Inhibitory Effect on Subcutaneous Xenograft Model in Nude Mice

Four- to 6-week-old female mice (BALB/c nu/nu, CLEA Japan, Tokyo) were used for subcutaneous xenograft models as described previously, under specific pathogen-free conditions in accordance with the institutional guidelines for animal care.[9] The doses and schedules of PTK/ZK and IFN/5-FU therapy were based on the results of previous studies.[9,17] Mice were assigned at random to one of the following four groups (5 mice per group): a) mice of the PTK/ZK group were administered PTK/ZK as an oral instillation (PO) at 20 mg/kg daily; b) each mouse of the IFN/5-FU group was injected subcutaneously (SC) 20,000U of IFN-α, three times per week, and an injected intraperitoneally (IP) 30 mg/kg 5-FU three times per week; c) mice of the IFN/5-FU+PTK/ZK group were administered PTK/ZK PO combined with IFN/5-FU SC/IP; and d) mice of the control group were administered SC/IP and PO injections of PBS. Tumor volume (TV) and body weight were measured twice per week, and TV was calculated using the following formula: (longest diameter) x (shortest diameter)^2 x 0.5. Four weeks after the initial treatment, all mice from each group were killed and tumors were harvested for examination.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). The unpaired Student's *t* test was used to examine differences in growth inhibitory effects in vitro. $P < 0.05$ was considered statistically significant.

RESULTS

VEGFR Expression and VEGF Secretion in Human HCC Cells

Flt-1 (VEGFR1) and KDR/Flk-1 (VEGFR2) were both expressed and in similar amounts in the two human HCC cells, PLC/PRF/5 and HuH7 (Fig. 1a). Incubation of both cells with IFN/5-FU alone and IFN/5-FU plus PTK/ZK, resulted in significant reductions of supernatant VEGF to 68.5% and 65.8%, respectively (Fig. 1b). These results indicated that PTK/ZK did not enhance the effect of IFN/5-FU on VEGF secretion through VEGFRs.

Inhibitory Effects of PTK/ZK and IFN/5-FU on Human HCC Cells In Vitro

To evaluate whether the combination of PTK/ZK and IFN/5-FU has an antiproliferative effect on human HCC

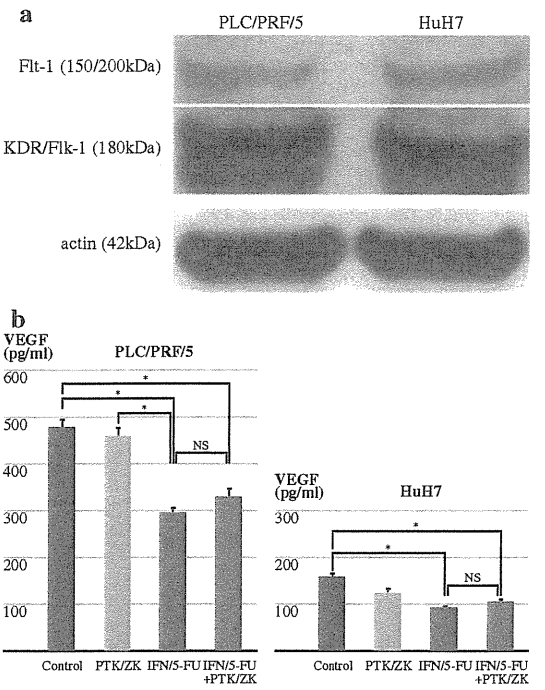

FIG. 1 VEGFRs expression and VEGF secretion in human HCC cells. **a** Expression of Flt-1 (VEGFR1) and KDR/Flk-1 (VEGFR2) in human HCC cells. **b** Secretion of VEGF from human HCC cells. PTK/ZK combined with IFN/5-FU significantly reduced the concentration of secreted VEGF in culture supernatants compared with the control group, whereas IFN/5-FU alone had no such effect. Data are mean ± SEM of triplicate assays; * $P < 0.05$

cells, we measured first the growth inhibition by the MTT assay. The data showed PTK/ZK concentration-dependent inhibition of cell growth, and PTK/ZK augments the inhibitory effect of IFN/5-FU; the addition of PTK/ZK (10 μM) to IFN/5-FU reduced the percentage of viable cells by 22.2% and 45.9% in PLC/PRF/5 and HuH7 cells, respectively (Fig. 2). The cooperative effect was statistically significant ($P < 0.05$).

PTK/ZK and IFN/5-FU Therapy Enhances Apoptosis of Human HCC Cells

To determine the mechanism of the antiproliferative effects of PTK/ZK combined with IFN/5-FU therapy on human HCC cells and whether it is related to the induction of apoptosis, we evaluated the extent of apoptosis using the annexin V assay to detect pre-apoptotic cells. The combination of PTK/ZK and IFN/5-FU increased the rate of apoptotic PLC/PRF/5 cells in a concentration-dependent manner and to a greater extent than IFN/5-FU alone (Fig. 3a, b; $P < 0.05$). The HuH7 cells showed similar results (data not shown).

Next, we assessed the expression levels of Bcl-xL and Bcl-2 proteins for anti-apoptosis and Bax for pro-apoptosis

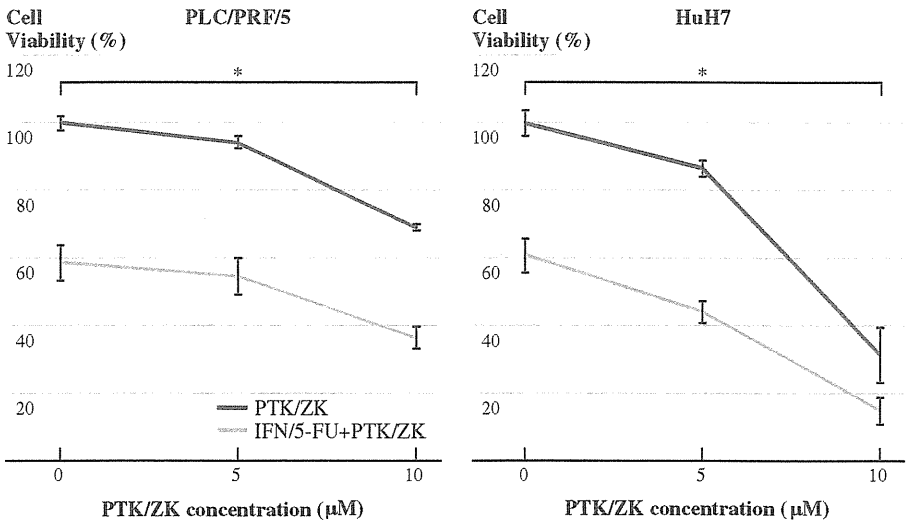

FIG. 2 Effect of PTK/ZK combined with IFN/5-FU on cell growth inhibition in human HCC cells lines. PLC/PRF/5 and HuH7 cells were incubated with PTK/ZK and/or IFN/5-FU, and then assayed using the MTT method. The proportion of viable cells incubated without drugs was defined as 100% viability. Data are mean ± SEM of four assays per condition.
* $P < 0.05$. PTK/ZK combined with IFN/5-FU reduced cell growth more than IFN/5-FU alone in both PLC/PRF/5 and HuH7 cells

(Fig. 3c), which were reported previously to be associated with the apoptotic effect of IFN/5-FU therapy.[20] Figure 3d shows the relative expression levels of each protein compared with actin. PTK/ZK combined with IFN/5-FU decreased the expression levels of Bcl-xL and Bcl-2 to half of those in control, although there was no statistical difference in Bcl-2 expression of PLC/PRF/5 cells. In Bcl-2 expression, the addition of PTK/ZK to IFN/5-FU alone attenuated 10–30%, although there was no statistical difference. On the other hand, PTK/ZK combined with IFN/5-FU upregulated the expression of Bax compared with control by 1.5–3.4 times. The effect of the additional PTK/ZK to IFN/5-FU was 10–30% increments, although there was no statistical difference.

Effects of PTK/ZK and IFN/5-FU on the Cell Cycle in Human HCC Cells

Flow cytometric analysis was used to examine cell cycle progression of treated and untreated human HCC cells. Before any treatment, all cells were synchronized in G0-G1 phase by serum starvation for 72 hours. The cells were then put back in the growth medium with 10% FBS and the treatments were started. Cells were collected 12, 24, 48, and 72 hours later. Flow cytometric data confirmed that after serum starvation, the majority of cells (PLC/PRF/5, 77.9%; HuH7, 50.3%) were in G0-G1 phase. At 24 hours after the addition of either PTK/ZK combined with IFN/5-FU or IFN/5-FU alone, the PLC/PRF/5 cells showed increased S-phase-DNA content. This S phase accumulation and G0/G1 phase degradation was maintained at 48 and 72 hours (Fig. 4a). In contrast, HuH7 cells treated with PTK/ZK combined with IFN/5-FU showed more cells with G0/G1-phase-DNA content at 24, 48, and 72 hours; a similar pattern was seen with PTK/ZK alone (Fig. 4b). We evaluated the cell cycle-related protein expression levels: cyclin D1 for promotion of cell cycle, p27 and p21 for delay (Fig. 4c). PTK/ZK combined with IFN/5-FU decreased the expression level of cyclin D1 in PLC/PRF/5 cells by 0.84 times that in control cells and increased the expression of p27 by 0.65 times than in control HuH7 cells; however, the differences were not significant. On the other hand, the expression of p21 protein was significantly different between PLC/PRF/5 and HuH7 cells. PTK/ZK combined with IFN/5-FU significantly decreased the expression of p21 by 0.71 times than in control PLC/PRF/5 cells, whereas the relative decrease was only small in HuH7 cells.

Inhibitory Effects of PTK/ZK and IFN/5-FU on Human HCC Xenografts In Vivo

The serial changes in implanted tumor volume in each treatment group are shown in Fig. 5. HuH7 cells were injected SC into nude mice, which were then treated for 4 weeks according to their group (n = 5 each). On day 30, the mean TV in the control group was 4.8 ± 1.1 cm^3, whereas TVs in the single-treatment groups were 3.2 ± 0.1 cm^3 (PTK/ZK group) and 2.0 ± 0.4 cm^3 (IFN/5-FU group). PTK/ZK combined with IFN/5-FU therapy significantly reduced the mean TV to 1.3 ± 0.3 cm^3 at 30 days. There were no significant differences in body weights among the different mice groups after removing the xenografts on the 27th day in each group compared with the respective pretreatment weight. Considered together, the above findings indicate that the combination of PTK/ZK and IFN/5-FU therapy inhibited the growth of human HCC cells both in vitro and in vivo, and that PTK/ZK enhanced the inadequate effect of IFN/5-FU therapy.

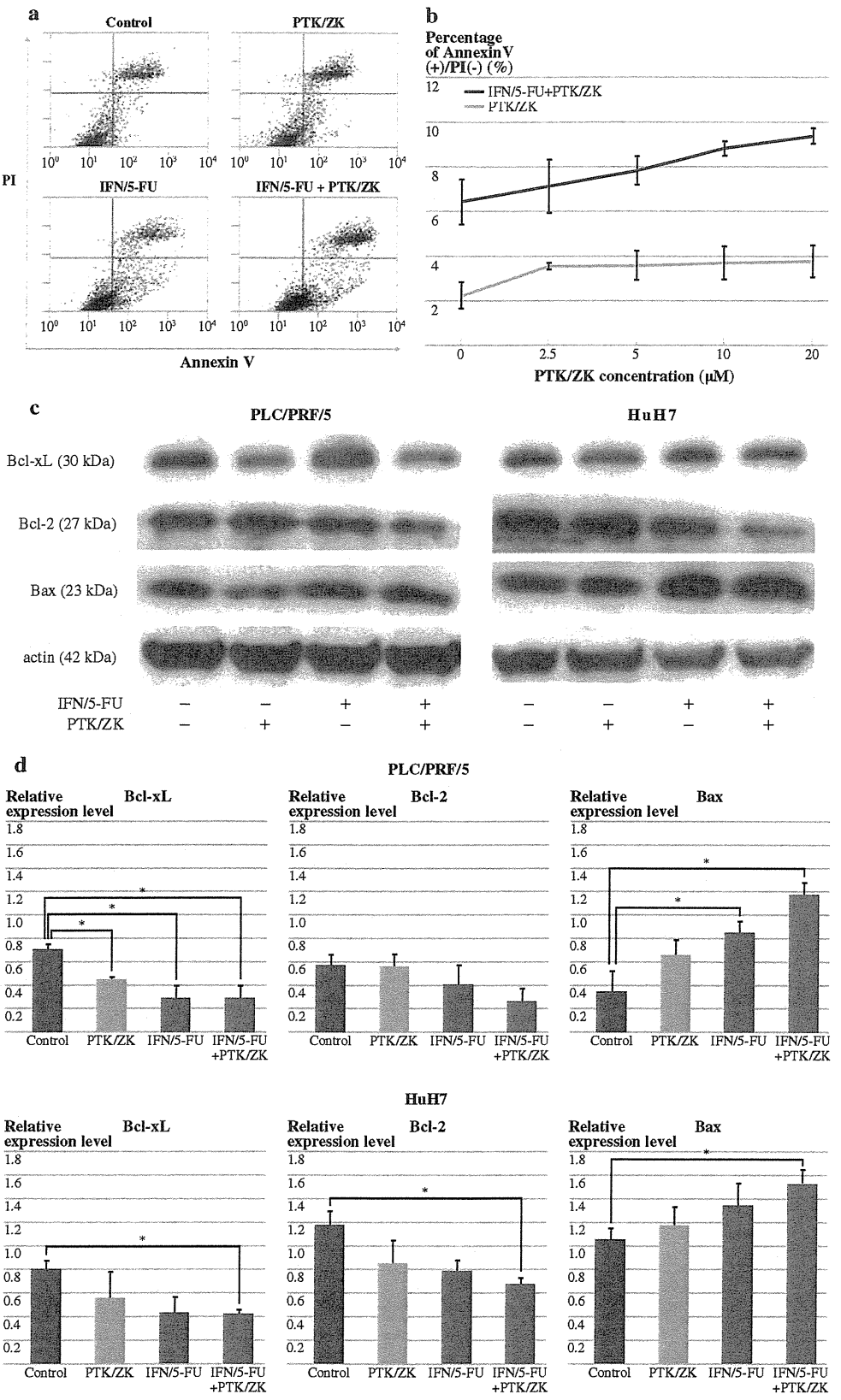

FIG. 3 Effects of PTK/ZK combined with IFN/5-FU on cell apoptosis, using the annexin V assay. Cells were harvested and double stained for annexin V-FITC and PI, with apoptosis defined by annexin V-positive/PI-negative cells. **a** Representative figure of flow cytometry. **b** Percentage of apoptosis at the indicated PTK/ZK concentration with or without IFN/5-FU. Data are mean ± SEM of triplicate assays. PTK/ZK combined with IFN/5-FU increased apoptosis in a concentration-dependent manner in PLC/PRF/5 cells to a greater extent than IFN/5-FU alone ($P < 0.05$). **c** Expression of apoptosis-related proteins (Bcl-xL, Bcl-2, Bax) in PLC/PRF/5 and HuH7 cells treated for 6 hours, assessed by Western blot analysis. **d** Relative expression levels of apoptosis-related proteins. The band intensities were analyzed by densitometry and expressed relative to actin. Data are mean ± SEM of triplicate assays. * $P < 0.05$

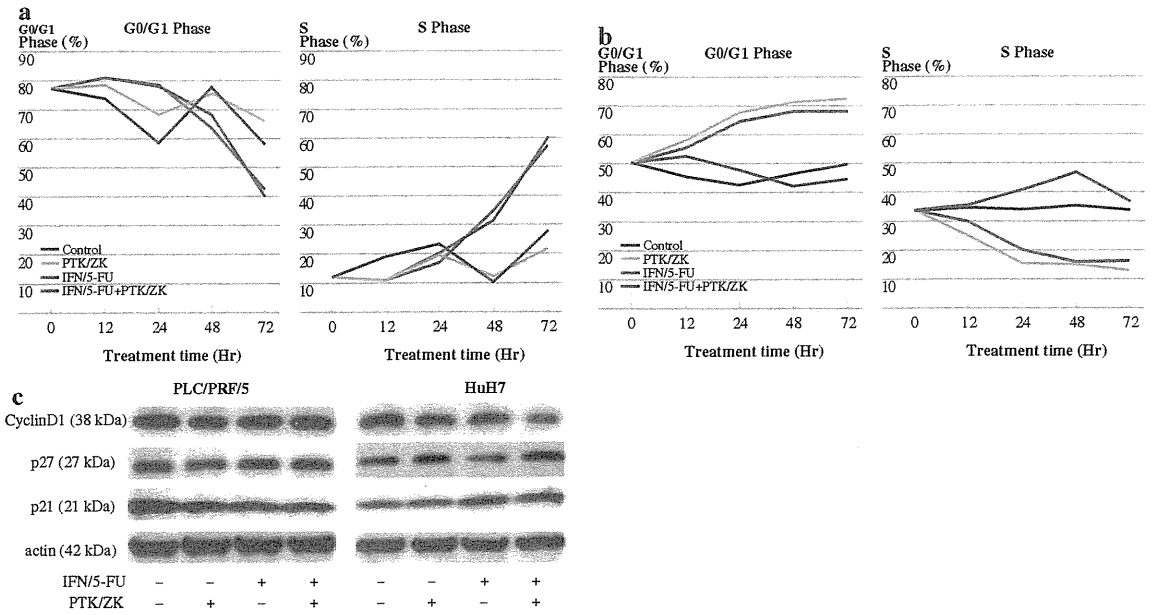

FIG. 4 Effects of PTK/ZK combined with IFN/5-FU on cell cycle in PLC/PRF/5 (**a**) and HuH7 (**b**) cells. The percentages of cells in G0-G1 phase (*left panel*) and S phase (*right panel*) are indicated for each treatment time course. **c** Expression of cell cycle-related proteins (cyclin D1, p27, p21) in PLC/PRF/5 and HuH7 cells treated for 12 h, assessed by Western blot analysis

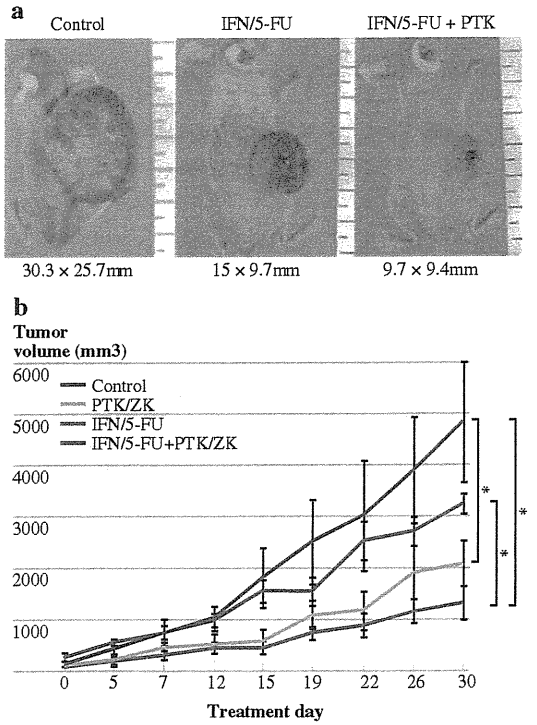

FIG. 5 Effect of PTK/ZK combined with IFN/5-FU therapy on tumor volume in xenografted nude mice. **a** Representative figure. **b** Change of tumor volume in each treatment. Data are mean volume of tumors ± SEM. * $P < 0.05$. Tumor volume of the IFN/5-FU+PTK/ZK therapy group was significantly decreased compared with the other two groups (control and PTK/ZK group); however, there was no significant difference between the IFN/5-FU and IFN/5-FU+PTK/ZK groups

DISCUSSION

We reported previously the potential mechanisms of the antitumor effects of IFN/5-FU therapy, both in vitro and in vivo, namely, the synergistic inhibition of cell proliferation and induction of apoptosis. The cell growth inhibition, including regulation of cell cycle progression, was orchestrated by increasing the S-phase fraction and thereby cell cycle arrest, whereas the apoptotic effect was mediated via IFNAR2 signaling to regulate the expression of apoptosis-related molecules.[18,20] Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and/or the Fas/Fas-L pathway also seemed to partially mediate the antitumor effects of IFN/5-FU therapy.[19,21] In addition, this therapy showed significant antitumor activity through the inhibition of angiogenesis in vitro and in vivo.[8,9] On the other hand, IFNAR2 protein is expressed on the cell surface in human HCC cell lines, although it is relatively weak in HuH7 cells and no cooperative effects were seen in these cells.[18] There are some nonresponders to IFN/5-FU therapy clinically, possibly due to activation of Wnt/β-catenin signaling pathway inducing chemoresistance to the IFN/5-FU therapy.[22] Thus, it is desirable to further examine the antitumor effects of this therapy to identify different potential targets.

Our previous study also showed that IFN/5-FU therapy inhibits VEGF secretion by tumor cells.[8] In the current study, we anticipated a stronger inhibitory effect on VEGF signaling with supplemented IFN/5-FU therapy, therefore we chose PTK/ZK because it potently inhibits all known VEGFR tyrosine kinases (including Flt-4, which is

associated with the lymph system), selectively.[23] PTK/ZK works by binding to the ATP-binding sites of VEGFRs inhibiting tyrosine kinase phosphorylation.[23]

The major findings of the present study were as follows: a) PTK/ZK did not enhance the effect of IFN/5-FU therapy on secreted VEGF; b) PTK/ZK combined with IFN/5-FU inhibited cell growth in vitro and in vivo; c) the combination of PTK/ZK and IFN/5-FU enhanced the induction of apoptosis, but had different effects on cell cycle between two cell lines; and d) Bcl-2 family protein-related apoptosis plays a key role in the effect of these therapies, and the expression of p21 protein related to the cell cycle changed adversely in cells incubated with PTK/ZK and IFN/5-FU.

The findings suggested that pathways other than the VEGF secretion could be involved in the antitumor effects of PTK/ZK combined with IFN/5-FU. In considering the mechanisms of this effect related to apoptosis and cell cycle, we found that the addition of PTK/ZK increased the apoptotic effect of IFN/5-FU therapy dose-dependently. To elucidate the molecular mechanisms underlying this additional effect in human HCC cells, we examined the expression of key apoptotic regulators, Bcl-xL, Bcl-2, and Bax, which were regulated by IFN/5-FU therapy in the previous report.[20] Our findings suggested that VEGF binding to VEGFR activates a phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway leading to the upregulation of Bcl-2 protein.[24,25] On the other hand, evidence suggests that IFN induces apoptosis with activation of the Bcl-2-family members Bak and Bax, and that it activates several signaling pathways, including mainly the canonical Janus tyrosine kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, but also the p38 mitogen-activated protein kinase (MAPK) and PI3K/Akt pathways.[26] In addition, since PTK/ZK potently inhibits the activities of all known VEGFR tyrosine kinases, it also is active against other receptors, such as platelet-derived growth factor receptor beta and c-kit, although at higher concentrations.[23] It is therefore probable that PTK/ZK acts in different apoptotic pathways following IFN/5-FU treatment and that the combined therapy tested herein enhanced such effect.

Second, we evaluated the effect of PTK/ZK combined with IFN/5-FU on the cell cycle. Interestingly, we obtained different results on cell cycle analysis between the two cell lines tested, with the findings suggesting that IFN/5-FU has a stronger effect on PLC/PRF/5 cells than on HuH7 cells, whereas PTK/ZK works the other way. This phenomenon could be related to p21 protein in the cells, which reacted adversely in the present study. This protein is a cyclin-dependent kinase inhibitor acting mainly to induce G1 arrest,[27–29] thus our results could be understood within this functional context. A number of studies showed that p21 plays a role in cell cycle progression and growth via p53-dependent and -independent pathways.[27,28,30] The two cell lines used in the present study harbor p53 mutations, and therefore such effects must be p53-independent via stimuli, such as IFNs and transforming growth factor-β (TGF-β).[31–33] However, HuH7 cells are associated with a p53 point mutation, which results in mutated proteins expressed with a prolonged half-life and thus protracted effects on apoptosis and cell cycle arrest.[31,34,35] Such properties could explain the observed changes in cell response and action site among the different drug treatment of the two cell lines. Thus, combining several drugs that act by different mechanisms should enhance these effects, and expand the possibility of potent therapy against HCC.

In these results, PTK/ZK could synergistically enhance the antitumor effects of IFN/5-FU, particularly in nonresponders, but the potential side effects must be managed. In our in vivo experiments, we used PTK/ZK alone at 20 mg/kg. Although this dose was lower than the effective doses used previously [50 or 100 mg/kg; effective concentration for human HCC cells xenografts as a single agent[17]], IFN/5-FU therapy combined with low-dose PTK/ZK therapy would sufficiently inhibit tumor growth and had no obvious adverse effects on the mice. Such combination therapy for advanced HCC might provide sufficient antitumor effects with fewer side effects. Sorafenib, multikinase inhibitor including VEGF, is widely used against HCC, and this also may be useful in this combination therapy.

In conclusion, we showed that PTK/ZK combined with IFN/5-FU therapy had antitumor effects on human HCC cells in vitro and in vivo, and that these effects were related to upregulated apoptosis and the complementary effects on cell cycle delay, without any change on VEGF secretion.

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The Roles of Surgical Oncologists in the New Era – Minimally Invasive Surgery for Early Gastric Cancer and Adjuvant Surgery for Metastatic Gastric Cancer

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Key Words
Gastric cancer · Laparoscopic surgery · Chemotherapy · Adjuvant surgery

Abstract
In the new era of technical development in surgery, operative devices, molecular targeting and chemotherapeutic agents, surgical oncologists have two main roles in the treatment of gastric cancer. One is to provide patients with minimally invasive surgery, including laparoscopy- or robot-assisted surgery in early gastric cancer patients, and the new concept of surgical intervention toward advanced and metastatic disease. Since recently, laparoscopy-assisted distal gastrectomy has become prevalent in Japan as a surgery which is minimally invasive for the patients and provides them with a good quality of life afterwards. However, the provision of advanced surgical techniques, including lymph node dissection and reconstruction, is more important for patient survival. The second role of surgical oncologists is to evaluate the significant values of the aggressive treatment which we term 'adjuvant surgery' for stage IV gastric cancer patients who have successfully responded to initial chemotherapy for curative intent. Stage IV gastric cancer patients are now being informed about the possibility of longer survival with the new chemotherapeutic and surgical strategic approach.

Introduction

Gastric cancer is the fourth most commonly diagnosed cancer and the second highest in terms of mortality rate. It is a global disease and a type of cancer frequently found in Asian countries. Recent demographic surveys have demonstrated that the mortality rate is notably decreasing, in spite of an only gradual decrease of the occurrence rate [1, 2]. The major causes of this phenomenon in Japan might be the broad reach of the general screening system of gastric cancer, and secondly, the innovation of newly developed diagnostic systems for the early detection of cancer and the high standard of operative techniques and chemotherapy [3].

According to the Japanese General Rules and Guidelines for gastric cancer [4, 5], intramucosal cancers are treated by endoscopic submucosal dissection or endoscopic mucosal resection, and minimally invasive surgery, including laparoscopic gastrectomy, is often performed for the rest of the early gastric cancers which are limited to within the submucosal layer [6–8].

The surgical treatments for stage II and III gastric cancer are well established, as demonstrated by Songun et al. [9] after a 15-year follow-up of the randomized nationwide Dutch D1D2 trial. That is to say, D2 lymphadenectomy is the recommended surgical approach for

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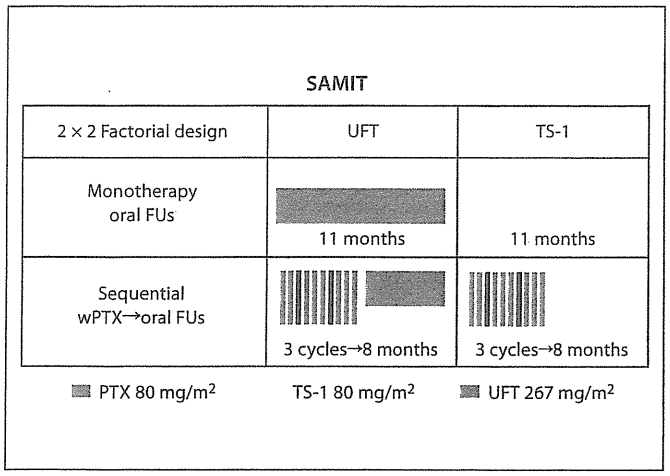

Fig. 1. The rationale of the SAMIT trial. PTX = Paclitaxel.

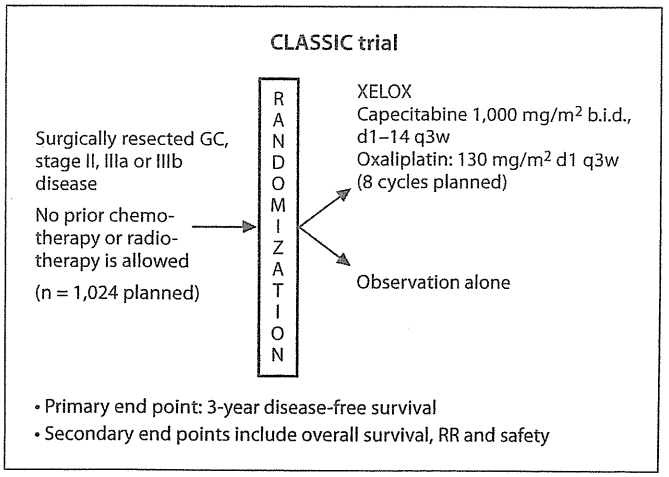

Fig. 2. The rationale of the CLASSIC trial. GC = Gastric cancer; XELOX = capecitabine in combination with oxaliplatin.

Table 1. Consensus of perioperative chemotherapy of gastric cancer

USA	SWOG 9008/Intergroup 0116 5-FU/leucovorin + radiation
Europe	MAGIC trial Perioperative ECF
Japan	ACTS-GC Postoperative S-1

patients with resectable (curable) gastric cancer, and is popular in Japan, Korea and other Asian countries. However, postoperative or perioperative treatments remain a controversial issue between the East and the West. Perioperative ECF (epirubicin/cisplatin/5-FU) therapy is regarded as the standard treatment in the UK and in some European countries [10] and intraoperative radiation with postoperative chemotherapy is the widely accepted treatment in the USA [11]. D2 lymph node dissection was not performed in most of the cases in these trials. What was interesting is that the postoperative survival of the patients who underwent D2 lymph node dissection without postoperative chemotherapy in Japan was far better than for those in the Medical Research Council Adjuvant Gastric Cancer Infusional Chemotherapy (MAGIC) trial and Intergroup study [12]. According to the results of the standard procedure of curative surgery, the Adjuvant Chemotherapy Trial of Thymidine Synthase (TS-1) for Gastric Cancer (ACTS-GC) was performed for 1 year on stage II and III patients to establish the postoperative S-1 treatment; it was accepted with significant survival benefit of the treatment group in Japan [13, 14]. The consensus of the perioperative strategies is summarized in table 1. The Stomach Cancer Adjuvant Multi-Institutional Trial (SAMIT) is currently ongoing; it compares the benefits of S-1 and UFT and also the benefits of adding paclitaxel as adjuvant chemotherapy for curatively resected patients with serosal invasion of a tumor [15]. In Korea, the CLASSIC trial is underway to establish a standard postoperative adjuvant chemotherapy with capecitabine in combination with oxaliplatin after curatively resected stage II and III gastric cancer patients have undergone D2 lymph node dissection [16] (fig. 1, 2).

There is no established global standard chemotherapy for metastatic or recurrent gastric cancer. A combination therapy of fluoropyrimidine and platinum is commonly used [17]. Data is also available about a triplet regimen. ECX (epirubicin/cisplatin/capecitabine) [18], EOX (epirubicin/oxaliplatin/capecitabine) [19] and DCF (docetaxel/cisplatin/5-FU) [20] (or modified DCF [21]) are used as standard care in certain areas of the US and the UK. S-1 combination chemotherapy (S-1 + CDDP) is currently regarded as the standard first-line treatment in metastatic gastric carcinomas in Japan [22]. The median survival time (MST) was prolonged to 13.0 months in the SPIRITS trial conducted in Japan.

As reported at ASCO 2009, a targeted therapy for HER2, Herceptin, was approved for HER2-positive gastric cancer in Europe, Korea and other areas [23]. Other targeted therapies are now under investigation in clinical

trials. Under these circumstances, we need, via clinical trials, to provide a new treatment which is more effective and has fewer adverse events throughout the world and especially in Asian countries. The data relating to gastric cancer should be obtained by collaboration between Asian countries and transmitted globally to establish a standard treatment because gastric cancer is the most prevalent and common disease in this part of the world.

What is interesting in the recent trend of chemotherapeutic treatment in stage IV gastric cancer is that downstaging of the tumors is often observed with high response rate (RR) regimens with newly developed chemotherapeutic agents, and as a result, R0 resection (complete resection with no residual microscopic tumor) has been performed on quite a few patients after chemotherapy [24, 25]. These cases can broadly be called 'adjuvant surgery' or oncosurgery (conversion therapy as it is often described in the treatment of liver metastasis in colorectal surgery) after neoadjuvant chemotherapy [26–28].

Considering the present observations described above, minimally invasive surgery, including laparoscopic surgery or robotic surgery for early gastric cancer [29], and aggressive surgery with curative intent in stage IV, or recurrent gastric cancer with perioperative chemotherapy are the main themes of surgical oncology in the new era. These points are highlighted in this article.

Minimally Invasive Surgery

Laparoscopic Surgery and Its Indication for Gastric Cancer

Laparoscopy-assisted distal gastrectomy (LADG), a minimally invasive surgery, has recently become prevalent in Japan, and provides patients with a good quality of life [30–32]. However, it is more important to provide them with advanced surgical techniques including lymph node dissection and reconstruction. According to the Japanese guidelines for gastric cancer treatment, LADG is not regarded as the standard procedure. In order to establish the safety and noninferiority of the method compared to open surgery, randomized control studies in Japan and Korea are ongoing [33, 34]. For the technical assurance of the laparoscopic surgery, a certification system has been adopted by the Japanese Society of Endoscopic Surgery.

In our institution, the indication for the operation is restricted to patients with early gastric cancer which includes: carcinomas of the mucosal layer (T1), no evidence of lymph node metastasis (N0), not suitable for endoscopic mucosal resection (with a size of more than 2 cm and with ulcer scar formation), or invaded to the submucosal layer with no clinical lymph node metastasis. Up to October 2010, we performed 204 laparoscopic gastrectomies including 152 LADG, 8 laparoscopy-assisted pylorus-preserving gastrectomies, 21 laparoscopy-assisted proximal gastrectomies, 6 laparoscopy-assisted total gastrectomies and 17 simple resections of the stomach.

Surgical Techniques

In order to perform complete laparoscopic gastrectomy, resection and anastomosis should be performed in the abdominal cavity. In this section, we describe our standard procedure of LADG and gastroduodenostomy.

We performed 103 cases of gastroduodenostomy using the delta anastomosis technique [30, 35]. Under general anesthesia, the patient was placed in the supine position with legs apart. Initially, a trocar was inserted under the umbilical portion via a 2-cm incision by the open method. Flexible laparoscopy (Olympus) was used in the operation and the camera operator stood between the legs of the patient. Four other trocars were inserted in the flank and subcostal regions.

The operation consisted of 9 parts: (1) ligation of the left gastroepiploic artery and vein [dissection of lymph node (LN) 4d and 4sb], (2) ligation of the right gastroepiploic artery and vein (LN 6), (3) transection of the duodenum, (4) ligation of the right gastric artery (LN 5), (5) dissection of LN 8a, (6) ligation of the left gastric artery and vein (LN 7, 9 and 11p), (7) dissection of LN 1 and 3, (8) transection of the stomach and (9) reconstruction by the Billroth I method with the delta anastomosis technique. The surgeon stood on the right side except during step (2) and (3).

(1) Ligation of the Left Gastroepiploic Artery and Vein (Dissection of LN 4d and 4sb)

The greater omentum was dissected with harmonic scalpel (Ethicon) about 5 cm away from the epiploic vessels; LN 4d and 4sb were removed. The left gastroepiploic artery and vein were dissected with clips.

(2) Ligation of the Right Gastroepiploic Artery and Vein (LN 6)

The surgeon stood on the left side of the patient to continue the procedure. The greater omentum of the right side was divided in the same manner towards the hepatic flexure of the colon and the gastroduodenal artery was visualized. Dissection of LN 6 was performed, with liga-

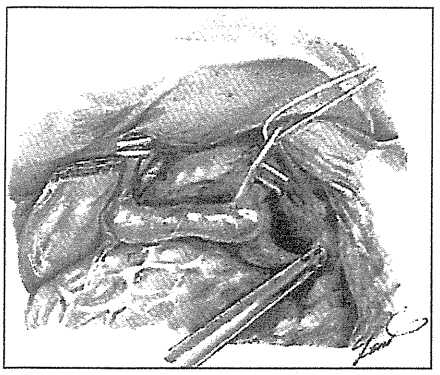

Fig. 3. Lymph node dissection by laparoscopic approach (illustrated by Leon Sakuma [30]).

tion and division of the right gastroepiploic artery and vein by harmonic scalpel.

(3) Transection of the Duodenum and (4) Ligation of the Right Gastric Artery (LN 5)

The surgeon stood on the right side of the patient again. The antrum was lifted and the duodenum was transected close to the pylorus ring using an Echelon (Ethicon) from the left lower port and then the right gastric artery was divided and dissected with the harmonic scalpel with clips cleaning the LN 5.

(5) Dissection of LN 8a

The stomach was lifted towards the left flank and the lesser omentum was divided visualizing the hepatic branch of the vagus nerve near the liver bed. Preserving the branch, the dissection was performed towards the cardia. The serosa of the right crus was dissected with the harmonic scalpel.

LN 8a was dissected using the harmonic scalpel visualizing the common hepatic artery preserving the hepatic plexus of the autonomic nerve. The dissection was performed from the right side towards the celiac axis (fig. 3).

(6) Ligation of the Left Gastric Artery and Vein (LN 7, 9 and 11p)

The fat tissue and connective tissues of LN 8a, 7, 9 and 11p were dissected with the harmonic scalpel. The left gastric artery and vein were visualized, then ligated with 2 clips and divided with the harmonic scalpel. The dissection was performed along the curus towards the esophagogastric junction.

(7) Dissection of LN 1 and 3

The dissection of the LN 1 and 3 was performed along the lesser curvature of the stomach, dissecting the anterior and posterior branch of the vagus nerves toward the stomach. The dissection was performed towards the transection line of the stomach.

(8) Transection of the Stomach and
(9) Reconstruction by the Billroth I Method with the Delta Anastomosis Technique in the Abdominal Cavity

The proximal resection margin was estimated by the serosal side carbon ink color which was injected in the submucosal layer the day before the operation by endoscopy and transected from the left lower port using Echelon (60 mm). The resected stomach was captured by the end catch and taken out through the camera port with an additional abdominal muscle fascia incision but without an additional skin incision.

For the gastroduodenostomy, the edge of greater curvature of the remnant stomach and the duodenum were opened with the harmonic scalpel and the linear stapler (endcutter 45 mm) was inserted via each hole and connected and fired. The V-shape anastomosis of gastroduodenostomy was performed with the entry hole opened. The final step was the closure of the hole with 3 firings of the linear stapler by lifting up the 3 stitches (3-0 monocryl) of incomplete closure of the entry hole (fig. 4).

The mean operative time was 253 min and blood loss was 50 ml. Thirty-seven lymph nodes were harvested. Patients started to walk the next day, started the oral intake treatment on day 3 after the operation and were discharged on day 9. Among 103 cases of delta anastomosis, there was no anastomotic leakage and no reoperation; there were, however, 2 cases of anastomotic stenosis.

Although this procedure requires time and the precise knowledge of the anatomy of the upper abdominal regions, it provides patients with several advantages including improved cosmetics, shorter hospitalization, minimal operative pains and a low incidence of bowel movability and pancreas functions (demonstrated elsewhere). Moreover, the postoperative complications can be reduced. The LADG with lymphadenectomy can be one of the most effective therapeutic methods for early gastric cancer patients.

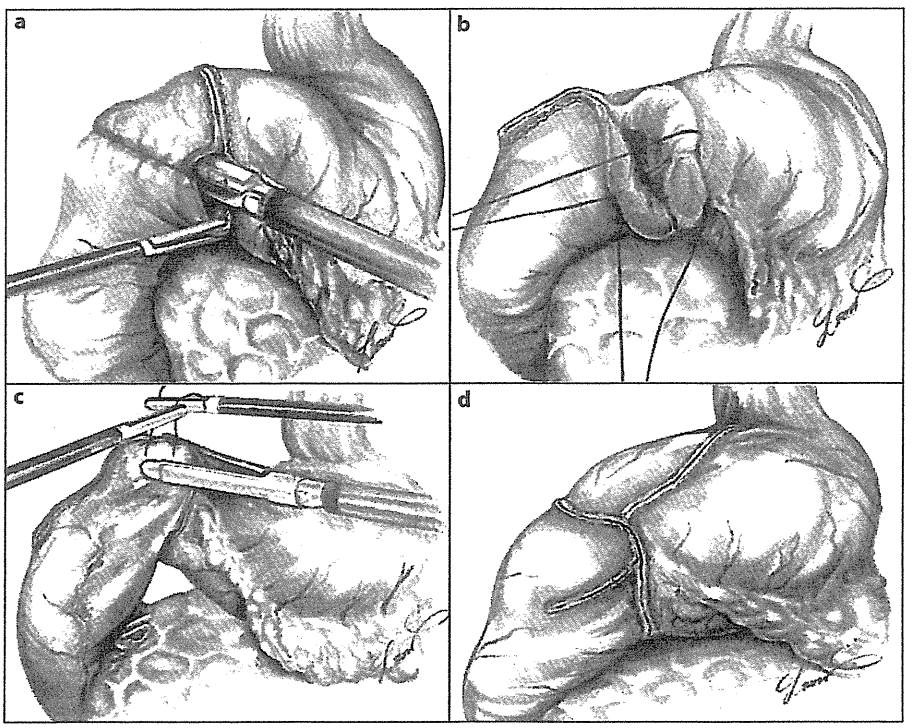

Fig. 4. Delta anastomosis (illustrated by Leon Sakuma [30]). **a** Anastomosis by linear stapler. **b** Ligation of entry hole. **c** Closure of entry hole by linear stapler. **d** Final anastomosis image.

New Therapeutic Approach for Stage IV Gastric Cancer

Establishment of New Chemotherapeutic Regimens for Gastric Cancer

Several combination regimens with S-1 have been established in Japan in this decade and randomized phase III studies have been conducted. They are S-1 + CDDP, S-1 + CPT-11 and S-1 + docetaxel as reported by Fujii et al. [36].

Cisplatin at a dose of 60 mg/m^2 on day 8 was combined with S-1 for 3-weeks-on and 2-weeks-off treatment [37]. This was repeated every 5 weeks, unless disease progression was observed. The RR was 74% (14/19; 95% confidence interval (CI) 54.9–90.6) and the MST was 383 days. Komatsu et al. [38] reported the results of a phase I/II study with CPT-11 + S-1 (IRIS study) in AGC patients. S-1 was given orally twice a day for 14 days and CPT-11 was administered as a 90-min intravenous infusion on days 1 and 15. This regimen was repeated every 4 weeks. The overall RR was 54.2% in the phase II study. The MST achieved with this regimen was 581 days. Yoshida et al. [39, 40] performed a phase I study and a phase II study of docetaxel in combination with S-1 in patients with AGC. In the phase II study, the RR was 52.1% and the MST was 434 days. Moreover, the biochemical modulations of docetaxel enhanced the sensitivity of 5-FU in vitro and in vivo [41]. More interestingly, the mTOR inhibitor downregulated the expression of TS and enhanced the reactivity of 5-FU on TMK-1 gastric cancer cells [42].

Based on the results obtained in the above phase II studies, 3 large randomized phase III studies, the SPIRITS trial [22], the TOP-002 trial [43] and the JACCRO GC03 trial [36, 44] were conducted independently to compare the data with that of S-1 monotherapy, the results of which are summarized in table 2.

In the SPIRITS trial, chemotherapy-naïve patients with AGC were randomly assigned to receive either S-1 plus cisplatin or S-1 alone. The primary end point was overall survival and the secondary end points were progression-free survival, proportion of responders and safety. Median overall survival was significantly longer in the patients assigned to receive S-1 plus cisplatin than in those assigned to receive S-1 alone (13.0 vs. 11.0 months, respectively; hazard ratio (HR) 0.77; 95% CI 0.61–0.98; $p = 0.04$). Progression-free survival was significantly longer in the patients assigned to receive S-1 plus cisplatin than in those assigned to receive S-1 alone (median progression-free survival 6.0 vs. 4.0 months, respectively; $p < 0.0001$). Moreover, of the 87 patients with target tumors assigned to receive S-1 plus cisplatin, 1 showed a complete response (CR) and 46 showed a partial response

Table 2. Review of phase III clinical trials in Japan

	S-1/ S-1 + CPT-11 (GC0301/TOP-002)	S-1/ CPT-11 + CDDP (JCOG 9912)	S-1/ S-1 + CDDP (SPIRITS trial)	S-1/ S-1 + docetaxel (START trial)
MST, months	10.5/12.8	11.4/12.3	11.0/13.0	11.0/13.0
1-year survival rate	45.0%/52.0%	49.7%/52.5%	46.7%/54.1%	46.0%/52.5%
2-year survival rate	22.5%/18.0%	-/-	15.3%/23.6%	20.6%/23.7%

(PR) (total RR 54%). Of the 106 patients with target tumors assigned to receive S-1 alone, 1 showed a CR and 32 showed a PR (total RR 31%). Based on this trial, S-1 plus cisplatin became regarded as a new standard first-line treatment for patients with AGC in Japan.

A randomized phase III trial was conducted to evaluate the efficacy and safety of IRIS (S-1 + CPT-11) versus S-1 alone for AGC. Patients with previously untreated AGC were randomized to arm A (oral S-1, 80 mg/m^2 on days 1–28, every 6 weeks) or arm B (IRIS: oral S-1, 80 mg/m^2 on days 1–21; intravenous CPT-11, 80 mg/m^2 on days 1 and 15, every 5 weeks) by dynamic allocation. As a result, 326 patients were randomized to arm A (162 patients) or arm B (164 patients), with a final 315 evaluable patients (160 in arm A and 155 in arm B). Although the MST of the arm A patients was 318 days (95% CI 286–395) and that of the arm B patients was 389 days (95% CI 324–458), arm B did not show significant superiority to arm A. The RRs were significantly different, being 26.9% in arm A versus 41.5% in arm B in 187 RECIST (Response Evaluation Criteria in Solid Tumors)-evaluable patients. Based on this trial, IRIS achieved MST and was better tolerated; however, it did not show significant superiority to S-1 alone in terms of the overall survival, and could thus not become a first-line treatment for AGC.

A randomized phase III study comparing S-1 alone with the S-1 + docetaxel combination was conducted through the JACCRO GC03 trial. This study was a prospective, multicenter, multinational (Korea and Japan), nonblinded, randomized, phase III study of patients with AGC. Patients were randomly assigned to receive 3-week cycles of treatment arm A (docetaxel and S-1) or 6-week cycles of treatment arm B (S-1 only). The primary objective of the study was to compare the median overall survival of the test arm (docetaxel and S-1) with that of the control arm (S-1 only). The secondary objectives were to assess the time to tumor progression (defined as the time from randomization to the date of first documentation of progressive disease), to determine the clinical response/RR (defined as the sum of the CR and PR according to RECIST criteria) and to evaluate the safety of the 2 regimens. It was expected that 628 patients (314 in each treatment arm) would be enrolled in this trial and this was exceeded, with confirmation of 628 patients from 103 centers in September 2008. Although the primary end point was not met, PR and RR were superior in the combination arm [44]. What is more interesting in this combination is that the docetaxel enhances the cytotoxic effect of 5-FU via biochemical modulations through decreased expression and activity of TS and dihydropyrimidine dehydrogenase and increased activity of orotate phosphoribosyltransferase [41]. It was recently reported that these effects can be modulated even more by molecular targeting agents including mTOR inhibitor [42].

The Role of Surgical Intervention in Stage IV Gastric Cancer Patients

Palliative and Volume Reduction Surgery

Gastric bypass, jejunostomy, ileostomies and colostomies are sometimes performed because of the pyloric stenosis of the primary tumor and/or tumors of the peritoneal disseminated disease of gastric cancer, and often, even if not by R0 resection, primary tumors are removed because of bleeding or obstruction of the stomach and bowels, all of which are regarded as palliative surgery. In the 1980s, the resection of the primary tumors and the removal of metastatic disease were often conducted as tumor volume reduction surgery. However, the prognosis of patients was not satisfactory because although the main treatment tool was palliative chemotherapy, the RR of chemotherapy regimens in those days was 20–30% and in the end, the patients died due to the tumor burden in spite of the reduction surgery. In order to improve the survival of the patients, new regimens or new chemotherapeutic

agents with more effective and reduced adverse effects were called for, but until recently, palliative chemotherapy was regarded as the standard strategy in stage IV or recurrent gastric cancer patients.

Adjuvant Surgery

As described in the previous section, after the new chemotherapeutic agents were developed including S-1, docetaxel, paclitaxel, irinotecan, oxaliplatin and molecular targeting agents, the RR and survival of patients have improved dramatically. Interestingly, it was often reported that with newly developed chemotherapeutic regimens, the tumors were downstaged and the curative resections or R0 resections were performed in stage IV gastric cancer patients [24]. It is only recently that those cases were often found successful after treatment with S-1 + CDDP and S-1 + docetaxel regimens [45]. These operations are called 'adjuvant surgery' as previously reported [24]. The indications for adjuvant surgery are that curative resection (not palliative) can be expected, based on the response to chemotherapy, the absence or CR of other distant metastases such as peritoneal dissemination, extensive lymph node metastases or lung metastasis. The macroscopically complete removal of liver deposits is feasible, and minimal residual tumors after chemotherapy in distant lymph nodes can be extensively removed. Palliative chemotherapy is the standard strategic approach for stage IV gastric cancer. However, if treatment has been successful with CR or PR and the tumors are considered resectable or R0 resection is deemed possible, it could be feasible to perform aggressive operations to remove the residual tumors, although these operations can be regarded as adjuvant. Of course, it might be required to continue chemotherapy after these surgeries, even after R0 resections, because these cases were treated as stage IV gastric cancer. This chemotherapeutic strategy is called perioperative chemotherapy [10]. In other words, so-called neoadjuvant chemotherapy (NAC) was performed, downstaging of the tumors followed, and as a result of this, the R0 resections could take place. It must be clarified that, strictly speaking, NAC is the chemotherapy which is conducted in patients with potentially curative resectable tumors before treatment [46]. NAC is performed in order to improve the prognosis or improve the resectability of the tumors. For aggressive operations in stage IV gastric cancer patients, it can be termed adjuvant surgery with perioperative chemotherapy. The merit of adjuvant surgery in stage IV gastric cancer with a favorable response to chemotherapy is that the compliance with chemotherapy is better before surgery compared to afterwards, and secondly, it can be regarded as an in vivo sensitivity test. Thirdly, tumors definitely acquire resistance to chemotherapy, which is why aggressive operations are preferred while the tumor growth is well controlled with chemotherapy, because it is well known that tumor growth is enhanced by the cytokines after surgical treatment [47]. The best timing for the operation is when the best response of the tumor to chemotherapy is observed, not when the tumor is increasing in size or has acquired the ability to regrow. Generally, we estimate the best timing for the removal of the tumor to be when the CR or PR is detected when 4–6 cycles of S-1 + CDDP or S-1 + docetaxel regimens have been performed. This strategy is regarded as rescue surgery, oncosurgery or conversion therapy (recently conducted in metastatic liver tumors from colorectal cancer) [48–52]. In the REGATTA trial, palliative surgery followed by chemotherapy for stage IV gastric cancer is now being conducted in Japan and Korea in order to evaluate the significant roles of tumor volume reduction and interesting results are expected.

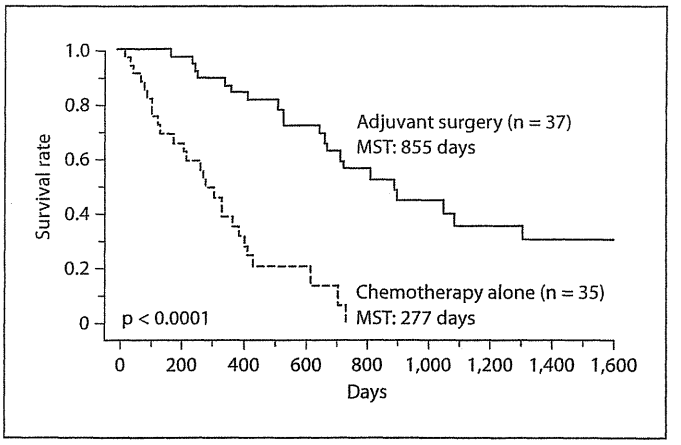

Fig. 5. Survival of the patients with adjuvant surgery in stage IV gastric cancer.

From 2001 to 2009, we treated 158 stage IV gastric cancer patients who had received S-1 + CDDP and S-1 + docetaxel treatment. We performed adjuvant surgery aiming at R0 resection of the primary and metastatic disease on 37 of these patients. The median survival of the patients who underwent surgery was 855 days after the initial start of the chemotherapy, while for those without an operation it was 277 days (fig. 5). As we reported in a preliminary retrospective analysis [24], this type of surgery might be effective in patients diagnosed as stage IV

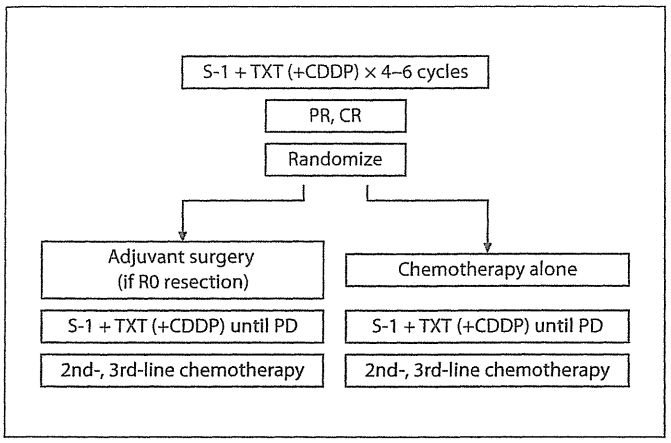

Fig. 6. Future trial of adjuvant surgery. Perioperative chemotherapy in stage IV gastric cancer: a randomized controlled trial of S-1 + docetaxel with or without CDDP. PD = Progressive disease; TXT = docetaxel.

due to liver metastasis or distant lymph node metastasis, but not for cases of peritoneal dissemination. Of course, there is a bias that the adjuvant surgery group had a good response to chemotherapy and the others not. In order to prove the significance of the adjuvant surgery, further analysis will be needed. Under investigation by a randomized phase II/III study, using S-1 + docetaxel and/or CDDP among patients who had had CR or PR and were considered curatively resectable, patients were randomized to a 'continuation of chemotherapy' group or an 'adjuvant surgery followed by chemotherapy (perioperative chemotherapy)' group (fig. 6).

Salvage Surgery

Salvage surgery is regarded as the surgery that is performed after curative radiation or chemoradiation therapy to remove the residual or regrown tumors which have invaded adjacent organs (as described in the Japanese guidelines of esophageal cancer [53, 54]). Salvage surgery is conducted in locally advanced tumors, but adjuvant surgery is conducted in metastatic cancer. Indeed, using the term 'adjuvant' in palliative surgery, even if it is after successful chemotherapy in stage IV gastric cancer, might be criticized. Because, in general, the term 'adjuvant' can be used when the tumor does not exist macroscopically, the term 'adjuvant chemotherapy' is used for chemotherapy when an R0 resection has been performed. In this sense, the term 'adjuvant surgery' could be defined as the curative surgery after CR was detected by chemotherapy in stage IV cancer. Further discussion might be required to determine the most appropriate terminology.

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Docetaxel, Nedaplatin, and S-1 (DGS) Chemotherapy for Advanced Esophageal Carcinoma: A Phase I Dose-escalation Study

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Abstract. *Aim: More effective regimens are urgently needed for treatment of esophageal carcinoma; therefore, we conducted a phase I trial of a combination of docetaxel, nedaplatin, and S-1 (DGS) to determine the optimal dose in patients with advanced esophageal carcinoma. Patients and Methods: We studied 14 patients with previously untreated advanced cervical esophageal carcinoma with T3-4 tumors and/or M1 staging and esophageal carcinoma with cervical lymph node metastasis. The patients received an infusion of docetaxel at different dose levels (levels 1, 2, 3, 4: 25, 30, 35, 40 mg/m^2, respectively) and an infusion of nedaplatin (40 mg/m^2) on day 8 plus oral administration of S1 (80 mg/m^2/day) for two consecutive weeks at two-week intervals. Results: Dose-limiting toxicities (DLTs) included febrile neutropenia and leukopenia. DLTs occurred in 2 out of 5 patients at level 4. The response rate was 78.6 (11/14)%, including a complete response rate of 35.7(5/14)%. Conclusion: The DGS regimen reported here was well tolerated and toxicities were manageable. The maximum tolerated dose was level 4, and the recommended dose was determined to be docetaxel at 35 mg/m^2 with nedaplatin at 40 mg/m^2 plus S1 at 80 mg/m^2. We found that our regimen, administered on an outpatient basis, showed high activity and tolerance. A phase II study has been started.*

Locally advanced or widespread metastatic esophageal carcinoma is difficult to treat and is often thought to progress rapidly. Quick deterioration of respiratory and nutritional states makes outpatient care impossible and leads to an extremely poor prognosis. It is necessary to establish effective and safe outpatient chemotherapy that provides survival benefits and improvements in quality of life compared with best supportive care.

Over the past several decades, patients with unresectable or inoperable esophageal disease have usually been treated with various chemotherapy strategies, and prognosis is extremely poor, with a mean survival time of less than 8.1 months with current chemotherapies used singly or in combination with 5-fluorouracil (5-FU), vindesine, mitomycin, docetaxel, paclitaxel, cisplatin, irinotecan, vinorelbine, or capecitabine (1-3).

Standard chemotherapy is fluorouracil and cisplatin combination therapy (FP), for which the median survival time is reported to be 9.2 months for responders and 5.3 months for nonresponders (4, 5). The response rates reported with FP range from 35 to 40%, whereas two-year survival rates of patients with locally advanced esophageal cancer range from 8 to 55% (mean 27%) (6-8).

To improve both local and distant tumor control in patients with esophageal carcinoma, new therapeutic combinations must be developed. Recently, favorable antitumor effects of combination therapy with fluorouracil and taxanes were reported. Many studies have shown that taxanes have significant activity in patients with locally advanced and metastatic esophageal carcinomas (9). For advanced esophageal carcinoma, a combination of docetaxel and 5-FU with concurrent radiotherapy had good efficacy (10).

Docetaxel, cisplatin, and 5-FU (DCF) have exhibited different mechanisms of activity in upper gastrointestinal malignancies. In a randomized phase III study from the V325 study group, advanced gastric or gastroesophageal junction cancer patients receiving DCF not only had statistically significantly improved overall survival and time to tumor progression, but they also had better preserved quality of life compared with patients receiving FP therapy (11, 12).

We previously reported a phase I study of DCF for advanced esophageal squamous cell carcinoma. To minimize toxicity and maximize dose intensity, we investigated a

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biweekly regimen. This regimen was tolerable and highly active. The response rate was 88.9%, including a complete response rate of 33.3% (13). However, hospitalization is necessary with this regimen, and cisplatin requires hydration and is thus not easily used if renal dysfunction is present.

The combination of docetaxel and S1 is highly active and well tolerated for advanced or recurrent gastric cancer (14), and synergy of this combination has been reported *in vitro* (15). S1 (TS1®; Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) was developed by the biochemical modulation of tegafur, a 5-FU prodrug; gimeracil, a dihydropyrimidine dehydrogenase inhibitor; and oteracil, which inhibits pyrimidine phosphoribosyl transferase specifically in the gastrointestinal tract and thereby reduces the phosphorylation of 5-FU in the intestine. S1 is a well-designed oral formulation, with the dual actions of reinforcing antitumor activity and reducing gastrointestinal toxicity (16).

In a late phase II study of nedaplatin (*cis*-diammineglycolatoplatinum) in patients with advanced head and neck cancer, the response rate was 37.5%, higher than that reported for cisplatin, and carboplatin (17-19). Nedaplatin is a less nephrotoxic analogue of cisplatin. Drug secretion and re-absorption in the convoluted tubules are not seen, and it is less toxic to the gastrointestinal tract mucosa than is cisplatin, which is a second-generation platinum derivative that has demonstrated potent antitumor activity against lung, testicular, esophageal, gynecological, and head and neck cancers. Platinum primarily acts as an alkylating agent, whereas docetaxel stabilizes microtubules and inhibits mitosis; therefore, a combination of docetaxel and platinum should be expected to result in additive antitumor effects and non-overlapping toxicity profiles.

A phase II study of induction chemotherapy with docetaxel and nedaplatin for oral squamous cell carcinoma showed a good response rate of 33.3% (20). Hydration is not required before or after nedaplatin administration, thus allowing use of the drug on an outpatient basis.

We therefore conducted a phase I clinical trial of the triplet combination of docetaxel, nedaplatin, and S-1 (DGS) in patients with advanced cervical esophageal carcinoma with T3-4 tumors and/or M1 staging and esophageal carcinoma with cervical lymph node metastasis. The goal of this trial was to determine the recommended dose (RD) for use in phase II trials on the basis of the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT). Secondary objectives were treatment-related toxicity and efficacy.

Patients and Methods

Patient eligibility criteria. Patients eligible for the present study had to be ≥20 years of age at the time of registration and have histologically or cytologically confirmed squamous cell carcinoma (SCC) or either T3/T4 or recurrent adenocarcinoma. An Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 was required, as were a life expectancy of >12 weeks and adequate liver, bone marrow, renal, and cardiovascular function as evidenced by the following measures: serum bilirubin ≤1.5 mg/dl, neutrophil count ≥1,500/mm^3, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of less than or equal to twice the upper limit of normal range, platelet count ≥100,000/mm^3, hemoglobin ≥8.0 g/dl, and serum creatinine ≤1.5 mg/dl or creatinine clearance rate >60 ml/min. In addition, the latest chemotherapy treatment must have been at least 4 weeks before trial enrollment. Major exclusion criteria included the following: previous treatment with taxane therapy for recurrent disease or irradiation to major bone areas; serious concomitant malignancy; active infectious disease with fever; severe drug allergy; symptomatic peripheral neuropathy; uncontrolled diabetes mellitus, hypertension, angina pectoris, arrhythmia or congestive heart failure; and interstitial pneumonia or lung fibrosis. Prior to study entry, all patients were required to sign an informed consent form approved by the Ethical Committee of Gifu University Hospital. Ultimately, 14 patients were enrolled in the study, and all fully underwent DGS therapy.

Study design. The primary objectives of this phase I dose-escalation study were to determine the MTD and toxicity of escalating doses of docetaxel combined with a fixed dose of nedaplatin and S-1 in patients with advanced esophageal carcinoma. The secondary objective of the study was to obtain preliminary data regarding clinical response. This study of DGS was conducted at the Department of Surgical Oncology, Gifu University School of Medicine.

At least three patients were entered at each docetaxel dose level. No dose escalation for individual patients or within a dose level was permitted. All three patients at a given dose level had to complete the first two cycles of treatment without DLT before further patients were enrolled in the next dose level. If DLT did not occur, the next dose level was explored. Doses were increased in sequential groups of three patients until the MTD was established or the highest intended dose levels were reached. If any of the three patients experienced DLT, an additional three patients were treated at the same dose level. If more than three out of the six patients at a given dose level experienced DLT, that dose level was defined as the MTD. The dose level one step below the MTD was set as the RD for further evaluation in a phase II study.

Treatment plan. The patients received an intravenous infusion of docetaxel at different dose levels (level 1, 25 mg/m^2; level 2, 30 mg/m^2; level 3, 35 mg/m^2; and level 4, 40 mg/m^2) and an intravenous infusion of nedaplatin (40 mg/m^2) followed by 500 ml hydration on day 8 plus oral administration of S1 (80 mg/m^2/day) twice daily (within 30 minutes after the morning and evening meals) for two consecutive weeks at two-week intervals (one cycle).

On day 8, patients received docetaxel diluted in 250 ml of normal saline at the assigned dose. It was infused intravenously over 2 hours. Then nedaplatin was prepared in normal saline at a dose of 40 mg/m^2 and administered intravenously over 2 hours followed by 500 ml hydration. If the patient had upper digestive tract obstruction, S-1 was administered through a 6-8 Fr nasogastric tube inserted in the stomach. The dose-escalation scheme is described in Table I. The initial dose of docetaxel was 25 mg/m^2 (dose level 1), and this was increased up to a maximum of 40 mg/m^2 in 5-mg/m^2 steps.

Table I. *Dose-escalation scheme.*

Dose level	Docetaxel (mg/m^2)	Nedaplatin (mg/m^2)	S1 (mg/m^2)
1	25	40	80
2	30	40	80
3	35	40	80
4	40	40	80

Supportive therapy for treatment and prophylaxis for expected side-effects were administered. All patients were premedicated with intravenous administration of 2 mg of granisetron. Hypersensitivity reactions were treated with prophylactic use of intravenous dexamethasone at 8 mg, which was infused 1 hour prior to the administration of docetaxel. Further dexamethasone was prescribed at a dose of 8 mg orally for 2 days after administration of docetaxel to reduce the risk of hypersensitivity reaction and fluid retention. Diuretics were added at the discretion of the treating physician. Additional antiemetics were recommended on subsequent days as needed.

Granulocyte colony-stimulating factor (G-CSF) was administered once a day if the neutrophil count was below 500/μl or if febrile neutropenia (fever ≥38°C and neutrophil count of <1,000/μl) were observed. G-CSF was stopped if the neutrophil count was >5,000/μl. To avoid severe mucositis, L-glutamine at 8 g was administered orally to all patients.

Patient monitoring and response criteria. Complete staging procedures for documentation of disease extent, which included assessment of ECOG performance status, medical history, and physical examination, were performed on all patients. Laboratory evaluations were obtained within one week before initiation of treatment and at the start of each treatment cycle and included the following: complete blood cell count; serum electrolytes; urea; creatinine and 24-hour creatinine clearance; bilirubin; alkaline phosphatase and transaminases; carcinoembryonic antigen (CEA), squamous cell carcinoma-related antigen (SCC), carbohydrate antigen 19-9 (CA19-9) and cytokeratin 19 fragment (CYFRA) measurements, and electrocardiogram. For baseline reference, either computed tomography (CT) or magnetic resonance imaging (MRI) and positron-emission tomography CT were performed within two weeks prior to study entry. During chemotherapy, a complete blood count was measured in all patients every week, and levels of electrolytes, serum creatinine, transaminases, alkaline phosphatase and bilirubin, and plasma urea were measured every two weeks. We used the Common Terminology Criteria for Adverse Events (v3.0) to grade the medical history, which included physical examination and toxicity assessment, every two weeks during the study. Tumor measurements were made from radiographic films or scans taken to document treatment response during therapy and were repeated at every second cycle of treatment or sooner if the patient appeared to show disease progression. We assessed tumor response according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (21). A barium meal study, endoscopy, ultrasonography, and CT or MRI was used to evaluate the response status of measurable lesions.

We defined complete response as complete disappearance of all clinically detectable malignant disease and partial response as a ≥30% decrease in the sum of the perpendicular diameters of all measurable lesions present for at least 4 weeks. We defined progressive disease as either a ≥20% increase in the sum of the products of measurable lesions over the smallest sum observed or as the appearance of new lesions. Stable disease did not qualify as complete response, partial response, or progressive disease.

Table II. *Characteristics of patients.*

Characteristic	
No. of patients	14
Age, years	
Median	65.9
Range	40-81
Gender	
Male	11
Female	3
Performance status	
0-1	14
Histology	
SCC	11
Adenocarcinoma	3
Disease status	
Locally advanced	7
Locally advanced and metastatic	7
Stage of disease	
T3N1M0	2
T3N2M0	2
T4N3M0	3
T3N4M1	4
T4N1M1	1
T4N2M1	2
Site of primary disease	
Ut	3
Mt	6
Lt	5
Differentiation	
Well differentiated	3
Moderately differentiated	5
Poorly differentiated	6

SCC: Squamous cell carcinoma; Ut: upper lesion of thorasic esophagus; Mt: middle lesion of thorasic esophagus; Lt: lower lesion of thoracic esophagus.

Definition of DLT and criteria for dose modifications. The Common Terminology Criteria for Adverse Events (v3.0) was used to evaluate and score toxicity. We defined DLT to include the following: febrile grade 3 neutropenia, grade 4 neutropenia lasting >7 days, grade 3 leucopenia, grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding tendency, or any grade 3 or 4 non-hematological toxicity other than nausea/vomiting, anorexia, diarrhea, alopecia, and general fatigue. Occurrence of hematological toxicity of ≥grade 3 resulted in delay of therapy until the platelet count was at least $100,000/mm^3$ and absolute neutrophils were ≥2,000/μl. Occurrence of gastrointestinal toxicity of ≥grade 3 resulted in delay of chemotherapy until the optimum dose could be tolerated. Treatment was repeated every 4 weeks or as soon as the patient had recovered from the toxicity of the previous chemotherapy. However, the patient was removed from the study if toxicity persisted for more than two weeks following the time of planned treatment. Delay in

Table III. *Dose-escalation scheme in relation to dose-limiting toxicity and response.*

Dose level of docetaxel	Patients	No. of cycles	DLT	Type	Response
25 mg/m^2	3	24	0	–	2 CR, 1 SD
30 mg/m^2	3	15	0	–	1 CR, 2 PR
35 mg/m^2	3	16	0	–	2PR, 1 SD
40 mg/m^2	5	17	4	2 Leucopenia 2 Febrile neutropenia	2 CR, 2 PR, 1 SD
Total	14	72	4		Response rate: 78.6%

DLT: Dose-limiting toxicity; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

administration of the second cycle of therapy of longer than two weeks was also considered a DLT. Dose modifications for the next dose were based on the most severe toxicity observed since the previous treatment course. If DLT occurred, treatment was interrupted until toxicity resolved to ≤grade 1.

Results

Patient characteristics. Between November, 2008, and January, 2010, 14 patients were enrolled in the present study. Demographic and clinical characteristics of the study population are summarized in Table II. Four docetaxel dose levels were evaluated. A total of 72 courses of chemotherapy were administered, with the median number of courses administered per patient being 5.1 (range, 2-10). All patients had locally advanced esophageal carcinoma or metastatic lesions. Median patient age was 65.9 years (range, 40-81 years). All patients had an ECOG performance status of 0-1. Histology showed well-differentiated carcinoma in 21.4% of the patients and poorly differentiated carcinoma in 42.9%. Only two patients were hospitalized and administered S-1 by nasogastric tube until finishing their second courses. After the second course, S-1 was administered orally to these two patients in the outpatient setting. The other 12 patients were treated solely as outpatients.

Toxicity and dose-finding study. Data on the dose-escalation scheme, DLT, and response are summarized in Table III. Only ≥grade 2 toxicity data were collected and reported, and especially for neutropenia and leucopenia, only ≥grade 3 toxicity data were reported. Patient characteristics were well balanced across all dose levels. No treatment-related deaths were observed.

The level 1 dose (docetaxel 25 mg/m^2) was initially administered to three patients. No patient had grade 3-4 neutropenia lasting five days with fever. Of the three patients treated at dose level 1, all had grade 2 anorexia, one had grade 2 fatigue, and one had grade 2 nausea. Twenty-four courses of chemotherapy were evaluated, and two responders were observed.

Three patients were initially enrolled at dose level 2 (docetaxel 30 mg/m^2). One patient had grade 2 anemia. One patient experienced grade 2 nausea, three experienced grade 2 anorexia, and one patient experienced grade 2 hypersensitivity reaction. Fifteen courses of chemotherapy were administered at dose level 2. All three patients were responders.

At dose level 3 (docetaxel 35 mg/m^2), no patients developed grade 3/4 hematologic toxicity. One patient experienced grade 2 anemia, and one patient had grade 2 thrombocytopenia. Two patients experienced grade 2 anorexia, one experienced grade 1 nausea, one had grade 1 mucositis, and one had grade 1 pericardial effusion. Sixteen courses of chemotherapy were evaluated, and two responders were observed.

At dose level 4 (docetaxel 40 mg/m^2), one out of two patients developed grade 3 toxicity characterized by febrile neutropenia lasting five days with fever, so three patients were added to the cohort at this dose level. Of the five patients treated at this dose level, two had grade 3 leucopenia, two had grade 3 febrile neutropenia lasting five days with fever, one experienced grade 2 nausea, one experienced grade 2 anorexia, and one had grade 2 mucositis. The febrile neutropenia of the two patients was resolved within five days by G-CSF support. Seventeen courses of chemotherapy were evaluated. Among the five patients entered at this dose level, four responders were observed. This dose (docetaxel 40 mg/m^2) was considered the MTD; therefore, the dose of docetaxel for further phase II studies was determined to be 35 mg/m^2.

The frequency of treatment-related toxicities is summarized in Table IV. Grade 3 leucopenia occurred in 2 out of 14 patients, and grade 3 febrile neutropenia also occurred in 2 out of 14 patients (14.3%). Alopecia was the most frequent non-hematologic toxicity with an incidence of 13/14 of patients, followed by anorexia (9/14) and nausea (4/14). Edema (3/14) and hypersensitivity reaction (1/14), which are known toxicities attributed to docetaxel, were observed, but these side-effects were manageable and reversible. Grade 1/2 mucositis occurred in 2 out of 14