Molecular Pathology of Lung Cancer

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This overview of the molecular pathology of lung cancer includes a review of the most salient molecular alterations of the genome, transcriptome, and the epigenome. The insights provided by the growing use of next-generation sequencing (NGS) in lung cancer will be discussed, and interrelated concepts such as intertumor heterogeneity, intratumor heterogeneity, tumor mutational burden, and the advent of liquid biopsy will be explored. Moreover, this work describes how the evolving field of molecular pathology refines the understanding of different histologic phenotypes of non-small-cell lung cancer (NSCLC) and the underlying biology of small-cell lung cancer. This review will provide an appreciation for how ongoing scientific findings and technologic advances in molecular pathology are crucial for development of biomarkers, therapeutic agents, clinical trials, and ultimately improved patient care.

The goal of this review is to provide a focused review of the most clinically relevant aspects of thoracic molecular pathology. Historically, genomic alterations (GAs) that were considered drivers in lung cancer were interrogated in panels using an à la carte approach with techniques such as Sanger sequencing, pyrosequencing, immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH), which limited reportable GAs to select genes (Yu et al. 2009; Kim et al. 2013a; Sholl et al. 2013). Mutations in epidermal growth factor receptor (*EGFR*) and translocations in the anaplastic lymphoma kinase (*ALK*) gene and the *ROS1* gene were the first three alterations recognized as clinically actionable drivers, and comprised the majority of the initial clinical genetic testing in lung cancer (Gaughan and Costa 2011).

As the use of next-generation sequencing (NGS) became more cost-effective and widely used, it revealed additional actionable GAs. Evidence has accumulated to prove that NGS is an accurate technology with performance that is concordant or better than conventional methods (Hinrichs et al. 2015; Ali et al. 2016). Advances in information about actionable GAs from comprehensive genomic profiling (CGP) has propelled precision medicine forward with the aim to provide evidence-based interpretation of GAs for targeted therapy (Coco et al. 2015; Suh et al. 2016).

The increase in the number of genes and GAs covered by NGS has expanded the variety of GAs identified as actionable in lung cancer. A shift toward NGS-based testing in advanced lung cancer has decreased the number of lung

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cancer cases where a driver is not identified. Increasingly, case reports describe patients with novel drivers identified by NGS that would not have been identified if they were evaluated solely by hotspot-based panels (Capelletti et al. 2014).

Historically, lung cancer was divided categorically into non-small-cell lung cancer (NSCLC) (∼85%) and small-cell lung cancer (SCLC) (∼15%) by pathologist review of hematoxylin and eosin (H&E) stained slides. NSCLC is a heterogeneous category of entities that includes lung adenocarcinoma (LUAD) as the most common histologic cancer type (∼50%), squamous cell carcinoma of the lung (SqCCL) as the second most common type (∼30%), large-cell lung carcinoma (LCLC) (∼10%), and combinations of histologic phenotypes such as adenosquamous carcinoma (ASC), and rare histologic entities, such as atypical carcinoid (AC) tumor, bronchial gland carcinoma, and sarcomatoid carcinoma, collectively accounting for the remaining types of NSCLC (∼10%) (Pikor et al. 2013; Chan and Hughes 2015; Stevic and Milenkovic 2016; Chirieac and Kobzik 2017; Li and Lu 2018; Melosky 2018; Weissferdt 2018).

In clinical practice, lung cancer patients typically undergo an initial biopsy to determine whether there are histomorphologic features on H&E that are able to diagnostically distinguish between NSCLC or SCLC. Performance of IHC typically will assist in determining the subtypes of NSCLC. IHC that favors NSCLC that is LUAD is typically positive for CK7 and TTF-1, and negative for p63, CK5/6, and p40. IHC that favors NSCLC that is SqCCL is typically positive for p63, CK5/6, and p40 and negative for TTF-1 and CK7. The art of establishing a diagnosis may be challenging as many pathologists are aware that, in practice, some tumors might not have "read the book" regarding conventional IHC staining patterns. In such cases, other special stains such as mucin can assist (e.g., mucin is typically positive for LUAD, and negative for SqCCL). ASCs are a rarely encountered tumor that is biphasic, meaning there is a component that is LUAD and a component that is SqCCL. These components by definition have at least 10% each of malignant squamous and glandular components. Neuroendocrine IHC markers (CD56, chromogranin, and synaptophysin) are typically positive for neuroendocrine carcinomas such as SCLC and LCLC. In NSCLC, the tumor proportion score (TPS) for programmed death ligand-1 (PD-L1) as determined by IHC (e.g., PD-L1 IHC 22C3 pharmDx) assists in determining eligibility for immunotherapy. While tissue is submitted for NGS, rapid testing for mutations in *EGFR*, *KRAS*, and *BRAF* are selectively tested via a rapid method such as pyrosequencing, while rearrangements in *ALK* and *ROS1* can be rapid identified via FISH. When NGS results are made available, these results can corroborate the results of rapid testing, and potentially provide other GAs that may be actionable targets.

The Centers for Medicare & Medicaid Services (CMS) regulates all laboratory testing via the Clinical Laboratory Improvement Amendments of 1988 (CLIA) certification process. Commercial laboratories and leading cancer centers that develop their molecular panels for obtaining analyses to guide patient care must have laboratories that are CLIA-certified. For instance, the PD-L1 IHC 22C3 pharmDx assay was approved by the FDA for identifying NSCLC patients eligible (TPS $\geq$ 1%) for treatment with pembrolizumab, an assay available at NeoGenomics Laboratories, which is CLIA-certified.

Particularly in lung biopsies, there is a balance between the need to acquire adequate tissue for H&E, IHC, rapid testing, and/or NGS, which are weighed against mitigating the risk of adverse events such as iatrogenic pneumothorax. A discussion between the clinician and patient must occur to communicate the risk balanced with the diagnostic benefit gained from a biopsy to determine that the patient is clearly providing an informed consent to undergo a procedure with the goal to retrieve tissue for diagnostic purposes.

Given the sheer volume, breadth, and rapid evolution of current literature describing the molecular pathology of lung cancer to date, the intention of this review is to distill only the most salient points to complement the other reviews in this collection. One general caveat about our understanding of lung molecular pathology is that clear molecular distinctions be-

tween different subtypes of lung cancer is complicated by some studies that molecularly characterize NSCLC without distinguishing between histologic types.

Herein, we describe the genomic, transcriptomic, epigenomic, and proteomic differences between different histologic types of lung cancer with relevance to research and therapeutic strategies.

ONCOGENOTYPES

Oncogenotypes are molecular alterations that include genomic mutations, alterations that cause dysregulation in mRNA expression, dysregulation in expression of miRs, and epigenomic changes.

Mutations

As NGS-based DNA sequencing has become increasingly available, the knowledge about different types of mutations that are potentially actionable beyond the genetic biomarkers with associated FDA-approved targeted therapies has expanded (Chan and Hughes 2015; Tafe et al. 2016). An oncogene is a gene in which activating mutations confer gain-of-function to a gene, which ultimately promotes oncogenesis. Conversely, a tumor-suppressor gene (TSG) is a gene in which inactivating mutations confer loss-of-function to a gene, which ultimately promotes oncogenesis. Significant oncogenes and TSGs in lung cancer are summarized in Table 1.

Molecular characteristics of lung cancer are critical for patient care and are included in the National Comprehensive Cancer Network (NCCN) guidelines (National Comprehensive Cancer Network 2021). They are also fundamental for epidemiological studies, such as genome-wide association studies (GWAS) (Chang et al. 2015; Schabath et al. 2016; Inamura 2017; Bossé and Amos 2018; de Groot et al. 2018; Li et al. 2019b; Tam et al. 2019; Tang et al. 2019). The advancement of the use of clinically relevant GAs (CRGAs) for patient care is the consequence of evidence-based studies and clinical trials.

Efforts to characterize the diversity of GAs recurrently observed in lung cancer are motivated by the clinical need to determine the likelihood of response to specific therapies based on specific lung cancer genotypes (Shim et al. 2017). An advantage of NGS testing is that it can identify GAs, such as NTRK fusions that are rare in lung cancer, but have proven benefits in other tumor types (Tafe et al. 2016).

Table 1. Summary of reported oncogenes and tumor-suppressor genes in lung cancer

Oncogenes		Tumor-suppressor genes
AKT1	*KIT*	*BRCA2*
AKT2	*KRAS*	*CDKN2A*
ALK	*MET*	*KEAP1*
BRAF	*NRAS*	*MLL2*
DDR2	*NRF2*	*NF1*
EGFR	*NTRK1/2/3*	*PTEN*
ERBB2	*PIK3CA*	*RB1*
FGFR1	*RET*	*STK11*
HRAS	*RICTOR*	*TP53*
JAK3	*ROS1*	*TSC1*
	SOX2	*VHL1*

Oncogenes are depicted in red to signify promotion of oncogenesis when overexpressed. Tumor-suppressor genes are depicted in green to signify promotion of oncogenesis with loss of expression. Of note, some genes have the dichotomous capability of being oncogenic or tumor-suppressive depending upon the context, such as the nature of the dysregulated signaling pathways, tumor type, or cell type.

The interpretation of NGS-based test results to determine the most appropriate course of therapy often requires a review of the most recent literature and guidelines. Many current clinical trials for patients with lung cancer require knowledge about mutated or wild-type genes for eligibility.

As an overall class, tyrosine kinase inhibitors (TKIs) have been effective as targeted therapies for a variety of genotypes of NSCLC. Different TKIs have activity specifically targeted for mutations in *EGFR*, *ERBB2 (HER2)*, *BRAF*, and rearrangements in *ALK*, *RET*, and *ROS1*. The most common genes with clinically relevant GAs are summarized below.

EGFR Family

In LUAD, the most clinically relevant and prevalent GAs are in exons 19 and 21 of the *EGFR*

gene. The most prevalent and well characterized of these are *EGFR* exon 19 deletions and the L858R mutation in exon 21 (Sholl et al. 2009; Yu et al. 2009). These GAs are predictive of response to targeted therapy with *EGFR*-targeted TKIs, such as osimertinib, gefitinib, erlotinib, and afatinib, to abrogate the constitutive TKI-activating effect conferred by these *EGFR* mutations. Osimertinib was historically provided once patients developed *EGFR* T790M as a resistance mechanism to the other *EGFR* inhibitors; however, more recently, the phase III FLAURA study established osimertinib as first-line standard of care (SoC) for *EGFR*-mutated NSCLC in patients with locally advanced or metastatic NSCLC (Nan et al. 2017; Soria et al. 2018). In the FLAURA trial, frontline use of osimertinib was associated with better outcome than other *EGFR* TKIs (erlotinib or gefitinib). Frontline use of osimertinib results in a different portfolio of resistance mutations, such as *EGFR* C797S and *MET* amplification, which will be discussed in more depth later in the context of therapy-induced tumor heterogeneity (Sequist et al. 2011).

Other drivers in the *EGFR* family include *ERBB2 (HER2)* alterations, predominantly exon 20 insertions, but also point mutations, and amplifications, and *VEGFR* amplifications. *ERBB2* (*HER2*) up-regulation is typically associated with low PD-L1 expression; whereas *VEGFR* up-regulation correlates positively with PD-L1 expression, although this has been mainly characterized in other cancer types (Xue et al. 2017). Monocertinib and amivantamab are FDA approved for NSCLC with *ERBB2* exon 20 insertions. Monoclonal antibodies that target *VEGFR* include bevacizumab and ramucirumab (Remon et al. 2019).

ALK Rearrangements

In 3% to 7% of NSCLC, carcinogenesis is driven by a translocation that causes the fusion of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene promoter region, with the *ALK* gene tyrosine kinase domain known as the *EML4-ALK* fusion oncogene (Shroff et al. 2018). *EML4-ALK* fusions are more prevalent in younger (≤35 yr) patients with LUAD (16.9%) and in patients with a never-smoker history (Chen et al. 2019). Current FDA-approved TKIs that target *ALK* fusions include crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib; while entrectinib has been under investigation (Awad and Shaw 2014). Recent NCCN guidelines for NSCLC (Version 6.2021) clarified brigatinib (category 1) as a preferred first-line therapy option for patients with *ALK* rearrangement-positive metastatic NSCLC (National Comprehensive Cancer Network 2021). Moreover, brigatinib and lorlatinib are currently FDA approved for *ALK* fusions.

KRAS Alterations

Mutations in the small GTPase Kirsten Rat Sarcoma (*KRAS*) are identified in ∼25% of NSCLCs (Sholl et al. 2015). The overall importance of developing *KRAS* inhibitors is highlighted in a pan-cancer analysis reporting general *RAS* mutations (*KRAS*, *NRAS*, and *HRAS*) as a driver in ∼25% of all cancer types. *KRAS* gene mutations are the most common type of *RAS* gene mutation, with a prevalence of 85% out of all *RAS*-mutated cancer types (Hobbs et al. 2016; Li et al. 2018b). Although development of successful *KRAS*-targeted therapies has been challenging, recent studies demonstrate evidence of clinical activity with the *KRAS* G12C inhibitor AMG 510 targeted against LUADs harboring the *KRAS* G12C mutation (Cox et al. 2014; Chan and Hughes 2015; Canon et al. 2019; Fakih et al. 2019; Moore et al. 2020). In May 2021, the FDA approved sotorasib (AMG 510) (Amgen), which is a first-in-class *KRAS*-G12C inhibitor for NSCLC, with an ongoing phase 3 study (NCT04303780) for the comparison of AMG 510 and docetaxel *KRAS*-G12C-mutated NSCLC subjects. Further exploration includes combinations that pair sotorasib with PD1/PDL1 blockers, MEK inhibitors, and SHP2 inhibitors (Mullard 2021).

KRAS mutation testing has clinical utility in other ways. For example, based on the BATTLE-2 trial, the presence of a *KRAS* mutation predicts lack of response to EGFR inhibition. The BATTLE-2 trial stratified NSCLCs based on *KRAS*

mutational status to determine its effect on clinical responsiveness to erlotinib and reported that patients with *KRAS*-mutated tumors experienced a statistically longer progression-free survival (PFS) if not treated with the *EGFR* TKI erlotinib, although a significant impact on overall survival (OS) was not observed (Papadimitrakopoulou et al. 2016). In addition, as *KRAS* mutations tend to be a mutually exclusive driver in LUAD prior to first-line therapy and can often be identified quickly by a rapid tissue test or liquid biopsy, it can lead to more rapid initiation of therapy or clinical trial enrollment.

ROS1 Rearrangements

The c-ROS oncogene 1 (*ROS1*) is a receptor tyrosine kinase gene that acts as a driver in 1% to 2% of NSCLC, typically in never-smokers or light smokers. FDA-approved TKIs for *ROS1*-rearranged NSCLC include crizotinib and entrectinib (Remon et al. 2019). *ROS1*-rearranged NSCLC is associated with PD-L1 expression, whereas *EGFR*-mutated NSCLC is negatively associated with PD-L1 expression (Lee et al. 2019).

MET Alterations

The mesenchymal-to-epithelial transition (*MET*) factor transmembrane tyrosine kinase receptor gene is a proto-oncogene that binds hepatocyte growth factor (*HGF*). Activating *MET* GAs predominantly consist of *MET* gene amplification or *MET* gene exon 14 (*MET*ex14) splice-site mutations. NSCLCs with *MET*ex14 mutations often have concomitant *MDM2* and cyclin-dependent kinase 4 (*CDK4*) amplifications (Frampton et al. 2015; Paik et al. 2015b; Awad et al. 2016; Schrock et al. 2016).

MET GAs are identified as primary changes and as resistance mechanisms to targeted therapy, such as resistance to *EGFR* inhibitor therapy in *EGFR*-mutated LUADs and NSCLCs harboring MET GAs often respond to *MET* inhibition (Paik et al. 2015a). For example, *MET* inhibition of NSCLCs with concurrent *EGFR* and *MET* abnormalities may respond to dual blockade of *EGFR* and *MET* (Pérez-Ramírez et al. 2015a; Shi et al. 2016b). Activation of *ERBB3* signaling via *MET* amplification may mediate resistance to *EGFR* TKIs (Engelman et al. 2007). Cigarette smoke is a factor that enhances *c-MET* signaling in lung cancer and consequently may limit responsiveness to EGFR TKI therapy (Tu et al. 2018).

BRAF Alterations

A *BRAF* inhibitor, vemurafenib, can be used if dabrafenib and trametinib combination therapy is unable to be tolerated in lung cancers harboring *BRAF* V600E mutations. *BRAF* mutations and *BRAF* fusions, such as between *AGK* and *BRAF*, may arise as resistance mechanisms to therapy with *EGFR* or *ALK* inhibitors (Vojnic et al. 2019; Boyle et al. 2020). The potential for BRAF-targeted therapy after a *BRAF* GA is identified as the resistance mechanism is under investigation (Vojnic et al. 2019). Trametinib, a *MEK* inhibitor that has been studied in combination with the *BRAF* inhibitor, dabrafenib, is a combination therapy that has shown activity against NSCLC, and is FDA approved for *BRAF*-mutated NSCLC (Solassol et al. 2019).

RET Rearrangements

RET rearrangements have been reported in 1.2% of NSCLCs, predominantly LUADs. *RET* rearrangements have been found to be mutually exclusive with both *EGFR* mutations and *ALK* rearrangements. *RET* rearrangements have been associated with younger age, nonsmoking history, papillary morphology, and mucin production (Tsuta et al. 2014). Success with treating *RET*-rearranged lung cancer with targeted therapies may depend on use of highly selective *RET* inhibitors (Bronte et al. 2019). Selpercatinib and pralsetinib are FDA approved for NSCLCs that harbor *RET* fusions.

NTRK Fusions

Neurotrophic receptor tyrosine kinase (*NTRK*) fusions in the *NTRK1*, *NTRK2*, and *NTRK3* genes are identified in <1% of NSCLCs (Farago et al. 2018). Identification of *NTRK* fusions in lung cancer has been limited by availability of

assays that accurately and comprehensively detect these fusions. However, given the potent clinical activity and FDA approval of TRK inhibitors in *NTRK*-mutated solid tumors, identification of such fusions with multiplexed NGS-based fusion assays is encouraged (Amatu et al. 2016; Cocco et al. 2018; Garinet et al. 2018; Kummar and Lassen 2018; Yan and Zhang 2018; Huang and Feng 2019). Recent NCCN guidelines for NSCLC (Version 6.2021) clarified that *NTRK1/2/3* gene fusions are established predictive molecular biomarkers (National Comprehensive Cancer Network 2021). Larotrectinib and entrectinib are FDA approved for *NTRK* fusion-positive cancers.

PI3K/AKT1/PTEN /mTOR Pathway

Up-regulation of the *PI3K/AKT1/PTEN* pathway is important in that it can be a mechanism of resistance to TKIs. In the event of this up-regulation, *PI3K*, *AKT*, *mTOR*, and *NF-κB* are potential targets for effective inhibition (Pérez-Ramírez et al. 2015b).

Beyond the Genome

Beyond the characterization of the underlying GAs enriched in different lung cancer subtypes, it is also important to understand the dysregulation in cell signaling attributed to these GAs (Zhang et al. 2019d). Dysregulation in cell signaling with oncogenic consequences may also result from transcriptomic or epigenomic alterations (Duruisseaux and Esteller 2018; Kim and Kim 2018; Teixeira et al. 2019).

mRNA Expression

RNA sequencing, also referred to as "RNA-seq" or gene expression profiling (GEP), is a method for evaluating the sequences and/or expression levels of the mRNA transcriptomes of biological specimens. In some scenarios, RNA sequencing provides more accurate identification of GAs that are difficult to comprehensively detect by DNA sequencing, such as splice variants, gene fusions, or dysregulated signaling pathways that may not have a clearly defined underlying driver (Dhanasekaran et al. 2014; Fernandez-Cuesta et al. 2015; Li et al. 2017b). Moreover, comparison of GEP results in a significant number of different histologic tumor types, which provides insight into which genes are typically dysregulated in a given tumor type (Lim et al. 2018b; Cai et al. 2019a).

Single-cell RNA-sequencing (scRNA-seq) is a recent approach that has been shown to provide insight into the lung tumor microenvironment (TME). With scRNA-seq, it is possible to characterize different stromal cell and immune cell subtypes (Lambrechts et al. 2018; Ma et al. 2019; Tian et al. 2019). scRNA-seq can also provide insight into the heterogeneity of tumor subpopulations, or identify the presence or absence of therapy-responsive or therapy-resistant subpopulations (Kim et al. 2015; Levitin et al. 2018; Damiani et al. 2019).

Comparison of the RNA sequence of lung tumor with paired nontumor lung tissue facilitates studies of transcriptomic dysregulation, such as changes in PD-L1, PD-L2, and *VEGFR* expression in NSCLC tumors. Furthermore, recurrent patterns of dysregulation specifically observed in tumor tissue have prognostic implications in NSCLC (Bang et al. 2017; Uhlen et al. 2017).

GEP is also a useful adjunct to determine the anatomic site of origin in cancers of unknown primary (CUP) origin, which traditionally relies heavily on IHC to deduce the most probable anatomic site of origin (Lin and Liu 2014; Kandalaft and Gown 2016; Selves et al. 2018). Oftentimes, selection of treatment options is reliant on confirming the anatomic site of origin (Greco and Pavlidis 2009; Tomuleasa et al. 2017). GEP can reveal distinct differential patterns of RNA expression in different cancer types and subtypes oftentimes with use of less tumor tissue than the extensive IHC panels used in challenging CUP cases (Greco et al. 2010, 2012; Hainsworth et al. 2013; Handorf et al. 2013; Varadhachary 2013; Bentley et al. 2014; Hainsworth and Greco 2014; Kerr et al. 2014; Wei et al. 2014; Greco et al. 2015; Hoadley et al. 2018; Lyu and Haque 2018). Although traditionally IHC has been the main method for diagnosis and biomarker evaluation in CUP, recent data demonstrate a commensu-

rate level of performance compared to evaluation by DNA-seq and/or RNA-seq (Pavlidis et al. 2015; Conway et al. 2019).

MicroRNAs (miRs)

Knowledge is growing about the importance and diversity of the regulatory roles that noncoding RNAs (ncRNAs) play in normal cellular function, as well as the consequences of dysregulation of ncRNAs for the development of lung cancer (Guan et al. 2012; Siegler et al. 2012; Ludwig et al. 2016; Wang et al. 2016; Dhawan et al. 2018; Li et al. 2018a; Liu et al. 2018a,b; Sonea et al. 2018; Deng et al. 2019; Li and Fu 2019; Ling and Yuen 2019; Tutar and Tutar 2019). miRs are a significant portion of ncRNAs. miRs range from ~21 to 25 nucleotides in length, and inhibit target mRNAs through sequestration and subsequent degradation via the RNA-induced silencing complex mRNA, a process effectively referred to as RNA interference (Chipman and Pasquinelli 2019). miRs can promote or suppress oncogenesis and metastasis in the context of transcriptomic dysregulation (Griffiths-Jones et al. 2006; Chan and Wang 2015; Coebergh van den Braak et al. 2018; Kim et al. 2018a; Lamichhane et al. 2018; Wu et al. 2019a).

Some miRs have oncogenic behavior, such as oncomiRs, which inhibit TSGs when up-regulated. Conversely, other miRs are oncogenic when down-regulated, such as tumor-suppressive miRs (TS-miRs or anti-oncomiRs) (Liu et al. 2019). A significant number of miRs are capable of functioning as either tumor-suppressive or oncomiRs depending on the context of the dysregulated tumor type (Svoronos et al. 2016). Given the nonstringent range of an average of ~100–200 targets that typically bind to a given miR, it is not uncommon for a miR that predominantly functions as an oncomiR in most contexts, to occasionally serve as a TS-miR in rare instances. Conversely, a miR that predominantly functions as a TS-miR in most contexts could occasionally function as an oncomiR (Svoronos et al. 2016). However, some miRs are known to consistently function as oncomiRs, such as miR-21, and others, such as miR-126 and miR-153, consistently function as TS-miRs (Hamada et al. 2012; Chen et al. 2015b; Bica-Pop et al. 2018; Huang et al. 2018b; Liu et al. 2019).

miR expression profiling is an approach that uses differentially expressed miRs to offer additional diagnostic and/or prognostic information, particular in cases where traditional pathologic data is limited or noncontributory. For instance, miR expression profiling with a panel of 92 differentially expressed miRs can help identify the site of origin in cases of CUP, which is valuable in that many treatment decisions and clinical trials are based on tumor type/site of origin (Rosenfeld et al. 2008; Barker et al. 2009; Rosenwald et al. 2010; Varadhachary et al. 2011; Meiri et al. 2012; Pentheroudakis et al. 2013). Moreover, miR-17, miR-190b, and miR-375 can accurately differentiate between SCLC and NSCLC. Other differentially expressed miRs have been specifically attributed to epithelial-to-mesenchymal transition (EMT) and metastatic potential (Wu et al. 2019b). Progress has been made in characterizing differentially expressed miRs in lung cancer to serve as the basis for development of miR profiles with potential for diagnostic, prognostic, or predictive clinical utility (Hu et al. 2010; Guan et al. 2012; Gallach et al. 2017; Yerukala Sathipati and Ho 2017).

miRs also are being considered in the development of therapeutic strategies for lung cancer. In general, a therapeutic approach to mitigate miR dysregulation either targets an up-regulated oncomiR by using antisense oligonucleotides; or alternatively, the down-regulation of a TS-miR may be mitigated by administering a synthetic miR that mimics the deficient TS-miR (Lu et al. 2018; Weidle et al. 2019). Notable miRs that are consistently dysregulated in lung cancer include oncomiRs miR-192 and miR-662 and TS-miR miR-374a (Võsa et al. 2011; Kuo et al. 2013; Chen et al. 2015a; Shu et al. 2016; Filipska et al. 2018). Significant miRs in lung cancer are summarized in Table 2.

Epigenomic Changes

Epigenomic or epigenetic changes are generally defined as changes in gene expression that are not the result of an underlying alteration in the genomic sequence, but rather an external mod-

Table 2. Summary of microRNAs (miRs) in lung cancer

miRs	Lung adenocarcinoma	Squamous cell cancer of the lung	Non-small-cell lung cancer	Atypical carcinoid	Neuroendocrine carcinoma
OncomiRs	miR-155 miR-16 miR-19a miR-20a miR-26a miR- 31 miR-93 miR-99a miR-100 miR-106b miR-135b miR-200b miR-200c (Molina-Pinelo et al. 2014)	miR-192 (Filipska et al. 2018) miR-662 (Filipska et al. 2018) miR-21 PTEN JAG1 and WNT1 (Gao et al. 2011) miR-205	miR-138 Sox4 (Bai et al. 2019) miR-21 PTEN JAG1 and WNT1 (Zhang et al. 2010; Bica-Pop et al. 2018) miR-96 GPC3 (Fei et al. 2018) miR-224 TNFAIP1 and SMAD4 (Cui et al. 2015)	miR-100 mTOR miR-34a SIRT1 and TP53 (Demes et al. 2016)	miR-21 JAG1 and WNT1 (Demes et al. 2016)
TS-miRs	miR-374a TGFA (Shu et al. 2016) miR33a-5p (Kuo et al. 2013)		miR-374a TGFA (Võsa et al. 2011) miR-345-5p (Chen et al. 2015a) miR-143 EGFR (Zhang et al. 2016) miR-134 EGFR (Qin et al. 2016) miR-182 Cortactin (Li et al. 2018d) miR-4317 FGF9 and CCND2 (He et al. 2018) miR-181a-5p HMGB2 (Li et al. 2018c) miR-183 MTA1 (Yang et al. 2018) miR-200c (Ceppi et al. 2010) miR-153 AKT (Chen et al. 2015b; Yuan et al. 2015)		

MicroRNAs (miRs) with select representative complementary targets in lung cancer. MiRs are short RNA sequences that can promote or suppress oncogenesis, and metastasis in the context of transcriptomic dysregulation. OncomiRs target tumor-suppressor genes, whereas tumor-suppressor miRs (TS-miRs) target oncogenes. Although this table represents miRs and examples of select relevant targets, miRs often have multiple targets.

ification. The prefix "epi" signifies that the changes observed are not in the genome proper; rather they are changes made "over" the genome.

Modifications that result from epigenomic changes include regulation by transcriptional deactivation mediated by miRs, methylation "silencing" of DNA by DNA methyltransferases (DNMTs), or transcriptional deactivation by chromatin condensation via histone deacetylation by histone deacetylases (HDACs). Conversely, transcriptional activation is regulated by chromatin loosening via histone acetylation by histone acetyltransferases (HATs) (Langevin et al. 2015).

Therapeutic strategies to alter epigenomic features of DNA are under investigation (Jakopovic et al. 2013; Duruisseaux and Esteller 2018). For instance, the role of *HDAC* inhibitors (HDACis) and *DNMT* inhibitors (DNMTis) to effectively restore the expression of epigenetically silenced TSGs is actively under investigation in lung cancer in preclinical models and clinical trials (Schiffmann et al. 2016; Zheng et al. 2016; Chen et al. 2018b; Damaskos et al. 2018; Jia et al. 2018; Yamada et al. 2018). HDACis in combination with immune checkpoint inhibition (ICI) and other targeted therapies have demonstrated clinical benefit (Beg and Gray 2016; Zheng et al. 2016; Terranova-Barberio et al. 2017; Gandhi et al. 2018; Suraweera et al. 2018; Banik et al. 2019). HDACis cause therapeutic benefit in lung cancer due to transcriptional activation in cancer cells and components of the TME, such as T cells (Zheng et al. 2016; Gandhi et al. 2018).

Single-cell chromatin immunoprecipitation followed by sequencing (scChIP-seq) is a technique that is useful in identifying areas of epigenomic dysregulation (Grosselin et al. 2019). Studies are underway to analyze sputum with droplet digital PCR (ddPCR) to develop a methylation-specific ddPCR profile for identification of early-stage lung cancer (Leng et al. 2017b; Su et al. 2018).

Studies are increasingly offering an integrated view of the genomic, transcriptomic, epigenomic, and proteomic alterations that occur in lung cancer (Lazar et al. 2013; Chooback et al. 2017; Stewart et al. 2019; Teixeira et al. 2019). Comprehensive integration of multi-omics data has revealed important findings, such as distinct mutation profiles and GEP findings observed in heavy smokers compared with nonsmokers (Li et al. 2015; Cardona et al. 2019). Multi-omics data also enables an evidence-based approach to identify site of origin in tumors with unknown site of origin by clinical history and histopathology (Hoadley et al. 2018).

Another noteworthy study used whole-genome sequencing (WGS) to reveal recurrent noncoding alterations in LUADs that did not have alterations in the *RTK/RAS/RAF* pathway genes such as *EGFR*, *BRAF*, *ALK*, *RET*, *ROS1*, and *KRAS*. The LUADs in this study had a high frequency of *TP53* mutations and high mutation burden, as well as focal deletions targeting the promoter or transcription start site of *STK11* and *KEAP1* ($n = 3$), along with promoter mutations associated with the increased expression of ILF2 (Carrot-Zhang et al. 2021).

A large-scale study from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) performed WGS with integrative analyses of 2658 whole-cancer genomes and their matching normal tissues across 38 tumor types (Campbell et al. 2020).

INTERTUMOR HETEROGENEITY AND DEVELOPMENT OF ACQUIRED VULNERABILITIES

Recurrently observed types of dysregulation by different tumor types serve as an underlying biologic explanation for different clinical behaviors of specific tumor types. Pathways that are uniquely up-regulated or down-regulated are potentially clinically relevant when considering clinical response to a given therapy. For instance, SqCCL and SCLC cell lines with up-regulated squalene epoxidase (*SQLE*) have been shown to be particularly responsive to squalene epoxidase inhibition (SQLEi) (Cirmena et al. 2018; Mahoney et al. 2019).

Intertumor heterogeneity is defined as differences in tumors with the same histologic type in separate patients. Intertumor heterogeneity accounts for the observation that the same tumor

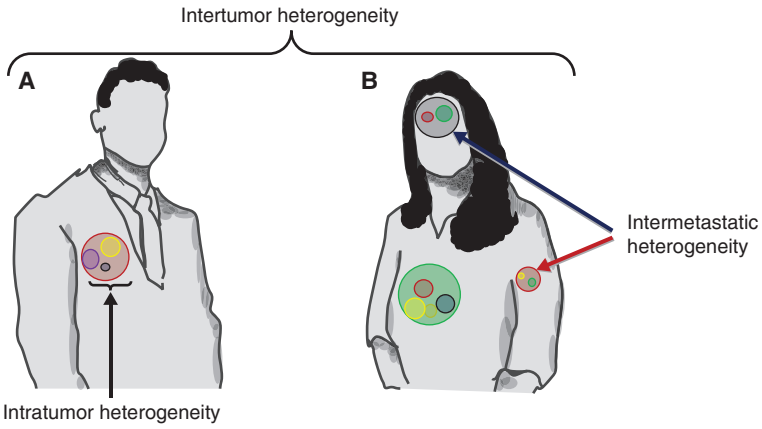

Figure 1. Intertumor heterogeneity, intratumor heterogeneity (ITH) and intermetastatic heterogeneity. Patient A and Patient B both share the diagnosis of lung adenocarcinoma (LUAD). Intertumor heterogeneity is defined as the difference between the molecular alterations in the primary LUAD from Patient A and Patient B. ITH is defined as the molecular alterations between subpopulations within the same tumor as depicted in Patient A as represented by the yellow, purple, and gray spheres within the tumor. Intermetastatic heterogeneity is defined as the difference in the molecular alterations that comprise the subpopulations when comparing metastatic sites as depicted in Patient B with metastases in her brain (dark blue arrow) and in her left humerus (red arrow).

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type may behave differently in different patients (Fig. 1 highlights different types of heterogeneity). Differences in behavior may include distinguishing metrics such as traditional pathologic features when comparing morphologic appearance; potential for invasion and/or metastases; TME, which includes the immune TME (ITME); proliferative index; and growth patterns (Sun et al. 2014; Donnem et al. 2015; Busch et al. 2016; Soo et al. 2018; DeNardo and Ruffell 2019; Shaul and Fridlender 2019; Togashi et al. 2019). Determination of critical differences in intertumor heterogeneity is critical to understanding why different patients with the same histologic tumor type have different responses when all given the same therapy. These critical differences in intertumor heterogeneity motivate the need to further explore the variety of molecular subtypes that comprise tumor types (The Cancer Genome Atlas Research Network 2012a; Müller et al. 2016).

Other distinguishing features between tumors may be differences in mutational status, gene expression, microsatellite instability (MSI) status, extent of chromosomal instability (CIN), viral status, and/or epigenomic differences, such as methylation status (Perez-Moreno et al. 2012; Clinical Lung Cancer Genome Project (CLCGP); Network Genomic Medicine (NGM) 2013; Pikor et al. 2013; Stinchcombe 2013; Hashida and Daibata 2014; Fang et al. 2015; Robinson et al. 2016; Guo et al. 2017; Inamura 2017; Zhang et al. 2017; Kim et al. 2018b; Testa et al. 2018; Cai et al. 2019b; Hu et al. 2019a; Rudin et al. 2019b; Cirenajwis et al. 2020). Protein expression studies via IHC have been performed to provide a more comprehensive context of the proteomic outcome of GAs (Lee et al. 2019). Protein expression that is up-regulated and down-regulated in lung cancer has also been characterized by mass spectrometry (MS) (Carvalho et al. 2017; Cheung and Juan 2017).

Overall, studies of intertumor heterogeneity focus on key biomarkers that offer insight for clinically meaningful outcomes, such as response to chemotherapy or ICI responsiveness. Moreover, there has been an effort to provide clarity about the extent to which lung cancer can be meaningfully further subdivided into molecular subtypes. For instance, molecular subtypes of LUAD have been proposed based upon established mutations, such as in *EGFR* and *TP53*, in addition to differentially expressed genes, such as these five differentially expressed genes with prognostic significance (*RTKN2*,

ADAM6, *SPINK1*, *COL3A1*, and *COL1A2*) (West et al. 2012; Chen et al. 2017; Hu et al. 2019a). The actionable GAs specifically enriched in a given subtype may collectively confer responsiveness to a targeted agent or specific therapeutic approach (Kim et al. 2013b; Minna et al. 2014; Derks et al. 2018; Huang et al. 2018a; Barazas et al. 2019; Rudin et al. 2019a).

Subtypes that have GAs that inactivate TSGs are unable to be approached via direct targeting the inactivated TSG. However, inactivation of a TSG may confer a vulnerability that is exploitable by using an approach using a targeted agent that is especially responsive for a TSG-inactivated subtype, a concept called synthetic lethality. For instance, cases of NSCLC with *SMARCA4* loss are specifically sensitive to *CDK4/6* inhibitors. Synthetic lethality is also an approach that may be considered for inhibiting oncogenes that are difficult to inhibit with traditional therapeutic strategies, such as *KRAS*. Consequently, agents that are able to exploit the synthetic lethality imparted by *KRAS*-mutated NSCLC are currently under investigation (Shimomura et al. 2019).

Non-Small-Cell Lung Cancer

The major histologic types of NSCLC as described in the 2015 WHO classification are summarized below (Kim et al. 2013b; Travis et al. 2015; Inamura 2017). Figure 2 highlights the

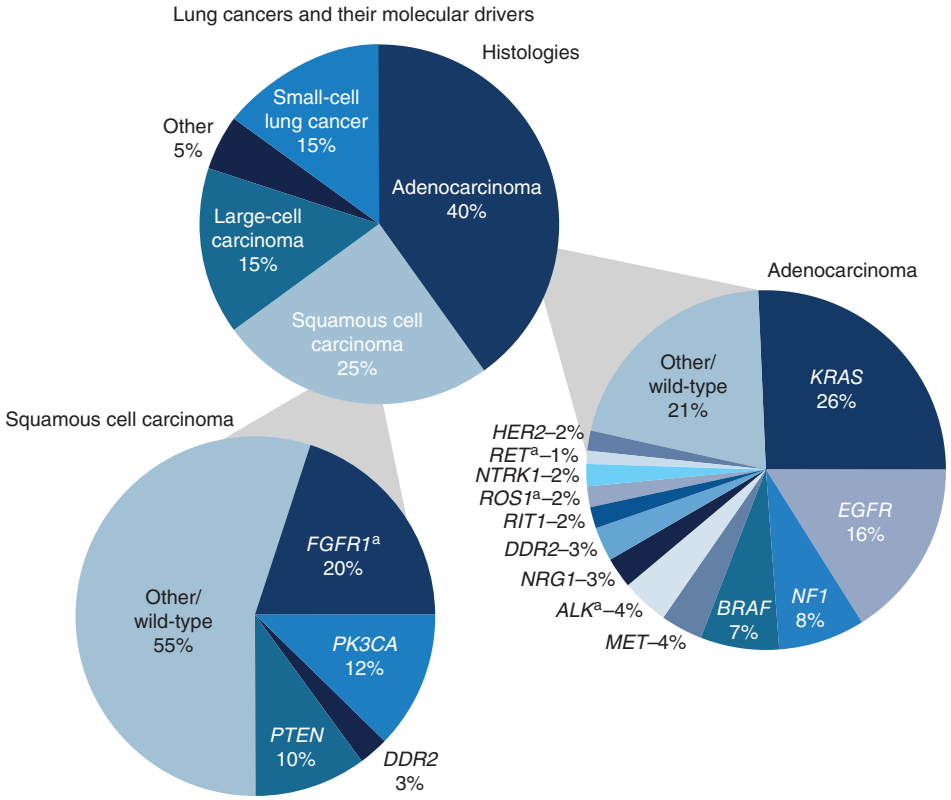

Figure 2. Comparison of drivers in lung adenocarcinoma and squamous cell carcinoma of the lung (SqCCL). The *top* pie chart shows the categorization of lung cancer into subtypes. The *bottom right* pie chart shows the most common drivers reported in lung adenocarcinoma. The *bottom left* pie chart shows the most common drivers reported in squamous cell carcinoma of the lung. ([a]) Includes both gene amplications and mutations. For additional information, see Rosell et al. (2016). (Figure is freely available from the American Cancer Society website.)

drivers in lung adenocarcinoma and squamous cell carcinoma of the lung.

Lung Adenocarcinoma

LUAD is the most common histologic cancer type of NSCLC, and is typically comprised of a combination of several major morphologic subtypes, which include solid, papillary, micropapillary and lepidic growth patterns (Solis et al. 2012). Molecular characterization of LUADs focuses on actionable drivers that predict potential response to targeted therapy, such as LUADs with *EGFR*, *BRAF*, and *ERBB2* mutations, and *ALK*, *ROS1*, *RET*, and *NTRK* fusions, and *MET*ex14/amplification; while mutations that have proven to be more challenging to target, such as those in *KRAS*, *TP53*, and *PIK3CA*, are also characterized in a significant subset of LUAD studies (Jordan et al. 2017). In untreated LUAD, *KRAS* mutations, *EGFR* mutations, and *ALK* rearrangements tend to be mutually exclusive (Dong et al. 2016).

A TCGA comprehensive study using whole-exome sequencing (WES) of 230 LUADs reported common mutations in *KRAS* (33%), *EGFR* (14%), *BRAF* (10%), *PIK3CA* (7%), *MET* (7%), and *RIT1* (2%). Mutated TSGs included *TP53* (46%), *STK11* (17%), *KEAP1* (17%), *NF1* (11%), *RB1* (4%), and *CDKN2A* (4%). Mutations in chromatin-modifying genes included *SETD2* (9%), *ARID1A* (7%), and *SMARCA4* (6%) and mutations in the RNA splicing genes included *RBM10* (8%) and *U2AF1* (3%). Recurrent mutations in *MGA* (8%) were primarily loss-of-function (frameshift and nonsense) and were mutually exclusive with focal *MYC* amplification (The Cancer Genome Atlas Research Network 2014). Other studies have reported *AXL* and *GAS6* coexpression to have potential prognostic implications in LUAD (Seike et al. 2017).

The major histologic patterns of LUAD subtypes with characterized GAs are summarized below.

Mucinous Lung Adenocarcinoma

A study of mucinous LUADs reported a higher prevalence of *KRAS* mutations (62%) in mucinous LUADs than in general LUADs (46%), reducing options for targeted therapy (Dong et al. 2016). Nonmucinous LUADs had a lower prevalence of *KRAS* mutations (13%) and a higher prevalence of *EGFR* mutations. *NRG1* fusions that activate the *ERBB2/ERBB3* signaling pathway were also enriched in mucinous LUADs (Garinet et al. 2018).

Mucinous and Nonmucinous Micropapillary Lung Adenocarcinoma Subtypes

Mucinous micropapillary LUADs have a higher prevalence of *ERBB2* mutations, *ALK* rearrangements, and a lower prevalence of *EGFR* mutations when compared to LUADs with a nonmucinous micropapillary component (Choi et al. 2013; Kamata et al. 2016). LUADs with a nonmucinous micropapillary component are enriched with *EGFR*, *KRAS*, and *BRAF* mutations (Ninomiya et al. 2009; Travis et al. 2011; Cai et al. 2016).

Papillary, Lepidic, Acinar, and Hobnail Lung Adenocarcinoma Subtypes

LUADs with papillary, lepidic, acinar, or hobnail morphological components are enriched with *EGFR* mutations (Shim et al. 2011; Travis et al. 2011; Cai et al. 2016; Dong et al. 2016).

Solid Lung Adenocarcinoma Subtype

KRAS mutations are frequently found in tumors with a solid growth pattern (Rekhtman et al. 2013; Dong et al. 2016). *ALK* rearrangements are enriched in LUADs with solid or cribriform patterns (Dong et al. 2016).

Signet Ring Cell Lung Adenocarcinoma Subtype

ALK rearrangements are enriched in LUADs with a signet ring cell component (Dong et al. 2016).

Squamous Cell Carcinoma of the Lung

SqCCL is the second most common histologic cancer type (~30%) of NSCLC and enriched with mutations in *FGFR1*, *NRF2*, *AKT1*, and *DDR2* with comparison to LUAD (Pikor et al. 2013).

A genomic study by TCGA of 178 SqCCLs by CGP determined that the most common mutations were in the *TP53* (81%) *MLL2* (20%), *PIK3CA* (16%), and *PTEN* (15%) genes, and other phosphoinositide 3-kinase (PI3K) pathway genes. Inactivating mutations in the *CDKN2A* gene that encode the p16INK4A and p14ARF proteins were identified in 72% of SqCCL. Squamous differentiation gene mutations were reported in 44% of SqCCL cases, with 8% identified in the *NOTCH1* gene. Other genes with prevalent mutations were *KEAP1* (12%), *NFE2L2* (19%), and *HLA-A* (3%) (The Cancer Genome Atlas Research Network 2012b).

TP53 mutations and *PIK3CA*, *FGFR1*, and *SOX2* amplifications are more common in SqCCLs compared with LUADs; whereas *EGFR* mutations are much less common in SqCCLs (8% in nonsmokers with SqCCL and 2.1% of smokers with SqCCL compared with LUADs (Heist et al. 2012; Perez-Moreno et al. 2012; Rooney et al. 2013). Rare targetable GAs in SqCCL, such as fusions in *RET* and *ALK*, have been reported, but it is difficult to know whether or not the SqCCL cases with these rare changes have an undetected adenocarcinoma component to them since tumors can be morphologically heterogeneous and the often small samples used for diagnosis may not fully represent the morphological subtypes within the tumor (Kenmotsu et al. 2014; Hirsch et al. 2018; Zhang et al. 2019c). The *MS4A1* gene has been found to be significantly up-regulated in SqCCLs that arise due to exposure to asbestos (Wright et al. 2012).

Large-Cell Lung Carcinoma

A genomic study that evaluated 78 samples of LCLC reported the most frequent mutations in *TP53* (71%) and *RB1* (26%). Mutations in the PI3K/AKT/mTOR pathway were identified in 15% of LCLCs with specific mutations in the *PIK3CA* (3%), *PTEN* (4%), *AKT2* (4%), *RICTOR* (5%), and *MTOR* (1%) genes. Activating mutations were detected in the *KRAS* (6%), *FGFR1* (5%), *KIT* (4%), *ERBB2* (4%), *HRAS* (1%), and *EGFR* (1%) genes (Miyoshi et al. 2017). A study of neuroendocrine carcinomas reported that *FGFR2* mutations were exclusively found in LCLC and not in SCLC (Vollbrecht et al. 2015). A study of 17 LCLCs reported 24% with *KRAS* mutations (Naidoo et al. 2016). A study that stratified LCLC based upon mutational status of *RB1* concluded that the *RB1* wild-type LCLCs had a more favorable response with a platinum-gemcitabine chemotherapy combination when compared with a platinum-etoposide combination (Derks et al. 2018).

A recent study positively correlated LCLC with PD-L1 expression compared with other histologic types of NSCLC, which supports investigation of effectiveness of ICI in LCLC (Chan et al. 2019a).

Typical and Atypical Carcinoid

Typical carcinoid (TC) and atypical carcinoid (AC) of the lung are characterized by GAs that inactivate genes affecting histone methylation, such as SWI/SNF complex subunit mutations (*ARID1A*, *SMARC1*, *SMARCA2*, *SMRCA4*). Additional alterations include mutations of *CBX6*, *EZH2*, *EIF1AX*, and *E3 ubiquitin ligase* genes (Pelosi et al. 2017).

Adenosquamous Carcinoma

ASC of the lung likely has a monoclonal origin for the SqCCL and LUAD components because microdissection of these components in two studies of 53 total ASCs demonstrated a highly convergent mutation rate between components (Vassella et al. 2015; Shi et al. 2016c). Mutations typically associated with LUADs and SqCCLs are identified in ASCs, including a first report of a novel *TPD52L1-ROS1* fusion variant in ASC (Zhu et al. 2016).

Pulmonary Sarcomatoid Carcinoma

Activating GAs in *MET* are particularly prevalent in pulmonary sarcomatoid carcinomas,

namely, *MET*ex14 splice-site mutations (22%) (Liu et al. 2016).

TP53 mutations have been reported in up to 74% of pulmonary sarcomatoid carcinomas, and *KRAS* mutations in 34%. Other less common mutations that are consistently reported are in the *EGFR*, *BRAF*, *ERBB2 (HER2)*, *RET*, *ALK*, *AKT1*, *JAK3*, *NRAS*, and *PIK3CA* genes. A high percentage of pulmonary sarcomatoid cancers have high PD-L1 expression (from 53% to 69%), warranting further investigation regarding the potential benefit of ICI in this cancer subtype (Karim et al. 2018; Weissferdt 2018).

Bronchial Mucoepidermoid Carcinoma

Bronchial mucoepidermoid carcinoma is extremely rare. It is characterized by a specific translocation, t(11;19)(q21;p13), which results in an *MECT1-MAML2* fusion. This same fusion is also a characteristic of salivary mucoepidermoid carcinoma (Fois et al. 2017; Szymanski et al. 2018).

Small-Cell Lung Cancer

In a study of 110 SCLCs, nearly all tumors had concomitant inactivation of the *TP53* and *RB1* genes resulting from complex genomic rearrangements. In two SCLCs, chromothripsis, which is defined as a massive genomic rearrangement during a single catastrophic event, was identified as an oncogenic cause with overexpression of cyclin D1 leading to effective inactivation of *Rb1* (George et al. 2015).

A study of 98 undifferentiated SCLCs reported the most common mutations in the *TP53* (86%), *RB1* (54%) and *MLL2* (17%), *RICTOR* (10%), *KIT* (7%), *PIK3CA* (6%), *EGFR* (5%), *PTEN* (5%), *KRAS* (5%), *MCL1* (4%), *FGFR1* (4%), *BRCA2*, (4%), *TSC1* (3%), *NF1* (3%), *EPHA3* (3%), and *CCND1* genes (Ross et al. 2014). Mutations in *JAK3*, *NRAS*, *RB1*, and *VHL1* were exclusively found in SCLC and not in LCLC (Vollbrecht et al. 2015). A study of SCLCs in Chinese patients demonstrated fewer mutations in Wnt and Notch signaling pathways compared to Western studies, emphasizing the importance of diverse patient cohorts for the genomic understanding of lung cancer (Hu et al. 2019b). Molecular subtypes within SCLC have been proposed based upon the differential expression of *ASCL1*, *NeuroD1*, *YAP1*, and *POU2F3* (Tsai et al. 2017; Chang et al. 2018; Ujhazy and Lindwasser 2018; Rudin et al. 2019a).

Malignant Pleural Mesothelioma

Malignant pleural mesothelioma (MPM) is characterized by frequent inactivation of TSGs, including enrichment of inactivating mutations in the cyclin-dependent kinase inhibitor 2A/alternative reading frame (*CDKN2A/ARF*) gene. An association between *CDKN2A* copy number loss in MPM tumor cells, stromal p16 immunoreactivity, and high pulmonary asbestos fiber count was reported with the suggestion that *CDKN2A* alterations in MPMs might indicate a history of asbestos exposure (Sekido 2013; Kettunen et al. 2019). The NF2/Hippo and BRCA-associated protein 1 (*BAP1*) pathways are also frequently mutated in MPM (Arzt et al. 2014; Miyanaga et al. 2015; Joseph et al. 2017; Felley-Bosco 2018). Overall, MPM is enriched in mutations in pathways involving *TP53*/DNA repair, the cell cycle, MAPK, and PI3K/AKT (Hylebos et al. 2016). In cases of MPM that are challenging to diagnose due to complex morphology and nonconclusive IHC results, molecular testing may provide additional diagnostic clues.

In a TCGA study of 74 MPMs, epithelioid MPMs had strong expression of the *VISTA* immune-checkpoint gene. WES revealed a group of MPMs with *TP53* and *SETDB1* mutations as well as concomitant loss of heterozygosity (LOH) that affected more than 80% of the genome (Hmeljak et al. 2018). In another TCGA study of 42 malignant mesotheliomas (including pleural [55%], peritoneal [26%], and pericardial [5%]), the most common aberrations were in the *BAP1* (47.6%), *NF2* (38.1%), *CDKN2A/B* (35.7%), and *TP53* (16.7%) genes. *BAP1* aberrations consisted of mutations (50%), loss (25%), rearrangements (5%), and multiple alterations (20%). *NF2* aberrations consisted of mutations (81.3%), loss (12.5%), and multiple alterations (6.3%) (Kato et al. 2016). Current strategies under investigation for the treatment

of MPM include poly-ADP ribose polymerase (PARP), CDK, and mTOR inhibitors as well as ICI and epigenetic modulators (Tolani et al. 2018).

Loss-of-function mutations in *BAP1* and DNA repair genes predict improved OS following platinum chemotherapy (Hassan et al. 2019). A six-miR signature including miR-21-5p, miR-23a-3p, miR-30e-5p, miR-221-3p, miR-222-3p, and miR-31-5p can also predict improved OS in MPM (Ahmadzada et al. 2018).

INTRATUMOR HETEROGENEITY AND CLONALITY

GAs shared by the majority or all regions of a tumor are called trunk mutations, or clonal mutations. In contrast, branch mutations are unique to a subclonal population within a tumor. Clonal mutations tend to reflect early events in tumor evolution, in contrast to subclonal mutations that tend to occur later (see Fig. 3). Clonal evolution is characterized as GAs becoming more "branched" as the tumor evolves from early precursor lesions, such as adenocarcinoma in situ (AIS), to progressively more advanced lesions, such as minimally invasive adenocarcinoma (MIA), then invasive LUAD (Vinayanuwattikun et al. 2016). One study reported that mutations in the *TP53*, *KEAP1*, *STK11*, and *EGFR* genes tend to be clonal as oposed to subclonal with the conclusion that these drivers have an earlier role in progression (Fig. 1; Shi et al. 2016a).

In lung cancer, intratumor heterogeneity (ITH) has been quantified using the ITH index (ITHi) (Zhang et al. 2019f). Factors associated with high ITH include higher numbers of mutations, both clonal and subclonal, and copy number alterations. High ITH is associated with exposure to exogenous mutagens (e.g., cigarette smoker). Studies of pre-neoplastic non-

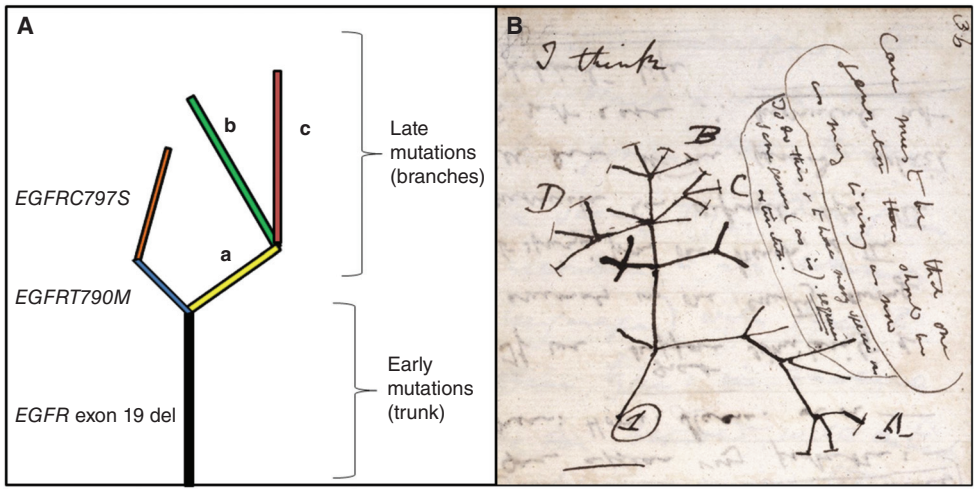

Figure 3. Tumor evolution and consequential intratumor heterogeneity in lung cancer. (*A*) Early clonal mutations that are shared between high numbers of the tumor subpopulations are depicted in the trunk. These early mutations are often described as founder mutations, which are commonly found in genes such as *EGFR*, *MET*, *KRAS*, *BRAF*, and *TP53*. Later subclonal mutations are depicted in the branches, which are limited to specific tumor subpopulation(s). Later mutations are often described as private mutations when only reported in a unique tumor subpopulation. Later mutations are commonly found in genes such as *PIK3CA*, *NF1*, and *EP300* and can occur as resistance mutations. In this figure, the early truncal *EGFR* exon 19 deletion is clonal. The *EGFR* T790M and *EGFR* C797S are later subclonal resistance mutations, which can be identified after treatment with early-generation EGFR inhibitors. For simplicity, mutations in other branches are referred to as a, b, and c. (*B*) A sketch by Charles Darwin similarly depicting evolutionary relationships with a trunk and branches. (Panel *B*, which is in the public domain in the UK, was reprinted from Darwin 1837–1838.)

tumor tissue with mutagen exposure have shown areas of clonal expansion with higher numbers of mutations compared to nonexposed tissue (Martincorena et al. 2015, 2018; Martincorena 2019; Yizhak et al. 2019). The U.S. Cancer Moonshot initiative has recommended the development of a pre-cancer atlas. This initiative and others are supporting efforts to better understand the evolution of pre-neoplastic "nontumor" tissue, to clones/subclones, to early precursor tumor tissue, to late tumor tissue with a goal to help define sentinel genomic events and enable earlier detection of cancer (Spira et al. 2017; Janiszewska and Polyak 2018; Lippman et al. 2018; Srivastava et al. 2018a,b).

Enrichment of specific GAs in subpopulations that comprise ITH may be informative in anticipating tumor behavior. For instance, in LUADs that were post-therapy with *EGFR* TKIs, identification of biallelic inactivation of the *TP53* and *RB1* genes predicted transformation into SCLC, which is an established resistance mechanism to *EGFR* TKIs (Fig. 4; Sequist et al. 2011; Lee and Um 2017).

Prior to the FDA approval of osimertinib as a first-line treatment of EGFR-mutated NSCLC, treatment of NSCLC with first- or second-generation *EGFR* TKIs (first-/second-generation *EGFR* TKIs) frequently became ineffectual due to resistance to treatment via a mutation at *EGFR* T790M. Consequently, *EGFR* T790M-mutated NSCLCs were treated with osimertinib, a third-generation *EGFR* inhibitor that binds irreversibly to C797; however, a concomitant *cis*-oriented mutation at C797S could then occur and confer resistance to osimertinib (Mitsudomi and Yatabe 2010; Niederst et al. 2015; Yu et al. 2015). A therapeutic strategy to minimize the heterogeneity of populations with *EGFR* resistance mutations at T790M and C797S with agents that are synthetic lethal is actively under investigation (Vyse et al. 2017).

Although mutation at *EGFR* T790M is the most common mechanism attributed to development of resistance to first-/second-generation EGFR TKIs, other resistance mechanisms, such as MET amplification and histologic transformation to SCLC, can occur. Transformation to SCLC is characterized by enrichment with mutations in retinoblastoma 1 gene (*RB1*), tumor protein p53 gene (*TP53*), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α gene (*PIK3CA*) with retention of the original *EGFR* mutation (Yu et al. 2013; Zhang and Yuan 2016; Lee et al. 2017; Liu 2018; Remon et al. 2019).

The AURA3 trial reported resistance mechanisms to osimertinib, such as loss of *EGFR*

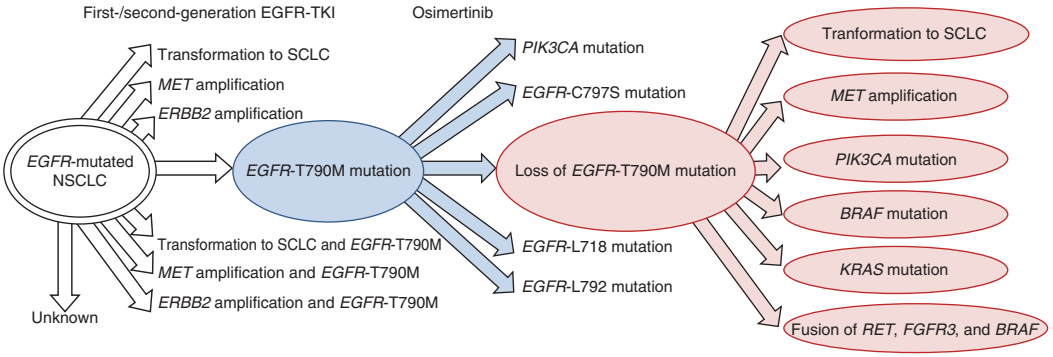

Figure 4. Mechanisms of resistance after therapy with epidermal growth factor receptor (*EGFR*) inhibitors. This overview shows the most commonly reported resistance mechanisms (white arrows) that confer resistance to treatment with first-/second-generation EGFR tyrosine kinase inhibitors in *EGFR*-mutated non-small-cell lung cancer (NSCLC). In lung tumors harboring an *EGFR* T790M mutation, subsequent treatment with osimertinib imparts a selective advantage to subpopulations with resistance to osimertinib (blue arrows), which includes EGFR wild-type NSCLC with loss of *EGFR* T790 with other concomitant alterations (red connectors) (Remon et al. 2019).

T790M in ~50% of patients, acquired *EGFR* mutations in 21%, *MET* amplification in 19%, *ERBB2* (*HER2*) amplification in 5%, *PIK3CA* mutation and/or amplification in 5%, oncogenic fusions (*RET*, *NTRK*, and *FGFR*) in 4%, cell-cycle mutations in 4%, and *BRAF* V600E mutations in 3% (Papadimitrakopoulou et al. 2018).

Any changes in the evolution of the populations of clones/subclones that reflect ITH are of great importance when considering therapeutic agents, particularly if a clonal/subclonal population has a gene mutation that confers resistance to therapy (Amirouchene-Angelozzi et al. 2017; Sheng et al. 2018; Testa et al. 2018; Williams et al. 2018). "Game theory" is a conceptual approach to understanding tumor evolution. "Game theory" strategies can enable studies of the dynamic changes in subpopulations contributing to a tumor's ITH and the impact of specific treatments and their timing in an effort to determine treatment conditions that are beneficial to patients. Variations in treatment include duration of treatment, breaks in treatment, the sequence of agents given in a treatment regimen, or use of collateral agents. The dynamic changes to consider include how the cancer cells and the TME change following treatment (Kaznatcheev et al. 2019).

The identification of resistance mutations that arise as a consequence of therapies emphasize the importance of serial biopsies, as the mutation(s) that account for the majority of tumor cells that drive the progression of cancer may behave fluidly, as opposed to static tumor populations that remain fixed over time. Frustratingly, this presents a challenge to clinicians and pathologists alike to seek to determine the "moving target" of the driver mutation that would be most amenable to therapy.

The TRAcking non-small cell lung Cancer Evolution through therapy (Rx) (TRACERx) study aims to characterize recurrent patterns of evolution in ITH that are observed in multiple patients with NSCLC to determine or "track" populations with actionable drivers or resistant populations (Jamal-Hanjani et al. 2017; Negrao et al. 2018). Ongoing studies are actively investigating agents that demonstrate a response in *EGFR* inhibitor-resistant tumor cell populations, such as poly(ADP-ribose) polymerase 1 (*PARP-1*) inhibitors (Marcar et al. 2019).

Intermetastatic heterogeneity is defined as the entirety of heterogeneity of clones/subclones within all of the metastatic lesions that arise from an original primary lung cancer (Caswell and Swanton 2017). Intrametastatic heterogeneity is defined as the heterogeneity within a single metastasis (Vignot et al. 2013; Roper et al. 2019; Zhang et al. 2019f). The determination of whether there are therapy-resistant clones/subclones within metastatic sites is a clinically relevant consideration when making management decisions to continue a given therapy or initiate a new therapeutic option (Sosa Iglesias et al. 2018; Zhang et al. 2018a).

In summary, the evolution of the overall heterogeneity of the primary lung tumor and metastatic sites ideally should be used to inform the type(s) of therapy, and sequence of therapy administration in lung cancer (Pakkala and Ramalingam 2018).

TUMOR MUTATIONAL BURDEN (TMB)

High TMB is under investigation as a biomarker for patients with lung cancer to predict clinical benefit from ICI (Rizvi et al. 2015; Goodman et al. 2017; Gandara et al. 2018; Greillier et al. 2018; Hellmann et al. 2018; Hendriks et al. 2018; Maleki Vareki 2018; Chan et al. 2019b; Ready et al. 2019; Samstein et al. 2019). TMB is defined as the number of somatic nonsynonymous single-nucleotide variants (SNVs), insertions, and deletions per megabase covered by a genomic panel. Currently, the PD-L1 TPS, which is defined as the percentage of viable tumor cells with at least partial membrane staining for PD-L1, is the only biomarker with FDA-approved indications to predict response to ICI in lung cancer. However, assessment of PD-L1 expression in the collected specimen is difficult to standardize and does not always comprehensively reflect the PD-L1 expression in the entire lung cancer due to possible complicating factors such as heterogeneity of PD-L1 expression and small biopsies with a paucity of available tumor cells for evaluation (McLaughlin et al. 2016; Terra et al. 2017; Khunger et al. 2018; Velcheti

et al. 2018; O'Malley et al. 2019). Accurate assessment is further complicated by interassay and interobserver variability. Even with high quantity and quality tissue for PD-L1 assessment, PD-L1 assessments do not clearly predict which patients will or will not respond to ICI. Consequently, alternative biomarkers for ICI responsiveness, such as TMB, are desirable. Regardless of biomarker, the benefit imparted by ICI needs to be considered in context of the risk of immune-related adverse events (irAEs) (Puzanov et al. 2017; Martins et al. 2019; Ricciuti et al. 2019).

When TMB is evaluated based upon a given targeted sequencing panel, the TMB reported may vary from other targeted sequencing panels given factors such as differences in the areas of genomic coverage (Büttner et al. 2019). An approach that addresses this challenge is for targeted sequencing panels to correlate the extent to which a targeted TMB is commensurate with the TMB determined by WES (Meléndez et al. 2018; Butler et al. 2019). Efforts for standardization and harmonization of TMB to minimize variability across NGS assays are ongoing (Chang et al. 2019; Stenzinger et al. 2019).

A comparison of single-region and multiregional tissue tumor mutational burden (tTMB) with blood TMB (bTMB) in a series of LUAD, SqCCL, and lymphoepithelioma-like carcinoma (LELC) concluded that single-region tTMB correlated more closely with multiregional tTMB than bTMB (Zhang et al. 2019e). Another study proposed that the quality of the mutations that comprise TMB should be considered to provide context rather than solely focusing on the TMB number. This study found that relatively more widespread clonal mutations had greater potential in eliciting T-cell immunoreactivity and predicting sensitivity to ICI than relatively more private subclonal mutations (McGranahan et al. 2016). The extent to which mutational status, such as *MET*-activating and *STK11*-inactivating mutations, contribute to tumor immunotolerance is another ongoing area of investigation (Saigi et al. 2019).

High TMB (TMB-H) may result from GAs that cause an underlying deficiency in DNA damage response (DDR) pathways, such as tumors with homologous recombination deficiency (HRD), deficient DNA polymerase ε or DNA polymerase δ1 (*POLE* or *POLD1*) proofreading, alterations of the base excision repair (BER) pathway, deficient mismatch repair (dMMR), and deficient *ERCC1*-mediated repair (Warth et al. 2016; Takamochi et al. 2017; Bever and Le 2018; Heeke et al. 2018; Knijnenburg et al. 2018; Penault-Llorca and Radosevic-Robin 2018; Song et al. 2018b; Chabanon et al. 2019; Chae et al. 2019b; Park and Pursell 2019; Diossy et al. 2021).

Increased TMB has also been associated with carcinogen exposure in tobacco smokers that develop lung cancer (Alexandrov et al. 2016; Davis et al. 2017; Nagahashi et al. 2018; Norum and Nieder 2018; Phillips 2018; Song et al. 2018a; Blons et al. 2019; Cardona et al. 2019; Chae et al. 2019b). Of note, TMB-H is associated with global DNA hypomethylation, increased CIN, and GAs in the *TP53* and *APOBEC* genes. TMB-H is not often observed in lung cancers with *EGFR* GAs (Cai et al. 2018; Nagahashi et al. 2018; Phillips 2018; Zhong et al. 2018).

Increased TMB is not the only measure of overall tumor antigenicity. Viral-related antigenicity is another cause of up-regulation of host immune response and immunogenicity. Viral-related antigenicity is associated with responsiveness to ICI, even if the corresponding TMB is relatively low.

Whether the underlying cause of immunogenicity is mutant protein antigenicity or viral, there are specific histologic findings that correspond with a strong immunogenic response to the tumor, such as tumor-infiltrating lymphocytes (TILs), peritumoral stromal lymphocytes, and/or tumor-adjacent lymph node–like structures termed tertiary lymphoid structures (TLSs) (Al-Shibli et al. 2008; Donnem et al. 2015; Schalper et al. 2015; Anichini et al. 2018; Soo et al. 2018; Blons et al. 2019).

A digital pathology-based method to quantify tumor immunogenic response was developed by the Society for Immunotherapy of Cancer (SITC), which was initially defined as an immunoscore predictive biomarker in colon cancer (Pagès et al. 2018). Ongoing studies are investigating the potential of higher immunoscores to predict response to ICI in patients

with multiple other tumor types (Kirilovsky et al. 2016; Gnjatic et al. 2017; Zeng et al. 2018), including NSCLC (Donnem et al. 2015, 2016; Schalper et al. 2015; Paulsen et al. 2017; Brahmer et al. 2018; Soo et al. 2018).

CELL-FREE DNA (cfDNA) AND CIRCULATING TUMOR CELLS (CTCs)

Circulating tumor DNA (ctDNA) derived from circulating cell-free DNA (cfDNA) and circulating tumor cells (CTCs) are detectable in most cases of advanced stage lung cancer and evidence supports their clinical utility in guiding therapy (Duréndez-Sáez et al. 2017; Razavi et al. 2017; Lim et al. 2018a; Zhang et al. 2018c; Cheng et al. 2019; Zhao et al. 2019). Liquid biopsies with assessment of mutations in ctDNA are a particularly helpful approach for identifying mutations in tumors that are not amenable to biopsy (Calabuig-Fariñas et al. 2016; Foy et al. 2017). Quantification of CTCs in lung cancer may have prognostic value with correlation between post-therapy decreases in CTCs and therapeutic response. With advanced technologies, CTCs can be isolated for sequencing, tested with other methods such as IHC, or even be used to create patient-derived xenografts (PDX) (Hofman et al. 2011a,b, 2019; Tan et al. 2016; Carter et al. 2017; Drapkin et al. 2018).

In a clinical context, ctDNA results can be complementary to solid tumor biposies, since both methods have advantages and disadvantages; however, the cost for testing with multiple methods may be too cost-prohibitive for widespread simultaneous testing with debatable improved clinical benefit (Aggarwal et al. 2018). An advantage of ctDNA over solid tissue testing is the ability to detect clonal and subclonal mutations arising from the primary and all metastatic sites if ctDNA is released from all tumor sites into the circulating blood. This advantage provides value for detecting mutations in tumors with heterogeneity among metastatic sites and/or within the primary site. Traditional biopsies of solid tumor are limited to mutational analysis of only the sampled tumor due to the impractical costs and risks of performing multiple biopsies in patients with multiple metastases (Santarpia et al. 2018a; Stanta and Bonin 2018). After initiation of targeted therapy, ctDNA testing of blood samples can be performed to evaluate for resistance mutations, reducing the risks and costs of repeat biopsies (Zill et al. 2018).

Detection of TMB and PD-L1 with ctDNA and CTCs, respectively, is being explored with comparison to tumor tissue biopsy TMB and PD-L1 results and evaluation as potential biomarkers for response to ICI in NSCLC (Chae et al. 2019a). Significant PD-L1 heterogeneity was observed in the CTCs (Koh et al. 2019).

Liquid biopsies can also be a reliable method for detecting circulating miRs, which has led to the development of miR profiles to detect circulating differentially expressed miRs in lung cancer (Hu et al. 2010; Deng et al. 2014; Huang et al. 2014; Wu et al. 2015; Leng et al. 2017a; Yang et al. 2017; Zaporozhchenko et al. 2018; Calabrese et al. 2019). Methylation patterns of ctDNA have potential to differentiate between cancer types and tumor tissue site origins (Moss et al. 2018).

The chronologic appearance of GAs throughout the evolution of lung cancer has been characterized in solid tumor tissue, and the evaluation of ctDNA data from serial liquid biopsies has helped to enrich the understanding of the evolution of different tumor subpopulations (Sequist et al. 2011; Vinayanuwattikun et al. 2016; Jamal-Hanjani et al. 2017; Ma et al. 2017; Klein et al. 2018). Detection of GAs by ctDNA analysis that are observed relatively early in tumor evolution, such as *KRAS* mutations in pancreatic ductal adenocarcinoma (PDAC), in combination with blood testing for CA19-9 is a promising strategy to enable screening and earlier diagnosis of PDAC, a cancer that is typically not detected until presentation of symptoms at an advanced stage (Cohen et al. 2017). Given that NSCLC is also not usually diagnosed until symptoms present at an advanced stage, efforts to enable earlier diagnosis have likewise been invested in studies using ctDNA as an adjunct to other specific biomarkers such as miRs (exosomal and non-exosomal), DNA methylation patterns, protein markers, metabolites and CTCs to enable early-stage diagnosis of NSCLC (Deng

et al. 2014; Liang et al. 2018b; Oxnard et al. 2018; Santarpia et al. 2018b; Calabrese et al. 2019).

Serial liquid biopsies offer the benefit of determining any changes in the intermetastatic heterogeneity during the post-therapy evolution of the populations of resistant clones/subclones (e.g., *EGFR* TKI resistance) (Imamura et al. 2016; Oxnard et al. 2016; Lim et al. 2018a). An approach using serial liquid biopsies to track the decrease of relevant mutant allele fractions (MAFs) in ctDNA is under investigation as a surrogate metric to determine the presence/extent of a therapeutic response after chemotherapy, radiotherapy, or targeted therapy (Raja et al. 2018; Gao et al. 2019; Phallen et al. 2019). Clearance of detectable ctDNA mutations occurs rapidly after effective therapy with the MAFs representing a snapshot of the immediate rate of tumor cell death at the time of liquid biopsy (Gao et al. 2019). Postoperative increases in ctDNA and/or CTCs may serve as predictive surrogate metrics for early tumor recurrence (Liang et al. 2018a; Moding et al. 2018).

The fragmentation profile of the overall ctDNA is a feature that may have potential utility in distinguishing cancer patients from healthy patients. A machine learning-based study that evaluated the fragmentation lengths from WGS of cfDNA demonstrated shorter ctDNA fragmentation in lung cancer patients than cfDNA in healthy patients (Cristiano et al. 2019).

CLINICAL TRANSLATIONS

Recent advances in molecular pathology and associated technologies outpace their translation to clinical care. Triage is needed to focus clinical translation efforts on technologies most likely to truly benefit patients. Recent guidelines have focused on recommending a translational approach informed by the benefits of using targeted TKIs (Kalemkerian et al. 2018; Lindeman et al. 2018; Planchard et al. 2018; Zhang et al. 2019a). ICI for standard of care is typically most beneficial when no drivers are identified in the cancer to indicate targeted therapy (Herbst et al. 2016; Zago et al. 2016; Carbone et al. 2017; Bironzo and Di Maio 2018; Bylicki et al. 2018; Hirsch et al. 2018; Osmani et al. 2018; Shroff et al. 2018; Dong et al. 2019; Forde et al. 2019; West et al. 2019). For instance, the KEYNOTE 024 and KEYNOTE 042 trials support the first-line use pembrolizumab in advanced NSCLC with a level of PD-L1 expression of at least 50%, only in the absence of *EGFR* and *ALK* alterations (Mok et al. 2019; Reck et al. 2019).

A therapeutic approach in lung cancer that is gaining momentum is the use of additive or synergistic therapy ICI combinations with other complementary treatments such as chemotherapy, radiotherapy or targeted agents, such as *EGFR* TKIs, *VEGFR* TKIs, or HDACis, as well as the use of dual agents for ICI such as concomitant PD-1/PD-L1 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibition (Le et al. 2017; Fukumura et al. 2018; Qiao et al. 2018a,b; Drapkin and Farago 2019; Jiang et al. 2019; Pavan et al. 2019; Rocco et al. 2019). Studies in other cancer types have supported potential clinical benefit with ICI combination strategies (Bastos and Zaharcu 2016; Chen et al. 2018a; Tang et al. 2018; Burrack et al. 2019; Merhi et al. 2019; Rini et al. 2019; Sicklick et al. 2019; Taberna et al. 2019; von der Grün et al. 2019).

Moreover, pathologic analyses of major pathological response in NSCLC after neoadjuvant treatment is actively under investigation, which have corroborated the prognostic significance of major pathological response (MPR), which has been determined by assessment of the percentage of residual tumor cells (65% for LUAD and 10% for non-LUAD) to assign a prognostic score to NSCLC cases that are post-neoadjuvant therapy. Assessment of a tumor regression grade (TRG) to quantify the percentage of residual tumor cells has been used in the setting of other tumor types, and clinical utility of TRG in NSCLC will likely assist in determination of prognosis.

Incorporation of NGS testing in a way that is complementary to other methods, such as PCR, FISH, and IHC, may optimally benefit treatment decisions (Dorward et al. 2017; Sholl 2017; Osmani et al. 2018; Dong et al. 2019). The NCCN provides guidance about molecular testing in their guidelines, which are derived from an evidence-based approach based on publications and professional consensus of expert panels (National Comprehensive Cancer Network 2021).

Despite efforts by the NCCN to provide guidelines and recommendations, universal incorporation of these recommendations has not been adopted across all clinical practice patterns (MacLean et al. 2016; Gutierrez et al. 2017; Gierman et al. 2019). The National Lung Cancer Round Table (NLCRT) has enacted task groups, such as the Triage for Appropriate Treatment (TAT) group, to advocate for patients in the interest of guideline-adherent diagnosis and treatment. This effort includes gathering of data to better understand barriers to guideline-recommended care (Kim et al. 2019).

The NCCN guidelines acknowledge the reality of clinical practice in resource-limited situations and have developed general guidelines for best patient care in such situations. The NCCN guidelines also recognize the need for balance between simpler but rapid testing with more comprehensive (but often slower and more expensive) testing, such as NGS, to identify GAs that may be less common but are nonetheless actionable with targeted therapy or may qualify patients for clinical trials or off-label therapies (Jordan et al. 2017). Another important consideration about molecular testing in lung cancer addressed by the NCCN guidelines is the need to triage the limited amount of tumor tissue often obtained with biopsies and fine needle aspirations for the most clinically needed diagnostic tests (Bubendorf et al. 2017; Brainard and Farver 2019).

Furthermore, studies such as the Interrogating Cancer Etiology Using Proactive Genetic Testing (INTERCEPT) study determined that pathogenic germline variants contributed to cancer in 13.3% of the 3000 patients in the study (Mandelker and Zhang 2018; Lincoln et al. 2020; Fiala et al. 2021; Samadder et al. 2021). The importance of studies such as the INTERCEPT study helped to inform a joint consensus recommendation to establish standards and guidelines for the interpretation and reporting of sequence variants in cancer as published by the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. This study emphasized that NGS reporting should include germline variants with known evidence of clinical impact (Li et al. 2017a).

ARTIFICIAL INTELLIGENCE, DEEP LEARNING, AND FUTURE DIRECTIONS

Development of research tools to confirm, challenge, and expand our understanding of lung cancer is critical (Washetine et al. 2018). Artificial intelligence (AI), with a deep learning (DL)-based approach, is a potentially useful tool to facilitate interpretation of the massive amounts of data that are being produced by research and clinical genetic testing, data that ordinarily requires a labor-intensive effort for humans to interpret (Yoon et al. 2018; Zhou et al. 2018; Al-Quraishi 2019; Amin et al. 2019; Clark et al. 2019; Kuleshov et al. 2019; Li et al. 2019a; Shah et al. 2019; Thormann et al. 2019). Studies involving DL had initially focused on digital pathology applications (Cruz-Roa et al. 2013; Wang et al. 2014, 2018b; Xie et al. 2015; Djuric et al. 2017; Nalisnik et al. 2017; Saha et al. 2017; Sharma et al. 2017; Xu et al. 2017; Ehteshami Bejnordi et al. 2018; Khosravi et al. 2018; Liu et al. 2018c; Rakhlin et al. 2018; Saltz et al. 2018; Venkat et al. 2018; Yan et al. 2018; Zhang et al. 2018b; Hu and Yu 2019; Pell et al. 2019; Shapcott et al. 2019). DL via convolutional neural networks (CNNs) has set a precedent for its diagnostic predictive utility of histomorphologic and radiologic features, commensurate with the performance of pathologists and radiologists in some contexts (Fig. 5; Cruz-Roa et al. 2013, 2015; Yu et al. 2016; Teramoto et al. 2017; Alsubaie et al. 2018; Borkowski et al. 2018; Coudray et al. 2018; Hosny et al. 2018; Wang et al. 2018a; Ardila et al. 2019; Gertych et al. 2019; Wei et al. 2019; Zhang et al. 2019b,g,h).

Historically, correlation of histomorphologic features with underlying molecular alterations has been limited by the amount of work required to complete such studies (Shim et al. 2011; Song et al. 2013; Hu et al. 2014). As DL-based studies using CNNs has advanced, the potential to provide an enhanced understanding of the relationship between histomorphologic features and molecular alterations has expanded (Coudray et al. 2018; Couture et al. 2018; Mobadersany et al. 2018; Saltz et al. 2018; Cheerla and Gevaert 2019; Kather et al. 2019; Montalto and Edwards 2019). This approach may provide unique insights into how to best detect DL-pre-

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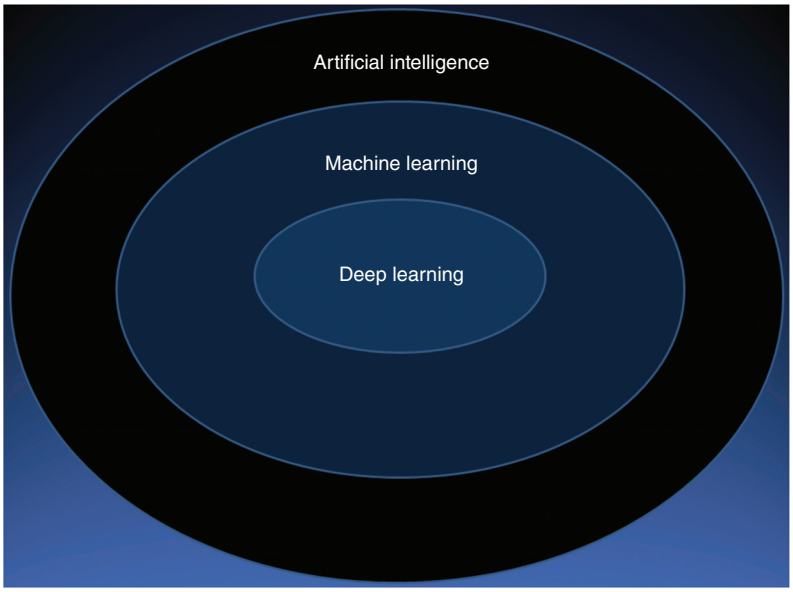

Figure 5. Artificial intelligence, machine learning, and deep learning. Artificial intelligence is a broadly defined technique that uses logic to mimic human intelligence. Machine learning is a subset of artificial intelligence that uses algorithms to improve effectiveness of performance. Deep learning is a subset of machine learning that uses neural networks to process identified data to train optimal performance with respect to a specific task (e.g., recognition of histologic features in a particular tumor type).

dicted drivers, and which signaling pathways are activated as a method to predict response to targeted therapy (Way et al. 2018; Chiu et al. 2019; Han et al. 2019; Luo et al. 2019). Moreover, DL may provide insight into the accurate identification of molecular subtypes and recurrent mutational signatures (Huang et al. 2018c; Yu et al. 2020). DL has also demonstrated promise in classifying tumor types based on RNA-seq analysis (Lyu and Haque 2018). CancerSEEK is an innovative approach that uses a DL-based method to determine tumor-type specific mutations in liquid biopsies to determine the anatomic site of origin in CUP cases (Cohen et al. 2018). Availability of high-quality genomic data via reliable databases or data repositories will be crucial for future DL-based studies (Avsec et al. 2019).

With appropriate supervision and quality control, the ongoing technical advances in AI have potential to enable and refine the interpretation of clinical data. Advancement in these tools to perform meaningful analyses of large datasets may enhance our ability to practice precision medicine (Boersma et al. 2019).

CONCLUDING REMARKS

The art of translating advances in science and technology into improvement in patient care involves leveraging available tools and knowledge. Although this review represents only a snapshot in our understanding of the molecular pathology of lung cancer, it also offers a contextual view of the ongoing advances in lung cancer to improve patient care and the development of more accurate biomarkers to inform therapeutic decisions.

COMPETING INTEREST STATEMENT

The authors declare no relevant conflicts of interest.

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