Efficacy of Combination Chemotherapy Using a Novel Oral Chemotherapeutic Agent, TAS-102, with Oxaliplatin on Human Colorectal and Gastric Cancer Xenografts

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Abstract. TAS-102 is a novel oral nucleoside antitumor agent consisting of trifluridine (FTD) and the thymidine phosphorylase inhibitor tipiracil hydrochloride (at a molar ratio of 1:0.5) that was approved in Japan in 2014 for the treatment of unresectable advanced or recurrent colorectal cancer. In the present study, the enhancement of therapeutic efficacy using a combination of TAS-102 and oxaliplatin was evaluated in a xenograft-bearing nude mouse model of colorectal and gastric cancer. TAS-102 was orally administered twice-a-day from day 1 to 14, and oxaliplatin was administered intravenously on days 1 and 8. The in vivo growth-inhibitory activity was evaluated based on the tumor volume and the growth-delay period, was estimated based on the period required to reach a tumor volume five-times greater than the initial volume (RTV5). The tumor growth-inhibitory activity and RTV5 in mice administered TAS-102 with oxaliplatin were significantly superior to those associated with either monotherapy in mice with colorectal (HCT 116, SW-48; p<0.001) and gastric cancer (SC-2, MKN74; p<0.001). MKN74/5FU, a 5-fluorouracil-resistant MKN74 sub-line, was sensitive to both FTD and oxaliplatin in vitro. In vivo, TAS-102 alone was effective in MKN74/5FU, and its anti-tumor activity was significantly enhanced in combination with oxaliplatin (p<0.001). No significant decrease in body weight or toxicity was observed compared to either monotherapy. The present pre-clinical findings indicate that combination of TAS-102 and oxaliplatin is a promising treatment option for colorectal or gastric cancer, and can be utilized in both chemo-naïve tumors and recurrent tumors after 5-fluorouracil treatment.

Colorectal cancer is the third most common cancer in men (10%) and the second most common in women (9.2%) worldwide. It was also the fourth leading cause of cancer-related death in 2012 (1). Unresectable metastatic colorectal cancer is treated with systemic chemotherapeutic agents, including fluoropyrimidines, irinotecan hydrochloride, and oxaliplatin, and targeted-agents, such as bevacizumab (a monoclonal antibody to vascular endothelial growth factor), cetuximab, and panitumumab (a monoclonal antibody to the epidermal growth factor receptor), that have improved the patient survival rate (2-6). Although these standard therapies are initially effective, many patients experience relapse due to onset of drug resistance, and are subsequently treated with salvage chemotherapy. In 2013, the multikinase inhibitor regorafenib was shown to prolong overall survival, compared to placebo, in patients with unresectable refractory colorectal cancer (7).

TA2-102 is comprised of an antineoplastic thymidine-based nucleoside analog, trifluridine (FTD), and the thymidine phosphorylase inhibitor, tipiracil hydrochloride (TPI), at a molar ratio 1:0.5 (weight ratio, 1:0.471). FTD is the active anti-tumor component of TAS-102. The monophosphate form of FTD inhibits thymidylate synthase, whereas the triphosphate form is incorporated into DNA in tumor cells (8-10). DNA incorporation is known to have anti-tumor effects, since oral FTD-induced inhibition of thymidylate synthase disappears rapidly after drug elimination (8, 9).

Orally-administered FTD undergoes rapid first-pass metabolism to its inactive form by thymidine phosphorylase in the intestines and liver (11). Consequently, TPI was synthesized to maintain adequate plasma concentrations of orally-administered FTD and to potentiate its anti-tumor
activity (12). The optimal molecular ratio of FTD to TPI is 1:0.5 (13). In pre-clinical studies, FTD and TAS-102 exhibited unique antitumor effects, including efficacy against 5-fluorouracil (5-FU)-resistant colorectal tumor cells in vitro and in vivo (14-16), and a persistent effect after the end of drug administration (10, 17).

In a randomized phase II trial of patients with metastatic colorectal cancer refractory to or intolerant to standard chemotherapies, the overall survival (OS) period of those receiving TAS-102 with best supportive care (9.0 months) was significantly longer than that of those treated with placebo and best supportive care (6.6 months, hazard ratio=0.56, p=0.0011) (18). TAS-102 also significantly improved overall survival period (median OS=7.1 months, 95% CI=6.5-7.8 months) for TAS-102, and 5.3 months (95% CI=4.6-6 months for placebo) and progression-free survival, and had a favorable safety profile compared to placebo in patients with metastatic colorectal cancer refractory to standard chemotherapies in an International multi-center randomized double-blind phase III study (RE COURSE) (19). TAS-102 was approved for clinical use in Japan in March 2014.

We previously reported that combination TAS-102 and irinotecan hydrochloride therapy inhibited colorectal and gastric cancer, including 5-FU-resistant colorectal tumors, in vivo (20). Oxaliplatin is a key anticancer drug used to treat gastrointestinal cancer. Although it cannot be said that anti-tumor effect of oxaliplatin alone is enough, it has been widely used in combination with 5-FU and folic acid (FOLFOX4 and mFOLFOX6) (21, 22); with capecitabine (XELOX) (23); with epirubicin, and capecitabine (EOX) (24); and with S-1 (SOX) (25-27).

In the present study, we evaluated the antitumor effects of TAS-102 in combination with oxaliplatin against gastrointestinal tumor xenografts, including a 5-FU-resistant sub-line, using a nude mouse model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Schedule</th>
<th>RTV (mean±SD)</th>
<th>TGI (n, %)</th>
<th>RTV5 (median, days)</th>
<th>BWC (mean±SD, g, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>26.6±2.85</td>
<td>6</td>
<td>0.0</td>
<td>11.6</td>
</tr>
<tr>
<td>TAS-102</td>
<td>150</td>
<td>Day 1-14 (b.i.d.)</td>
<td>9.12±0.95</td>
<td>6</td>
<td>65.7</td>
<td>18.1</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>12</td>
<td>Day 1, 8</td>
<td>16.1±2.96</td>
<td>6</td>
<td>39.5</td>
<td>15.4</td>
</tr>
<tr>
<td>Combination</td>
<td>150+12</td>
<td>Day 1-14+1, 8</td>
<td>5.16±0.94</td>
<td>6</td>
<td>80.6</td>
<td>27.0</td>
</tr>
</tbody>
</table>

RTV: Relative tumor volume on day 29; TGI: tumor growth-inhibition ratio on day 29; RTV5: time at which RTV reached 5. *p<0.05, **p<0.01, ***p<0.001 vs. Control using two-sided Aspin-Welch t-test; ^p<0.001 by closed testing procedure using two-sided Aspin-Welch t-test; #p<0.001 vs. Control using log-rank test; $p<0.001 vs. either monotherapy using log-rank test, NS: not significant vs. Control using two-sided Aspin-Welch t-test.
Materials and Methods

Chemicals. FTD and TPI were synthesized at YUKI GOSEI KOGYO LTD (Tokyo, Japan) and Taiho Pharmaceutical, Co., Ltd. (Tokyo, Japan), respectively. 5-FU, dimethylsulfoxide (DMSO), and crystal violet were purchased from Wako Pure Chemicals Co., Ltd (Osaka, Japan). Oxaliplatin (Elplat injection®) was purchased from Sanofi Aventis (Paris, France). Hydroxypropyl methylcellulose (HPMC) and glucose injections (5%) were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and Otsuka Pharmaceutical Factory, Inc. (Naruto, Japan), respectively. Fetal calf serum (FCS) and RPMI-1640 were purchased from Sigma-Aldrich Japan Co. LLC. (Tokyo, Japan). The other materials were commercially available products of the highest grade.

Tumor cells. The human colorectal cancer cell lines HCT 116 (28) and SW48 (29) were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and the American Type Culture Collection (Rockville, MD, USA), respectively. The human gastric cancer cell line SC-2 (30) and MKN74 (31) were obtained from the Central Institute for Experimental Animals (Kawasaki, Japan) and RIKEN BRC Cell Bank (Tsukuba, Japan), respectively. The 5-FU-resistant cell line MKN74/5FU was established from MKN74 cells after long-term culture in the presence of 5-FU as reported previously (32).

HCT 116, and SW48 cells were maintained by implantation into the right axilla of nude mice at 3-week intervals. MKN74 and MKN74/5FU were maintained in RPMI-1640 supplemented with 10% FCS, at 37˚C in a humidified atmosphere of 95% air and 5% CO2. HCT 116, SW48, MKN74, and MKN74/5FU were authenticated by short tandem repeat analysis in 2014.

Growth-inhibitory activity of 5-FU, FTD, and oxaliplatin in MKN74 and MKN74/5FU cells in vitro. Cell lines were seeded (2×10³ cells/well) in 96-well plates on day 0. 5-FU and FTD were dissolved in DMSO and diluted with culture medium (final DMSO concentration was less than 0.5%). The oxaliplatin injections were diluted with culture medium. Different drug concentrations were added to the medium on day 1, and the growth-inhibitory activity was evaluated using crystal violet staining and spectroscopic analysis on day 6 (33). The concentration that inhibited cell growth by 50% (IC₅₀) was calculated from the regression lines.

In vivo anti-tumor activity. Male nude mice were purchased from CLEA Japan Inc. (Tokyo, Japan) and were housed under specific conditions.

Figure 1. Relative tumor volume (RTV) of HCT 116 human colorectal tumors (a) and body weight change (BWC) (b) in HCT 116-bearing nude mice. Mice were randomized according to the tumor volumes on day 0. The mice were treated with 0.5% hydroxypropyl methylcellulose (control) or TAS-102 (150 mg/kg), administered orally twice daily from days 1 to 14. Oxaliplatin (12 mg/kg) was administered intravenously, alone or in combination with TAS-102, on days 1 and 8. The tumor volume and body weight were measured twice per week. The values indicate the mean and SD (n=6). The horizontal dotted line indicates an RTV of 5.
pathogen-free conditions, with food and water provided ad libitum. After the animals had been placed in quarantine for one week, they were implanted subcutaneously with a solid human tumor (HCT 116, SW48, and SC-2), the volume of which was approximately 8 mm³. MKN74 or MKN74/5FU culture cells were suspended in saline and implanted subcutaneously (4×10⁶ cells/mouse).

To evaluate the antitumor activity, the mice were randomized according to the tumor volume once the mean tumor volume had reached approximately 150 to 200 mm³ (day 0). Each group consisted of six mice.

TAS-102 was prepared by mixing FTD and TPI at a molar ratio of 1:0.5 in 0.5% HPMC. The TAS-102 dose was expressed according to the amount of FTD. TAS-102 was administered orally (150 mg/kg/day) from day 1 to 14, twice per day, with approximately 6 h between doses (8, 13). For the control group, 0.5% HPMC alone (10 ml/kg) was administered according to a similar schedule. The dose of oxaliplatin was determined by a preliminary test. Oxaliplatin (12 mg/kg) was diluted with 5% glucose injection and administered intravenously once per day on days 1 and 8.

The tumor diameters were measured twice per week, and the tumor volume was estimated as 0.5×length×width². The relative tumor volume (RTV) was calculated using the following formula:

\[ RTV = \frac{\text{tumor volume on the measured day}}{\text{tumor volume on day 0}}. \]

On day 29, the tumor growth-inhibition (TGI) ratio was calculated using the following formula:

\[ TGI = \left[ 1 - \frac{\text{RTV of the treated group}}{\text{RTV of the control group}} \right] \times 100 \%(\%) \]

The antitumor activity was also evaluated according to the RTV5 value, which indicates the period of time required for the RTV to reach 5. The values represent the mean and SD for 3-4 independent experiments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Schedule</th>
<th>RTV (mean±SD)</th>
<th>n</th>
<th>TGI (%)</th>
<th>RTV5 (median, days)</th>
<th>BWC (mean±SD, g)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>41.21±2.28</td>
<td>6</td>
<td>0.0</td>
<td>9.1</td>
<td>2.71±1.22</td>
<td>10.82</td>
</tr>
<tr>
<td>TAS-102</td>
<td>150</td>
<td>day 1-14 (b.i.d.)</td>
<td>14.34±1.03c</td>
<td>6</td>
<td>65.2</td>
<td>15.6⁵</td>
<td>0.36±0.52b</td>
<td>1.42</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>12</td>
<td>day 1, 8</td>
<td>25.25±2.81c</td>
<td>6</td>
<td>38.7</td>
<td>14.4⁶</td>
<td>0.85±0.70a</td>
<td>3.42</td>
</tr>
<tr>
<td>Combination</td>
<td>150+12</td>
<td>day 1-14+1, 8</td>
<td>4.79±0.69c,d</td>
<td>6</td>
<td>88.4</td>
<td>26.0c</td>
<td>1.02±0.94h</td>
<td>3.97</td>
</tr>
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</table>

RTV: Relative tumor volume on day 29; TGI: Tumor growth-inhibition ratio on day 29; RTV5: time at which RTV reached 5. *p<0.05, †p<0.01, ‡p<0.001 vs. Control using two-sided Aspin-Welch t-test; §p<0.001 by closed testing procedure using two-sided Aspin-Welch t-test; ¶p<0.001 vs. Control using log-rank test; ††p<0.001 vs. either monotherapy using log-rank test; gnot significant vs. Control using two-sided Aspin-Welch t-test.

The tumor diameters were measured twice per week, and the tumor volume was estimated as 0.5×length×width². The relative tumor volume (RTV) was calculated using the following formula:

\[ \text{RTV} = \frac{\text{tumor volume on measured day}}{\text{tumor volume on day 0}}. \]

On day 29, the tumor growth-inhibition (TGI) ratio was calculated using the following formula:

\[ \text{TGI} = \left[ 1 - \frac{\text{RTV of the treated group}}{\text{RTV of the control group}} \right] \times 100 \%(\%). \]

The antitumor activity was also evaluated according to the RTV5 value, which indicates the period of time required for the RTV to reach 5. To estimate the RTV5, the RTV change for each mouse was plotted, and the date on which the RTV reached 5 was estimated using linear-regressions (34).

To evaluate toxicity, the body weight change (BWC) was calculated using the following formula:

\[ \text{BWC} = \frac{\text{body weight on last day} - \text{body weight on day 0}}{\text{body weight on day 0}} \times 100 \%(\%). \]

In cases where the mean body weight loss was more than 20%, or in which toxic death was observed as a result of the treatment, the treatment was designated as toxic.

All the animal studies were performed according to the guidelines of and with the approval of the Institutional Animal Care and Use Committee of Taiho Pharmaceutical Co., Ltd. (Approval Number: 14PB07).

Statistical analysis. Significant differences in the mean RTV values between the treated and control groups on day 29 were analyzed using the Aspin-Welch two-sided t-test. The combined effect of TAS-102 and oxaliplatin on the antitumor activity was analyzed according to a closed testing procedure using the Aspin-Welch two-tailed t-test (35). The statistical analysis of RTV5 was performed using the log-rank test according to a previous manuscript (20). A p-value less than 0.05 was considered significant, as calculated using EXSUS (Ver. 8.1; Arm Systex Co., Ltd., Osaka, Japan).

Results

Increased anti-tumor activity of combined oxaliplatin and TAS-102 in human colorectal cancer in vivo. The antitumor activity of TAS-102 and oxaliplatin administered alone and in combination were evaluated. The change in

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Compound</th>
<th>IC₅₀ μM</th>
<th>Ratio⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKN74</td>
<td>5-FU</td>
<td>3.41±1.95</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Trifluridine</td>
<td>2.25±0.73</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Oxaliplatin</td>
<td>0.377±0.106</td>
<td>–</td>
</tr>
<tr>
<td>MKN74/5FU</td>
<td>5-FU</td>
<td>11.6±3.52</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Trifluridine</td>
<td>1.87±0.35</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Oxaliplatin</td>
<td>0.292±0.141</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Half-maximal (50%) inhibitory concentration (IC₅₀); ⁴Ratio of IC₅₀ of MKN74/5FU to MKN74 cells.

Table IV. Cytotoxicity of 5-fluorouracil (5-FU), oxaliplatin and trifluridine against MKN74 and MKN74/5FU cells in vitro. The values represent the mean and SD for 3-4 independent experiments.
RTV and BWC in HCT 116 tumor-bearing nude mice is shown in Figure 1a and b, respectively. As shown in Table 1, monotherapy induced significant anti-tumor activity \((p<0.001)\). However, the anti-tumor activity of combination treatment was significantly superior to both monotherapies \((p<0.001)\). The mean RTV5 of the monotherapy was significantly \((p<0.001)\) longer than that of the control group. Furthermore, combination treatment significantly lengthened the RTV5 \((p<0.001)\), as compared to both monotherapies (Figure 1a, Table 1). The body weight loss

**Table V. Antitumor activity and body weight changes (BWC; day 0-29) in mice implanted with MKN74 human gastric tumor after treatment with TAS-102 and oxaliplatin.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Schedule</th>
<th>RTV (mg/kg/day)</th>
<th>TGI (%)</th>
<th>RTV5 (median, days)</th>
<th>BWC (mean±SD, g) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>15.97±0.73</td>
<td>0</td>
<td>12.2 –</td>
<td>3.54±0.77</td>
</tr>
<tr>
<td>TAS-102</td>
<td>150</td>
<td>day 1-14 (b.i.d.)</td>
<td>7.95±0.38b</td>
<td>60.2</td>
<td>21.7d</td>
<td>2.64±0.41NS</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>12</td>
<td>day 1, 8</td>
<td>10.25±0.87b</td>
<td>35.8</td>
<td>17.0d</td>
<td>2.90±0.92NS</td>
</tr>
<tr>
<td>Combination</td>
<td>150+12</td>
<td>day 1-14+1, 8</td>
<td>3.78±0.53b,c</td>
<td>76.3</td>
<td>29.0e</td>
<td>1.55±0.77a,f</td>
</tr>
</tbody>
</table>

RTV: Relative tumor volume on day 29; TGI: tumor growth-inhibition ratio on day 29; RTV5: time at which RTV reached 5. \(p<0.05\), \(p<0.01\) vs. Control using two-sided Aspin-Welch \(t\)-test; \(p<0.001\) by closed testing procedure using two-sided Aspin-Welch \(t\)-test; \(p<0.001\) vs. Control using log-rank test; \(p<0.001\) vs. either monotherapy using log-rank test; \(p>0.05\) vs. either monotherapy using two-sided Aspin-Welch \(t\)-test.
of the HCT 116 tumor-bearing nude mice was less than 20% throughout the experiment in all groups, and no significant difference in BWC was observed for either monotherapy.

The change in RTV and BWC in SW48 tumor-bearing nude mice are shown in Figure 2a and b, respectively. As shown in Table II, both monotherapies had significant anti-tumor activity ($p<0.001$). The anti-tumor activity of the combination therapy was significantly superior to both monotherapies ($p<0.001$). The mean RTV5 in mice receiving monotherapy was significantly ($p<0.001$) longer than that of the control group. However, the RTV5 in mice

Table VI. Antitumor activity and body weight changes (BWC; day 0-29) in mice implanted with 5-fluorouracil resistant human gastric tumor MKN74/5FU after treatment with TAS-102 and oxaliplatin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Schedule</th>
<th>RTV (mean±SD)</th>
<th>TGI (%)</th>
<th>RTV5 (median, days)</th>
<th>BWC (mean±SD, g)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>16.94±1.75</td>
<td>0.0</td>
<td>12.8 –</td>
<td>3.22±0.54</td>
<td>12.19</td>
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<tr>
<td>TAS-102</td>
<td>150</td>
<td>day 1-14 (b.i.d.)</td>
<td>6.93±0.63</td>
<td>65.1</td>
<td>12.1</td>
<td>1.79±0.95</td>
<td>6.82</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>12</td>
<td>day 1, 8</td>
<td>10.08±1.77</td>
<td>65.1</td>
<td>20.0</td>
<td>1.63±1.25</td>
<td>6.45</td>
</tr>
<tr>
<td>Combination</td>
<td>150+12</td>
<td>day 1-14+1, 8</td>
<td>3.42±0.424</td>
<td>66.8</td>
<td>29.0</td>
<td>1.83±0.59</td>
<td>6.87</td>
</tr>
</tbody>
</table>

RTV: Relative tumor volume on day 29; TGI: tumor growth-inhibition ratio on day 29; RTV5: time at which RTV reached 5. $^a$p<0.05, $^b$p<0.01, $^c$p<0.001 vs. Control using two-sided Aspin-Welch t-test; $^d$p<0.001 by closed testing procedure using two-sided Aspin-Welch t-test; $^e$p<0.001 vs. Control using log-rank test; $^f$p<0.001 vs. either monotherapy using log-rank test; $^g$not significant vs. either monotherapy using two-sided Aspin-Welch t-test.

Figure 3. Relative tumor volume (RTV) (a) and body weight change (BWC) (b) in SC-2 gastric cancer-bearing nude mice. Mice were randomized according to the tumor volumes on day 0. The mice were treated with 0.5% hydroxypropyl methylcellulose or TAS-102 (150 mg/kg), administered orally twice daily from days 1 to 14. Oxaliplatin (12 mg/kg) was administered intravenously, alone or in combination with TAS-102, on days 1 and 8. The tumor volume and body weight were measured twice per week. The values indicate the mean and SD (n=6). The horizontal dotted line indicates an RTV of 5.
administered combination therapy was significantly (p<0.001) longer than that with either monotherapy (Figure 2a, Table II). The body weight loss of the SW48 tumor-bearing nude mice was less than 20% throughout the experiment in all groups. These results indicate that this combination actively inhibits colorectal cancer growth, without increasing toxicity.

Increased anti-tumor activity of combined oxaliplatin and TAS-102 in human gastric cancer in vivo. The anti-tumor activity of TAS-102 and oxaliplatin administered alone and in combination was evaluated. The change in RTV and BWC in SC-2 tumor-bearing nude mice is shown in Figure 3a and b, respectively. Monotherapy significantly inhibited tumor growth (p<0.001). However, the combination therapy exerted a significantly greater growth-inhibitory effect than both monotherapies (p<0.001). Combination therapy significantly increased the RTV5 length as compared to those of monotherapy groups (p<0.001, Table III). There was no significant difference in BWC between the monotherapy and combination therapy groups. Furthermore, the body weight loss of the SC-2 tumor-bearing nude mice was less than 20% throughout the experiment.

In vitro growth-inhibitory activity on 5-FU-resistant cells. The growth-inhibitory activity of 5-FU, FTD, and oxaliplatin in MKN74 and MKN74/5FU cells is shown in Table IV. The IC50 values for 5-FU treatment were 3.4-fold higher for MKN74/5FU cells than in MKN74 cells. In contrast, the IC50 values for FTD and oxaliplatin were approximately equivalent in MKN74/5FU and MKN74 cells. These data indicate MKN74/5FU cells showed no cross-resistance to either FTD or oxaliplatin.

Increased anti-tumor activity of combined oxaliplatin and TAS-102 in human 5-FU resistant gastric cancer in vivo. The anti-tumor activity of TAS-102 and oxaliplatin alone and in combination was evaluated in vivo using xenograft models with MKN74, and MKN74/5FU cells. The change in RTV in mice with MKN74 and MKN74/5FU xenograft

![Figure 4](image-url)
tumors is shown in Figure 4a and 5a, respectively. Monotherapy inhibited MKN74 tumor growth significantly $(p<0.001)$, whereas combination therapy had superior antitumor activity compared to monotherapies $(p<0.001)$ (Table V). The mean RTV5 for monotherapies was significantly longer $(p<0.001)$ than that of the control. However, combination therapy significantly lengthened $(p<0.001)$ the RTV5 compared to both monotherapies (Table V).

Both TAS-102 and oxaliplatin monotherapies also exhibited antitumor activity against MKN74/5FU tumors. Moreover, combination therapy was significantly superior to both monotherapies $(p<0.001)$ (Table VI). The RTV5 value of the combined therapy was significantly longer $(p<0.001)$ than either monotherapy (Table VI). Furthermore, the body weight loss of the MKN74 and MKN74/5FU tumor-bearing nude mice was less than 20% throughout the experiment (Figure 4b and 5b). These results suggest that combination therapy was well-tolerated in MKN74 and MKN74/5FU tumor-bearing nude mice.

**Discussion**

In the present study, combination therapy consisting of TAS-102 and oxaliplatin was shown to be effective in nude mice xenografts derived from two colorectal (HCT 116, SW48), two gastric (SC-2, MKN74), and one 5-FU-resistant gastric tumor (MKN74/5-FU) cell line, as determined by tumor-growth inhibition and growth delay period.

To our knowledge, the present study is the first to report the *in vivo* effects of combined TAS-102 and oxaliplatin therapy, although it has been reported that FTD potentiated the cytotoxicity of oxaliplatin in colorectal cancer cells *in vitro* (36). Oxaliplatin exerts its effects via the formation of adducts with DNA, and followed by DNA fragmentation (37, 38). In contrast, few DNA strand breaks were detected in FTD-treated HCT 116 cells, despite FTD misincorporation into genomic DNA, suggesting that the antiproliferative effect of FTD is not due to the induction of DNA strand breaks (10). It was reported that more than...
80% of the FTD incorporated into DNA was retained 24 h after a washing-out procedure, even though the intracellular triphosphate form of FTD was rapidly eliminated with a half-life of 30 min or less (8). These data may indicate that FTD incorporated into DNA is resistant to DNA-repair enzyme DNA glycosidase (39). Temmink et al. reported that simultaneous treatment using FTD and oxaliplatin significantly increased the formation of platinum (Pt)-DNA adducts in H630 and SW116 in vitro (39). The Pt-DNA adducts were retained after removal of FTD (36). In addition more DNA-strand breaks were induced in the presence of FTD compared to oxaliplatin alone (36). Collectively, FTD incorporation into DNA might inhibit DNA repair after DNA fragmentation caused by oxaliplatin, leading to synergistic effects of this combination therapy.

FTD was active against MKN74/5FU and MKN74 cells in vitro (Table IV), and the antitumor activity of TAS-102 in MKN74/5FU cells was equivalent to MKN74 cells in terms of both tumor growth inhibition and growth delay period (Figure 4a and 5a, Table V and VI). As MKN74/5FU cells were established via long-term exposure to 5-FU, this cell line might mimic refractory tumors after 5-FU-based chemotherapy. 5-FU reportedly exerts its anti-tumor activity by inhibiting thymidylate synthase through phosphorylated 5-FU and by inducing RNA dysfunction when F-UTP is incorporated into RNA (40, 41). 5-FU resistance in MKN74/5FU cells likely occurs through the overexpression of thymidylate synthase, which is the main target enzyme of 5-FU (31). In contrast, the main anti-tumor mechanism of TAS-102 is thought to be the incorporation of FTD triphosphate into DNA (8, 9). Thus the mechanism of TAS-102 differs from that of 5-FU, possibly explaining the lack of cross-resistance.

Gastrointestinal and hematological adverse effects were most commonly observed in phase II and III clinical studies using TAS-102 (18, 19). In contrast, neurosensory disturbances were the most frequent grade 3 NCI-CTC toxicity, and their occurrence may be dependent on the cumulative oxaliplatin dose (42, 43). These toxicities are not common with TAS-102; therefore, increased toxicity seems unlikely when using this combination therapy. In vivo studies revealed tolerable toxicity and no significant difference in BWC between monotherapy and combination therapy. Nevertheless, other toxicities were not examined. Careful monitoring of side-effects, including neurotoxicity, and hematological and gastrointestinal toxicity, is required to evaluate the efficacy of this combination therapy in clinical studies.

In conclusion, we demonstrate that combination therapy using TAS-102 and oxaliplatin is active against colorectal and gastric cancer, including 5-FU-resistant gastric tumors. Similar to FOLFOX, this combination may be promising for front-line therapy against metastatic colorectal tumors. A clinical study of this combination therapy is warranted.

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References


