Trends in Molecular Medicine

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REVIEWS

Opinion

# Abandoning the Notion of Non-Small Cell Lung Cancer

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**Non-small cell lung cancers (NSCLCs) represent 85% of lung tumors. NSCLCs encompass multiple cancer types, such as adenocarcinomas (LUADs), squamous cell cancers (LUSCs), and large cell cancers. Among them, LUADs and LUSCs are the largest NSCLC subgroups. LUADs and LUSCs appear sharply distinct at the transcriptomic level, as well as for cellular control networks. LUADs show distinct genetic drivers and divergent prognostic profiles versus LUSCs. Therapeutic clinical trials in NSCLC indicate differential LUAD versus LUSC response to treatments. Hence, LUAD and LUSC appear to be vastly distinct diseases at the molecular, pathological, and clinical level. Abandoning the notion of NSCLC may critically help in developing novel, more effective subtype-specific molecular alteration-targeted therapeutic procedures.**

## Highlights

Lung cancers are classified as small-cell (SCLC) or non-small cell (NSCLC). Such distinction reflects the different clinical presentation, disease course, and therapeutic options of the two subgroups.

However, recent advances question the reason for categorizing together heterogeneous NSCLC subtypes such as adenocarcinomas (LUADs) and squamous cell carcinomas (LUSCs).

Experimental evidence indicates that LUADs and LUSCs are sharply distinct at the transcriptomic level. Distinct genetic drivers, control networks, and prognostic profiles were identified in the two tumor subgroups. Therapeutic clinical trials in NSCLC indicated differential LUAD versus LUSC response to chemotherapy, kinase mutation-targeted treatments, and immune checkpoint inhibitors.

LUAD and LUSC thus appear to be vastly distinct diseases at the molecular, pathological, and clinical level.

## Lung Cancer Identity

Lung cancers are traditionally classified as small cell (SCLC) or non-smallcell (NSCLC) [1,2]. SCLCs are malignant tumors that account for approximately 15% of lung cancers and can be identified through their neuroendocrine features [2]. NSCLCs account for about 85% of all lung cancers [1,3] and include any type of lung cancer other than SCLCs. Such distinction reflects the different histopathology, disease course, and therapeutic options of the two subgroups.

However, it is unclear whether NSCLC classifications may effectively categorize heterogeneous tumor subgroups and guide corresponding therapeutic strategies [4–6].

Recent influential reviews [3], key therapeutic clinical trials [7,8], the latest NCCN guidelines (V.3 2019, 12 February 2019) (www.nccn.org/professionals/physician_gls/pdf/nscl_blocks.pdf), and the current WHO classification of lung cancer [9,10] still refer to NSCLC as a tumor classification benchmark. However, experimental and clinical evidence is accumulating that indicates profound dishomogeneity among NSCLC subtypes, calling into question the founding reason for their joint categorization as NSCLC.

We have analyzed the **transcriptome** (see Glossary) profiles, prognostic markers, and genetic drivers versus the histopathology, biological history, and response to therapy of NSCLC subgroups. Vast diversity between subgroups was revealed for all such parameters (Figure 1, Key Figure). Such sets of indicators are founding elements for **disease classification**, as they associate closely with disease origin, biological history, and outcome. Hence, our findings indicate that the NSCLC classification comprises distinct diseases, which should be recognized as such.

## Lung Cancer Fundamentals

SCLCs show rapid growth and can develop paraneoplastic syndromes, such as Cushing's disease, carcinoid syndrome, inappropriate production of hormones, and neurodegenerative diseases such as progressive multifocal leukoencephalopathy and subacute cerebellar

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## Key Figure

### Non-Small Cell Lung Cancer (NSCLC) Subtype Feature Identification

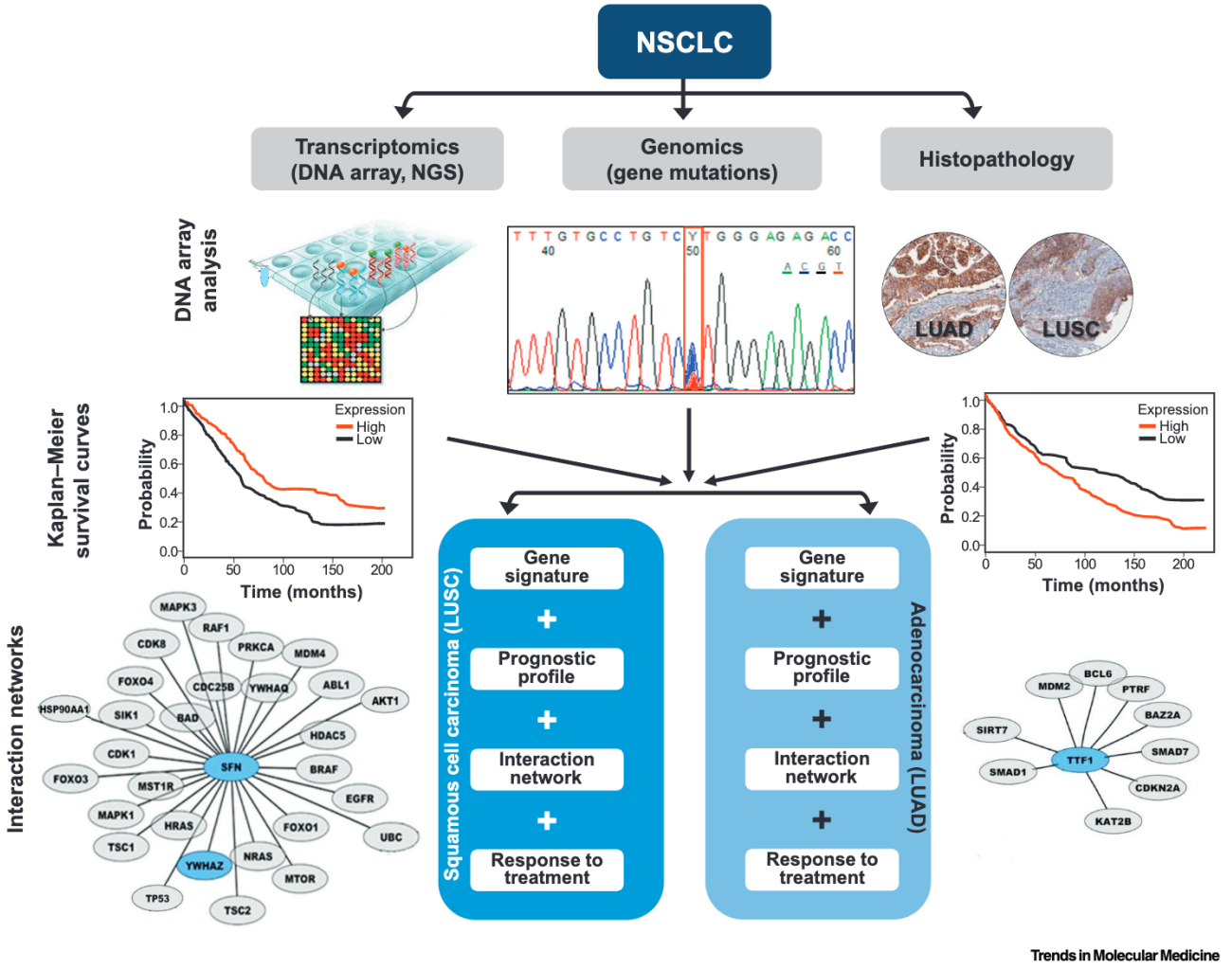


Figure 1. For a Figure360 author presentation of Figure 1, see the figure legend at https://doi.org/10.1016/j.molmed.2019.04.012

Top: Block diagrams of comparative analyses of NSCLC subgroups for diagnostic, prognostic, and therapeutic procedures. Transcriptomics: DNA array analysis flow chart. Genomics: gene mutation sequence analysis (*TP53* mutation chromatogram; wild type, blue peak; mutated, red peak). Histopathology: expression pattern of diagnostic/prognostic proteins in adenocarcinomas (LUADs) versus squamous cell carcinomas (LUSCs) (http://www.proteinatlas.org) (GLUT1 protein staining). Bottom: Tumor subtype-specific subgrouping. Prognostic profile: schematics of Kaplan–Meier survival curves for high (red) versus low (black) target gene expression (*DSG3* mRNA). Interaction networks: protein–protein interaction networks of overexpressed genes in LUADs versus LUSCs.

degeneration. SCLCs almost exclusively occur in smokers. Extensive exposure to carcinogens from tobacco smoke induces a high mutational load. *TP53* mutations occur in 75–90% of SCLCs and associate with frequent DNA amplifications and deletions [11,12], including nearly obligate loss of *RB1*. Loss of PTEN and activation of PI3K [13] are found in a substantial fraction of cases [2]. Most SCLC are already metastatic at presentation and require management primarily by chemotherapy and radiotherapy.

NSCLCs that are localized at the time of presentation can undergo surgery or radiotherapy with curative intent. However, NSCLCs do not respond to chemotherapy as well as SCLCs do. NSCLCs encompass multiple cancer types, such as LUADs, LUSCs, and large cell cancers, and mixed histotypes. Among them, LUADs and LUSCs represent the largest subgroups [1]. LUADs account for 40% of lung cancers. LUSCs represent about 25–30% of all lung cancers.

## Histopathology of LUAD and LUSC

LUADs originate from cells that secrete surfactant components. Morphological patterns of LUADs include acinar, papillary, solid, micropapillary, and invasive mucinous types. Lepidic components or pure lepidic patterns are found in noninvasive forms, previously classified as bronchoalveolar carcinoma, which can be associated with invasive mucinous or acinar LUAD. Less frequently, LUADs show colloid, fetal, or enteric features. When adenocarcinoma morphology patterns are not clearly apparent, the diagnosis can be supported by staining for thyroid transcription factor 1 (TTF-1) or napsin-A, both of which show approximately 80% sensitivity for LUAD identification.

By contrast, LUSCs originate from cells that line the inside of the lung airways. The WHO reclassified LUSCs into keratinizing, non-keratinizing, and basaloid subtypes [10]. LUSC diagnosis is based on the presence of squamous cell patterns, keratinization, and intercellular bridges [14]. When such patterns are not present, diagnosis of LUSC can be supported by staining for p40, p63, or cytokeratin 5/6, and the lesion is classified as a non-keratinizing LUSC.

## Clinicopathological Features of LUAD and LUSC

Although LUAD can occur in smokers, this is the most common type of lung cancer seen in nonsmokers. It is more common in women than in men and is more likely to occur in younger people than other types of lung cancer and to present at more advanced stages of disease [15]. In the past 25 years, for unknown reasons, LUADs have replaced LUSCs as the most frequent histological subtype.

LUSCs are linked to a history of smoking and are frequently found in the main bronchi, in central regions of the lungs. No significant differences have been detected across LUSC subtypes for clinicopathologic features, location, pleural involvement, lymphovascular invasion, age of appearance, molecular lesions (e.g., EGFR and ALK rearrangements [16]), or prognosis [17].

Extrathoracic metastatic disease is found at autopsy in ≈50% of patients with LUSC and 80% of patients with LUAD or large cell carcinoma versus >95% of patients with SCLC.

## Transcriptomic Profiles of LUAD versus LUSC

Whole-transcriptome analysis, through RNA-seq or array hybridization, provides a quantitative measure of the transcription rates of all expressed genes. This information gives insights into gene function and gene expression regulation, which in turn are associated with the biological processes that trigger the underlying disease. Correspondingly, distinct transcriptomes faithfully correlate with distinct tumor types [1].

Genes differentially expressed between LUAD and LUSC include main Gene Ontology subgroups [18–22], among them regulatory networks for cell proliferation, DNA replication, DNA repair, and RNA splicing. Cellular-structure determinants were also differentially expressed, such as for cytoskeleton assembly, exosome secretion and cell–cell junction formation, which play a key role in tumor-cell loss of differentiation and tissue invasion.

Whole-transcriptome profiling thus indicates vast diversity in LUAD versus LUSC. These findings are the cornerstone for the differential classification of LUAD and LUSC as distinct diseases. They

## Glossary

**Body mass index (BMI):** body weight divided by the square of body height. The BMI quantifies the amount of different tissue components (muscle, fat, and bone), and categorize that person as underweight (<18.5 kg/m$^2$), normal weight (18.5–25 kg/m$^2$), overweight (25–30 kg/m$^2$), or obese (>30 kg/m$^2$).
**Control pathways:** signal transduction cascades through which individual genes exert their effects. Control pathways are nonlinear and converge into networks of multiple, intertwined signaling paths. Key components of such networks are represented as nodes. Node–node interactions are represented by connecting lines.
**Disease classification:** consolidates knowledge on disease origin, pathogenetic mechanism, natural history, and response to therapy. This body of knowledge is utilized to classify diseases as separate entities.
**Hazard ratio (HR):** the ratio of the frequencies of adverse events in the two subgroups under comparison.
**Immunohistochemistry:** procedure for detecting antigens in tissue sections through antibody binding. Antibody-bound enzymes, such as horseradish peroxidase or alkaline phosphatase, are used to catalyze a color-producing reaction, which can be visualized and quantified under the microscope.
**Kaplan–Meier curves:** disease relapse curves, which indicate the time of any adverse event and compute the remaining cases as a percentage of patients that remain alive or disease-free at any given time. Kaplan–Meier curves depict cancer biological history as a cascade of disease events over time.
**Prognostic impact:** specific genetic changes or protein/mRNA biomarkers can show associations with distinct cancer groups or disease severity. The intensity of such association quantifies their impact on disease prognosis.
**Transcriptome:** the ensemble of all RNAs transcribed by the genome in a specific tissue or cell type. Transcriptome analysis, whether by RNA-seq or DNA array hybridization, thus provides quantitative details on the transcription of all expressed genes. This information is utilized to infer gene function and gene expression regulation.

also indicate distinct regulatory settings for tumor progression pathways in LUAD versus LUSC, such as for the regulation of cell proliferation and tissue invasion, which may have a direct impact on the course of the disease.

## Driver Genetic Changes in LUAD versus LUSC

Distinct driver genetic changes are associated with distinct neoplastic diseases [1]. In lung cancers, the type of mutated oncogene and the cells of origin dictate LUAD versus LUSC formation, tumor aggressiveness, and invasive capacity. Recent studies have identified several SNPs associated with increased risk for the development of lung cancers in never smokers, which are mostly LUAD [23]. EGFR mutations and EML4-ALK rearrangements were more frequently associated with LUAD in nonsmokers [15]. Overall, mutations in receptor tyrosine kinases were frequent in LUAD but rare in LUSC [3]. Systematic analysis of mutated tumor genes [24] identified distinct

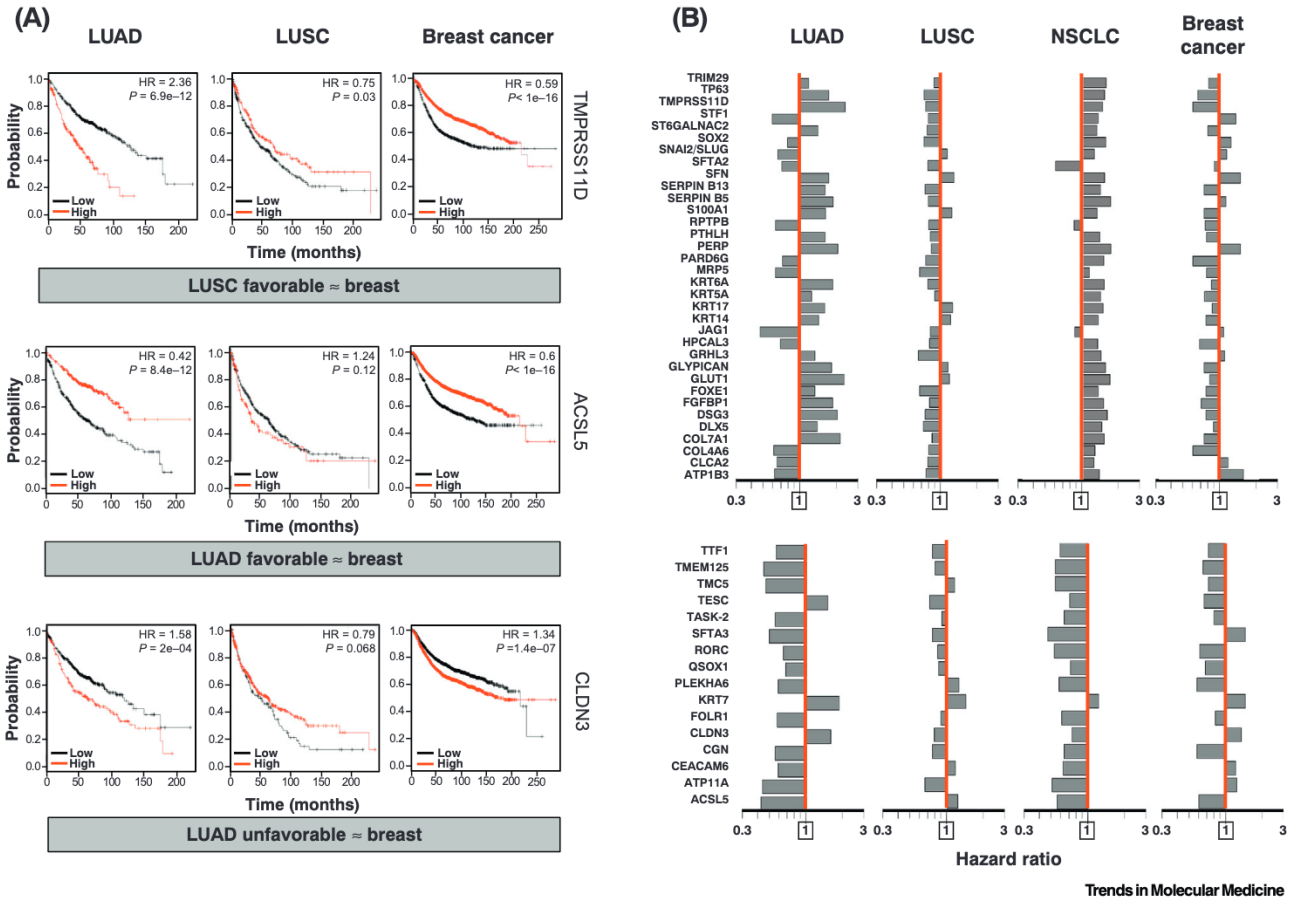


Figure 2. Analysis of Prognostic Determinants in Adenocarcinoma (LUAD) and Squamous Cell Carcinoma (LUSC) versus Breast Cancer. (A) Representative examples of Kaplan–Meier survival curves for LUAD-, LUSC-, and breast cancer-bearing patients, as obtained from Relli *et al.* [26]. Correlations between survival curves and tumor histology were computed. Tumor samples were analyzed for mRNA levels for each of the markers analyzed by DNA microarray hybridization or next-generation sequencing. Histopathology data and **immunohistochemistry** of randomly selected subsets of individual tumors were utilized for validation of gene expression at the protein level. Patient survival was compared for cases that showed high (red) versus low (black) tumor expression of the genes indicated on the right. Median survival, hazard ratios (HRs), and correlated *P* values are indicated. TMPRSS1D is a favorable prognostic determinant for LUSC that shows a corresponding impact on breast cancer, but not on LUAD. ACSL5 is a favorable prognostic determinant for LUAD that shows corresponding impact on breast cancer, but not on LUSC. CLDN3 is an unfavorable prognostic determinant for LUAD that shows a corresponding impact on breast cancer, but not on LUSC. (B) Bar plots show the HR/prognostic impact of the genes indicated on LUAD, LUSC, and benchmark breast cancers, as computed in Relli *et al.* [26]. Top: Diagnostic genes for LUSC. Bottom: Diagnostic Genes for LUAD. The genes are listed in alphabetical order. The red bars indicate HR = 1. The graphs are plotted on a log scale.

determinants in LUAD (*EGFR*, *MET*, *BRAF*, *TERT*) versus LUSC (*NOTCH* mutations and amplification of *FGFR1*, *SOX2*, *PIK3CA*). Late evolutionary genetic changes appeared to be correspondingly distinct [3,24], indicating distinct tumor progression trajectories. A notable example is that of the mutated tumor suppressor gene *TP53* [3], which is frequently found at early stages in LUSC but only at late stages in LUAD, suggesting distinct roles of *TP53* during the progression of the two tumor histotypes.

## Control Pathways and Signaling Networks

Distinct cancer types are associated with differential cell **control pathways** [1,4]. Keratins and other cytoskeletal components participate in the terminal differentiation of cornified epithelia [14]. Correspondingly, *KRT5* and *KRT6A* were shown to be associated with better prognosis in LUSC. By contrast, overexpression of most keratins was shown to be associated with tumor progression in LUAD (Figure 2) [25], whether by interference with differentiation processes or through perturbation of the regulation of tumor stem cells [14]. A driving p53/p63/p73 axis was found to be strongly associated with LUSC [26,27] but not with LUAD (Figure 3). Notably, invasion

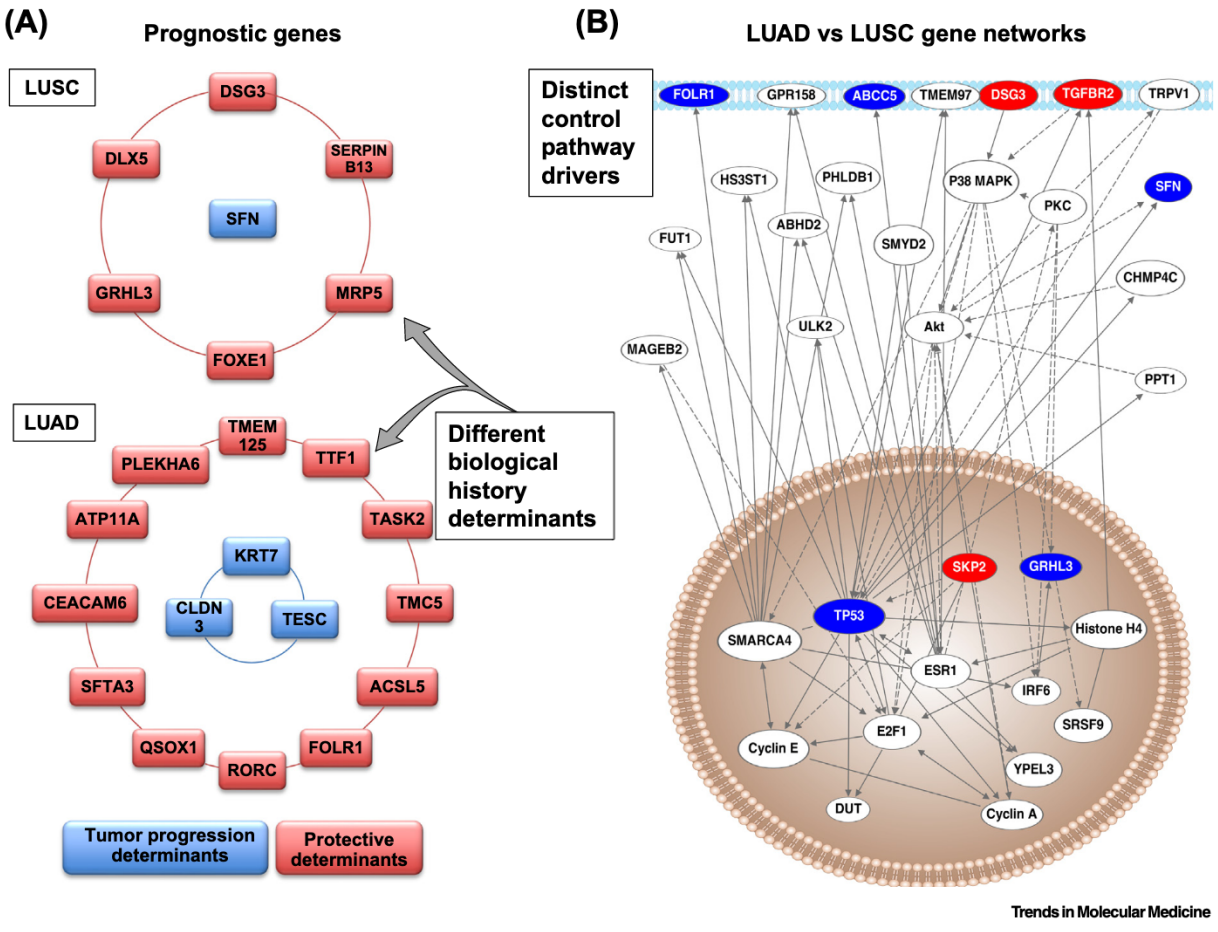


Figure 3. Adenocarcinoma (LUAD) versus Squamous Cell Carcinoma (LUSC) Control Gene Networks. (A) Prognostic determinant sets in LUAD versus LUSC. Genes with positive prognostic impact are highlighted in red; tumor progression determinants are in blue. (B) Graphical representation of control gene networks of LUAD versus LUSC, as modified from Relli *et al.*[26]. Genes are represented as nodes; biological relationships between nodes are represented as lines (network edges). Direct relationships, unbroken lines; indirect relationships, broken lines. Shared determinants between LUSC and LUAD networks are highlighted in red; genes identified in LUSC are in blue; gene interactors are in white.

determinants, such as *SERPINS*, are distinctly associated with lung cancer subtypes. *SERPINB5* overexpression is associated with poor prognosis of adenocarcinomas, such as LUADs and ductal pancreatic adenocarcinomas [26,28]. *SERPINB13* downregulation is associated with decreased survival in squamous tumors, such as LUSCs and head and neck cancers [26,29].

Overall network analysis [22,27,30,31] indicated that key signaling networks appear starkly different in LUSCs versus LUADs (Figure 3 and Table 1). Differentially activated pathways included those of growth factors and growth factor receptors, transcription factors, cell cytoskeleton, and cell–cell junction components, together with constituents of the intercellular matrix. Only three genes were found to be shared between LUSC and LUAD networks (i.e., *DSG3*, *TGFBR2*, and *SKP2*) (Figure 3). However, *DSG3* is a strong risk factor for LUAD, whereas it is a protective determinant in LUSC. *TGFBR2* is vastly protective for LUAD [**hazard ratio (HR)** 0.35; $P = 2.2e{-}16$] but much less so for LUSC (HR 0.76; $P = 0.021$). Thus, even the few shared determinants of LUSC/LUAD appear to play rather distinct roles in the two diseases [26].

## Risk Factors

Many risk factors are linked to the development of lung cancer, such as smoking, lung infections (HPV and *Mycobacterium tuberculosis*), hormonal factors, diabetes mellitus, radon exposure, occupational/domestic exposure to carcinogens, and preexisting lung disease [1,15]. The main ones among them are smoking and second-hand smoking [23]. LUSCs are tightly linked to a history of smoking.

McKay *et al.* [32] performed a genome-wide SNP lung cancer association study in 29 266 cases and 56 450 controls. They found a strikingly different genetic architecture in LUAD versus LUSC.

Table 1. Gene Sets Identified as Differentially Expressed in LUAD Versus LUSC

| Category | Expression/cell function | Gene | Refs |
|---|---|---|---|
| Diagnostic determinants by differential expression | Expression in LUAD | *ABCC5*, *YWHAS*, *TMPRSS11D*, *FOXE1*, *SNAI2*, *GRHL3*, *HsT19447*, *PARD6G*, *PTHLH*, *SOX2*, *S100A*, *CLCA2*, *DLX5*, *ST6GALNAC2*, *GPC1*, *PTPRZ1*, *JAG1*, *CSTA*, *DSG3*, *SERPINB13*, *VSNL1*, *TRIM29*, *ATP1B3*, *KRT14*, *PERP*, *KRT17*, *SERPINB5*, *PPKNEFD*, *KRT6A*, *KRT5*, *COL7A1*, *FGFBP1*, *SLC2A1*, *SFTA2*, *COL4A6* | [18] |
| | Expression in LUSC | *TMEM125*, *NKX2-1/TTF1*, *CLDN3*, *KCNK5*, *TMC5*, *CGN*, *ACSL5*, *TESC*, *FOLR1*, *RORC*, *QSOX1*, *KRT7*, *SFTA3*, *CEACAM6*, *ATP11A*, *PLEKHA6* | |
| Top discriminants of LUAD versus LUSC | Omnibus gene expression profiles | *HSP90AA1*, *BCL2*, *CDK2*, *KIT*, *HDAC2* | [49] |
| | Gene interaction networks | *E2F*, *CTGF*, *PDGF* | [21] |
| Pathway-based diagnostic gene signatures | Regulation of epidermis development | *HsT19447*, *COL7A1*, *KRT5*, *KRT14*, *KRT17*, *PTHLH*, *GRHL3* | [19,20,22] |
| | Regulation of intermediate filament components | *KRT5*, *KRT6A*, *KRT14*, *KRT17*, *PPKNEFD* | |
| | Regulation of exosome formation | *ATP1B3*, *CSTA*, *DSG3*, *YWHAS*, *GPC1*, *KRT5*, *KRT6A*, *KRT6B*, *KRT14*, *KRT17*, *SERPINB5*, *SERPINB13*, *SLC2A1*, *TMPRSS11D*, *PPKNEFD* | |
| | Regulation of cell proliferation | *IGF1R*, *GSK3B*, *ATR*, *SKP2*, *CDK1*, *CDK2*, *SMC3*, *PLK1*, *CCND3* | |
| | Regulation of DNA replication and repair | *RFC2*, *PRIM2*, *MCM4*, *MCM5*, *ATR* | |
| | Regulation of RNA splicing | *PRPF19*, *SRSF2*, *THOC4* | |
| | Regulation of cell–cell junction formation | *TGFBR2*, *CTNND1*, *CKD4*, *CASK*, *MPP5* | |

Altogether, 18 risk-enhancing loci were identified, many of which associated only with LUAD. Data from 1.6 million people and 23 732 incident lung cancer cases showed that **body mass index (BMI)** was associated with an overall decreased risk for NSCLC [33]. However, this association varied by histological type, as BMI was associated with lower risk for LUAD and higher risk for LUSC [33]. Hence, risk factor profiles appear profoundly different in LUAD versus LUSC.

## Prognostic Determinants in LUAD versus LUSC

**Prognostic impact** stems from the fundamental mechanics of tumor progression [13,34]. Patient prognosis is mostly assessed using **Kaplan–Meier curves** of disease relapse according to risk factors and associated HR. This analysis identified *TROP2*, a widespread driver of cancer growth [13,34], as having a negative bearing on unselected cases of NSCLC and LUAD [26]. However, Trop-2 expression did not have a negative impact on LUSC, where it is associated with terminal differentiation to cornified cells. This suggested that distinct determinants may be associated with distinct functional states and with differential impact on distinct lung cancer subgroups. A systematic analysis of a large series of genes differentially overexpressed in LUAD versus LUSC [18,22] revealed 69 genes that acted as prognostic determinants. These are summarized in Table 1 and are described in detail by Relli *et al.*[26]. Remarkably, prognostic impacts in LUAD versus LUSC were concordant in only 10% of the cases [26]. This figure is even lower than that of the concordance of impact versus benchmark breast cancers (25% prognostic concordance for LUAD parameters; 31% for LUSC) (Figure 2).

In summary, tumor progression trajectories in LUADs and LUSCs are distinct. Of note, the prognostic impact of individual determinants was often found to be blunted, if not entirely obscured, when LUAD and LUSC were categorized together as NSCLC [26] (Figure 2 and 3A), suggesting inappropriate averaging of starkly heterogeneous tumor parameters. This correspondingly implies that separate classification of LUAD and LUSC may lead to immediate improvement of clinical prognosis assessment procedures.

## Response to Therapy

### Chemotherapy

In 2002, a pivotal study by Schiller *et al.* [35] compared four different chemotherapy regimens for advanced NSCLC. The response rate and survival did not differ significantly between patients assigned to receive any of the four regimens, Based on those results, clinicians generally did not distinguish LUAD and LUSC, since the management was identical. However, in 2011 Scagliotti and colleagues reported a Phase III trial finding that pemetrexed/platinum was superior to gemcitabine/platinum in LUAD and equally inferior in LUSC. This trial formed the clinical basis for distinguishing between the two histologies [36].

More recently, the tumor suppressor FBW7 was found frequently mutated or downregulated in human LUSC, and FBW7-linked LUBAC-mediated NF-κB signaling was identified as a determinant of chemotherapy resistance. Inhibition of NF-κB activation using TAK1 or LUBAC inhibitors resensitized LUSC tumors to cisplatin, suggesting avenues for more effective chemotherapeutic management of LUSCs [37].

### Molecularly Targeted Therapy

Recently, targeted anti-VEGF bevacizumab therapy was found to improve the survival of LUAD-bearing patients [4], whereas it was contraindicated in patients with LUSC because of fatal hemoptysis [4]. By contrast, the anti-EGFR necitumumab was found to be effective only in LUSC [4]. *ALK* rearrangements, *ROS1* fusions, and *BRAF* mutations prevail in LUAD, whereby they provide actionable mutations for targeted therapy [4].

## Clinician's Corner

Lung tumors are classified as small-cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). The usefulness of distinguishing NSCLC from SCLC is clear. The NSCLC classification, however, is raising issues of appropriateness and usefulness, as mounting clinical and experimental data indicate great heterogeneity among NSCLC subtypes.

Vast diversity was found in genetic drivers of cell transformation in adenocarcinomas (LUADs) versus squamous cell lung cancers (LUSCs), suggesting distinct tumor progression trajectories. This was associated with great diversity of gene transcription profiles and of cellular control networks.

Consistent with profound diversity between tumor types, distinct biomarkers, prognostic indicators, and tumor progression paths were found in LUADs versus LUSCs. Correspondingly, joint categorization of LUAD and LUSC as NSCLC was shown to blunt prognostic impact estimates, due to averaging of heterogeneous tumor parameters. Hence, separate classification of LUAD and LUSC is expected to lead to immediate improvement of clinical prognostic determination procedures.

The therapeutic-response profiles of LUAD versus LUSC are correspondingly different. Targetable tyrosine kinase mutations essentially are only present in LUADs. Profoundly different responses of the two NSCLC subtypes to immune checkpoint inhibitors and to pemetrexed-based chemotherapy has also been shown.

Recent influential reviews, therapeutic clinical trials, and current NCCN and WHO guidelines still refer to NSCLC as a tumor classification benchmark. Hence, formal abandonment of the notion of NSCLC appears urgently needed. This is expected to critically help in developing novel, more effective, subtype-specific diagnostic, prognostic, and therapeutic procedures.

ASCO guidelines on systemic therapy for stage IV NSCLC recommended afatinib, erlotinib, or gefitinib for tumors bearing sensitizing EGFR mutations and crizotinib for those with ALK or ROS1 gene rearrangement. In the second-line setting, recommendations include: docetaxel, erlotinib, gefitinib, or pemetrexed for patients with LUAD; docetaxel, erlotinib, or gefitinib for those with LUSC; and chemotherapy or ceritinib for those with ALK rearrangement who experience progression after crizotinib [38].

The efficacy of osimertinib was amply assessed in EGFR T790M-bearing tumors [5–7,39–43]. In the FLAURA trial (NCT02296125), osimertinib showed efficacy superior to that of standard erlotinib/gefitinib in the first-line treatment of EGFR mutation-positive advanced NSCLC, with a similar safety profile and lower rates of serious adverse events [7]. The EGFR T790M mutation is more prevalent in LUAD than in other NSCLCs [39]. However, only a minority of studies on osimertinib utilized subgroup classification for therapy outcome evaluation [6,43]. EGFR mutations are found in 10–15% of patients with NSCLC. However, as essentially all of them occur in LUADs, they can account for up to a quarter of these cases. Hence, a subgroup analysis in NSCLC therapeutic clinical trials, based on both histology and mutation spectrum, is recommended.

### Immune Checkpoint Inhibitors

Immune checkpoint inhibitors, although being effective in multiple cancer types, appear to be differentially active in LUADs versus LUSCs [44], although ongoing large Phase III studies may shed additional light on this issue. Nivolumab, a monoclonal antibody that blocks programmed death 1 (PD-1) proteins, was recently approved by the FDA for use in patients with advanced LUSC. Pembrolizumab, an anti-PD-1 antibody, in combination with pemetrexed, and platinum chemotherapy was recently approved by EMA as first-line treatment of metastatic LUAD.

Atezolizumab in combination with carboplatin/paclitaxel/bevacizumab was recently granted FDA approval in untreated LUAD patients. Consistent, the addition of atezolizumab to bevacizumab plus chemotherapy significantly improved progression-free survival and overall survival among patients with metastatic LUAD, regardless of PD-L1 expression and EGFR or ALK genetic alteration status [45]. It should be noted that no responses were seen on pembrolizumab treatment in EGFR-mutated tumors. As high PD-L1 expression does not exclude the presence of a targetable mutation, if both are present the targetable mutation should thus be treated first.

Of note, durvalumab, an anti-PD-L1 antibody, was recently tested after treatment with chemoradiotherapy [8]. In contrast to the above evidence of subtype specificity of both immune checkpoint inhibitors and pemetrexed [36], as yet no subtype-specific data analyses have been made available in this seminal study, suggesting that reevaluation of current guidelines on the use of the NSCLC categorization is an urgent need.

## Concluding Remarks

A large body of experimental evidence indicates that LUAD and LUSC are vastly distinct diseases at the molecular, pathological, and clinical level. Hence, different diagnostic, prognostic, and therapeutic procedures should be followed in patients bearing LUAD or LUSC. Challenges remain, as adequately powered analyses will be required to assess corresponding parameters in the remaining NSCLC subgroups, the lesser incidence of which has as yet prevented correspondingly detailed analyses. A distinct need is that for large cell carcinomas, because of the severe clinical course of such a disease [1].

We envisage, however, that it will soon be possible to develop molecular signatures that would sharply distinguish among lung cancer subgroups, as driven by distinct clusters of activated

### Outstanding Questions

How can the notion of distinct NSCLC subgroups be best used for to develop novel, more effective therapeutic procedures?

Should clinical trials of lung cancer therapy be requested to adopt cancer subgroup analysis as a prespecified endpoint?

Can dissection of the basic mechanisms of the growth and differentiation of squamous cancers versus adenocarcinomas be used to provide novel molecular targets for diagnosis, prognosis, and therapy?

oncogenes, such as mutated *EGFR*, *ALK*, *ROS1*, *TP53*, *MET*, *BRAF*, *TERT*, *NOTCH*, *FGFR1*, *SOX2*, *PIK3CA*, and others [3,24].

Of note, MET amplification can mediate primary and secondary resistance of EGFR-mutant forms to targeted tyrosine kinase inhibitors [46,47], suggesting benefit for the simultaneous inhibition of the two genes. Correspondingly, combined EGFR and RET inhibition is performed in cases of acquired resistance to osimertinib in EGFR-mutant NSCLC carrying RET fusions [48]. Hence, cluster analysis of lung cancer oncogenic determinants may impart therapeutic indications. Mutated oncogene clusters occur with distinct frequency in LUADs versus LUSCs. It is thus expected that better knowledge of oncogenic drivers of LUAD and LUSC and of corresponding molecular signatures may rapidly lead to much more effective, subgroup-specific therapies.

Finally, as the genetic drivers and tumor control networks at work in LUAD versus LUSC are vastly diverse, a wealth of novel targets is provided for the development of novel, cancer-subgroup focused, molecularly targeted therapies. Hence, abandoning the notion of NSCLC for the adoption of a subtype-centered tumor classification is expected to critically help in developing better personalized diagnostic, prognostic, and therapeutic procedures (see Outstanding Questions).

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## References

1. De Vita, V.T. *et al.* (2015) *De Vita, Hellman & Rosenberg's Cancer: Principles & Practice of Oncology*, Lippincott Williams & Wilkins
2. Semenova, E.A. *et al.* (2015) Origins, genetic landscape, and emerging therapies of small cell lung cancer. *Genes Dev.* 29, 1447–1462
3. Herbst, R.S. *et al.* (2018) The biology and management of non-small cell lung cancer. *Nature* 553, 446–454
4. Kalemkerian, G.P. *et al.* (2018) Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology clinical practice guideline update. *J. Clin. Oncol.* 36, 911–919
5. Rybarczyk-Kasiuchnicz, A. and Ramlau, R. (2018) Current views on molecularly targeted therapy for lung cancer – a review of literature from the last five years. *Kardiochir. Torakochirurgia Pol.* 15, 119–124
6. Bui, K.T. *et al.* (2018) Targeted molecular treatments in non-small cell lung cancer: a clinical guide for oncologists. *J. Clin. Med.* 7, 192
7. Soria, J-C. *et al.* (2018) Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N. Engl. J. Med.* 378, 113–125
8. Antonia, S.J. *et al.* (2018) Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N. Engl. J. Med.* 379, 2342–2350
9. Osmani, L. *et al.* (2017) Current WHO guidelines and the critical role of immunohistochemical markers in the subclassification of non-small cell lung carcinoma (NSCLC): moving from targeted therapy to immunotherapy. *Semin. Cancer Biol.* 52, 103–109
10. Travis, W.D. *et al.* (2015) The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. *J. Thorac. Oncol.* 10, 1243–1260
11. Alberti, S. *et al.* (1994) DNA methylation prevents the amplification of *TROP1*, a tumor associated cell surface antigen gene. *Proc. Natl. Acad. Sci. U. S. A.* 91, 5833–5837
12. Nasr, A.F. *et al.* (2003) Mutations ofTP53 induce loss of DNA methylation and amplification of the *TROP1* gene. *Oncogene* 22, 1668–1677
13. Guerra, E. *et al.* (2016) Trop-2 induces tumor growth through Akt and determines sensitivity to Akt inhibitors. *Clin. Cancer Res.* 22, 4197–4205
14. Sánchez-Danés, A. and Blanpain, C. (2018) Deciphering the cells of origin of squamous cell carcinomas. *Nat. Rev. Cancer* 18, 549–561
15. de Groot, P. and Munden, R.F. (2012) Lung cancer epidemiology, risk factors, and prevention. *Radiol. Clin. N. Am.* 50, 863–876
16. Chen, R. *et al.* (2017) Correlation of clinicopathologic features and lung squamous cell carcinoma subtypes according to the 2015 WHO classification. *Eur. J. Surg. Oncol.* 43, 2308–2314
17. Kadota, K. *et al.* (2014) Comprehensive pathological analyses in lung squamous cell carcinoma: single cell invasion, nuclear diameter, and tumor budding are independent prognostic factors for worse outcomes. *J. Thorac. Oncol.* 9, 1126–1139
18. Charkiewicz, R. *et al.* (2017) Gene expression signature differentiates histology but not progression status of early-stage NSCLC. *Transl. Oncol.* 10, 450–458
19. Girard, L. *et al.* (2016) An expression signature as an aid to the histologic classification of non-small cell lung cancer. *Clin. Cancer Res.* 22, 4880
20. Hou, J. *et al.* (2010) Gene expression-based classification of non-small cell lung carcinomas and survival prediction. *PLoS One* 5, e10312
21. Daraselia, N. *et al.* (2012) Molecular signature and pathway analysis of human primary squamous and adenocarcinoma lung cancers. *Am. J. Cancer Res.* 2, 93–103
22. Liu, J. *et al.* (2014) Identifying differentially expressed genes and pathways in two types of non-small cell lung cancer: adenocarcinoma and squamous cell carcinoma. *Genet. Mol. Res.* 13, 95–102
23. Pallis, A.G. and Syrigos, K.N. (2013) Lung cancer in never smokers: disease characteristics and risk factors. *Crit. Rev. Oncol.Hematol.* 88, 494–503
24. Jamal-Hanjani, M. *et al.* (2017) Tracking the evolution of non-small-cell lung cancer. *N. Engl. J. Med.* 376, 2109–2121
25. Nisman, B. *et al.* (1999) Prognostic value of CYFRA 21-1, TPS and CEA in different histologic types of non-small cell lung cancer. *Anticancer Res.* 19, 3549–3552

26. Relli, V. *et al.* (2018) Distinct lung cancer subtypes associate to distinct drivers of tumor progression. *Oncotarget* 9, 35528–35540
27. Campbell, J.D. *et al.* (2018) Genomic, pathway network, and immunologic features distinguishing squamous carcinomas. *Cell Rep.* 23, 194–212.e196
28. Cao, D.F. *et al.* (2007) Prognostic significance of maspin in pancreatic ductal adenocarcinoma: tissue microarray analysis of 223 surgically resected cases. *Mod. Pathol.* 20, 570–578
29. de Koning, P.J.A. *et al.* (2009) Downregulation of SERPINB13 expression in head and neck squamous cell carcinomas associates with poor clinical outcome. *Int. J. Cancer* 125, 1542–1550
30. Chang, Y-H. *et al.* (2015) Pathway-based gene signatures predicting clinical outcome of lung adenocarcinoma. *Sci. Rep.* 5, 10979
31. Guerra, E. *et al.* (2016) p53, cathepsin D, Bcl-2 are joint prognostic indicators of breast cancer metastatic spreading. *BMC Cancer* 16, 649
32. McKay, J.D. *et al.* (2017) Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes. *Nat. Genet.* 49, 1126–1132
33. Yu, D. *et al.* (2018) Overall and central obesity and risk of lung cancer: a pooled analysis. *J. Natl. Cancer Inst.* 110, 831–842
34. Ambrogi, F. *et al.* (2014) Trop-2 is a determinant of breast cancer survival. *PLoS One* 9, e96993
35. Schiller, J.H. *et al.* (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N. Engl. J. Med.* 346, 92–98
36. Scagliotti, G. *et al.* (2011) Treatment-by-histology interaction analyses in three Phase III trials show superiority of pemetrexed in nonsquamous non-small cell lung cancer. *J. Thorac. Oncol.* 6, 64–70
37. Ruiz, E.J. *et al.* (2019) LUBAC determines chemotherapy resistance in squamous cell lung cancer. *J. Exp. Med.* 216, 450–465
38. Masters, G.A. *et al.* (2015) Systemic therapy for stage IV non-small-cell lung cancer: American Society of Clinical Oncology clinical practice guideline update. *J. Clin. Oncol.* 33, 3488–3515
39. West, H. (2017) Prioritizing molecular markers to test for in the initial workup of advanced non-small cell lung cancer: wants versus needs. *Ann. Transl. Med.* 5, 371
40. Cadranel, J. (2018) Osimertinib in first-line treatment – is a comparison not proof? *Ann. Transl. Med.* 6, 57
41. Wang, N. *et al.* (2019) Osimertinib (AZD9291) increases radiosensitivity in EGFR T790M non-small cell lung cancer. *Oncol. Rep.* 41, 77–86
42. Tan, C-S. *et al.* (2018) Third generation EGFR TKIs: current data and future directions. *Mol. Cancer* 17, 29
43. Ricciuti, B. *et al.* (2017) Osimertinib in patients with advanced epidermal growth factor receptor T790M mutation-positive non-small cell lung cancer: rationale, evidence and place in therapy. *Ther. Adv. Med. Oncol.* 9, 387–404
44. Forde, P.M. *et al.* (2018) Neoadjuvant PD-1 blockade in resectable lung cancer. *N. Engl. J. Med.* 378, 1976–1986
45. Socinski, M.A. *et al.* (2018) Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N. Engl. J. Med.* 378, 2288–2301
46. Camidge, D.R. and Davies, K.D. (2019) MET copy number as a secondary driver of epidermal growth factor receptor tyrosine kinase inhibitor resistance in EGFR-mutant non-small-cell lung cancer. *J. Clin. Oncol.* 37, 855–857
47. Lai, G.G.Y. *et al.* (2019) Clonal MET amplification as a determinant of tyrosine kinase inhibitor resistance in epidermal growth factor receptor-mutant non-small-cell lung cancer. *J. Clin. Oncol.* 37, 876–884
48. Piotrowska, Z. *et al.* (2018) Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov.* 8, 1529–1539
49. Lu, C. *et al.* (2016) Identification of differentially expressed genes between lung adenocarcinoma and lung squamous cell carcinoma by gene expression profiling. *Mol. Med. Rep.* 14, 1483–1490