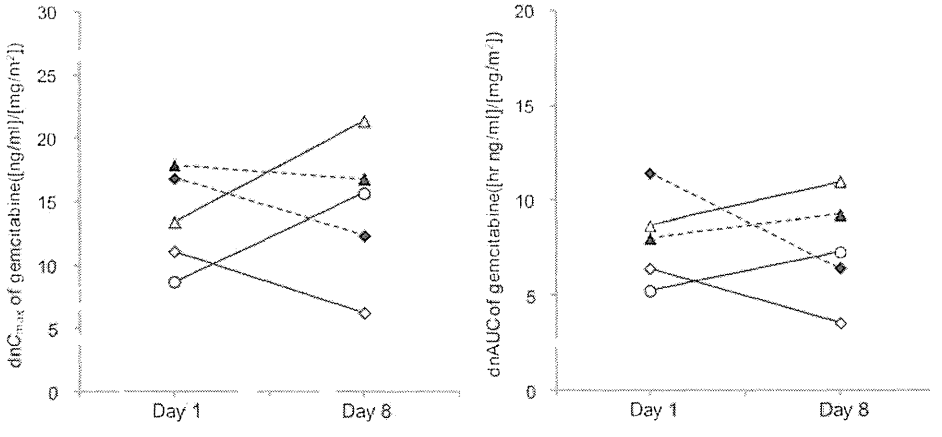


**Figure 2.** Individual values of dose-normalized $C_{max}$ and AUC of gemcitabine on Days 1 and 8. $dnC_{max}$, dose-normalized maximum observed concentration; dnAUC, dose-normalized area under the concentration–time curve; Day 1, after completing gemcitabine infusion, and before ganitumab infusion; Day 8, after completing gemcitabine infusion, and 7 days after ganitumab infusion.

Turner's syndrome has shown that sensorineural hearing loss was negatively correlated with the serum concentration of IGF-1 (15), which suggests that hearing loss may be associated with the use of IGF-1R inhibitors.

In the PK analysis, no apparent drug–drug interaction between ganitumab and gemcitabine was observed. Similar AUC values of ganitumab between our study and a Japanese Phase 1 study in patients with advanced solid tumors (9) indicated that exposure to ganitumab 20 mg/kg would not be affected by the administration of gemcitabine. The mean $C_{max}$ and AUC of gemcitabine in our study did not show any meaningful change between before and after administration of ganitumab. Gemcitabine is a small-molecule drug that is mainly eliminated by cytidine deaminase, whereas ganitumab is an immunoglobulin G1 monoclonal antibody considered to be mainly eliminated via catabolism. Therefore, a mechanism-based drug–drug interaction is not expected. The results on PK parameters in our study supported this expectation.

According to the exposure–response analysis, increased exposure to ganitumab was associated with prolonged progression-free survival and overall survival in patients with mPC (11). Since the ganitumab exposure at 20 mg/kg in our study appeared to be increased in a dose-dependent manner, when compared with that at 12 mg/kg in the Phase 2 study (10), further evaluation on the efficacy outcome at a ganitumab 20 mg/kg dose in patients with mPC is warranted.

No anti-ganitumab binding antibodies were detected in our study. In the previous single-agent study, anti-ganitumab binding antibodies were detected in one patient at Week 9, but no neutralizing antibodies were detected (8). In addition, the AUC values of ganitumab in this patient were similar after the first and third doses. Thus, we consider that the anti-ganitumab binding antibodies had no apparent effect on serum ganitumab concentrations.

Although assessment of efficacy was not a primary objective of our study, the combination of ganitumab and gemcitabine showed potential activity. Four patients (80%) achieved a best response of stable disease, and three (60%) had a time to progression longer than 100 days.

In conclusion, ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m$^2$ was tolerable and showed an acceptable safety profile in Japanese patients with untreated mPC. Exposure to ganitumab at 20 mg/kg in our study was higher than that at 12 mg/kg in the previous Phase 2 study. Appropriateness of using the dose level of ganitumab 20 mg/kg for patients with mPC was confirmed by these findings, and the efficacy and safety of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m$^2$ were evaluated in the randomized Phase 3 study (GAMMA [Gemcitabine and AMG 479 in Metastatic Adenocarcinoma of the Pancreas], Clinical Trials gov. NCT01231347). However, this Phase 3 study was stopped because of futility. Currently, the other clinical data on efficacy, safety and PK are under analysis.

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## Conflict of interest statement

Y.K., K.S. and T.T. are employees of Takeda Bio Development Center Ltd. J.G. is employed in a leadership position and owns stock of Amgen Inc. M.I. has no conflict of interest to disclose.

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ORIGINAL RESEARCH ARTICLE

# Efficacy of Prophylactic Minocycline Treatment for Skin Toxicities Induced by Erlotinib Plus Gemcitabine in Patients with Advanced Pancreatic Cancer: A Retrospective Study

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**Abstract**

*Background* Erlotinib has been reported as being associated with a high incidence of skin toxicities such as acneiform rash, paronychia, and xerosis.

*Objective* The aim of this study was to evaluate the efficacy of prophylactic minocycline treatment for the skin toxicities induced by erlotinib as compared with deferred minocycline treatment in patients with pancreatic cancer treated with erlotinib plus gemcitabine.

*Methods* A total of 96 patients were studied retrospectively, of whom 44 received prophylactic minocycline between August 2012 and June 2013 and 52 received deferred minocycline treatment between August 2011 and July 2012 at the National Cancer Center Hospital East, Kashiwa, Japan. In the prophylactic minocycline group, 200 mg/day oral minocycline was prophylactically administered during the treatment period.

*Results* The incidence rate of acneiform rash and xerosis of any grade during the first 6 weeks of treatment was significantly reduced in the prophylactic minocycline group compared with the deferred minocycline treatment group (47.7 vs. 80.8 %, $p < 0.001$; 2.3 vs. 19.2 %, $p = 0.01$). Multivariate analysis identified prophylactic minocycline as a significant independent factor associated with the incidence of acneiform rash and xerosis of any severity (odds ratio [OR] 0.16, 95 % confidence interval [CI] 0.06–0.46, $p < 0.001$; OR 0.11, 95 % CI 0.01–0.90, $p = 0.04$).

*Conclusion* Prophylactic minocycline appears to be useful for the management of erlotinib-related acneiform rash and xerosis during chemotherapy in patients with advanced pancreatic cancer.

**Key Points**

Skin toxicities induced by erlotinib, a small-molecular tyrosine kinase inhibitor of epidermal growth factor receptor (EGFR), impair quality of life and often necessitate treatment interruption, dose reduction, and discontinuation of treatment.

Tetracycline may have anti-inflammatory effect in addition to its antimicrobial effect.

Prophylactic minocycline appears to be useful for the management of erlotinib-related acneiform rash and xerosis.

## 1 Introduction

In recent years, epidermal growth factor receptor (EGFR) has become an important target for therapy in a variety of cancers. Erlotinib, a small-molecular tyrosine kinase inhibitor of EGFR, administered in combination with gemcitabine has been shown to significantly improve overall

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survival (OS) compared with gemcitabine alone in patients with advanced pancreatic cancer [1]. However, treatment with erlotinib plus gemcitabine has been reported as being associated with the development of an acneiform rash in approximately 70 % of patients [1, 2]. Erlotinib has also been reported as inducing paronychia and xerosis [3, 4]. Severe skin toxicity may impact the efficacy of chemotherapy combined with EGFR-targeted drugs by necessitating treatment interruption, dose reductions, and discontinuation of therapy. In addition, these skin toxicities may also impair the quality of life (QOL) of patients with advanced pancreatic cancer. Therefore, appropriate management of the skin toxicities induced by EGFR inhibitors is essential during combined treatment with chemotherapy in patients with advanced pancreatic cancer.

Tetracycline is a well known antimicrobial drug, but it is also often used to treat the skin toxicities induced by EGFR inhibitors. The efficacy of systemic tetracycline therapy combined with topical moisturizers and/or steroids has been reported for the skin toxicities, including acneiform rash, paronychia, and xerosis, induced by EGFR inhibitors [5, 6]. Thus, tetracycline may also have an anti-inflammatory effect in addition to its antimicrobial effect.

We initially introduced deferred minocycline treatment, initiated after the emergence of grade 2 or 3 skin toxicities, for patients with advanced pancreatic cancer receiving erlotinib plus gemcitabine treated between August 2011 and July 2012. However, skin toxicities induced by erlotinib in patients with advanced pancreatic cancer impaired patients' QOL and often necessitated treatment interruption, dose reductions, and discontinuation of therapy. Therefore, in August 2012, we introduced prophylactic minocycline treatment for the skin toxicities induced by erlotinib in patients with advanced pancreatic cancer. The objective of this study was to retrospectively analyze data from these two patient groups in order to evaluate the efficacy of prophylactic minocycline treatment for the skin toxicities induced by erlotinib as compared with deferred minocycline treatment in patients with advanced pancreatic cancer receiving treatment with erlotinib plus gemcitabine.

## 2 Methods

This retrospective study was conducted with the approval of the ethics committee of the National Cancer Center, and in accordance with epidemiological research guidelines (2013-126).

### 2.1 Patients

Study subjects were patients with advanced pancreatic cancer treated with erlotinib plus gemcitabine for more than 6 weeks at the National Cancer Center Hospital East, Kashiwa, Japan, between August 2011 and June 2013. Patients who did not receive erlotinib plus gemcitabine for more than 6 weeks were excluded from this study, because they had limited chance to develop erlotinib-related skin toxicities. Erlotinib was administered at a dose of 100 mg daily, and gemcitabine was administered intravenously over 30 min at a dose of 1,000 mg/m$^2$ once every week for 3 consecutive weeks; each treatment cycle lasted for 4 weeks. The doses of erlotinib and gemcitabine could be adjusted based on the development of adverse events. The treatment was continued unless disease progression became obvious or unacceptable toxicity occurred.

### 2.2 Skin Treatment Schedule

A total of 96 patients were treated with erlotinib plus gemcitabine between August 2011 and June 2013 at our hospital. Among them, 44 patients treated between August 2012 and June 2013 received prophylactic minocycline treatment, and 52 patients treated between August 2011 and July 2012 received deferred minocycline treatment (Fig. 1). In the prophylactic minocycline group, 200 mg/day oral minocycline was administered from day 1 of chemotherapy and continued. In the deferred minocycline treatment group, 200 mg/day oral minocycline was initiated after the emergence of grade 2 or 3 skin toxicities, in principle. In both groups, emollients such as heparinoids were applied to the susceptible regions (e.g., face, chest, and back) and portions of the skin that dry easily (e.g., hands and feet) from day 1, and skin treatment with topical steroids of strong and medium classes was initiated after the emergence of any skin toxicities. The steroids difluprednate 0.05 % and hydrocortisone butyrate 0.1 % were applied to the body and face, respectively.

### 2.3 Erlotinib and Gemcitabine Relative Dose Intensity

Erlotinib dose intensity was defined as the cumulative dose received divided by the duration of the study therapy in weeks. Erlotinib relative dose intensity was defined as the dose intensity divided by the dose prescribed for the duration of the first 6 weeks of erlotinib administration (100 mg $\times$ the number of days that a patient received treatment).

The total administered doses of gemcitabine were calculated as the total delivered dose of gemcitabine divided by the body surface area (mg/m$^2$). Gemcitabine dose intensity was defined as the total administered doses divided by the duration of the study therapy in weeks. Gemcitabine relative dose intensity was defined as the dose intensity divided by planned dose intensity for the duration of the first 6 weeks.

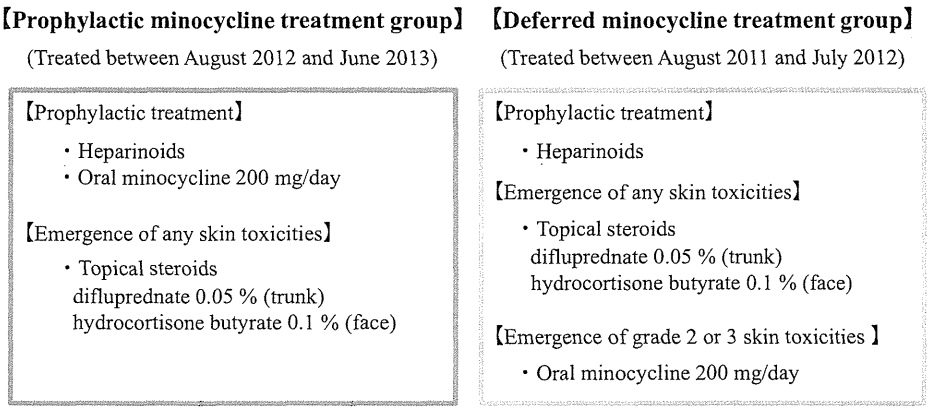


**Fig. 1** Treatment protocols in the prophylactic and deferred minocycline treatment groups

### 2.4 Statistical Analysis

All adverse events, including acneiform rash, paronychia, and xerosis were graded according to the Common Toxicity Criteria for Adverse Events (CTCAE, version 4.0). Skin toxicities were evaluated during the first 6 weeks of erlotinib plus gemcitabine treatment. Time to first occurrence of acneiform rash was defined as the period from the commencement of treatment to the date of the first occurrence of acneiform rash. Responses and progression were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). OS was determined as the period from the commencement of treatment to the date of death from any cause or the date of the last follow-up. Progression-free survival (PFS) was defined as the period from the commencement of treatment to the date of confirmation of disease progression or death. Time to first occurrence of skin toxicity, OS, and PFS were calculated by the Kaplan–Meier product-limit method. The cut-off for the follow-up data was 30 June 2013.

Univariate and multivariate analyses were undertaken to evaluate the relationships between the pretreatment clinical variables and the risk of development of acneiform rash and xerosis. The variables were selected by considering their possible relationships to the incidence of skin rash observed in our own clinical experience or in previous reports [7–9]. The univariate associations between the incidence of skin rash and the pretreatment clinical variables were tested using the $\chi^2$ test or Fisher's exact test. We entered factors identified as significant by univariate analysis with a significance level of $<0.2$ into the multivariate analysis. Multivariate analysis was undertaken using logistic regression to identify significant factors associated with the incidence of acneiform rash of any grade. We used SPSS software (version 17.00, SPSS, Inc., Chicago, IL, USA) for the statistical analysis. $p$ values $<0.05$ were considered to indicate statistical significance.

**Table 1** Patient characteristics

| Variable | Prophylactic minocycline treatment | Deferred minocycline treatment | $p$ value |
|---|---|---|---|
| No. of patients | 44 | 52 | |
| Age, years | 66 (41–82) | 67 (34–83) | 0.40 |
| Gender | | | |
| Female | 16 (36.4) | 23 (44.2) | |
| Male | 28 (63.6) | 29 (55.8) | 0.33 |
| UICC disease stage | | | |
| III | 8 (18.2) | 12 (23.1) | |
| IV | 36 (81.8) | 40 (76.9) | 0.72 |
| Primary tumor site | | | |
| Body or tail of pancreas | 29 (65.9) | 30 (57.7) | |
| Head of pancreas | 15 (34.1) | 22 (42.3) | 0.41 |
| Ascites present | 18 (40.9) | 16 (30.8) | 0.45 |
| ECOG PS | | | |
| 0 | 26 (59.1) | 35 (67.3) | |
| 1 | 18 (40.9) | 15 (28.8) | |
| ≥2 | 0 (0) | 2 (3.8) | 0.23 |
| Prior surgical resection of primary tumor | 2 (4.5) | 3 (5.8) | 0.58 |
| CEA (ng/mL) | 6.4 (0.4–80.3) | 4.2 (0.5–293.4) | 0.33 |
| CA19-9 (U/mL) | 658 (0.1–98,500) | 506.9 (0.1–453,000) | 0.17 |

Data are presented as $n$ (%) or median (range) unless otherwise indicated

*CA19-9* carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *ECOG PS* Eastern Cooperative Oncology Group performance status, *UICC* Union for International Cancer Control

## 3 Results

### 3.1 Patient Characteristics

The baseline characteristics of the study subjects are summarized in Table 1. No significant differences were

**Table 2** Incidence of skin toxicities during the first 6 weeks of treatment with erlotinib plus gemcitabine

| Variables | Prophylactic minocycline treatment | Deferred minocycline treatment | *p* value |
|---|---|---|---|
| Acneiform rash | | | |
| Any grade | 21 (47.7) | 42 (80.8) | <0.001 |
| Grade ≥2 | 9 (20.5) | 15 (28.8) | 0.34 |
| Paronychia | | | |
| Any grade | 7 (15.9) | 11 (21.2) | 0.51 |
| Grade ≥2 | 3 (6.8) | 3 (5.8) | 0.58 |
| Xerosis | | | |
| Any grade | 1 (2.3) | 10 (19.2) | 0.01 |
| Grade ≥2 | 0 (0) | 0 (0) | – |
| ERL dose intensity during first 6 weeks of tx with ERL plus GEM | 83.3 % (14.3–100) | 83.3 % (4.8–100) | 0.49 |
| GEM dose intensity during first 6 weeks of tx with ERL plus GEM | 80.7 % (35.4–100) | 77.0 % (37.9–100) | 0.57 |

Data are presented as *n* (%) or mean (range) unless otherwise indicated

*ERL* erlotinib, *GEM* gemcitabine, *tx* treatment

observed in the baseline characteristics between the prophylactic minocycline treatment group and the deferred minocycline treatment group. The median duration of erlotinib treatment during the first 6 weeks of chemotherapy was 5.1 and 5.2 weeks in the prophylactic and deferred minocycline treatment groups, respectively ($p = 0.66$). No significant differences were observed in the proportion of erlotinib treatment interruption between the prophylactic and the deferred minocycline treatment groups (56.8 vs. 57.7 %; $p = 0.93$). The proportion of erlotinib dose reduction tended to be lower in the prophylactic than the deferred minocycline treatment group, although it was not significant (6.8 vs. 17.3 %; $p = 0.12$). No patients in the prophylactic minocycline treatment group required erlotinib dose reduction for skin toxicities; however, three patients in the deferred group did.

### 3.2 Efficacy of Prophylactic Minocycline Treatment

The incidence rate of acneiform rash of any grade was significantly lower in the prophylactic than in the deferred minocycline treatment group (47.7 vs. 80.8 %; $p < 0.001$; Table 2). The incidence rate of acneiform rash of grade 1 severity was significantly lower in the prophylactic than in the deferred minocycline treatment group (27.3 vs. 51.9 %, $p = 0.01$) (Fig. 2). However, the incidence rate of acneiform rash of grade 2 severity was not significantly lower in the prophylactic than in the deferred minocycline treatment group (20.5 vs. 28.9 %, $p = 0.34$) (Fig. 2). In addition, the incidence rate of xerosis of any grade was also significantly lower in the prophylactic than in the deferred minocycline treatment group (2.3 vs. 19.2 %; $p = 0.01$). However, no significant difference was observed in the incidence rate of paronychia of any grade between the two treatment groups.

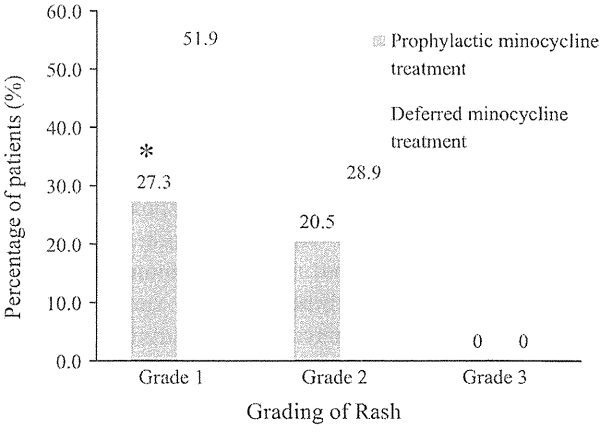


**Fig. 2** Incidence of acneiform rash of each grade of severity in the prophylactic minocycline treatment group and deferred minocycline treatment group. *$p = 0.01$

The median time to first occurrence of acneiform rash was 2.0 weeks (95 % confidence interval [CI] 1.4–2.6) in the deferred minocycline treatment group (hazard ratio [HR] 0.43; 95 % CI 0.26–0.73, $p < 0.001$) (Fig. 3). This was not reached in the prophylactic minocycline treatment group.

### 3.3 Risk Factors for the Development of Acneiform Rash and Xerosis

To identify risk factors for the development of acneiform rash and xerosis, associations between the incidence of acneiform rash or xerosis and patient characteristics were analyzed via univariate analysis (Table 3). The analysis identified prophylactic minocycline treatment as a significant factor associated with the incidence of acneiform rash ($p < 0.001$). In addition, the incidence rate of

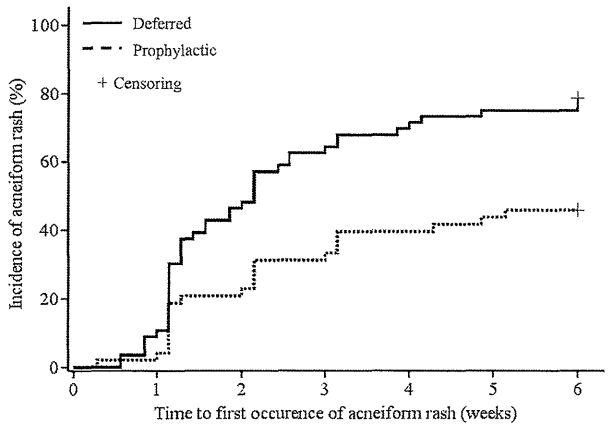


**Fig. 3** Time to first occurrence of acneiform rash of any grade of severity in the prophylactic minocycline treatment group and deferred minocycline treatment group during the first 6 weeks of treatment. Median time to first occurrence of acneiform rash was not reached in the prophylactic minocycline treatment group, as compared with the median time to first occurrence of rash of 2.0 weeks (95 % confidence interval 1.4–2.6) in the deferred minocycline treatment group (hazard ratio 0.43; 95 % confidence interval 0.26–0.73; $p < 0.001$)

acneiform rash was numerically lower in the female (55.0 %) versus male (73.2 %) group ($p = 0.06$); in the group receiving a lower dose intensity of erlotinib (57.5 %) versus the higher-dose intensity group (71.4 %; $p = 0.16$); in the group with Union for International Cancer Control (UICC) stage IV disease (61.0 %) versus those with stage III (84.2 %; $p = 0.06$); and in the group with Eastern Cooperative Oncology Group (ECOG) performance status $\geq 2$ (0 %) versus those with performance status 0–1 (67.0 %; $p = 0.12$) (Table 3).

Prophylactic minocycline treatment (odds ratio [OR] 0.16; 95 % CI 0.06–0.46; $p < 0.001$) and female gender (OR 0.30; 95 % CI 0.11–0.87; $p = 0.03$) were factors significantly associated with the incidence of acneiform rash in the multivariate logistic regression analysis (Table 4).

In addition, prophylactic minocycline treatment ($p = 0.01$) was also identified via univariate analysis as a factor significantly associated with the incidence of xerosis. The incidence rate of xerosis was numerically lower in the group with higher levels of serum carcinoembryonic antigen (CEA) (6.7 vs. 15.7 % in the group with lower serum levels; $p = 0.17$) (Table 3). Multivariate logistic regression analysis also identified prophylactic minocycline treatment as a significant independent factor associated with the incidence of xerosis (OR 0.11; 95 % CI 0.01–0.90; $p = 0.04$; Table 4).

### 3.4 Antitumor Efficacy

The response rate was 9.1 % in the prophylactic and 8.0 % in the deferred minocycline treatment group ($p = 0.85$). The disease control rate was also similar between the two groups (86.4 vs. 84.0 %, respectively; $p = 0.75$). The median OS was 18.0 months (95 % CI 9.5–26.5) in the deferred minocycline treatment group (HR 0.69; 95 % CI 0.30–1.57; $p = 0.38$) and was not reached in the prophylactic minocycline treatment group. The OS rates at 6 months were 76.2 % (95 % CI 60.4–92.1) in the prophylactic minocycline treatment group and 88.1 % (95 % CI 50.9–97.1) in the deferred treatment group. The median PFS time was 5.4 months (95 % CI 4.5–6.2) in the prophylactic group and 5.7 months (95 % CI 3.3–8.2) in the deferred group (HR 0.86; 95 % CI 0.51–1.47; $p = 0.59$).

### 3.5 Safety

The adverse events in the treatment period overall are summarized in Table 5. No significant differences were observed in adverse effects, excluding skin toxicities, between the two groups. In particular, the incidence of nausea, vomiting, vertigo, and liver dysfunction, which are known adverse events associated with the administration of minocycline, were similar between the groups.

## 4 Discussion

Skin toxicities, including acneiform rash, paronychia, and xerosis, are the most frequent adverse effects associated with the administration of EGFR inhibitors [1, 2, 10, 11]. Because neither univariate nor multivariate analysis identified the dose intensity of gemcitabine as a significant independent factor associated with the incidence of acneiform rash or xerosis of any grade of severity, skin toxicities seemed to be unrelated to the gemcitabine treatment. These skin toxicities may impair the QOL of patients and cause treatment interruption, dose reductions, or discontinuation of EGFR-targeted therapy, although they are not life-threatening toxicities. EGFR is normally expressed in undifferentiated, proliferating keratinocytes in the basal and suprabasal layers of the epidermis and regulate normal keratinocyte proliferation, differentiation, migration, and survival [12, 13]. Thus, the skin toxicities induced by EGFR inhibitors appear to involve inflammatory changes in the follicular epithelium [14, 15], although the mechanism is still not fully understood. Tetracyclines are antibacterial drugs; however, several studies suggest that they may also have anti-inflammatory effects. Inhibition of the secretion of tumor necrosis factor (TNF)-α and interleukin (IL)-6, phospholipase $A_2$, nitric oxide synthases, and/or caspase-1 by tetracyclines, with modulation of lymphocyte proliferation, neutrophil migration, and phagocytosis, may represent the mechanisms underlying

**Table 3** Univariate analysis to identify possible factors related to the development of acneiform rash and xerosis during the first 6 weeks of treatment with erlotinib plus gemcitabine

| Variables | *n* | Pts with acneiform rash | Pts without acneiform rash | *p* value | Pts with xerosis | Pts without xerosis | *p* value |
|---|---|---|---|---|---|---|---|
| Minocycline treatment | | | | | | | |
| Prophylactic | 44 | 21 (47.7) | 23 (52.3) | | 1 (2.3) | 43 (97.7) | |
| Deferred | 52 | 42 (80.8) | 10 (19.2) | <0.001 | 10 (19.2) | 42 (80.8) | 0.01 |
| Gender | | | | | | | |
| Female | 40 | 22 (55.0) | 18 (45.0) | | 6 (15.0) | 34 (85.0) | |
| Male | 56 | 41 (73.2) | 15 (26.8) | 0.06 | 5 (8.9) | 51 (91.1) | 0.27 |
| Age, years | | | | | | | |
| <70 | 40 | 25 (62.5) | 15 (37.5) | | 4 (10.0) | 36 (90.0) | |
| ≥70 | 56 | 38 (67.9) | 18 (32.1) | 0.59 | 7 (12.5) | 49 (87.5) | 0.48 |
| BSA ($m^2$) | | | | | | | |
| <1.585 (median) | 49 | 19 (38.8) | 30 (61.2) | | 7 (14.3) | 42 (85.3) | |
| ≥1.585 (median) | 47 | 14 (29.8) | 33 (70.2) | 0.35 | 4 (8.5) | 43 (91.5) | 0.38 |
| Dose intensity of ERL (%) | | | | | | | |
| <83.3 (median) | 40 | 23 (57.5) | 17 (42.5) | | 3 (7.5) | 37 (92.5) | |
| ≥83.3 (median) | 56 | 40 (71.4) | 16 (28.6) | 0.16 | 8 (14.3) | 48 (85.7) | 0.24 |
| Dose intensity of GEM (%) | | | | | | | |
| <79.4 (median) | 47 | 33 (70.2) | 14 (29.8) | | 7 (14.9) | 40 (85.1) | |
| ≥79.4 (median) | 49 | 30 (61.2) | 19 (38.7) | 0.35 | 4 (8.2) | 45 (91.8) | 0.24 |
| UICC disease stage | | | | | | | |
| III | 19 | 16 (84.2) | 9 (47.4) | | 3 (15.8) | 16 (84.2) | |
| IV | 77 | 47 (61.0) | 30 (38.9) | 0.06 | 8 (10.4) | 69 (89.6) | 0.38 |
| Primary tumor site | | | | | | | |
| Body or tail of pancreas | 59 | 37 (62.7) | 22 (37.3) | | 6 (10.2) | 53 (89.8) | |
| Head of pancreas | 37 | 26 (70.3) | 11 (29.7) | 0.45 | 5 (13.5) | 32 (86.5) | 0.43 |
| Ascites | | | | | | | |
| Present | 29 | 17 (58.6) | 12 (41.4) | | 2 (6.9) | 27 (93.1) | |
| Absent | 67 | 46 (68.7) | 21 (31.3) | 0.34 | 9 (13.4) | 58 (86.6) | 0.29 |
| ECOG PS | | | | | | | |
| 0–1 | 94 | 63 (67.0) | 31 (33.0) | | 11 (11.7) | 83 (88.3) | |
| ≥2 | 2 | 0 (0) | 2 (100) | 0.12 | 0 (0) | 2 (100) | 0.78 |
| CEA (ng/mL) | | | | | | | |
| <5.25 (median) | 51 | 35 (68.6) | 16 (31.4) | | 8 (15.7) | 43 (84.3) | |
| ≥5.25 (median) | 45 | 28 (62.2) | 17 (37.8) | 0.51 | 3 (6.7) | 42 (93.3) | 0.17 |
| CA19-9 (U/mL) | | | | | | | |
| <658 (median) | 51 | 36 (70.6) | 15 (29.4) | | 6 (11.8) | 45 (88.2) | |
| ≥658 (median) | 45 | 27 (60.0) | 18 (40.0) | 0.28 | 5 (11.1) | 40 (88.9) | 0.92 |

Data are presented as *n* (%) unless otherwise indicated

*BSA* body surface area, *CA19-9* carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *ECOG PS* Eastern Cooperative Oncology Group performance status, *ERL* erlotinib, *GEM* gemcitabine, *pts* patients, *UICC* Union for International Cancer Control

the anti-inflammatory effect of tetracyclines [16–19]. In addition, there were almost no minocycline-related adverse effects in this study. Based on these findings, we theorized that we could effectively counteract the inflammatory changes in the follicular epithelium with prophylactic systemic tetracycline treatment.

In this retrospective analysis, we clarified that prophylactic oral minocycline treatment significantly reduced the incidence of acneiform rash and xerosis induced by erlotinib in patients with advanced pancreatic cancer. Furthermore, no significant differences between the two groups were observed in the incidence of nausea, vomiting,