The Role of Endobronchial Ultrasound Guided Transbronchial Needle Aspiration Specimens for Next Generation Sequencing in Non-Small Cell Lung Cancer: A Clinical Perspective

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Introduction

The National Comprehensive Cancer Network (NCCN) 2017 Clinical Practice Guidelines for Non-Small Cell Lung Cancer (NSCLC) recommend concomitant diagnosis, staging and acquisition of adequate material for genetic testing [1]. Endobronchial ultrasound (EBUS) guided transbronchial needle aspiration (TBNA) is recommended as the best first test for diagnosis and staging of lung cancer [2]. In fact, the majority of patients with lung cancer are diagnosed by small volume biopsies or cytology specimens [3] and the majority of patients are diagnosed at an advanced stage for which broad molecular profiling is recommended [1].

Next Generation Sequencing (NGS) also known as massively parallel sequencing, is a form of broad molecular profiling utilizing a single test to identify thousands of somatic or germline mutations from hundreds of genes allowing for the examination of the entire cancer genome and transcriptome [4]. NGS identifies mutations targetable with approved drugs and may detect other genetic abnormalities, which allows enrollment in clinical trials. Published evidence suggests that NGS is more sensitive than conventional testing for potentially targetable driver-mutations (e.g. EGFR, KRAS, ALK, ROSI, RET) [5,6]. Furthermore, NCCN recommendations go beyond testing for EGFR, ALK, and ROS-1. NGS uncovers mutations for which a targeted agent is available and recommended by NCCN guidelines in 26% of patients; an additional 39% of patients tested with NGS may have a genomic alterations for which a targeted agent is available on a clinic trial [6]. Comprehensive genetic profiling is also warranted at the time of disease progression to evaluate for secondary mutations, which might be targetable by approved drugs, offer prognostic value or help guide referrals towards clinical trials [1,7,8]. Mechanisms of resistance to EGFR TKIs (T790 M mutation, MET amplification, BRAF and PIK3CA mutations) can be identified and targeted [7]. Resistance mechanisms to first generation ALK inhibitors (e.g. crizotinib) are increasingly discovered and may be targeted by newer ALK inhibitors which have been FDA approved (eg. ceritinib, alectinib) [9-11]. These resistance mutations are identified by testing large gene panels made feasible by NGS [11].

While NGS testing of formalin fixed paraffin embedded (FFPE) cell block samples is feasible [12,13], the DNA quality is likely to be better on cytology smears [14-16]. Moreover, cellularity and adequacy can be assessed at the time of the procedure on slides via rapid onsite examination (ROSE), whereas cell block adequacy cannot be assessed intra-procedurally. Prior studies demonstrate the feasibility of extracting DNA from fine needle aspiration (FNA) tumor samples preserved on cytology slides [17-21] and these studies show that there is a direct correlation between slide cellularity and successful NGS testing. Slides with greater than 5000 cells or at least 5 ng/mL of DNA are successful 95% of the time for sequencing of 50 target genes [17]. Notably, studies that evaluated NGS panels with >200 genes were all performed on histology samples or cell blocks, not on smears [6,13].

In this clinical perspective, we describe our institution’s workflow for NGS testing of 50 gene (OncoScreen) and >1000 gene (OncoPlus) panels on slide cytology samples obtained by EBUS-TBNA from metastatic mediastinal, hilar or interlobar lymph nodes in patients with NSCLC.

Specimen Acquisition, Handling and Processing

On EBUS examination, if a lymph node greater than 5mm is identified during a mediastinal staging procedure, an EBUS TBNA of the lymph node is performed using a 25-gauge needle (Figure 1). A minimum of three aspirates per node is performed during a routine EBUS-TBNA procedure. This process continues through the remaining lymph node stations. Following aspiration of a sample, the needle is removed from the scope and a drop of sample material is discharged onto a glass slide, first by advancing the needle stylet and, if insufficient material is discharged, then followed by injection of air using an empty syringe attached to the stylet hub. The material dispelled on the glass slide is then smeared with a second slide resulting in two smears. One of the slides is air-dried using Diff-Quik stain and undergoes (ROSE). The other slide is sprayed-fixed with alcohol for future Pap staining. After each pass, all remaining aspirate material from the needle is ejected onto a glass slide, first by advancing the needle stylet and, if insufficient material is discharged, then followed by injection of air using an empty syringe attached to the stylet hub. The material dispelled on the glass slide is then smeared with a second slide resulting in two smears. One of the slides is air-dried using Diff-Quik stain and undergoes (ROSE). The other slide is sprayed-fixed with alcohol for future Pap staining. After each pass, all remaining aspirate material from the needle is ejected onto a glass slide, first by advancing the needle stylet and, if insufficient material is discharged, then followed by injection of air using an empty syringe attached to the stylet hub. The material dispelled on the glass slide is then smeared with a second slide resulting in two smears. One of the slides is air-dried using Diff-Quik stain and undergoes (ROSE). The other slide is sprayed-fixed with alcohol for future Pap staining. After each pass, all remaining aspirate material from the needle is ejected

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treatment at time of diagnosis of advanced lung cancer or disease progression without requiring more invasive procedures for tissue acquisition.

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References


