CIRCULOGENE

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Interventional Pulmonologists and Liquid Biopsy Molecular Testing

The survival outcome benefit in advanced non-small cell lung cancer (NSCLC) from molecular testing is immense with a doubling of median overall survival (OS) and potential 5-year durability with targeted and immune-based therapies (1,2). Just as important as knowing the right therapy, it is equally important to avoid the wrong therapy. Not testing for or not knowing a driver mutation or fusion target in metastatic NSCLC is present, will miss the tremendous outcome benefit of the targeted therapy and lead to the potentially wrong therapy of chemoimmune therapy by default. Not knowing immune resistance mutations are present such as STK11 or KEAP1 and co-mutations will lead to ineffective immune-based therapy and potential disease hyperprogression (3,4). Not knowing radiation resistant mutations will lead to poorer survival in curative stage NSCLC. Not knowing the molecular tumor biology at the time of treatment decision making will miss a patient's best treatment and may lead to a wrong treatment with a much poorer survival outcome. Molecular testing is necessary in advanced lung cancer and is now becoming equally important in earlier curative stages of lung cancer. Not testing or not knowing the molecular tumor biology in the 'Precision Oncology' era of lung cancer before starting treatment is no longer an acceptable standard of care.

I. Advances have facilitated an ease of molecular testing in lung cancer

Next-generation technology (NGS) makes molecular testing complete, efficient, and more cost effective than individual sequential molecular testing approaches (5). Individual pathogenic driver mutations, gene rearrangement fusions, or gene amplifications do not need to be individually remembered and ordered. A broad all-encompassing NGS panel provides the complete molecular testing needed.

Liquid biopsy with plasma NGS molecular testing has further extended this needed full molecular testing with a simple blood test. Although tissue and plasma NGS testing remains complementary, completeness and timing of results have led the International Association for the Study of Lung Cancer (IASLC) to publish a consensus statement advocating and supporting a 'plasma first' molecular testing approach in NSCLC (6). Comparative simultaneous tissue and plasma NGS testing unexpectedly has indicated that tissue molecular testing will miss 33-43% of the mutations present, whereas testing plasma first will identify 80-87% of the targetable mutations/fusions (7,8,9). Tissue is still the 'gold standard' in making a diagnosis of cancer. However, given this data, the true 'gold standard' of molecular tumor biology testing has evolved to plasma. More complete molecular findings and a much quicker turnaround time of the

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molecular tumor biology results make a liquid biopsy with plasma NGS an ideal molecular testing approach.

II. Problems with the current model of molecular testing that need to be overcome

1. Molecular testing not getting done

The biggest problem with the current molecular testing approach is that the molecular testing is simply not getting done. Chart review data continues to show National Comprehensive Cancer Network (NCCN) guideline recommended molecular testing is not being performed by medical oncologists in the majority of patients. At the American Society of Clinical Oncology (ASCO) in 2019, a chart review of 1,203 advanced NSCLC patients from five community oncology practices of 289 oncologists, identified full NCCN guideline recommended biomarker testing in only 22% of advanced NSCLC patients (10). Even in the MYLUNG (Molecularly Informed Lung Cancer Treatment in a Community Cancer Network) consortium of US Oncology practices with a structured care pathway system, still under half of advanced NSCLC patients had recommended molecular testing performed (11).

This has led to multi-disciplinary thoracic tumor board discussions and treatment decisions being made without the full molecular tumor biology knowledge. This lack of knowing the full molecular tumor biology can lead to missing the best therapy for an individual and lead to a wrong treatment decision even in earlier stage NSCLC where the curative impact is more profound. How can a multidisciplinary thoracic tumor board begin to consider a treatment recommendation without knowing the molecular tumor biology?

Even more unsettling is medical oncologists are starting treatment without having knowledge of PD-L1, EGFR, or other mutations/fusions. In the MYLUNG consortium, only 35% of patients had the ordered tissue molecular testing results available before initiating first-line treatment (11). Testing but not knowing is no different than not testing and not knowing. The molecular tumor biology will be a guess and the right therapy will be a guess and thus potentially missed. The right therapy matters but also the right therapy first matters. Cross over treatment does not make up the lost survival that occurs when the best therapy is not undertaken first (12).

2. Time matters

The time from diagnosis to treatment matters. It is not the turnaround time of a molecular test that matters. It is the time from diagnosis of the cancer to starting treatment of the cancer that matters. Studies identify a 30-day window from the time from diagnosis to starting treatment as the critical period before survival outcomes begin to fall. Not because of any treatment

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difference, but simply the delay in starting a treatment. Kasymjanova et al reported that starting treatment within 30 days from diagnosis more than doubled the 4-year OS compared to a delay of more than 30 days across all stages of NSCLC (13). A meta-analysis of thirty-four studies across seven major cancer types, including NSCLC, noted a significant association between increased cancer mortality and delaying cancer treatment beyond 4 weeks from diagnosis (14).

3. Tissue only or first approaches

A tissue only or first approach of molecular testing is limiting implementing the therapeutic advances of treating lung cancer. With all advances comes new knowledge. New knowledge should spark new thinking and new ways of doing things. The current model of tissue testing only or first should no longer be an acceptable model of molecular testing in lung cancer. Tissue misses more mutations than plasma NGS testing. Tissue molecular testing is still not being performed in over half of advanced NSCLC patients. Tissue NGS testing takes too long with turnaround times of 3+ weeks leading to starting treatment greater than 30 days from diagnosis.

This 30-day time window starting from diagnosis to starting treatment is vitally important to survival outcome yet is not being achieved when tissue only or first molecular testing is performed. Tissue NGS testing typically has a 3-week turnaround time, when tissue is sufficient for molecular testing. With a needed pathology review for a histologic diagnosis of 3-5 days, followed by a request for tissue NGS testing, this delays initiation of treatment past the 30-day mark in the majority of patients (or patients start on treatment without knowing the molecular tumor biology findings). In the ASCO 2021 presentation and 2022 subsequent publication of the MYLUNG consortium study, the median time from diagnosis to treatment was 36 days (range 25-65 days) (11). Tissue testing also relies on sufficient tissue acquisition for full molecular testing. That in and of itself can be a limiting barrier to molecular testing in over 40% of patients (7,15).

4. Molecular testing impactful in earlier stage NSCLC

Molecular tumor biology also matters in earlier stage lung cancers. Knowing the molecular tumor biology to guide adjuvant treatment matters in resected NSCLC. The proof of principle ADAURA trial has shown a tremendous disease-free survival benefit of an EGFR TKI in EGFR mutant resected NSCLC compared to chemotherapy (16). The original adjuvant cytotoxic chemotherapy trials did not identify a survival benefit in the setting of a KRAS or TP53 mutation. In fact, there was a significant detrimental OS outcome with adjuvant chemotherapy if both KRAS and TP53 mutations were present (17).

Now in the ‘immune era’ of treating NSCLC, IMpower010 has shown a significant disease-free survival benefit in stage II and medically operable stage IIIA of adjuvant immune checkpoint

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inhibitor therapy with cytotoxic chemotherapy in PD-L1 expressing resected NSCLC (18). Pre-clinical data supports a better pre-operative immune-based therapy approach than post-operative approach due to the importance of the intact tumor draining lymph nodes for T-cell priming (19). SWOG S1801 in resectable stage III-IV melanoma showed a significantly improved 72% 2-year event free survival utilizing the same immune therapy in the neoadjuvant setting compared to 49% with the same therapy in the adjuvant setting (20). Given immune tumor biology and immune checkpoint inhibitor therapy, although yet to directly studied, this would also oncologically be expected in NSCLC.

The two phase 3 neoadjuvant chemoimmune therapy trials in operable NSCLC both show a significantly higher pathologic complete response (pCR) resulting in remarkably improved event free as well as overall survival compared to neoadjuvant chemotherapy, supporting a neoadjuvant immune therapy approach. (21,22). As the transition to neoadjuvant chemoimmune therapy evolves in resectable NSCLC, knowing the molecular tumor biology becomes critical in decision making. Unlike the adjuvant setting where surgical tissue is bountiful, small bronchoscopy biopsies may well be fraught with insufficient tissue for full molecular testing, bringing liquid biopsy plasma NGS molecular testing to the forefront just as in stage IIIB/IV disease. Neoadjuvant chemoimmune therapy can be greatly impacted by the presence of immune resistance mutations and potential hyperprogression closing the curative surgical window (4, 23). Knowing the immune checkpoint inhibitor sensitive and more importantly the potential resistant mutations is vital to know in neoadjuvant therapy decision making.

The number of pre-treatment ctDNA alterations are prognostic in advanced as well as earlier stage lung cancers (24-26). The greater the ctDNA shedding into the plasma, the more aggressive the tumor biology. Notably, in the neoadjuvant chemoimmune CheckMate 816 trial, the pCR doubled in those with complete clearance of any pre-treatment ctDNA shedding (21.) In the NADIM trial, clearance of any ctDNA shedding after the pre-operative chemoimmune therapy, was associated with remarkable OS outcomes that was as predictive as the surgical specimen pCR (26).

The molecular tumor biology also has an impact on the local modality treatment decision of NSCLC. A genomic landscape of radiation therapy sensitivity and resistant mutations are now being identified. Mutations in KEAP1, KRAS, PIK3CA, or MET amplification are associated with unfavorable stereotactic radiation treatment benefit in anatomical stage I NSCLC (27-30). The question of post-operative radiation therapy (PORT) with occult or persisting N2 disease is answered by the molecular tumor biology. Loco-regional control and even survival is extremely poor when the radiation resistant mutations of KEAP1, STK11, and PIK3CA are present, whereas PORT demonstrates complete locoregional control with radiation sensitive mutations of POLE, ARID1A, and ATM (31). Molecular tumor biology knowledge in all stages of lung cancer impacts treatment and survival outcomes. Not knowing or not testing for immune therapy and radiation therapy resistant alterations is clearly detrimental to individual patient outcomes in

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curable earlier-stage NSCLC. Liquid biopsy for plasma NGS testing in resectable NSCLC is important for patients to get there best curative outcome possible.

III. Programmatic molecular testing makes a difference across all stages of NSCLC

Implementing a programmatic approach to molecular testing of lung cancer is a vital foundation for a 'center of excellence' lung cancer program. The survival outcome benefits that precision oncology, immune oncology, and aggressive multi-disciplinary treatment of lung cancer provide will be lost if molecular testing is not fully done. With a consistent programmatic approach of molecular testing, all members of the lung cancer program team will know what needs to be done, when it needs to be done, and will ensure it gets done. This will provide the needed molecular tumor biology when treatment discussions and decisions are being made. A programmatic approach to molecular testing will allow one to see the molecular tumor biology never seen or known before. Just as we think and provide better lung cancer care and treatment together as a multi-disciplinary team, having a consistent programmatic approach to molecular testing will ensure the needed molecular testing gets done.

Anatomical staging may not be known at the time of diagnostic bronchoscopy. EBUS frequently identifies unexpected nodal involvement. Beyond the anatomical stage, the aggressiveness of the NSCLC can be identified by the number of ctDNA alterations being shed into the plasma. This impacts a decision regarding the aggressiveness of the treatment decisions as well as the liquid biopsy plasma NGS can guide the best treatment approach. Immune therapy sensitive and resistant mutations make a difference in decision making. Radiation therapy sensitivity and resistance mutations will impact a stereotactic radiation therapy or a PORT decision. Targeted therapy with no benefit of additional cytotoxic chemotherapy can be identified. No matter the stage, vital treatment information for each individual can be identified with liquid biopsy plasma NGS testing.

How can you have a multi-disciplinary treatment discussion, let alone any decision, without knowing the molecular tumor biology findings? Without molecular tumor biology findings, precision oncology and personalized cancer treatment does not exist.

IV. Interventional Pulmonologists as the Fourth Pillar of Lung Cancer

Just as interventional pulmonologists (IP) are integral in the diagnosis and staging of lung cancer, they are integral to a programmatic approach of molecular testing in NSCLC. Thoracic medical oncologists treat lung cancer with a variety of chemotherapy, targeted, and immune-based systemic therapies; yet never directly diagnose or stage the lung cancer. Thoracic radiation oncologists treat lung cancer with a variety of radiation therapy modalities for palliation, definitive local/locoregional control, and now immune modulation; yet never directly diagnose

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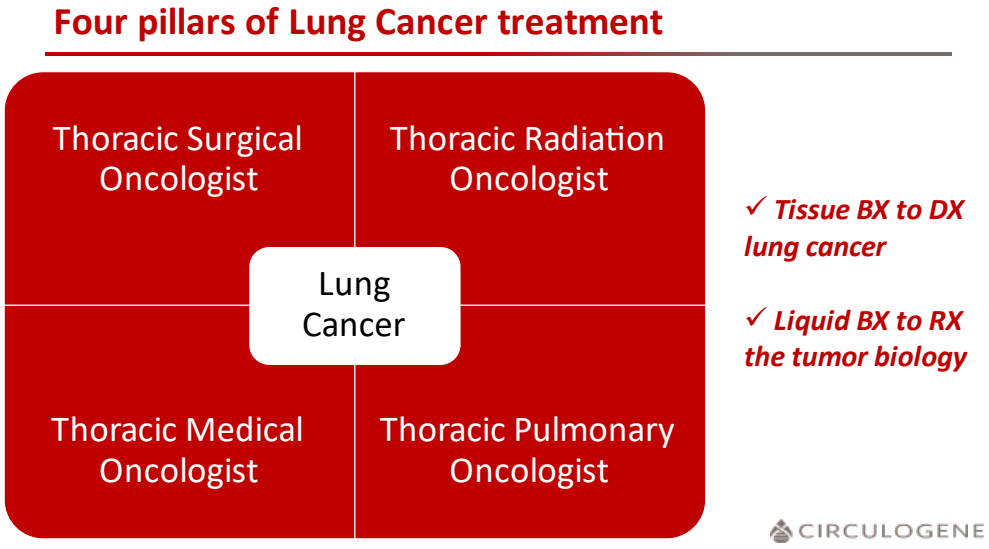
or stage the lung cancer. Thoracic surgical oncologists treat lung cancer with a variety of surgical resection techniques; and play a role in tissue acquisition for the diagnosis of lung cancer. Most frequently however, an interventional pulmonologist is tasked with obtaining tissue for confirming the diagnosis of lung cancer as well as staging the mediastinum with endobronchial ultrasound (EBUS). Integration of EBUS mediastinal staging at the time of diagnosis has been shown to shorten the time from diagnosis to treatment resulting in improved survival outcomes (32). This directly makes an IP a 'thoracic pulmonary oncologist' and a vital fourth pillar of managing and treating lung cancer.

A tissue diagnosis is requested of the IP. Staging of the mediastinum is expected from the IP. A request of the IP for sufficient tissue for molecular testing is implicitly implied. To complete the diagnosis, intra-thoracic staging, and to provide full molecular tumor biology testing, all to guide the treatment of lung cancer, both tissue biopsy sampling for a tissue NGS and a simple blood draw for a plasma NGS is within the role and expectations of an IP. Sufficient tissue is asked of the IP for needed molecular testing. A liquid biopsy for plasma NGS testing is complementary to tissue NGS molecular testing, and in fact can identify more mutations, and more mutations more quickly, than tissue. Why is drawing a liquid biopsy not a role of the IP? It is clearly not getting done nor getting done a timely manner in the majority of patients with the current model of molecular testing.

Just as not fully staging for extra-thoracic disease with PET and CNS imaging and not fully staging the mediastinum with endobronchial ultrasound (EBUS) can lead to a wrong treatment and poorer outcome, not knowing the molecular tumor biology of lung cancer, may miss the right and best treatment and potentially lead to a wrong treatment. And patient survival outcomes suffer.



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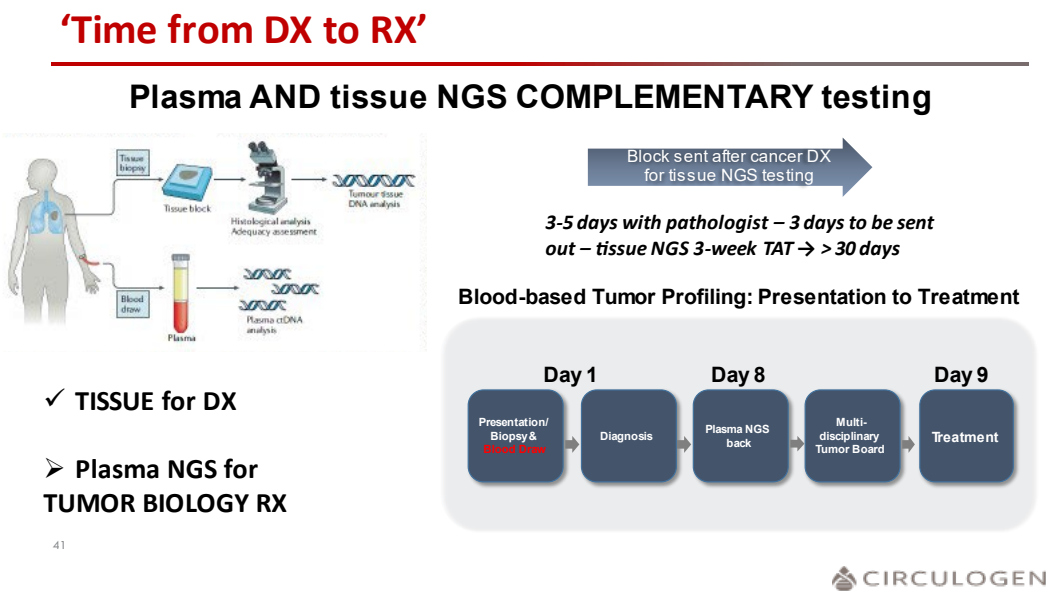
V. Programmatic molecular testing at the time of the tissue biopsy is the solution

The IP drawing a liquid biopsy for plasma NGS testing at the time of the confirming tissue diagnosis provides an efficient and effective programmatic molecular testing approach. That is part of their roles as thoracic pulmonary oncologists and the fourth pillar in the management and treatment of lung cancer. Adopting this programmatic approach of the IP drawing the liquid biopsy plasma NGS at the time of bronchoscopy tissue biopsy increased the molecular tumor biology being known in 85% of patients at the time of the initial oncologic evaluation. Previously it was known in just 9% of cases at this institution (33).

Even though the final stage is frequently unknown at the time of diagnosis, all stages of lung cancer need and can benefit from molecular testing. Just as not fully staging for extra-thoracic disease with PET and CNS imaging and not fully staging the mediastinum with endobronchial ultrasound (EBUS) can lead to a wrong treatment and poorer outcome, not knowing the molecular tumor biology of lung cancer, may miss the right and best treatment and potentially lead to a wrong treatment. And patient survival outcomes suffer.

A programmatic approach with a liquid biopsy for plasma NGS testing at the time of the tissue diagnosis and mediastinal staging by the IP provides the solution of making sure the needed molecular testing gets done. It provides the molecular tumor biology at the time of multi-disciplinary treatment decision making. It shortens the time from diagnosis to treatment. A best treatment can be identified. A wrong treatment avoided. And patient survival outcomes for all stages of lung cancer will improve.



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