CCR FOCUS

Lung Cancer in the Era of Precision Medicine

Katerina Politi[1,2,3] and Roy S. Herbst[2,3]

Abstract

The past decade has been transformative for lung cancer patients, physicians, and scientists. The discovery of *EGFR* mutations that confer sensitivity to tyrosine kinase inhibitors in lung adenocarcinomas in 2004 heralded the beginning of the era of precision medicine for lung cancer. Indeed, it precipitated concerted efforts by many investigators to define molecular subgroups of lung cancer, characterize the genomic landscape of lung cancer subtypes, identify novel therapeutic targets, and define mechanisms of sensitivity and resistance to targeted therapies. The fruits of these efforts are visible every day now in lung cancer clinics: Patients receive molecular testing to determine whether their tumor harbors an actionable mutation, new and improved targeted therapies that can overcome resistance to first-generation drugs are in clinical trials, and drugs targeting the immune system are showing activity in patients. This extraordinary promise is tempered by the sobering fact that even the newest treatments for metastatic disease are rarely curative and are effective only in a small fraction of all patients. Ongoing and future efforts to find new vulnerabilities of lung cancers, unravel the complexity of drug resistance, increase the efficacy of immunotherapies, and perform biomarker-driven clinical trials are necessary to improve outcomes for patients with lung cancer. *Clin Cancer Res; 21(10); 2213–20. ©2015 AACR.*

See all articles in this *CCR Focus* section, "Progress in Lung Cancer."

Introduction

Every year more than 200,000 new cases of lung cancer are diagnosed in the United States and 160,000 people succumb to this disease (1). Fortunately the incidence rate of lung cancer is decreasing in this country, for both men and women, largely due to decreased tobacco use. Lung cancers fall into several different histologic categories, including lung adenocarcinoma (~40%), squamous cell lung carcinoma (~25%), large cell carcinoma (~10%), and small cell lung carcinoma (SCLC, ~20%). Recent developments have highlighted how these lung cancer subtypes are, in addition to being morphologically distinct, molecularly distinct and have different therapeutic vulnerabilities. These findings have had profound implications for the diagnosis and treatment of lung cancer, where until recently treatment selection was largely based on whether the lung cancer fell into the broad categories of non–small cell lung cancers (NSCLC, encompassing lung adenocarcinomas, squamous cell lung carcinomas, and large cell carcinomas) or SCLC. Today, lung cancers are subtyped and some undergo molecular profiling to determine the best treatment options for individual patients. There also is an increasing appreciation for the fact that tumors evolve through treatment and that repeat biopsies at the time of disease progression can provide critical information to further inform subsequent treatment strategies. A consequence of this knowledge is that there is growing emphasis on biomarker-driven clinical trials some with adaptive and flexible designs that take into consideration new data emerging during the course of the trials. Collectively, these advances are rapidly changing the lung cancer landscape and have the potential to significantly affect outcomes for patients with this disease in coming years.

Molecular Profiling of Lung Cancer

The case for tumor profiling: oncogene-driven lung adenocarcinomas

EGFR mutations and *ALK*-rearrangements were the first molecular alterations in lung adenocarcinoma—discovered in 2004 and 2007, respectively—that were shown to confer sensitivity to specific targeted therapies, namely tyrosine kinase inhibitors (TKI, Fig. 1; refs. 2–6). The remarkable responses to TKIs observed in patients and the discoveries made studying these molecular subsets of lung cancer served as catalysts for further exploration of the lung cancer genome and led to the incorporation of molecular testing into routine clinical practice. As described in two reviews in this *CCR Focus* (7, 8), clinical trials have revealed that treatment of advanced *EGFR* and *ALK*-rearranged lung cancers with appropriate TKIs is superior to chemotherapy (NCT00322452, NCT00932893; refs. 9, 10). Conversely, it has also been shown that patients with non-EGFR mutant lung cancers rarely respond to EGFR TKIs and are more likely to benefit from chemotherapy supporting the importance of matching tumor genotype to therapy (9). However, it is important to consider that due to the emergence of resistance (discussed below) and the high cost of targeted therapies, some agents may not change outcomes sufficiently to be cost-effective, a factor that should be considered as more targeted therapies enter clinical practice (11). Nevertheless, spurred by these early successes, investigators have since identified and further characterized additional oncogenic driver mutations in lung adenocarcinoma. In addition to mutations in *KRAS*, that were first described in lung cancer in the 1980s (12, 13) and are observed in 25% to 30% of

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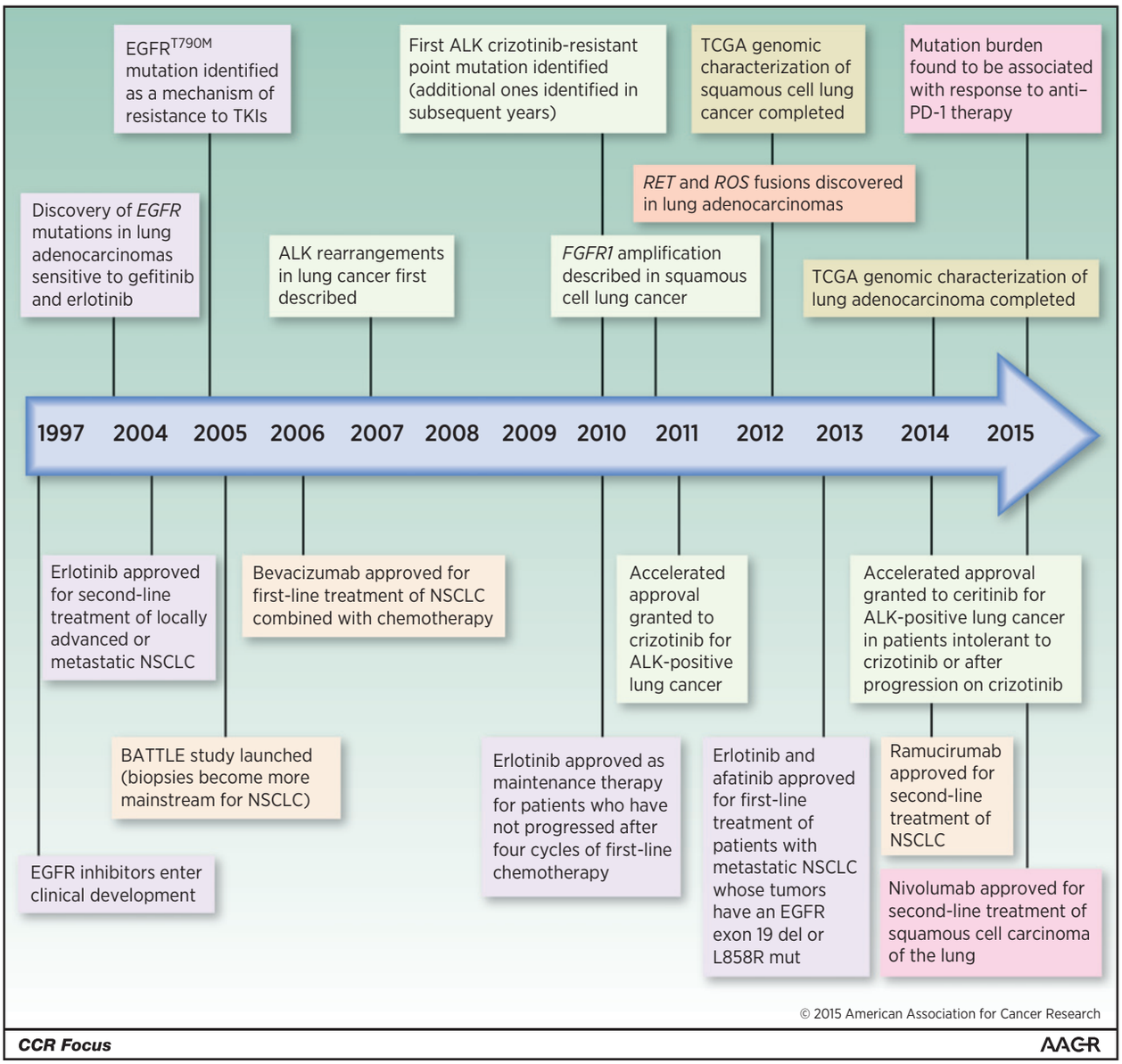

Figure 1.
Timeline of selected major discoveries in lung cancer in recent years (above the arrow) and related clinical trials (below the arrow).

lung adenocarcinomas (but uncommon in other subtypes), mutations in *ERBB2* (3%), *BRAF* (2%), *PIK3CA* (1%), *MAP2K1* (1%), and *NRAS* (1%) are also observed (14). *KRAS*-mutant cancers, including lung cancers, have been historically especially difficult to target and are the focus of a new NCI initiative directed toward tackling RAS-mutant cancers. RAS is also the topic of a recent *CCR Focus* on targeting RAS-driven cancers (April 2015). Clinical trials to assess the efficacy of targeted therapies in tumors harboring the less common oncogenic driver mutations are ongoing (e.g., NCT01336634).

One of the most surprising revelations of the past decade has been the discovery of recurrent gene fusions in NSCLC in addition to ALK-rearrangements. Gene fusions involving the tyrosine kinases *ROS1* and *RET* are found in 1% to 2% of lung adenocarcinomas (15–17). Tumors harboring *ROS1* fusions were recently shown to have a 72% response rate to crizotinib demonstrating the sensitivity of these tumors to TKIs (NCT00585195; ref. 18). Preliminary data in *RET* fusion positive lung cancers suggest that these tumors may also be responsive to TKIs like cabozantinib that can inhibit RET (NCT01639508; ref. 19). Fusions involving the receptor tyrosine kinase NTRK1 were also recently reported in lung adenocarcinoma (20). Whether these tumors are sensitive to TRKA inhibitors remains to be determined; however, preclinical work in cell lines indicates that such drugs can inhibit phosphorylation of the NTRK1 fusion and reduce cell growth (20). Finally,

CD74-NRG1 fusions have been detected in invasive mucinous lung adenocarcinomas and could potentially be targeted using drugs to block ERBB receptors and their downstream signaling molecules (21).

Collectively, these data demonstrate how the majority (~60%–70%) of lung adenocarcinomas harbor potentially actionable alterations in oncogenic drivers. Indeed, a recent study performed by the Lung Cancer Mutation Consortium (a consortium of 14 institutions) profiled 733 lung adenocarcinomas and found alterations in at least one of ten oncogenes tested in 64% of the tumors (22). Interestingly, patients who were matched to genotype-directed therapy had better survival than those who were not highlighting the promise of matching tumor-genotype to therapy, although confirmatory studies remain to be conducted.

Is there a role for molecular profiling in lung squamous cell carcinoma and SCLC?

Gandara and colleagues review molecular alterations found in lung squamous cell carcinoma and their implications for treatment in this *CCR Focus* (23). To date, none of the recurrent molecular alterations—including amplification and/or mutation of *FGFR* family members, which are commonly altered in lung squamous cell carcinoma—have proven to be as predictive for response to therapy as *EGFR* or *ALK* alterations in lung adenocarcinoma. We await the results of clinical trials like the The Lung Master Protocol trial (Lung-MAP; NCT02154490) that are designed to investigate the relationship between driver mutations and response to therapy to determine whether treatment stratification based on molecular profiles is useful in lung squamous cell carcinoma (24).

Similar to lung squamous cell carcinoma, the relationship between specific molecular alterations and response to therapy in SCLC remains to be determined (25). As described by Pietanza and colleagues, subgroups of SCLC with distinctive genotypic features, may be sensitive to certain drugs (25). These include tumors with *FGFR1* amplification, PARP overexpression or *MYC* amplification that could be responsive to FGFR, PARP, and Aurora kinase inhibitors, respectively.

In summary, although tumor profiling is well-established for lung adenocarcinomas, its clinical benefits for other histologic subtypes of lung cancer such as lung squamous cell carcinoma and SCLC are still unclear.

Lung cancer -omics and new targets

Although driver mutations in oncogenes are prevalent and play a critical role in lung adenocarcinoma, their role is not as clear in other lung cancer subtypes. Moreover, even in oncogene-driven lung cancers, targeted therapies are usually only partially effective. To better understand the biological landscape of lung cancers, national and international large-scale -omic studies were undertaken shortly after the discovery of *EGFR* mutations. The NIH selected lung adenocarcinoma as one of the cancer types to study in the Tumor Sequencing Project, a pilot demonstration project for the developing TCGA, The Cancer Genome Atlas (26). The successful completion of this project paved the way for genomic efforts, including both the lung squamous cell carcinoma and lung adenocarcinoma TCGA and the Clinical Lung Cancer Genome Project (27–29). Independent of TCGA efforts, comprehensive genomic analyses of SCLC have also been performed (30, 31). Collectively, these efforts have contributed to the identification of new driver mutations and potential therapeutic targets in lung cancer. They have also increased our understanding of these diseases and have revealed that, across subtypes, lung cancers are among the tumors with the highest mutational burden along with melanoma and bladder cancer (32). Although this finding alone is not surprising, given the high carcinogen exposures of these cancers, it poses a challenge for distinguishing driver versus passenger alterations.

In addition to comprehensively identifying mutations in genes that encode members of RTK-induced signaling cascades, the genomics efforts have uncovered mutations in genes involved in other important cellular processes. Chromatin-modifying genes are recurrently mutated in lung adenocarcinoma, lung squamous cell carcinoma, and SCLC (27, 28, 30, 31), and represent potential therapeutic targets in these diseases. A recent report described the increased sensitivity of lung cancer cell lines with *SMARCA4* mutations to inhibition of the methyltransferase EZH2 and etoposide, highlighting how targeting epigenetic regulators in the appropriate genomic context could represent a valid therapeutic strategy (33).

Other pathways altered in lung cancer, however, may be even more challenging to target. For example, according to data from the genomic characterization of lung squamous cell carcinoma by the TCGA, mutations in the KEAP1-CUL3-NFE2L2 oxidative stress response pathway are found in 34% of these cancers (28). Activation of this pathway, which is also frequently observed in lung adenocarcinomas, can promote cell proliferation and survival by stimulating the metabolism of cancer cells and regulating redox balance, processes that may contribute to chemo- and radioresistance (34). Similarly, alterations in genes involved in lung differentiation and lineage-specification are also commonly observed although the functional relevance of these changes still remains to be well understood and targeting differentiation pathways is challenging. In lung squamous cell carcinoma, for example, close to half of the tumors examined in the TCGA had alterations in genes that regulate squamous differentiation like *TP63* and *SOX2* (28). *SOX2* is also frequently amplified in SCLC and has been shown to be a potential driver in these tumor as *SOX2* knockdown in SCLC cell lines with high SOX2 expression, led to a decrease in cell viability (30, 31, 35). In lung adenocarcinoma, amplification of the lineage-specific transcription factor *NKX2.1* has been described, although recent data indicate that it may have dual oncogenic and suppressive functions depending on the context complicating therapeutic considerations (36–39).

The comprehensive genomic studies have set the stage for our understanding of the mutational, expression, epigenetic, and proteomic changes present in the different lung cancer subtypes. This very valuable information underscores the complexity of lung cancer and is playing a crucial role in the prioritization of functional studies that will allow the identification of *bona fide* therapeutic targets in coming years.

Beyond mutations: the future of molecular profiling for lung cancer

The integration of genomic data, functional studies, and data from biomarker-driven clinical trials will shape molecular profiling of lung cancer in the near future. It is likely that this will at a minimum include mutational analysis of a panel of cancer genes, along with determination of copy-number alterations and rearrangements. One area of particular excitement in this regard is that

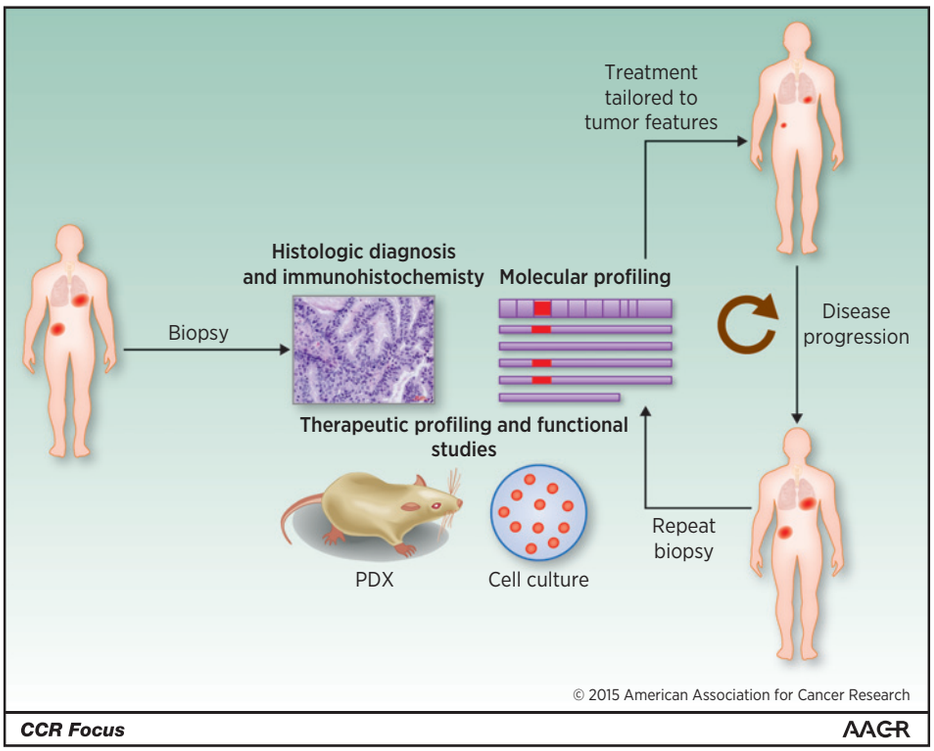

Figure 2.
Schema of a potential process for incorporating molecular profiling and repeat biopsies into the treatment of lung cancer. The initial diagnostic biopsy is evaluated using histopathology, immunohistochemistry, and molecular profiling to determine therapy. A repeat biopsy is collected at the time of acquired resistance and reanalyzed using the same methods to identify changes between the pretreatment specimen and that collected at resistance. The process is repeated through each different line of therapy. When possible cell lines and patient-derived xenografts should be generated to facilitate functional studies of resistance mechanisms and therapeutic testing.

of developing biomarkers of response to drugs that target the immune system. These therapies, and in particular immune checkpoint inhibitors, are showing remarkably durable responses in subsets of lung cancer patients (40) and the first immunotherapy, nivolumab, was approved for second-line treatment of lung squamous cell carcinoma recently in March 2015 (41). Advances in this field are reviewed by Soria and colleagues in this *CCR Focus* (42). Given that only approximately 20% of tumors respond to immune checkpoint inhibitors, investigators and pharmaceutical companies are prioritizing the identification of biomarkers of response and resistance to these agents. Expression of the ligand for the immune checkpoint molecule PD-1, PD-L1, on tumor cells, and/or other immune cells in the microenvironment is being explored as a marker predictive of response to immunotherapies (43). Moreover, just recently, it was shown that response to anti–PD-1 therapy is correlated with a higher mutational load (44). Although it still remains to be determined which biomarker will eventually be most predictive of response to immunotherapies, it is likely that an assessment of such a marker will be incorporated into future molecular profiling of lung cancer.

Tackling Drug Resistance

Today advanced lung cancer remains an incurable disease due to the inevitable emergence of drug resistance even in cases when tumors initially respond well to therapy. Efforts to delay/prevent or overcome drug resistance require an understanding of the mechanisms of acquired resistance. Current technologies for genomic and functional studies of tumors paired with the increasing appreciation for the importance of repeat biopsies of tumors at the time of disease progression have contributed, in recent years, to our understanding of acquired resistance, especially to targeted therapies. Harnessing and expanding these efforts to study tumor evolution through treatment, with chemotherapy, targeted therapy or immunotherapy, is likely to shape the landscape of clinical trials and treatment strategies for patients in coming years.

Repeat biopsies in lung cancer

Much of our current understanding of acquired drug resistance has come from the molecular analysis of repeat biopsies at progression in patients with *EGFR*-mutant or *ALK*-rearranged lung cancer following treatment with a TKI.

In 2004 when *EGFR* mutations were discovered, it was very uncommon to perform a rebiopsy if a patient with lung cancer developed progressive disease. Rebiopsies were seen as potentially harmful and unlikely to provide beneficial information for further treatment. Shortly after the discovery of *EGFR* mutations in EGFR TKI-sensitive lung adenocarcinomas, groups at Memorial Sloan Kettering Cancer Center (MSKCC) and Harvard described a secondary mutation in EGFR, the $EGFR^{T790M}$ mutation, in tumors that had acquired resistance to TKIs in patients who had undergone a repeat biopsy at the time of progression (45, 46). Now we know that this mutation accounts for more than 50% of cases of acquired resistance to EGFR TKIs and drugs that can inhibit the activity of $EGFR^{T790M}$ mutants are currently under clinical investigation (47–49). Additional mechanisms of resistance to EGFR TKIs were also uncovered through analysis of repeat biopsies, including the transformation to SCLC, *HER2*, and *MET* amplification, *PIK3CA* and *BRAF* mutations and *NF1* downregulation, as described in this *CCR Focus* by Riely and Yu (7, 50–55).

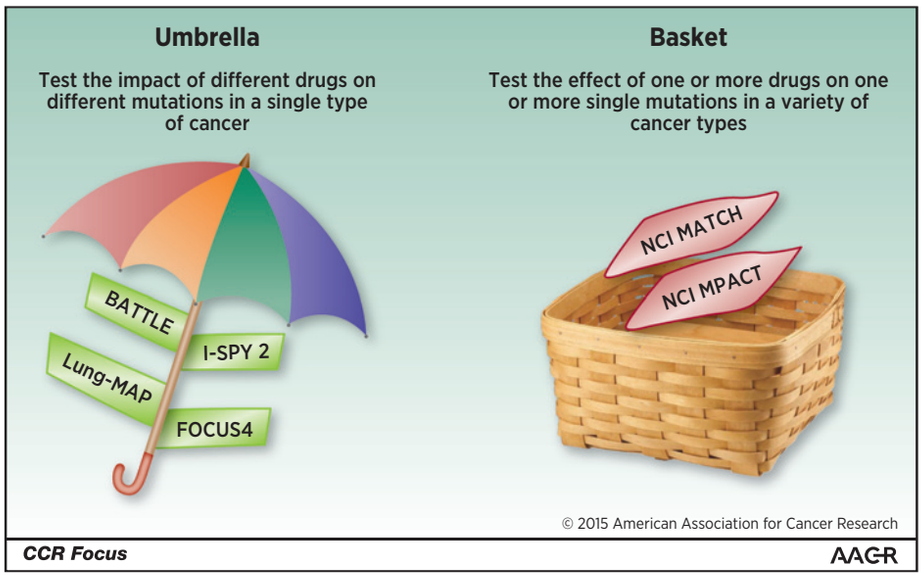

Figure 3.
Novel clinical trial designs to test precision-medicine approaches in cancer. Examples of trials that fall under the "umbrella" and "basket" trial categories are shown. Reprinted from Herbst et al. (24).

Repeat biopsies have also been very informative to track the evolution of disease in ALK-rearranged lung cancers as discussed by Katayama and colleagues (8). Approximately 25% of crizotinib-resistant tumors harbor secondary mutations in ALK (56). Newly developed agents with higher potency, including alectinib, ceritinib, and AP26113, have the ability to inhibit the activity of several crizotinib-resistant mutants (including the L1196M gatekeeper mutation). In this regard, a recent study showed that tumors resistant to ceritinib harbored mutations that confer resistance to this drug even though these were not detected post-crizotinib in the same patient (57).

Incorporating rebiopsies at disease progression into clinical practice

The NCCN guidelines indicate that it is reasonable to perform a biopsy at the time of disease progression in EGFR-mutant TKI-resistant lung cancers. Indeed, as transformation to SCLC is a mechanism of resistance, it is important to exclude its presence given that the treatment of SCLC is so different from that of lung adenocarcinomas. Moreover, the presence of $EGFR^{T790M}$ has been shown to have prognostic value with $EGFR^{T790M}$ mutations portending to better outcomes (58, 59). Finally, it is likely that third-generation TKIs will be used in the setting of $EGFR^{T790M}$-positive disease, where they are showing the best results. Similarly, in ALK-rearranged lung cancer the presence of a secondary ALK mutation at the time of acquired TKI resistance could determine whether the patient receives an ALK inhibitor in second-line or is treated with a different regimen altogether. Furthermore, recent findings have unveiled how new ALK inhibitors have unique patterns of specificity; therefore, the nature of the specific secondary ALK mutation present could further narrow down the choice of subsequent TKI therapy (60).

The results from studies of TKI-resistant EGFR and ALK-mutant lung cancer support the use and incorporation of repeat biopsies into routine clinical practice to assist with determination of future treatment strategies (Fig. 2). Concerns regarding the feasibility of performing such biopsies in lung cancer in the past have been mitigated by successful programs at several institutions. In 2013, investigators at MSKCC reported on the molecular analysis of 155 cases of EGFR-mutant TKI-resistant lung cancer (61). Underscoring the feasibility of this approach, only 3% of specimens had to be excluded due to insufficient material and specimens were collected from a wide range of procedures, most commonly lung, liver, or lymph node biopsies and brain metastasis resections. Additional series of rebiopsies have been reported, further testifying to the increasing acceptance of this approach (51, 62).

The future of repeat biopsies at the time of disease progression

Currently, repeat biopsies are mostly performed in cases of acquired resistance to a targeted- or immuno-therapy with some tissue being used for routine molecular studies and the majority being stored to be used for research purposes. One of the pitfalls of this approach, however, is that specimens are small, therefore, limited studies can be performed. Furthermore, extensive analysis of signaling pathways is not easily feasible in these specimens. However, with improvements in cell culture techniques and in the establishment of patient-derived xenografts, scientists are attempting to propagate resistant tumors (63). This allows for a virtually unlimited supply of tumor that can be analyzed and also used to test the drug sensitivities of resistant cancers. Resources like these will allow the identification of rational treatment strategies to prevent/delay or overcome resistance. Future efforts to improve our understanding of drug resistance should also focus on applying similar approaches to tumors resistant to chemotherapy and making sure that all clinical trials incorporate biopsies at the time of disease progression into their protocols.

Clinical Trial Design in the Era of Precision Medicine

New trial designs have been used to match the right drug to the right patient at the right time and are playing an increasingly prominent role in cancer studies, including lung cancer (64). Two major categories of studies follow this design (Fig. 3): "Basket" studies examine the effect of specific therapeutic agent(s) on a

defined molecular target regardless of the underlying tumor-type. This design facilitates a particular targeted therapeutic strategy (i.e., inhibition of an oncogenically mutated kinase) across multiple cancer types. Examples are NCI's **M**olecular **A**nalysis for **T**herapy **Ch**oice (MATCH) and the **M**olecular **P**rofiling based **A**ssignment of **C**ancer **T**herapeutics (MPACT, NCT01827384) trials (65). The second type, "Umbrella" studies, evaluate multiple targeted therapeutic strategies in a single type of cancer. Examples are **I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**l**ecular Analysis **2** (I-SPY TRIAL 2, I-SPY 2, NCT01042379; ref. 66), the FOCUS4 study in advanced colorectal cancer (67), and the phase II adaptive randomization design **B**iomarker-integrated **A**pproaches of **T**argeted **T**herapy for **L**ung Cancer **E**limination (BATTLE; ref. 68) and BATTLE-2 (64, 69) in NSCLC (NCT00409968 and NCT01248247).

Lung-MAP is a recently initiated umbrella trial specifically for patients with advanced lung squamous cell carcinoma and is described extensively in the review on lung squamous cell carcinoma in this *CCR Focus* (23). It is built on the principles and approaches of the previously mentioned trials. Particularly, I-SPY 2 established infrastructure for conduct of a Master Protocol (including development of the Master Investigational New Drug application with the FDA). Although based on concepts developed in I-SPY 2 and the BATTLE trials, Lung-MAP has a different overall strategy. In this study, each modular arm is designed to take a drug from phase II to phase III (if it meets an interim phase II analysis). This trial has been described in a recent review (24). Importantly, since nivolumab has been approved in this setting, this trial remains adaptive and is evolving, allowing for the potential to study new immunotherapy combination regimens. The Master Protocol approach has also been adopted to determine which of the several ALK TKIs recently developed should be used as first-line therapy for this disease as described by Katayama and colleagues (8). Whether such approaches are more widely adopted in the future will depend on outcomes of these studies.

Conclusions

Developments in lung cancer research over the past decade have galvanized the community and stimulated studies that are changing the way lung cancer is treated. Despite progress, metastatic lung cancer remains incurable. Challenges for the coming decade are to harness our knowledge of the biology of lung cancer to combat drug resistance and to develop novel durable, cost-effective therapeutics to improve survival of patients with this disease.

Disclosure of Potential Conflicts of Interest

K. Politi reports receiving a commercial research grant from AstraZeneca and Kolltan; is listed as an inventor on a patent application for $EGFR^{T790M}$ mutation testing, which is licensed to MolecularMD by Memorial Sloan Kettering Cancer Center; and is a consultant/advisory board member for Takeda. R.S. Herbst is a consultant/advisory board member for Biothera, Diatech, Kolltan, and N-of-One. No other potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K. Politi, R.S. Herbst
Development of methodology: K. Politi, R.S. Herbst
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Politi, R.S. Herbst
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Politi, R.S. Herbst
Writing, review, and/or revision of the manuscript: K. Politi, R.S. Herbst
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Politi, R.S. Herbst

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