Overcoming Platinum Resistance Through the Use of a Copper-Lowering Agent

Siqing Fu, Aung Naing, Caroline Fu*, Macus Tien Kuo§, and Razelle Kurzrock

Departments of Investigational Cancer Therapeutics (Phase I Clinical Trials Program), and §Department of Molecular Pathology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030; and ´College of Arts and Sciences, New York University, New York, NY 10012

Corresponding authors: Siqing Fu, MD, PhD, Department of Investigational Cancer Therapeutics, Unit 0455, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, E-mail: siqingfu@mdanderson.org; Telephone: 713-792-4318; and Fax: 713-745-3855, and Macus Tien Kuo, PhD, Department of Molecular Pathology, Unit 951, the University of Texas MD Anderson Cancer Center, 7435 Fannin Boulevard, Houston, Texas 77054, E-mail: tkuo@mdanderson.org; Telephone 713-834-6038; and Fax: 713-834-6025.

No financial support is listed.

COI Disclosure: All authors have no potential conflict of interest to disclose except for Razelle Kurzrock who discloses commercial research grant from Merck (Major) and honoraria from speakers bureau from Merck (Minor).
RUNNING TITLE:
Overcoming Platinum Resistance Using a Copper-Lowering Agent

KEY WORDS:
platinum resistance, carboplatin, trientine, copper-lowering agent, resensitization, ovarian cancer, copper, ceruloplasmin, hCtr1, human copper transporter 1, clinical trial

ABBREVIATIONS:
hCtr1: human copper transporter 1
AUC: area under the concentration curve
RECIST: response evaluation criteria in solid tumors
CTCAE: common terminology criteria for adverse events
IV: intravenous
ABSTRACT

Low levels of human copper transporter 1 (hCtr1) mRNA are associated with a shorter progression-free survival after platinum-based therapy. Pretreatment with a copper-lowering agent such as trientine enhanced hCtr1-mediated platinum uptake. Therefore, we conducted a pilot study (NCT01178112) of carboplatin and trientine with the goal of resensitizing advanced cancer patients to platinum chemotherapy. This case report reviews the outcomes of five patients with platinum-resistant high-grade epithelial ovarian cancer enrolled on the study to date. Overall, they tolerated treatment well. Severe adverse events that occurred in two patients were myelosuppression, notably anemia requiring transfusion. Dose-limiting toxicity was not observed within the first 28 days (cycle 1). After two cycles of therapy, partial remission was achieved in one patient (10+ months), stable disease in three patients (2, 3.5+ and 5 months, respectively), and one patient had progressive disease. These cases provide preliminary clinical evidence that the role of decreasing copper levels in reversing platinum resistance merits additional clinical investigation. Evaluation of this novel strategy is warranted in larger studies to assess the efficacy of this approach for treating platinum-resistant advanced epithelial ovarian cancer in patients with high copper levels.
INTRODUCTION

The standard first-line treatment for advanced epithelial ovarian cancer is cytoreductive surgery followed by adjuvant therapy with a platinum-based regimen. Despite a salutary initial response, many patients relapse and eventually succumb to their disease following development of drug resistance. Among the mechanisms that mediate platinum resistance (1, 2), elevated copper level-induced downregulation of the major copper influx transporter hCtr1 (human copper transporter 1) plays a major role (3). Recent discoveries revealed that hCtr1 regulates intracellular copper homeostasis, which in turn, controls hCtr1 expression via a homeostatic feedback loop (4). Copper-lowering agents increased the expression of hCtr1, subsequently resensitizing tumor cells to platinum therapy (5). Here, we report preliminary evidence that a copper-lowering agent may be able to, at least partially, reverse platinum resistance in patients with platinum-resistant high-grade epithelial ovarian cancer.
METHODS

To test the hypothesis that resistance to platinum therapy can be reversed through the use of a copper-lowering agent, we are conducting a pilot study (NCT01178112) at MD Anderson Cancer Center (6) in which carboplatin is combined with trientine (triethylenetetramine: N,N'-bis(2-aminoethyl)ethane-1,2-diamine; Figure 1), a copper-lowering agent (5), to treat patients with advanced malignancies. Five patients enrolled to date with a histologically proven diagnosis of platinum-resistant high-grade epithelial ovarian cancer are reviewed (Table 1). Platinum resistance was defined as radiographic disease progression within 6 months of completion of a platinum-based regimen. After giving informed consent, patients received the study treatment (intravenous [IV] carboplatin AUC 4 [dose level 1] or AUC 6 [dose level 3] once every four weeks, plus oral trientine 500 mg four times a day [two times with meals, and two times without meals] initially, with dose adjustment to maintain serum ceruloplasmin levels at 5 to 15 mg/dL). Tumor responses were evaluated using RECIST 1.1 (Response Evaluation Criteria in Solid Tumors version 1.1) (7), and toxicity was assessed using CTCAE version 4.0 (8). Serum ceruloplasmin and copper levels were monitored periodically (weekly initially, and then less frequently as appropriate). This study was conducted in accordance with MD Anderson Institutional Review Board guidelines.
CASE REPORTS

Patient 1: A 69-year-old white woman status post cytoreductive surgery for stage IIIC high-grade serous ovarian cancer in November 2004, received five lines of systemic therapy: paclitaxel plus carboplatin (becoming platinum resistant in 5 months), letrozole, topotecan, liposomal doxorubicin, and bevacizumab plus temsirolimus. In July 2010, she was enrolled at dose level 1. After two cycles of therapy, the patient was removed from the study for grade 3 hyperbilirubilemia caused by tumor-related intrahepatic biliary duct obstruction. The patient’s tumor bulk increased by 14% and the tumor marker CA-125 increased by 42%, while her serum ceruloplasmin and copper levels decreased slightly, as shown in Table 1 and Figure 2.

Patient 2: A 55-year-old white woman with a six-year history of high-grade serous ovarian cancer initially underwent cytoreductive surgery and subsequently received 11 lines of systemic therapy: paclitaxel plus carboplatin, topotecan, liposomal doxorubicin, gemcitabine, etoposide, paclitaxel plus carboplatin (demonstrating platinum resistance in 6 weeks), docetaxel, bevacizumab plus cyclophosphamide, paclitaxel, letrozole, and docetaxel plus sirolimus. In January 2011, the patient enrolled at dose level 3. She experienced grade 3 thrombocytopenia and anemia, and grade 2 fatigue, which improved significantly after carboplatin was decreased to AUC 5 two cycles later. Her overall tumor was reduced by 35%, 59%, and 70% after 2, 4 and 6 cycles, respectively, as shown in Figure 3. The patient’s CA-125 level decreased by 98% (Figure 4A). After 10+ months, the patient continues on the study uneventfully. Her ceruloplasmin copper levels decreased significantly (Figures 4B and 4C).
**Patient 3:** A 60-year-old African American woman status post cytoreductive surgery for stage IIIC high-grade mixed epithelial ovarian cancer in February 2005, received 11 lines of systemic therapy: carboplatin and interferon plus filgrastim, carboplatin and gemcitabine (becoming platinum resistant in 6 weeks), letrozole, liposomal doxorubicin, bevacizumab and cyclophosphamide, topotecan and bevacizumab, topotecan, paclitaxel, docetaxel, tamoxifen, a c-MET inhibitor, and palliative pelvic radiation for vaginal bleeding. In May 2011, the patient was enrolled at dose level 3. She experienced grade 4 newly diagnosed acquired sideroblastic anemia, which required transfusion. The patient achieved a 10% tumor reduction lasting 5 months, associated with reduced serum ceruloplasmin and copper levels (Table 1 and Figure 2). The tumor marker CA-125 decreased by 64%. The patient was removed from the study for vaginal bleeding.

**Patient 4:** A 57-year-old white woman underwent cytoreductive surgery for stage IIIC high-grade endometrioid ovarian cancer four years ago. She subsequently received five lines of systemic therapy: paclitaxel and carboplatin plus bevacizumab, liposomal doxorubicin plus carboplatin (becoming platinum resistant in 4 weeks), liposomal doxorubicin, docetaxel plus vandetanib, and an AKT inhibitor plus a MEK inhibitor before she was enrolled at dose level 3 in June 2011. No grade 2 or higher treatment-related adverse events occurred. After two months, she was removed from the study for tumor progression by 120%. Her CA-125 increased by 409% and her ceruloplasmin and copper levels decreased slightly.
Patient 5: A 49-year-old Asian woman with a known BRCA-1 mutation underwent cytoreductive surgery for stage IIIIC high-grade serous ovarian cancer in September 2006, and subsequently received five lines of systemic therapy: paclitaxel plus carboplatin (becoming platinum resistant in 6 months), topotecan, liposomal doxorubicin, bevacizumab plus gemcitabine, and bevacizumab plus cyclophosphamide. In July 2010, she was enrolled at dose level 3. The patient had stable disease for 3.5+ months with moderately reduced serum ceruloplasmin and copper levels. Her tumor marker CA-125 decreased by 45%.
DISCUSSION

Decreased platinum uptake due to excessive copper levels may serve as a critical step in the development of platinum resistance (4, 9, 10). Elevated copper levels in cancer cells and higher serum ceruloplasmin and copper levels have been observed in patients with advanced epithelial ovarian cancer (11). Low levels of hCtr1 mRNA have been associated with a shorter progression-free survival after platinum-based therapy (12).

Prolonged use of a copper-lowering agent to keep serum ceruloplasmin levels between 5 and 15 mg/dL did not cause clinical toxicity since critical copper-dependent cellular processes were not affected (4). Treatment with copper-lowering agents alone in advanced cancer patients produced no tumor responses (13-17). One study with tetrathiomolybdate, another copper-lowering agent, plus irinotecan, fluorouracil and leucovorin demonstrated a 25% response rate in metastatic colorectal cancer patients (18). However, no clinical trial has yet been reported using a copper-lowering agent plus a platinum agent in advanced cancer patients.

Salvage therapy in platinum-resistant patients generally produces low response rates (less than 10%) and durable responses are rare (19, 20). Clearly, a novel strategy is needed to improve treatment of platinum-resistant ovarian cancer. Here, we report five patients with platinum-resistant high-grade epithelial ovarian cancer who were treated with carboplatin plus trientine. Our preliminary clinical findings support the hypothesis that decreased copper levels in patients with platinum-resistant epithelial ovarian cancer may resensitize cancer cells to carboplatin therapy. The patients whose copper levels after one and two cycles of therapy with trientine were decreased the most had the
greatest reduction in tumor bulk, as seen in patients 1, 2 and 3. In contrast, the fourth patient did not respond to carboplatin and the concentration of copper in her body, as reflected by levels of surrogate biomarkers (serum ceruloplasmin and copper levels), did not change significantly after treatment with trientine. It is of great interest that patient 5, who had a BRCA-1 mutation, achieved a minor tumor response, suggesting that even mildly increased platinum uptake leads to increased antitumor activity since BRCA-1 mutated cancer is sensitive to platinum agents (21).

The major limitation is the small number of patients, which abrogates drawing definitive conclusions. Other mechanisms possibly at play include antiangiogenesis. Another limitation is that, rather than being caused by decreased copper levels, the decreases observed in serum ceruloplasmin and copper levels might be a predictive prognostic marker since patients who achieve rapid decreases in these levels after trientine treatment are most likely to respond to chemotherapy. Another possible mechanism accounting for our observations could be that decreased serum ceruloplasmin and copper levels in response to trientine treatment reflect the efficacy of chemotherapy in responding patients due to post-therapy improved acute phase reactions.

To the best of our knowledge, this report provides first-in-human preliminary data showing that at least partial resensitization of cancer cells to platinum therapy may be achieved through the use of a copper-lowering agent. These interesting preliminary clinical findings suggest that a larger study using a copper-lowering agent in combination with a platinum-based regimen is warranted for evaluation in treating platinum-resistant epithelial ovarian cancer patients with high copper levels.
Acknowledgements

The authors thank Thuan Nguyen and Adrienne Howard in the Department of Investigational Cancer Therapeutics at MD Anderson Cancer Center for coordinating this clinical trial and Joann Aaron in the Department of Investigational Cancer Therapeutics at MD Anderson Cancer Center for editing this manuscript.
Grant Support

This is an investigator-initiated study without a grant support.
REFERENCES


Table 1: Tumor responses and changes in serum ceruloplasmin and copper levels

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor response after 2 cycles</th>
<th>Pre-Dose</th>
<th>After 1 cycle</th>
<th>After 2 cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ceruloplasmin (mg/dL)</td>
<td>Copper (μg/mL)</td>
<td>Ceruloplasmin (mg/dL)</td>
</tr>
<tr>
<td>1</td>
<td>+ 14%</td>
<td>39.1</td>
<td>2.01</td>
<td>32.9 (16%)</td>
</tr>
<tr>
<td>2</td>
<td>- 35%</td>
<td>34</td>
<td>1.71</td>
<td>23.4 (31%)</td>
</tr>
<tr>
<td>3</td>
<td>- 10%</td>
<td>26</td>
<td>1.02</td>
<td>20.5 (21%)</td>
</tr>
<tr>
<td>4</td>
<td>+ 120%</td>
<td>42.2</td>
<td>1.96</td>
<td>39.8 (6%)</td>
</tr>
<tr>
<td>5</td>
<td>- 2%</td>
<td>31</td>
<td>1.57</td>
<td>26.8 (14%)</td>
</tr>
</tbody>
</table>

The brackets indicate percent reductions compared to baseline levels.
FIGURE LEGENDS

Figure 1: Structure of trientine [triethylenetetramine: N,N'-bis(2-aminoethyl)ethane-1,2-diamine].

Figure 2: Shown are changes in tumor sizes, CA-125, serum ceruloplasmin and copper levels in five patients with platinum resistant high-grade epithelial ovarian cancer who had received two cycles of therapy with trientine and carboplatin. All patients received carboplatin at AUC 6 except for Patient 1 who received carboplatin at AUC 4. Patient 5 had a BRCA-1 mutation.

Figure 3: Tumor responses in Patient 2 are demonstrated in two panels of computed tomography (CT) scans of chest, abdomen and pelvis. Tumor resolution of mediastinal lymphadenopathy, and reduction of abdominal wall mass, peritoneal implants and right inguinal lymphadenopathy were indicated by red arrows. The left panels represent CT scans prior to study enrollment, and the right panels are of corresponding sites after six cycles of therapy with carboplatin plus trientine, respectively.

Figure 4: Changes in the levels of tumor marker CA-125, serum ceruloplasmin and copper after treatment with carboplatin plus trientine in Patient 2 are demonstrated in panels A, B and C, respectively. Decreases in the CA-125 tumor marker, as shown in panel A, were associated with decreases in serum ceruloplasmin levels, as shown in panel B, and serum copper levels, as shown in panel C.
Figure 1

H2N
\( \text{HN} \)
\( \text{HN} \)
\( \text{NH}_2 \)
Figure 2
Figure 3
Figure 4
Molecular Cancer Therapeutics

Overcoming Platinum Resistance Through the Use of a Copper-Lowering Agent

Siqing Fu, Aung Naing, Caroline Fu, et al.

Mol Cancer Ther Published OnlineFirst April 5, 2012.

Updated version

Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-11-0864

Author Manuscript

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pub@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.