DCF (docetaxel, cisplatin and 5-fluorouracil) chemotherapy is a promising treatment for recurrent advanced squamous cell anal carcinoma

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Background: Squamous cell carcinoma of the anal canal (SCCA) is a rare disease, mostly diagnosed at early stage. After concurrent chemoradiation (CRT) with mitomycin C and 5-fluorouracil (5FU), local or metastatic recurrences occur in >20% of the patients. After treatment failure, cisplatin (CDDP)-based chemotherapy is the standard option, but complete response (CR) is a rare event and the prognosis remains poor.

Patients and methods: Eight consecutive patients with advanced recurrent SCCA after CRT were treated with DCF regimen (docetaxel 75 mg/m²/day 1, CDDP 75 mg/m²/day 1 and 5FU at 750 mg/m²/day for 5 days every 3 weeks). Tumour samples were analysed for human papillomavirus (HPV) genotyping, as well as p16 and p53 expression.

Results: After a median follow-up of 41 months, the overall survival rate at 12 months was 62.5% (95% CI 22.9–86.1 months). Four patients achieved a complete remission and remain relapse-free at the time of analysis with a progression-free survival of 19, 33, 43 and 88 months. Three of these patients underwent surgery for all involved metastatic sites. For all of them, pathological CR was confirmed. DCF regimen appeared feasible in these patients previously exposed to pelvic CRT, and no grade IV toxicity occurred. All patients in complete remission had HPV-16-positive SCCA, while HPV could only be detected among 50% of the non-responding patients. Of interest, immunohistochemical study revealed a p16+/p53− phenotype in these patients, while none of non-responders expressed p16.

Conclusion: The high level of complete and long-lasting remission among SCCA patients treated with DCF regimen supports the assessment of this strategy in prospective cohorts.

Key words: anal cancer, squamous cell carcinoma, taxane, HPV, docetaxel, DCF

introduction

Squamous cell carcinoma of the anal canal (SCCA) is a rare disease, representing only 1%–5% of gastrointestinal malignancies. Its incidence is steadily increasing in men and women: 30 000 new cases are diagnosed worldwide each year [1, 2].

Concurrent chemoradiation (CRT) with mitomycin C (MMC) and 5-fluorouracil (5FU) is the standard of care for localized anal cancers. Nevertheless, >20% of the patients will develop locally advanced recurrences or metastases [2]. The treatment of metastatic disease relies on systemic chemotherapy. Nevertheless, evidence to define the appropriate therapy is lacking. Decision making is based on small retrospective trials including a limited number of patients [3–6]. A combination of cisplatin (CDDP) and 5FU has become a standard regimen for advanced anal carcinoma, as illustrated by the recently revised guidelines of the National Comprehensive Cancer Network, which acknowledged that no other regimen has shown to be effective [2, 6, 7]. However, complete remission is a rare event and SCCA patients who are not candidates for surgery and resistant to radiotherapy are currently considered to be eligible only for a palliative therapy.

The absence of curative therapeutic option available to treat relapsing patients led to the initiation of clinical trials to intensify preoperative CRT and decrease primary treatment failure. However, in the ACCORD03 and RTOG 98-11 studies, CDDP and 5FU chemotherapy before and in combination with radiotherapy did not improve local and distant recurrence rates [8, 9]. Then, overall outcomes for patients with non-resectable or metastatic relapses remain dismal with overall 5-year survival rate below 20% for stage IV disease, and the development of
Table 1. Patients’ characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Year of diagnosis</th>
<th>Stage of primary SCCA</th>
<th>Delay of recurrence (months)</th>
<th>WHO performance status</th>
<th>Recurrence sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>Female</td>
<td>2002</td>
<td>T1N0M0</td>
<td>36</td>
<td>0</td>
<td>Local relapse</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>Male</td>
<td>2006</td>
<td>T2N2M0</td>
<td>3</td>
<td>1</td>
<td>Regional relapse (N3)</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>Female</td>
<td>2008</td>
<td>T3N3M0</td>
<td>8</td>
<td>0</td>
<td>Mediastinal and supra-clavicular lymph nodes</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>Female</td>
<td>2009</td>
<td>T2N2M0</td>
<td>3</td>
<td>2</td>
<td>Local relapse</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>Male</td>
<td>2008</td>
<td>T2N0M0</td>
<td>8</td>
<td>0</td>
<td>Regional (N3) and lung metastases</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>Female</td>
<td>2009</td>
<td>T3N3M0</td>
<td>12</td>
<td>0</td>
<td>Liver metastases and lymph nodes</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Male</td>
<td>2010</td>
<td>T1N0M0</td>
<td>6</td>
<td>0</td>
<td>Local relapse</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>Female</td>
<td>2010</td>
<td>T3N1M0</td>
<td>6</td>
<td>0</td>
<td>Liver metastases, local relapse, pelvic and lomboaortic lymph nodes</td>
</tr>
</tbody>
</table>

Patients 1, 3, 6 and 8 (grey lines) are in complete remission following DCF therapy.

effective salvage therapies is still a relevant issue for these patients.

The docetaxel antitumor agent stabilizes tubulin polymerization leading to mitosis arrest and cell death. It has been previously proposed that a loss of normal p53 function confers sensitization to taxane chemotherapy by increasing G2/M arrest and apoptosis [10]. Since the association between anal carcinoma and human papilloma virus (HPV) infection is especially strong [1] and E6 oncoprotein encoded by HPV type 16 and 18 induces the degradation of p53 [11], we hypothesized that SCCA might be sensitive to taxane-containing chemotherapies.

The addition of docetaxel to CDDP plus 5FU (DCF) was already shown to improve overall survival without any impact on toxic death in advanced squamous cell carcinoma of the head and neck [12]. Then, considering the lack of efficacy of CDDP–5FU in relapsing patients previously exposed to 5FU, we decided to treat metastatic SCCA patients using DCF chemotherapy.

Here, we report a retrospective analysis of the clinical outcomes achieved in eight consecutive metastatic SCCA patients for whom our regional multidisciplinary committee indicated the initiation of DCF chemotherapy. This regimen triggered an unexpected high level of long-term complete remission, and we decided to set up a translational research study. Interestingly, a p16+ and p53− phenotype was observed in all patients in remission.

patients and methods

patients and previous treatments

Between 2005 and 2012, all metastatic SCCA patients referred to our regional cancer institute, aged <75 years and with a creatinine clearance of >60 ml/min, were treated using DCF regimen. Here, we report the eight consecutive patients considered eligible to this therapy by the regional multidisciplinary committee and treated in one university and one general hospital. All patients included were previously exposed to MMC-5FU CRT (45–59 Gy) for the treatment of their primary disease. Six patients were initially treated with up-front CRT, while patients 4 and 5 received MMC-5FU CRT following incomplete surgical resection of the primary SCCA.

SCCA relapses were all histologically confirmed, and all patients had negative HIV blood tests. Patients 1, 4, 7 and 8 had a relapsing disease occurring in irradiated field. Patient’s characteristics are described in Table 1.

treatment

DCF consisted of docetaxel (75 mg/m² day 1), CDDP (75 mg/m² day 1) and 5FU (750 mg/m² by 24-h continuous infusion for 5 days) delivered every 21 days for a maximum of six cycles. CT scans were planned after three and six cycles or if a symptomatic progression was suspected. G-CSF prophylaxis was prescribed for all patients. Three cycles of chemotherapy were scheduled before CT scan assessment.

Tumour assessment was carried out according to RECIST v1.0 criteria using CT scan every 3 months until disease progression or death.

tumour samples and immunohistochemistry

Epitope-HPV01 study was conducted in accordance with French laws and after approval by the local ethic committee. HPV genotyping assays were carried out using the INNO-LIPA HPV Genotyping Extra Test (Innogenetics, Gent, Belgium) and immunohistochemistry was carried out on a Ventana automate (Benchmark XT, Roche-Ventana, Tucson) with the specific antibodies anti-p16 (clone E6H4, previously diluted, CINtec, Heidelberg) and anti-p53 (clone DO-7, Dako, working dilution 1:200), as previously described [13].

results

treatment administration and side-effects

DCF chemotherapy was stopped for clinical or radiological progression in four patients (patients 2, 4, 5 and 7). Patient 3 experienced grade 3 neuropathy leading to DCF discontinuation after the third cycle. Patients 1, 6 and 8 discontinued treatment in order to undergo metastasis surgical resection.

Of note, no grade IV toxicity was observed. Four patients presented different grade III toxicities: anaemia, asthaenia, neutropenia, stomatitis and neuropathy. Main haematological toxicities were anaemia (63%, including a grade 3 for patient 1 and grade 2 anaemia for patient 6) and neutropenia (50%, including a grade 3 for patient 6 and a grade 2 for patients 1 and 8). Main non-haematological toxicities were stomatitis (38%, including a grade 3 for patient 8 and a grade 2 for patient 1) and asthaenia (75%).

efficacy

A long-term remission was achieved in four out of the eight patients treated by DCF chemotherapy.
Patients 1, 6 and 8 achieved a radiological partial response after three DCF administrations (Figure 1), and salvage abdominoperineal resection of all involved metastasis sites was then decided after four cycles of DCF for patient 1 and following five cycles for patients 6 and 8. A pathological complete response (CR) was observed in all patients (Figure 2). These patients were then followed up without any complementary treatment.

Patient 3 was treated for supra-clavicular and mediastinal lymph node metastases by three cycles of DCF. A CR was observed on the CT scan carried out following the third DCF treatment, and a complementary radiotherapy was administered. Indeed, the progression-free survival is 88, 43, 33 and 19 months for patients 1, 3, 6 and 8, respectively. All these patients are alive without any relapse at the time of analysis (Table 2).

In contrast, DCF failed to provide any clinical benefit in four patients. A symptomatic progression was observed in patients 2 and 4 after the first cycle of DCF.

In patient 2, DCF was prescribed as induction chemotherapy to treat an inguinal bulky relapse diagnosed 3 months after the initial CRT. An extensive surgical resection supported by skin graft was then carried out. This patient is still in complete remission 78 months after the first DCF cycle. Patient 4 died because of disease progression 1 month after DCF discontinuation.

Patient 5 was treated for a locoregional relapse occurring in the irradiated area and for lung metastases 8 months after treatment of the primary SCCA. A disease progression was
identified by CT scan after three cycles of DCF. None of the palliative chemotherapies prescribed thereafter influenced the clinical outcomes. Patient 7 was treated by DCF for a locally advanced and unresectable relapse diagnosed 6 months after the end of the initial chemoradiation. A disease progression was observed after three cycles of DCF.

HPV genotyping and immunostaining for p16 and p53

Then, the tumour samples collected before DCF initiation were analysed for HPV, p16 and p53 expression. All tumours of complete responders harboured HPV type 16 genotype, while HPV genome was detected in only two out of the four tumours of non-responding patients (supplementary Table S1, available at Annals of Oncology online). A strong and diffuse immunoreactivity for p16 was observed in tumour samples of all patients in CR, while p16 expression was not detected in tumours of all non-responders. While absent in all patients in long-term remission, p53 was expressed in two out of the four patients who progressed rapidly after DCF initiation.

discussion

There are currently no curative medical treatments for metastatic SCCA. Therapeutic options are limited, and the recommended CDDP and 5FU chemotherapy generates a low rate of CRs in relapsing SCCA. Moreover, CDDP–5FU combination failed to improve the clinical outcomes of non-previously pre-treated SCCA.
patients in two randomized clinical trials [8, 9]. Then, the identification of alternative chemotherapies is a relevant issue in this disease. The results presented here suggest that DCF might provide a high level of long-term remission in metastatic SCCA.

The low number of patients is a main limitation of this study. However, recurrent or metastatic SCCA is acknowledged to be infrequent, and the low number of patients possible to include precludes the implementation of clinical trials. Nevertheless, several results reported here sustain the potential clinical benefit of DCF chemotherapy.

First, the clinical efficacy of DCF was confirmed by the pathological assessment of three patients (patients 1, 6 and 8) who underwent the surgical removal of all their metastases. In these patients, a complete histological response was observed in all metastases removed.

Another observation supporting the clinical interest of DCF is the long-term remission rate of 50% achieved with this regimen. Indeed, patients 1, 3, 6 and 8 are alive without disease with a follow-up of 88, 43, 33 and 19 months, respectively.

Table 2. Treatment and clinical outcomes

<table>
<thead>
<tr>
<th>Case</th>
<th>Number of cycles</th>
<th>Complementary treatment</th>
<th>Response</th>
<th>PFS(a) (months)</th>
<th>OS(a) (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Surgery</td>
<td>pCR</td>
<td>88(b)</td>
<td>88(b)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td></td>
<td>PD</td>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>CRT</td>
<td>cCR</td>
<td>43(b)</td>
<td>43(b)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>–</td>
<td>PD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>–</td>
<td>PD</td>
<td>2.5</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>Surgery</td>
<td>pCR</td>
<td>33(b)</td>
<td>33(b)</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>–</td>
<td>PD</td>
<td>2.5</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>Surgery</td>
<td>pCR</td>
<td>19(b)</td>
<td>19(b)</td>
</tr>
</tbody>
</table>

\(a\)From the first DCF cycle.  
\(b\)Patient still progression-free at the time of analysis. Rows in grey indicate the patients in complete remission after DCF chemotherapy.

CRT, chemoradiotherapy; pCR, pathological complete response; cCR, clinical and radiological complete response; PD, progression disease.

*These patients are still alive without disease.

Table 3. Phase II trials and retrospective analyses of chemotherapy in advanced SCCA patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment protocol</th>
<th>Treatment line</th>
<th>Number of patients</th>
<th>Overall response rate</th>
<th>Complete response rate</th>
<th>Number of patients responding to chemotherapy and in remission at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faivre et al. [6]</td>
<td>5FU + CDDP</td>
<td>First and second lines</td>
<td>18</td>
<td>66%</td>
<td>6%</td>
<td>Three patients (follow-up of 4, 5 and 7 years)</td>
</tr>
<tr>
<td>Jhawer et al. [4]</td>
<td>MMC, Adriamycin, CDDP (MAP) + bleomycine-CCNU</td>
<td>Not reported</td>
<td>20</td>
<td>60%</td>
<td>0%</td>
<td>Not reported</td>
</tr>
<tr>
<td>Wilking et al. [3]</td>
<td>Bleomycin, vincristine, high-dose methotrexate</td>
<td>First line (n = 14)</td>
<td>15</td>
<td>25%</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Lukan et al. [16]</td>
<td>Cetuximab + irinotecan</td>
<td>First line (n = 4)</td>
<td>7</td>
<td>57%</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second line (n = 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Third line (n = 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hainsworth et al. [5]</td>
<td>Paclitaxel + Carboplatin</td>
<td>First and second lines</td>
<td>7 (60)(a)</td>
<td>57%</td>
<td>29%</td>
<td>Two patients (21%) (follow-up of 33 and 62 months)</td>
</tr>
<tr>
<td>Abbas et al. [14]</td>
<td>Paclitaxel</td>
<td>Second line (n = 2)</td>
<td>7</td>
<td>60%</td>
<td>0%</td>
<td>Not reported</td>
</tr>
<tr>
<td>Alcindor [15]</td>
<td>Paclitaxel</td>
<td>First line (n = 2)</td>
<td>5</td>
<td>60%</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Golub et al. [17]</td>
<td>Paclitaxel, ifosfamide, CDDP</td>
<td>First line (n = 3)</td>
<td>3</td>
<td>25%</td>
<td>0%</td>
<td>One patient (33%) (follow-up of 28 months)</td>
</tr>
<tr>
<td>Kim et al. (2013)</td>
<td>Taxotere + CDDP + 5FU</td>
<td>First line</td>
<td>8</td>
<td>50%</td>
<td>50%</td>
<td>Four patients (50%)(b) (follow-up of 19, 33, 43 and 88 months)</td>
</tr>
</tbody>
</table>

\(a\)Seven of 60 patients included in the study had SCCA.  
\(b\)Patients still alive and free of disease.
Furthermore, our results indicate that DCF might be feasible in patients previously exposed to pelvic chemoradiation. Of note, no grade IV toxicity occurred and DCF did not hamper the possibility of salvage surgery in patients 1, 6 and 8.

Finally, our results are in line with the clinical efficacy reported with paclitaxel, a potent microtubule-stabilizing agent. Paclitaxel, was also shown to be active in CDDP–5FU refractory SCCA patients [14]. In this study, seven patients were treated with weekly paclitaxel after failure of CDDP and 5FU. Three partial and one CRs were observed. In another study, paclitaxel was used in five metastatic patients and induced three partial responses [15]. The potential interest of paclitaxel combined with 5FU and carboplatin was also assessed in advanced squamous cell carcinoma from several origins [5]. Seven SCCA patients were included in this phase II clinical trial. Two partial and two CRs were reported with long remissions. Altogether, including the patients reported in our study, 30 advanced or metastatic SCCA patients were treated with a taxane-based chemotherapy. Seven of these patients (23.3%) treated in different institutions achieved a CR and a long-lasting complete remission (Table 3).

The development of specific biomarkers will be critical to better select patients eligible to taxane-based chemotherapy. p16 is a cyclin-dependent kinase inhibitor known to disrupt the cyclinD1/cyclin-dependent kinase 4/6 complex and frequently observed following immortalization of epithelial cells by HPV. In the present study, all patients in complete remission had HPV16+ tumours with a high level of p16 and no expression of p53. In contrast, DCF was not effective in all the tumours with a p16+ phenotype. Indeed, the prognosis value of p16 expression was thoroughly assessed in head and neck cancers where it was associated with an improved prognosis and a better locoregional control [18–20]. Then, the potential interest of p16 and p53 immunohistochemistry to select patients eligible to DCF therapy should be investigated prospectively. Therefore, these results show that taxane-containing chemotherapy might be effective in advanced SCCA leading to unexpected complete and long-lasting remissions. Further investigations are warranted to confirm the benefit of DCF in SCCA patients.

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disclosure

The authors have declared no conflicts of interest.

references