Large-Cell Neuroendocrine Carcinoma: Controversies in Diagnosis and Treatment

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Key Words
Large-cell carcinoma, small-cell carcinoma, neuroendocrine carcinoma

Abstract
The diagnosis and treatment of large cell neuroendocrine carcinoma are controversial, difficult, and clearly still evolving. Diagnosing this particular entity can be hampered by the limitations and restrictions imposed by its own definition in the current WHO classification. These complexities in the semantics of diagnostic criteria can puzzle not only the pathologist but also the treating physician, and lead to difficulties in choosing treatment for individual patients. Because of its low incidence (2%–3% of non–small cell carcinomas) and the difficulties in diagnosis, data regarding treatment outcomes are based on series in which the diagnosis is frequently made retrospectively in reclassification, the numbers of patients are small, and the determinants of therapy choice (e.g., treatment with or without adjuvant chemotherapy postresection) cannot be known. Thus, the evidence on which to base recommendations for stage-based treatment paradigms is flawed in many respects. This article discusses these difficult issues for pathologists and oncologists, offers a perspective regarding approaches in treatment, and suggests ways in which prospective data on this uncommon cancer can be gathered to inform treatment guidelines and improve patient outcomes. (JNCCN 2011;9:1122–1129)

Large-cell neuroendocrine carcinoma (LCNEC) is one of the most challenging diseases to diagnose in the practice of diagnostic surgical pathology, and is consequentially challenging for oncologists to determine the best treatment. The knowledge of a subgroup of tumors with large cell histology and neuroendocrine differentiation or morphology has been recognized for more than 25 years. However, as other tools have become available in the practice of pathology, like the ever-expanding list of immunohistochemical markers, new difficulties arise, restricting the diagnosis of these tumors. Some of these problems, limitations, and restrictions have been presented in the literature.1–8 As a result of these limitations, the treatment of patients may not be standardized, because much of what is known derives from retrospective series of cases in which a reclassification has occurred.

Definition
The WHO currently defines LCNEC as a tumor with:
- histological features such as organoid nesting, trabecular growth, rosettes and perilobular palisading patterns
- the tumor cells are generally large, with moderate to abundant cytoplasm
- Nucleoli are frequent, prominent and their presence facilitates separation from small cell carcinoma
- Mitotic counts are typically 11 or more…per 2 mm² of viable tumor
- Confirmation of neuroendocrine differentiation is required using immunohistochemical markers such as chromogranin, synaptophysin and NCAM (CD56).
- One positive marker is enough… ²

However, this definition applies to resected tumors and not to small biopsies.

Classification
There is not unanimous agreement regarding the classification of neuroendocrine carcinomas. In general, these tumors could be classified into the following groups:
Large-Cell Neuroendocrine Carcinoma

• Low-grade neuroendocrine carcinoma (carcinoid tumor)
• Intermediate-grade neuroendocrine carcinoma (atypical carcinoid)
• High-grade neuroendocrine carcinoma
  ➤ Small cell carcinoma (SCC)
  ➤ LCNEC

The presence of mitotic activity in these tumors is one point for which unanimous agreement does not exist. According to the WHO, low-grade carcinomas should not have mitotic activity exceeding 1 mitotic figure per 10 high-power fields, whereas the number of mitotic figures for high-grade tumors should exceed 10 per 10 high-power fields. As noted before, these criteria were established in resected specimens and not in small biopsies, in which the number of mitotic figures, or even the number of high-power fields, may not exceed 10. Some experts disagree with the WHO classification regarding the cut-point on mitoses for low-grade versus intermediate-grade tumors, believing that a low-grade tumor may have as many as 5 mitoses in 10 high-power fields. Moran et al.\textsuperscript{1} reviewed this controversial topic and recommend a classification system presented in Table 1.

In addition, the diagnosis of LCNEC can be further compromised because the presence of neuroendocrine differentiation must be documented, either through immunohistochemistry or electron microscopy. Thus, LCNEC is not a light microscopic diagnosis but rather one that requires the use of ancillary tools. In contrast, the diagnosis of SCC can be made in the absence of staining for the common neuroendocrine markers.

In view of the restriction to the definition of LCNEC, the following scenarios can be encountered in practice:

• A tumor that has the histopathologic features of a neuroendocrine tumor (e.g., trabecular pattern, rosettes), and the tumor cells show positive staining for one of the purported neuroendocrine markers. This tumor will be classified as LCNEC (Figure 1).

• A tumor that shows the histopathologic features of a neuroendocrine tumor but which lacks the positive staining for neuroendocrine markers. This tumor will be classified as large cell carcinoma with neuroendocrine morphology or pattern. This particular tumor, although very likely the same as the neoplasm described first, falls short of the LCNEC designation because of negative staining for neuroendocrine markers.

• A tumor that does not show the histopathologic features of a neuroendocrine carcinoma but which shows positive staining for one of the purported neuroendocrine markers. This tumor will be classified as large cell carcinoma with neuroendocrine differentiation. This particular tumor, although not likely belonging to the family of neuroendocrine tumors, is classified as having neuroendocrine differentiation because of positive neuroendocrine markers.

### Diagnosis

The observation that some non-SCCs may show neuroendocrine features is not new. In 1988, Mooi et al.\textsuperscript{9} described 11 pulmonary tumors that were previously diagnosed as large cell or squamous cell carcinoma and showed the presence of dense secretory granules on ultrastructural studies and positive staining for neuron-specific enolase. In 1997, Dressler et al.\textsuperscript{10} studied 40 cases of LCNEC and suggested that

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Necrosis</th>
<th>Nuclear Atypia</th>
<th>Mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade NE Ca (carcinoid tumor)</td>
<td>Absent</td>
<td>Absent or mild</td>
<td>&lt; 3 x 10 hpf</td>
</tr>
<tr>
<td>Intermediate-grade NE Ca (atypical carcinoid)</td>
<td>Present (comedo-necrosis)</td>
<td>Moderate</td>
<td>3–10 x 10 hpf</td>
</tr>
<tr>
<td>High-grade NE Ca large cell type</td>
<td>Present</td>
<td>Prominent</td>
<td>&gt; 10 x 10 hpf</td>
</tr>
<tr>
<td>High-grade NE Ca small cell type</td>
<td>Present</td>
<td>Prominent</td>
<td>&gt; 10 x 10 hpf</td>
</tr>
</tbody>
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Abbreviations: Ca, carcinoma; hpf, high-power field; NE, neuroendocrine.

these tumors have a poor prognosis. In addition, the authors pointed out that some of these tumors might not show positive staining using neuroendocrine markers, and suggested the term large cell carcinoma with occult neuroendocrine differentiation. When considering that the morphologic separation of LCNEC from SCC is based on the presence of prominent nucleoli in the cases of large cell carcinoma, this distinction may not be as easy to determine as stated. In that regard, Marchevsky et al., using morphometric analysis, evaluated 28 surgically resected high-grade pulmonary neuroendocrine carcinomas (16 SCCs and 12 large cell carcinomas). The authors concluded that considerable overlap exists between these entities, which, in their experience, helped them to separate only 9 of the 28 cases studied.

Because of these controversial issues surrounding the diagnosis of LCNEC, publications have proliferated that, rather than shedding light on this issue, have generated more misconception and controversy. An example is the addition of terms such as small cell–like large cell neuroendocrine carcinoma, large cell neuroendocrine carcinoma with neuroendocrine morphology, large cell carcinoma with neuroendocrine features, large cell carcinoma with neuroendocrine differentiation, small cell variant of large cell neuroendocrine carcinoma, and large cell variant of SCC. These terms clearly confuse an already confusing subject. A review of previous publications on the subject of LCNEC shows that most series consist of retrospective studies of cases that were previously called non-SCC or large cell carcinomas and, after positive immunohistochemical neuroendocrine studies, were classified according to the current WHO definition of these tumors. Importantly, studies dealing exclusively with non-SCCs have shown the presence of neuroendocrine differentiation in as many as 36% of these cases, and some have argued that this differentiation has no prognostic value.12–15

The diagnosis of LCNEC is not as straightforward as one would expect. However, to properly diagnose these tumors, one must keep in mind that the tumor is a poorly differentiated non-SCC that shows a trabecular growth pattern, or ribbons of malignant cells, and sometime rosettes. If that determination can be made, then the diagnosis becomes easier on morphologic grounds.

Immunohistochemical and Ultrastructural Features
Currently, immunohistochemical markers are used routinely in tumor diagnosis. This practice is important for diagnosing neuroendocrine tumors, and is more so for LCNEC. Therefore, chromogranin, synaptophysin, and neuronal adhesion cell molecule (NCAM [CD56]) are commonly used to determine the neuroendocrine origin of these tumors. In the case of LCNEC, the positive stain for one of these markers will be enough to classify the tumor as neuroendocrine origin. In addition, the use of ultrastructural studies to determine the presence of neurosecretory granules in tumor cells is an alternative. Nevertheless, electron microscopic studies are rarely performed in current practice because of the availability of immunohistochemical studies and the requirement of having fresh tissue (preferably).

Molecular Biology
Iyoda et al. compared stage I resected LCNECs (n = 20) and other large cell carcinomas (n = 13) for a variety of markers. Mitotic rate, Ki-67 labeling index, and bcl-2 were all higher in LCNEC, similar to findings in separate studies of SCC.16 In a large se-
eries of 83 cases, LCNEC was also found to strongly express the receptor tyrosine kinases KIT, platelet-derived growth factor receptor (PDGFR-α, PDGFR-β, and Met, again similar to findings in SCC.17

In a study of 92 pulmonary neuroendocrine carcinomas, Rusch et al.18 identified increased expression of Ki-67 and p53, and loss of Rb as discriminating factors to delineate LCNEC and SCC from both atypical and typical carcinoids. Onuki et al.19 compared loss of heterozygosity (LOH) at 10 chromosomal regions and mutations in p53 in a similar study of 59 neuroendocrine tumors. The incidence of LOH and p53 gene mutation progressively increased with increasing grade of tumor, again separating the high-grade LCNEC and SCC from the lower-grade carcinoids.

Confirming the impression that LCNEC and SCC are closely related biologically, Jones et al.20 found that gene expression profiles of LCNEC (n = 8) and SCC (n = 17) were indistinguishable but clearly demarcated from those of carcinoids (n = 13), large cell carcinoma (n = 13), adenocarcinoma (n = 12), and normal lung (n = 30), all of which had distinct profiles. Overall, these data support a shared histogenesis for LCNEC and SCC.

**Epidemiology, Clinical Presentation, and Staging**

As is true for SCC, a history of cigarette smoking is found in more than 85% of patients diagnosed with LCNEC.21,22 Men predominate in most series, and median age ranges from 62 to 68 years.

Most primary LCNECs (> 67%) occur in the lung periphery, in contrast to the central location of SCC, and therefore symptoms related to central mass affecting the main and lobar bronchi are less common.23 Patients may instead have an asymptomatic nodule or nonspecific symptoms, such as malaise, sweats, chest pain, and dyspnea.

No prospective data are available on the results of staging; in the preoperative setting, the diagnosis of LCNEC can be problematic based on fine needle aspirates and small biopsies. Therefore, staging guidelines for non–small cell lung cancer are commonly applied.

**Prognosis**

Many series have been reported regarding survival of patients undergoing resection of LCNEC. These data, which are reviewed by Fernandez and Battafarano24 were from an era before the common use of adjuvant chemotherapy. The rates for 5-year overall survival and for stage I vary widely, from 13% to 57% and 18% to 88%, respectively. Notably, most of these series include 50 patients or fewer, with the most favorable outcomes seen in a series including only 18.24 Outcomes have also been directly compared with other non-SCCs or with large cell carcinoma, specifically, and identified worse prognosis for LCNEC.21,25 For example, Battafarano et al.25 reported a 5-year survival of 30% for LCNEC compared with 71% for large cell carcinoma.

Somewhat at odds with these findings are those recently reported by Varlotto et al.26 Similar 4-year survival rates for LCNEC (41%) and other large cell carcinomas (42%) were found in 1444 patients treated with surgery without radiation and who were registered in the SEER database-17. Although the authors report a numerically worse 4-year survival rate for SCC (32%) treated with surgery without radiation (n = 355), multivariate analysis showed no significant difference in overall or lung cancer–specific survival between LCNEC and SCC, nor between LCNEC and other large cell carcinomas. Based on these observations and similar presenting patient and tumor characteristics of LCNEC and other large cell carcinomas, the authors concluded that LCNEC should be classified and treated as a large cell carcinoma.

Although a complete review of these data is beyond the scope of this review, many factors lessen the strength of their conclusion, including an increased percentage of LCNEC among large cell carcinomas (from 8%–21%) during the study period suggesting evolving recognition of this entity, and previous grouping of it with the large cell category, thus blurring the ability to identify a difference in outcome. Furthermore, the pathologic diagnosis cannot be independently confirmed as has been done in most other series because of the difficulties inherent in making the diagnosis, as discussed earlier. Because of limitations in the database, no information is available regarding the use of adjuvant chemotherapy, which may have influenced outcomes in any of the categories. The findings in this report seem to beg the question of how LCNEC should be treated given that, except for stage Ia, stage-specific outcomes are not reported, and it is only known that
patients underwent resection without radiation. This is despite the fact that approximately 40% of the patients were stage II or III. Especially in this group and the patients with SCC, the complete absence of data regarding chemotherapy and the reasons for not treating with radiation patients staged IIIa by virtue of N2-positive nodes is problematic.

Because LCNEC is part of the spectrum of pulmonary neuroendocrine tumors, Asamura et al.22 compared outcomes postresection for 141 patients with LCNEC, 113 patients with SCC, 9 with atypical carcinoid, and 55 with typical carcinoid tumors. The survival curves of patients with LCNEC and SCC were superimposed and far worse than those of the patients with carcinoid tumors, with 5-year survival of 40% and 36% for LCNEC and SCC, respectively, compared with 88% and 78% for the typical and atypical carcinoids. Other smaller series have identified the same trends, reporting that LCNEC and SCC have similarly worse survival compared with the better-differentiated carcinoid tumors.27,28

Treatment

LCNEC is generally believed to be similar to SCC in histogenesis, biology, and clinical behavior. Efforts to obtain prospective data to define stage-based treatment recommendations have been stymied by the relative rarity of LCNEC and the difficulties with pathologic diagnosis. For patients with locally advanced or metastatic disease (stage III or IV), extrapolation from the treatment paradigms for both non-SCC and SCC, with chemoradiation and chemotherapy in stage III, and chemotherapy and palliative radiation in stage IV, seems reasonable. Regarding drug choice, based on the weight of the data that will be reviewed later, regimens based on efficacy in SCC (e.g., etoposide and a platinating agent) are preferred.

Recommendations for the management of patients with stage I or II LCNEC are more problematic, specifically regarding the use of adjuvant chemotherapy and, in node-positive patients, thoracic radiation, as would be considered for SCC. Later sections discuss the mainly retrospective experience with the use of perioperative/adjuvant chemotherapy in patients who underwent resection and, from one report, response rates obtained when chemotherapy was given for patients with evaluable disease.

Rossi et al.17 reviewed 83 patients who had undergone surgery for LCNEC. Despite the fact that 65% of the cohort had pathologic stage I disease, the median survival was only 17 months; 5-year survival rates for stage I, II, and III patients were 33%, 23%, and 8%, respectively. Adjuvant chemotherapy was administered to 28 of the 83 patients; 13 received a regimen generally used in SCC (e.g., etoposide/platin); 15 patients treated were treated with regimens generally used in non–small cell lung cancer (i.e., platin with either gemcitabine, paclitaxel, or vinorelbine). Flawed as this comparison may seem given the small number of patients and the nonrandomized nature of treatment selection, survival outcomes were significantly better for patients undergoing therapy based on SCC, with a median survival of 44 versus 11 months (P < .0001). Surprisingly, median survival of the 15 patients treated with regimens used for non–small cell lung cancer was equal to that of the 58 patients who had no adjuvant chemotherapy. Similar findings were obtained in patients who underwent chemotherapy after the development of metastatic disease. In multivariate analysis, therapy with etoposide and a platin was the strongest predictor of survival.

A very small prospective study by Iyoda et al.29 of adjuvant etoposide/cisplatin post–complete resection of LCNEC included 15 patients who were compared with a historical group of 23 patients who underwent resection only. Patient characteristics, types of resection, and stage distribution were similar between the groups. Again, given the small number of patients and comparison with historical controls, firm conclusions are not possible. However, outcomes were significantly better for the patients treated with chemotherapy, with 5-year disease-free and overall survival rates of 87% versus 35%, and 89% versus 47%, respectively.

These same investigators subsequently published a retrospective series of 72 patients postresection of LCNEC, comparing 30 who were treated with platin-based adjuvant chemotherapy with 42 treated with resection only or with resection and adjuvant nonplatin-based chemotherapy.30 Recapitulating the findings from their small prospective trial, this series also showed cisplatin-based adjuvant chemotherapy was an independent prognostic factor in multivariate analysis, with 5-year disease-free survival rates of 59% versus 33%.
Saji et al.\textsuperscript{31} recently reported their retrospective experience with 45 patients with LCNEC and large cell carcinoma with neuroendocrine morphology or differentiation treated surgically. Neoadjuvant or adjuvant chemotherapy was administered to 23 patients; 21 were treated with platin-based regimens—14 with irinotecan or etoposide and 7 with a taxane. Comparing outcomes in the 23 chemotherapy-exposed patients and the 22 treated with resection only, survival benefit for perioperative chemotherapy was identified in the overall population and in stage-specific subsets. The 5-year survival rates were 87% versus 58%, the favorable outcome perhaps reflecting the predominance of stage I disease (61%). Benefit was observed across stages. In multivariate analysis, chemotherapy treatment was strongly predictive of survival; the hazard ratio for risk of death in patients undergoing resection only was 9.5 ($P = .0457$).

Twenty patients with stage III or IV or recurrent LCNEC were retrospectively analyzed by Yamazaki et al.,\textsuperscript{32} 6 of whom had prior exposure to nedaplatin ($n = 1$) or cyclophosphamide-based therapy ($n = 5$). Overall response rates to combination chemotherapy were as follows: etoposide/cisplatin 5 of 9 (55%) and cisplatin/vindesine with or without mitomycin 5 of 10 (50%). All but one of the responses were observed in the group of 14 patients who were chemotherapy-naïve.

In a retrospective series of 18 patients reported by Filosso et al.,\textsuperscript{33} 10 patients with LCNEC and positive preoperative indium-111 pentetreotide scintigraphy were treated with adjuvant octreotide alone or with radiation. Only 1 of these patients experienced recurrence, whereas 8 of 8 patients with pentetreotide-negative imaging died of recurrent disease. Given the small number of patients and the retrospective nature of this report, the data require confirmation. However, in the 6 years since this publication, no prospective trial or larger series has been reported, leading to some skepticism regarding the reproducibility of the results. Thus, therapeutic use of octreotide in LCNEC remains the province of clinical investigation. Similarly, the value of radio-labeled pentetreotide scintigraphy in staging has not been investigated.

Although accepting all the weaknesses inherent in conclusions from small, nonrandomized, mainly retrospective series, the weight of the evidence does not seem to indicate benefit for chemotherapy in resected LCNEC and supports the use of regimens that have proven effective in SCC. The reader should be aware that this represents a departure from the recommendations of the NCCN Guidelines Panel to manage LCNEC as non–small cell lung cancer.

**Brain Metastases/Prophylactic Cranial Irradiation**

Limited data on the incidence of brain metastases in patients with LCNEC are available from a subset of retrospective series. The rate in patients undergoing surgery was 15\%, 22\%, 28\%, and 39\% in 4 series, respectively.\textsuperscript{17,30,33,34} Among patients with recurrence, metastasis to the brain occurred in 30\%, 42\%, 44\%, and 54\%, similar to rates in patients with extensive SCC. However, with these limited data, recommendations cannot be made regarding the use of prophylactic cranial irradiation. This should be studied further in prospective trials.

**Conclusions**

The following points summarize the complexities faced in diagnosing and treating this unique malignancy and offer recommendations for future studies that will inform the imperfect knowledge base and hopefully improve outcomes for patients.

- LCNEC as currently defined must display both neuroendocrine morphology and positive staining for neuroendocrine markers. The authors believe that the behavior of large cell carcinomas with neuroendocrine morphology only (i.e., marker-negative) is likely similar.
- Distinguishing LCNEC from non-SCC in biopsy specimens can be challenging for pathologists. Analysis of specimens from surgical procedures frequently leads to revision in diagnosis.
- The molecular genotype and phenotype of LCNEC and SCC are similar.
- Because of the rarity of LCNEC, and vagaries in diagnosis because of the complexity of current pathologic classification, current treatment recommendations must be based on retrospective data, which are imperfect at best.
- The current knowledge base supports extrapolation from treatment paradigms for SCC in managing patients with LCNEC. General recommendations are as follows:
  - Adjuvant chemotherapy for resected patients (e.g., etoposide/platin 4 courses)
Chemoradiation for patients with positive nodes postresection or those with unresectable stage III (etoposide/platin 4–6 courses concurrent with radiation followed by additional chemotherapy to complete a total of 4 courses)

Chemotherapy for patients with stage IV disease (etoposide/platin 1–2 courses)

Palliative radiation as clinically indicated

Prophylactic cranial irradiation cannot be recommended as a routine practice

• Given shared characteristics and similar recommendations for therapy, it seems reasonable to include patients with this uncommon cancer and unresectable disease in clinical trials for SCC; prospective data will otherwise be difficult to obtain. Initially, data can be reported separately for this subgroup. Eventually, investigators may discover that outcomes are similar, stage by stage, with uniform therapy. In that case, the clinical and pathologic distinction between these high-grade malignancies may become insignificant.

• Regarding evaluation of adjuvant therapy (both chemotherapy and radiation) in patients eligible for resection, a registry trial can be envisioned, perhaps run through the National Cancer Institute Cooperative Group Program. Central pathology review and uniform stage-based treatment algorithms could then be applied and evaluated prospectively.

References


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