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Response to standard therapies and comprehensive genomic analysis for patients with lung adenocarcinoma with *EGFR* exon 20 insertions

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Abstract

Purpose: *EGFR* exon 20 insertions (ex20ins) are an uncommon genotype in non-small cell lung cancer (NSCLC) for which targeted therapies are under development. We sought to describe treatment outcomes and genomic and immunophenotypic characteristics of these tumors.

Experimental Design: We identified sequential patients with NSCLC with *EGFR* ex20ins and compared their clinical outcomes and pathologic features with other NSCLC patients.

Results: Among 6,290 patients with NSCLC, 106 (2%) had *EGFR* ex20ins. Patients with *EGFR* ex20ins were more likely to be Black (14 vs 6%, p<0.001) or Asian (22 vs. 10%, p<0.001) compared to all other patients with NSCLC. Median tumor mutational burden (TMB) (3.5 vs. 5.9, p<0.001) and proportion of tumors with PD-L1 expression ≥1% (22 vs. 60%, p<0.001) were lower in *EGFR* ex20ins compared to other NSCLC (TMB n=5851, PD-L1 expression n=282) and *EGFR* del 19/L858R (median TMB 3.5, p=0.001; 39% PD-L1≥1%, p=0.02). Compared to a 2:1 cohort of patients with metastatic NSCLC without targetable alterations (n=192), *EGFR* ex20ins patients had longer overall survival (median 20 vs. 12 mo, HR 0.56, p=0.007) and longer time to treatment discontinuation (TTD) for platinum chemotherapy (median 7 vs. 4 mo, HR 0.6, p=0.02) and no improvement in TTD for immune checkpoint inhibitors (ICI) (HR 1.75, p=0.05).

Conclusions: With better outcomes on platinum chemotherapy, patients with *EGFR* ex20ins NSCLC have improved prognosis, lower PD-L1 expression and TMB, and derive less benefit from ICI compared to NSCLC patients without targetable oncogenes. Improving molecularly targeted therapies could provide greater benefit for patients with *EGFR* ex20ins.

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Keywords

EGFR exon 20 insertions; non-small cell lung cancer; lung adenocarcinoma; platinum chemotherapy

INTRODUCTION:

The treatment of patients with metastatic non-small cell lung cancer (NSCLC) has evolved rapidly in recent years, as next-generation sequencing (NGS) has facilitated identification of new molecular targets and development of multiple generations of effective targeted therapies. The effectiveness of immune checkpoint inhibitors (ICI) as monotherapy or combination therapy has further added to the repertoire of approved treatment options for NSCLC(1–5). Prior work has shown that molecular subtypes of NSCLC further influence response to standard treatments(6–8). In particular, patients with some oncogene-driven lung cancers have improved responses to chemotherapy compared to patients without oncogene-driven cancers(9,10).

Epidermal growth factor receptor (*EGFR*) exon 20 insertions (ex20ins) are driver alterations that comprise approximately 4–10% of *EGFR*-mutant NSCLC (11–13) and 2% of all NSCLC (14–16). *EGFR* ex20ins preferentially maintain the regulatory C-helix element of EGFR in its active, outward conformation (17), while *EGFR* exon 19 deletions (del 19) and L858R alterations permit constitutive receptor activation by destabilizing the inactive form of EGFR and inducing greater affinity for ATP than wild-type EGFR (18,19). Due to these conformational and mechanistic differences between classical sensitizing *EGFR* mutations and exon 20 insertions, NSCLC with *EGFR* ex20ins are generally insensitive to currently approved EGFR tyrosine kinase inhibitors (TKIs) at standard doses(20–22), although limited responses to standard dosing osimertinib have also been reported(23,24). A notable exception is *EGFR* A763_Y764insFQEA, an alteration found in the C-helix predicted to activate EGFR in a manner closely resembling classic sensitizing alterations, which has demonstrated sensitivity to multiple EGFR TKIs in both *in vitro* models and a limited number of patients(25–27).

While there are no currently approved targeted therapies for *EGFR* ex20ins, it is an active area for drug development with multiple promising molecularly targeted strategies in clinical trial testing. Several investigational agents, including mobocertinib (28) and amivantamab (29), have shown encouraging activity against *EGFR* ex20ins in early clinical trials. Osimertinib 160 mg, twice the standard dose, has shown activity in patients with *EGFR* ex20ins from preliminary trial results(30). The role and effectiveness of standard therapy in *EGFR* exon20ins remains unclear. A better understanding of the effectiveness of standard therapies in *EGFR* ex20ins is needed to assess whether investigational agents offer substantial benefit.

We sought to describe the clinical outcomes and response to standard therapies, including ICI, platinum-based chemotherapy, and combination chemo-ICI. We identified all patients with NSCLC and *EGFR* ex20ins detected by next-generation sequencing (NGS) using

MSK-IMPACT (31) at our institution and retrospectively evaluated their clinical outcomes compared to a historical cohort of NSCLC without targetable alterations.

METHODS:

Patient identification:

We identified all patients with NSCLC whose tumors underwent genomic profiling with MSK-IMPACT(31) prior to July 2020 using the MSK Clinical Sequencing Cohort in cBioPortal(32,33). Patients with *EGFR* ex20ins were identified from this cohort, with *EGFR* exon 20 insertion status verified by a diagnostic molecular pathologist. A 2:1 control cohort was selected consecutively from the remaining cases after removing all cases with known driver alterations in *EGFR, ALK, RET,* and *BRAF V600E*. All patients with *EGFR* ex20ins and the 2:1 control cohort underwent medical record review to obtain treatment history, pathology, and basic demographic information. Basic patient (age sequencing was performed, sex, race) and tumor characteristics (histology, genomic results, and TMB) for all patients was collected from cBioPortal. As patient smoking histories were not collated for the entire cohort but were of interest to us, we used a subgroup of patients with NSCLC included in the MSK-IMPACT clinical sequencing cohort where smoking histories was available from a previously published study(6), after removing patients with *EGFR* exon20ins. The study was conducted in accordance with recognized ethical guidelines and was approved by the MSK Institutional Review Board/Privacy Board.

Somatic alterations were determined using MSK-IMPACT as has been previously described (31). Somatic alterations, including copy number alterations, were assessed for enrichment in *EGFR* ex20ins cases compared to unselected NSCLC cohort and a separate cohort of *EGFR* L858R/del 19 cases using Fisher's exact test. To reduce false discovery in multiple testing, a false discovery rate q value <0.05 was applied using Benjamini-Hochberg procedure. Tumor mutational burden (TMB) was calculated across each version of the MSK-IMPACT panel (341, 410 or 468 genes) and is defined as the total number of mutations divided by the coding region analyzed. TMB is reported as mutations/megabase (Mb). Only one sample was used per patient. If patients had multiple samples available, the sample with the highest tumor purity was selected for TMB analysis. PD-L1 expression was performed as part of routine clinical care and was scored as the percentage of tumor cells with membranous staining

Statistical Methods:

Patient and tumor characteristics were compared using Wilcoxon rank-sum test, chi-square test of independence, or Fisher's exact tests. Overall survival was defined from the date of first-line metastatic treatment to the date of death or last follow-up, with a data lock on July 15, 2020. Time to treatment discontinuation (TTD) was defined from the first date of treatment to the decision date of treatment termination or last follow-up; patients were censored if they remained on treatment by July 15,2020. Overall survival and TTD probabilities were computed using Kaplan-Meier estimates with left truncation to account for the time of MSK-IMPACT. For the delayed entry Kaplan-Meier analyses, patients may enter the risk set post-baseline if their IMPACT data were recorded after the start of

treatment. Patients were also excluded if full treatment details, such as date of first-line therapy or reason for therapy discontinuation, were unknown. The comparative analysis for the time to event endpoints with respect to *EGFR* exon 20 mutation status was computed using the log-rank test with left truncation.

RESULTS:

Patient and tumor characteristics:

From July 2014 to July 2020, 106 patients with *EGFR* ex20ins were identified out of 6,290 (2%) NSCLC patients with MSK-IMPACT results and 1,507 (7%) with any *EGFR* mutation. Of these, 59 (56%) were diagnosed in the metastatic setting and 47 (44%) at an earlier stage, with 17 disease recurrences during the study period (supplemental Fig 1). Compared to the remaining 6,184 patients with NSCLC without *EGFR* ex20ins, *EGFR* ex20ins patients were younger (median age 66 vs. 69, p<0.001), were more frequently women (69 vs 58%, p=0.03) and Black (14 vs 6%, p=0.001) or Asian (22 vs 10%, p<0.001) (Table 1). As has been described previously (11,13), the majority of *EGFR* ex20ins patients had adenocarcinoma histology (96 vs 76%, p<0.001) and were never or light former smokers (88 vs. 52%, p<0.001) compared to a subgroup of patients with smoking history available (n=985).

We identified 15 distinct exon 20 insertion alterations. The most commonly observed were S768_D770 duplication (n=22, 21%), A767_V769 duplication (n=20, 19%), and N771_H773 duplication (n=13, 12%) (Fig. 1).

Clinical outcomes and response to therapy:

We next evaluated survival from initiation of first-line metastatic treatment. With a median follow-up of 1.3 years (range 0.2 to 17 years), 62 patients with *EGFR* ex20ins and 192 patients without driver alterations were included. Median survival for patients with *EGFR* ex20ins was 20 months (95% confidence interval [CI] 17 months to not reached), compared to 12 months for patients without targetable alterations (95% CI 10 to 15 months), with HR 0.56 (95% CI 0.37 to 0.86, p=0.007, Fig. 2A).

Given well-established data that patients with *EGFR* ex20ins do not benefit from currently approved EGFR TKIs at standard doses, we focused on time to treatment discontinuation (TTD) for standard therapies for metastatic NSCLC: platinum-based doublet therapy +/− bevacizumab, ICI and chemo-ICI. In this analysis, 31 patients with *EGFR* ex20ins and 94 NSCLC patients without targetable alterations who received platinum chemotherapy in the metastatic setting (Supplemental Table 1) were included. The most common reason for treatment discontinuation was progressive disease (PD), which occurred in 65% of patients with EGFR ex20ins and 70% of those patients without targetable oncogenic drivers. Patients with *EGFR* ex20ins had longer TTD on platinum-based chemotherapy compared to patients without targetable alterations with median time of 7 vs. 4 months (HR 0.60, 95% CI 0.39 to 0.93, p=0.02) (Fig. 2B).

The next most common standard therapy received was ICI treatment with anti-PD-1 or anti-PD-L1 antibodies, with 15 *EGFR* ex20ins and 99 control patients included. Among *EGFR*

ex20ins, 6 (40%), 5 (33%), and 4 (27%) of patients received ICI as the first, second, or third or greater line of treatment, compared to 72% of control patients receiving ICI as second-line treatment. All *EGFR* ex20ins patients discontinued ICI for PD, compared to 77 (78%) control patients, with the remaining 10 control patients discontinuing ICI for toxicity. Duration of treatment with ICI was not different for patients with and without *EGFR* ex20ins (median TTD 2.8 vs. 2.8 mo, HR 1.75, 95% CI 1.0 to 3.1, p=0.05, Fig. 2C). We also assessed outcomes for the small subset of patients who received chemo-ICI, including12 patients with *EGFR* ex20ins and 36 control patients. The most common treatment given was carboplatin, pemetrexed, and pembrolizumab. The median time on chemo-ICI was similar for patients with *EGFR* ex20ins and control patients (median 7 vs. 5 months, HR 1.1, 95% CI 0.52 to 2.41, p=0.8, Fig. 2D).

Genomic and Immunophenotypic Characteristics:

To determine whether known prognostic and predictive factors were different in patients with *EGFR* ex20ins, we compared genomic and immunophenotypic characteristics of *EGFR* ex20ins tumors to all NSCLC without *EGFR* ex20ins and tumors with classical *EGFR* alterations (del 19 and L858R). Of the 6,290 unique NSCLC patients with MSK-IMPACT available at the time of analysis, 1,088 patients had *EGFR* del 19 or L858R. The tumor mutational burden (TMB) in tumors with *EGFR* ex20ins (median TMB 3.4, interquartile range [IQR] 1.8 to 4.6, n=106) was lower than that observed in *EGFR* del 19/L858R (median 3.5, IQR 2.6 to 5.6, p=0.001, n=1058) and NSCLC without *EGFR* ex20ins (median 5.9, IQR 3.0 to 10.0, p<0.001, n=5,851) (Fig. 3A). Among available tumor samples, a higher proportion of tumors with *EGFR* del 19/L858R had PD-L1% expression ≥1% compared to *EGFR* ex20ins tumors (39% vs. 22%, p=0.02, Fisher's exact). A higher proportion of tumors without *EGFR* ex20ins also had PD-L1 expression ≥1% compared to *EGFR* ex20ins tumors (60% vs. 22%, p<0.001, Fisher's exact) (Fig. 3B).

We next evaluated the frequency of co-occurring genomic alterations. Co-mutations that were observed in the *EGFR* ex20ins cohort at a frequency ≥5% were in *TP53* (48%), *CTNNB1* (6%), and *U2AF1* (6%). Copy number alterations (CNA) observed at ≥5% frequency were *EGFR* amplifications (17%), deletions in *CDKN2A* (17%) and *CDKN2B* (16%), and amplifications in *NKX2–1* (12%), *FOXA1* (8%), and *TERT* (8%) (Fig. 4A). We identified that alterations in *KRAS* (27%, any alteration), *STK11* (13%), *KEAP1* (13%), *NF1* (7%), *PTPRT* (7%), *RBM10* (10%), *KMT2D* (8%), *SETD2* (5%), *and PTPRD* (9%) occur more frequently in tumors without *EGFR* ex20ins, while mutations in *EGFR* (100 vs. 24%) and *CTNNB1* (9 vs. 3%) were more common in tumors with *EGFR* ex20ins (p<0.001, q<0.05 by Benjamini-Hochberg) (Fig. 4B). *EGFR* (15 vs. 6%) and *RBM10* (4 vs. 0.3%) amplifications were also enriched in tumors with *EGFR* ex20ins (p<0.001, q<0.03) (Fig 4C). We next compared the genomic landscape of *EGFR* ex20ins to classical *EGFR* del 19 and L858R cases, but there were no somatic alterations associated with either cohort that met our statistical thresholds. There were no CNAs enriched in *EGFR* ex20ins cases compared to *EGFR* del 19/L858R cases.

DISCUSSION:

In this analysis, we have described the clinical outcomes of patients with *EGFR* ex20ins NSCLC, an uncommon driver alteration in NSCLC, as well as the molecular features of these tumors. We found that *EGFR* ex20ins occurred in 2% of all patients with NSCLC and 7% of patients with *EGFR*-mutant lung cancers. Overall, we found that *EGFR* ex20ins were more prevalent in Black patients, Asian patients and never smokers and that patients with *EGFR* ex20ins have a somewhat greater benefit with platinum-based chemotherapy than NSCLC patients without a targetable alteration.

Prior reports on clinical outcomes of *EGFR* ex20ins patients have largely focused on the lack of response to EGFR TKIs and have provided limited data on response to cytotoxic and immune-based NSCLC therapies. Our analysis may serve as a benchmark to assess the efficacy of multiple investigational agents targeting *EGFR* ex20ins in single-arm clinical trials. In other molecularly defined NSCLC populations, targeted therapies for EGFR and ALK alterations have shown clear survival and response benefits over chemotherapy, as would be expected of oncogene-addicted cancers dependent on driver alterations (34–41). In our North American cohort, we found that patients with *EGFR* ex20ins have encouraging responses to platinum chemotherapy, with median TTD of 7 months that was superior to responses observed in an NSCLC cohort without driver alterations. These results are in concordance with these previously published studies. A study of Chinese patients with *EGFR* ex20ins reported a progression-free survival (PFS) of 6 months on first-line platinum-based chemotherapy(42). A study of 22 Korean patients reported a 50% objective response rate (ORR)with platinum chemotherapy(43). One possible explanation for this finding is that oncogene-addicted NSCLC is more sensitive to pemetrexed, with which all patients with *EGFR* ex20ins were treated, than other NSCLC. This has been demonstrated with other oncogene-driven NSCLC, including *ALK*(9,44), *ROS1*(45) and *RET*(10). Given the low number of patients in our cohorts treated with chemo-ICI, future studies are required to evaluate whether patients with *EGFR* ex20ins derive greater clinical benefit from chemo-ICI compared to platinum chemotherapy. This remains an important question to answer as it will enable clinicians to appropriately sequence therapies, but of note, combination platinum chemotherapy and pembrolizumab is not FDA approved for patients with *EGFR* mutations(46)

The suboptimal response to ICI observed among patients with *EGFR* ex20ins aligns with previous observations that ICI have poor activity in patients with NSCLC with driver alterations. A series of *EGFR*-mutant lung cancers previously reported poor responses to ICI(47). In the IMMUNOTARGET registry, patients with the common *EGFR* exon 19 deletion or L858R mutations, or fusions in *RET, ROS1* or *ALK* had ORRs to ICI <20% and PFS less than 3.5 months (48). This may be explained partially by low tumor PD-L1 expression and low TMB, which have been consistently reported in NSCLC tumors with driver alterations(49). However recent work suggests that PD-L1 expression does not predict responsiveness to immune checkpoint blockade in patients with *EGFR* exon 19 deletion or L858R cancers (50)and may be of limited utility in predicting responsiveness to ICI in *EGFR*-mutant lung cancer. A further consideration for potentially avoiding ICI in patients with *EGFR* ex20ins is the risk for severe immune-related adverse events if osimertinib is

given following ICI, as has been demonstrated in patients with sensitizing *EGFR* alterations(51). Overall, our results suggest that ICI may not be a fruitful later-line therapy for patients with *EGFR* ex20ins.

Given the rarity of *EGFR* ex20ins, the overall number of patients receiving each line of therapy is a limitation of this single-institution retrospective study. In this retrospective analysis, we used TTD rather than RECIST-based response rate or PFS to assess clinical efficacy, although TTD may approximate PFS(52). Our study population was also heterogeneous and received each category of treatment at varying time points of metastatic disease, which may confound the responses reported. Finally, a source of potential bias is that all patients included in our study underwent genomic profiling with MSK-IMPACT, which resulted in fewer patients with squamous cell carcinoma included in the comparator cohort (estimated real-world prevalence 30%, compared to 11% prevalence in our cohort). Despite these limitations, this study remains among the largest cohorts of patients with *EGFR* exon 20 insertions reported. The distribution of unique *EGFR* exon 20 insertions in our cohort is similar to previous reports, with the majority of insertions occurring in the far loop region following the C-helix (26,53,54). However, our cohort included only one patient with A763_Y764insFQEA—an alteration sensitizing to EGFR TKIs—while other studies have cited frequencies of 5–10%.

In summary, we describe here comprehensive genomic, immunophenotypic, and clinical outcomes of patients with *EGFR* ex20ins. We anticipate that patients with *EGFR* ex20ins will be increasingly recognized and understanding the response to standard therapies will help clinicians determine what treatments to offer to patients unable to enroll in clinical trials or who have exhausted trial options. Our analysis demonstrates that with low TMB and low PD-L1, *EGFR* ex20ins tumors are similar to *EGFR* del 19/L858R in genomic landscape and have relatively few genomic or immunophenotypic vulnerabilities to exploit with standard therapy options after progression on platinum-based chemotherapy. Given the promising activity of several investigational targeted therapies for *EGFR* ex20ins, these remain the preferred option for patients with *EGFR* ex20ins over later line ICI or non-platinum chemotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRANSLATIONAL RELEVANCE:

An uncommon NSCLC genotype, *EGFR* exon 20 insertions are the subject of active drug development, although no targeted therapies have yet been approved. The response to standard therapies for these cancers has not been well characterized and is needed to serve as a benchmark to assess the efficacy of investigational agents in single-arm trials. We sought to describe the response to standard treatments for these patients and provide a comprehensive analysis of the molecular features of *EGFR* exon 20 insertion NSCLC. While responses to platinum chemotherapy are encouraging compared to NSCLC without targetable alterations, responses to immune checkpoint inhibitors are shorter. We report that *EGFR* exon 20 insertion tumors have low PD-L1 tumor expression, low TMB and infrequent co-alterations. Our results highlight the need for targeted therapies in this patient population.

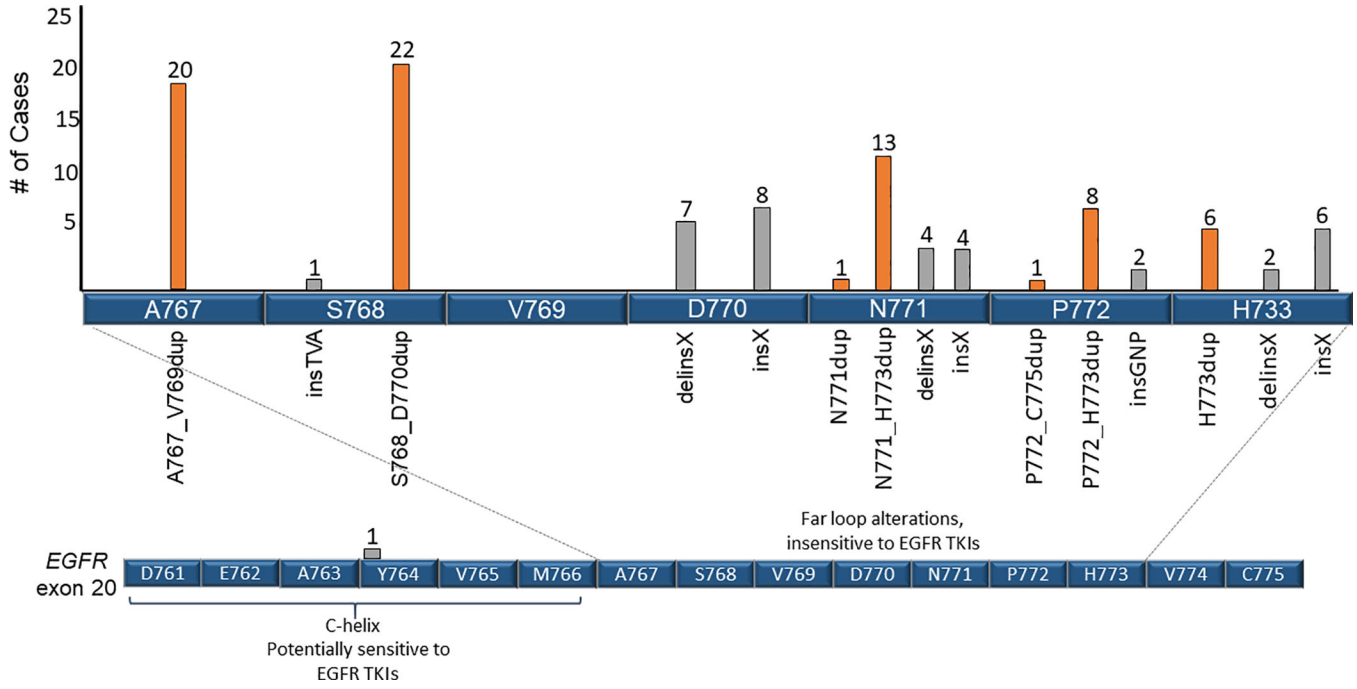

Figure 1: Exon 20 insertions.

15 distinct *EGFR* ex20ins were identified. The locations and number of each insertion are identified, along with amino acids 761D to 766M, which comprise the regulatory C-helix. Only one insertion (A763_Y764insFQEA) was found in the C-helix; this alteration is predicted to be sensitizing to EGFR TKIs. Amino acids 767A to 775C compose the loop following the C-helix (far loop alterations) where the majority of *EGFR* ex20ins events occur. Duplication events are labeled in orange and other insertion events are in gray.

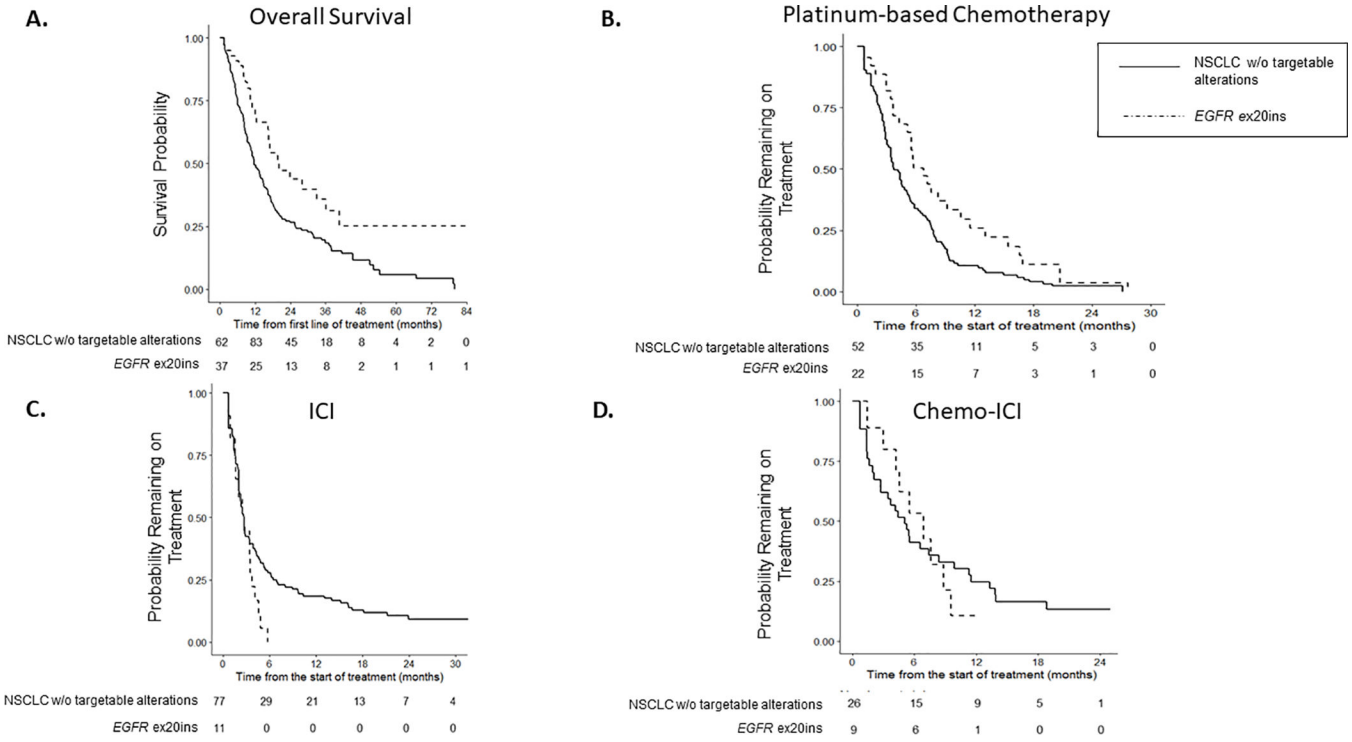

Figure 2: Clinical Outcomes.

A. Overall survival for *EGFR* ex20ins cohort was compared to patients with NSCLC without targetable driver alterations **B**. Time to treatment discontinuation (TTD) on platinum chemotherapy **C.** TTD for immune checkpoint inhibitor (ICI) **D.** TTD for chemo-ICI. To account for left truncation, any cases where MSK-IMPACT resulted after end of treatment, date of death, or last clinic follow-up were excluded. For the delayed entry Kaplan-Meier analyses, patients may enter the risk set post-baseline if their IMPACT data were recorded after the start of treatment.

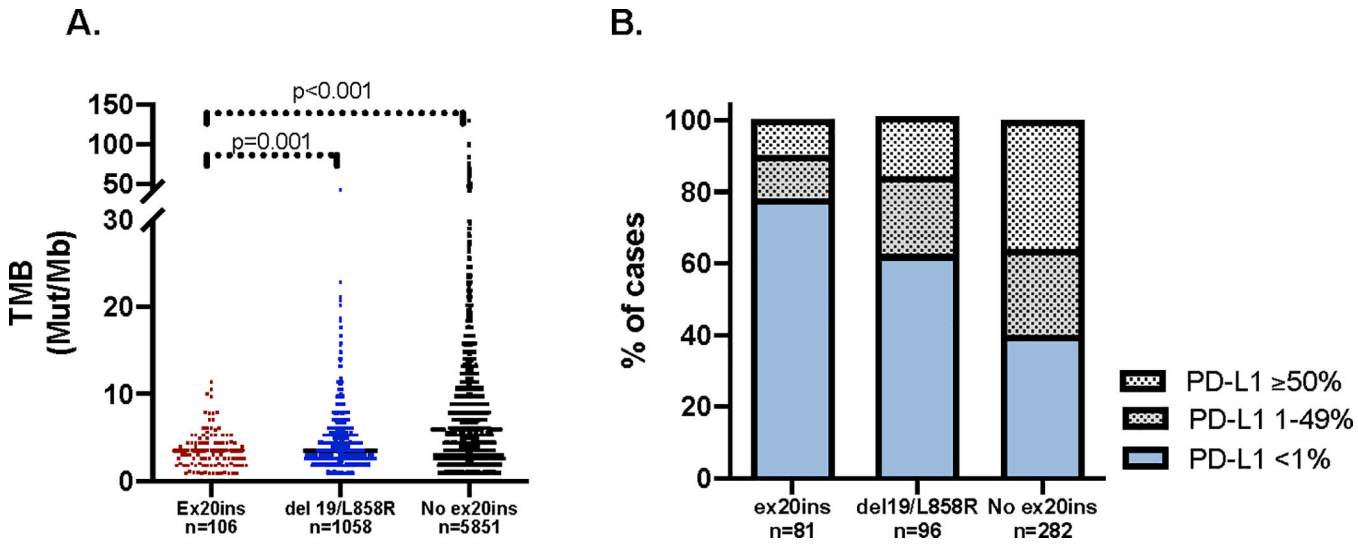

Figure 3: Immunophenotype of exon 20 insertion cases.

A. Median tumor mutational burden (TMB) from available cases is significantly lower in *EGFR* exon 20 insertion (ex20ins) cases compared to both *EGFR* del 19/L858R tumors and NSCLC tumors without *EGFR* ex20ins **B.** Tumor PD-L1 expression was quantified as low (<1%), intermediate (1–49%) and high (≥50%) for available cases and tabulated across ex20ins, *EGFR* del19/L858R cases, and NSCLC without *EGFR* ex20ins tumors.

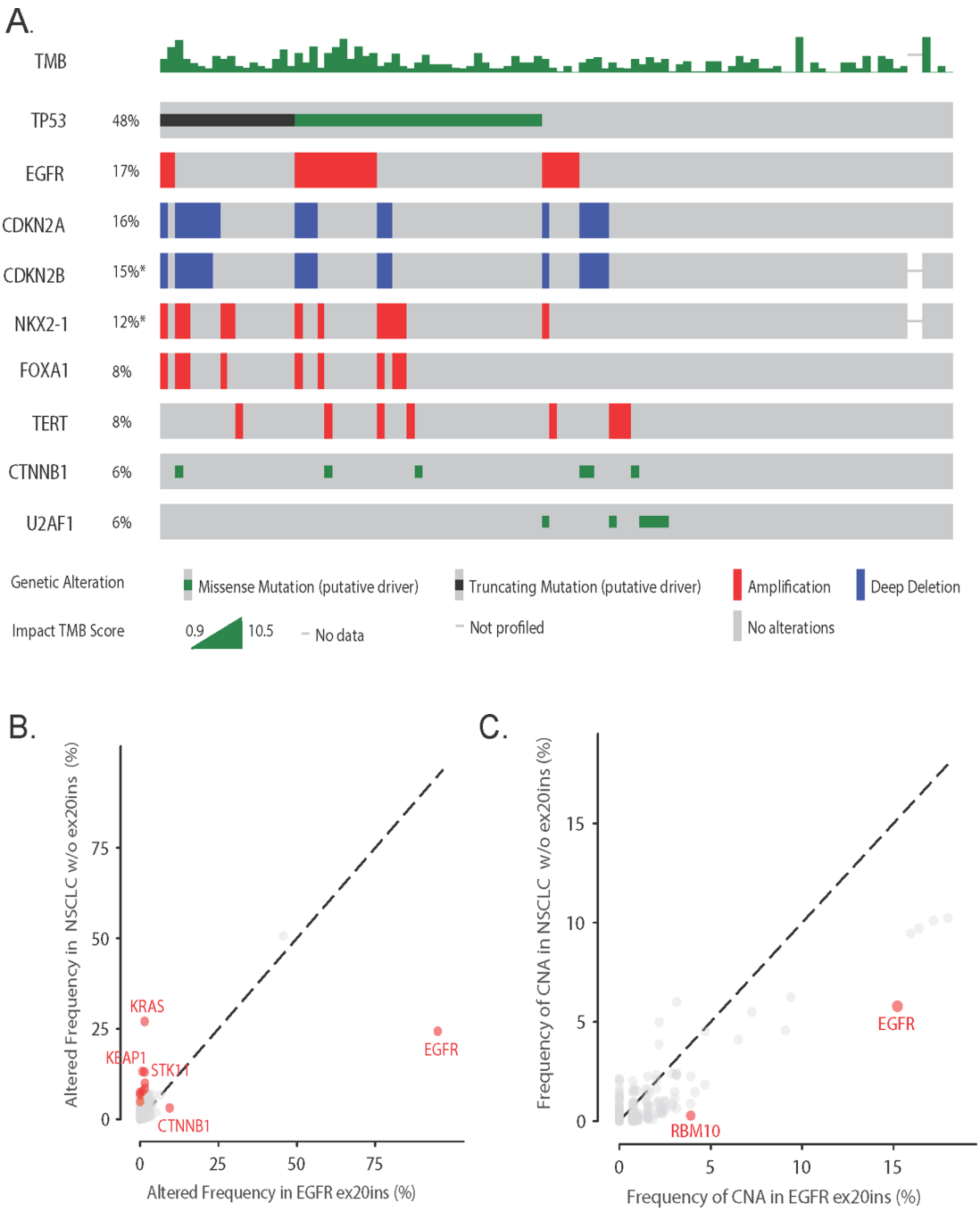

Figure 4:

Genomic landscape of *EGFR* ex20ins. **A.** Oncoprint of the mutations and copy number alterations found at ≥5% frequency among ex20ins cases, with TMB quantified at top of graph **B**. Frequency of altered genes in *EGFR* ex20ins cohort compared to NSCLC without *EGFR* ex20ins. Genes highlighted in red designate q-value <0.05. **C**. Frequency of CNA in the *EGFR* ex20ins cohort compared to other NSCLC cases.

Table 1:

Patient characteristics:

Basic demographic information was compared between the 106 patients with *EGFR* ex20ins and all other patients with non-small cell lung cancer (NSCLC) who underwent genomic profiling with MSK-IMPACT. Py indicates pack-years of smoking history. “Other” histologies include carcinoid, sarcomatoid, adenosquamous, lymphoepithelial, and basaloid, among other rare histologies.

	***EGFR* ex20ins n=106 n (%)**	**NSCLC without *EGFR* ex20ins n=6,184 n (%)**	
Age, median (range)	66 (30,−90)	69 (13, 90)	p<0.001
Sex			p=0.03
Male	33 (31)	2586 (42)	
Female	73 (69)	3585 (58)	
Not stated	0 (0)	13 (<1)	
Race			
White	61 (62)	4875 (84)	p<0.001
Asian	22 (22)	583 (10)	p<0.001
Black	14 (14)	318 (6)	p=0.001
Native American	0 (0)	9 (<1)	
Hawaiian/Pacific Islander	1 (1)	5 (<1)	
Not known	8 (8)	394 (6)	
Histology			p<0.001
Adenocarcinoma	102 (96)	4735 (76)	
Squamous	0 (0)	655 (11)	
Large cell/neuroendocrine	0	142 (2)	
Poorly differentiated	4 (4)	387 (6)	
Other	0 (0)	277 (4)	
Smoking history		n=985	p<0.001
Never smoker	63 (59)	314 (32)	
≤15 py	31 (29)	192 (20)	
>15 py	12 (11)	468 (48)	
Not known	0	11 (1)	