From targets to targeted therapies and molecular profiling in non-small cell lung carcinoma

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Although tumor molecular-profile-directed therapy appears promising in early clinical studies, there are many practical challenges to its successful clinical application in non-small-cell lung cancer (NSCLC). These challenges may be broadly classified as those relating to tumor (heterogeneity), tissue (acquisition and processing), testing (assays for molecular profiling) and trials (clinical evaluation of molecular markers and drugs). Strategies to overcome these challenges include (i) understanding the biological basis of tumor heterogeneity and of carcinogenesis in the large subset of patients with no currently evident driver events; (ii) technological advances in minimally invasive acquisition of tumor and next-generation sequencing (NGS) which would enable single-platform analysis of molecular alterations in limited tissue at a reasonable turnaround time (TAT); (iii) deliberation in early stages of drug development as well as clinical trial design to identify, validate and assess the clinical utility of biomarkers in conjunction with drugs and (iv) collaboration to improve understanding of and accrual to trials enrolling patients with rare molecular alterations.

Key words: molecular profiling, next-generation sequencing, non-small-cell lung cancer, tumor heterogeneity

introduction

Improved understanding of the molecular mechanisms of non-small-cell lung cancer (NSCLC) which are essential for carcinogenesis and tumor progression has led to the development of drugs targeting these malignant-cell-specific vulnerabilities. However, these drugs are most efficacious in patients whose tumors harbor specific molecular alterations and their effectiveness may go undetected in unselected study groups. Clinical features alone have proven insufficient to predict the presence or absence of these genetic alterations.

Molecular profiling, the prospective analysis of tumor genetic expression, proteomic profile, deregulated cellular pathways and/or somatic mutations [1] could identify patients who are most likely to benefit from a specific drug and thereby, potentially improve outcomes, minimize toxic effects and abbreviate drug development. However, the increasing number of driver mutations in ever smaller subsets of patients and the availability of an array of candidate drugs (Table 1) have made clinical application of this paradigm challenging. In this review, we will discuss molecular profiling in NSCLC—the opportunities, challenges and potential strategies to overcome them.

clinical application of molecular profiling

Molecular profiling has been found feasible and of potential clinical benefit in patients with refractory metastatic solid tumors (Table 2) [2,3]. A pilot trial in refractory metastatic cancers demonstrated clinical benefit, defined as progression-free survival (PFS) ratio (PFS on molecular-profile-directed treatment/PFS on prior treatment) of ≥1.3 in 27% (18 out of 66) of patients who received molecular-profile-directed therapy (95% CI, 17%–38%; one-sided \( P = 0.007 \)) [2]. A phase I program reported longer time to treatment failure compared with prior therapy (median 5.3 versus 3.2 months, \( P = 0.0003 \)) and higher overall response rate (ORR) (29% versus 8%; \( P = 0.0001 \)) in 161 patients with advanced malignancies and one genetic alteration who received molecular-profile-directed therapy compared with patients who received treatment not directed by the molecular profile [3].

In NSCLC, several reports have demonstrated the feasibility of molecular analyses in a majority of patients (Table 2) [4–7]. These studies used either archival tissue or fresh biopsies and multiple assays—fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR)-based direct sequencing and/or multiplexed genotyping platforms. The median turnaround time (TAT) for results was 2 to 4 weeks and at least one genetic alteration was identified in 51%–84% of patients. The Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE-1) trial was the first completed prospective, molecular-profile driven trial.
in NSCLC [7]. Chemo-refractory patients underwent mandatory pretreatment biopsies. Based on the tumor molecular profile, they were assigned to five biomarker groups (EGFR, KRAS/BRAF, VEGF/VEGFR2, RXR/Cyclin D1, None) and randomly assigned initially equally and later adaptively to four treatment groups (erlotinib, sorafenib, vandetanib or erlotinib plus bexarotene). The overall 8-week disease control rate (DCR) was 46% and eight of the 20 biomarker-treatment matches met the predefined criterion for efficacy, i.e. >80% probability of achieving a >30% 8-week DCR.

Drawing from these and our own experiences of a pilot trial of molecular profiling in lung cancer, we will discuss the challenges to clinical application of molecular profiling under four broad categories: tumor (heterogeneity), tissue (acquisition and processing), testing (assays for molecular profiling) and trials (clinical evaluation of molecular markers and drugs).

### Table 1. Frequency of common genetic alterations in advanced non-small-cell lung cancer (NSCLC), their clinico-pathologic correlates and the drugs targeting them

<table>
<thead>
<tr>
<th>Genetic alteration</th>
<th>Gene</th>
<th>Frequency (%)</th>
<th>Major clinico-pathological correlates</th>
<th>Selected drugs targeting the gene/s (additional targets) (phase of clinical trial evaluation in NSCLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>EGFR</td>
<td>10–35</td>
<td>Asian, female, never smoker, adenocarcinoma</td>
<td>Erlotinib (approved) Gefitinib (approved) Afatinib (EGFR/HER2) (phase III) Dacomitinib (Pan HER) (phase III)</td>
</tr>
<tr>
<td></td>
<td>HER2</td>
<td>2–4</td>
<td>Never smoker, female, adenocarcinoma</td>
<td>Lapatinib (EGFR/HER2) (phase III) Dacomitinib (Pan HER) (phase III) Afatinib (EGFR/HER2) (phase III)</td>
</tr>
<tr>
<td></td>
<td>PI3K</td>
<td>1–3</td>
<td>Squamous cell carcinoma</td>
<td>BKM120 (phase II) Pictilisib (phase II) PX866 (phase II) XL147 (phase II) XL765 (PI3K/MTOR) (phase II) BEZ235 (PI3K/MTOR) (phase II) BYL719 (phase II) Perifosine (PI3K/AKT) (phase II) PF04691502 (phase II) PKI587 (PI3K/MTOR) (phase I)</td>
</tr>
<tr>
<td>AKT1</td>
<td></td>
<td>1–2</td>
<td>Not described</td>
<td>None</td>
</tr>
<tr>
<td>KRAS</td>
<td></td>
<td>15–25</td>
<td>Former/current smokers</td>
<td>Pazopanib (multiple kinases) (phase III) Dabrafenib (phase II) Selumetinib (phase II) Trametinib (phase II)</td>
</tr>
<tr>
<td>BRAF</td>
<td></td>
<td>2–3</td>
<td>Former/current smokers</td>
<td>None</td>
</tr>
<tr>
<td>MEK</td>
<td></td>
<td>1</td>
<td>Adenocarcinoma</td>
<td>None</td>
</tr>
<tr>
<td>DDR2</td>
<td>ALK</td>
<td>3–7</td>
<td>Squamous cell carcinoma</td>
<td>Dasatinib (multiple kinases) (phase II) Crizotinib (MET/ALK/ROS) (approved) AP26113 (ALK/EGFR) (phase II) LDK 378 (phase I)</td>
</tr>
<tr>
<td>ROS1</td>
<td></td>
<td>1</td>
<td>Younger age, never/light-smokers, adenocarcinoma</td>
<td>Crizotinib (MET/ALK/ROS) (phase II)</td>
</tr>
<tr>
<td>KIF5B-RET</td>
<td></td>
<td>1</td>
<td>Younger age, never/light-smokers, adenocarcinoma</td>
<td>Sunitinib (multiple kinases) (not in clinical trials)</td>
</tr>
<tr>
<td>Amplification</td>
<td>MET</td>
<td>3</td>
<td>EGFR mutant tumors following prior treatment with EGFR TKI</td>
<td>Onartuzumab (phase III) Tivantinib (phase III) Cabozantinib (multiple kinases) (phase II) Lenvatinib (multiple kinases) (phase II) Brivanib alaninate (multiple kinases) (phase II) BG398 (pan-FGFR) (phase I)</td>
</tr>
<tr>
<td>FGFRI</td>
<td></td>
<td>2</td>
<td>Squamous cell carcinoma</td>
<td>None</td>
</tr>
</tbody>
</table>

NSCLC, non-small-cell lung cancer; TKI, tyrosine kinase inhibitor.
Table 2. Summary of reports of clinical application of molecular profiling

<table>
<thead>
<tr>
<th>Author, year of publication</th>
<th>N</th>
<th>Type of study</th>
<th>Patient characteristics</th>
<th>Tissue used</th>
<th>Targets interrogated</th>
<th>Technology used</th>
<th>Median turnaround time (TAT) in weeks</th>
<th>Success rate (all proposed tests carried out) (%)</th>
<th>Frequency of at least one genetic alteration (%)</th>
<th>Treated with a matched-targeted agent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic malignancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Von Hoff et al. 2010 [2]</td>
<td>86</td>
<td>Prospective multi-institutional trial with central laboratory</td>
<td>Refractory metastatic cancers</td>
<td>Mandatory fresh biopsy</td>
<td>11 proteins 51 genes</td>
<td>IHC, FISH, Gene expression microarray</td>
<td>NR</td>
<td>98</td>
<td>98</td>
<td>77</td>
</tr>
<tr>
<td>Tsimberidou et al. 2011 [3]*</td>
<td>955</td>
<td>Prospective single institution trial</td>
<td>Refractory metastatic cancers</td>
<td>NR</td>
<td>NR</td>
<td>PCR, IHC, FISH</td>
<td>NR</td>
<td>89</td>
<td>41.5</td>
<td>19</td>
</tr>
<tr>
<td>Non-small-cell lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kris, 2010 [5]*</td>
<td>301</td>
<td>Prospective single institution trial</td>
<td>Adenocarcinoma</td>
<td>Previously obtained tissue</td>
<td>EGFR, KRAS, BRAF, HER2, PIK3CA, MBIK1 AKT1, ALK</td>
<td>PCR-based direct sequencing and Sequenom&lt;sup&gt;a&lt;/sup&gt;, FISH</td>
<td>NR</td>
<td>92</td>
<td>58</td>
<td>17</td>
</tr>
<tr>
<td>Ortiz et al. 2011 [6]*</td>
<td>226</td>
<td>Prospective single institution trial</td>
<td>Non-squamous NSCLC</td>
<td>NR</td>
<td>EGFR, KRAS, BRAF, PIK3CA, HER2, ALK</td>
<td>PCR-based direct sequencing, FISH</td>
<td>4</td>
<td>89</td>
<td>54</td>
<td>14</td>
</tr>
<tr>
<td>Sequist et al. 2011 [4]</td>
<td>589</td>
<td>Retrospective single institution experience</td>
<td>NSCLC</td>
<td>NR</td>
<td>AKT1, APC, BRAF, CTNNB1, EGFR, ERBB2, FLT3, IDH1, IAK2, KIT, KRAS, NOTCH1, NRAS, PIK3CA, PTEN, TP53, ALK EGFR, KRAS, BRAF, Cyclin D1, VEGF, VEGFR-2, RXRs α, β, γ</td>
<td>SNaPshot&lt;sup&gt;b&lt;/sup&gt;, FISH</td>
<td>2.8 (range 1.0–8.9 weeks)</td>
<td>95</td>
<td>51</td>
<td>22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kim et al. 2011 [7]</td>
<td>255</td>
<td>Prospective single-institution-randomized trial</td>
<td>Advanced pre-treated NSCLC</td>
<td>Mandatory fresh biopsy</td>
<td>PCR-based direct sequencing, FISH, IHC</td>
<td>&lt;2</td>
<td>NR</td>
<td>84</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

*Abstract only.
<sup>a</sup>Sequenom: a multiplexed mass spectrometry-based assay.
<sup>b</sup>SNaPshot: multiplexed PCR-based assay.
<sup>c</sup>78 (22%) of the 353 patients with advanced disease were candidates for targeted therapy.

N, number of patients; PCR, polymerase chain reaction; NSCLC, non-small-cell carcinoma; NR, not reported.
TTNB, which is employed for more peripheral and mediastinal lesions. The reported rates of successful molecular profiling with TTNB vary depending on the fixation method and the extent of analysis carried out (Table 3) [8–11].

The need to minimize biopsy-related complications has prompted interest in the use of less invasive sources of tissue like cytological specimens, circulating tumor cells (CTCs) and free serum DNA [12–14]. Although molecular profiling of cytological samples (for e.g. aspirates like those obtained using endobronchial ultrasound-guided transbronchial needle aspiration and pleural effusions) was discouraged in the past, recent studies demonstrate the feasibility of using an adequately cellular tumor cytology sample [12, 15, 16]. Depending on the frequency of mutations and tumor cell proportion, cytological material can provide results comparable with surgical specimens [16]. CTCs captured using a microfluidic-based device containing epithelial-cell adhesion molecule-coated microposts successfully identified the expected EGFR activating and resistant mutations in 92% and 55% of patients with EGFR-mutated NSCLC [13]. In 12 patients for whom primary tumor samples, CTC, and plasma were all available, CTC-based detection had a sensitivity of 92%. Further studies are needed to clarify whether this potential source of cancer cells is representative of the primary tumor.

Once tissue is obtained, its strategic management, i.e. processing to preserve as much as possible for molecular testing, is essential. This involves planning and coordination between members of the thoracic oncology multidisciplinary team to choose the optimal tissue acquisition procedure as well as allocation of tissue for morphological diagnosis and prioritization of molecular studies [17]. Based on the assay used, amount and type of tissue available and the specific needs in individual cases, each laboratory must determine its own priority of tests. Figure 1 shows a schema which is based on the available data to facilitate optimal use of available tissue for treatment decisions and exploratory studies. It is conceivable that this model would be simplified in the near future with the clinical use of comprehensive clinical genomic analyses.

Other important considerations after tissue acquisition include standardization of fixation and processing methods, TAT and quality control. Formalin-fixed paraffin-embedded (FFPE) specimens, the most common source of tissue, may yield poor-quality DNA due to cross-linking and degradation which leads to decreased amplicon length and artifactual mutations [18]. Cytological specimens may afford better preservation of nucleic acids and nuclear structure due to the use of alcohol-based fixation and direct smearing of cells as opposed to formalin fixation and tissue sections in FFPE specimens [19]. TAT, the interval from genotype requisition to result finalization, is an important consideration in advanced NSCLC due to the rapid clinical course. TAT may be prolonged, for example in cases where an alternative sample is requested from an outside institution when it is not feasible to obtain a new biopsy or when the initial specimen was of poor quality and retesting is needed. Guidelines have been issued

### Table 3. Selected reports evaluating common primary tumor acquisition methods in NSCLC

<table>
<thead>
<tr>
<th>Author, year of publication</th>
<th>Type of study</th>
<th>N</th>
<th>Procedure used</th>
<th>Type of tissue obtained</th>
<th>Molecular analyses carried out</th>
<th>Complications</th>
<th>Successful molecular analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill et al. 2012 [11]a</td>
<td>Single-institution retrospective review</td>
<td>81</td>
<td>CT-guided TTNB</td>
<td>Formalin-fixed paraffin embedded (FFPE)</td>
<td>PCR-sanger sequencing for hotspot mutations; EGFR, KRAS, BRAF, PIK3CA HER2; FISH: ALK</td>
<td>Pneumothorax: 23 (28.3%) Chest tube: 6 (7%) Hospitalization: 9 (11%)</td>
<td>Sequencing: 64 (79%) FISH: 60 (71%)</td>
</tr>
<tr>
<td>Solomon et al. 2010 [9]</td>
<td>Selected consecutive patients from a phase II single-institution trial</td>
<td>18</td>
<td>CT or fluoroscopy guided TTNB</td>
<td>FFPE</td>
<td>EGFR by direct sequencing or PCR; KRAS by direct sequencing</td>
<td>No chest tube placement or hospitalization</td>
<td>16 (89%)</td>
</tr>
<tr>
<td>Cheung et al. 2010 [10]</td>
<td>Retrospective review</td>
<td>47</td>
<td>CT-guided TTNB</td>
<td>Fresh frozen</td>
<td>EGFR by PCR</td>
<td>Pneumothorax: 6 (13%) Chest tube: 2% Hospitalization NR</td>
<td>Hemoptysis: 3 (6%)</td>
</tr>
</tbody>
</table>

*aAbstract only.

NSCLC, non-small-cell lung cancer; N, number of patients; CT, computed tomography; TTNB, transthoracic needle biopsy; FFPE, formalin-fixed paraffin embedded; PCR, polymerase chain reaction; FISH, fluorescence in situ hybridization; NR, not reported.
regarding timelines for delivery of archived tissue (both from in-house and outside institutions) for testing of EGFR and ALK [20]. The Clinical Laboratory Improvement Amendments (CLIA) serve as a regulatory standard for tests which will be used for clinical decision making to ensure the accuracy, reliability and timeliness.

Alterations in signaling pathways following successive lines of treatment warrant molecular analysis on biopsies obtained at the time of progression rather than using the original diagnostic biopsy. The importance of assessing cancers throughout the disease course with repeated biopsies is exemplified by studies of patients with EGFR-mutant NSCLC with acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) [21]. The mechanisms of resistance included genetic (e.g. EGFR T790M mutation, PIK3CA mutation) and histologic [e.g. transformation of NSCLC to small-cell lung cancer (SCLC)] alterations with significant impact on the choice of further treatment.

The pursuit of potentially improved outcomes should be balanced against the risks associated with the use of invasive procedures for tissue acquisition. Moreover, ethical concerns apply to mandatory biopsies for research participation [22]. Voluntary informed consent and close monitoring to minimize and manage procedure-related complications are imperative.

**testing: assays for molecular profiling**

Most laboratories use direct sequencing of PCR-amplified exon sequences for identification and confirmation of mutations, which are currently relevant to treatment (e.g. EGFR and
The need for standardization of pre-analytic conditions and cost-effective surrogate for genetic testing and a potential choice of treatment, prognosis and emergence of resistance. Although the feasibility of targeted NGS of FFPE specimens to detect actionable mutations has been demonstrated, at this time, it may be neither feasible nor affordable to use NGS in a clinical setting.

**Tumor: heterogeneity**

Heterogeneity exists between primary and metastatic NSCLC, within an individual tumor and following successive lines of therapy. Tumor heterogeneity assessments in NSCLC to date are limited by the number of genes evaluated (only EGFR and KRAS in most cases) and inconsistent techniques, often with limited sensitivity. The frequencies of heterogeneity reported in these studies have ranged between 0% and 29% for EGFR and 9% and 25% for KRAS mutations. Moreover, biopsy specimens contain a mixture of malignant cells, adjacent normal cells and stroma, and infiltrating normal cells. While small core biopsies may not be representative of clonal heterogeneity of the entire tumor, conventional sequencing of a large sample may miss heterogeneity by representing only the dominant clone.

Intratumor heterogeneity may have implications on the choice of treatment, prognosis and emergence of resistance. The presence of a mixed population of EGFR-mutated and wild-type cells has been reported to result in reduced response to gefitinib. Heterogeneity in the EGFR mutation status between the primary lung tumors and their metastases may explain the mixed response to EGFR TKIs in some patients. The presence of low frequency of pretreatment extreme sensitivity, but may yield false-positive results due to contamination, are difficult to perform on poor-quality RNA extracted from FFPE specimens, need multiplexed assays to detect all of them. The existing approach to drug development also involves development of a companion diagnostic assay in conjunction with a drug which entails optimization of the assay and its analytical and clinical validation, i.e. evidence that the result of the analytically validated assay correlates with the clinical outcome of interest and assessment of clinical utility for the intended use. This approach is costly, especially when the molecular alterations are rare and large numbers of patients have to be screened as exemplified by RET fusions and DDR2 mutations which occur in 1% of NSCLC patients.

Moreover, it is time consuming and requires a large amount of tissues to perform all the different assays. Clinical application of NGS-based platforms may offer a potential solution to this quandary by providing faster, cheaper, yet comprehensive assessment of tumor molecular landscape using limited tissue. Although the feasibility of targeted NGS of FFPE specimens to detect actionable mutations has been demonstrated, at this time, it may be neither feasible nor affordable to use NGS in a clinical setting.
**trial: clinical evaluation of molecular markers and drugs**

Many anticancer therapies benefit only a subset of patients and the benefit may be overlooked by the traditional broad eligibility approach in clinical trials. Hence, the paradigm has shifted towards restricting study enrollment based on the presence of a biomarker which potentially identifies a population that is likely to respond to a given drug. Negative results of several phase III trials of EGFR TKI in unselected patients [48–52] and the rapid clinical development of crizotinib in patients with ALK-rearranged NSCLC [53, 54] provide contrasting examples of the importance of this strategy. Considering the increasing number of drugs and potentially druggable genetic alterations, newer clinical trial designs are needed to simultaneously develop multiple drugs and drug combinations in molecular-profile-defined subsets of patients [55]. There are several ongoing trials which employ novel clinical trial designs to evaluate in parallel multiple-targeted therapies in selected NSCLC patients.

An ongoing pilot trial at the National Cancer Institute is evaluating the feasibility of carrying out fresh biopsies for MP and the efficacy of multiple molecular-profile-directed therapies in 600 patients with advanced thoracic malignancies (NCT01306045). Real-time molecular analysis using multiple platforms (pyrosequencing, NGS, CGH and FISH) identifies oncogenic mutations, insertions, deletions, gene amplifications and translocations of 12 genes to guide treatment allocation while analysis of >190 cancer-related genes is used for the discovery of new biomarkers. Patients with ALK-rearranged NSCLC receive crizotinib, whereas the remaining patients are assigned to one of five experimental arms based on biomarker assessment (EGFR mutation or indel: erlotinib; KRAS, NRAS, HRAS or BRAF mutations: selumetinib; PIK3CA, AKT or PTEN mutations, PIK3CA amplification: MK2206; ERBB2 mutation/amplification: lapatinib; KIT mutation, PDGFRA mutation/amplification: sunitinib) or standard treatment if not eligible for any of the experimental arms. For each of the 15 possible treatment arms (three disease types—NSCLC, SCLC and thymic malignancies; five drugs), the study uses the optimal two-stage phase II design [56].

The BATTLE-1 trial randomized an initial cohort (n = 97; 40%) equally to each of the four treatment arms [7]. Subsequent patients (n = 158; 60%) were randomly assigned according to the Bayesian adaptive algorithm [57], which used the prior probability and 8-week DCR of the initial cohort to generate a ‘posterior’ probability of DCR for a given treatment which was, in turn, used to increasingly assign patients to treatment arms with the greatest efficacy. The Lung Cancer Mutation Consortium Protocol (LCMC), a collaborative effort which involves 14 cancer centers in the United States, aims to determine the frequency of oncogenic mutations in 1000 patients with advanced lung adenocarcinoma (NCT01014286) [58]. The linked clinical and mutational analyses are used to determine the frequency of each mutation, its association with clinical features and outcome and its association with other mutations. As therapeutic protocols specific for these mutations are developed, patients are notified of their eligibility for these studies. A secondary goal of LCMC is to establish a consortium of sites that have the capability of conducting multiple mutation testing in a CLIA-certified lab.

There are several hurdles to molecular-profile-driven patient selection in early-phase clinical trials. This approach runs the risk of discounting the efficacy of a drug when an incorrect biomarker is used for patient selection, which often results from the complexity of signaling pathways, especially in early drug development. A molecular-profile-driven approach also raises issues of validating assays, especially when the assay is used to guide treatment decisions [32]. The efficacy of a drug may be overlooked in biomarker ‘negative’ population. For example, crizotinib resulted in ORR of 50% and 61%, respectively, in two multicenter single-arm studies in patients with ALK-translocated (defined as >15% cells with ALK rearrangement) locally advanced or metastatic NSCLC which led to its Food and Drug Administration (FDA) approval [53, 54]. However, in an expansion cohort of 19 ALK-negative patients, crizotinib resulted in five partial responses (ORR 26%, 95% CI, 9%–51%) [59]. It is unclear whether the responses to crizotinib in ALK-negative patients are a result of its activity in other genetic alterations (e.g. MET amplification or ROS rearrangement) [60] or due to issues with validation of assay performance. To address these questions, FDA has recommended a clinical trial to explore the activity of crizotinib in ALK-negative patients, adequacy of current assay cut-off and the role of additional biomarkers. Alternative clinical trial designs which do not require biomarker selection before initiation of study may address some of these issues [61, 62]. Accrual to trials enrolling rare subgroups is also a challenge in molecular-profile-driven patient selection. LCMC is an example of a nationwide initiative, which could identify and maximize accrual to trials of rare molecular subtypes.

The remarkable responses seen in early-phase clinical trials of some molecular-profile-directed therapies such as crizotinib have also raised questions regarding the necessity and feasibility of randomized phase III trials before regulatory approval [63]. Forgoing phase III trials would expedite drug development, improve patient access and mitigate cost, but may yield less definitive safety and efficacy data [64]. Despite its potential risks, early efficacy results of selected drugs in a molecular-profile-defined population may potentially be used to forgo pre-marketing phase III trials [63, 64].

**conclusion and future directions**

Among the several molecular targets that are being investigated in NSCLC, inhibition of only a few in selected patients has led to meaningful clinical benefit (e.g. EGFR, ALK), whereas many others have not proven useful (e.g. IGF1R). A lack of validated
biomarkers and patient selection has led to many drugs showing no efficacy or even detrimental effects in clinical studies, despite strong rationale and pre-clinical activity. Molecular-profile-driven patient selection has demonstrated the potential to improve outcomes in NSCLC. The practical challenges to clinical application of molecular profiling may be conceptualized as those relating to ‘tumor’ (heterogeneity), ‘tissue’ (acquisition and processing), ‘testing’ (assays for molecular profiling) and ‘trials’ (clinical evaluation of molecular markers and drugs).

Multidisciplinary determination of the least invasive procedure to obtain adequate specimen, standardization of sample collection, processing and strategic management of available tissue can increase the success rate of molecular profiling. CTC and free serum DNA may be potential alternatives to invasive tissue acquisition in the future. Further studies are needed to understand the biological basis and implications of tumor heterogeneity as well as the role of tumor suppressor genes and epigenetic events in the large implications of tumor heterogeneity as well as the role of tumor suppressor genes and epigenetic events in the large subset of NSCLC patients (~40%) with no known driver mutations. Clinical use of NGS-based platforms could provide faster, cheaper and comprehensive assessment of tumor molecular landscape using limited tissue. Regulatory mechanisms should adapt to the need for such platforms. Beginning from the early stages of drug development and clinical trial design, efforts should focus on identifying, validating and assessing the clinical utility of biomarkers in conjunction with drugs. Collaborative efforts are needed to improve patient accrual to trials of drugs targeting less common genetic alterations. It is anticipated that comprehensive clinical genomic analysis will be a more viable molecular profiling of the vast majority of patients who are cared for in a general oncology setting.

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references
Annals of Oncology


62. Bergethon K, Shaw AT, Ignatius Ou SH et al. ROS1 rearrangements de...