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Letter to the Editor

Dear Editor

I read with interest the original article titled “A study of therapy targeted EGFR/ALK mutations in Indian patients with lung adenocarcinoma: A clinical and epidemiological study” by Rana et al. published in *Med J Armed Forces India* 2018;74:148–153.¹ This topic is close and dear to me because during my study period in the Tata Memorial Center, Mumbai, and Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), I was involved in molecular studies and followed up the whole procedure of mutational analysis and sequencing of KRAS in lung adenocarcinomas.

In this particular article, it was stated that the molecular studies of 152 cases were outsourced; therefore, I want to highlight the salient features of the type and adequacy of specimens; the type of biopsy such as trucut biopsy, the use of bronchoscopic biopsy or transthoracic needle biopsy in bigger specimens, or resection specimens in some cases; and difficulties encountered in DNA extraction, and the overall mutation rate was found in literature to be high in resection specimens.² We had studied lobectomy blocks in lung adenocarcinomas, and DNA extraction was very difficult; there was noise in signals, and repeated analysis was required in almost all the cases.

The genetic abnormalities in non-small-cell lung carcinoma (NSCLC; adenocarcinoma and squamous carcinoma) include EGFR^{3–8} (exon 21 L858R mutation and exon 19 deletion) mutations; HER2 mutations (1%); BRAF mutations (2%); exon 14 MET mutations (3.3%); and ALK,^{9–11} ROS1, RET, and NTRK rearrangements. Targeted therapy with gefitinib, erlotinib, and afatinib was used for EGFR-mutated advanced NSCLC; the ALK inhibitors crizotinib and ceritinib were used for ALK-positive NSCLC, and for ROS1 rearrangement-positive NSCLC, crizotinib was used.

In small centers, where lobectomies and metastasectomies are not performed for lung cancers, small biopsies and cytology specimens are the only material available for almost 80% of patients with NSCLC, and usually, the molecular tests are outsourced. In that case, the tissue should be optimal and it is essential that the tissue sample size is maximized. The biopsy specimen while performing bronchoscopy or transthoracic needle biopsy should not be one or two haphazard cores. At least six biopsy specimens should be taken and many blocks prepared in the laboratory, which can be used separately for EGFR, ALK, KRAS mutations, and so forth. For polymerase chain reaction-based next-generation

sequencing (NGS) technique, a minimum of 5–10 ng of DNA (1000 cells; including malignant and normal DNA) is required. Hybrid capture-based NGS technologies, encompassing RNA sequencing, require 100–200 ng of RNA or DNA. There is always a possibility of false-negative and false-positive results that occur because of artificial base pair changes. Whole-genome sequencing analyses require more DNA (10⁶ tumor cells) and are not adapted for small samples.

In this era of targeted therapy, small samples (biopsy and cell blocks from fine needle aspiration cytology (FNAC) or fluid cytology) may play an important role in the management and prioritization of tumor material for multiple predictive marker testing in NSCLC and are preferable to obtain the largest biopsy possible for better molecular results and correct targeted treatment.

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Reply

Dear Editor,

It is highly gratifying to see the keen interest shown by the reader in our article.¹ One of the objectives of our article was to impress upon the importance of adequate processing of the tissue samples which are most of the times very limited. The reader has totally echoed our sentiments in this arena. In our study more than 90% of samples of lung adenocarcinoma were Trucut tissue biopsies either through Bronchoscope or CT guided. In today's time of early screening and accessible medical facilities, early diagnosis is being achieved on minimally invasive procedures and smaller biopsies.² Minimally invasive diagnostic techniques are only beneficial if adequate tissue can be obtained for detailed molecular characterization.³ The cases in our study which we rejected due to inconclusive molecular testing results are primarily due to lack of adequate tissue. We cannot agree more with the reader for the need of adequate tissue in cases of suspected lung malignancies for the maximum benefit to the patient with already established targeted therapies. In fact in our article we have impressed upon the separate processing of smaller biopsies so that to limit extra exposure to formalin fixation and for faster careful processing.⁴ Furthermore during grossing many a times pathologists mark tiny biopsies with eosin stain, this should be discouraged for tissues likely to be subjected to immunofluorescence as eosin has auto fluorescence. Interaction between the pathologist and the treating physician is paramount in ascertaining the type

of diagnostic specimen and understanding the limitations of the assay when only a small sample is available. We thank the reader for sharing his/ her experience in this field and whole heartedly agree that wherever possible tissue sample should be maximized and at least six core biopsies should be taken in suspected cases of lung malignancies to help in molecular studies which are essential for targeted therapy.

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