Therapy, outcome and analysis of c-kit expression in patients with extrapulmonary small cell carcinoma

Uludag University Medical School, Bursa, Ege University Medical School, Department of Medical Oncology, Izmir, Uludag University Medical School, Bursa, Ege University Medical School, Department of Pathology, Izmir, Uludag University Medical School, Department of Biostatistics, Bursa, Turkey

SUMMARY

In this study, we aimed to investigate the clinicopathological characteristics with special emphasis on c-kit expression and the treatment results of patients with extrapulmonary small cell carcinoma (EPSCC). The medical records of the patients with EPSCC were reviewed, and the data regarding patient and tumour characteristics, treatment and clinical outcome were retrieved and analysed. A total of 28 patients with the diagnosis of EPSCC were identified. There were 19 males and 9 females, with a mean age of 56.5 years. Patients with limited disease (LD) \((n = 13)\) were treated with surgery, chemotherapy (CT) and radiotherapy with different sequences. Patients with extensive disease (ED) \((n = 15)\) were mainly treated with combination CT. The median overall survival was 14.5 months in patients with LD compared to 11 months in those with ED \((p = 0.029)\). Ten patients (36%) showed c-kit overexpression. There was no significant difference between the survival of c-kit-positive and c-kit-negative patients \((p = 0.367)\). In conclusion, our study demonstrates that the prognosis of EPSCC is poor despite currently available treatments. C-kit may be considered as a potential target for novel therapeutical approaches.

Keywords: Extrapulmonary; small cell carcinoma; therapy; c-kit

INTRODUCTION

Extrapulmonary small cell carcinoma (EPSCC) has been increasingly recognised as a clinicopathological entity, distinct from SCC of the lung (SCLC). These tumours have been reported to arise from a wide variety of sites including the prostate, bladder, oesophagus, colon and rectum, stomach, gallbladder, pancreas, breast, kidney, salivary glands, larynx, endometrium, ovary and uterine cervix (1–8).

Although variations in the prognosis of EPSCC from different sites have been observed, the clinical behaviour of these tumours is generally aggressive like their pulmonary counterparts (9). Therapy varies according to the stage and the organ site. Platinum-based chemotherapy (CT) in extensive disease (ED) and multimodality treatment including CT, surgery and radiotherapy (RT) in limited disease (LD) are commonly employed for the treatment of EPSCC (9–12). CT seems to be the cornerstone of treatment (13). Nevertheless, responses are ordinarily short-lived, and more effective agents are needed for the treatment of these tumours (11).

The c-kit proto-oncogene encodes a transmembrane tyrosine kinase receptor that serves as the receptor for KIT ligand and is closely related to the receptors for platelet-derived growth factor, macrophage colony-stimulating factor and FMS-like receptor tyrosine-kinase ligand (14,15). The kinase activity of c-kit has been implicated in the pathophysiology of many tumours such as gastrointestinal stromal tumours (GIST), mast cell leukaemia, seminoma-dysgerminoma, acute myelogenous leukaemia, melanoma, ovarian carcinoma, neuroblastoma and SCLC. In SCLC, activation of this kinase is secondary to ligand binding rather than activating mutations that commonly occur in GIST (14).

Immunohistochemical studies have shown that c-kit overexpression varies between 27.9 and 91% of SCLC specimens (16,17). Also, the prognostic value of c-kit expression in SCLC has been investigated in several studies and contradictory results have been found. While some studies suggested an unfavourable (18) or a favourable (19) prognostic role of c-kit in SCLC, c-kit was not regarded as a significant prognostic factor in the other studies (16,20). However, most of the studies regarding the analysis of c-kit expression in SCLC postulated that c-kit might be used as a potential target for the treatment of this aggressive disease (16,18,21).
Evaluation of c-kit expression in EPSCC has been carried out in fewer studies. Yamasaki et al. (22) reported a case with primary SCC of the breast whose tumour demonstrated approximately 80% c-kit positivity. Akintola-Ogunremi et al. (23) detected c-kit overexpression in 23% of the cases with colorectal neuroendocrine carcinomas. On the other hand, a recent study found that c-kit is overexpressed in only 5% of the cases with SCC of the uterine cervix (24).

In the light of these observations, it seems that there is still a need for more information about EPSCC, especially in terms of pathophysiology and treatment considerations. The aim of the current study was to evaluate the clinicopathological characteristics with special emphasis on c-kit expression and the treatment results of patients with EPSCC.

PATIENTS AND METHODS

Patients

The medical records of patients with EPSCC who were treated in the medical oncology departments of Uludag University Medical School and Ege University Medical School were reviewed and the following data were noted for each age, gender, smoking habit, anatomic location of tumour, extent of disease, treatment details and clinical course. The patients included in the analysis were required to have a normal chest radiograph, computed tomography scan of the chest, sputum cytology and/or negative bronchoscopy. Patients with well-differentiated neuroendocrine tumours, mixed histological types or Merkel cell carcinoma of the skin were excluded from the analysis. Patients were staged as LD or ED. LD was defined as a tumour confined to the primary site with or without regional lymph node involvement. Any tumour spread beyond that described for LD was classified as ED.

Review of Pathologic Specimens and Immunohistochemical Studies

Formalin-fixed, paraffin-embedded tissue specimens were available for each case. The diagnosis of EPSCC was based on the histological criteria that were carried out for SCLC (25). Histological diagnosis was further confirmed by the immunohistochemical detection of the neuroendocrine markers including chromogranin (Cg), synaptophysin (SYN) or neuron-specific enolase (NSE). Immunohistochemical staining for c-kit was performed using 1:200 dilution of the rabbit polyclonal antibody for c-kit (NeoMarkers; catalogue no. RB-1670). Appropriate positive and negative controls were used. For c-kit scoring, the percentage of c-kit positive cells (completely negative: <5% of the tumour cells positive, 1+: 5–25%, 2+: 26–50%, and 3+: >50%) and the intensity of staining (weak or strong) were recorded in each case. Strong staining intensity was defined as the intensity comparable to that seen in GIST. C-kit was considered positive if >50% of the neoplastic cells exhibited positive reactivity with a strong staining intensity (24).

Statistical Methods

Overall survival (OS) was defined as the length of time from the date of diagnosis to death or to the latest follow-up. Deaths from causes other than the tumour were regarded as withdrawals. Failure-free survival (FFS) was defined as the length of time from the date of diagnosis to progression or death. Survival times were estimated using the Kaplan–Meier method, and comparisons between groups were carried out using the log-rank test. Categorical variables and age distribution between c–kit-positive and c–kit-negative groups were compared with Fisher’s exact test and independent samples t-test, respectively. A p value of <0.05 was considered statistically significant.

RESULTS

Clinicopathological Features

A total of 28 patients with the diagnosis of EPSCC were identified (Table 1). There were 19 males and 9 females, with a mean age of 56.5 years (range, 34–73). Fourteen patients (50%) had a positive history of smoking. Thirteen and 15 patients were classified as LD and ED, respectively. The most common location of the primary tumour was the genitourinary system including the urinary bladder (five cases) and prostate (four cases). Paraneoplastic syndromes were observed in three (11%) cases. Two cases with SCC of the prostate (case 14) and ovary (case 18) had paraneoplastic hypercalcaemia, and one case with SCC of the bladder (case 3) had systemic AA amyloidosis and nephrotic syndrome. Immunohistochemical studies revealed that NSE was the most prominent neuroendocrine marker being positive in 22 cases (79%). SYN and Cg were positive in 19 (68%) and 14 (50%) cases, respectively.

Therapeutic Approaches and Outcome

Among 28 patients with EPSCC, 20 patients had died of disease by the time of this analysis, and six patients were still living with disease. One patient had been lost to follow-up (case 9), and one patient had died of myocardial infarction 1 month after surgery (case 4).

Attempted therapeutic approaches in LD included surgery, CT and RT with different sequences (Table 1). Curative surgery was performed in four cases, and it was the sole treatment modality for one patient (case 1). The latter case developed distant metastasis 3 months after operation and he died of progressive disease 14 months after the diagnosis. Other patients who had undergone curative surgery received additional treatments and these patients died of disease 11, 15

### Table 1 Summary of clinicopathologic characteristics, c-kit expression and treatment results of patients with extrapulmonary small cell carcinoma (EPSCC)

<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Sex</th>
<th>Site</th>
<th>Stage</th>
<th>Metastasis</th>
<th>c-kit</th>
<th>Treatment/response</th>
<th>Site of failure and time (months)</th>
<th>Status/survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>Male</td>
<td>Bladder</td>
<td>LD</td>
<td>–</td>
<td>2/weak</td>
<td>Cystectomy/CR</td>
<td>Liver, LN (3)</td>
<td>DOD (14)</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>Male</td>
<td>Bladder</td>
<td>LD</td>
<td>–</td>
<td>Neg</td>
<td>TUR, CAV ×6/SD</td>
<td>Brain, LN (6)</td>
<td>DOD (7)</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>Male</td>
<td>Bladder</td>
<td>LD</td>
<td>–</td>
<td>3/strong</td>
<td>TUR, CAV ×6/SD</td>
<td>Liver, adrenal (6.5)</td>
<td>DOD (12)</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>Female</td>
<td>Bladder</td>
<td>LD</td>
<td>–</td>
<td>2/strong</td>
<td>TUR</td>
<td>–</td>
<td>DUC (1)</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>Male</td>
<td>Bladder</td>
<td>LD</td>
<td>–</td>
<td>Neg</td>
<td>TUR, CAV ×6, RT/CR</td>
<td>Brain (15)</td>
<td>DOD (16)</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>Male</td>
<td>Prostate</td>
<td>LD</td>
<td>–</td>
<td>3/strong</td>
<td>CAV ×6, RT/PR</td>
<td>Liver (8)</td>
<td>DOD (9)</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>Female</td>
<td>Breast</td>
<td>LD</td>
<td>–</td>
<td>Neg</td>
<td>FEC ×3, MRM, CE-FEC ×4, RT/CR</td>
<td>LN (9)</td>
<td>DOD (11)</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>Male</td>
<td>Larynx</td>
<td>LD</td>
<td>–</td>
<td>1/weak</td>
<td>TL, CE ×3, RT, CE ×3/CR</td>
<td>Bone (12)</td>
<td>DOD (15)</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>Male</td>
<td>Salivary gland</td>
<td>LD</td>
<td>–</td>
<td>2/weak</td>
<td>Refusing treatment</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>Male</td>
<td>UO (LN)</td>
<td>LD</td>
<td>–</td>
<td>Neg</td>
<td>CAP ×6, RT/CR</td>
<td>Liver, LN (42)</td>
<td>DOD (44)</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>Female</td>
<td>UO (LN)</td>
<td>LD</td>
<td>–</td>
<td>3/strong</td>
<td>CE ×6, RT/CR</td>
<td>Bone (13)</td>
<td>DOD (14.5)</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>Female</td>
<td>Mediastinum</td>
<td>LD</td>
<td>–</td>
<td>Neg</td>
<td>CE ×7, RT/PR</td>
<td>Bone (18)</td>
<td>AWD (25)</td>
</tr>
<tr>
<td>13</td>
<td>71</td>
<td>Male</td>
<td>Thymus</td>
<td>LD</td>
<td>–</td>
<td>3/strong</td>
<td>Total Resection, FEP ×6/CR</td>
<td>Local, pleura (17)</td>
<td>DOD (3)</td>
</tr>
<tr>
<td>14</td>
<td>61</td>
<td>Male</td>
<td>Prostate</td>
<td>ED</td>
<td>Liver</td>
<td>Neg</td>
<td>CE ×4/PR</td>
<td>Liver (6.5)</td>
<td>DOD (9)</td>
</tr>
<tr>
<td>15</td>
<td>62</td>
<td>Male</td>
<td>Prostate</td>
<td>ED</td>
<td>LN</td>
<td>3/strong</td>
<td>CE ×6/PR</td>
<td>Abdomen, LN (9.5)</td>
<td>AWD (12)</td>
</tr>
<tr>
<td>16</td>
<td>73</td>
<td>Male</td>
<td>Prostate</td>
<td>ED</td>
<td>Brain, LN</td>
<td>3/strong</td>
<td>Top ×1/PD</td>
<td>Brain, LN (1.5)</td>
<td>DOD (2)</td>
</tr>
<tr>
<td>17</td>
<td>48</td>
<td>Female</td>
<td>Breast</td>
<td>ED</td>
<td>Bone</td>
<td>2/weak</td>
<td>CE ×1/PD</td>
<td>Bone (2)</td>
<td>DOD (2.5)</td>
</tr>
<tr>
<td>18</td>
<td>34</td>
<td>Female</td>
<td>Ovary</td>
<td>ED</td>
<td>Peritoneum</td>
<td>Neg</td>
<td>CE ×4/PD</td>
<td>Local, peritoneum (5)</td>
<td>DOD (15)</td>
</tr>
<tr>
<td>19</td>
<td>70</td>
<td>Female</td>
<td>Ovary</td>
<td>ED</td>
<td>Peritoneum</td>
<td>1/weak</td>
<td>CbT ×2/PD</td>
<td>Liver, peritoneum (3), brain (10)</td>
<td>DOD (11.5)</td>
</tr>
<tr>
<td>20</td>
<td>56</td>
<td>Female</td>
<td>Endometrium</td>
<td>ED</td>
<td>Bone</td>
<td>Neg</td>
<td>Histerectomy, wCb ×14/PR</td>
<td>–</td>
<td>AWD (6.5)</td>
</tr>
<tr>
<td>21</td>
<td>37</td>
<td>Male</td>
<td>UO</td>
<td>ED</td>
<td>Liver, bone</td>
<td>2/weak</td>
<td>CbE ×7/SD</td>
<td>Liver (6.5)</td>
<td>DOD (7)</td>
</tr>
<tr>
<td>22</td>
<td>51</td>
<td>Male</td>
<td>UO</td>
<td>ED</td>
<td>Brain, bone</td>
<td>3/strong</td>
<td>RT to brain, CE ×3/SD</td>
<td>–</td>
<td>AWD (5)</td>
</tr>
<tr>
<td>23</td>
<td>55</td>
<td>Male</td>
<td>UO</td>
<td>ED</td>
<td>Liver</td>
<td>3/strong</td>
<td>5-FU + 1 Fa/PD</td>
<td>Liver, peritoneum (9)</td>
<td>DOD (11)</td>
</tr>
<tr>
<td>24</td>
<td>64</td>
<td>Male</td>
<td>UO</td>
<td>ED</td>
<td>Liver, LN</td>
<td>Neg</td>
<td>CE ×7/PR</td>
<td>Bone (9.5)</td>
<td>DOD (13)</td>
</tr>
<tr>
<td>25</td>
<td>39</td>
<td>Male</td>
<td>Ileum</td>
<td>ED</td>
<td>Peritoneum</td>
<td>3/strong</td>
<td>CE ×4/SD</td>
<td>Peritoneum (4.5)</td>
<td>DOD (6)</td>
</tr>
<tr>
<td>26</td>
<td>59</td>
<td>Male</td>
<td>Rectum</td>
<td>ED</td>
<td>LN, lung, bone</td>
<td>Neg</td>
<td>CE ×6/PR, RT to bone</td>
<td>–</td>
<td>AWD (10)</td>
</tr>
<tr>
<td>27</td>
<td>61</td>
<td>Male</td>
<td>Gallbladder</td>
<td>ED</td>
<td>Liver</td>
<td>Neg</td>
<td>CE ×4/PR</td>
<td>–</td>
<td>AWD (5.5)</td>
</tr>
<tr>
<td>28</td>
<td>60</td>
<td>Male</td>
<td>Thymus</td>
<td>ED</td>
<td>Bone, lung</td>
<td>3/strong</td>
<td>RT to bone, CE ×6/PR</td>
<td>Bone, lung (8)</td>
<td>DOD (9)</td>
</tr>
</tbody>
</table>

LD, limited disease; ED, extensive disease; UO, unknown origin; LN, lymph nodes; RT, radiotherapy; CE, cisplatin + etoposide; CbT, carboplatin + paclitaxel; CAV, cyclophosphamide + adriamycin + vincristine; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; Top, Topotecan; FEP, 5-fluorouracil + epirubicin + cisplatin; CAP, cyclophosphamide + adriamycin + cisplatin; wCb, weekly carboplatin; CbE1, carboplatin + etoposide + ifosfamide; MRM, modified radical mastectomy; TUR, transurethral resection; TL, total laryngectomy; SD, stable disease; PD, progressive disease; PR, partial response; CR, complete response; DOD, dead of disease; AWD, alive with disease; DUC, dead of unrelated cause.
and 31 months after the diagnoses. Sequential chemo-radiotherapy (CRT), without curative surgery, was given to five patients, one of which received it after palliative surgery. The survival times of these patients were 9, 14.5, 16, 25 and 44 months. The other patients were treated with palliative surgery alone (one case) and palliative surgery plus CT (two cases). One patient refused the treatment. A total of 10 patients with LD received CT. Of the eight evaluable patients, two complete (25%) and four partial responses (50%) were observed, with an overall objective response rate of 75%. In one of the partial responders, complete response could be achieved by the addition of RT (case 11). The median FFS of all patients with LD was 12 months (95% CI, 6.61–17.39), and the median OS was 14.5 months (95% CI, 11.26–17.74).

Various chemotherapeutic regimens were used in the treatment of ED, the majority of which consisted of platinum-based combinations (Table 1). A partial response was achieved in seven patients (47%), with a median duration of 4.5 months. Three patients with ED were treated with newer agents (paclitaxel, topotecan and CPT-11) initially or after the disease had progressed. None of these patients demonstrated any response. The median FFS of the patients with ED was 6.5 months (95% CI, 3.48–9.52), and the median OS was 11 months (95% CI, 6.84–15.16). There was a significant difference in survival between patients with LD and those with ED (p = 0.029; Figure 1). For all patients included in the survival analysis, the median OS was 12 months (95% CI, 9.17–14.83).

**Analysis of C-Kit Expression**

The results of c-kit expression analysis are shown in Table 1. Positive (3+/strong) c-kit expression was observed in 10 (36%) patients (Figure 2). The distribution of age, gender and disease stage were not significantly different between c-kit positive and c-kit negative groups (p = 0.714, p = 0.417 and p = 0.705, respectively). The median OS was 11 months (95% CI, 5.22–16.78) for c-kit positive group compared to 13 months (95% CI, 8.77–17.23) for c-kit negative group (p = 0.367; Figure 3). Also, there was no significant difference between the survival of c-kit-positive and c-kit-negative patients, when the tumours showing 1+, 2+ or weak staining were excluded from the negative group (p = 0.187).

**DISCUSSION**

EPSCC occurs rarely, with an overall incidence of approximately 0.1 to 0.4% in extrapulmonary sites (9). At present, there is no consensus regarding the optimal treatment strategy of EPSCC. Although local treatments alone can result in satisfactory long-term survival (10,11,26), multimodality treatments including definitive local treatments together with CT have been increasingly proposed for LD
OVEREXPRESSION OF c-kit HAD NO EFFECT ON SURVIVAL (p = 0.367, log-rank test)

According to the data of the current study, it is difficult to make a definitive conclusion regarding the best therapeutic option for LD, because of the presence of few cases in the each treatment arm. However, our results indicate that the disease is characterised by the high rate of systemic recurrence, and the distant metastases are the main cause of death, even for patients with localised disease. Both the systemic nature and the chemoresponsiveness of the disease, particularly to the platinum-based combinations, justify the use of CT in the treatment of LD. Therefore, we recommend that platinum-based CT should be added to definitive local treatments in LD. Also, we think that CRT is an effective alternative treatment modality that permits the preservation of organ functions, as emphasised before (10,29,30). This treatment modality could be a more suitable approach than curative surgery for certain sites such as the urinary bladder and larynx, where the preservation of organ functions is desired. Although the best sequence has not been determined in EPSCC, it is plausible to treat these patients with concurrent and early CRT, which is proposed for the treatment of LD SCLC (31).

Being an essential part of the multimodality treatment for LD, CT is the most important treatment modality for ED EPSCC. Response rates vary between 43 to 73% (10,11). Nevertheless, the responses obtained with CT are usually short-lived. In the study of Galanis et al. (11), the median response duration for platinum-based regimens and doxorubicin-based regimens were 8.5 and 4.5 months, respectively. Kim et al. (27) reported a 57% response rate to CT, with a median time to failure of 6.4 months in ED. Our results were in agreement with these observations. Though nearly half of the patients with ED responded to CT, the median duration of response was only 4.5 months. Taking into account the median survival times of our patients with ED (11 months) and also LD (14.5 months), which were comparable to the same stages of SCLC (32), these results reflect the aggressive nature of EPSCC and imply that newer therapeutical approaches are needed for the treatment of this fatal disease. Although our data regarding the effectiveness of newer agents such as the topoisomerase-I inhibitors and taxanes were limited, we think that incorporation of these agents into the treatment of EPSCC may result in improvements in patient outcomes. Similar suggestions have been made for SCLC (31).

During recent years, the c-kit receptor has become one of the most attractive therapeutic targets for the treatment of SCLC. Initial studies reported that up to 81% of SCLC cell lines showed c-kit expression by Northern blot hybridisation (33). As a practical method to detect c-kit expression, immunohistochemistry (IHC) has been frequently used in many studies (34). However, the results were variable as delineated in the study of Rohr et al. (19). One of the possible explanations of these variations is the paucity of consensus regarding the intensity of staining and the cut-off level for scoring in the definition of c-kit positivity. In the study of Micke et al. (18), clear cytoplasmic staining, including membrane staining in ≥10% of all tumour cells was required for c-kit positivity, and 37% of SCLC specimens were found to be positive by this definition. On the other hand, c-kit was designated as positive if >50% of the tumour cells showed positive staining with equal- or decreased-staining intensity compared to the positive controls in the study of Naeem et al. (21), and c-kit was found to be positive in 53% of SCLC in that study. Wang et al. (24) used more stringent criteria for the definition of c-kit positivity, and they found that 5% of cervical SCC and 36% of SCLC specimens showed c-kit positivity. In our study, we used the same protocol with the study of Wang et al. (24). The reason for this choice was that these criteria, that defined c-kit positivity as the staining extent and intensity were comparable to GIST, could accurately identify the patients who might benefit from the treatment with selective tyrosine kinase inhibitors. By this definition, we found that 36% of our patients showed c-kit overexpression. Therefore, it appears that c-kit is overexpressed in a considerable proportion of patients with EPSCC as similar to that seen in SCLC; however, this may change according to site of origin.

One limitation of our study is concerned with the effect of c-kit on the survival of patients with EPSCC, because there were only 10 cases in the c–kit-positive group, and the tumours in the groups consisted of different sites of origin. Although our results were in agreement with the previous studies that were accomplished in a subset of EPSCC (23) and SCLC (20), it would be better to examine this issue in each primary site. However, our results make c-kit more relevant to a potential target for a novel form of treatment, because more than one-third of our patients exhibited c-kit overexpression. In fact, similar observations led to a recent phase II study in SCLC, which failed to demonstrate any antitumour activity of imatinib (35). However, the study
included only 14 patients with the confirmed diagnosis of SCLC and only four of them were c-kit positive. Additionally, because SCLC is characterised by a multistep carcinogenic process, in which the exact role of c-kit has yet to be understood as opposite to GIST (32), we believe that c-kit tyrosine-kinase inhibitors would likely play an adjunctive role rather than a sole treatment modality in the treatment of SCC, as proposed before (14,15).

In conclusion, EPSCC is characterised by aggressive clinical behaviour and dismal outcome with currently available treatment strategies. Because a notable proportion of the patients exhibit c-kit overexpression, c-kit may be considered as a potential target for novel therapeutic approaches.

REFERENCES


*Paper received August 2004, accepted October 2004*