

Effects of Alkalization Therapy on Chemotherapy Outcomes in Metastatic or Recurrent Pancreatic Cancer

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Abstract. *Background/Aim:* The acidic tumor micro-environment is associated both with the progression and drug resistance of cancer. We aimed to investigate the effects of alkalization therapy performed concurrently with chemotherapy on the survival of advanced pancreatic cancer patients (study registration: UMIN 000035659). *Patients and Methods:* Twenty-eight patients with metastatic or recurrent pancreatic cancer were assessed in this study. Alkalization therapy consisted of an alkaline diet with supplementary oral sodium bicarbonate (3.0-5.0 g/day). *Results:* The mean urine pH was significantly higher after the alkalization therapy (6.85 ± 0.74 vs. 6.39 ± 0.92 ; $p < 0.05$). The median overall survival from the start of alkalization therapy of the patients with high urine pH (> 7.0) was significantly longer than those with low urine pH (≤ 7.0) (16.1 vs. 4.7 months; $p < 0.05$). *Conclusion:* An alkalization therapy may be associated with better outcomes in advanced pancreatic cancer patients treated with chemotherapy.

Pancreatic cancer is a highly aggressive disease in which current standard chemotherapy regimens have limited effects. The median overall survival (OS) of metastatic pancreatic cancer patients in a prospective study of European and American populations was reported to be 11.1 months, using a combination regimen consisting of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX), and 8.5 months in the group receiving nab-paclitaxel plus gemcitabine (1, 2). In a Japanese patient population with metastatic pancreatic cancer, the median OS was 10.7 months in FOLFIRINOX-treated patients and 13.5 months in nab-paclitaxel plus

gemcitabine-treated patients (3, 4). Cancer cells tend to produce adenosine triphosphate (ATP) using aerobic glycolysis, even in the presence of sufficient oxygen, although normal cells usually generate ATP via oxidative phosphorylation. This alteration in energy metabolism is characteristic of cancer cells. Increased glycolysis causes the overproduction of lactic acid, and the export of protons from tumor cells into the extracellular space by acid-base regulators, such as Na^+/H^+ exchangers and monocarboxylate transporters, creates an acidic tumor microenvironment (5, 6). An acidic tumor microenvironment is associated both with the progression and drug resistance of solid tumors (7, 8). An *in vitro* study of pancreatic ductal adenocarcinoma cells demonstrated that the acidic microenvironment activates proton-sensing G-protein-coupled receptors, which increase the expression of IL-6 and may be associated with cancer cell proliferation (9).

Several *in vivo* and *in vitro* studies have demonstrated that tumor acidity can be altered through bicarbonate administration, and suggested that systemic buffering may lead to antitumor effects. In mouse models of metastatic breast cancer, neutralization of the acidic tumor microenvironment was shown to suppress the metastasis of cancer cells and improve survival (10). Similarly, in mouse models of pancreatic cancer, trishydroxymethyl aminomethane (tris base) buffer was reported to have similar effects as bicarbonate in reducing tumor volume and increasing survival (11). Recently, our group reported that prolonged progression-free survival (19.5 months) and OS (28.5 months) were observed in advanced lung cancer patients ($n=11$) treated with epidermal growth factor receptor tyrosine kinase inhibitor and an alkaline diet (eating fruit and vegetables and limiting meat and milk). We also reported that an alkaline diet resulted in the alkalization of urine pH (6.95 ± 0.05) (12). A prospective clinical trial in healthy volunteers demonstrated that the long-term consumption of sodium bicarbonate (0.5 g/kg/day) was feasible and safe, and an increase in urine pH was observed following bicarbonate intake (13). In cancer patients, a pilot phase I clinical study that investigated the safety of the long-term consumption of sodium bicarbonate in patients with cancer was conducted by a group at the University of Arizona

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(ClinicalTrials.gov Identifier: NCT02531919), although the detailed results have not yet been reported. It remains unclear whether intervention with alkalization therapy, results in better outcomes in advanced pancreatic cancer patients treated with standard chemotherapy. We hypothesized that alkalization therapy induced by an alkaline diet, with or without bicarbonate, would lead to survival benefits in pancreatic cancer patients in a pH-associated fashion. Therefore, we conducted a retrospective study to investigate the effects of urine pH on the survival of advanced pancreatic cancer patients receiving alkalization therapy and chemotherapy.

Patients and Methods

Patients. This study was retrospectively conducted to investigate the effects of alkalization therapy on chemotherapy outcomes in advanced pancreatic cancer patients. A total of 50 patients with metastatic or recurrent pancreatic cancer were treated at the Karasuma Wada Clinic between April 2015 and December 2017. Alkalization therapy was initiated in all patients as described below. Written informed consent from each patient was obtained. Patients were allowed to receive all appropriate concomitant chemotherapy treatments during the study. Other interventional therapies were not recommended in this study. All patients received supplementary intravenous (*i.v.*) vitamin C (25-50 g/day once every 1 or 2 weeks). If patients refused to follow an alkaline diet owing to their dietary preference, they were excluded from the study population. Therefore, 22 patients who did not follow the alkalization therapy or visited our clinic less than 3 times were excluded from the study. Finally, 28 pancreatic cancer patients who were given alkalization therapy together with chemotherapy were analyzed. All procedures were performed in accordance with the ethical principles stated in the 1995 Declaration of Helsinki. This study was approved by the Institutional Review Board of the Japan-Multinational Trial Organization and was registered with UMIN Clinical Trials (UMIN 000035659).

Alkalization therapy. We defined alkalization therapy as treatments that have an alkalinizing effect, such as an alkaline diet and bicarbonate therapy. In this study, all patients had an alkaline diet to increase their urine pH, with more vegetables and fruits and less meat and dairy products. Patients were instructed to take at least 400 g of fruits and vegetables per day and not to take meat and dairy products, although the actual diet was decided by the patients at home. All patients recorded their daily meals during at least the first 4 weeks from the start of the alkaline diet, to confirm whether the meals were appropriate or not, and they were given advice according to their records. At every visit, a doctor or nurse provided patients with instructions on an alkaline diet and assessed whether patients had been following the alkaline diet regularly. Oral bicarbonate (3.0-5.0 g/day) was given when urine pH did not increase above 7.0 or when patients wished to take it.

Assessment procedures. OS from either the time of diagnosis or recurrence and the start of the alkalization therapy in each patient was calculated. Urine pH was analyzed at regular visits, at least once every 2 months, or up to twice a month. Overall tumor burden was assessed by calculating the sum of the diameters of all measurable diseases based on computed tomography scans taken

Table I. *Patient characteristics.*

Characteristics	Value
No. of patients	28
Age (years)	63.6 (47-82)
Gender	
Men	15
Women	13
Performance status	
0	2
1	24
2	1
3	1
Clinical stage	
Metastatic	19
Postoperative recurrence	9
Chemotherapy before first visit	
Yes	23
No	5

within 2 months before the start of alkalization therapy, according to the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 criteria.

Statistical analyses. Data were analyzed on June 30, 2019. Mean urine pH values were calculated for each patient before and after initiation of the alkalization therapy. Urine pH data before alkalization therapy included urine samples during the 6 months before the initiation of alkalization therapy. Urine pH data after alkalization therapy included all urine samples after the initiation of alkalization therapy. The mean urine pH of each patient was compared using the paired *t*-test between before and after alkalization therapy. OS from either the time of diagnosis or recurrence and the start of alkalization therapy was calculated using Kaplan–Meier estimates, and compared between patients with a urine pH of 7.0 or less and those with a urine pH of higher than 7.0. OS from the start of the alkalization therapy was also compared between patients with a urine Δ pH of 1.0 or less and those with a urine Δ pH of more than 1.0. OS from the time of initiation of the alkalization therapy was also compared among patients with a urine Δ pH of 0 or less, those with a Δ pH of more than 0, but less than or equal to 1.0, and those with a Δ pH of more than 1.0. Urine Δ pH was calculated by subtracting the mean urine pH before alkalization therapy from the mean urine pH after alkalization therapy. Mean data set values were presented with \pm standard deviation. All *p*-values were two-sided and *p*-values of less than 0.05 were considered to indicate a statistically significant difference between two groups. All statistical analyses were performed with EZR (version 1.32) (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface that is a modified version of R (The R Foundation for Statistical Computing, Vienna, Austria) (14).

Results

Patient characteristics. The patients analyzed included 15 men and 13 women, and the mean age at the start of the alkalization therapy was 63.6 (range=47-82) years. Daily

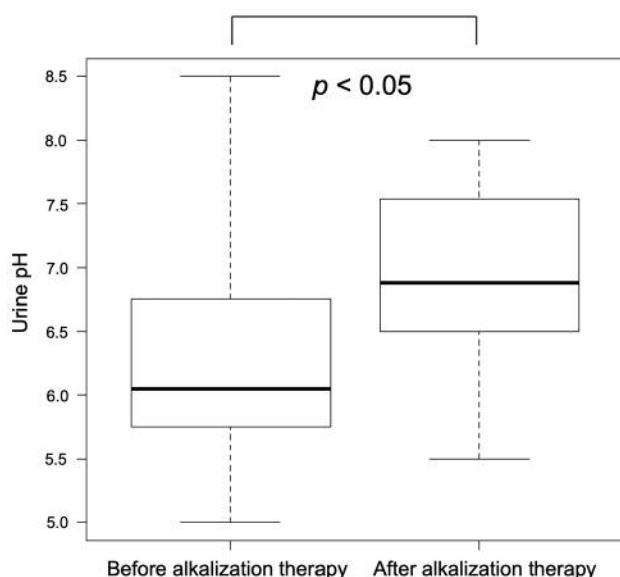


Figure 1. Effect of alkalinization therapy on urine pH. The mean urine pH before and after initiation of alkalinization therapy is shown.

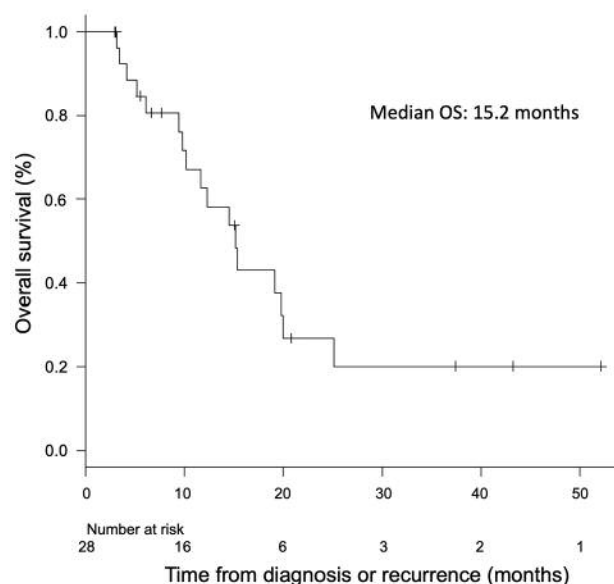


Figure 2. Overall survival of the patients from the time of diagnosis or recurrence. Kaplan–Meier curve of the overall survival of the patients from the time of diagnosis or recurrence.

living ability of each patient was evaluated according to the Eastern Cooperative Oncology Group scale of performance status (PS). Two patients had PS 0, 24 had PS 1, and 1 each had PS 2 and 3. Nineteen patients were clinical stage IV and 9 had recurrent disease. Twenty-three patients had received chemotherapy before their first visit to our clinic, whereas 5 had not. Twenty out of the 28 patients took supplementary bicarbonate. Patient characteristics are presented in Table I.

Urine pH analysis. The mean urine pH of the patients before and after starting alkalinization therapy are shown in Figure 1. A significant difference was observed between mean urine pH before and after initiation of alkalinization therapy (6.39 ± 0.92 vs. 6.85 ± 0.74 ; $p < 0.05$).

Overall survival. The median OS from the time of diagnosis or recurrence was 15.2 months [95% confidence interval (CI)=9.8-20], and the median OS from the start of alkalinization therapy was 8.4 months (95%CI=4.2-17.6), as shown in Figures 2 and 3. The mean time from diagnosis or recurrence to the start of alkalinization therapy was 5.13 ± 4.55 months. Seventeen of the 28 patients have died as of June 2019. After the start of the alkalinization therapy, the median OS of patients with a urine pH of higher than 7.0 was 16.1 months [$n=11$, 95%CI=5.5–not available (NA)] as compared with 4.7 months for patients with a urine pH of 7.0 or lower ($n=17$, 95%CI=3.4–9.2; $p < 0.05$) (Figure 4). The median OS of patients with a urine Δ pH of more than 1.0 was 16.1 months ($n=9$,

95%CI=5.5-NA) as compared with 4.3 months for patients with a urine Δ pH of 1.0 or lower ($n=19$, 95%CI=2.8-8.4; $p < 0.05$) (Figure 5). The Kaplan–Meier curves of OS from the time of start of the alkalinization therapy for patients with a Δ pH of 0 or less, those with a Δ pH of more than 0 but less than or equal to 1.0, and those with a Δ pH of more than 1.0, are shown in Figure 6. Patient characteristics according to urine pH values are shown in Table II. The mean time from diagnosis or recurrence to the start of alkalinization therapy was not significantly different between the 2 groups; however, it tended to be shorter in patients with a urine pH of higher than 7.0, than in those with a urine pH of 7.0 or lower. Overall tumor burden was not significantly different between the 2 groups with either a urine pH of higher than 7.0 or lower than 7.0. Regarding non-measurable disease according to RECIST version 1.1, 1 patient had peritoneal dissemination with ascites and 1 had peritoneal dissemination in the group with a urine pH of higher than 7.0. In contrast, 3 patients had peritoneal dissemination in the group with a urine pH of 7.0 or lower. The regimens of chemotherapy that patients received together with their alkalinization therapy, as well as other patient characteristics did not differ remarkably between the two groups.

Discussion

Our study demonstrated that alkalinization therapy, consisting of an alkaline diet with or without bicarbonate supplementation, significantly increased the urine pH of patients compared with

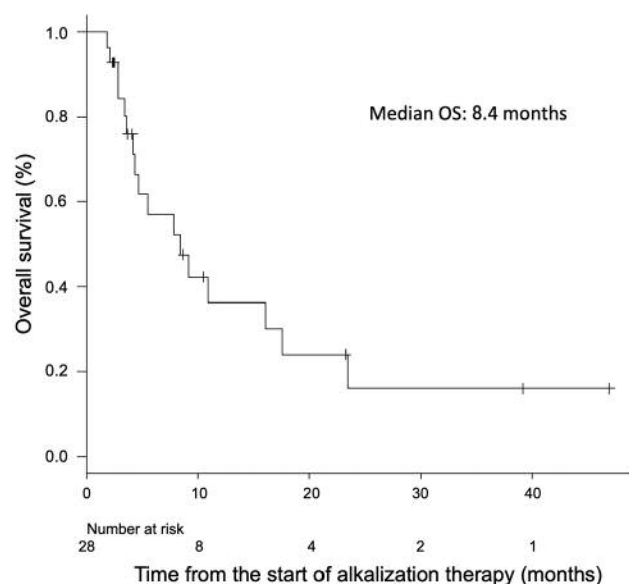


Figure 3. Overall survival of the patients from the start of alkalization therapy. Kaplan–Meier curve of the overall survival of the patients from the time of start of alkalization therapy.

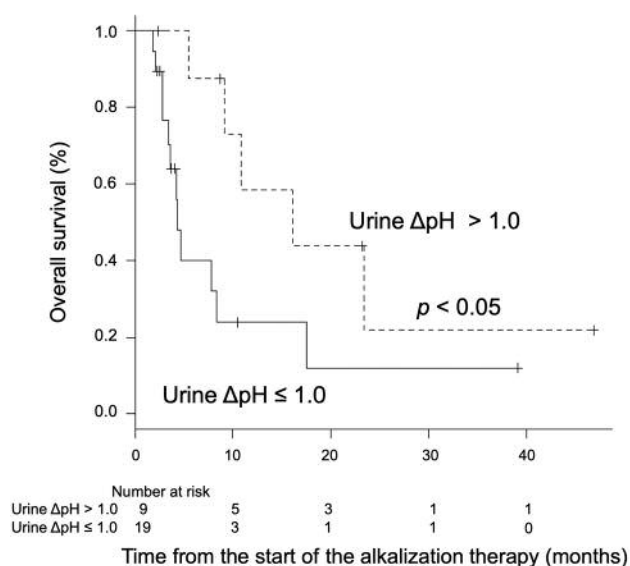


Figure 5. Association between overall survival and urine ΔpH. Kaplan–Meier curves of the overall survival from the start of alkalization therapy between patients with a urine ΔpH of higher than 1.0 and 1.0 or lower.

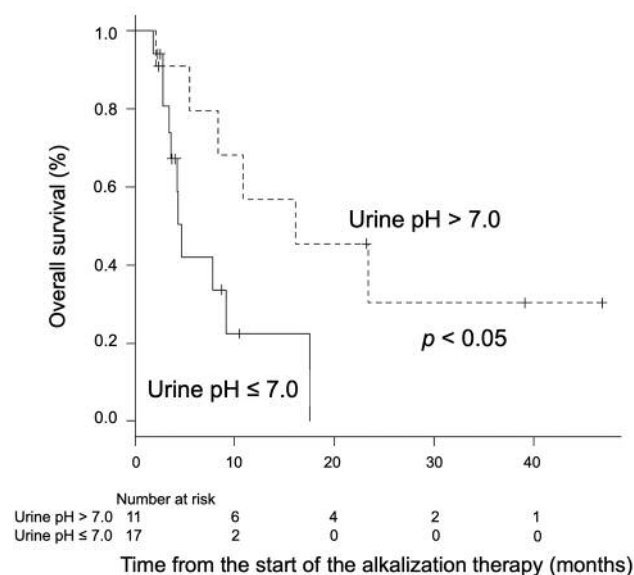


Figure 4. Association between overall survival and urine pH. Kaplan–Meier curves of the overall survival from the start of the alkalization therapy between patients with a urine pH of higher than 7.0 and 7.0 or lower.

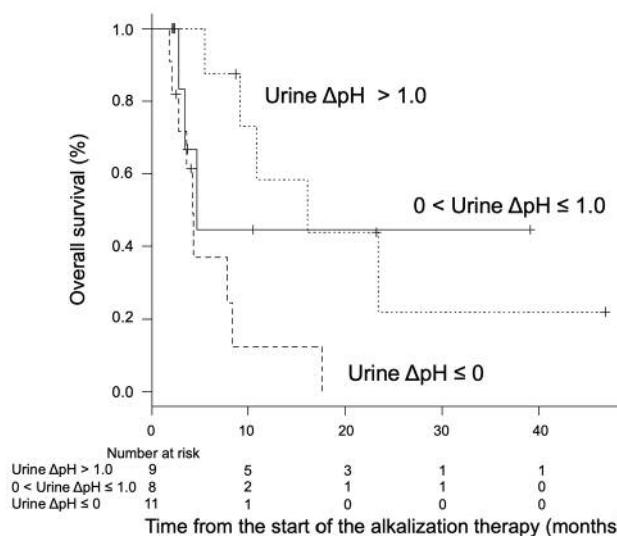


Figure 6. Overall survival of patients with different urine ΔpHs. Kaplan–Meier curves of the overall survival from the start of alkalization therapy of patients with a ΔpH of 0 or less, those with a ΔpH of more than 0 but less than or equal to 1.0, and those with a ΔpH of more than 1.0.

that at the initiation of treatment. Although the effects of chemotherapeutic drugs on urine pH are not well known, a clinical study reported that cisplatin did not affect urine pH (15). Robey *et al.* reported that sodium bicarbonate

consumption of 0.5 g/kg/day (*i.e.*, 25 g/50 kg body weight) was associated with an increase in urine pH (12). In our study, all patients were instructed to follow an alkaline diet, and 20 out of the 28 patients also consumed bicarbonate at a dose of only

Table II. Comparison of the characteristics of patients with different urine pH values.

	Urine pH >7.0 (N=11)	Urine pH ≤7.0 (N=17)
Age (years)	67.6 (54-82)	61.0 (47-77)
Gender		
Men	5	10
Women	6	7
Performance status		
0	2	0
1	8	16
2	0	1
3	1	0
Time from diagnosis or recurrence to alkalization therapy (months)	3.45±4.04	6.21±4.65
Mean urine pH		
Urine pH before alkalization therapy	6.41±1.02	6.37±0.87
Urine pH after alkalization therapy	7.53±0.26	6.38±0.54
Clinical stage		
Metastatic	8	11
Postoperative recurrence	3	6
Overall tumor burden		
Sum of measurable diseases (mm)	126.6±164.0 (N=10)	121.0±161.0 (N=13)
Chemotherapy		
Nab-paclitaxel plus gemcitabine	5	6
Gemcitabine plus erlotinib	1	4
Gemcitabine	3	1
S-1	1	3
Erlotinib	1	2
Unknown	0	1

3.0-5.0 g/day, which was much lower than that of previous reports (12). An epidemiological study reported that high fruit and vegetable consumption and low meat intake was significantly associated with more alkaline urine (16). Our previous study showed a significant increase in urine pH after an alkaline diet alone compared with before intervention (n=11) (11). Fruits and vegetables are known to have an alkalinizing effect on urine pH, and meat has an acidifying effect on urine pH, as demonstrated from renal net acid excretion, which was calculated to predict the acid-base balance (17). Therefore, we speculated that the combination of an alkaline diet and bicarbonate in our study may have had a stronger effect to alkalize urine pH than a single intervention of either an alkaline diet or bicarbonate.

The median OS of advanced pancreatic cancer patients in our study from diagnosis or the detection of recurrence was 15.2 months, which was not inferior to that of the current standard treatment using FOLFIRINOX or nab-paclitaxel plus gemcitabine (1-4), although this comparison should be interpreted with caution. Alkalization of the tumor microenvironment is thought to be associated with improved results of cancer treatment (7). Mathematical models and computer simulation studies confirmed that bicarbonate raised tumor extracellular pH (18, 19). A low pH in the extracellular

environment of cancer cells causes multidrug resistance *in vitro* and *in vivo* (7, 20, 21). In MCF-7 human breast cancer cells, doxorubicin cytotoxicity was reported to decrease with lower pH, shown by the data that the half maximal effective concentration (EC₅₀) of doxorubicin was 0.12 μm at an extracellular pH 7.4, compared to 0.27 μm at pH 6.8 (22). In R3327-AT1 prostate carcinoma cells, the cytotoxicity of daunorubicin was reported to decrease after incubation at pH 6.6, compared with that at pH 7.4 (23). Two mechanisms of multidrug resistance were suggested. First, some drugs, mainly weak-base chemotherapeutic drugs, are positively charged in environments of acidic extracellular pH, and become trapped in extracellular compartments, reducing cellular uptake and efficacy (24, 25). Second, the activity and expression of the multidrug transporter p-glycoprotein is increased by acidic extracellular pH (23, 26). It was also reported that the acidity of the tumor microenvironment affects cancer immunity. An *in vitro* study demonstrated that an acidic environment suppressed the T-cell response and decreased the secretion of IFN-γ and TNF-α. In mouse models of melanoma, alkalization of bicarbonate was reported to enhance the effects of anti-programmed cell death 1 therapy (27). These studies support our speculation that alkalization therapy may be associated with a more favorable chemotherapeutic effect on

advanced pancreatic cancer patients; however, further studies are required to investigate the correlation between alkalization therapy and improved OS.

In this study, we demonstrated that a urine pH of more than 7.0 was significantly associated with prolonged OS in advanced pancreatic cancer patients, compared with a urine pH of 7.0 or less. We showed that the initial urine pH before alkalization therapy between the two groups with a urine pH of more than 7.0 and a urine pH of 7.0 or less after alkalization therapy did not differ remarkably. Therefore, the difference in OS after alkalization therapy might be associated with alkalization effects. We also demonstrated that the median OS of patients with a urine Δ pH of higher than 1.0 was significantly longer than those with a urine Δ pH of 1.0 or lower, and there was a tendency of correlation between an increase in Δ pH and prolonged OS. It has been reported that an acidic environment affects the early stages of the neoplastic process. In Syrian hamster embryo cells cultured at pH 6.7, multistage neoplastic transformation was observed to be at least 10- to 20-fold greater than that at pH 7.35 (28). Wojtkowiak *et al.* from the group of the Lee Moffitt Cancer Center demonstrated that chronic low pH growth conditions induced autophagy associated with cancer survival. In MDA-MB-231 human breast cancer cells, the markers of autophagy were significantly increased when cultured at pH 6.7, compared with those at pH 7.4 (29). These data support the idea that the viability of cancer cells is associated with pH. To our knowledge, the present study is the first to report the association between an increase in urine pH and longer OS of advanced pancreatic cancer patients.

Activated mutations in the *KRAS* oncogene are observed in more than 90% of invasive and metastatic pancreatic cancer cases (30). Oncogenic *KRAS* mutations promote tumor metabolism through the stimulation of glucose uptake (31). In mouse models of colorectal cancer with *KRAS* mutations, it has been reported that high-dose vitamin C inhibits glyceraldehyde 3-phosphate dehydrogenase in highly glycolytic *KRAS* mutant cells and leads to cell death (32). In the present study, all patients received supplementary *i.v.* vitamin C, which may also have affected the results of pancreatic cancer patient treatment.

We acknowledge that there are several limitations to this study. First, this study was a single-center retrospective analysis and the sample size was small. Second, the timing of the start of alkalization therapy was not consistent and the details of the patients' daily diet were not meticulously controlled. Therefore, a further prospective study is necessary to validate our results. Third, although we showed increases in urine pH of the patients in this study, we did not analyze the extracellular pH in the environment surrounding cancer cells. However, it is difficult to measure the extracellular pH of cancer cells in the actual clinical setting. As there are no sufficient lines of evidence that urine pH

reflects the pH of the tumor microenvironment, further investigation of the association between extracellular pH and urine pH is necessary. Hence, we could not rule out that our poor OS group with a urine pH of 7.0 or less might be simply a cohort of patients with more aggressive disease owing to factors other than the alkalization process, because patients with advanced pancreatic cancer with very limited OS may be associated with a more acidic microenvironment that is more difficult to control by alkalization therapy. Finally, alkalization by eating more vegetables and fruits and less meat and dairy products may not be the only effect in this study, because a diet that is high in vegetables and fruits may have some potential effects on the metabolism of cancer cells, such as *via* anti-inflammatory effects, caloric restriction, and changes in insulin levels and the microbiome.

Conclusion

We demonstrated that the combination of chemotherapy with alkalization therapy is associated with more favorable outcomes in advanced pancreatic cancer patients and patients with recurrent pancreatic cancer who had increased urine pH after alkalization therapy. Further studies are required to investigate whether alkalization therapy is associated with alkalization of the tumor microenvironment and with more favorable outcomes.

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Conflicts of Interest

The Authors declare that they have no conflicts of interest associated with this study.

Authors' Contributions

Reo Hamaguchi performed the literature review, analyzed the data, and wrote the article. Ryoko Narui performed the acquisition of data. Hiromi Wada supervised the study. All Authors conceived and designed the study and gave final approval for publication.

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