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Review

Large cell carcinoma of the lung: A tumor in search of an author. A clinically oriented critical reappraisal

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ABSTRACT

Large cell carcinoma (LCC) is a merely descriptive term indicating a subtype of lung cancer with no specific features of small-cell lung cancer (SCLC), adenocarcinoma (ADC) or squamous cell carcinoma (SQC). This diagnosis is allowed on surgical specimens only, whereas its counterpart in biopsy/cytology samples is non-small-cell lung carcinoma (NSCLC), not otherwise specified (NOS). Although these two terms do not fulfill the same concept, they can be interchangeable synonyms at the clinical level, reflecting, in different ways, the inability to define a specific subtype. Immunohistochemistry (IHC), next generation sequencing (NGS) analysis and, historically, electron microscopy have been unveiling diverse cell differentiation lineages in LCC, resulting in LCC-favor ADC, LCC-favor SQC and LCC-favor large-cell neuroendocrine carcinoma (LCNEC), the latter hopefully to be included into the neuroendocrine tumor (NET) group in the future. Paradoxically, however, the interpretation issues of LCC/NSCLC-NOS are not diminishing, but even increasing albeight an accurate diagnosis is oncologically required and crucial. Also, rare LCC/NSCLC-NOS cases exhibiting null/unclear phenotype, are difficult to classify, and this terminology could be maintained for the sake of classification (basically these tumors are serendipitous ADC, as also confirmed by the lack of p40). In this review article, seven relevant issues to LCC have been addressed by using a question–answer methodology, with final key points discussing major interpretation issues. In conclusion, most LCC/NSCLC-NOS may be eventually re-classified and addressed by exploiting IHC and/or molecular testing to satisfy the criteria of precision medicine (the right drug, to the right patient, at the right time).

1. Background

Large cell carcinoma (LCC) is probably the main controversial issue in the current lung cancer classification, clearly requiring significant changes in the next future. In this review article, we organized the discussion on LCC answering to the most frequent questions (Q) emerging from the pertinent literature and the oncologists' community.

Q1: LARGE CELL CARCINOMA EXISTENCE: TO BE, OR NOT TO BE? THAT IS THE QUESTION!

Answer: YES, it still survives to indicate lung undifferentiated non-small-cell tumors, but its own diagnostic criteria and terminology are under refinement according to improved lung cancer biology understanding. Its prevalence is destined to hopefully vanish.

Discussion: Large cell carcinoma (LCC) of the lung is an uncommitted term describing a group of primary pulmonary carcinomas having undifferentiated features, without any neuroendocrine (NE), squamous or glandular differentiation and without specific clinical characteristics [1]. LCC diagnosis is restricted to surgical specimens only, once meticulous sampling

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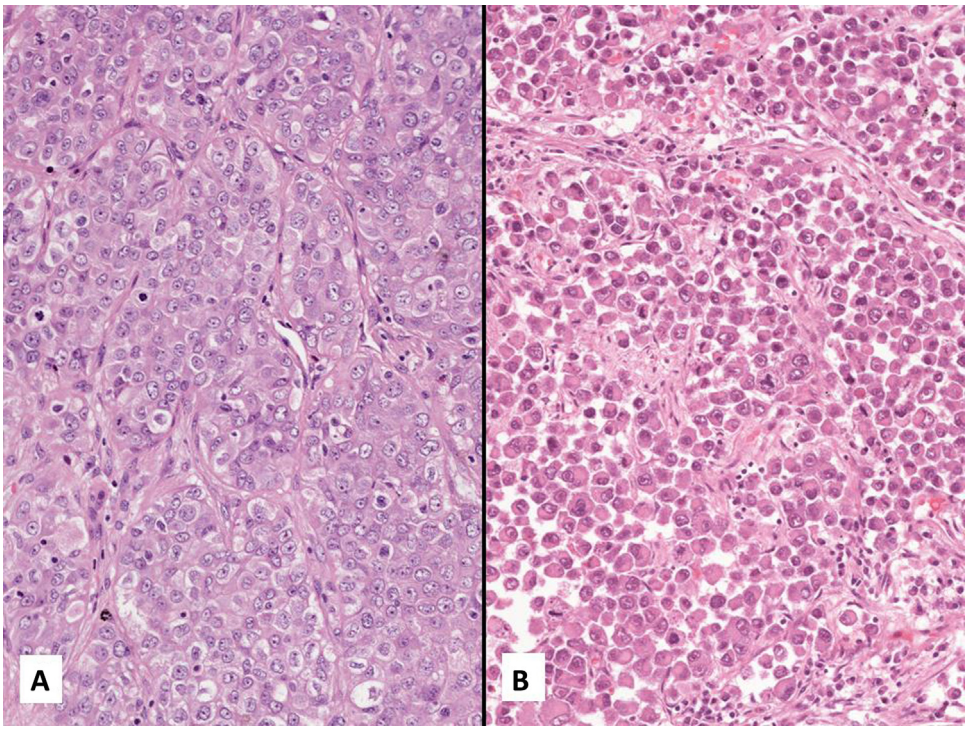

Fig. 1. Examples of undifferentiated LCC with a solid (A) and discohesive (B) growth pattern.

and immunophenotyping has been conducted to exclude the presence of areas featuring ADC or SQC differentiation. A diagnosis of LCC cannot be made on biopsies or cytological samples, where non-small-cell lung cancer (NSCLC) lacking any specific morphologic criteria must be diagnosed as NSCLC-NOS [2,3]. Although the term LCC was adopted in lung cancer classifications to compensate the impossibility of further classifying undifferentiated NSCLC [1,4–6], most LCC are probably more related to lung ADC than SQC, at least from a clinical viewpoint [7]. At light microscopy, LCC is basically a tumor featuring large, atypical, polygonal cells, arranged in cohesive or even discohesive sheets or nests with vesicular nuclei and prominent nucleoli, and a moderate amount of cytoplasm, in the absence of specific signs of differentiation (Fig. 1). Before drawing such a diagnostic conclusion, mucin histochemistry (Alcian-blue/PAS stain, PAS-diastase, Kreyberg or mucicarmine stain) is required to exclude the "solid with mucin production" variant of ADC. Apart from the entirely undifferentiated LCC, the current 2004-WHO classification recognizes five different variants of LCC, namely clear cell carcinoma (CCC), lymphoepithelioma-like carcinoma (LELC), LCC with rhabdoid phenotype (LCC-R), basaloid carcinoma (BC) and large cell neuroendocrine carcinoma (LCNEC) [1]. In our and others' views [5–12], this sub-classification of LCC is a source of confusion, because BC shows a SQC lineage and should be classified accordingly, LELC should be restricted to Epstein–Barr virus-related neoplasms with SQC lineage (as seen in the relevant head & neck tumors), most LCC-R and at least two third of CCC are definitely poorly-differentiated ADC, and LCNEC belongs to the spectrum of NET. A rare subset of LCC do not react with any of the specific lineage markers ("null phenotype") or shows immunohistochemistry (IHC) negativity for ADC markers in the presence of only focal positivity for squamous or NE markers ("unclear phenotype") and remain part of the LCC-NOS category when dealing with surgical specimens, and of the NSCLC-NOS group in the case of cytology/biopsy samples, provided that metastatic or other uncommon pulmonary tumors (e.g., sarcomatoid or NUT midline carcinomas) have been reasonably excluded (Fig. 2).

KEY MESSAGES: *(A) The LCC (on surgical specimens only) category should be reduced as much as possible, subtyping all undifferentiated (mucin-negative, and NE marker negative) carcinomas by IHC and reporting cases defined by immunophenotype in the relevant category with the terminology "LCC-NOS, favor adenocarcinoma" or "LCC-NOS, favor squamous cell carcinoma". (B) Immunohistochemically negative or ambiguous cases ("null/unclear phenotype") should enter in the group of LCC/NSCLC-NOS, hopefully not exceeding 5% in the routine practice.*

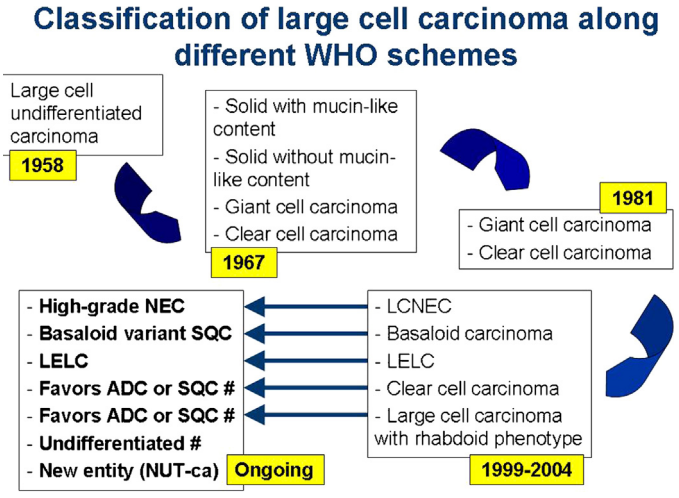

Fig. 2. A summary of the previous and ongoing classifications of large cell carcinoma of the lung according to the WHO schemes (#, after immunostains and molecular investigations).

Q2: ARE NSCLC-NOS DIAGNOSES IN BIOPSY & CYTOLOGY THE SAME AS LARGE CELL CARCINOMA?

Answer: YES from a practical point of view, when dealing with clinical decisions to be taken, although they reflect different contexts, which this terminology originates from.

Discussion: This question seems tautological, but in the daily practice the general pathologist is not perfectly aware of the difference between a diagnosis of LCC on a resected tumor specimen and the report on "NSCLC-NOS" versus "NSCLC-NOS, favors squamous" or "NSCLC-NOS favors adenocarcinoma", according to the IHC profile in a small biopsy or cytology. NSCLC-NOS on small specimens may be ultimately classified as ADC or SQC in surgical specimens, because only undifferentiated areas were stochastically assessable in biopsy/cytology samples. A diagnosis of NSCLC-NOS on small specimens does not conceptually correspond to a diagnosis of LCC on a surgical sample, but, from a practical point of view, has the same clinical implications. Unfortunately, in almost 70% of lung cancer patients, biopsy or cytology specimens will be the only tissue available for tumor diagnosis and it is possible that some of such diagnoses will be inconclusive (i.e., NSCLC-NOS), due to casual sampling of undifferentiated tumor areas, rather than to a real, totally undifferentiated carcinoma. Needless to say that any effort to refine the final diagnosis using IHC is well received to reduce the category of NSCLC-NOS cases [10,13,14]. This new approach to accurate eventual diagnoses on biopsy/cytology represents a revolution in the clinical landscape of lung cancer therapy [3]. Indeed, it determined a "paradigm shift" [15] able to expand immunophenotyping to extreme degrees of diagnostic responsibility.

KEY MESSAGES: *The application of the term LCC is restricted to undifferentiated primary non-small-cell lung tumor exhaustively analyzed on surgically resected specimens and totally lacking squamous, glandular or NE lineage. Similar neoplasms detected in small biopsy or cytological samples should be reported as non-small-cell lung carcinomas-not otherwise specified (NSCLC-NOS). IHC is recommended when facing NSCLC-NOS diagnosis and, if conclusive, an additional comment "favor SQC" or "favor ADC" should be introduced.*

Q3: IS LCNEC PART OF THE LCC CATEGORY?

Answer: YES, so far, but it is expected to be moved to the neuroendocrine tumor category in the near future

Discussion: LCNEC was described in 1991 [16] as part of undifferentiated carcinomas, but characterized by a solid, "organoid" pattern of growth with peripheral nuclear palisading and comedo-like or large "geographic" necrosis. The expression of NE cell markers (chromogranin A, synaptophysin, CD56) correctly identified LCNEC as a separate entity, which was tentatively incorporated in the LCC. This view was also confirmed in WHO 1999 and 2004 classifications, where IHC was included among the main diagnostic criteria required for a final LCNEC diagnosis. Indeed, LCNEC belongs to the spectrum of pulmonary NETs and should more appropriately be moved to this group, in consideration of common morphological, immunophenotypic, molecular, and clinical response to SCLC-based chemotherapy [17–24]. We expect these similarities will lead to a reclassification of LCNEC in the next WHO blue book. LCNEC ranges from tumors closely overlapping with SCLC [25–29], to tumors resembling atypical carcinoid or even to solid ADC [20–32]. The clinical impact of NE differentiation in morphology-overt NSCLC is still debated [33–35].

KEY MESSAGE: *LCNEC is currently listed as one of the LCC variants, but shares morphological, phenotypic, molecular and clinical features with SCLC. It is therefore expected its reclassification as part of the spectrum of NE neoplasms.*

Q4: HOW TO CLASSIFY NON-NEUROENDOCRINE UNDIFFERENTIATED LARGE CELL CARCINOMAS?

Answer: exclude solid ADC, metastases and sarcomatoid carcinoma, then proceed to immunoprofiling (see Q5)

Discussion: The exact categorization of undifferentiated LCC raises the possibility of a metastatic tumor to the lung or a non-epithelial lung primary neoplasm (e.g., sarcoma, lymphoma, melanoma) [5,6], as well as a sarcomatoid carcinoma, a malignancy characterized by extensive and stable epithelial-mesenchymal transition (EMT), which requires the recognition of a sarcomatoid (giant and/or spindle cells) component in at least 10% of the tumor or an heterologous component of metaplastic transdifferentiated sarcoma or blastematous cells [36,37]. With regard to LCC (or NSCLC) diagnosis, the current attitude is to force phenotype definition by means of IHC [38–44], in order to reduce the NOS category. Of course, the clinical issue remains, whether the response to specific treatments and survival of such cases is more akin to the corresponding better differentiated forms, rather than to LCC with null/unclear phenotype. IHC ultimately lead to an ADC phenotype in 80–90% of cases, while less than 20% of undifferentiated LCC demonstrate squamous cell differentiation [11–13,38–44]. The remaining tumors will be labeled LCC with unclear/null phenotype or NSCLC-NOS, according to the type of specimen under evaluation.

KEY MESSAGE: *In the presence of an undifferentiated tumor in the lung, the possibility of a sarcomatoid carcinoma, a metastasis or non-epithelial lung primary tumor (e.g., lymphoma, sarcoma, melanoma) should always be considered. At the end, immunophenotyping for glandular or squamous markers is recommended, then appropriately assigning the tumor to the respective histological categories (NSCLC-favor ADC or NSCLC-favor SQC). The remaining tumors will be classified as LCC with null/unclear phenotype or NSCLC-NOS, according to the type of specimen under assessment.*

Q5: IS IT POSSIBLE TO DEFINE DIAGNOSTIC ALGORITHMS BY IMMUNOHISTOCHEMISTRY?

Answer: YES, thus reducing the LCC category to those cases with null/unclear phenotype only.

Discussion: Undifferentiated NSCLC in biopsy or cytology samples or LCC in surgical specimens can be appropriately submitted to IHC, using markers highlighting the three main different cell differentiation lineages of lung cancer, namely glandular, keratinizing, and NE differentiation [45]. Nuclear markers appear to outperform especially in poorly cellular samples and, therefore, TTF-1 and p40 are the products of choice in the first diagnostic round [28]. In double negative cases, other lineage markers include napsin-A protein for ADC and CK5/6, desmocollin-3, desmoglein or SOX2 for SQC. A completely negative profile or a focal expression of squamous cell or NE markers could determine the assignment to the category of "null/unclear phenotype". Facing with double negative cases, pathologists should always consider a metastatic tumor, although most of them are likely to be ADC [5,11–14,24,38,41–44,46–48]. Each laboratory may establish different protocols and prefer one marker to another, mainly based on its own specific experience. Mucin stain [8,39], TTF-1 clone 8G7G3/1 over clones SPT24 and SP141 [49–51], p40 [52,53], napsin-A [54–56], cytokeratins 5/6 [57], desmocollin-3 and other desmosomal markers [58–62] are the most useful markers. Immunophenotyping highlights a cell differentiation lineage, based on the recapitulation of normal cell products involved in lung development, such as TTF-1 in pneumocytes and respiratory bronchiolar cells (terminal respiratory unit) or p40 in the basal layer cells of non-terminal bronchioles and bronchi. Carcinomas of the lung reproduce these settings quite faithfully [63,64]. To this respect, p40 is acting as a practical driver

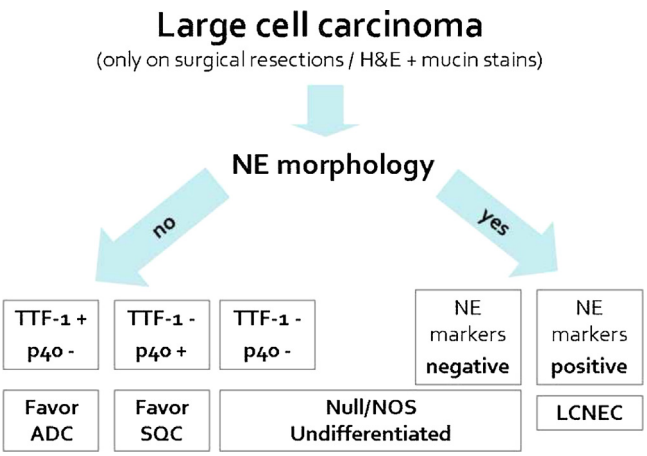

Fig. 3. A simple scheme summarizing the key points in the diagnosis and differentiation of LCC for clinical purposes.

marker in lung cancer characterization according to the axiom "*no p40, no squamous*" due to its extraordinary specificity for SQC [44,52]. So, p40/TTF-1 negative tumors are basically ADC, once sarcomatoid carcinoma or an unexpected metastasis were reasonably ruled out. Molecular data using extensive genetic characterization [11] or NGS analysis support this contention (Pelosi et al., manuscript in preparation). While the absence of TTF-1 expression in a NSCLC does not exclude an ADC lineage, the lack of p40 reactivity excludes by definition a squamous cell differentiation lineage. A double negative (TTF-1-/p40-) or unclear-phenotype (TTF-1-/p40±) tumor is more probably related to ADC. A practical flow-chart is depicted in Fig. 3. Vimentin proved to be useful in diagnosing sarcomatoid carcinomas (even on biopsy samples), when strict interpretation criteria were applied [65].

KEY MESSAGE: *A limited panel of immunomarkers will help to assign a tumor to either subtype (NSCLC-NOS, NSCLC favor ADC or NSCLC favor SQC, in the case of biopsy or cytology samples) or to the category of LCC (in case of resected specimens). Generally, TTF-1 and p40 are sufficient markers to define the phenotype, thus sparing tissue for molecular tests. For practical purposes, the rare NSCLC/LCC with a "null/unclear phenotype" is more probably related to ADC.*

Q6: IS THE SEARCH FOR PREDICTIVE MARKERS USEFUL IN THE LCC CATEGORY?

Answer: YES, since such markers in LCC-NOS/NSCLC-NOS, favor "ADC" or "SQC" are often expressed as in the conventional ADC or SQC groups.

Discussion: Massive sequencing of lung cancer is providing relevant profiles of single tumor subtypes with important information for prognostic and predictive purposes [66,67]. Data on LCC are currently scant, but it is reasonable to expect that many if not all LCC will display a genetic profile (including microRNAs) [68,69] aligned to either ADC or SQC. The same holds true for known predictive markers of response to specific therapies, as thymidylate synthase in LCC [70]. After immunotyping 102 LCC with TTF-1 and p40, Rekhtmann and coworkers [11] investigated the molecular alterations characteristic of ADC and found that they occurred in favor-ADC or marker-null LCC, only, whereas the sole *PIK3CA* mutation occurred in LCC featuring squamous profile. In another study on 121 LCC of different subtypes, including the NOS group [12], a high prevalence of *KRAS* mutations (approximately 40%) and single *EGFR* mutations or *ALK* translocations were identified in the group of LCC having an ADC-oriented phenotype, but not in other subtypes. Similar results have recently been obtained in the LCC category by using NGS analysis based on the assessment of 28 different non-random gene alterations in lung cancer [71–75]. Anyhow, the above data support the recommendation of testing molecular markers also in LCC when planning a targeted treatment to optimally stratify the patients' subgroups. In this line, LCC (or NSCC-NOS) are often incorporated with ADC into clinical studies, when a trial or a treatment is to apply using drugs designed for ADC, thus creating an "umbrella" *non-pathologic* category, referred as to "non-squamous carcinoma". This approach is apparently reasonable from a practical point of view, since most LCC share genetic changes with ADC. Several driver gene alterations are emerging in the SQC group [67] and there is no reason for excluding from discussion the undifferentiated LCC featuring squamous cell properties. These tumors may be treated as much as poorly differentiated SQC with specific targeted treatments according to a mutational repertoire preferentially affecting SQC with a predictive role of therapy response [5]. While *EGFR* and *KRAS* seem restricted to ADC phenotype [76], with uncommonly reported mutations in SQC being possibly related to adenosquamous carcinoma, pure SQC (and reasonably also NSCLC-favor SQC) were shown to bear *PI3K/AKT* mutations, which together with *TP53* mutations and *FGFR* amplification represent the most common genetic alterations found in pulmonary, as well as in head & neck, SQC [67,76].

KEY MESSAGE: *After defining the immunophenotype of LCC and the preferential differentiation lineage, a further characterization of predictive markers may be performed. Most LCC/NSCLC "favor adenocarcinoma" show genetic features paralleling those of conventional ADC. However, a minority of LCC is phenotypically and genetically linked to SQC.*

Q7: IS SURVIVAL OF LCC THE SAME AS THAT OF DIFFERENTIALLY MORE REFINABLE TUMORS?

Answer: No, there seems to be some differences between LCC exhibiting clear-cut histological differentiation at IHC and undifferentiated LCC with "null" phenotype at IHC, this latter resulting basically more akin to ADC.

Discussion: The clinical significance of LCC and their categorization in few subsets according to IHC profiles is still controversial [77]. The main question is now whether LCC/NSCLC have a different biological behavior according to IHC profile. In other words, if immunophenotyping is a clinically relevant exercise for stratifying LCC/NSCLC groups with different outcome. As forced immunoprofiling is time consuming and not costless, it should be compensated by a clear-cut demonstration of clinical usefulness on such subtyping, which leaves a reasonable figure of 5% of null/unclear phenotype LCC. Some authors have proposed a further step after IHC for these totally undifferentiated cases by using well-known microRNA or molecular markers specific to ADC and SQC [5]. To address the question on the clinical relevance of subtyping, the response to treatment and subsequent survival, LCC cases should be compared with control groups of stage-matched ADC and SQC treated in the same way. In a study by Rekhtman and coworkers [11] it was found that 20 of 102 surgical LCC had a "marker-null" profile (after TTF-1 and p40), which was associated with a significantly inferior disease-free and overall survival as compared to LCC-favored ADC and LCC-favored SQC by IHC. The higher aggressive behavior of undifferentiated lung carcinomas was confirmed in a study on high stage (III–IV) NSCLC cytology/biopsy patients treated with chemo- or targeted therapy [78]. Compared to the control group of ADC, patients with NSCLC confirmed as ADC/null phenotype by IHC tended to have shorter overall and progression-free survival than ADC defined by morphology, similar to that of adenosquamous carcinoma or SQC, likely due to poorer differentiation. In another larger study dealing with 224 advanced "non-SQC" NSCLC diagnosed on biopsy/cytology samples and homogeneously

treated, 67% of cases were classified as ADC and the remaining 33% further characterized by IHC [79]. Apart from very few (10%) tumors with SQC phenotype, almost half of these poorly differentiated tumors featured an ADC immunophenotype, with the remaining exhibiting marker-null phenotype. At survival analysis, similar outcome was observed for differentiated ADC and the NSCLC-favor ADC category, while "null" phenotype NSCLC showed significantly worse response to therapy and outcome [80,81]. These preliminary data support the clinical usefulness of immunophenotyping at least for lung ADC, as the "NSCLC, favor ADC" category has a similar clinical behavior to conventionally diagnosed (better differentiated) ADC. A major effort in accurately subtyping all apparently undifferentiated lung carcinomas, using a combined morphological and immunophenotypic approach seems to be clinically justified in both surgical specimens and small biopsy/cytology samples.

KEY MESSAGE: *The few available data support the clinical usefulness of further LCC/NSCLC subtyping by IHC. In fact, the clinical behavior of NSCLC-favor ADC is similar to that of control better-differentiated ADC, while "null" phenotype NSCLC had a significantly worse response to therapy and outcome. Therefore, subtyping of resected LCC/NSCLC-NOS by IHC from every case of advanced NSCLC patients seems clinically warranted.*

2. Conclusion

Rather than fighting for the disappearance of LCC, it seems safer and wiser to accept that a minority of primary lung cancers completely devoid of signs of differentiation may elude any eventual classification. This includes the lack of either morphological, immunophenotypical or even genetic specific profiles. While this category should be dramatically reduced in surgical specimens, the group of hard-to-subtype lung cancer cases is likely to be more numerous in small biopsy/cytology samples for at least two reasons: (a) the impossibility of looking at the whole tumor mass and (b) the need to preserve as much diagnostic material as possible for predictive molecular testing. These latter cases will reflect the intrinsic difficulties of obtaining an accurate classification of lung cancer because of uncommitted morphology, null/unclear immunophenotype or genetic alterations not clearly assessable to either tumor type.

Conflict of interest statement

All the authors have no conflicts of interest to declare.

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