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Review Article

Can Next Generation Sequencing Be the Standard of Care for all Patients With Metastatic Non-Squamous Non-Small Cell Lung Cancer

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Introduction

The genetic alterations of most lung cancers are enormous. There are approximately 20,667 genes in the human genome comprised of over 3 billion base pairs. Smokers with lung adenocarcinoma have, on average, 8-10 mutations per 1 million bases (base pairs), which equates to over 50,000 single nucleotides [1]. The current standard of care in the treatment of stage IV non-squamous non-small cell lung cancer (NSCLC) is based on tailoring treatment on just a handful of actionable genetic drivers. Specifically, the International Association for the Study of Lung Cancer (IASLC), the College of American Pathologists (CAP), and the Association for Molecular Pathology (AMP) issued a joint evidence-based guideline establishing recommendations for molecular diagnostic testing in lung cancer. In summation, their recommendations were to test all patients with lung adenocarcinoma for EGFR and ALK abnormalities regardless of clinical variables such as smoking history, gender, or ethnicity [2]. These guidelines are dated, and the landscape of actionable targets, as well as the availability and cost of technology, have changed dramatically since the recommendation. In light of the explosion of newly discovered gene targets, advances in sequencing technology, and development of novel pharmaceuticals, it is time to reexamine these guidelines and recommend every tumor be sequenced.

The current standard of care for metastatic NSCLC is dependent on if the tumor has EGFR, ALK, ROS-1, BRAF V600, or PDL-1 gene alterations. If the tumor harbors an activating mutation in EGFR exon 19 or 21, an EGFR tyrosine kinase inhibitor is preferred as the first-line therapy [3]. In tumors harboring a ROS-1 or similarly ALK gene alterations, Crizotinib is preferred first-line therapy [4]. In carcinomas with BRAF V600E mutations, the FDA recently approved the combination of Dabrafenib plus Trametinib [5]. If a tumor is PDL-1 positive with a tumor proportion score of ≥50%, single-agent Pembrolizumab is preferred, but only if the tumor does not contain EGFR, ALK, or ROS1 alterations [6]. In the scenario that a tumor has none of the above actionable biomarkers, standard of care is treatment with carboplatin plus pemetrexed or carboplatin plus paclitaxel with or without bevacizumab [7].

Recently, mutations in the MET exon 14 and have been described as a potential target. The MET oncogene has been shown to be a sufficient cause of carcinoma exclusive of other oncogenic drivers including EGFR, KRAS, and BRAF mutations in addition to being exclusive to ALK ROS-1 and RET gene rearrangements [8]. Uniquely, MET mutations are not related to smoking history [8]. Treatment with MET Tki Crizotinib in MET exon 14 positive tumors have shown very high response rates. A single-arm study showing 34 patients with metastatic NSCLC with a MET mutation had median overall survival of 8.1 months, whereas those treated with a MET inhibitor had mean overall survival of 24.6 months [9]. While more evidence is needed, one should consider using a MET Tki as first or second line in patients with MET exon 14 skipping mutations. Despite 3% of lung adenocarcinomas being driven by MET exon 14 skip mutations, a target with known pharmacologic agents, the current standard of care does not currently require genetic testing for MET at diagnosis [10].

The list of possible drug targets continues (see Table 1), and new targets are emerging at a faster pace than ever before. In addition to identifying genes and proteins that are oncogenic drivers, there is another field of small molecules that are emerging as potential biomarkers. JAK and STK11 mutations are potential predictive biomarkers for resistance to immunotherapy, while KRAS mutations, tumor mutational burden, and PDL-1 status may

be markers of increased immunotherapy effectiveness [11]. With the simultaneous rise of advanced genomics and an increase in specialized pharmaceuticals, there has been an explosion of newly designed target therapies. When and if a clinician will use these targeted therapies, however, hinges on profiling the carcinoma genome for both breadth and depth of mutations, this is a task most suited for next genome sequencing (NGS). Therefore, as more molecular and gene targets emerge, is it now time to perform NGS on all patients with NSCLC rather than current standard single molecular target testing? (Table 1).

Table 1: List of Actionable Targets.

Actionable Target	Tier/FDA approval	Drug
EGFR mutations	1st/ Yes	Erlotinib, Gefitinib and Afatinib [12]
ROS1 translocations	1st/Yes	Crizotinib [4]
ALK translocations	1st/Yes	Alectinib [13], Crizotinib [4], Brigatinib[14] and Certinib [15]
Brafv600e mutations	1st/Yes	Dabrafenib and Trametinib [5]
PD-L1 (tumor proportion score >50%)	1st/Yes	Pembrolizumab [16]
Met mutations or amplifications	2nd/No	Crizotinib [17]and Cabozantinib [18]
Her2 mutations	2nd/No	Afatinib, Trastuzumab, and Ado-trastuzumab emtansine [19]
RET mutation or translocation	2nd/No	Cabozantinib [20], Vandetani [21] and Alectinib [22]
LMNA-NTRK1 fusion	2nd/No	LOXO-101[23]

From a clinical perspective, acting on this widespread menu of new actionable targets is only possible if those targets are pursued. Traditional DNA analysis is done with Sanger sequencing (SS), pyrosequencing, or traditional single-gene PCR assays [24]. The major issue with these technologies is only a few genes can be sequenced per run leading to exorbitant cost when looking at more than handful of genes [25]. Additionally, these technologies fail to incorporate intratumor heterogeneity with low sensitivities in identifying oncogenic drivers present at low frequency [26,27]. NGS is a technology in which the entire genome can be analyzed simultaneously, making it an ideal tool when the list of actionable targets is substantial [28].AdditionallyNGS is characterized by high coverage also called "deep sequencing," which allows for the characterization and identification of intratumor heterogeneity of low-frequency variants [29].

The first task for a clinician using NGS is with obtaining a sample. Analyzing samples can currently originate from FFPE tumor blocks, a diagnostic biopsy sample, or, more recently a simple blood draw (liquid biopsy). FFPE tumor blocks are common and allow the sample to undergo further pathological staining later. Storage of FFPE is also commonplace making it easier for further testing in the future.

With liquid biopsy, one obstacle is yield of sufficient tumor. Currently, this process yields, on average, one cancer cell in 106-108 cells, which is insufficient genetic material without undergoing an additional enrichment step [30].

This Enrichment step adds another layer of manageable complexity, which is yet another factor a physician needs to consider when interpreting the results. Important aspects to consider when circulating tumor DNA is used for NGS is both the possibility of excluding possible copy number variations though can catch evolving alterations in tumor heterogeneity that would be missed with a solid tissue biopsies [31,32]. Liquid biopsies do offer several advantages, particularly in late-stage disease, where the amount of ctDNA is sufficient. Benefits include being noninvasive and as cheap to run as tumor-based NGS. Liquid biopsies can also be used as a complementary test in addition to tissue testing especially in detecting low-frequency mutation in clonal evolutions or when tissue-based testing is not possible due to risk of biopsy location [33]. Advances in bioinformatic analysis are now at the point where as long as the sample has adequate coverage, liquid NGS is as sensitive as traditional based PCR in identifying mutations [34].

Additional factors include the availability and access to an NGS platform and the amount of time a clinician must wait for sample processing. While ten years ago, NGS was limited to large, wealthy, private institutions, and large university health systems, NGS technology has since been democratized across the continental US. This has been accomplished as all the major NGS business platforms, including Paradigm, Nantomics, and Foundation, have partnered with UPS and other shipping centers and designed specific and affordable NGS-specific shipment packages. While access has increased, turnaround time is still company dependent with one to 4 weeks being typical for NGS [35].

From a cost perspective, Scluckebier has studied the current standard of care for NGS versus EGFR, ALK, and ROS-1. The current standard is to first test for EGFR mutation and follow up a negative test with a FISH for ALK; if ALK is also negative, the rule is to then look at ROS1. NGS is both more sensitive and more specific, with a 24% increase in positive mutations identified; this comes at an extra cost of $400-800.76 compared to the standard [36] Currently there are numerous patient assistance programs supplied by the

major companies themselves that cover the costs. Of note, the cost of NGS has fallen by an order of magnitude in the last decade, and a budget impact analysis led by Rosa A. van Amerongen shows cost continuing to drop in the coming years; NGS is already cheaper than if one were to send more than two genes for individual testing [37].

The largest advantage of performing NGS over single mutation testing is the reduction of missing information that would yield an actionable target. Single mutation testing gives just that single gene. When a clinician maps out a course for a patient, having every actionable target at hand is incredibly important. Too much information, however, can present its own set of problems. Often NGS reports are excessively extensive and can be somewhat difficult to interpret due to a large number of mutations and alterations of unknown significance. This presents a catch-22 in data science. One cannot correlate and connect these mutations with clinical outcomes if one does not know they exist; conversely, physicians will not order these tests if there is not a known clinical impact. Be that as it may, the current repertoire of known actionable targets is significant enough to warrant NGS over single mutation testing (Table 2) Depends on gene and stage. Early-stage NSCLC ALK 68.8%, 91.7% ALK 100% EGFR. Fraction of ctDNA is also above 0.4% [42,43] Platform-specific.

Table 2: NGS comparison with Single Mutation Testing.

	Tissue- based NGS	Liquid-Based NGS	Single Mutation Testing
Costs	$450[38]	$686[39]	$94-$287 (per test) [38]
Likelihood missing info helpful for RX	Low	Low	High
PPV	100% (95% CI = 86.7, 100.0) [24]	100% (95% CI = 99-100)* [30]	100% for common specific alteration [2]
Amount of sample needed	1mm3 (excisional preferred) [40]	5-10ml peripheral blood/ cfDNA 5-20 ng/ml plasma**	1mm3 or less. Can use FNA, Core, or resection. Sample must consist of at least 50% tumor [2]
Interpretation of Results	Intermediate	Intermediate- Challenging	Simple
Turnaround Time	7-21 days [40,38]	10 Days [41]	0-3 days [2]

Advances in treatment for NSCLC are being driven by an ever-deepening and expanding knowledge of the biology of tumor cells on an individual level. Current guidelines are based on the treatment of just a few genes, but one must wonder why not all genes? Every passing year has brought numerous discoveries in gene targets and subsequent successes with novel pharmaceuticals. For instance, this past year saw the identification of pan tumor aberrations, such as NTRK, while extraordinary rare was able to be detected and targeted [44]. The breadth, ease, and cost of testing for these mutations, which can have profound impact on management, needs to be commonplace. Furthermore, making precision genetics part of the standard of care will further drive down costs, and the data generated from NGS will be a boon to knowledge, providing even more insight into other yet undiscovered genes. We believe we are at the point now both from a medical and economic standpoint to recommend that panel testing be routine for patients with NSCLC.

Conflict of Interest

None.

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