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Feasibility of endobronchial ultrasound transbronchial needle aspiration for massively parallel next-generation sequencing in thoracic cancer patients☆

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ABSTRACT

Introduction: Next-generation sequencing (NGS) allows for the identification of a growing number of therapeutic and prognostic molecular targets. However, NGS typically requires greater quantities of DNA than traditional molecular testing does. Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive procedure used to sample central thoracic lesions, but it is not well established whether this technique provides sufficient material for NGS.

Methods: We performed a retrospective review of EBUS-TBNA at our institution (3/1/14-9/28/16). NGS was performed using a comprehensive hybrid-capture based assay (MSK-IMPACT) that detects > 340 gene mutations. Samples found to be diagnostic for malignancy and for which MSK-IMPACT had been attempted were identified. Pathologic and clinical data were obtained from the medical record, and the results of MSK-IMPACT were examined.

Results: In total, 784 EBUS-TBNA procedures were performed during the study period. MSK-IMPACT was requested for 115 malignant samples and was successful for 99 (86.1%), identifying an average of 12.7 mutations at a mean coverage depth of 806X. NGS was performed on paraffin-embedded cell blocks in 93 cases (93.9%) and on cell-free DNA in needle rinse fluid in 6 cases. The success rate of the assay improved significantly from the first third of cases (76.3%), to 92.3% for the final one-third of cases ($p < 0.05$).

Conclusions: EBUS-TBNA reliably provided adequate tissue for hybrid capture NGS, and is a suitable option for comprehensive NGS testing in patients with thoracic malignancies.

1. Introduction

Next-generation sequencing (NGS) is an important tool in precision oncology, allowing improved prognostication and the identification of a growing number of clinically validated and investigational therapeutic molecular targets [1]. In non-small cell lung cancer (NSCLC) and, more specifically, lung adenocarcinoma, targeted therapies for *EGFR* mutations and *ALK-EML4* or *ROS1* rearrangements now compose part of the standard of care [2–5]. In addition, several alterations found in a subset of patients—such as *BRAF* V600E, *RET* fusions, *MET* exon 14 splice site mutations, and *ERBB2* amplification or activating mutations—may predict response to targeted therapies, such as those already approved for other tumor types or those approved on the basis of preclinical studies [6–13]. Several studies have identified *KRAS* mutations as an important negative prognostic marker [14,15], and, more recently, targeted approaches have been described for *KRAS*-mutant NSCLC [16,17]. In this setting, comprehensive genomic profiling using NGS allows for the identification of a wide spectrum of genomic alterations, resulting in personalized cancer treatment of an increasing number of patients, as well as the inclusion of patients in clinical trials, including so-called basket trials, organized by oncogenic driver alterations instead of by tissue of origin. The results of the recently reported ProfiLER trial demonstrate that large-scale NGS screening of cancer patients is both feasible and associated with a survival benefit [18].

Abbreviations: cfDNA, cell-free DNA; EBUS-TBNA, endobronchial ultrasound transbronchial needle aspiration; MSK, Memorial Sloan Kettering Cancer Center; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; ROSE, rapid on-site cytology evaluation

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Memorial Sloan Kettering Cancer Center (MSK) has developed a next-generation massively parallel molecular sequencing assay known as Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT). This hybrid capture-based NGS assay detects point mutations, small insertions/deletions, copy number alterations, and selected fusions in > 340 cancer-related genes [19,20]. Of these, 19 are clinically validated, while the rest are considered investigational. A complete description of the panel has been published [19]. Such a comprehensive assay is well-suited to the context of NSCLC, which includes a wide range of genomic alterations. In a recent study, 37% of lung cancer patients received matched treatment after molecular profiling using the MSK-IMPACT assay [21]. Compared with amplicon-based assays, however, hybrid capture-based assays, such as MSK-IMPACT, generally require a higher input of nucleic acids. The preferred source of material for molecular testing of NSCLC, in both presurgical and advanced-stage settings, is small specimens acquired with minimal invasiveness, including cytologic samples. In this setting, a potential limitation of NGS-based assays for comprehensive genomic profiling is the need for more DNA than would be required for traditional molecular assays, which perform well using cytologic samples without the requirement for major technical adjustments [22,23]. As such, there is a debate regarding whether minimally invasive, fine-needle aspiration biopsy techniques are appropriate when comprehensive NGS assays are planned.

Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive procedure for sampling mediastinal and pulmonary lesions. It is used to diagnose mediastinal lymphadenopathy and central lung masses, to stage mediastinal lymph nodes in patients with NSCLC and other malignancies, and to acquire tissue in the setting of malignant disease progression despite medical therapy. EBUS-TBNA is generally performed as an outpatient procedure and may be done with or without the use of general anesthetic. Morbidity is minimal and compares favorably with that of more-invasive techniques, such as mediastinoscopy or thoracoscopy [24]. The reported sensitivity, specificity, and diagnostic accuracy of nodal staging for NSCLC are all > 90% [25,26] and have been shown to be comparable or even superior to those of surgical staging [27]. The utility of EBUS-TBNA for molecular testing can be further improved with the use of rapid on-site cytologic evaluation (ROSE), which allows a cytopathologist to provide real-time feedback regarding specimen adequacy during the EBUS procedure [28]. Several studies have confirmed the feasibility of using EBUS-TBNA samples for traditional molecular assays such as EGFR, ALK and KRAS testing [22,29,30], and even small multi-gene panels [31], but the suitability of the technique for NGS assays, that can test for hundreds of genes, has only recently been established [32]. The Papanicolaou Society of Cytopathology guidelines discuss the use of EBUS-TBNA for NGS, but the evidence supporting this guideline consists of a single, five-patient series including both EBUS and CT guided fine-needle samples and a 27-gene NGS panel [33,34]. Concerns remain regarding the ability of EBUS to reliably obtain sufficient material for NGS, leading some oncologists to still prefer surgical specimens or core biopsies. Since only limited evidence exists to support the performance of comprehensive NGS panels on EBUS-TBNA specimens [32], we sought to address the question of whether EBUS-TBNA can reliably provide sufficient material for large hybrid capture NGS.

2. Materials and methods

We performed a retrospective review of a prospectively maintained thoracic surgery database, including all patients who underwent EBUS-TBNA by the Thoracic Surgery Service at MSK between March 1, 2014, when MSK-IMPACT was introduced for EBUS samples, and September 28, 2016. A waiver for the use of patient medical records was obtained from the MSK Institutional Review Board. Samples found to be positive for malignancy that had undergone MSK-IMPACT testing were identified. The results of the MSK-IMPACT assay were examined. Samples that either contained borderline tumor content or for which the assay was not successful (see below) were re-reviewed pathologically. Student's *t*-test, Fisher's exact test, and the χ^2 test were used to identify statistical differences between groups. *P* values refer to Student's *t*-test, except where noted.

2.1. EBUS-TBNA technique

In our group, all EBUS-TBNA procedures are performed under general anesthesia with either laryngeal mask airway or single lumen endotracheal intubation. An Olympus BF-UC180F EBUS (Olympus, Tokyo, Japan) scope is used, with a 21- or (infrequently) 22-gauge EBUS needle. ROSE is used to assess specimen adequacy in all cases. Based on a recent quality assurance survey of our surgeons, typically, 3–5 passes are made in each node, guided by ROSE assessment of adequacy, with approximately 10–15 strokes of the needle within the node per pass.

2.2. Genomic and pathologic sample analysis

Cell block material was obtained from CytoLyt-fixed, paraffin-embedded 5-μm-thick cell block sections on glass slides (range 5–21 slides/case). For all cases, 20 recuts were prepared for DNA extraction and at least 1 hematoxylin and eosin–stained (H&E) section was reviewed for assessment of specimen adequacy and estimation of tumor fraction. Macrodissection, by circling the area of enriched tumor on the slide, was performed when needed. Cases were rejected if < 10% of tumor content was present (i.e., if tumor cells represented < 10% of total nucleated cell cellularity, which included lymphocytes, bronchial cells, and stromal cells based on the H&E review). For positive samples in which cell blocks failed to capture a sufficient number of tumor cells, molecular testing was performed on cell-free DNA (cfDNA) from the needle rinse fluid in CytoLyt. Retrieving the cfDNA from the CytoLyt fluid was achieved by collecting the supernatant fluid present after cells were centrifuged from needle rinse fluid to form a cell block. The details of this procedure were described recently [35,36].

Sequencing of tumor and blood DNA was performed using the clinically validated MSK-IMPACT assay [20]. In brief, DNA was extracted using the DNeasy Tissue KIT (Qiagen, Valencia, CA), with either Citrasolv or (starting in March 2016) mineral oil for deparaffinization [34]. After elution, quantification was performed using a Qubit DNA high-sensitivity assay kit (Life Technologies, Carlsbad, CA). Samples for which 50 ng of DNA input was reached were processed; those below this cutoff were tested on alternative platforms (mass spectrometry genotyping [Sequenom, San Diego, CA]) downstream. Fragment analysis of exon 19 and 20 of *EGFR* and exon 20 of *ERBB2* was performed before MSK-IMPACT testing on all lung adenocarcinoma samples. DNA was submitted to shearing and processed to generate bar-coded libraries, pooled, and subjected to exon capture using custom-designed probes. Captured DNA fragments were sequenced on an Illumina HiSeq2500 system before being subjected to the bioinformatics analysis pipeline. In this assay, matched normal DNA from blood is simultaneously processed with tumor DNA for all samples, allowing identification and filtering out of single-nucleotide polymorphisms.

Over the course of the study, the number of genes included in the assay was increased from 341 to 469. Most samples (88%) were processed with the intermediate version (including 410 genes); only 3% were processed with the initial version (including 341 genes). All versions included a total of 19 genes with full clinical validation for which the cutoff for variant calling was set at 2%; it was set at 5% for all other genes in the panel (called "investigational")—meaning that mutations are reported only if they reached this frequency. Tumor samples were required to be sequenced to $\geq 200\times$ coverage.

At the time of reporting, samples meeting the assay performance criteria (i.e., > 10% proportion of tumor cells by visual estimate and > 50 ng of extracted DNA) but in which tumor cells