Beyond epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) testing in advanced non-small cell lung cancer: Is the picture as "ROS1" as it appears?

Lung cancer remains the most common cause of cancer-related mortality globally. The last couple of decades and in particular the last 5 years has been a very exciting period for both oncologists and patients alike. This has primarily been due to the advent of targeted therapies and subsequently immunotherapy (PD-1/PD-L1 inhibitors) as the 4th and 5th pillars for the treatment of lung cancer, respectively (based on the timelines for their development and approval).[1] Both of these have become very useful additions to the previously available treatment options [Figure 1], namely, surgical resection, chemotherapy, and radiation therapy. A large proportion of these treatment advances have been in advanced/metastatic (Stages IIIB/IV) non-small cell lung cancer (NSCLC) – the most frequent disease stage at presentation and the most frequent histological type respectively.[2-4] This has been particularly true in the case of non-squamous NSCLC (essentially lung adenocarcinoma) – which is the most common histological type seen in female nonsmokers.

The discovery of epidermal growth factor receptor (EGFR) sensitizing mutations was followed by the demonstration that EGFR tyrosine kinase inhibitors (TKIs) are superior to platinum-based doublet (two drug) chemotherapy as first-line treatment of patients with advanced/metastatic NSCLC harboring these sensitizing mutations (the most common being exon 19 deletions and the point L858R mutation on exon 21).[5] This was established in seven large randomized trials featuring all the three EGFR-TKIs that are currently available for use (the first generation reversible blockers gefitinib and erlotinib as well as the second generation irreversible blocker afatinib).[6-12] A similar and perhaps greater magnitude of benefit was shown in patients with rearrangements of the anaplastic lymphoma kinase (ALK) gene with the use of crizotinib (a small molecule ALK inhibitor) namely its superiority over standard platinum based doublet chemotherapy both in the first- and second-line settings.[13,14] Second generation ALK inhibitors, alectinib[15-17] and ceritinib,[18-20] initially researched and approved for patients intolerant to or having disease progression on crizotinib (especially brain metastases) are moving to the front line setting now.[21-23] Both EGFR sensitizing mutations and ALK rearrangements occur predominantly in adenocarcinoma histology with further enrichment being possible in female nonsmokers.[24,25]

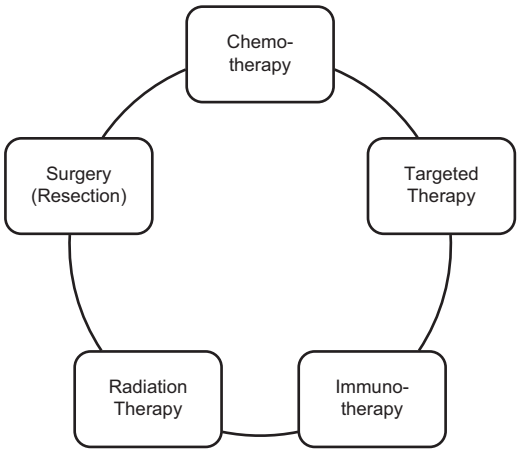

Figure 1: The five pillars of lung cancer treatment

The role of methodology employed for testing for these druggable genetic mutations and rearrangements needs special mention. As per our own experience (unpublished data), EGFR mutation prevalence was 5% higher with the more sensitive method of real-time amplification refractory mutation system polymerase chain reaction (ARMS-PCR) as compared to the initially employed conventional gene (Sanger) sequencing (23% vs. 18%). In case of ALK gene rearrangements, the prevalence is almost double (9% vs. 5%) with the more sensitive immunochemistry (D5F3 clone) as compared to BreakApart fluorescence *in situ* hybridization (FISH) method. The more sensitive techniques listed above are also associated with a significant increase in the percentage of interpretable results as these require lesser tissue. With the use of real-time PCR for EGFR and of the D5F3 immunochemistry for ALK, almost 95% of our samples tested had interpretable results as compared to around 82% with the initially used techniques. Since EGFR mutations and ALK rearrangements are mutually exclusive, we currently expect **one of every two females** with advanced/metastatic lung adenocarcinoma tested to be a candidate for targeted therapy (37% EGFR and 13% ALK prevalence in our setting). Similarly, for **nonsmoker males**, this translates into **three of every eight patients** being tested to be positive for either EGFR or ALK (26% and 10.5%, respectively) and thus being eligible for EGFR/ALK targeted therapies. Even for **current/ex-smoking males** (historically believed to be not associated with the presence of druggable genetic alterations), the expected prevalence at our center for them

to be candidates for targeted therapy is **one of every six** patients tested (11% EGFR and 6% ALK prevalence). For patients, the implications are enormous. It not only means that they can be treated initially with targeted therapy alone (without being subjected to chemotherapy) but also that this comes at the convenience of taking an oral drug at home with much lesser toxicity and much greater efficacy than conventional chemotherapy.

We are now moving fast forward to an era wherein commercially available highly sensitive methods such as digital droplet PCR and next generation sequencing (NGS) platforms are being used to test for these targetable mutations/rearrangements in tissue- and nontissue-based specimens as well.[26] In the case of EGFR, using peripheral blood [popularly called 'liquid biopsy'] for mutation testing in circulating tumor DNA (ctDNA; also sometimes referred to as circulating cell-free tumor DNA or cfDNA) is already approved and validated for routine clinical practice. As compared to DNA within circulating tumor cells, ctDNA or cfDNA is more frequently and easily detectable. This platform is used for detecting sensitizing EGFR mutations in treatment naïve patients with advanced/metastatic NSCLC (for offering therapy with first/second generation EGFR TKIs) and subsequently also once these patients have experienced disease progression to test for the acquired T790M resistance conferring mutation on exon 20 (to offer therapy with the third generation EGFR TKI – osimertinib).[27] The ultra-sensitive NGS platforms are also being utilized to find targetable genetic alterations other than EGFR/ALK in particular ROS1 rearrangements, HER2 mutations, $BRAF^{V600E}$ mutations, MET amplification, and MET exon 14 skipping mutations.

The article by Suryavanshi *et al*. (accompanying this editorial) in the current issue of this journal is an attempt to identify the prevalence of ROS1 rearrangements in a cohort of 105 lung adenocarcinoma patients which is commendable.[28] The finding of 3 patients positive by FISH is in concordance with world literature as the published prevalence is 2%–3%. Would the prevalence have been higher had the cohort had been screened with immunochemistry first followed by FISH for confirmation (something analogs to what happened to ALK testing) or if a more sensitive technique like NGS been employed? These are questions that may need to be addressed in future studies. From a treatment perspective, crizotinib, a small molecule ALK inhibitor, is equally efficacious, if not more, for patients with ROS1 gene rearrangements with objective responses ranging from 72% to 80%.[29,30] For routine clinical practice, what does this study do? **Does it make ROS1 testing as "ROS1" (rosy) as it appears to be?**

The answer to this is both yes and no. **Yes** because we are delighted to find that Indian patients have at least a similar prevalence of ROS1 rearrangements as reported from other parts of the world. ROS1 is another "druggable" genetic alteration in advanced/metastatic NSCLC (apart from EGFR and ALK) whose occurrence is mutually exclusive from the other driver mutations/rearrangements.[31] **No** because of two reasons – first, the drug crizotinib is marketed only by the innovator and costs approximately 75,000 Indian rupees (1175 US$) per month in a country with a GDP per capita of approximately 1800 US$ although the compassionate access program that is currently being offered by the company is enabling ALK +ve patients living below the poverty line to have access to this drug without paying for it. Second, for 100 lung cancer patients presenting to the clinic, the actual statistics turn out to be as shown in Table 1. This has been aptly called **"Targeting the 1% in Lung Cancer."**[32]

So what does it mean – should we really invest our resources and efforts in finding out patients with targetable genetic mutations/alterations in lung cancer who constitute

Table 1: Prevalence of targetable genetic mutations and rearrangements - percentage wise and actual numbers

	Prevalence (percentage)*	Actual numbers in 100 lung cancer patients
All histological types		
Squamous (NSCLC)	36%	36
SCLC	19%	19
Adenocarcinoma (NSCLC)	36%	36
Other NSCLC subtypes (including NSCLC-NOS)	9%	9
Non-squamous NSCLC	(36% + 9%) = 45%	(36 + 9) = 45
Advanced/metastatic disease (stages IIIB-IV)	84% (of all NSCLC)	(84 × 81/100) = 68
Advanced/metastatic (stages IIIB/IV) non-squamous NSCLC		(84 × 45/100) = 38
EGFR gene mutations	21% (of advanced/metastatic non-squamous NSCLC)	(21 × 38/100) = 8
ALK gene rearrangements	9% (of advanced/metastatic non-squamous NSCLC)	(9 × 38/100) = 3
ROS1 gene rearrangements	2% (of advanced/metastatic non-squamous NSCLC)	(2 × 38/100) = 1
Other targetable mutations and rearrangements (including BRAF, HER2, MET, RET)	1%-2% (of advanced/metastatic non-squamous NSCLC) each	(6 × 38/100) = 2
All patients with targetable mutations and rearrangements		(8 + 3 + 1 + 2) = 14
No targetable mutations or rearrangements		(100 - 14) = 86

*Calculations and prevalence percentages based on references 3-4. NSCLC: Nonsmall cell lung cancer, SCLC: Small cell lung cancer, NOS: Not otherwise specified, EGFR: Epidermal growth factor receptor, ALK: Anaplastic lymphoma kinase, BRAF: B-Rapidly Accelerated Fibrosarcoma, HER2: Human Epithelial growth factor Receptor-2, MET: Mesenchymal Epithelial Transition factor receptor, RET: REarranged during Transfection

a minority (15% or less)?[33] The answer is clearly a **BIG YES** – the effort is definitely worth it. The phenomenal radiological responses, as well as clinical benefit (including improvement in survival) observed with administration of a targeted drug in advanced/metastatic NSCLC patients with a driver mutation/rearrangement, is not matched by any type of chemotherapy, radiation or its combination. It needs to be clarified here that the **benefit of PD-1/PD-L1 immune check point inhibitors (immunotherapy) is largely restricted to patients WITHOUT a targetable genetic mutation/rearrangement** in whom first and often second line therapies are targeted drugs. Testing for EGFR mutations and ALK rearrangements is part of normal protocol for diagnostic evaluation and management of all patients with advanced/metastatic lung adenocarcinoma. This awareness is required to be disseminated among ALL physicians (pulmonologists/medical oncologists/radiation oncologists) involved in the treatment of this disease as well as for those working in the laboratory (pathologists/molecular biologists). It is mandatory for them not only to advise patients about the need for such testing but also to guide them regarding the existence of facilities where such testing is available/offered. Occasionally (as in the case of ROS1 and other relatively uncommon mutations/rearrangements), this exercise amounts to "finding a needle in a haystack." However, due to mutual exclusivity from EGFR and ALK, the option to test ROS1 (and the others druggable targets) should be offered to patients especially if (a) both of the former have already been tested and are negative and (b) the patient is a young never/light smoker with nonsquamous histology – all three being clinical surrogates that enrich the probability for their presence/detection. Ultimately, the use of targeted therapies in the presence of a positive predictive biomarker improves both the quality and quantity of life in the setting of advanced/metastatic NSCLC – a benefit which is something no health-care professional treating this disease can remain oblivious to. For a given patient, it often implies a world of difference and gives hope of leading a meaningful life for a reasonable period – something that perhaps he/she had completely given up when the diagnosis was shared with him/her.

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