

Trends in Molecular Medicine – Opinion

## Abandoning the notion of non-small cell lung cancer

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### Author Expertise

V.R. is expert in cancer NGS transcriptome profiling and DNA microarray data meta-analysis in silico and in prognostic profiling of lung cancer subtypes.

M.T. has a long-standing experience in cancer-driving genetic and epigenetic alterations and in the functional analysis of driver-gene interaction networks.

E.G. has identified key cancer drivers and regulatory signalling pathways, during normal development and in transformed tissues. E.G. has extensive expertise in cluster analysis of determinants of cancer progression with prognostic impact in cancer patients.

S.A. has discovered novel classes of oncogenes and of metastatic drivers, together with novel epigenetic and post-transcriptional regulatory mechanisms of tumor progression. S.A. has long-standing expertise in the prognostic analysis of large cancer patient case series and in the development of targeted anti-cancer therapies.

**Keywords:** lung cancer, prognosis, therapy, tumor classification.

34   **Abstract**

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

44

## 45 Glossary

46 **Body mass index (BMI):** body weight divided by the square of body height. The BMI quantifies  
47 the amount of different tissue components (muscle, fat, and bone), for categorizing that person as  
48 underweight (under 18.5 kg/m<sup>2</sup>), normal weight (18.5 to 25), overweight (25 to 30) or obese (over  
49 30).

50 **Control pathways:** signal transduction cascades, through which individual genes exert their  
51 effects. Control pathways are non-linear, and converge into networks of multiple, intertwined  
52 signaling paths. Key components of such networks are represented as nodes. Node-node  
53 interactions are represented by connecting lines.

54 **Disease classification:** disease classification consolidates knowledge on disease origin,  
55 pathogenetic mechanism, natural history and response to therapy. This body of knowledge is  
56 utilized to classify diseases as separate entities.

57 **Hazard ratio (HR):** the ratio of the frequency of adverse events in the two subgroups under  
58 comparison.

59 **Immunohistochemistry (IHC):** procedure for detecting antigens in tissue sections through  
60 antibody binding. Antibody-bound enzymes, such as horseradish peroxidase or alkaline  
61 phosphatase, are used to catalyze a color-producing reaction, which can be visualized and  
62 quantified under the microscope.

63 **Kaplan-Meier curves:** disease relapse curves, which indicate the time of any adverse event and  
64 compute the remaining cases as a percentage of patients that remain alive or disease-free at any  
65 given time. Kaplan-Meier curves depict cancer biological history as a cascade of disease events  
66 over time.

67 **Prognostic impact:** specific genetic changes or protein/mRNA biomarkers can show association to  
68 distinct cancer groups or to disease severity. The intensity of such association quantifies their  
69 impact on disease prognosis.

70 **Transcriptome:** the ensemble of all RNAs transcribed by the genome in a specific tissue or cell  
71 type. Transcriptome analysis, whether by RNA sequencing or DNA array hybridization, thus  
72 provides quantitative details on the transcription of all expressed genes. This information is utilized  
73 to infer gene function and gene expression regulation.

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76

## 77 **Lung cancer identity**

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

84 On the other hand, it is unclear whether NSCLC classifications may effectively categorize  
85 heterogeneous tumor subgroups and guide corresponding therapeutic strategies [4-6].

86 Recent influential reviews [3], key therapeutic clinical trials [7, 8], latest NCCN guidelines  
87 (V.3 2019, Feb. 12, 2019) ([www.nccn.org/professionals/physician\\_gls/pdf/nscl\\_blocks.pdf](http://www.nccn.org/professionals/physician_gls/pdf/nscl_blocks.pdf)) and the  
88 current WHO classification of lung cancer [9, 10] still refer to NSCLC as a tumor classification  
89 benchmark (Box 1). On the other hand, experimental and clinical evidence is accumulating, that  
90 indicates profound dishomogeneity among NSCLC subtypes, calling into question the founding  
91 reason for their joint categorization as NSCLC.

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

98

## 99 **Lung cancer fundamentals**

100 SCLC show rapid growth and can develop paraneoplastic syndromes, such as Cushing's disease,  
101 carcinoid syndrome, inappropriate production of hormones, neurodegenerative diseases, such as  
102 progressive multifocal leukoencephalopathy and subacute cerebellar degeneration. SCLC almost  
103 exclusively occur in smokers. Extensive exposure to carcinogens from tobacco smoke induces a  
104 high mutational load. *TP53* mutations occur in 75-90% of SCLC, and associate with frequent DNA  
105 amplifications and deletions [11, 12], including a nearly obligate loss of *RBI*. Loss of PTEN and  
106 activation of PI3K [13] are found in a substantial fraction of cases [2]. Most SCLC are already  
107 metastatic at presentation and require to be managed primarily by chemotherapy and radiotherapy.

108 NSCLC that are localized at the time of presentation can undergo surgery or radiotherapy  
109 with curative intent. On the other hand, NSCLC do not respond to chemotherapy as well as SCLC  
110 do. NSCLC encompass multiple cancer types, such as adenocarcinomas (**LUAD**), squamous cell

111 (LUSC) and large cell cancers, or mixed histotypes. Among them, LUAD and LUSC represent the  
112 largest subgroups [1]. LUAD account for 40% of lung cancers. LUSC squamous cancers represent  
113 about 25-30% of all lung cancers.

114

### 115 **Histopathology of LUAD and LUSC**

116 LUAD originate from cells that secrete surfactant components. Morphologic patterns of LUAD  
117 include acinar, papillary, solid, micropapillary and invasive-mucinous types. Lepidic components or  
118 pure lepidic patterns stand for noninvasive forms, previously classified as bronchoalveolar  
119 carcinoma, which can be associated to an invasive mucinous or acinar LUAD. Less frequently,  
120 LUAD show colloid, fetal or enteric features. When adenocarcinoma morphology patterns are not  
121 clearly apparent, diagnosis can be supported by staining for thyroid transcription factor 1 (TTF-1)  
122 or napsin-A, both of which show approximate 80% sensitivity for LUAD identification.

123 On the other hand, LUSC originate from cells which line the inside of lung airways. WHO  
124 reclassified LUSC into keratinizing, nonkeratinizing, and basaloid subtypes [10]. LUSC diagnosis  
125 is based on the presence of squamous cell patterns, keratinization and intercellular bridges [14].  
126 When such patterns are not present, LUSC diagnosis can be supported by staining for p40, p63 or  
127 cytokeratin 5/6, and the lesion is classified as a non-keratinizing LUSC.

128

### 129 **Clinicopathological features of LUAD and LUSC**

130 Although LUAD can occur in smokers, this is the most common type of lung cancer seen in non-  
131 smokers. It is more common in women than in men, and it is more likely to occur in younger people  
132 than other types of lung cancer, and to present at more advanced stages of disease [15]. In the past  
133 25 years, for unknown reasons, LUAD have replaced LUSC as the most frequent histologic  
134 subtype.

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

139 Extrathoracic metastatic disease is found at autopsy in  $\approx 50\%$  of patients with LUSC, 80% of  
140 patients with LUAD and large cell carcinomas, versus  $>95\%$  of patients with SCLC.

141

### 142 **Transcriptomic profiles of LUAD versus LUSC**

143 Whole **transcriptome** analysis, through RNA sequencing or array hybridization, provides a  
144 quantitative measure of actual transcription rates of all expressed genes. This information allows to  
145 gain insight into gene function and gene expression regulation, which, in turn, associate to the

146 biological processes that trigger the underlying disease. Correspondingly, distinct transcriptomes  
147 faithfully correlate to distinct tumor types [1].

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

153 Whole-transcription profiling thus indicates vast diversity in LUAD versus LUSC. These  
154 findings are cornerstone for differential classification of LUAD and LUSC as distinct diseases.  
155 They also indicate distinct regulatory settings for tumor progression pathways in LUAD versus  
156 LUSC, such as for regulation of cell proliferation and tissue invasion, which may have direct impact  
157 on the course of the disease.

158

### 159 **Driver genetic changes in LUAD versus LUSC**

160 Distinct **driver genetic changes** associate to distinct neoplastic diseases [1]. In lung cancers, the  
161 type of mutated oncogene and the cells of origin dictate LUAD versus LUSC formation, tumor  
162 aggressiveness and invasive capacity. Recent studies have identified several single nucleotide  
163 polymorphisms associated with increased risk for lung cancer development in never smokers, which  
164 are mostly LUAD [23]. EGFR mutations and EML4-ALK rearrangements were more frequently  
165 associated with LUAD in nonsmokers [15]. Overall, mutations in receptor tyrosine kinases were  
166 frequent in LUAD, but rare in LUSC [3]. Systematic analysis of mutated tumor genes [24]  
167 identified distinct determinants in LUAD (*EGFR*, *MET*, *BRAF*, *TERT*) versus LUSC (*NOTCH*  
168 mutations and amplification of *FGFR1*, *SOX2*, *PIK3CA*). Late evolutionary genetic changes  
169 appeared correspondingly distinct [3, 24], indicating distinct tumor progression trajectories. A  
170 notable example is that of the mutated tumor suppressor gene *TP53* [3], which is frequently found  
171 at early stages in LUSC, but only at late stages in LUAD, suggesting a distinct role of *TP53* during  
172 progression of the two tumor histotypes.

173

### 174 **Control pathways and signaling networks**

175 Distinct cancer types associate to differential cell **control pathways** [1, 4]. Keratins and other  
176 cytoskeletal components take part to terminal differentiation of cornified epithelia [14].  
177 Correspondingly, *KRT5*, *KRT6A* were shown to associate to better prognosis in LUSC. On the other  
178 hand, overexpression of most keratins was shown to associate to tumor progression in LUAD  
179 (Figure 2) [25], whether by inference with differentiation processes or through perturbation of  
180 regulation of tumor stem cells [14]. A driving p53/p63/p73 axis was found strongly associated to

181 LUSC [26, 27], but not to LUAD (Figure 3). Notably, invasion determinants, such as *SERPINS*,  
182 distinctly associated to lung cancer subtypes. *SERPINB5* overexpression associates to bad prognosis  
183 of adenocarcinomas, such as LUAD and ductal pancreatic adenocarcinomas [26, 28]. *SERPINB13*  
184 down regulation associates with decreased survival in squamous tumors, such as LUSC and head  
185 and neck cancers [26, 29].

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

195

## 196 **Risk factors**

197 Many risk factors are linked to the development of lung cancer, e.g. smoking, lung infections (HPV  
198 and *Mycobacterium tuberculosis*), hormonal factors, diabetes mellitus, radon exposure,  
199 occupational/domestic exposure to carcinogens and pre-existing lung disease [1, 15]. Main ones  
200 among them are smoking and second-hand smoking [23]. LUSC are tightly linked to a history of  
201 smoking.

202 McKay et al. [32] performed a genome-wide SNP lung cancer association study in 29,266  
203 cases and 56,450 controls. They found a strikingly different genetic architecture in LUAD versus  
204 LUSC. Altogether, 18 risk-enhancing loci were identified, many of which only associated with  
205 LUAD. Data from 1.6 million people and 23,732 incident lung cancer cases showed that body mass  
206 index (BMI) associated with an overall decreased risk for NSCLC [33]. However, this associations  
207 varied by histological type, as BMI associated with lower risk for LUAD and with a higher risk for  
208 LUSC [33]. Hence, risk factor profiles appear profoundly different in LUAD versus LUSC.

209

## 210 **Prognostic determinants in LUAD versus LUSC**

211 **Prognostic impact** stems from fundamental mechanics of tumor progression [13, 34]. Patient  
212 prognosis is mostly assessed using **Kaplan-Meier curves** of disease relapse according to risk  
213 factors and associated **hazard ratios** (HR). This analysis identified *TROP2*, a widespread driver of  
214 cancer growth [13, 34], as having a negative bearing on unselected cases of NSCLC and LUAD  
215 [26]. On the other hand, Trop-2 expression did not have a negative impact on LUSC, where it

216 associates to terminal differentiation to cornified cells. This suggested that distinct determinants  
217 may associate to distinct functional states and to differential impact on distinct lung cancer  
218 subgroups. A systematic analysis of a large series of genes differentially overexpressed in LUAD  
219 versus LUSC [18, 22] revealed 69 genes, that acted as prognostic determinants. These are  
220 summarized in Table 1 and are described in detail in Relli et al. [26]. Remarkably, prognostic  
221 impacts in LUAD versus LUSC were only concordant in 10% of the cases [26]. This figure is even  
222 lower than that of concordant impact versus benchmark breast cancers (25% of prognostic  
223 concordance for LUAD parameters; 31% for LUSC) (Figure 2).

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

230

## 231 **Response to therapy**

### 232 *Chemotherapy*

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

240 More recently, the tumor suppressor FBW7 was found frequently mutated or down-  
241 regulated in human LUSC, and FBW7-linked LUBAC-mediated NF- $\kappa$ B signaling was identified as  
242 a determinant of chemotherapy resistance. Inhibition of NF- $\kappa$ B activation using TAK1 or LUBAC  
243 inhibitors resensitized LUSC tumors to cisplatin, suggesting avenues for more effective  
244 chemotherapeutic management of LUSC [37].

245

### 246 *Molecular-targeted therapy*

247 Recently, targeted anti-VEGF bevacizumab therapy was found to improve the survival of LUAD-  
248 bearing patients [4], whereas it ended up as contraindicated in patients with LUSC because of fatal  
249 hemoptysis [4]. On the other hand, the anti-EGFR necitumumab was only found effective in LUSC

[4]. *ALK* rearrangements, *ROS1* fusions and *BRAF* mutations prevail in LUAD, whereby they provide actionable mutations for targeted therapy [4].

ASCO guidelines on systemic therapy for stage IV NSCLC recommended afatinib, erlotinib, or gefitinib for tumors bearing sensitizing EGFR mutations; 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].

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 NSCLC [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 LUAD, they can account for up to a quarter of these cases. Hence, a subgroup analysis in NSCLC therapeutic clinical trials, as based on both histology and mutation spectrum, is recommended.

#### *Immune-checkpoint inhibitors*

Immune-checkpoint inhibitors, albeit being effective in multiple cancer types, appear differentially active in LUAD versus LUSC [44], though 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 upon 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 with the above evidence of subtype specificity of both immune

285 checkpoint inhibitors and pemetrexed [36], as yet no subtype-specific data analyses have been made  
286 available in this seminal study, suggesting that reevaluation of current guidelines on the use of the  
287 NSCLC categorization is an urgent need.

288

289 **Concluding Remarks**

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

297 We envisage, though, that it will soon be possible to develop molecular signatures that  
298 would sharply distinguish among lung cancer subgroups, as driven by distinct clusters of activated  
299 oncogenes, such as mutated *EGFR*, *ALK*, *ROS1*, *TP53*, *MET*, *BRAF*, *TERT*, *NOTCH*, *FGFR1*,  
300 *SOX2*, *PIK3CA* and others [3, 24].

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

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

314

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321

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438

439      **Table 1. Genes sets identified as differentially expressed in LUAD versus LUSC**

Category	Expression/Cell function	Genes	Refs.
<b>Diagnostic determinants by differential expression</b>	Expression in LUAD	<i>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</i>	[18]
	Expression in LUSC	<i>TMEM125, NKX2-1/TTF1, CLDN3, KCNK5, TMC5, CGN, ACSL5, TESC, FOLR1, RORC, QSOX1, KRT7, SFTA3, CEACAM6, ATP11A, PLEKHA6</i>	
<b>Top discriminants of LUAD versus LUSC</b>	Omnibus gene expression profiles	<i>HSP90AA1, BCL2, CDK2, KIT, HDAC2</i>	[49]
	Gene interaction networks	<i>E2F, CTGF, PDGF</i>	[21]:
<b>Pathway-based diagnostic gene signatures</b>	Regulation of Epidermis development	<i>HsT19447, COL7A1, KRT5, KRT14, KRT17, PTHLH, GRHL3</i>	[19, 20, 22]
	Regulation of intermediate filament components	<i>KRT5, KRT6A, KRT14, KRT17, PPKNEFD</i>	
	Regulation of Exosome formation	<i>ATP1B3, CSTA, DSG3, YWHAS, GPC1, KRT5, KRT6A, KRT6B, KRT14, KRT17, SERPINB5, SERPINB13, SLC2A1, TMPRSS11D, PPKNEFD</i>	
	Regulation of cell proliferation	<i>IGF1R, GSK3B, ATR, SKP2, CDK1, CDK2, SMC3, PLK1, CCND3</i>	
	Regulation of DNA replication and repair	<i>RFC2, PRIM2, MCM4, MCM5, ATR</i>	
	Regulation of RNA splicing	<i>PRPF19, SRSF2, THOC4</i>	
	Regulation of cell-cell junction formation	<i>TGFBR2, CTNND1, CKD4, CASK, MPP5</i>	

440

441 **Figure legends**

442 **Figure 1. Key Figure. NSCLC subtype-feature identification.**

443 *Top: block diagrams of comparative analyses of NSCLC subgroups for diagnostic, prognostic and*  
444 *therapeutic procedures.*

445 Transcriptomics: DNA array analysis flow chart; Genomics: gene mutation sequence analysis  
446 (*TP53* mutation chromatogram; wild-type: blue peak; mutated: red peak); Histopathology  
447 expression pattern of diagnostic/prognostic proteins in adenocarcinomas (LUAD) versus squamous  
448 cell carcinomas (LUSC) ([www.proteinatlas.org](http://www.proteinatlas.org)) (GLUT1 protein staining).

449 *Bottom: tumor-subtype-specific subgrouping.*

450 Prognostic profile: Schematics of Kaplan-Meier survival curves for high (red) versus low (black)  
451 target gene expression (*DSG3* mRNA). Interaction networks: protein-protein interaction networks  
452 of overexpressed genes in LUAD versus LUSC.

453

454 **Figure 2. Analysis of prognostic determinants in LUAD and LUSC versus breast cancer.**

455 (A) Representative examples of Kaplan-Meier survival curves of LUAD, LUSC and breast cancer-  
456 bearing patients, as obtained from Relli et al. [26]. Correlation between survival curves and tumor  
457 histology was computed. Tumor samples were analyzed for mRNA levels for each of the markers  
458 analyzed by DNA microarray hybridization or next-generation sequencing. Histopathology data and  
459 immunohistochemistry analysis of randomly selected subsets of individual tumors were utilized for  
460 validation of gene expression at the protein level. Patient survival was compared for cases that  
461 showed high (red) versus low (black) tumor expression of the genes indicated on the right. Median  
462 survival, hazard ratios and correlated P values are indicated.

463 *TMPRSS1D* is a favorable prognostic determinant for LUSC, that shows corresponding impact on  
464 breast cancer, but not on LUAD.

465 *ACSL5* is a favorable prognostic determinants for LUAD, which shows corresponding impact on  
466 breast cancer, but not on LUSC.

467 *CLDN3* is an unfavorable prognostic determinants for LUAD, which show corresponding impact  
468 on breast cancer, but not on LUSC.

469 (B) Bar plots show the hazard ratio/prognostic impact of the genes indicated on LUAD, LUSC and  
470 benchmark breast cancers, as computed in Relli et al. [26].

471 (top) diagnostic genes for LUSC.

472 (bottom) diagnostic genes for LUAD.

473 The genes are listed in alphabetical order. The red bars indicate hazard ratios = 1. The graphs are  
474 plotted on a log scale.

475

476 **Figure 3. LUAD versus LUSC control gene networks.**  
477 (A) Prognostic determinant sets in LUAD versus LUSC. Genes with positive prognostic impact are  
478 highlighted in red, tumor progression determinants are in blue.  
479 (B) Graphical representation of control gene networks of LUAD versus LUSC, as modified from  
480 Relli et al. [26]. Genes are represented as nodes, biological relationships between nodes are  
481 represented as lines (network edges). Direct relationships, solid lines; indirect relationships, dashed  
482 lines. Shared determinants between LUSC and LUAD networks are highlighted in red; genes  
483 identified in LUSC are in blue; gene interactors are in white.  
484  
485

486 **Clinician's Corner (Box 1)**  
487

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

492  
493 Vast diversity was found in genetic drivers of cell transformation in adenocarcinomas (LUAD)  
494 versus squamous cell lung cancers (LUSC), suggesting distinct tumor progression trajectories. This  
495 associated to great diversity of gene transcription profiles and of cellular control networks.

496  
497 Consistent with profound diversity between tumor types, distinct biomarkers, prognostic indicators  
498 and tumor progression paths were found in LUAD versus LUSC. Correspondingly, joint  
499 categorization of LUAD and LUSC as NSCLC was shown to blunt prognostic impact estimates,  
500 due to averaging of heterogeneous tumor parameters. Hence, separate classification of LUAD and  
501 LUSC is expected to lead to immediate improvement of clinical prognostic determination  
502 procedures.

503  
504 The therapeutic-response profiles of LUAD versus LUSC are correspondingly different. Targetable  
505 tyrosine kinase mutations essentially are only present in LUAD. Profoundly different response of  
506 the two NSCLC subtypes to immune check-point inhibitors and to pemetrexed-based chemotherapy  
507 has also been shown.

508  
509 Recent influential reviews, therapeutic clinical trials, and current NCCN and WHO guidelines still  
510 refer to NSCLC as a tumor classification benchmark. Hence, formally abandoning the notion of  
511 NSCLC appears urgently needed. This is expected to critically help develop novel, more effective,  
512 subtype-specific diagnostic, prognostic and therapeutic procedures.

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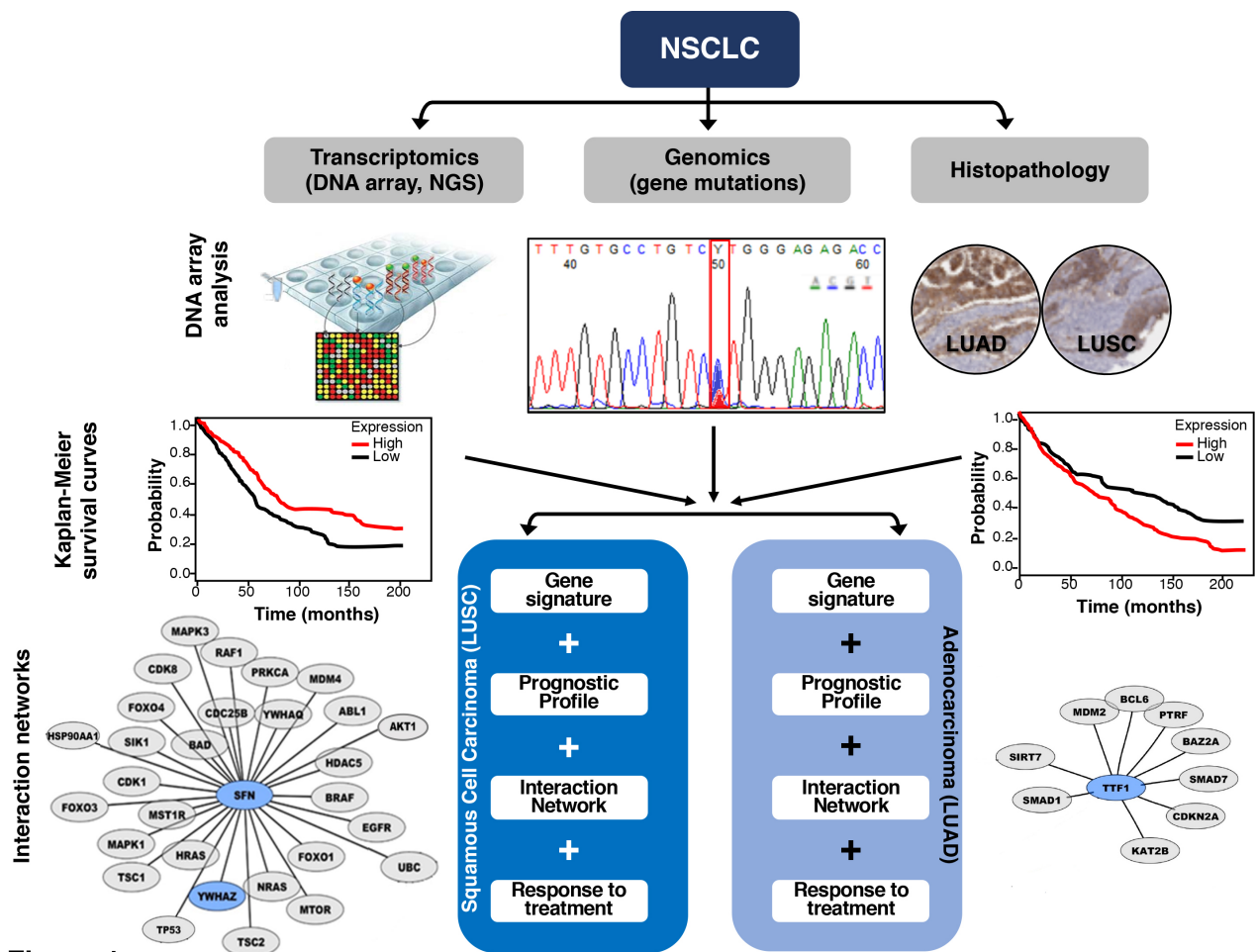


Figure 1

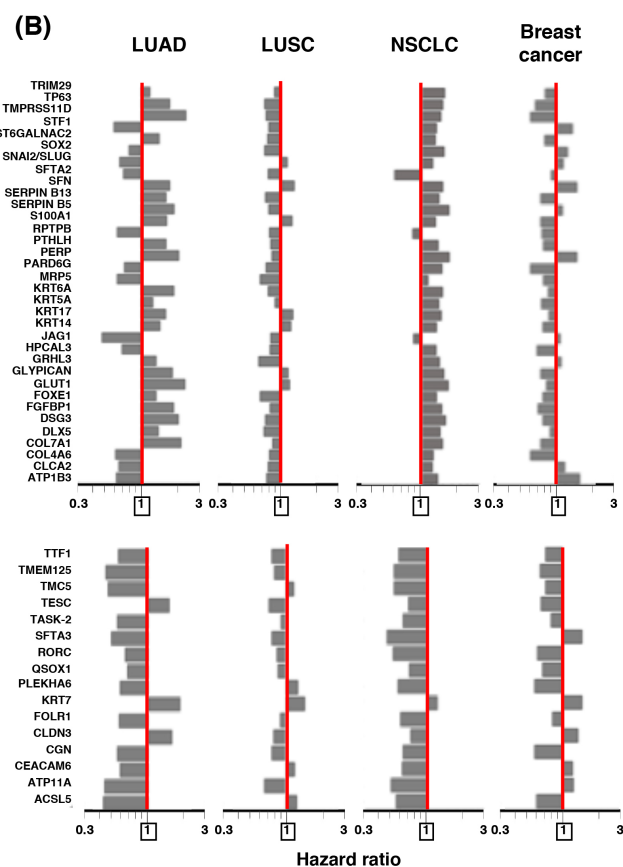
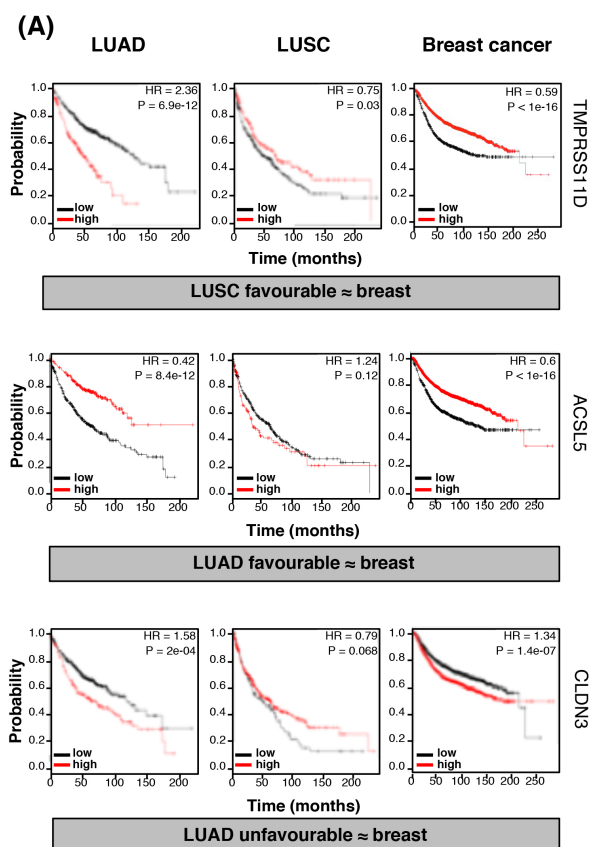


Figure 2

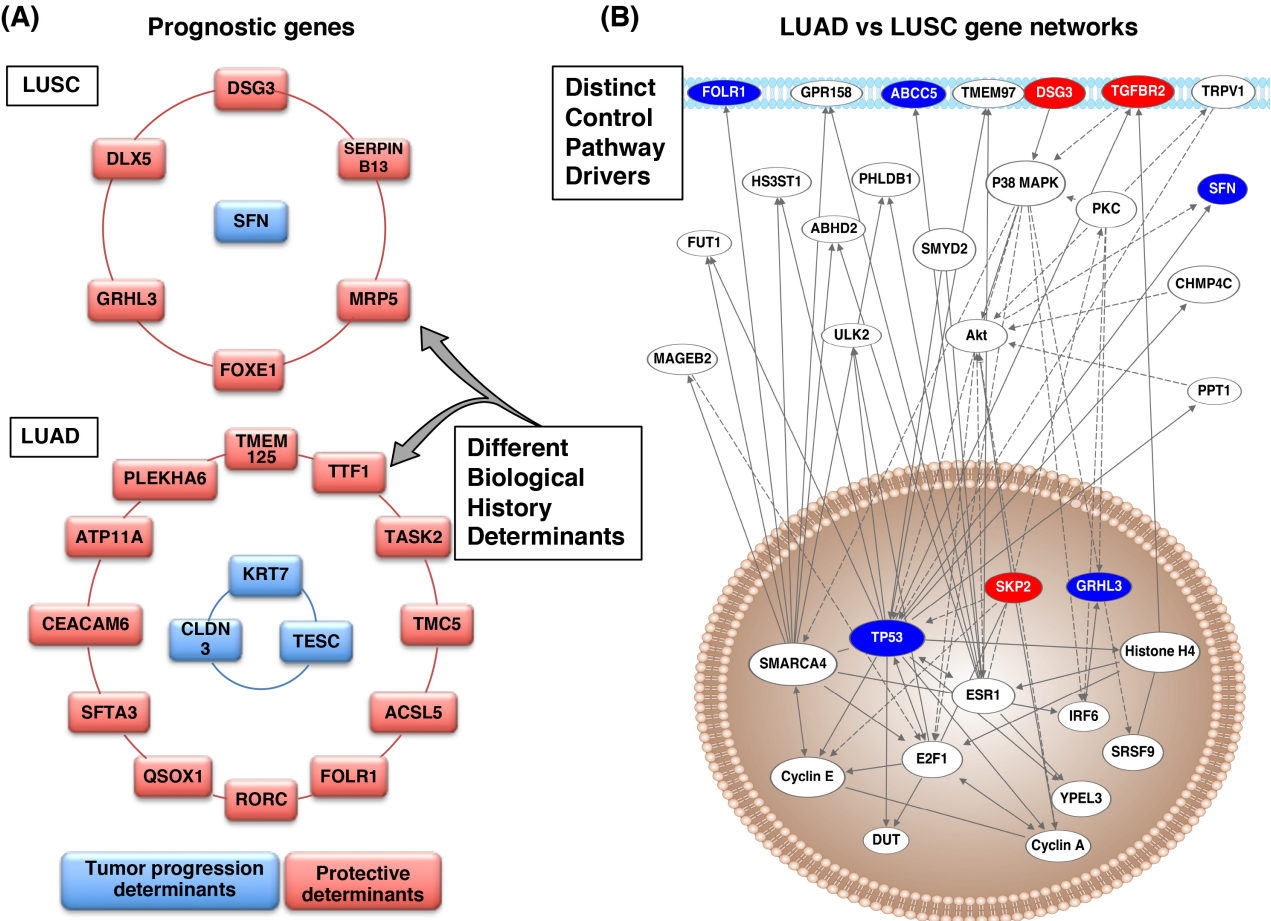


Figure 3