ORIGINAL ARTICLE

Diverse *EGFR* Exon 20 Insertions and Co-Occurring Molecular Alterations Identified by Comprehensive Genomic Profiling of NSCLC

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ABSTRACT

Introduction: *EGFR* exon 20 insertions (*EGFR*ex20ins) comprise an uncommon subset of *EGFR*-activating alterations relatively insensitive to first- and second-generation EGFR tyrosine kinase inhibitors (TKIs). However, recent early clinical data suggests these patients may benefit from newer-generation EGFR-TKIs. Comprehensive genomic profiling (CGP) identifies a broad spectrum of *EGFR*ex20ins and associated co-occurring genomic alterations (GAs) present in NSCLC.

Methods: Hybrid capture-based CGP was performed prospectively on 14,483 clinically annotated consecutive NSCLC specimens to a mean coverage depth of greater than 650X for 236 or 315 cancer-related genes.

Results: Of 14,483 NSCLC cases, CGP identified 263 (1.8%) cases with *EGFR*ex20ins, representing 12% (263 of 2251) of cases with *EGFR* mutations. Sixty-four unique *EGFR*ex20ins were identified, most commonly D770_N771>ASVDN (21%) and N771_P772>SVDNP (20%). *EGFR* amplification occurred in 22% (57 of 263). The most common co-occurring GAs effected tumor protein p53 (*TP53*) (56%), cyclin dependent kinase inhibitor 2A (*CDKN2A*) (22%), cyclin dependent kinase inhibitor 2B (*CDKN2B*) (16%), NK2 homeobox 1 (*NKX2-1*) (14%) and RB transcriptional corepressor 1 (*RB1*) (11%); co-occurring GAs in other known lung cancer drivers were rare (5%). Average tumor mutational burden was low (mean 4.3, range 0 to 40.3 mutations/Mb). Clinical outcomes to first- and second-generation EGFR TKIs were obtained for five patients and none responded.

Conclusions: In the largest series of *EGFR*ex20ins NSCLC, diverse *EGFR*ex20ins were detected in 12% of *EGFR*-mutant NSCLC, a higher frequency than previously reported in smaller single-institution studies. Clinical outcomes showed lack of response to EGFR TKIs. Tumor mutational burden

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was low, consistent with non–smoking associated NSCLC. Comprehensive sequencing revealed increased proportion and wide variety of *EGFR*ex20ins, representing a population of patients significant enough for focused efforts on effective interventions.

Keywords: EGFR exon 20 insertion; Genomics; Tumor mutational burden

Introduction

EGFR exon 19 deletions and *EGFR* exon 21 L858R represent the vast majority of EGFR-activating mutations, and are exquisitely sensitive to approved EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib, and afatinib.[1-3] Less common *EGFR* mutations have variable sensitivity to EGFR inhibitors, although many still show clinical sensitivity to EGFR TKIs (e.g., EGFR G719X, L861Q, and S768I).[4] *EGFR* exon 20 insertions (*EGFR*ex20ins) are a collection of *EGFR* driver mutations characterized by in-frame insertions that typically serve to constitutively upregulate EGFR kinase activity similar to sensitizing mutations (but are insensitive to first- and second-generation EGFR-TKIs). These alterations have previously been reported to comprise approximately 4% to 10% of all *EGFR* mutant lung cancers.[5-8] The diverse array of exon 20 insertions and the challenges associated with identifying them may lead to underestimation of their true frequency. Although *EGFR*ex20ins usually have the same transforming ability as more common EGFR-activating mutations and are thus considered driver mutations, they are typically unresponsive to first- and second-generation EGFR TKIs due to the modified structures of their kinase domains.[5-8] Very few of these *EGFR*ex20ins, such as A763_Y764insFQEA, have shown sensitivity to first- and second-generation EGFR TKIs.[9]

Several next-generation EGFR TKIs (some with pan–human epidermal growth factor receptor activity) have shown pre-clinical activity against *EGFR*ex20ins and are in clinical development (EGF816, AP32788, osimertinib, and poziotinib).[5,10,11] In particular, a strong signal of clinical activity was shown with poziotinib with an overall response rate of 64% in the first 11 patients in an early phase clinical trial.[12] However, no EGFR-TKIs are currently approved for *EGFR*ex20ins and their diversity of structures suggests that different insertion events may have divergent responsiveness to various EGFR TKIs. Thus, identifying the full array and scope of *EGFR*ex20ins is of paramount importance.

Studies examining *EGFR*ex20ins have generally been limited to cases from single institutions. Certain next-generation sequencing approaches such as comprehensive genomic profiling (CGP) permit sequencing of the entire *EGFR* gene to broadly assess for diverse *EGFR*ex20ins and co-existing genomic alterations (GAs) that may be relevant to the pathogenesis of *EGFR*ex20ins-positive NSCLC. In addition to characterizing the clinical and pathologic characteristics of *EGFR*ex20ins-positive NSCLC from a large dataset with molecular testing performed in the course of clinical care from multiple institutions, the purpose of this study was to assess the frequency and diversity of *EGFR*ex20ins in NSCLC by their pattern of sequence alterations and co-occurring GAs, which may impact the development of targeted treatments for these patients.

Methods

DNA was extracted from 40-μm formalin-fixed paraffin-embedded sections. *EGFR*ex20ins and co-occurring GAs were identified by hybrid capture-based CGP performed during the course of clinical care on 14,483 consecutive NSCLC specimens to a mean coverage depth of greater than 650X for 236 (version 1, July 2012 to August 2014) or 315 (version 2, August 2014 to June 2016) cancer-related genes plus selected introns from 19 or 28 genes frequently rearranged in cancer. *EGFR* amplification was defined as estimated copy number greater than 6 copies.[13] Clinical data such as age, sex, stage, and histologic subtype were abstracted from the accompanying pathology report submitted by the ordering physician. Treatment outcomes from these patients were included where available. Testing was performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited reference laboratory (Foundation Medicine, Inc., Cambridge, Massachusetts). Patient samples were evaluated for GAs, including base-pair substitutions, insertions/deletions (indels), copy number alterations, and rearrangements, as described previously. Tumor mutational burden (TMB) was characterized as the number of somatic base substitution or indel alterations per megabase (Mb) per previously described methods.[14] Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

Results

CGP performed on 14,483 NSCLC cases in the course of clinical care identified 2251 cases with *EGFR* mutations; 263 of these cases were *EGFR*ex20ins, representing 12% of all *EGFR*-mutant NSCLC and 1.8% of all NSCLC cases tested. *EGFR*ex20ins were the third most common type of *EGFR* mutation detected following *EGFR* exon 19 deletions (47%) and EGFR L858R (32%). Other less common *EGFR* mutations detected in this large case