Department of Oncogenomics

CASE 01

A Comprehensive Analysis Of EGFR mutated Advanced Non-Small Cell Lung Cancer (NSCLC) With Oncolife Lung Extended Panel With PDL-1 : A Ray Of Hope In Deciphering Therapies.

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Highlights of the case:

In view of Poorly differentiated Adenocarcinoma (Advanced NSCLC) with multiple cavitations, the prognosis of the mentioned clinical cancer type holds controversial. However, in a EGFR co-mutated NSCLC and with presence of ERBB2 copy number gains (below the determined cut off) acquired resistance to EGFR TKIs is anticipated. To monitor the molecular landscape in real time for disease progression and response, liquid biopsy is highly recommended. A ray of hope is noticed with Immune checkpoint inhibitors as the patient is observed with high PDL-1 expression (Clone PD-L1 22C3 (DAKO)) which should be used with caution in view of the achieved molecular landscape and potential therapy toxicities.

Therapeutic Facts in concordance with Molecular Landscape: Targeting Immunotherapy alone, literature has witnessed low efficacy in the treatment of patients with advanced NSCLC with oncogenic-driven tumors. Various clinical studies across the globe have proposed the use of combination regimens of Immune Checkpoint Inhibitors with Tyrosine Kinase Inhibitors which has led to excess toxicities with no additional efficacy, whereas Immunotherapy in combination with Chemotherapy, and especially with antiangiogenic drugs, looks promising for previously treated patients with targeted therapies.

Outcome of the case study:

Optimal identification, targeting and sequencing of molecular markers for targeted therapies, immunotherapy, and chemotherapy are essential to continue to improve patient outcomes in advanced NSCLC.
***The case was discussed with the prescribing Medical Oncologist: for Prognosis, Risk Assessment and better clinical outcome.

CASE PRESENTATION

Clinical Findings: Fever, Dizziness since a day.

Radiographic Findings: PET scan revealed ill defined soft tissue mass lesion in right lung upper lobe, mass is extending medially to right paratracheal region. Multiple cavitations seen.

Endobronchial Ultrasound Bronchoscopy Findings: Negative for AFB smear.

Endobronchial Ultrasound (EBUS) Transbronchial Needle Aspiration (TBNA) Findings: Consistent with Non small cell lung carcinoma: Poorly Differentiated Carcinoma (Adenocarcinoma).

Patient was referred for: OncoLife Targeted Lung Panel Extended with PDL1 DAKO

Tumor content: 30%

PANEL COVERAGE

Hotspot genes: ALK, BRAF, CTTNB1, GFR, EGFR, ERBB2, ERBB3, ERBB4, KIT, KRAS, MAP2K1, MET, NRAS, PIK3CA, RET, ROS1

Copy Number Genes: EGFR, ERBB2, MET, MYC

Fusion genes: ALK, BRAF, FGFR1, FGFR2, FGFR3, RET ROS1, MET exon 14 skipping, NTRK1,2,3,

IHC: PDL1 22c3 Dako

MOLECULAR FINDINGS

Pathogenic mutations in

a) EGFR gene Hotspot mutations (Exon 18: p.Gly719Ala and Exon 20: p.Ser768Ile) Figure 1 and 2.

b) CNV: Copy number gain in ERBB2 gene (Below the determined cut off threshold)

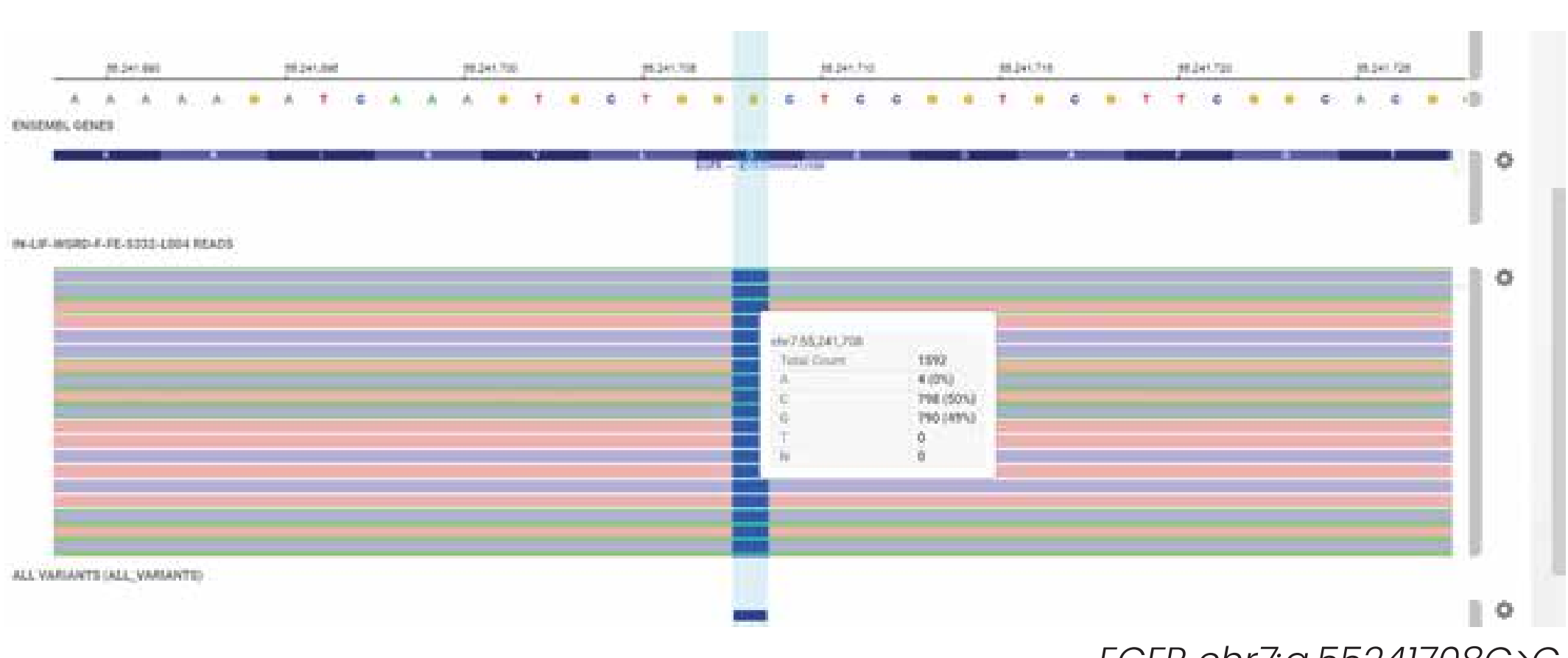
Gene	Variant Location	Variant Consequence	Clinical significance	Varian! Type	Reference
EGFR	chr7:g.55241708G>C, ENST00000275493, Exon 18	c.2156G>C, p.Gly719Ala, 50%	Pathogenic	Nonsynonymous SNV	rs121913428. VCV000045225.6
	chr7:g.55240005GA", ENST00000275493, Exon 20	c.2303G›T. p.Ser788lle. 50%	Pathogenic	Nonsynonymous SNV	rs121913485, VCV000045251.6

Gene (Variant) - Drug association	Drug Name	Summary
EGFR (p.Gly719Ala, p.Ser768lle) Responsive	Afatinib	The drug Afatinib has been approved for the first line treatment of metastatic non-small cell lung cancer (NSCLC) patients harbouring non-resistanEGFRmutations. The approval was based on response to Afatinib in a subset of patients (n=32) harbouring uncommon mutations p.Glu719, p.Leu861Gin and p.Ser768lle enrolled in one of the 3 clinical studies LUX-Lung 2 (NCT00525148), LUX-Lung 3 (NCT00949650) and LUX-Lung 6 (NCT01121393). The confirmed overall respon was 66% (95% a, 47.81)_ Response duration lasted for 12.18 months or more among the 21 responders (52% 212 months, 33% 218 months).

Due to presence of Copy number gain in ERBB2 gene (below the determined cut off threshold), we anticipated acquired resistance to anti EGFR therapy report was released with disclaimer (**)

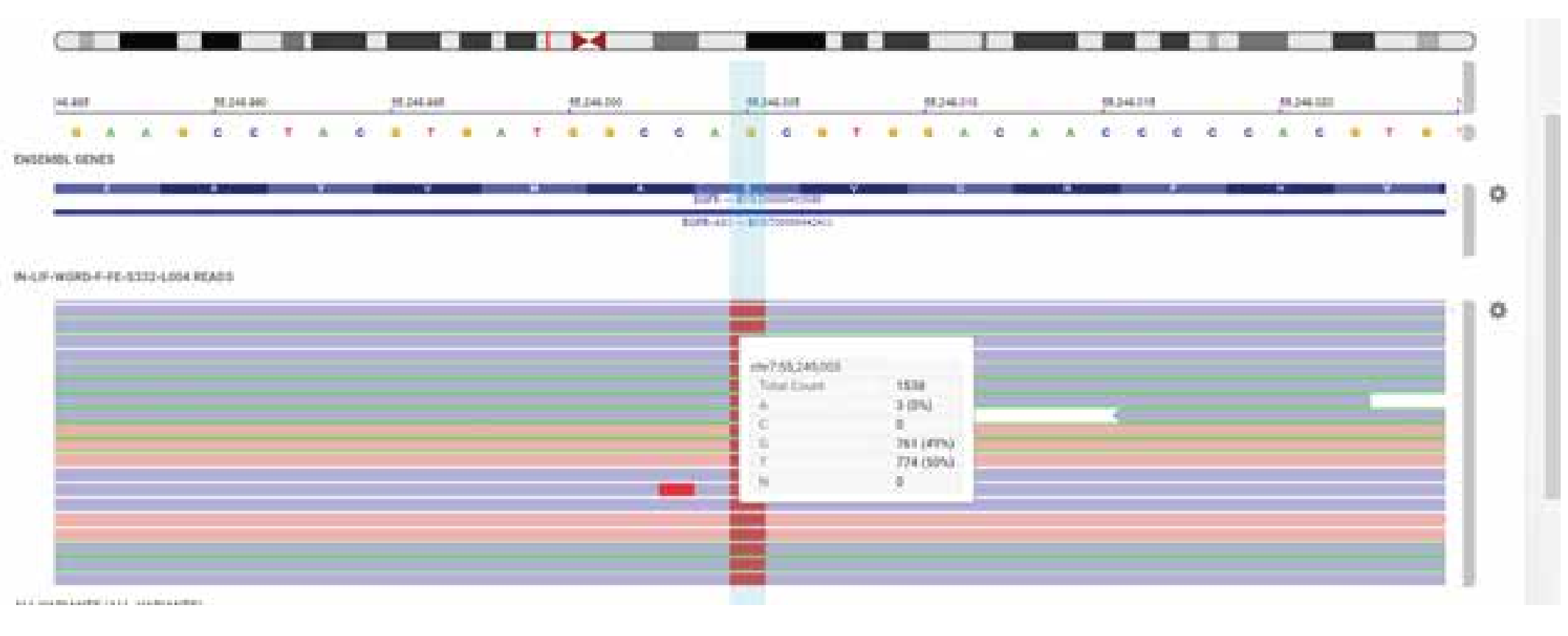
**Few recent research studies have shown that ERBB2 alterations (mutation/and or amplification) are associated with poor survival NSCLC and can lead to EGFR tyrosine kinase inhibitors. However, further studies in larger cohort are warranted to establish the pathogenicity of the copy number gain with ERBB2 gene and the therapeutic response in the mentioned molecular findings in NSCLC. [PMID: 34801409, 33606136, 28167203].

Recommendations: Follow up with liquid biopsy for further therapeutic response insights.



EGFR chr7:g.55241708G>C

Figure 1



EGFR chr7:g.55249005G>T

Figure 2

IMMUNOHISTOCHEMISTRY FINDINGS:

PDL-1 (Clone) PD-L1 22C3 (DAKO) (Figure 4)

Results:

Score:

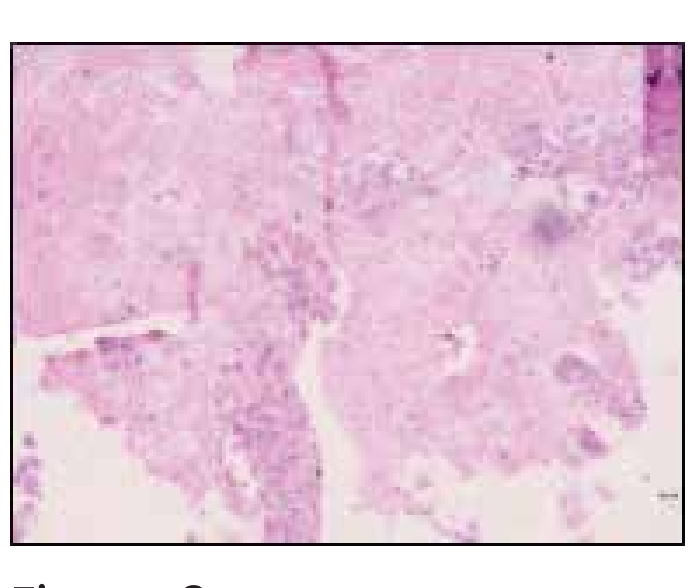


Figure 3

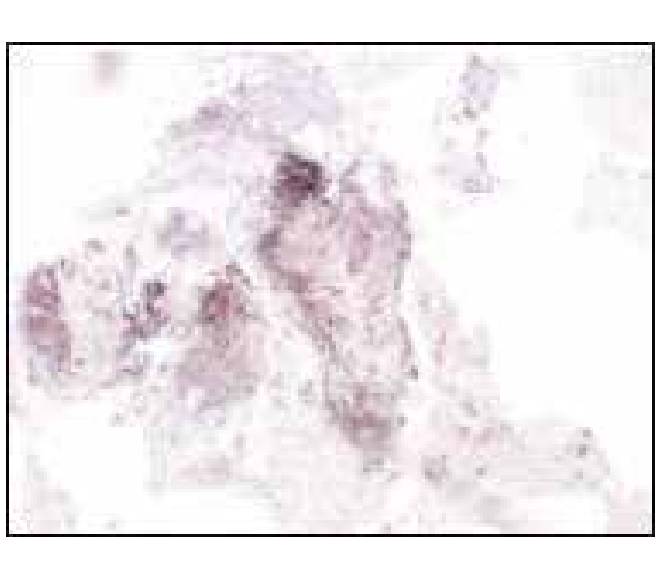


Figure 4

Tumor proportion score: Around 70%

TPD-L1 22C3 assay is indicated as a **Companion Diagnostic** aid in identifying patient for treatment with **KEYTRUDA (pembrolizumab)** therapy in following tumors.
Recommendations in Tumor Type:

✓ Non-small cell Lung carcinoma (NSCLC)

TUMOR	SCORING USED	INTERPRETATION GUIDELINES
NSCLC	TPS	TPS ≥ 50% - High PD-L1 expression TPS 1-49% - PD-L1 expression TPS < 1% - No PD-L1 expression
Other Sites	CPS	CPS ≥ 1 - PD-L1 expression CPS < 1 - No PD-L1 expression

CASE DISCUSSIONS

Perspective 1: Discussion with clinical relevance (EGFR+ERBB2 below the determined cut off):

- ERBB2 alteration (mutation and/or amplification) is associated with poor survival in EGFR Mutated Non Small Cell Lung Carcinoma (NSCLC) and can mediate resistance to EGFR tyrosine kinase inhibitors [PMID: 34801409, 30766753].
- In a clinical research study conducted in California (2017), four out of nine patients responded to targeted therapy (duration of response: three to ten months). The authors additionally identified a denovo PIK3CA mutation and an increase in ERBB2 copy number. The presence of these mutations was recognized as potential resistance mechanisms to targeted therapies. Disease progression was noticed upon prescribing targeted therapies [PMID: 28167203].

Perspective 2: Discussion with clinical relevance (EGFR+PDL-1): Acquired drug resistance to various tyrosine kinase inhibitor (TKI) inevitably develops in almost all epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC).

- Immune checkpoint inhibitors (ICIs), represented by programmed cell death receptor-1 (PD-1)/programmed death receptor ligand-1 (PD-L1) inhibitors, have drawn more attention in recent years due to their long-lasting clinical benefits and low toxicity in NSCLC patients. According to recent studies, the tumor microenvironment (TME), copy number variations, tumor-specific mutations and gut bacteria can all affect how well ICIs work.
- According to reports, the epidermal growth factor receptor (EGFR) plays a crucial role in EGFR-driven lung cancers by upregulating the expression of the immune escape protein programmed death ligand 1 (PD-L1). These aspects' exact limits and interrelationships still need to be established and should be investigated further especially in Indian cohort.
- Drugs used for conventional chemotherapy can help tumor patients regain immune surveillance function. Therefore, it is hypothesized that in patients with NSCLC who have EGFR mutations, adding ICIs to chemotherapy would result in the best clinical outcome. Bevacizumab has been discovered to mediate immunological modulation in addition to its well-known antiangiogenic action.
- PFS and OS in the EGFR-mutant group receiving combination therapy with nivolumab and chemotherapy in the first-line scenario of CheckMate 012 were 4.8 and 20.5 months, respectively, while they were 7.5 and 24.5 months in the EGFR wild-type group. In a different phase II research, NCT03513666, EGFR-mutant NSCLC patients who were treated with toripalimab with chemotherapy following TKI resistance experienced an ORR of 50% and a median PFS of 7 months. Due to the small sample size, it is unclear what the consequences of combining chemotherapy and ICIs will be. Therefore, larger clinical investigations are necessary to further establish the therapies using molecular discoveries.
- The good response of patients with not common EGFR mutations in NSCLC, such as those with exon 20 insertion or G719X, L861Q, or S768I mutations, was demonstrated by Chen et al. (2019) and was related to the concurrent expression of PD-L1 in the TME and the high prevalence of CD8+ tumor-infiltrating lymphocytes (TILs). Additionally, it was found that the diversity of the TME caused by various EGFR mutations produces various immunological reactions to ICIs. We may be able to choose the patient who will benefit from ICIs with the aid of further investigation into the immunological and pathological traits of various NSCLC subtypes.

CONCLUSION

- Non-small cell lung cancer in its advanced stages: Adenocarcinoma with Poorly Differentiated Carcinoma: Thankfully, with molecular results in hand, an NSCLC with poor prognosis is eligible to receive and get the benefit of superior treatment alternatives/combination therapies.
- Mutated EGFR (Co-mutations)
- ERBB2 Copy number gain (Below the lower limit): Indication for possibility of Acquired Resistance Mechanism (Recommendations: Liquid Biopsy Monitoring)
- PD-L1: 70% High Expressions: Indication for Combined therapy: Targeted Therapy/Chemotherapy + Immunotherapy for better outcomes but should be used with caution in view of ERBB2 copy number gain. Must be co-related with liquid biopsy on timely basis to monitor the CNV.
- The case was discussed with the prescribing Medical Oncologist: for Prognosis, Risk Assessment and better clinical outcome.
- A holistic picture is a win win situation : Patients, Clinicians, Molecular Pathologists and Molecular Scientists.

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