# Complementary Tissue and Liquid Biopsy Plasma NGS Molecular Testing

Getting patients on the right treatment, faster.

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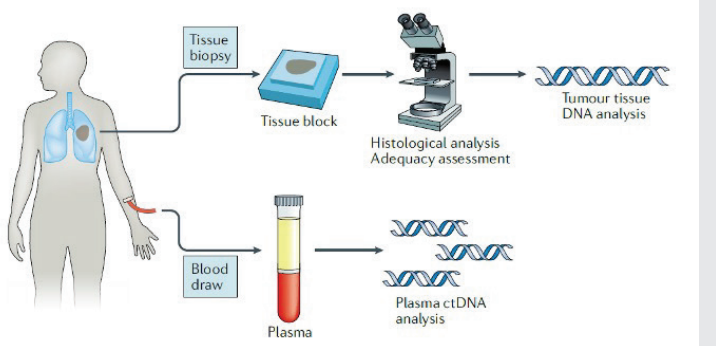
Tissue has long been held as the "gold standard" for cancer molecular testing. Now, in the "liquid biopsy" era of cancer medicine, that dogma has changed. Tissue genomic heterogeneity and differential plasma ctDNA/RNA shedding are limitations of tissue or plasma molecular testing alone. Tissue acquisition and sufficient sampling can also preclude complete tissue-only molecular testing. Studies are now very clear that both tissue and plasma NGS molecular testing are complementary. **Only concurrent tissue and plasma NGS testing can provide the best and most comprehensive genomic assessment of a cancer to provide the best precision oncology treatment and outcome for your patient.**

## Nat Rev Clin Oncol.

https://doi.org/10.1038/s41571-020-0423-x

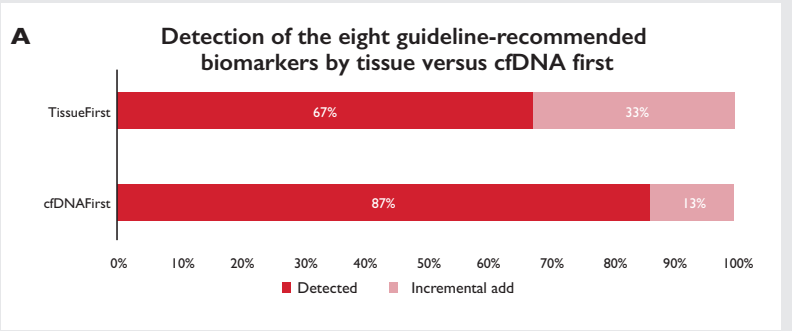
Strategies for the successful implementation of plasma-based NSCLC genotyping in clinical practice




## Clin Cancer Res. 2019

DOI: 10.1158/1078-0432.CCR-19-0624

Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non–small Cell Lung Cancer




Tissue-only NGS testing missed one-third of guideline mutations

Plasma NGS testing identified more mutations than tissue NGS testing

Tissue and plasma NGS testing are complementary, and both are needed to identify all treatable mutations

## JAMA Oncol.

2019;5(2):173-180. DOI:10.1001/jamaoncol.2018.4305

Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non–Small Cell Lung Cancer

**Plasma NGS testing added to tissue NGS testing nearly doubled the number of identified targetable mutations**

Nearly one-half of patients had tissue acquisition and sufficiency for complete tissue molecular testing limitations

### Key Points

**Question** Does adding plasma-based sequencing to tissue next-generation sequencing improve mutation detection for patients with non-small cell lung cancer?

**Findings** In this single-center cohort study of 323 patients with non–small cell lung cancer, 229 had concurrent plasma and tissue next-generation sequencing or were unable to complete tissue testing. Tissue alone detected targetable mutations for 47 patients (20.5%), whereas plasma sequencing increased targetable mutation detection to 82 (35.8%); 36 of 42 patients (85.7%) who received plasma next-generation, sequencing-indicated therapy achieved a complete or a partial response or stable disease.

**Meaning** Adding plasma next-generation sequencing testing to the routine management of metastatic non-small cell lung cancer appears to increase targetable mutation detection and improve delivery of targeted therapy.

## The ASCO Post

2018 SAN ANTONIO BREAST CANCER SYMPOSIUM (ABSTRACT GS3-08) SABCS 2018: SOLAR-1: Liquid Biopsies in Predicting Benefit of Alpelisib in PIK3CA-Mutant Breast Cancer

### Key Points

- While patients with *PIK3CA* mutations as evaluated in tissue samples had a 35% reduction in risk for disease progression, the risk reduction was 45% for patients with *PIK3CA* mutations as evaluated in circulating tumor DNA.
- Assessing mutational status by liquid biopsy resulted in even larger clinical benefit than tissue biopsy, improving median progression-free survival from 3.7 to 10.9 months.

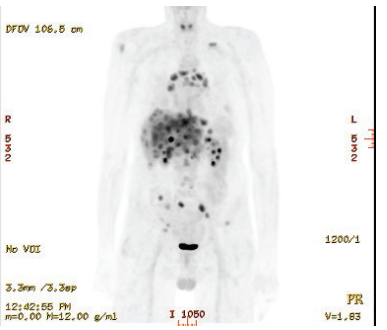
Liquid biopsy assessment of PIK3CA mutations achieved a longer targeted treatment PFS than tissue PIK3CA results

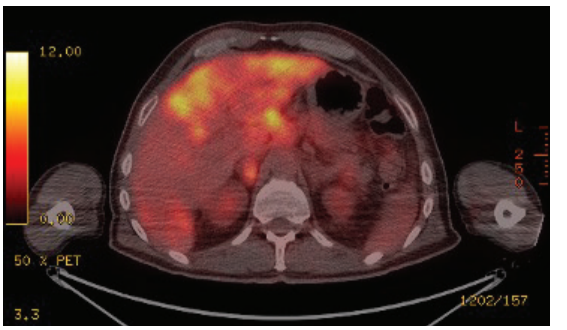
Plasma NGS can identify the dynamic and more aggressive clonal evolution of a cancer

# Tissue and Liquid Case Study

## 56-Year-Old male with metastatic TTF-1 and lung adenocarcinoma

Tissue testing is no longer the gold standard for molecular testing. Tissue is fraught with biopsy acquisition difficulties, often with insufficient tissue quantity precluding complete NGS testing and with sampling heterogeneity limitations. Plasma NGS testing is easily obtainable and accessible for complete molecular testing but can be limited when cancers are not shedding ctDNA/RNA. This case is a prime example of the importance and patient care benefit of concurrent tissue and plasma NGS testing.



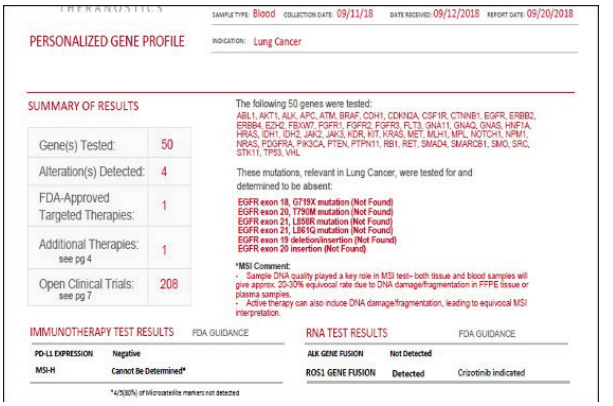




At the time of diagnostic bronchoscopy, tissue for histologic diagnosis as well as molecular testing was obtained. At the same time, a liquid biopsy for plasma NGS testing was drawn in the bronchoscopy suite. Thirty days later the tissue NGS testing finally returned but with QNS/quantity not sufficient for any targetable RNA fusion testing. However, the CIRCULOGENE liquid biopsy plasma NGS returned in just 8 days identifying a ROS-1 fusion and allowing immediate treatment initiation with crizotinib.

**BIOMARKER HIGHLIGHTS** (SEE PAGE 3 AND APPENDIX FOR MORE DETAILS)

| Biomarker | Method | Result |
|---|---|---|
| **Lineage Relevant Biomarkers** | | |
| MSI | NGS | Stable |
| Tumor Mutational Burden | | Low \| 6 Mutations/Mb |
| ALK | FISH | Negative |
| | RNA-Seq | QNS |
| | NGS | Mutation Not Detected |
| ROS1 | FISH | Negative |
| | RNA-Seq | QNS |
| PD-L1 | IHC | Positive, Low Expression, TPS: 1% |
| EGFR | NGS | Mutation Not Detected |
| KRAS | NGS | Mutation Not Detected |
| BRAF | NGS | Mutation Not Detected |
| PIK3CA | NGS | Mutation Not Detected |

| Biomarker | Method | Result |
|---|---|---|
| **Lineage Relevant Biomarkers (cont)** | | |
| ERBB2 (Her2/Neu) | NGS | Mutation Not Detected |
| MET | RNA-Seq | QNS |
| | NGS | Mutation Not Detected |
| | NGS | Amplified |
| RET | RNA-Seq | QNS |
| NTRK1 | RNA-Seq | QNS |
| **Other Notable Biomarker Results** | | |
| Mismatch Repair Status* | | Proficient |
| MLH1 | IHC | Positive \| 1+, 10% |
| MSH2 | IHC | Positive \| 1+, 50% |
| MSH6 | IHC | Positive \| 1+, 10% |
| PMS2 | IHC | Positive \| 1+, 90% |



PERSONALIZED GENE PROFILE

Sample Type: Blood Collection Date: 09/11/18 Date Received: 09/12/2018 Report Date: 09/20/2018

Indication: Lung Cancer

SUMMARY OF RESULTS

| | |
|---|---|
| Gene(s) Tested: | 50 |
| Alteration(s) Detected: | 4 |
| FDA-Approved Targeted Therapies: | 1 |
| Additional Therapies: see pg 4 | 1 |
| Open Clinical Trials: see pg 7 | 208 |

The following 50 genes were tested:
ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL

These mutations, relevant in Lung Cancer, were tested for and determined to be absent:

EGFR exon 18, G719X mutation (Not Found)
EGFR exon 20, T790M mutation (Not Found)
EGFR exon 21, L858R mutation (Not Found)
EGFR exon 21, L861Q mutation (Not Found)
EGFR exon 19 deletion/insertion (Not Found)
EGFR exon 20 insertion (Not Found)

*MSI Comment:
- Sample DNA quality played a key role in MSI test– both tissue and blood samples will give approx. 20-30% equivocal rate due to DNA damage/fragmentation in FFPE tissue or plasma samples.
- Active therapy can also induce DNA damage/fragmentation, leading to equivocal MSI interpretation.

IMMUNOTHERAPY TEST RESULTS — FDA GUIDANCE

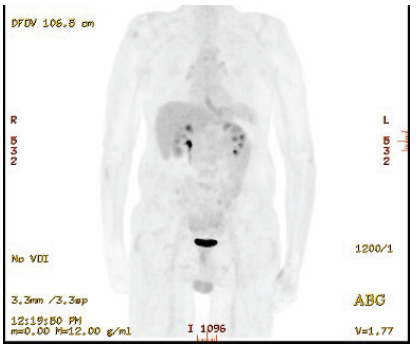
| | |
|---|---|
| PD-L1 EXPRESSION | Negative |
| MSI-H | Cannot Be Determined* |

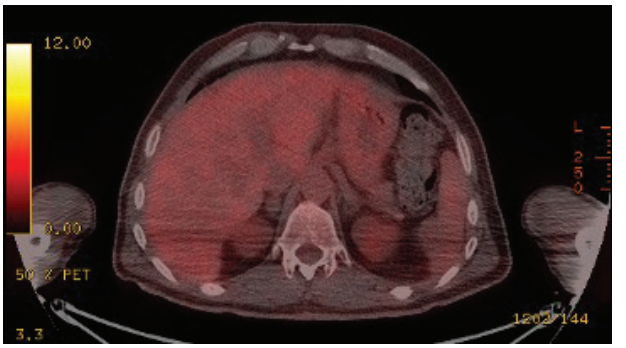
*4/5(80%) of Microsatellite markers not detected

RNA TEST RESULTS — FDA GUIDANCE

| | | |
|---|---|---|
| ALK GENE FUSION | Not Detected | |
| ROS1 GENE FUSION | Detected | Crizotinib indicated |

Treating the identified targetable oncogene driver achieved such a rapid radiographic response and clinical improvement, this patient reported to the nurse navigator, "I came in needing a wheelchair, and now I am dancing a jig."




Medical oncologists and patients with cancer deal with "what ifs" all the time. Both want the "what ifs" to become a "what is" as quickly and as certainly as possible. The "what ifs" here are profound. What if the plasma NGS were not drawn? What if the ROS-1 fusion were missed? What if immune-based systemic therapy would have been initiated? What if the only molecular testing results were tissue QNS for fusions? Do you repeat a tissue biopsy after one month of waiting? Or do you empirically start systemic treatment? He is very symptomatic. The only way he is to have a chance at getting better is to respond to treatment, and the only way he can respond to treatment, is to get treatment. Time makes a difference, and without treatment, time is wasting. By default, the best systemic therapy in IHC PD-L1 1% metastatic TTF-1 positive lung adenocarcinoma would be chemoimmune therapy—except when there is an EGFR mutation or RNA fusion. Then, immune-based therapy is inferior to standard chemotherapy alone. Worse yet, a potential prolonged survival from targeted therapy is lost. The "what if" of not having concurrent tissue and liquid biopsy plasma NGS test results available would have been a "what is" of missing the right and best treatment and stepping forward with the wrong cancer treatment.

Only concurrent tissue and plasma NGS testing can provide the best and most comprehensive genomic assessment of a cancer to provide the best precision oncology treatment and outcome for your patient.

Case Study Prepared by Doctor Paul Walker
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