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Success and failure rates of tumor genotyping techniques in routine pathological samples with non-small-cell lung cancer

Paul A. VanderLaan[a,1], Norihiro Yamaguchi[b,1], Erik Folch[b,c], David H. Boucher[b], Michael S. Kent[c], Sidharta P. Gangadharan[c], Adnan Majid[b,c], Michael A. Goldstein[b], Mark S. Huberman[b], Olivier N. Kocher[a], Daniel B. Costa[b,*]

[a] Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States
[b] Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States
[c] Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States

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ABSTRACT

Introduction: Identification of some somatic molecular alterations in non-small-cell lung cancer (NSCLC) has become evidence-based practice. The success and failure rate of using commercially available tumor genotyping techniques in routine day-to-day NSCLC pathology samples is not well described. We sought to evaluate the success and failure rate of *EGFR* mutation, *KRAS* mutation, and *ALK* FISH in a cohort of lung cancers subjected to routine clinical tumor genotype.
Methods: Clinicopathologic data, tumor genotype success and failure rates were retrospectively compiled and analyzed from 381 patient-tumor samples.
Results: From these 381 patients with lung cancer, the mean age was 65 years, 61.2% were women, 75.9% were white, 27.8% were never smokers, 73.8% had advanced NSCLC and 86.1% had adenocarcinoma histology. The tumor tissue was obtained from surgical specimens in 48.8%, core needle biopsies in 17.9%, and as cell blocks from aspirates or fluid in 33.3% of cases. Anatomic sites for tissue collection included lung (49.3%), lymph nodes (22.3%), pleura (11.8%), bone (6.0%), brain (6.0%), among others. The overall success rate for *EGFR* mutation analysis was 94.2%, for *KRAS* mutation 91.6% and for *ALK* FISH 91.6%. The highest failure rates were observed when the tissue was obtained from image-guided percutaneous transthoracic core-needle biopsies (31.8%, 27.3%, and 35.3% for *EGFR*, *KRAS*, and *ALK* tests, respectively) and bone specimens (23.1%, 15.4%, and 23.1%, respectively). In specimens obtained from bone, the failure rates were significantly higher for biopsies than resection specimens (40% vs. 0%, $p = 0.024$ for *EGFR*) and for decalcified compared to non-decalcified samples (60% vs. 5.5%, $p = 0.021$ for *EGFR*).
Conclusions: Tumor genotype techniques are feasible in most samples, outside small image-guided percutaneous transthoracic core-needle biopsies and bone samples from core biopsies with decalcification, and therefore expansion of routine tumor genotype into the care of patients with NSCLC may not require special tissue acquisition or manipulation.

1. Introduction

In the United States in 2013, the expected number of new cases of and deaths from lung cancer will exceed 220,000 and 159,000, respectively [1]. The overall, of all stages combined, five-year survival for the most prevalent form of lung cancer – non-small-cell lung cancer (NSCLC) – does not exceed 15% despite use of surgical resection, radiotherapy and systemic chemotherapy [1]. The last decade of research in lung cancer has yielded important advances in the development of targeted therapies that target driver oncogenes [2]. The most prevalent mutated or rearranged oncogenes identified in non-small cell lung cancers (NSCLCs) are v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*), epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), *ROS1*, *BRAF*, *ERBB2* and *RET* [3]. Specifically, mutations in *EGFR* and rearrangements (either inversions or translocations) involving *ALK* are part of the pathogenesis of some lung adenocarcinomas, predominantly

* Corresponding author at: Division of Hematology/Oncology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, United States. Tel.: +1 617 667 9236; fax: +1 617 975 5665.
E-mail address: dbcosta@bidmc.harvard.edu (D.B. Costa).
[1] These authors contributed equally.

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in never and/or light smokers, and predict for improved outcomes with tyrosine kinase inhibitors (TKIs), such as erlotinib and crizotinib, respectively, that target these aberrant kinases [4–6]. As such, molecular testing of lung cancer specimens has become part of routine clinical practice in the care for patients with advanced NSCLC.

Currently, the proposed testing guidelines from the College of American Pathologists, the International Association for the Study of Lung Cancer, the Association for Molecular Pathology, and the National Comprehensive Cancer Network recommend testing all advanced NSCLCs with an adenocarcinoma component at the time of diagnosis for *EGFR* mutation and for *ALK* fluorescence in situ hybridization (FISH) analysis [7,8]. These specimens are more frequently encountered as small biopsy or cytology specimens derived from either the primary tumor or from lymph node or distant metastatic sites, and processed as formalin-fixed, paraffin embedded tissue samples. Limited tumor cellularity in small biopsy or cytology specimens can lead to molecular testing failure. Additionally, it is recognized that different tissue processing techniques, including acid decalcification or heavy metal fixatives, can cause DNA degradation and impede molecular analysis [9]. These issues can influence the selection and success rates of specimens submitted for mutational analysis when considering sampling of bony lesions. Practically, this can pose a dilemma for the clinician and pathologist alike, in determining whether an initial biopsy used to establish the diagnosis of NSCLC is sufficient for additional molecular testing, or whether another specimen must be procured for such a purpose.

As molecular testing of NSCLCs has become a standard of clinical practice, various institutions have reported their testing protocol and experience [10–13]. From these studies, one can occasionally glean the failure rates of their specimens, though to our knowledge a systematic study of genetic testing failure rates for NSCLC using commercially available testing in a typical clinical practice setting has not been published to date. Here, we present the molecular testing efficiency for *EGFR*, *KRAS*, and *ALK* FISH analysis of clinical specimens from NSCLC patients from our institution over a five year time period, reflecting real-life clinical practice experience.

2. Materials and methods

2.1. Patient selection

Patients with a diagnosis of lung cancer who were seen by our providers and whose tumors were genotyped for at least *EGFR* mutations were identified through an ongoing Institutional Review Board (IRB) approved protocol at Beth Israel Deaconess Medical Center (BIDMC2009-P-000182). Patients and tumor pairs were excluded if genotyping was not performed. There were 381 patient-tumor specimens that were submitted to a commercial vendor for tumor genotype techniques between 2007 and 2012. The data cut off for analyses was December 19th, 2012. Study data were collected and managed using REDCap electronic data capture tools hosted at BIDMC.

2.2. Tumor processing and genotype

Surgical (i.e., either incisional or excisional biopsies that required a surgical procedure) and core needle biopsies were processed using standard techniques: 10% neutral buffered formalin fixation and paraffin-embedding. For any bone specimens that could not be cut with a scalpel at the grossing bench, an acid decalcification was performed using RDO rapid decalcifier solution (Apex Engineering Products Corporation, Aurora, IL) following formalin fixation. Core needle and small biopsy specimens typically underwent a brief 15–30 min decalcification, whereas larger surgical resection specimens were decalcified for 2–6 h depending on the amount of calcified bone in the chosen sections. Cell aspirates or cell concentrates from fluid samples were collected into a methanol–water fixative (CytoLyt, Hologic Corp., Marlborough, MA) and a single ThinPrep slide prepared, with residual material used to create a cell block using a plasma-thrombin method prior to formalin-fixation and paraffin embedding. Once a diagnosis was established on histologic and/or immunohistologic staining profiles as per evidence-based recommendations [8], the residual material in the formalin-fixed paraffin-embedded (FFPE) tissue blocks was submitted for molecular analysis. When multiple tissue blocks were available, the one with the highest tumor cellularity was chosen, without additional tumor microdissection or enrichment. Molecular analysis of tumor specimens was performed by a commercial vendor, Integrated Oncology (LabCorp, Esoterix Genetic Laboratories, LLC). *EGFR* mutation analysis was performed using standard DNA sequencing techniques with exons 18–21 sequenced [14,15]. For *KRAS* mutation analysis, DNA from exon 2 was amplified and subjected to single nucleotide primer extension to detect mutations at codons 12 and 13. *ALK* translocation status was analyzed using the Vysis ALK Break-Apart fluorescence in situ hybridization (FISH) probe (Abbott Molecular, Inc., Des Plaines, IL), as previously described [16]. Failure of the assays was defined as insufficient/unusable material to isolate DNA or inability to perform/complete sequencing for *EGFR* and *KRAS* mutations, and lack of hybridization signals after two attempts for *ALK* FISH.

2.3. Data collection

Clinical, pathologic, radiographic and tumor genotyping information was collected from chart extraction. The site of biopsy (lung, lymph node, pleura, bone, brain, liver, pericardium, or adrenal) and the type of biopsy (surgical specimen [both excisional and incisional], core needle biopsy, or cell block from aspirate/fluid) were extracted from the medical record. Slides from all specimens that failed molecular testing, as well as a subset of the successfully genotyped cases were re-reviewed by a pathologist (PAV), with data compiled on tumor cellularity, use of ancillary studies, histopathologic features, and specimen processing including decalcification.

2.4. Statistical methods

Logistic regression was used for the univariable and multivariable analyses to capture the influence of each clinical predictive factor on the failure rate of tumor genotyping. The clinically relevant predictive factors were included into the multivariable analysis regardless of their statistical significance in the univariable analysis. All categorical variables were dichotomized in the regression analyses. We report odds ratio (OR) and 95% confidence interval (95%CI) to each predictive factor. Fisher's exact test was performed to compare categorical variables. p-value < 0.05 was considered as statistically significant. All p-values we reported were two-sided. We performed our statistical analyses with STATA version 12 (STATA Corp, College Station, TX).

3. Results

3.1. Patient and tumor characteristics

Table 1 summarizes the clinical and pathological characteristics of the 381 patient-tumor pairs that were included in our cohort.

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