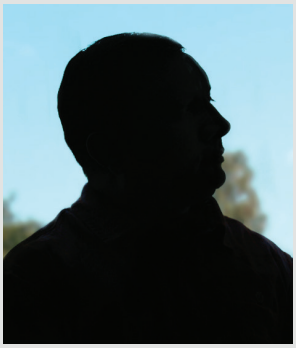
CASE STUDY

Tumor Monitoring Using Liquid Biopsy and Choice of Targeted Therapeutics Enabled by NGS Analysis Of Lung Cancer

Quick Summary



- Mr. Waradkar*, aged 59 years, was diagnosed with NSCLC.
- Genetic profiling of the tumor led to the identification of an *EML4-ALK* translocation in the tumor tissue.
- Absence of mutations in other genes – *BRAF, EGFR, ERBB2* and *RET*- that also play a role in the development of NSCLC was also indicated by the StrandAdvantage 152-gene test, used in this case.
- Choice of suitable targeted therapies - Crizotinib, Brigatinib, Alectinib and Ceritinib - in a sequential manner, is indicated by the identification of the *EML4-ALK* translocation.
- Strand has developed a customized liquid biopsy test that enables detection of **mRNA** transcribed from the translocated *ALK* gene, thereby expanding the scope and clinical utility of the liquid biopsy technique.
- It is possible to monitor the patient's response to delivered therapy, using this new liquid biopsy test.
- Clinical utility of kinase inhibitor drugs that act against mutations in *BRAF, EGFR, ERBB2* and *RET* was clearly negated.

Introduction

Lung cancers are notorious for fast progression and limited time of progression-free as well as overall survival (Murali et al. 2017). A significant hurdle posed by the low average overall survival time window of just 9 months is that there is very little time to choose appropriate therapies with the lowest failure rates. This is further complicated by the difficulty in obtaining tumor samples in some lung cancers. Genetic analyses of lung cancers, leading to accurate identification of molecular markers, can therefore provide valuable insights for choosing the most appropriate therapies. Genetic tests that can rapidly assess the presence or absence of most frequently mutated genes in solid tumors can help build a profile of lung cancer and enable guided choice of suitable therapeutics.

The StrandAdvantage 152-gene test is a comprehensive, pan-cancer test used to scan a tumor genome for picking up actionable genetic variants. Additionally, the availability of liquid biopsy to track the identified mutations also provides further monitoring and treatment opportunities in case of recurrence.

Patient Profile

Aniket Waradkar, aged 59 years, had been responding well to the chemotherapy treatment for early stage Non-Small Cell Lung Cancer (NSCLC) diagnosed the previous year, when a routine follow-up PET scan revealed a possible recurrence. However, the identified masses on the scan could not be biopsied due to their location to ascertain whether the growths were malignant or not.

Both patient and doctor wished to explore the possibilities of a blood-based liquid biopsy, a technique that had recently become available in India, to establish whether the growths bore the same molecular signature as the previously diagnosed cancer and should therefore be treated as a recurrence. To obtain a genetic profile of the tumor sample obtained during the original cancer diagnosis, the oncologist chose the StrandAdvantage 152-gene test as it offered the widest range of potential mutations to identify. This choice also increased their chances of finding a mutation that could be tracked by liquid biopsy, too.

** Name changed to protect patient privacy*

Results of the StrandAdvantage 152-gene Test

Profiling somatic cancers using multi-gene panel tests can help to identify mutations in proteins that are engaged in regulation of cell division. Targeted drugs, designed specifically to bind to and inactivate only mutated proteins, are now available. These drugs confer a tremendous advantage of inactivating tumor cells while sparing other normal tissues in the body.

Conversely, if mutations in specific genes are not identified in a tumor sample, then therapy with a set of drugs is contraindicated. Therefore, the StrandAdvantage 152-gene test can support the choice as well as rejection of chemotherapeutic agents. This decision support mechanism then translates into significant savings of cost and time for cancer patients, especially for lung cancer patients.

In Mr. Waradkar's case, significant genomic rearrangements were detected in the *ALK* gene in his NSCLC biopsy.

Therapy	*Tested Marker(s)*	*Relevant Marker(s)*	*Likelihood of Response***
Alectinib	*ALK*	*ALK* ^{trans}	*Enhanced*
Brigatinib	*ALK*	*ALK* ^{trans}	*Enhanced*
Crizotinib	*ALK, MET, ROS1*	*ALK* ^{trans}	*Enhanced*
Ceritinib	*ALK*	*ALK* ^{trans}	*Enhanced*

Table 1. Identification of Potentially Therapeutic Molecules

Translocations as well as other genomic re-arrangements of the *ALK* gene have been noted in NSCLC patients and are, paradoxically, associated with a better outcome for NSCLC in smokers (Sgambato et al. 2018; Wang et al. 2017).

Rearrangements in the *ALK* gene determined by NGS analysis indicated that targeted therapeutic drugs – Crizotinib, Brigatinib, Alectinib and Ceritinib - may be used for his therapy. Crizotinib is the first-line therapy drug usually prescribed for NSCLC patients presenting with *ALK* gene rearrangements (Ou et al. 2012). The therapeutic drugs Brigatinib, Alectinib and Ceritinib are other options to treat NSCLC, if resistance to Crizotinib develops in a patient (Sabari et al. 2017; Sullivan & Planchard 2016; Friboulet et al. 2014).

Another significant advantage of NGS analysis using the StrandAdvantage 152-gene test is the elimination of drugs that are NOT likely to provide therapeutic benefit. In Mr. Waradkar's case, mutations in *BRAF, EGFR, ERBB2* and *RET* genes were not detected in the tumor biopsy.

Since mutations in these genes were not identified, drugs such as Dabrafenib, Vemurafenib, Afatinib, Erlotinib, Gefitinib, Trastuzumab and Cabozatinib are unlikely to be effective in the treatment of NSCLC in this case. These indications were communicated to Mr. Waradkar's oncologist. The next step was to ascertain if the identified mutation could be detected in a liquid biopsy using blood.

Monitoring Patient Progression Using Liquid Biopsy

Liquid biopsy is a powerful technique for detection of genetic signatures of solid tumors using a blood sample provided by the patient (Marmarelis et al. 2017; Pécuchet et al. 2016; Bettegowda et al. 2014). In NSCLC, *EML4-ALK* translocations have been characterized and most common translocations have been mapped (Figure 1).

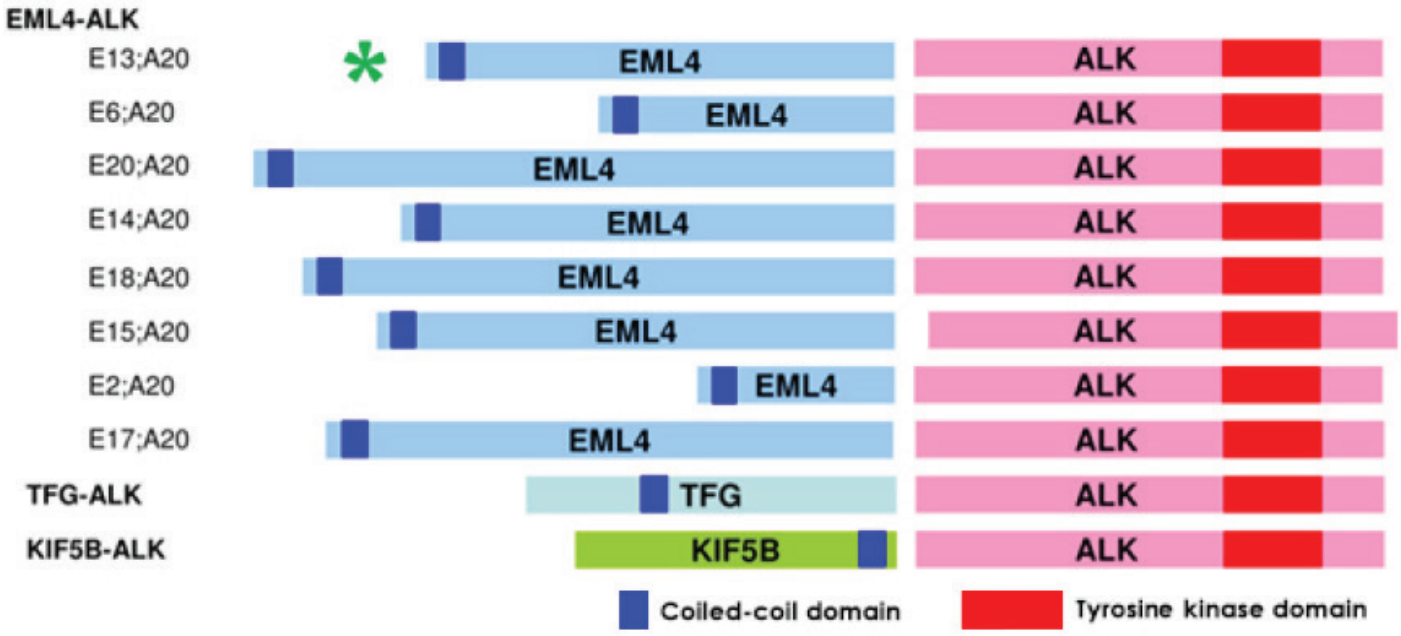


Figure 1. EML4-ALK translocations in NSCLC (Sasaki et al. 2010)

In Mr. Waradkar's case, variant no 1 (green asterisk in Figure 1) was identified in DNA extracted from the solid tissue biopsy. This variant has a breakpoint across exons 19 and 20 in the *ALK* gene (Sasaki et al. 2010). In order to confirm the translocation and also to detect the presence of this translocation in a liquid biopsy, a customized PCR-based test was designed at Strand.

RNA isolated from exosomes present in the plasma fraction of the liquid biopsy was transcribed into cDNA using RT-PCR. Primers designed for detection of the *EML4-ALK* translocation were instrumental in the detection of this gene rearrangement in the amplified cDNA.

Therapy Options

The genetic profile provided by the StrandAdvantage 152-gene test has been communicated to Mr. Waradkar's oncologist. He is currently being treated with Crizotinib (January 2018).

The possibility of using liquid biopsy to understand the patient status at subsequent stages of therapy has also been communicated to the oncologist.

Conclusions

- Genetic profiling of NSCLC using a comprehensive pan-cancer test, the StrandAdvantage 152-gene test, led to the identification of a genomic translocation of the *ALK* gene.
- A characteristic *EML4-ALK* translocation was identified in the solid tissue biopsy.
- Inhibitors of the *ALK* kinase protein - Crizotinib, Brigatinib, Alectinib and Ceritinib - are suitable targeted therapeutics for Mr. Waradkar's NSCLC.
- Absence of mutations in *BRAF, EGFR, ERBB2,* and *RET* genes has helped to eliminate other targeted drugs from the therapeutic regimen. This represents significant savings in cost of cancer therapy as well as time spent in guesswork for the patient.
- The *EML4-ALK* rearrangement was also detected using RNA extracted from a liquid biopsy sample.
- The availability of other nucleic acid tracers for tumor status assessment (in addition to cell-free and circulating tumor DNA) has expanded the scope of liquid biopsy, significantly.

StrandAdvantage 152-gene Test

The StrandAdvantage 152-gene test is a pan-cancer test that is designed to identify mutation hotspots in 152 genes that are frequently mutated in most solid tumors. This is a laboratory-developed test that has been designed and tested at the Strand Center for Genomics and Personalized Medicine, Bangalore, India.

This test has been benchmarked by an independent organization against similar tests from three other leading US laboratories. Results from the StrandAdvantage 152-gene test have been found to be at par with those provided by other US genomic diagnostic tests (Mori et al. 2016).

References

Bettegowda, C. et al., 2014. Detection of circulating tumor DNA in early- and late-stage human malignancies. Science translational medicine, 6(224), p.224ra24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24553385 [Accessed January 7, 2017].

Friboulet, L. et al., 2014. The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. Cancer discovery, 4(6), pp.662–673. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24675041 [Accessed December 13, 2017].

Marmarelis, M. et al., 2017. Emerging uses of circulating tumor DNA in advanced stage non-small cell lung cancer. Annals of translational medicine, 5(18), p.380. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29057240 [Accessed November 22, 2017].

Mori, Y., Levenson, V. & Otto, J., 2016. Tumor Genomic Profiling Reports from Different Vendors: A Comparison with Respect to Clinical Action Ability of the Provided Data. Adv Mol Diag, 1(2), pp.110–121. Available at: https://www.omicsgroup.org/journals/tumor-genomic-profiling-reports-from-different-vendors-a-comparisonwith-respect-to-clinical-action-ability-of-the-provided-data-.php?aid=78713 [Accessed December 16, 2016].

Murali, A.N. et al., 2017. Outcomes in Lung Cancer: 9-Year Experience From a Tertiary Cancer Center in India. Journal of Global Oncology, 3(5), pp.459–468. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29094084 [Accessed November 27, 2017].

Ou, S.-H.I. et al., 2012. Crizotinib for the treatment of ALK-rearranged non-small cell lung cancer: a success story to usher in the second decade of molecular targeted therapy in oncology. The oncologist, 17(11), pp.1351–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22989574 [Accessed December 13, 2017].

Pécuchet, N. et al., 2016. Base-Position Error Rate Analysis of Next-Generation Sequencing Applied to Circulating Tumor DNA in Non-Small Cell Lung Cancer: A Prospective Study. M. Ladanyi, ed. PLoS medicine, 13(12), p.e1002199. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28027313 [Accessed April 24, 2017].

Sabari, J.K. et al., 2017. The activity, safety, and evolving role of brigatinib in patients with ALK-rearranged non-small cell lung cancers. OncoTargets and therapy, 10, pp.1983–1992. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28435288 [Accessed December 13, 2017].

Sasaki, T. et al., 2010. The biology and treatment of EML4-ALK non-small cell lung cancer. European journal of cancer (Oxford, England : 1990), 46(10), pp.1773–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20418096 [Accessed January 10, 2018].

Sgambato, A. et al., 2018. Targeted therapies in non-small cell lung cancer: a focus on ALK/ROS1 tyrosine kinase inhibitors. Expert Review of Anticancer Therapy, 18(1), pp.71–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29187012 [Accessed January 9, 2018].

Sullivan, I. & Planchard, D., 2016. ALK inhibitors in non-small cell lung cancer: the latest evidence and developments. Therapeutic advances in medical oncology, 8(1), pp.32–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26753004 [Accessed December 13, 2017].

Wang, Z. et al., 2017. Anaplastic lymphoma kinase gene rearrangement predicts better prognosis in NSCLC patients: A meta-analysis. Lung Cancer, 112, pp.1–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29191580 [Accessed January 9, 2018].

Strand Life Sciences Pvt. Ltd.
5th Floor, Kirloskar Business Park, Bellary Road, Hebbal, Bangalore - 560 024
Phone: 1800-1022-695, support.strandx@strandls.com, www.strandls.com

#StayAheadOfCancer

Strand ID: LB-245122012018