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Genomic Tissue Analysis and Liquid Biopsy Profiles from Patients Diagnosed with Advanced Adenocarcinoma of the Lung

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Abstract

Background: Several genomic abnormalities have been discovered in adenocarcinoma of the lung in the last years; however, adequate quantity of tumor tissue for molecular analysis is a major handicap to offer most of the patients a personalized medicine. Reasons such as difficulty to perform tumor biopsy either because of tumor location or patient's co-morbid conditions are among the common factors for lack of molecular profiling results. Hence, liquid biopsy has emerged as a potential alternative to detect these genomic alterations.

Methods: We analyzed 81 consecutive patients to whom a liquid biopsy using Guardant 360 test was ordered in our thoracic oncology clinics at Lynn Cancer Institute, Boca Raton, Florida and Memorial Cancer Institute, Hollywood, Florida. Results from tissue genomic molecular profiling from each subject was obtained or recovered for comparison. Molecular genomic profiling results from this cohort were developed by different CLIA laboratories (e.g. Response Genetics, Caris Life Sciences, Foundation Medicine, BioTheranostics, and Genoptix). For liquid biopsy analysis, only Guardant 360 test was considered. The Guardant 360 test assays a panel of 70 genes. To identify genomic alterations in cancer-associated somatic variants with high sensitivity, cell-free DNA is extracted from plasma and genomic alterations are analyzed by massively parallel sequencing of amplified target genes.

Results: The distribution by gender was: 56 female patients and 25 males; median age 71 (range, 27-99). Eighty percent (65/81) of the patients had at least 1 genomic alteration by liquid biopsy (range, 1-10). Most common abnormalities found in liquid biopsy were: TP53 (49%), EGFR (42%), NF1 (25%), KRAS (17%), MET (15%). From this 65 pts with positive liquid biopsy results, 49 patients (75%) had tumor tissue molecular profiling results for comparison. Major reason for lack of tumor tissue molecular profiling results: insufficient tumor (18/81; 22%). For comparison between the 2 modalities, we considered all patients with available results in both tests; hence, sixty-three patients were used to compare tumor biopsy with liquid biopsy. Thirty-three patients out of 63 (52%) had at least 1 similar genomic abnormality or molecular profiling results found in both tumor and liquid biopsy. Most of the concordance was in EGFR alterations (17/22; 77%). Liquid biopsy caught 10 additional EGFR genomic aberrations not being identified by tumor biopsy (a total of 27 EGFR genomics aberrations were identified in liquid biopsy). Fourteen EGFR found in liquid biopsy were actionable; three out of 10 EGFR mutations found only in liquid biopsy were actionable.

Conclusion: Liquid biopsy using Guardant 360 evaluation offers an alternative to identify genomic alterations including actionable mutations; still, insufficient tumor is the major reason for lacking of tumor molecular profiling results and more advances to obtain tumor biopsy are needed. Our cohort had 77% concordance between liquid and tumor biopsies for EGFR alterations despite multiple factors against discussed in this manuscript.

Introduction

Lung cancer continues to be the number one cause of cancer related mortality in the United States [1]. It is also a disease state which has seen a major therapeutic development in the last years. Just in 2015, six different agents were approved by the US Food and Drug Administration from checkpoint

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inhibitors (e.g., nivolumab and pembrolizumab), to targeted agents (e.g., gefitinib, osimertinib, and alectinib), and biological agents (e.g., necitumumab). It is important that we are able to identify the specific target (molecular biomarker) for these novel targeted agents so patients can get the benefit from personalized medicine. In many instances, it is cumbersome to do a lung biopsy due to location of tumor, size of the tumor, high risk patients from pneumothorax (e.g. emphysema), patients who have several co-morbid conditions or taking anticoagulation, fragile patient, difficult access to healthcare professional experts in interventional procedures, high cost, and many others. Moreover, a tissue biopsy sometimes is just good enough to give us a histologic diagnosis, but the amount of tumor cells (percent of cellularity) present is not enough for molecular testing. Hence, other diagnostic alternatives have been used in the last two years to identify these molecular biomarkers, helping us to increase our patients' therapeutic options if a target is identified. Nowadays, we have diagnostic platforms using blood and urine known as liquid biopsies that are the focus of our manuscript is liquid biopsy using tumor-derived cell-free DNA (cfDNA) in the blood.

The analysis of circulating tumor DNA (ctDNA) is a challenge and requires highly sensitive techniques due to the small fraction of tumor-specific DNA masked within background levels of "wild-type" cell-free DNA. The initial methods to analyze cell-free DNA include quantitative real-time polymerase chain reaction (PCR)-based, fluorescence-based, and spectrophotometric approaches [2]. From that point, we have moved into digital genomic methods which have improved the identification of genetic alterations in ctDNA; among these novel genomic methods, digital PCR has emerged as a sensitive tool to detect point mutations in ctDNA at low allele fractions [3]. Digital PCR-based methods include droplet-based systems [4], microfluidic platforms for parallel PCR [5], and BEAMing (beads, emulsions, amplification and magnetics [6]. Another high-throughput sequencing technology is able to identify widespread ctDNA alterations across wide genomic regions [7]; Next-Generation Sequencing (NGS) technologies are currently being applied to plasma DNA analysis. Among these NGS techniques are targeted deep sequencing approaches (e.g., TamSeq [8], SafeSeq [9], Ion-AmpliSeq [10], and capture-based targeted deep sequencing such as CAPP-Seq [11]. Another platform will be the identification and characterization of circulating tumor cells (CTCs) per se, and from there many diagnostic tests can take place such as PCR, fluorescent in situ hybridization (FISH) analysis, and immunohistochemistry (IHC) [12,13]. Recently, the analysis of exosomes has shown to identify epidermal growth factor receptor (EGFR) alterations by analyzing microRNA [14]. Exosomes are nano-sized vesicles of endolysosomal origin which are released by several cytotypes in physiological and pathological conditions; tumor derived exosomes were recently proposed as excellent biomarkers for disease monitoring and prognosis in cancer patients [15]. All these novel diagnostic technologies have not been compared head-to-head. Thus, there is not a standard consensus of which one is better over the other; standardization is lacking at this time.

In our study, we analyzed the usefulness of liquid biopsy in the "real world" in two clinical practices in the community setting looking to ding how we can integrate these novel technologies routinely and into our therapeutic making decision. We used a test called Guardant 360 developed by Guardant Health; Guardant 360 is a panel of 70 genes which have shown to be cancer-associated somatic variants. In Guardant 360, the cfDNA is extracted from plasma and genomic alterations are analyzed by massively parallel sequencing of amplified target genes using the Illumina® HiSeq® 2500 or Illumina® NextSeq® 500 platforms and hg19 as the reference genome.

In this original article, we use NSG in blood trying to identify "druggable" genomic alterations in patients diagnosed with recurrence or metastatic adenocarcinoma of the lung, and compare these results with their phenotypic genomic profiles in tumor tissue.

Materials and Methods

We analyzed 81 patients with stage IV or recurrent adenocarcinoma of the lung using Guardant 360 test; sample collections were done at Lynn Cancer Institute, Boca Raton, Florida and Memorial Cancer Institute, Hollywood, Florida, USA. The alterations detected by Guardant 360 test included single nucleotide variations, amplifications, ALK, FGFR2, FGFR3, RET, ROS1, NTRK1 genes, and short insertions/duplications/deletions in exons 19 and 20 of the EGFR and ERBB2 genes as well as exon 14 skipping of the MET gene and others. Then, we compared these results with their tumor tissue biomarkers analysis in each individual, to assess their correlation.Guardant360 cannot discern the source of the circulating cfDNA and for some variants in the range of ~40 to 60% cfDNA, the test cannot easily distinguish germline variants from somatic alterations.Guardant360 is not validated for the detection of germline or *de novo* variants that are associated with hereditary cancer risk.

Results and Discussion

The distribution by gender was 56 females and 25 males; median age 71 (range, 27-99). 65 patients (80%) had at least one genomic alteration by liquid biopsy (range, 1-10). Most common abnormalities found in liquid biopsy were: EGFR (42%), TP53 (49%), and NF1 (25%). From this 65 patients with positive liquid biopsy results, 49 patients (75%) had tumor tissue molecular profiling results for comparison. Major reason for lack of tumor tissue molecular profiling results: insufficient tumor (18/81; 22%). For comparison between the two modalities, we considered all patients with available results in both tests (n= 63); 33 patients out of 63 (52%) had at least one same genomic abnormality or no abnormality identified in both type of "biopsies". Most of the concordance was in EGFR mutations (17/22; 77%). Twenty-seven EGFR genomics alterations were identified in liquid biopsy; 14 of them were actionable mutations. Liquid biopsy caught ten EGFR alterations not present or available by tumor biopsy molecular profiling results.

In our cohort, we observed more genomic alterations in liquid biopsy than tissue counterpart. Our study has several weaknesses, but we also need to keep in mind that today it is not standard of care to check at the same time of diagnosis tissue molecular profile as it probably should be done in an ideal world once both of them become standard, liquid biopsy using both blood and urine to increase the chances of identifying the presence of a driver mutation. It is not uncommon to have a negative tissue molecular profile and a positive blood and/or urine molecular profile or viceversa. These tests are not mutually exclusive but complementary to each other. This study gives us a sense of what exactly medical oncologists are doing in the routine clinical setting and how it can help our patients to be treated. The liquid biopsies in our cases were taken at a different time from diagnosis and most of the patients were already treated with one or several lines of therapies. Thus, this will certainly affect the concordance between tissue and liquid biopsy in a significantly manner.

Where are we know in the development of these novel technologies? There is no question that targeted therapies have their solid platform of development based on the acquisition of tumor tissue either before initiation of therapy or after the onset of resistance [16]. But, at the end of the day, everything relies on the identification of ctDNA from tumor cells for analysis. In this regard, there are many barriers for the acquisition of malignant tissue such as being invasive, high cost for procedure, potential complications which may require hospitalization, tumor heterogeneity, single snapshot in time, and others. Biopsies are not without clinical complications; adverse events have been reported up to 17.1% for investigative biopsies in the thoracic area; hence, location of the tumor is crucial when trying to minimize risk for the patient [17]. Something that we are certainly struggling with is tumor heterogeneity which characterizes most metastatic cancer. Tumors have shown different genetic profiles in different areas of the same lesion (e.g., intratumoral heterogeneity); similarly, there is heterogeneity in the metastatic sites in the same patient (e.g., intermetastatic heterogeneity) [18,19]. Herein, liquid biopsy may be complimentary for tissue biopsy as it will analyze the ctDNA which can come from primary or metastatic site. A tissue biopsy (which basically is a section of the tumor) will miss not only the molecular intratumoral but also the intermetastatic heterogeneity.

In theory, ctDNA can provide the same genetic information as a tissue biopsy necessary to interrogate driver pathway in oncogenes is [20]. This novel technology has several advantage over tissue biopsy including non-invasive, less cost, easy access, the opportunity to assess response and in principle, to seek for early recurrence (disease monitoring). Nonetheless, this approach is dependent on fragment of ctDNA shed into the bloodstream from dying cells during cellular turnover or apoptotic and necrotic cells [21,22].

Close attention has to be made when we talk about cfDNA. cfDNA can be derived from normal cells who undergo apoptosis and cancer cells. ctDNA is the portion of circulating DNA specifically derived from cancer cells, and is similarly present both unbound and bound to leukocytes and erythrocytes [23]. To be more precise and avoid ambiguity, when we refer to the unbound ctDNA in the plasma, we should coin the term "tumor-derived cfDNA". There are several techniques how to measure and analyze ctDNA. One of them is the identification of CTCs. Studies in different epithelial cancer types have demonstrated that the number of CTCs detected is related to prognosis [24].

CTC identification in some cases is key to guide therapy.

Tumor-derived cfDNA originates from lytic, apoptotic or necrotic tumor cells, or by active secretion from macrophages that have phagocytized necrotic cells [25]. The available techniques nowadays target certain genetic mutations to guarantee that cancer cells are in fact the source of cfDNA; hence, they focus on activated oncogenes, chromosomal disorders, and others. As we mentioned before, tumor-derived cfDNA not only may help us to detect early progression but also novel mutations which confer resistance to therapeutic agent. This mechanisms can also be detected from tumor-derived cfDNA at the time of progression [16,26]. As an example of this, we have the case of T790M mutation which confers resistance to first and second generation tyrosine kinase inhibitors. In this cohort of patients reported herein, we have four patients which were enrolled into the expanded access for osimertinib prior to its approval by the US FDA. Those patients responded well to the targeted agent based on their liquid biopsy molecular profiling. Today, technologies such as PCR-based, digital PCR, targeted deep sequencing, and whole genome sequencing are available to analyze tumor-derived cfDNA.

From our study, we were able to see that initial EGFR mutation found in the original tissue biopsy persists at the time of progression [27]. This goes accordingly with prior publications where EGFR mutations trend to persist despite progression of disease. What is new in these patients is the development of mechanisms of resistance which could be either a novel EGFR mutation (e.g., T790M), c-Met amplification, histologic transformation or other. Despite we obtain liquid biopsy months after diagnosis, Guardant 360 was able to have a EGFR concordance of 77% with tissue biopsy molecular profiling which is high, and more importantly, able to find actionable mutations not detected in the tumor tissue analysis which tell us about the tumor heterogeneity. The biopsy will give us only a result which represents a piece of the entire genetic picture of the patient's lung cancer.

Conclusion

This study showed that liquid biopsy is not only an alternative for patients with lung cancer when tumor biopsy is not feasible but also a complimentary test when tumor biopsy has insufficient tumor cells to perform an optimal predictive biomarker profile. EGFR and its variants are the most common driver mutations found in liquid biopsy; also, it has a high correlation with tumor biopsy (77%) despite being perform months after diagnosis and one or more lines of treatment delivered. We need to consider that our cohort has several weakness but also represents a reality of how liquid biopsy is being used in the "real world", in the routine clinical practice. With factors such as being a retrospective analysis, liquid biopsy not done at the same time of tumor biopsy, patients already treated and a cohort of patients who included different line of treatments, the authors consider that 77% is clinical relevant in this study. Moreover, cfDNA from plasma using Guardant 360 test identified more gene abnormalities than tumor biopsy; the clinical significance of each of them in metastatic adenocarcinoma of the lung needs prospective and well controlled clinical trial.

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