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Monitoring FTD in the peripheral blood mononuclear cells of elderly patients with metastatic colorectal cancer administered FTD plus bevacizumab as first-line treatment

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Abstract

Trifluridine/tipiracil (FTD/TPI) is an orally administrated anticancer drug with efficacy validated for patients with metastatic colorectal cancer (mCRC) or gastric cancer. FTD, a key component of FTD/TPI, exerts antitumor effects via its incorporation into DNA. Using specific antibodies against bromodeoxyuridine, FTD incorporation into DNA is detected in tumors and peripheral blood mononuclear cells (PBMC) of patients with mCRC who are administered FTD/TPI. The proportion of FTD-positive PBMC fluctuates according to the schedule of treatment, although the association between the proportion of FTD-positive PBMC and the clinical outcomes of patients is unknown. To answer this question, here we monitored the FTD-positive PBMC of 39 elderly patients with mCRC enrolled in KSCC1602, a single-arm phase 2 trial of FTD/TPI plus bevacizumab as a first-line treatment, for 1 month, during the first cycle of treatment. The median values and interquartile ranges of the percentage of FTD-positive PBMC on days 8, 15, and 29 were 39.3% (30.7%-52.2%), 66.9% (40.0%-75.3%), and 13.5% (5.7%-26.0%), respectively. Receiver operating characteristic analysis revealed that the percentage of FTD-positive PBMC on day 8 (the end of the first week of treatment) had moderate ability to accurately diagnose the occurrence of severe neutropenia and leukopenia within 1 month (area under the curve = 0.778 [95% confidence interval, 0.554-0.993]). This result suggests that excess FTD incorporation into PBMC at the initial phase of FTD/TPI plus bevacizumab treatment is a risk factor for early onset of severe hematological adverse events.

KEYWORDS

bevacizumab, leukopenia, neutropenia, peripheral blood mononuclear cells, trifluridine/tipiracil

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1 | INTRODUCTION

Trifluridine/tipiracil (FTD/TPI) is an orally administrated anticancer drug with efficacy validated for patients with metastatic colorectal cancer (mCRC) or gastric cancer who are refractory or intolerant to initial standard therapy.[1,2] FTD, a thymidine analog, is a cytotoxic component of FTD/TPI, and TPI hydrochloride inhibits FTD degradation in vivo to maintain adequate plasma levels of FTD.[3,4] When FTD is incorporated into the DNA of tumor cells during DNA replication, it exerts antitumor activity[5] and induces DNA replication stress, which suppresses tumor growth irrespective of *TP53* mutations.[6,7] Furthermore, the incorporation of FTD into proliferating normal cells may trigger adverse events. In a mouse model, FTD is efficiently incorporated into bone marrow cells,[8] which is associated with hematological toxicity caused by bone marrow suppression (eg, neutropenia or leukopenia), representing the most frequent adverse event of FTD/TPI.[1,2,9,10] Intriguingly, neutropenia induced by FTD/TPI is associated with better prognosis of patients with refractory mCRC,[9-11] although the mechanism underlying this association is unknown.

FTD is structurally and electrostatically similar to bromodeoxyuridine (BrdU), which is specifically recognized by anti–BrdU antibodies.[12] Using these antibodies, we detected FTD in peripheral blood mononuclear cells (PBMC)[8] and tumors[13] of patients with mCRC who were administered FTD/TPI. Although the proportion of FTD-positive PBMC fluctuates according to the schedule of FTD/TPI treatment,[8] the correlation between the proportion of FTD-positive PBMC and the clinical outcomes of patients is unknown.

FTD/TPI is approved as a monotherapy for the treatment of patients with metastatic cancer. A recent phase 1b/2 clinical trial (C-TASK FORCE) found that FTD/TPI plus bevacizumab is effective and tolerable for patients with mCRC who are refractory or intolerant to standard chemotherapy.[9] We conducted an open-label, single-arm phase 2 trial (KSCC1602) of FTD/TPI plus bevacizumab for elderly patients without prior chemotherapy.[14] This post-hoc study monitored FTD-positive PBMC of patients with mCRC for 1 month, during the first cycle of treatment, and assessed their diagnostic ability to predict the occurrence of adverse events and as well as prognosis.

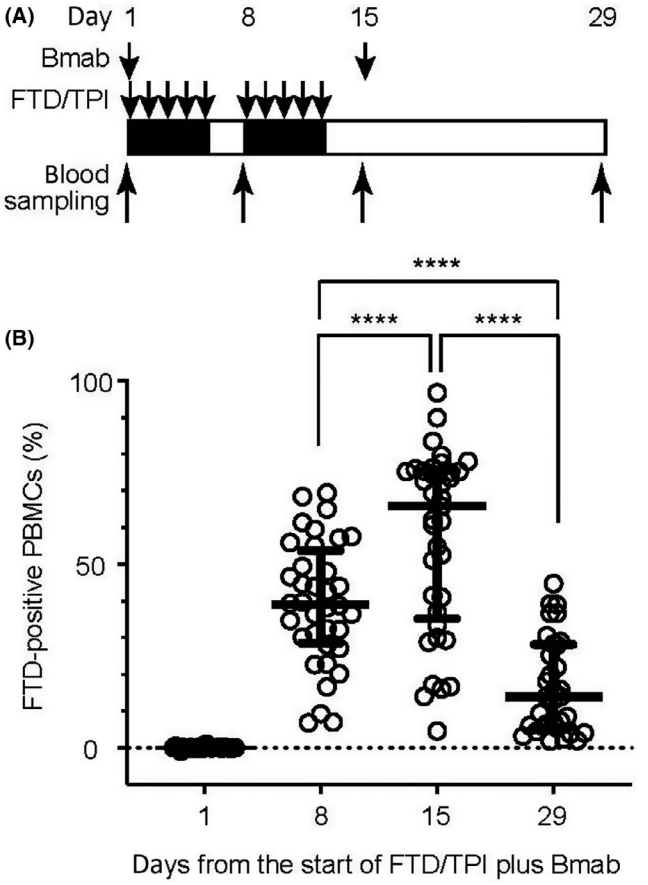

FIGURE 1 FTD-positive peripheral blood mononuclear cells (PBMC) of patients with mCRC treated with FTD/TPI plus bevacizumab. (A) Schedule of FTD/TPI plus bevacizumab treatment and blood sampling. Bmab: intravenous injection of 5 mg/kg bevacizumab. FTD/TPI: 70 mg/m^2 orally administered twice each day. (B) Scatter plots of FTD-positive PBMC. The median values and interquartile ranges are indicated by the bold horizontal bars. ****$P < .0001$, Wilcoxon signed-rank test

2 | MATERIALS AND METHODS

2.1 | Patients

We analyzed the records of 39 patients with mCRC who were enrolled in KSCC1602(UMIN000025241), an open-label, single-arm phase 2 trial of FTD/TPI plus bevacizumab for elderly patients not previously treated with chemotherapy.[14] Treatment comprised FTD/TPI (70 mg/m^2 orally, twice daily on days 1-5 and 8-12) plus bevacizumab (5 mg/kg intravenously on days 1 and 15) administered every 4 weeks (Figure 1A). Eligibility criteria were as follows: age ≥70 years, histologically confirmed unresectable metastatic colorectal adenocarcinoma, no history of chemotherapy, Eastern Cooperative Oncology Group performance status 0 or 1, at least one measurable lesion, and evaluable disease according to the Response Evaluation Criteria in Solid Tumours (version 1.1) (Table S1). The Institutional Review Board of Kyushu University Hospital approved the study protocol (number 28 056), which conforms with the ethical guidelines of the current version of the Declaration of Helsinki (2013). We requested that each patient grant written informed consent before participating in the study.

2.2 | Blood sampling and isolation of peripheral blood mononuclear cells

Blood was collected from patients on days 1, 8, 15, and 29 (Figure 1A), mixed with an anticoagulant solution containing heparin and citrate phosphate dextrose (NP-SC1000, Cat# 31-620, Nipro), and stored at room temperature. Within 3 days after blood sampling, PBMC were isolated by centrifugation with Lymphoprep (Cat #1114544, CosmoBio) and fixed with 70% ethanol.

2.3 | Fluorescence-activated cell sorting analysis of FTD-positive peripheral blood mononuclear cells

Fixed PBMC (1×10^6 cells) were depurinated for 30 minutes in 2N HCl, 0.5% Triton X-100 in PBS, blocked with 1% FBS, and reacted with an anti–BrdU antibody (B44; BD Bioscience) or control mouse IgG antibodies. Samples were incubated with an Alexa 488-conjugated goat anti–mouse IgG antibody (Thermo Fisher Scientific) with propidium iodide (5 μg/mL) and analyzed using a FACS Calibur (BD Bioscience).[8] The gated area of FTD-negative PBMC and the boundary between FTD-positive and FTD-negative PBMC were determined in each sample using a control mouse antibody against IgG.

2.4 | Statistical analysis

Comparisons between groups were performed using the Wilcoxon signed-rank test (Figures 1B and 2D) or Dunn's multiple comparison test (Figure S2A,B). Univariate analysis was performed using the Cox proportional hazards model (Table 3). Receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) was measured to evaluate the ability to diagnose the occurrence of severe neutropenia and leukopenia (Figure 2A-C and Figure S3A-C). When the AUC was between .7 and .8 (95% CI > .5), we judged that the diagnostic ability was reliable but with moderate accuracy. All statistical analyses were performed using EZR (Saitama Medical Centre, Jichi Medical University), a graphical user interface of R (The R Foundation for Statistical Computing), which includes a modified version of R commander designed to add statistical functions frequently used in biostatistics.[15]

3 | RESULTS

3.1 | The proportion of FTD-positive peripheral blood mononuclear cells increases during FTD/TPI administration and decreases thereafter

Using PBMC isolated from blood samples of 39 patients with mCRC enrolled in KSCC1602,[14] the proportion of FTD-positive cells was monitored during the first cycle of treatment. The schedule of drug administration is shown in Figure 1A. The blood samples for PBMC isolation were collected during the first cycle of treatment as follows: on day 1; immediately before the first FTD/TPI administration; days 8 and 15, the ends of first and second weeks of treatment, respectively; and day 29, 2 weeks after treatment ceased. The percentage of FTD-positive PBMC were determined as previously described.[8] The PBMC from 3 patients were not analyzed because of the low quality of blood samples. Data for 7 patients were missing because of individual reasons. Blood tests of 21 patients were performed on day 22. The transitions of the percentage of FTD-positive PBMC of 36 patients are shown in Figure S1. Once FTD/TPI was administered, FTD-positive PBMC were detected in all samples throughout the first cycle of treatment (days 1-29). The median values and interquartile ranges of the percentage of FTD-positive PBMC on days 8, 15, and 29 were 39.3% (30.7%-52.2%), 66.9% (40.0%-75.3%), and 13.5% (5.7%-26.0%), respectively (Figure 1B). From days 8 to 15, during FTD/TPI administration, the percentage of FTD-positive PBMC increased in 31 patients and decreased in 4 patients. Among

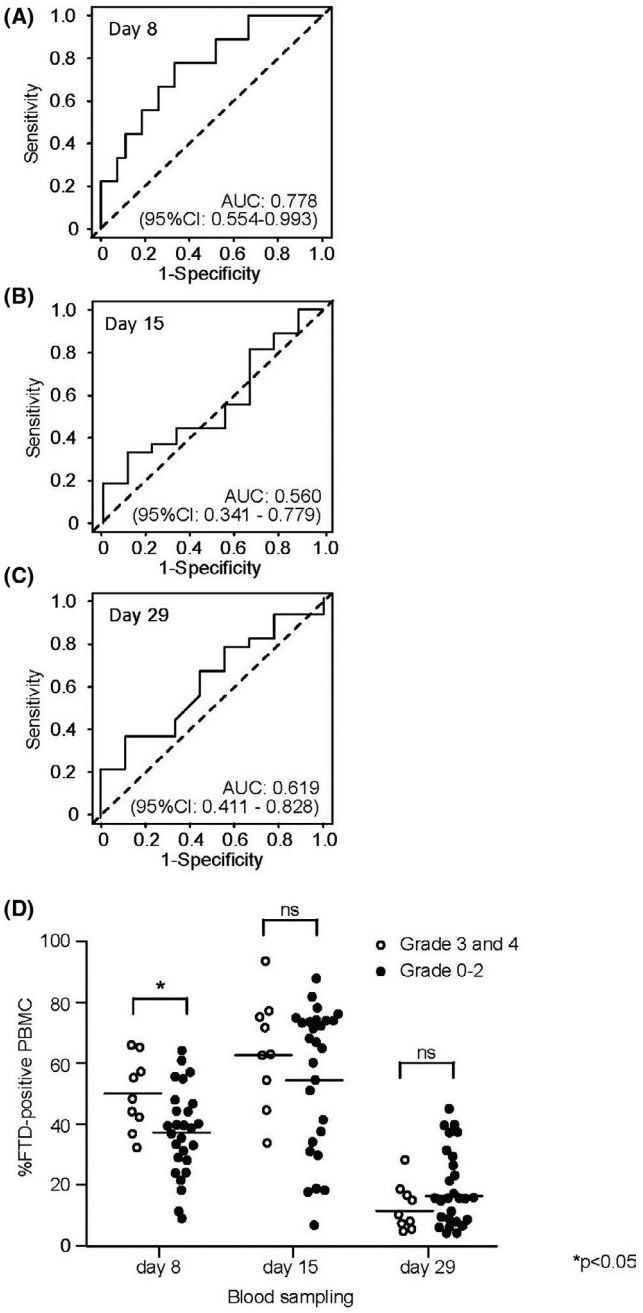

FIGURE 2 FTD-positive peripheral blood mononuclear cells (PBMC) and neutropenia. (A-C). The receiver operator characteristic curves of FTD-positive PBMC on days 8 (A), 15 (B), and 29 (C) as a function of the occurrence of grade 3 and 4 neutropenia during the first cycle of treatment. Area under the curve (AUC) values and their 95% confidence intervals (CI) are shown. (D) Scatter plots of FTD-positive PBMC of patients with grades 3 and 4 or grades 0-2 neutropenia. *$P < .05$, ns: not significant, Wilcoxon signed-rank test

the latter, 1 was not administered FTD/TPI during the second week because of fatigue, vomiting, and anorexia, although the other 3 patients were administered FTD/TPI during the second week as scheduled. In contrast, from days 15 to 29, the percentage of FTD-positive PBMC decreased in 32 patients.

The percentage of FTD-positive PBMC on day 15 was significantly higher than that on day 8 (median differences, 21.40%; $P < .0001$) or on day 29 (43.32%, $P < .0001$) (Figure 1B), confirming the fluctuation of the proportion of FTD-positive PBMC according to the schedule of FTD/TPI administration.[8] Furthermore, the percentage of FTD-positive PBMC on day 29 was significantly lower than that on day 8 (20.43%, $P < .0001$) (Figure 1B), indicating the effectiveness of 2 weeks' cessation of FTD/TPI administration.

3.2 | Association between FTD-positive peripheral blood mononuclear cells and hematological adverse events

The most frequent adverse event related to FTD/TPI therapy is bone marrow suppression, which leads to hematological adverse events, including neutropenia and leukopenia.[1,2,16] Among 36 patients, 29 (80.6%) and 23 (63.9%) experienced neutropenia and leukopenia, respectively. The frequencies and distributions of grades were consistent with those of a previously published clinical study (Table 1).[9]

The densities of white blood cells (WBC) (Figure S2A) and neutrophils (Figure S2B) started decreasing from day 15, and severe leukopenia (grades 3 and 4, <2,000 WBC/μL) and neutropenia (grades 3 and 4, <1,000 neutrophils/μL) mainly occurred on days 22 and 29.

TABLE 1 Adverse events

	Grade 1-2	Grade 3	Grade 4
Hematological			
Neutropenia	8 (22%)	16 (44%)	9 (25%)
Leucopenia	16 (44%)	15 (42%)	1 (3%)
Anemia	30 (83%)	4 (11%)	2 (6%)
Thrombocytopenia	25 (69%)	1 (3%)	0
Non-hematological			
Febrile neutropenia	0	2 (6%)	0
Anorexia	21 (58%)	6 (17%)	0
Hypertension	19 (53%)	5 (14%)	0
Fatigue	17 (47%)	3 (8%)	0
Diarrhoea	11 (30%)	2 (6%)	0
Nausea	19 (53%)	1 (3%)	0
Vomiting	12 (33%)	1 (3%)	0
Malaise	27 (75%)	0	0
Fever	7 (19%)	0	0
Bleed	6 (17%)	0	0
Oral mucositis	6 (17%)	0	0
Thrombosis	1 (3%)	0	0

FTD-positive PBMC were readily detected from day 8 (Figure 1B), suggesting that FTD incorporation into PBMC caused cytotoxicity, depleted FTD-positive WBC and neutrophils from the bloodstream, and subsequently led to severe hematological adverse events.

To test whether the proportion of FTD-positive PBMC correlated with the occurrence of severe hematological adverse events, we performed ROC analysis to assess the ability of the percentage of FTD-positive PBMC on each day to diagnose the occurrence of severe neutropenia or leukopenia within 1 month. The AUC of the percentage of FTD-positive PBMC on day 8 vs severe neutropenia was .778 (95% CI: .554-.993) (Figure 2A), indicating moderate ability to accurately diagnose the occurrence of severe neutropenia. On days 15 or 29, however, the AUCs were .560 (95% CI: .341-.779) and .619 (95% CI: .411-.828), respectively, suggesting no diagnostic ability (Figure 2B,C). Similar results were obtained for the occurrence of severe leukopenia (Figure SA-C).

We next compared the percentages of FTD-positive PBMC between patients with (grades 3 and 4) or without (grades 0-2) severe neutropenia. The percentage of FTD-positive PBMC on day 8 was significantly higher in those with severe neutropenia, while no significant difference was observed on days 15 or 29 (Figure 2D).

3.3 | Association between FTD-positive peripheral blood mononuclear cells and prognosis

Neutropenia induced by FTD/TPI is associated with better prognosis of patients with refractory mCRC.[9-11] We next evaluated the association between the percentage of FTD-positive PBMC and prognosis. First, when we evaluated the reliability of the percentage of FTD-positive PBMC to predict the best response rate (Table 2), we were unable to achieve a reliable prediction (data not shown). Univariate Cox regression analysis revealed that the percentage of FTD-positive PBMC on days 8, 15, or 29 did not show significant prognostic value for progression-free survival (PFS) or overall survival (OS) (Table 3). Severe neutropenia (grades 3 and 4) during the first cycle of treatment was not a prognostic factor for PFS or OS (Table 3).

TABLE 2 Best response to treatment

	n = 36
Complete response	0 (0%)
Partial response	14 (39%)
Stable disease	17 (47%)
Progression disease	3 (8%)
Not evaluable	2 (6%)
Overall response	14 (38.9%, 23.1-56.5)
Disease control	31 (86.1%, 70.5-95.3)

Note: Data in parentheses: % or %, 95% confidence interval

4 | DISCUSSION

Here we monitored FTD-positive PBMC of elderly patients with mCRC enrolled in KSCC1602, a single-arm phase 2 clinical trial of FTD/TPI plus bevacizumab as a first-line treatment. We detected FTD-positive PBMC in all blood samples and found that the percentage of FTD-positive PBMC on day 8, 1 week after initiating FTD/TPI plus bevacizumab treatment, had moderate ability to accurately diagnose the occurrence of severe neutropenia (grades 3 and 4) and leukopenia (grades 3 and 4) within 1 month (Figure 2A and Figure S3A). In contrast, patients who suffered severe hematological adverse events during the first cycle of treatment tended to exhibit a higher percentage of FTD-positive PBMC on day 8 (Figure 2D). These results suggest that excess FTD incorporation into PBMC at the initial phase of FTD/TPI plus bevacizumab treatment may represent a risk factor of severe hematological adverse events during the first cycle of treatment.

Our analysis revealed that the proportion of FTD-positive PBMC increased during FTD/TPI administration, peaked on day 15, and decreased during the subsequent 2 weeks cessation of treatment (Figure 1). These observations are consistent with previous data for FTD/TPI monotherapy,[8] confirming that the proportion of FTD-positive PBMC rapidly reflects the schedule of FTD/TPI treatment. We note that the median percentage of FTD-positive PBMC on day 15 (66.9%) was 1.7-fold higher than that on day 8 (39.3%).

In mice, FTD is incorporated into tumors as well as WBC in a dose-dependent manner.[17] In contrast, FTD persists longer in tumors than in bone marrow.[13] One possible adjustment to reduce the incidence of hematological adverse events may be biweekly administration of FTD/TPI, which may suppress the peak of FTD-positive PBMC but retain its tumor-suppressive effect. For example, in the phase 1b/2 BiTS study, biweekly treatment with FTD/TPI plus bevacizumab of patients with mCRC refractory to standard therapies,[18] the frequency of severe neutropenia (grades 3 and 4) was 11.4% (in the C-TASK FORCE trial and the present study, it was 72% and 69%, respectively), whereas the median PFS (4.25 months) reported by the BiTS study was not significantly inferior to that of the C-TASK FORCE trial (5.6 months).[9,14] The median PFS of the present study was 7.98 months (Figure S4) , possibly because we focused solely on patients undergoing first-line treatment.

The results of univariate Cox regression analysis indicated that there was no significant prognostic association of the percentage of FTD-positive PBMC with PFS or OS (Table 3). Furthermore, severe neutropenia (grades 3 and 4) was not significantly associated with PFS or OS. Previous studies, however, found that neutropenia is associated with better prognosis of patients administered FTD/TPI.[9-11] The number of patients (n = 39) in our study may have been too small to evaluate the prognostic value of FTD-positive PBMC or neutropenia. A validation cohort study with more patients is necessary to evaluate their prognostic values of FTD/TPI plus bevacizumab as a first-line treatment of elderly patients.

What are the factors that affect the proportion of FTD-positive PBMC and hematological adverse events? Thymidine kinase 1 (TK1) mediates FTD incorporation into the DNA of tumor cells,[19-21] and the level of TK1 is associated with the efficacy of FTD/TPI treatment.[22] Nucleoside transporters (hENT1, hENT2, and MATE1/OCT2) contribute to the incorporation of FTD into DNA.[12,19,23] The expression levels or single nucleotide polymorphisms of the genes encoding these proteins may determine the incorporation rate of FTD into PBMC, which would affect the frequency and severity of hematological adverse events. Plasma concentrations of thymidine may affect FTD incorporation, because thymidine concentrations in culture media affect FTD incorporation into human cancer cell lines.[6,12] These variables may determine the percentage of FTD-positive PBMC during the early phase of FTD/TPI treatment and subsequently affect the occurrence of severe hematological adverse events.

There are several limitations to our study. First, its sample size was insufficient to convincingly evaluate the prognostic value of FTD incorporation into PBMC. Second, we solely focused on FTD incorporation into DNA of PBMC. The risk of cytotoxicity is usually assessed by the blood concentration of a drug. The correlation between FTD incorporation into DNA of PBMC and its blood concentration should, therefore, be assessed. Third, analysis of FTD

TABLE 3 Univariate Cox hazard analysis

	PFS			OS		
	HR	95% CI	*P* value	HR	95% CI	*P* value
%FTD-positive cells at day 8	1.03	1.00-1.06	.056	1.02	.99-1.05	.199
%FTD-positive cells at day 15	1.01	.99-1.03	.481	1.01	.98-1.03	.529
%FTD-positive cells at day 29	1.01	.98-1.03	.669	1.01	.97-1.04	.717
Neutropenia (grades 3,4 vs grades 0,1,2) in cycle 1	1.22	.54-2.77	.631	1.10	.39-3.14	.855

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression free survival.

incorporation into tumor cells and PBMC in the same cohort is required to evaluate the correlation between hematological adverse events and the antitumor effect of FTD/TPI-based treatment.

In conclusion, FTD was incorporated into PBMC upon the initiation of FTD/TPI plus bevacizumab treatment. Monitoring FTD-positive PBMC at the initial phase of treatment may help to predict the occurrence of severe hematologic adverse events. Moreover, these findings have the potentially important clinical implication that FTD incorporation into bone marrow-derived cells suppresses their proliferation and survival, leading to hematological adverse events. The prognostic value of FTD incorporation into PBMC or neutropenia should be re-evaluated in future studies of a sufficient number of patients.

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DISCLOSURE

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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