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SHORT COMMUNICATION

# Phase 1 study of sulfasalazine and cisplatin for patients with CD44v-positive gastric cancer refractory to cisplatin (EPOC1407)

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**Abstract** A previous dose-escalation study of sulfasalazine (SSZ), an inhibitor of cystine-glutamate exchange transporter xc (–), in the variant form of CD44 (CD44v)-positive cancer stem cells (CSCs) suggested that administration of SSZ induces the reduction of CD44v-positive cells and intracellular reduced glutathione (GSH) levels in patients with advanced gastric cancer (AGC). Here we report a study to evaluate SSZ in combination with cisplatin in patients with CD44v-expressing AGC refractory to cisplatin. SSZ was given by oral administration four times daily with 2 weeks on and 1 week off. Cisplatin at 60 mg/m$^2$ was administered every 3 weeks. Of the 15 patients who underwent prescreening of CD44v expression, 8 patients were positive, and 7 patients were treated with the dose level of SSZ at 6 g/day. One patient experienced dose-limiting toxicity (DLT) as grade 3 anorexia. Although no other patients experienced DLT, 4 patients required dose interruption or reduction of SSZ; thus, we terminated further dose escalation. No patient achieved objective response, but 1 patient completed six cycles with stable disease for more than 4 months as well as reduction of intratumoral GSH level. The combination of SSZ plus cisplatin was manageable, although dose modification was frequently required during a short observational period.

**Keywords** Cancer stem cell · CD44v-positive · Gastric cancer · Sulfasalazine · xCT

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## Introduction

Cancer stem cells (CSCs) are associated with characteristics resistant to conventional therapies [1–3], with one mechanism being dependent on an enhanced ability for self-protection against reactive oxygen species (ROS) [4]. Splice variant isoforms of CD44 (CD44v) stabilize the xCT subunit of the cystine-glutamate exchange transporter xc (−) at the cell membrane, thereby promoting the cellular uptake of cystine and the consequent synthesis of reduced glutathione (GSH) [3, 5]. Thus, CD44v-positive cancer cells are resistant to ROS and possess stem-like properties [5, 6]. The presence of CD44v-positive cells was shown to be associated with poor outcome after local treatment [7, 8] as well as resistance to platinum-based therapy [9].

Sulfasalazine (SSZ) is a well-characterized specific inhibitor of xCT-mediated cystine transport and has been

shown to selectively suppress the proliferation of CD44v-positive cancer cells [10, 11]. We previously demonstrated that combination therapy of SSZ and cisplatin synergistically suppresses the growth of xenograft tumor models in comparison with either treatment alone [5, 9]. Recently, we also showed that administration of SSZ at a dose of 8 g/day in patients with advanced gastric cancer (AGC) induced reduction of CD44v-positive cells and GSH abundance in tumor cells, consistent with the mode of action of this drug in CSCs [12]. However, a reduction in CD44v-positive cells did not translate into an apparent reduction in tumor size. Because SSZ selectively inhibits the antioxidant system that is specifically activated in chemo-resistant CD44v-positive cells without affecting the chemo-sensitive CD44-negative cells, combination therapy with SSZ and ROS-generating cytotoxic anticancer agents is required for tumor shrinkage. Our preclinical study showed that the combination of cisplatin with SSZ induced a greater level of tumor cell death and tumor growth suppression compared with SSZ alone [5]. Here, we report a dose-escalation study to evaluate the feasibility of SSZ in combination with cisplatin in patients with CD44v-positive AGC that was refractory to cisplatin-based chemotherapy.

## Materials and methods

### Study design

The primary objective of this single-arm, dose-escalation study was to determine a recommended dose of SSZ in combination with cisplatin for patients with CD44v-positive AGC refractory to cisplatin. Secondary objectives included adverse events, objective response rate, progression-free survival (PFS), and the change of CD44v expression before and after treatment. The study protocol was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (protocol ID UMIN000015595).

### Patient eligibility

Main inclusion criteria included (1) the presence of histopathologically proven unresectable or recurrent gastric adenocarcinoma, (2) the presence of an archival tumor specimen with CD44v-positive cells, (3) the presence of a biopsiable tumor before and after one cycle of treatment, (4) disease progression during or within 3 months after previous treatment with cisplatin for AGC, and (5) adequate general status or organ function, as in our previous study [12]. Patients with a history of severe adverse events with cisplatin were excluded. All patients provided written informed consent to participate in the study, which was approved by the institutional ethics committee of National Cancer Center Hospital East.

### Drug administration and dose-escalation procedure

Eligible patients received SSZ (Salazopyrin, 500 mg; Pfizer, Karlsruhe, Germany) by oral administration four times daily of 1.5 or 2 g per dose (i.e., level 1, 6 g/day; level 2; 8 g/day) with 2 weeks on and 1 week off schedule. Cisplatin at 60 mg/m$^2$ was administrated every 3 weeks with adequate intravenous hydration and antiemetic premedication using palonosetron, dexamethasone, and an oral aprepitant. The dose of cisplatin was same as that of the S-1 plus cisplatin combination, which is one of the Japanese standard regimens [13]. Treatment was continued for a maximum of six cycles or until disease progression or the development of intolerable toxicity. Dose-limiting toxicity (DLT) was evaluated from the initial dose to the end of cycle 1, the same definition as in our previous study [12]. The dose level was escalated according to a typical 3 + 3 design. The data center of the Exploratory Oncology Research and Clinical Trial Center at the National Cancer Center (NCC-EPOC) confirmed patient eligibility, and the dose level was then assigned. Data collection, data analysis, and data interpretation were also performed by the NCC-EPOC (study number, EPOC 1407).

### Assessment

Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.0). Tumor response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST, v 1.1). Tumor measurements were obtained by computed tomography at baseline and every 6 weeks thereafter. Relative dose intensity was defined as the ratio of delivered dose intensity to the planned dose intensity.

### Evaluation of CD44v expression in tumor specimens and intratumoral GSH level

The expression status of CD44v in tumor tissue was evaluated by immunohistochemistry (IHC), as described previously [5, 9, 12]. CD44v positive was defined to be an average percentage of CD44v-positive cancer cells more than 10% per field, which was centrally evaluated by investigators (O.N., Y.M., H.S.) and confirmed by a pathologist (T.K.). The intratumoral abundance of GSH was determined with the use of a boron-doped diamond microelectrode as described previously [12, 15].

## Results

### Patient characteristics

Among the 15 patients who underwent prescreening for CD44v expression, 8 were positive. Thus, 7 patients were enrolled for this study between January and April 2015 (Table 1). All patients had experienced disease progression during previous cisplatin-containing chemotherapy.

Because one of the three patients in level 1 experienced DLT, we added three patients. One of them was excluded from DLT evaluation because of early treatment discontinuation as a result of progressive disease (patient 6) and replaced with one additional patient (total, seven patients in level 1).

### Tolerability and adverse events

One patient completed six cycles without disease progression (patient 3). Four patients discontinued treatment because of disease progression. One patient was discontinued because of clinically judged disease progression with enlarged primary tumor (patient 6). Another patient stopped because of tumor bleeding (patient 1). Patient 1 experienced anorexia of grade 3 as a DLT. Among the five patients who proceed to cycle 2, SSZ was interrupted in two patients (patients 2 and 5) and reduced in two other patients (patients 3 and 7) because of sustained grade 2 gastrointestinal toxicities (Table 1). We terminated further dose escalation as the result of frequent interruption or reduction. Hematological toxicity was rare, most of these events being of grade $\leq 1$. Cisplatin was reduced in one patient (patient 3) from cycle 4.

### Tumor response

No patients achieved objective response. One patient achieved stable disease for more than 4 months with survival duration of 9.9 months (patient 3; Fig. 1). Three other patients showed stable disease of limited duration (Table 1).

### Changes in intratumoral CD44v expression and GSH level

Among the other five patients, two showed significant reduction (>10%) proportion of CD44v-positive cancer cells (patients 1 and 5). The intratumoral level of GSH declined in patient 3, although no other patient showed clear reduction.

## Discussion

In this study, the 6-g dose of SSZ in combination with cisplatin is considered to be feasible based on predefined DLT criteria. Dose modification after cycle 1 was frequently required because of gastrointestinal toxicity, which are major side effects as in previous reports of SSZ or cisplatin [12, 13]. Although no obvious tumor shrinkage was observed, one patient with HER2-positive AGC competed six cycles of treatment with stable disease for more than 4 months. This patient also showed reduction of GSH after treatment. However, reduction of the proportion of CD44v-expressing cancer cells was not observed, and this warrants some explanation. Given that cancer cells often adapt oxidative stress through the upregulation of antioxidant ability or the suppression of endogenous ROS production [3], it can be speculated that, in this study, CD44v+ tumor cells of which the xCT activity is inhibited by SSZ might have adapted oxidative stress through the suppression of cellular metabolic activities concomitant with ROS production to evade cell death. Thus, the remaining CD44v+ tumor cells after SSZ treatment, while retaining CD44 expression, might have been deprived of some biological activities including GSH synthesis and mitochondrial metabolism. There is another possibility that the levels of GSH may not be valid as a biomarker in cancer tissues with a low proportion of CD44v+ cells in which GSH may be the product of other cell types. For some reason, positivity of CD44v in the current study at the average of 23% was lower than that observed in our previous study, and this might have been the cause of discordance in that the levels of GSH did not correlate with the tumor response to SSZ. The reasons for the limited efficacy in this study or insufficient reduction of CD44v-positive AGC cells should be discussed. First, the dose of SSZ when combined with cisplatin did not eventually reach the level tested in the previous study, in which SSZ was used as a single agent, as a result of the early termination of the dose-finding process in this study. Second, the attempt to treat cisplatin-resistant tumors with another cisplatin-containing regimen might have been too challenging. Our assumption that the SSZ might reverse the resistance against cisplatin acquired by the previous treatment could have been too optimistic, given the complex mechanism of cisplatin resistance [15]. Third, the patients registered for this study had a relatively low proportion of CD44v+ cells, of which the highest proportion was 41%. This point might explain the poor response to the SSZ treatment as, in our previous study, efficacy in terms of reduction of CD44v+ cells was observed among cases with a CD44v+ population greater than 76%. The fact that most of the orally administered SSZ is metabolized in the intestines before absorption and loses the xCT inhibitory effect has become of substantial concern after this study. We are currently in the process of developing a water-soluble xCT inhibitor that can be administered intravenously.

One limitation of the current study is the small number of cases evaluated following the decision to terminate the

**Table 1** Patient characteristics and treatment outcomes

| Patient | Age (years) | Gender | PS | HER2 | Prior lines of chemotherapy | Duration of previous cisplatin treatment (months) | Treatment cycle | DLT | Major adverse events (≥G2) | RDI of SSZ (%) | ORR | PFS (months) | CD44v-positive cancer cells (%) | | GSH level (mM) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Before | After | Before | After |
| 1 | 64 | F | 0 | – | 3 | 5.0 | 1 | Yes | G3 anorexia, bleeding<br>G2 fatigue, rash, nausea, malaise | 38 | SD | 1.6 | 22.1 | 2.0 | 8.7 | 18.8 |
| 2 | 56 | F | 1 | – | 3 | 5.6 | 3 | No | G3 anemia<br>G2 anorexia | 78 | SD | 2.1 | 25.8 | 30.8 | 14.3 | 14.6 |
| 3 | 71 | M | 1 | + | 4 | 6.6 | 6 | No | G2 nausea<br>G2 hyperbilirubinemia | 82 | SD | >4.0 | 5.2 | 23.4 | 20.2 | 5.3 |
| 4 | 77 | M | 0 | – | 3 | 1.8 | 2 | No | None | 99 | PD | 1.2 | 23.7 | 16.5 | 19.0 | 16.6 |
| 5 | 70 | F | 0 | + | 2 | 4.1 | 2 | No | G2 rash<br>G2 anemia | 86 | PD | 1.0 | 41.1 | 20.1 | 1.7 | 12.6 |
| 6 | 54 | M | 0 | + | 3 | 7.7 | 1 | NE* | G2 anorexia<br>G2 ALT elevation | 21 | NE[a] | 0.5* | 31.4 | 47.8 | 0.6 | 0.1 |
| 7 | 65 | M | 0 | + | 3 | 6.5 | 4 | No | G2 nausea | 76 | SD | 2.6 | 12.3 | 32.5 | 8.2 | 8.7 |

*F* female, *M*, male, *PS* performance status, *DLT* dose-limiting toxicity, *G* grade, *ALT* alanine aminotransferase, *RDI* relative dose intensity, *SSZ* sulfasalazine, *PD* progressive disease, *SD* stable disease, *NE* not evaluable, *ORR* objective response

[a] Clinically judged progressive disease

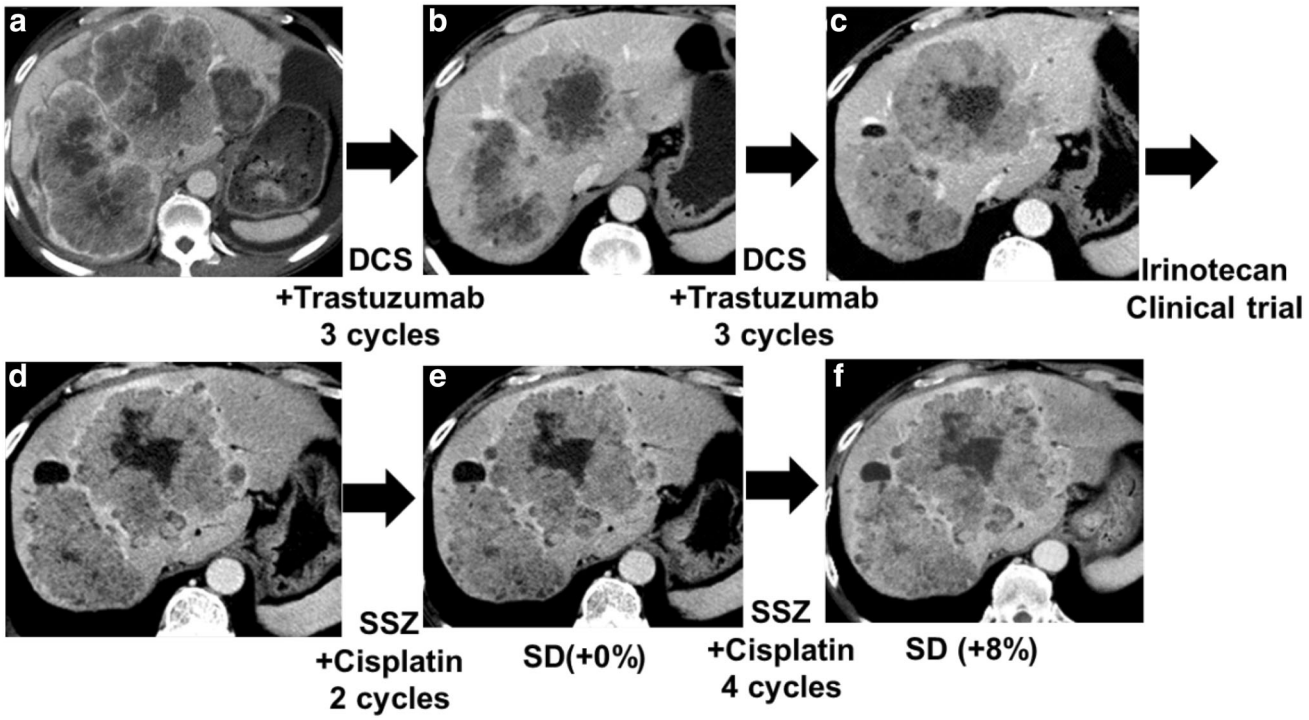


**Fig. 1** Patient with durable stable disease by sulfasalazine and cisplatin. Patient 3 was a 71-year-old man with HER2-positive gastric carcinoma with multiple liver metastasis (**a**). Combination of S-1, cisplatin, docetaxel (DCS), and trastuzumab achieved remarkable response after three cycles (**b**), but disease finally progressed after six cycles (**c**). After subsequent treatments with irinotecan, an investigational agent, and combination with S-1 plus oxaliplatin, he was enrolled into this clinical trial (**d**). Liver metastases remained as stable disease (SD) after six cycles of sulfasalazine (SSZ) and cisplatin (**e**, **f**)

dose-escalation process, which renders any statistical calculations impossible. Another weakness is that the pharmacokinetic interactions between SSZ and other drugs involved (e.g., cisplatin, palonosetron, dexamethasone, aprepitant) was not examined. These factors will be explored in an ongoing study of SSZ with cisplatin and pemetrexed for chemo-naive patients with non-small cell lung cancer.

In summary, the combination of SSZ plus cisplatin is manageable and could be explored in other settings, but did not show sufficient efficacy to proceed to the next step for the treatment of cisplatin-resistant AGC. Treatment of CD44v+ cells remains a compelling target of research, but a novel xCT inhibitor that can be administered intravenously may be needed.

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**Compliance with ethical standards**

**Conflicts of interest** No financial and personal relationships with other people or organizations are involved.

**Human rights statement** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

**Informed consent** Informed consent or a substitute for it was obtained from all patients for being included in the study.

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