BIOMARKERS: WHAT IS NGS?

Christine M. Lovly, MD, PhD @Christine_Lovly

Associate Professor of Medicine, Division of Hematology and Oncology

Ingram Associate Professor of Cancer Research

Co-Leader, Translational Research and Interventional Oncology Program

Vanderbilt University Medical Center

Nashville, TN USA

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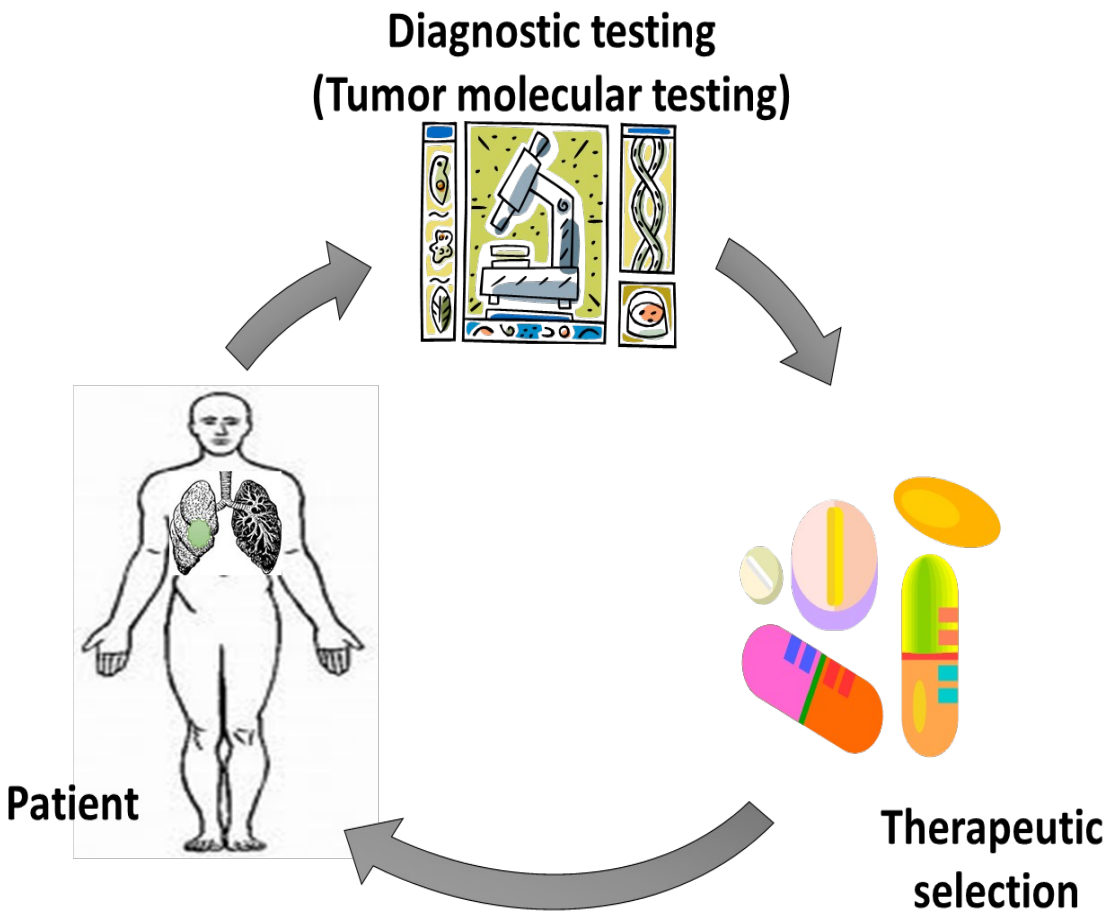
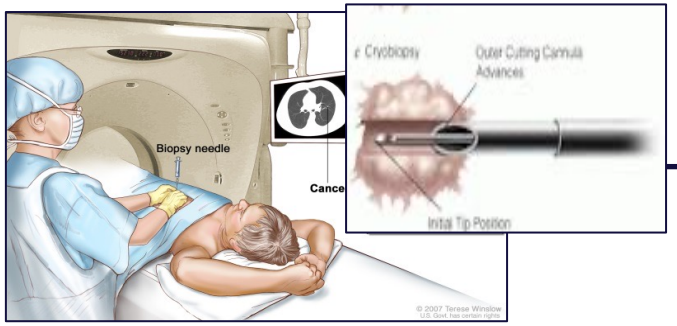
Why Biomarker Testing?



- Biomarker testing is the foundation for personalized (precision) cancer medicine.
- Goal: Individualize care for each patient.
- Prescribe treatments based upon the unique nature of each patient's cancer.
  - Target the tumor specific vulnerabilities.
  - Goal to maximize response, minimize toxicity, and improve outcomes.
- Avoid treatments that are likely to be ineffective.

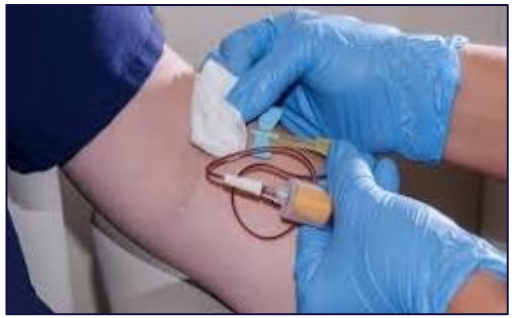
Biomarker testing – what sources of tissue can we evaluate?

Biopsy
Surgical resection specimen

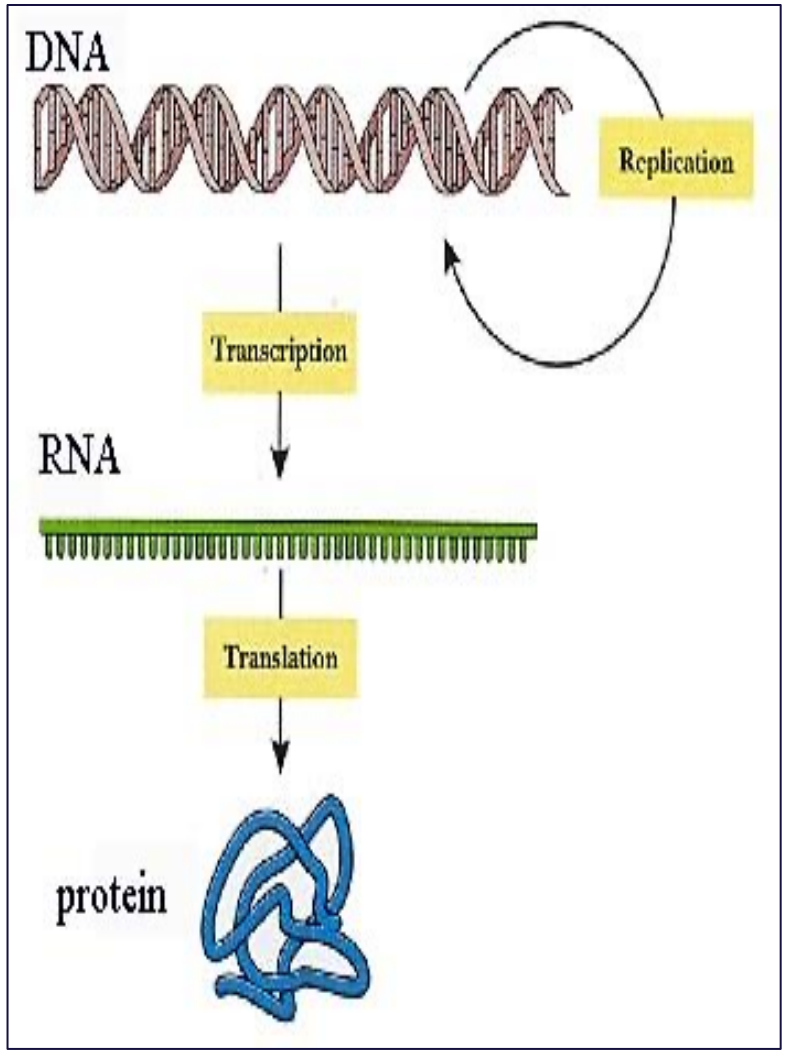


FFPE tissue (Formalin-Fixed Paraffin Embedded)

Blood



Biomarker testing – what analytes can we evaluate?

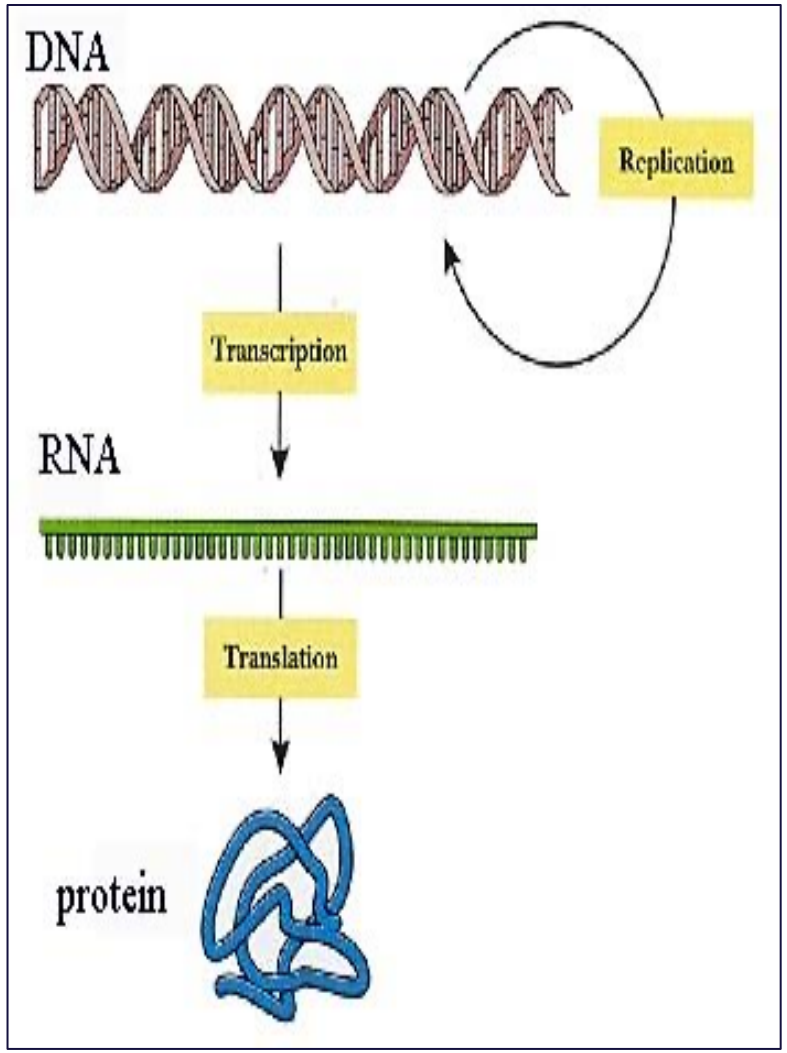


DNA:
- Point mutations
- Insertions/Deletions
- Gene amplification/deletion
- Gene fusions
- Epigenetic changes (methylation status)

RNA:
- Gene expression levels
- Gene fusions
- Point mutations (if you make cDNA)

PROTEIN:
- Absence/presence of a certain protein
- Relative expression levels
- Localization

Biomarker testing – how do we test?



DNA:
- PCR
- Next Generation Sequencing
- FISH (fluorescence in situ hybridization)
- Karyotyping

RNA:
- RT-PCR
- Microarray
- Next Generation Sequencing

PROTEIN:
- IHC (immunohistochemistry)
- Western blot
- Mass specometry
- RPPA (Reverse Phase Protein Array)

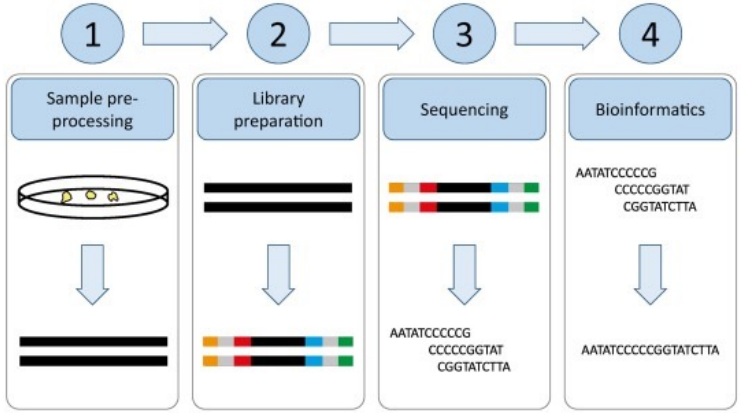
What is Next Generation Sequencing (NGS)?

- NGS refers to the METHOD for sequencing DNA or RNA. “NGS” is not a diagnostic test itself.
- NGS can be performed on many different types of samples: surgical resection specimens, biopsies, cell free DNA found in plasma, normal white blood cells found in whole blood, etc.
- One of the main benefits of NGS method is that multiple genes are sequenced at the same time
  - ✓ “Panel Size” – refers to how many genes are sequenced in a given NGS test – for example, “small gene panel” ~30-50 genes vs. “large gene panel” ~300-600 genes vs. whole exome sequencing.
- Many different commercial NGS platforms are available in the CLIA space for diagnostic testing.
- For a recently publicly available review of this technology, see: https://link.springer.com/chapter/10.1007/978-3-030-27994-3_8

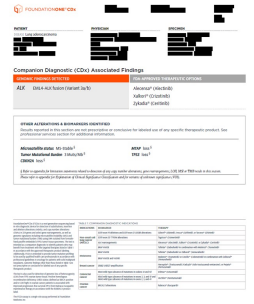
How does NGS work?

- Basic steps in NGS

DNA extracted from FFPE tumor sample

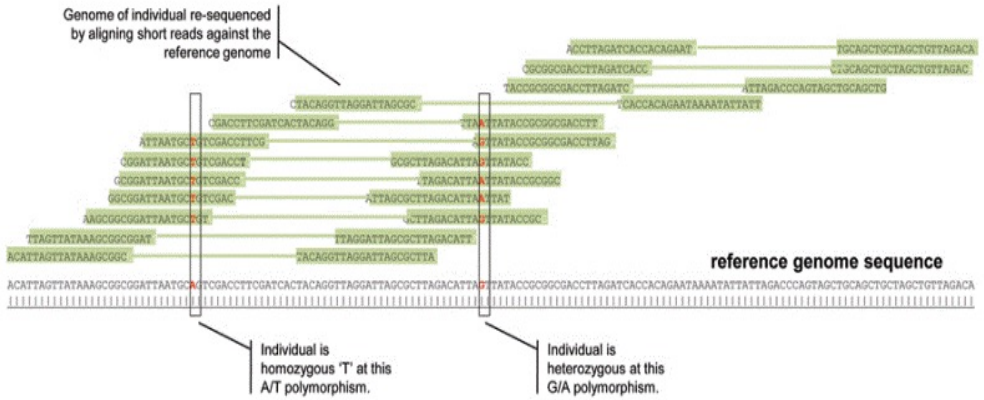


Report generated for patient care



- NGS requires “redundant” sequencing to obtain adequate “coverage”, which means how many times a piece of DNA is sequenced.

- Can detect ‘rare’ mutant DNA populations.
  - ✓ Allelic frequency: refers to how frequently an allele is found
  - ✓ In this case, how many times the tumor mutation are found relative to the ‘normal’ allele



References:
https://link.springer.com/chapter/10.1007/978-3-030-27994-3_8
https://doi.org/10.1016/j.biotechadv.2020.107537

Some commonly asked questions about NGS

When do I order biomarker testing for my lung cancer patients? Does it have to be NGS?

For patients with advanced / metastatic disease:

- PD-L1 testing for all histologies (PD-L1 tested for by IHC)
- Molecular testing is recommended for non-squamous (category 1) and should be considered for squamous histology.
- "when feasible, testing be performed via a broad, panel-based approach, most typically performed by NGS".
- For patients who, in broad panel testing don't have identifiable driver oncogenes (especially in never smokers), consider RNA based NGS if not already performed, to maximize detection of fusion events.

For neoadjuvant and adjuvant therapies, NCCN guidelines recommend "Test for PD-L1 status, EGFR mutations, and ALK rearrangements (stages IB–IIIA, IIIB [T3,N2])."

Current NCCN NSCLC - Version 2.2023 — February 17, 2023

Why is it important to think about NGS for both DNA and RNA? How do I know if the test I am using does both?

- DNA only NGS could possibly miss detection of fusions like ALK, ROS1, RET, NTRK. NGS of RNA from the tumor can maximize detection of therapeutically actionable kinase fusions.
- The methods section of the NGS report should tell you what is sequenced, or you can ask your institutional molecular pathologist or a representative from the vendor company you are using.

Some commonly asked questions about NGS

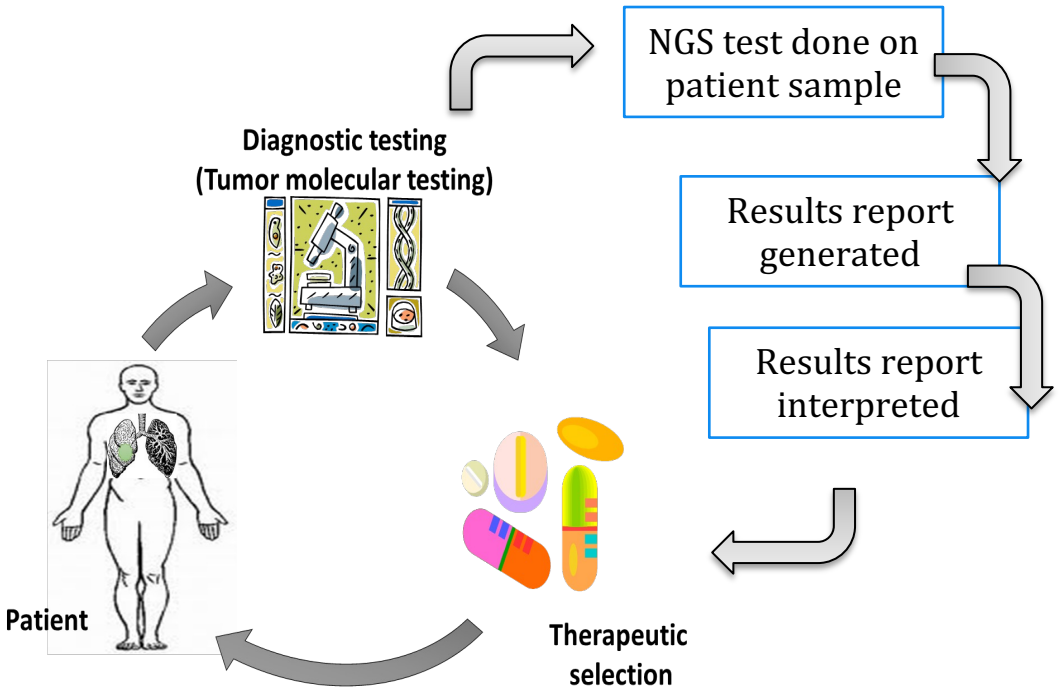
How many genes do I need to test for on my NGS panel?

- Per current NCCN guidelines, the biomarker testing at minimum should evaluate for EGFR, ALK , KRAS, ROS1, BRAF, NTRK1/2/3, METex14 skipping, RET, ERBB2 (HER2).
- Tumor mutational burden (TMB) is an approximate measurement of the total number of mutations in the genome. In 2020, the NCCN NSCLC panel removed TMB as an immune biomarker for patients with metastatic NSCLC based on clinical trial data and concerns about variable TMB measurements.

Is NGS of the tumor ("tissue NGS") the same as NGS from the blood ("liquid biopsy")?

- No!
- Tissue NGS and liquid biopsy involve assessment of different analytes using different types of NGS methods.
- Tissue NGS from a biopsy or surgical specimen: sequences pieces of DNA/RNA from the tumor itself. The tumor sample is usually in an FFPE block. Tissue NGS typically may have a longer turn around time than liquid biopsy, due to handling of the FFPE sample.
- Liquid biopsy involves analysis of cell free DNA (cfDNA), of which a component is circulating tumor DNA (ctDNA). ctDNA is composed of small fragments of DNA shed from the tumor into the bloodstream. Analysis of ctDNA must be very sensitive, so the "read depth" is typically higher than tissue NGS.
- Tissue NGS testing and ctDNA analysis ("liquid biopsy") are complementary and not mutually exclusive.

The complexities of NGS reports



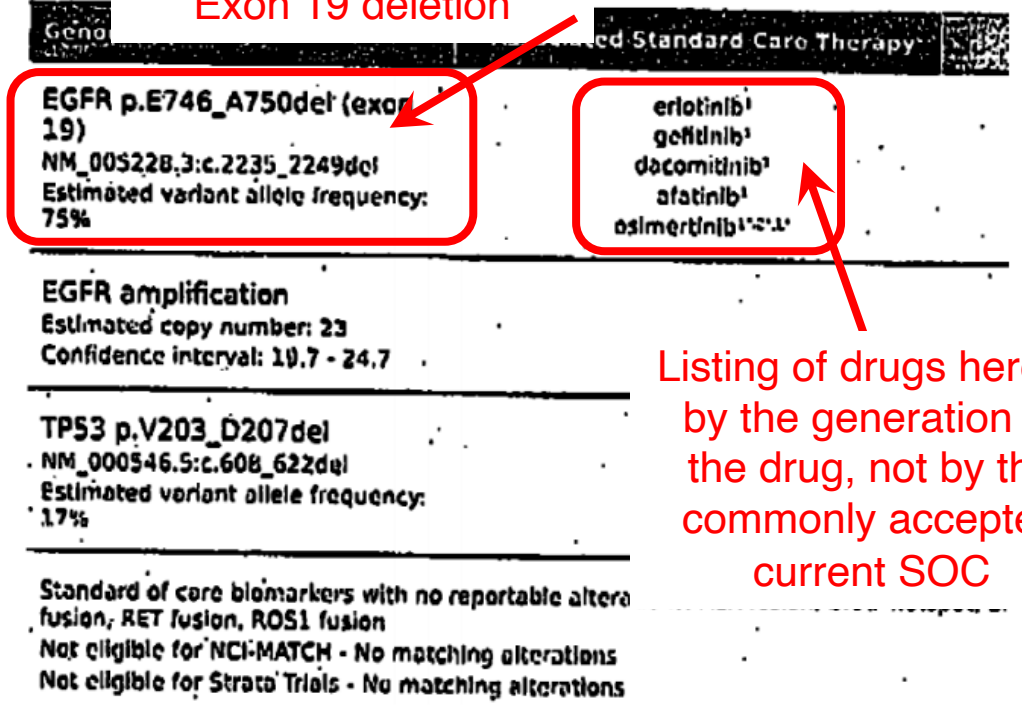
- Once the test has been ordered and the tumor (or ctDNA) sequenced, an important and complicated next step in the processing of obtaining NGS in the clinic remains → that is, reviewing and interpreting the NGS report.
- Interpreting complex genomic testing result is challenging.
- Reports are not harmonized with respect to content, format, length, standards for variant calling, what data are presented, standards for levels of evidence when making therapeutic assertions.

Reference: Schilsky and Longo NEJM 387;23 nejm.org December 8, 2022

The complexities of NGS reports – an example

- 62 year old male former smoker (< 10 pack year history, quit > 30 years ago) presented with progressive dyspnea on exertion over months.
- Work-up eventually revealed a large right hilar mass, mediastinal lymphadenopathy, liver metastases, and one small (< 1cm) metastases in the left temporal lobe.
- Biopsy of the right hilar mass revealed poorly differentiated adenocarcinoma of lung origin.
- PD-L1 by IHC (Dako 22C3) was 40%.
- NGS from the tumor biopsy was sent. There was a lack of clarity on the results. Patient received one cycle of carboplatin / pemetrexed.

Missing commonly used clinical vernacular "Exon 19 deletion"



Listing of drugs here is by the generation of the drug, not by the commonly accepted current SOC

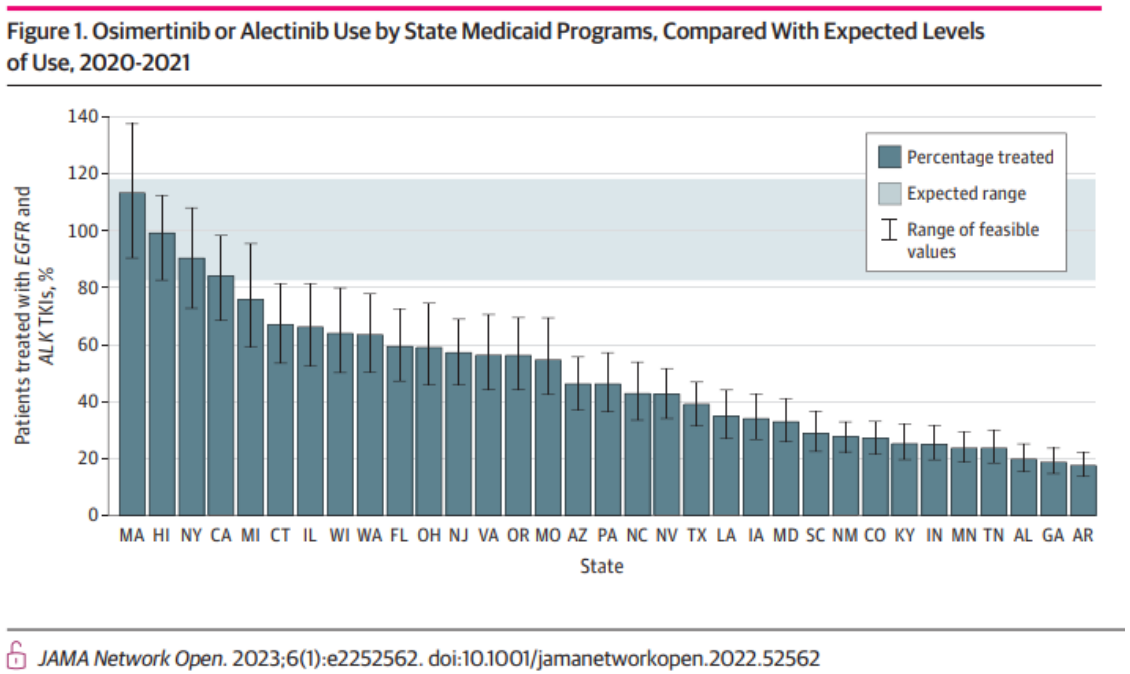
- This gentleman ultimately received osimertinib with clinical and radiographic benefit. But this course was delayed by challenges in interpreting the NGS testing report.

Resources to help decode the complexities of NGS reports

- Discussions with your institutional / local molecular pathologist.
- Molecular tumor boards.
- Many diagnostic companies host their own molecular tumor boards and have genomic scientists available to help with interpretation of the reports as questions arise.
- On-line resources to assist with decision support, such as OncoKB and MyCancerGenome.

Biomarker testing is accepted standard of care for patients with lung cancer, but we still have a long way to go…

- It has been 20 years since EGFR mutations were detected in lung cancer and 14 years since the pivotal IPASS study (Mok T NEJM 2009) was published.
- Despite this time frame, there are still compelling data that biomarker testing and prescription of targeted therapies are not reaching expected benchmarks.
- Analysis of Medicaid patients with metastatic NSCLC:
  - Only 3 states (9%) had dispensing volumes consistent with expected levels,
  - 18 states (55%) had dispensing volumes substantially below expected levels.
  - JAMA Network Open January 2023



- **To fully realize the promise of precision medicine and biomarker testing, progress in science / innovation must be matched with equal efforts in delivery and implementation of care in a thoughtful and equitable manner.**

Thank you for your time!

Please feel free to contact me by email, Twitter, or LinkedIn!


@Christine_Lovly

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