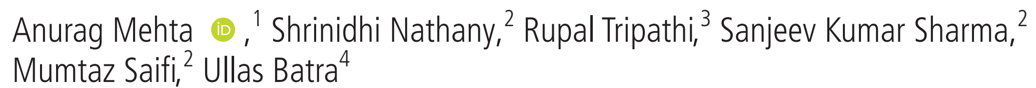
Non-amplification genetic alterations of *HER2* gene in non-small cell lung carcinoma



Anurag Mehta ,[1] Shrinidhi Nathany,[2] Rupal Tripathi,[3] Sanjeev Kumar Sharma,[2] Mumtaz Saifi,[2] Ullas Batra[4]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2020-206730).

[1]Deapartment of Laboratory Medicine, Transfusion Services and Molecular Diagnostics, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India
[2]Section of Molecular Diagnostics, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India
[3]Department of Research, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India
[4]Department of Medical Oncology, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India

Correspondence to
Dr Anurag Mehta, Rajiv Gandhi Cancer Institute and Research Centre, Delhi 110085, India; anumehta11@gmail.com

Received 7 May 2020
Revised 15 May 2020
Accepted 19 May 2020
Published Online First 11 June 2020

© Author(s) (or their employer(s)) 2021. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Mehta A, Nathany S, Tripathi R, *et al*. *J Clin Pathol* 2021;**74**:106–110.

ABSTRACT

Aims The present study investigated the incidence and spectrum of human epidermal growth factor receptor 2 (HER2) mutations, associated clinicopathological characteristics and the co-occurrence of *HER2* gene amplification in the *HER2* gene mutated cases in non-small cell lung cancer (NSCLC).

Methods All patients with advanced lung adenocarcinoma (LUAD) who underwent broad genomic profiling by next generation sequencing (NGS) from 2015 to 2019 were included in the study. *HER2* gene amplification was checked in all the *HER2* gene mutated cases. Tumour tissues of all the mutated cases were examined by fluorescent in situ hybridisation (FISH).

Results Fifty-four (37.2%) out of the 145 cases harboured tier 1 driver mutations comprising *EGFR* in 22.1%, ALK rearrangements in 7.6% cases, *ROS1* rearrangements and $BRAF^{V600E}$ in 3.5% cases each, and *NTRK* fusion in 0.7% cases. Nine (6.2%) cases exhibited a significant genetic alteration in *HER2* gene (tiers 2 and 3) on NGS. The most common alteration was exon 20 insertion of amino acid sequence AYVM in five cases (p.E770_A771insAYVM) followed by insertion of YVMA (p.A771_Y772insYVMA) in one case, insGSP (p.V777_G778insGSP) in one case and two missense mutations: p.G776C and p.QA795C (novel variant). The median copy number of the HER2 gene was 3.21 while on FISH, the median HER2/CEP17 ratio was 2.0.

Conclusions There is a relatively higher occurrence of *HER2* exon 20 mutations as primary oncogenic driver in NSCLC especially LUAD. Our cohort has demonstrated (p.E770_A771insAYVM) as the strikingly dominant insertion mutation against the most often globally reported (p.A771_Y772insYVMA).

INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is a transmembrane protein with 'receptor tyrosine kinase' function, that on activation initiates transcription of genes driving cell proliferation, migration and differentiation.[1] Gain-of-function genetic alterations in *HER2* gene release the regulated pathway to a constitutively activated oncogenic driver. Amplification of *HER2* gene and overexpression of *HER2* protein are well known in breast,[2] gastric,[3] bladder[4] and gall bladder[4] malignancies and act as a predictive marker for *HER2*-directed targeted therapy. *HER2* gene amplification has also been observed in lung adenocarcinoma (LUAD).[5 6] However, these have so far not been found actionable.[5] Contrarily, *HER2* missense and in-frame insertions in LUAD are being recognised as actionable.[6]

HER2 exon 20 mutations have been reported at a sizeable frequency of 4% of LUAD in The Cancer Genome Atlas (TCGA) database[5–7] and 3% in the lung cancer mutation consortium (LCMC) project.[6] In consideration of significant frequency and actionability, the most recent National Comprehensive Cancer Network guidelines[8] promote identification of *HER2* mutations and treating these cases with Ado-Trastuzumab. OncoKB,[9] the precision medicine database from Memorial Sloan Kettering Cancer Center, list *HER2* gene exon 20 insertion mutations as level 2 biomarker and the missense mutations as level 3 biomarker supported by published literature for biomarker-based therapy with adotrastuzumab and neratinib.[7 10 11] *HER2* mutations in non-small cell lung cancer (NSCLC) are specially observed in Asians, women, never smokers and in tumours with adenocarcinoma histology.[6 12] These mutations are located in the tyrosine kinase domain of *HER2* receptor.[5 6 10 12] A canonical 12 bp duplication/insertion at codon 771 in exon 20 that results in insertion of the YVMA amino acid sequence is the most recurrent of these mutations[5]; however, several other highly and potentially clinically significant mutations have also been detected in patients of NSCLC.[9 13]

In view of growing significance of *HER2* mutations as predictive biomarker, this study was conducted to identify the incidence and spectrum of *HER2* mutations, investigate the clinicopathological characteristics and identify the co-occurrence of *HER2* gene amplification in the *HER2* gene mutated cases in NSCLC.

MATERIALS AND METHODS

All patients with advanced LUAD and a few chosen by physician with other morphologies with strong likelihood of finding an oncogenic driver were included in the study conducted from 2015 to 2019. All patients underwent broad genomic profiling by next generation sequencing (NGS). Clinical data were collected and collated from the electronic medical records of the patients. Information related to the demographic profile and tumour details was recorded. Histological features were evaluated independently by two pathologists and reported in line with the WHO, 2015 criteria.[14]

A total of 145 patients were included in this study. Various mutations detected on NGS in these patients were classified into four tiers based on the

J Clin Pathol: first published as 10.1136/jclinpath-2020-206730 on 11 June 2020. Downloaded from http://jcp.bmj.com/ on March 11, 2021 at Seoul National University Medical Library and Hospital. Protected by copyright.

evidence of actionability as per American College of Medical Genetics and Genomics (ACMG).[15]

Next generation sequencing

The percentage of tumour cells relative to other cells (stromal, inflammatory and normal appearing epithelial cells) was estimated on one H&E stained tumour section by a pathologist, and an area with a maximum tumour cell content but not <20% was encircled for macrodissection to carry out nucleic acid extraction for NGS assay. Nucleic acids (DNA and RNA) were extracted each from 40 µM (4×10 µM) macrodissected Formalin Fixed Paraffin Embedded (FFPE) tissue section using the Recover All Total Nucleic Acid Isolation kit as recommended by the manufacturer (Thermo Fisher Scientific, San Francisco, USA). DNA and RNA concentrations were measured on Qubit fluorometer (Thermo Fisher Scientific, USA) employing dsDNA HS and the Qubit RNA HS Assay kits, respectively. RNA was reverse transcribed to cDNA using the SuperScript VILO (Thermo Fisher Scientific) cDNA synthesis kit (all from Thermo Fisher Scientific). Libraries were prepared using the Oncomine Focus assay and were equalised to 100 pm concentration using the Ion PGM Select Library Equalizer kit. Template preparation and enrichment were performed using the Ion OneTouch Select Template Kit (all from Thermo Fisher Scientific) on Ion OneTouch 2. The library was quality checked using Tape Station with High sensitivity DNA kits, to ensure adequate size and cleanup. The library was sequenced using the Ion PGM sequencer (Thermo Fisher Scientific) or the Ion S5 sequencer using a 520/530 chip.

Data analysis

Quality metrics were checked on Torrent suite (V.5.0) for mapped reads, uniformity of reads, reads with 100×, 500× and 1000× coverage, strand bias, number of amplicons read end-to-end, mapping quality, base quality and base coverage. The run was considered successful when the following quality metrics were met[16]: (1) mapped reads ≥300 000; (2) average base coverage depth ≥1000; (3) amplicons having at least 500 reads: ≥90%; (4) no strand bias:≥90%; (5) amplicons read end-to-end: ≥85%. Five thousand mapped reads for expression control genes were set as the limit for the RNA library. For the copy number alterations, an median absolute pairwise difference (MAPD) score of <0.5 was considered optimal. The NGS readouts and variants were called using Ion Reporter Software V.5.10. The final report, with possible treatment and trials available, was generated using the Oncomine Knowledge Reporter. The variants called were visualised on Integrative Genomics Viewer to ascertain validity of the variant call. Additionally, the functional effects were checked primarily on OncoKB.[9] Catalogue of Somatic Mutations in Cancer (COSMIC) database,[13] Varsome search engine,[17] Clinvar[18] and existing publications in PubMed were studied for further confirmation. The variants were classified as per the 'Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer (ACMG)[15]' into tiers 1, 2, 3 and 4 based on their clinical significance. The canonical transcript NM_004448.3 on assembly hg19 version of the human genome was used to call the *HER2* variants. The list of genes interrogated and the genetic alterations studied are cited in online supplementary table 1.

HER2 amplification

HER2 gene amplification was checked in all the *HER2* gene mutated cases by scrutinising the NGS data for *HER2* gene copy number alteration after due consideration of MAPD of <0.5[16]. The integrated genome viewer focused on *HER2* gene was examined for all the amplicons residing in *HER2* gene to assess the ploidy. In addition, FFPE sections from tumour of all the mutated cases were examined by fluorescent in situ hybridisation (FISH). The pretreatment was done using 0.2M hydrochloric acid followed by 1 M sodium thiocyanate and protease solution. The target and probes were denatured using heat treatment at 80°C for 10 min. Subsequently, hybridisation was done at 37°C for 16 hours using Spec *ERBB2*/CEN17 Dual colour probe (ZytoVision Germany; *ERBB2* labelled green and CEN17 labelled orange). Post hybridisation, first wash was done at 72°C using 0.3% NP40/2× Saline Sodium Citrate Buffer (SSC), and a second using 0.1% NP40/2× SSC at ambient temperature. Counterstaining was done using 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) II (Abbott, Chicago, Illinois, USA). The signals were analysed using DM6000B Leica Fluorescent microscope (Wetzlar, Germany), employing four filters; DAPI for nuclear localisation, Fluorescein isothiocyanate -FITC (green), Tetramethylrhodamine-5-Isothiocyanate (TRITC) (orange) and Dual. Images were captured using Cytovision software (Leica Biosystems, Wetzlar, Germany). The results were interpreted in accordance with American Society of Clinical Oncology - College of American Pathologists (ASCO-CAP) guidelines 2018 for invasive breast carcinoma.[2]

Statistical analysis

SPSS V.23 for Windows (SPSS) was used for statistical analysis. The categorical variables were presented in frequencies along with respective percentages. A two-sided p-value <0.05 was considered as significant.

RESULTS

Patient characteristics

Of the 145 patients of NSCLC who underwent NGS and included in this study, adenocarcinoma, squamous cell carcinoma and not otherwise specified (but likely carcinosarcoma) morphologies were reported in 133, 8 and 4 patients, respectively. The patient characteristics are shown in table 1. The median age of the patients was 60 years with a male-to-female ratio of 1.2:1.

NGS findings

Fifty-four (37.2%) out of the 145 cases harboured tier 1 driver mutations comprising *EGFR* in 22.1% (32/145), ALK rearrangements in 7.6% (11/145) cases, *ROS1* rearrangements and

Table 1 Clinical features of 145 patients with non-small cell lung cancer

Characteristics	N (%)
Median age in years (range)	60 (18–86)
Gender	
Male	78 (53.8)
Female	67 (46.2)
Smoking history	
Never smoker	58 (40)
Ever smoker	34 (23.4)
Not available	53 (36.6)
Histology	
Well differentiated	29 (20)
Moderately differentiated	86 (59.3)
Poorly differentiated	26 (17.9)
Carcinosarcoma	4 (2.8)

J Clin Pathol: first published as 10.1136/jclinpath-2020-206730 on 11 June 2020. Downloaded from http://jcp.bmj.com/ on March 11, 2021 at Seoul National University Medical Library and Hospital. Protected by copyright.

Table 2 Mutations detected on next generation sequencing in 145 patients with non-small cell lung cancer

Genetic alterations	N (%)
Tier 1	54 (37.2)
EGFR sensitising mutations	32 (22.1)
ALK fusion rearrangement	11 (7.6)
ROS1 fusion rearrangement	5 (3.5)
NTRK fusion rearrangement	1 (0.7)
BRAF(V600E) mutation	5 (3.5)
Tier 2	15 (10.3)
HER2 exon 20 insertions	7 (4.8)
MET exon 14 skipping	3 (2.1)
MET amplification	2 (1.4)
RET fusion rearrangement	3 (2.1)
Tier 3	28 (19.3)
KRAS mutations	25 (17.2)
MAP2K1 mutations	1 (0.7)
HER2 oncogenic mutations	2 (1.4)
Tier 4*	33 (22.8)
ATM mutations	2 (1.4)
BRAF mutations (others—not V600E)	2 (1.4)
Others	29 (20)
No mutation detected	15 (10.3)

*Nine cases which had level 4 mutations also had levels 1 and 2 mutations.
EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2.

BRAF^{V600E} in 3.5% cases each (5/145), and *NTRK* fusion in 1 case (0.7%). Ninety-one cases (62.7%) lacked tier 1 mutations.

Tier 2 alterations were seen in 15 (10.3%) cases including *RET* fusions in 3 cases, *MET* exon 14 skipping mutation in 3 cases, *MET* amplification in 2 cases and *HER2* exon 20 insertion in 7 cases. Besides, two cases had oncogenic missense mutations in exon 20 of *HER2* gene categorised as tier 3 *HER2* alterations. Overall, 9 out of the 145 (6.2%) cases exhibited a significant genetic alteration in *HER2* gene on NGS. All *HER2* mutations were mutually exclusive with other known oncogenic drivers of LUAD. Table 2 exhibits the mutational spectrum detected on NGS.

Spectrum of HER2 mutations

HER2 mutations were detected in 6.2% cases (9/145). Among the tier 2 mutations, *HER2* mutations accounted for an overwhelming 46.6% of the cases (7/15 cases). The clinicopathological features and genomic alterations in the *HER2* mutant cases have been profiled in table 3. The most common alteration was exon 20 insertion of amino acid sequence AYVM in five cases (p.E770_A771insAYVM) followed by insertion of YVMA (p.A771_Y772insYVMA) in one case, insGSP (p.V777_G778insGSP) in one case and two missense mutations: p.G776C and p.QA795C. Eight of the nine variants encountered were classified as variants of strong clinical significance, and one (p.Q795C) was reported as variant of unknown clinical significance. This variant is not reported in the COSMIC, ClinVar, dbSNP or PubMed databases, and hence is considered a novel variant.

The tumour fraction in the HER2 mutated group ranged from 50% to 90%, with the *HER2* variant allele fraction ranging between 22.9 and 67.4 and being arithmetically proportional to monoallelic somatic change of clonal/truncal nature. The p.E770_A771insAYVM mutant cases had a median age of 63 years and showed male preponderance. All these patients had distant organ metastases and nodular pleural involvement.

HER2 amplification status

The *HER2* amplification status using NGS was available for 5/9 (55.5%) cases. The median copy number of the *HER2* gene was 3.21. The copy number variation heat map of a representative

Table 3 Spectrum of *HER2* mutations

Case #	*HER2* mutation	TF/VAF	Variant class ACMG[17]	COSMIC ID[13]	Age/sex	Smoking status	Histology	Other concurrent mutations	*HER2* ploidy by NGS	*HER2*/CEP17 ratio by FISH
1	p.(V777_G778insGSP)	60/32.4	Tier 2	COSM303948	62/F	Never	Acinar	–	2.71	3.3/1.6=2.06
2	p.(E770_ A771insAYVM)	90/50.1	Tier 2	COSM20959	80/F	Never	Solid	–	3.73	4.6/1.7= 2.70
3	p.(E770_ A771insAYVM)	50/23.9	Tier 2	COSM20959	79/F	Never	Acinar	*STK11*	Not available	3.3/1.7=1.94
4	p.(Q795C)	60/24.3	Tier 3	NOVEL* Not in COSMIC Not in clinvar/ dbSNP	62/M	Never	CS	*KIT, ATM, GNAQ, SMAD4*	Not available	2.4/1.8=1.33
5	p.(A771_ Y772insYVMA)	90/67.4	Tier 2	COSM133608	54/M	Never	Acinar	–	Not available	3.1/1.9=1.63
6	p.(E770_ A771insAYVM)	70/33.2	Tier 2	COSM20959	64/M	Never	Acinar	*NOTCH1*	Not available	Not available
7	p.(G776C)	80/34.2	Tier 3	COSM303938	78/M	Never	Acinar	–	3.21	Not available
8	p.(E770_ A771insAYVM)	50/22.9	Tier 2	COSM20959	33/M	Never	Acinar	–	1.36	3.6/1.6= 2.25
9	p.(E770_ A771insAYVM)	60/25.3	Tier 2	COSM20959	57/M	Never	Acinar	–	4.14	Not available

The variants in HER2 have been reported using the NM_004448.3 isoform of the HER2 gene, with hg19 assembly.
*The mutation p.Q795C has two pathogenic moderate and one pathogenic supporting assertion and one benign supporting assertion as per American College of Medical Genetics and Genomics (ACMG) rules and is hence labelled as variant of uncertain significance.
A, alanine; C, cysteine; COSMIC, Catalogue of Somatic Mutations; CS, carcinosarcoma; E, glutamate; FISH, fluorescent in situ hybridisation; G, glycine; HER2, human epidermal growth factor receptor 2; M, methionine; NGS, next generation sequencing; P, proline; S, serine; TF, tumour fraction; V, valine; VAF, variant allele fraction; Y, tyrosine.

J Clin Pathol: first published as 10.1136/jclinpath-2020-206730 on 11 June 2020. Downloaded from http://jcp.bmj.com/ on March 11, 2021 at Seoul National University Medical Library and Hospital. Protected by copyright.

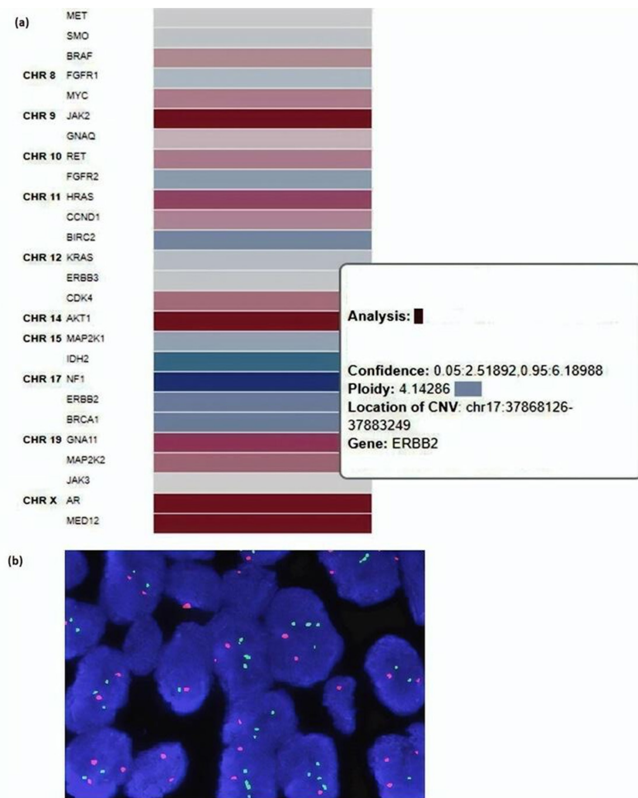


Figure 1 (A) Copy number variations heat map showing the ploidy for *ERBB2* (*HER2*) with an average ploidy of 4.14 in a representative case. (B) Fluorescent in situ hybridisation image showing HER2 signals in green and centromere 17 signals in red in a non-amplified case.

case is depicted in figure 1. Additionally, orthogonal testing for *HER2* amplification was performed employing FISH in 6/9 (66.6%) cases. The other three cases could not be tested due to exhaustion of tissue in the FFPE blocks. On FISH, the median HER2/CEP17 ratio was 2.0 (1.3–2.7). A comparison of the NGS copy number, the *HER2* mutant and FISH *HER2*/CEP17 ratio is also shown in table 3. *HER2* ploidy status by NGS and *HER2/CEP17* ratio by FISH was available in five and six cases, respectively. Interestingly, all these nine cases were non-smokers and seven out of nine cases had acinar histology.

DISCUSSION

Lung cancer is one of the most frequently occurring cancer globally and advanced NSCLC carries a bad prognosis. While there is enough information on incidence and treatment benefits for tier 1 biomarkers in LUAD, the information is still being gathered for tier 2 biomarkers. The current study identifies the incidence and nature of *HER2* mutations and looks at the coexistence of *HER2* gene amplification in the *HER2* gene mutated cases of NSCLC especially LUAD.

In the present study, tier 1 alterations involving *EGFR*, *ALK*, *ROS1*, *NTRK* and *BRAF*$^{p.V600E}$ were identified at a frequency of 37.24% which is similar to the incidence reported from the Indian subcontinent.[19] The tier 2 mutations comprising *MET* amplification, *MET* exon 14 skipping mutation, *RET* fusion rearrangement and *HER2* exon 20 insertion mutation were observed in 10.3% cases of the tested cohort. Of the tier 2 mutations, the *HER2* exon 20 insertion mutations were the most dominant. *HER2* exon 20 insertions categorised as tier 2 biomarker is known to be mutually exclusive with other tier 1 driver alterations[5 6 10 12] and our study reiterates the same. We have observed that *HER2* exon 20 insertion mutations are truncal, and a primary oncogenic driver in NSCLC as reported previously.[5 6 20]

HER2 alterations have been reported in 4% cases of NSCLC in the TCGA database[5] and in 3% cases of LCMC cohort.[6] The tier 2 *HER2* mutations in our cohort were 4.8%. The literature on frequency of *HER2* exon 20 mutation in Indian subcontinent is non-existent but our report suggests that unlike *EGFR* and *KRAS*, the incidence of *HER2* exon 20 insertion mutations is similar with the global data. Many studies conducted prior to the use of NGS, however, have shown lesser frequencies varying from 1% to 2.6%[12 21–24] (table 4). Geographical differences and diverse platforms with lower analytical sensitivity used in these studies like Sanger sequencing, single strand conformational polymorphism, fragment length analysis and mass spectrometry (table 4) possibly account for this difference. On the basis of the observed high frequency of *HER2* insertions mutations in the current study, even more than combined frequency of ROS*1*, NTRK and *BRAF*$^{p.V600E}$ tier 1 mutations, the authors propose that all patients should be tested for this genetic alteration with the ultimate goal of receiving targeted therapy.

The variants reported in databases and literature largely bunch in the tyrosine kinase domain of the *HER2* gene spanning exons 18–21. All the seven insertion variants and two level 3 missense mutations identified in our cohort resided in exon 20. The most common among these accounting for five of the nine *HER2* gene alterations is a duplication/insertion of 12 bp leading to insertion of four additional amino acids AYVM (p.E770_A771insAYVM). Previous studies have reported insertion of YVMA (p.A771_Y772insYVMA)[5–7 25] as the most common alteration; however, in the present study, it was reported in a single case only. This is ascribed to either the racial difference or a limited sample size of the present study. Of the two missense mutations in exon 20 of *HER2* gene, the p.Q795C in exon 20 was seen in a case of NSCLC—not otherwise specified that had carcinosarcomatous

Table 4 Comparison with other contemporary large studies

Study	Number of subjects	Method of detection	*HER2* exon 20 insertion mutation rate	Most common variant	Concurrent mutation
Shigematsu *et al*[21]	671	Sangers sequencing	1.6%	YVMA	None
Arcila *et al*[12]	560	Sizing assay and mass spectrometry	2% enriched population	YVMA	None
Barlesi *et al*[23]	11 723	Assorted platforms in 28 hospitals	1%	YVMA	One with *BRAF* and one with ALK
Buttitta *et al*[24]	403	Single-strand conformation polymorphism	2.2%	YVMA	None
Tomizawa *et al*[22]	504	Sangers sequencing	2.6%	YVMA	Not Reported
Present study, 2020	145	Next generation sequencing	4.8%	AYVM	None

A, alanine; HER2, human epidermal growth factor receptor 2; M, methionine; V, valine; Y, tyrosine.

J Clin Pathol: first published as 10.1136/jclinpath-2020-206730 on 11 June 2020. Downloaded from http://jcp.bmj.com/ on March 11, 2021 at Seoul National University Medical Library and Hospital. Protected by copyright.

morphology on small biopsy. p.Q795C classifies as variant of 'uncertain clinical significance' by the ACMG rules.[15] It may be added that another amino acid change at the same codon (Q795R (COSM85896))[13] has also been categorised as a variant of uncertain clinical significance strengthening the current call.

HER2 amplification status as determined on NGS yielded a median ploidy of 3.21. None of the cases with *HER2* mutation showed concurrent significant gene amplification, highlighting their independent status as oncogenic driver mutations. On orthogonal testing by FISH for *HER2*/CEP17 ratio, the median ratio was 2.0. Previous reports suggest that concurrent higher levels of HER2 gene amplification or higher HER2/CEP17 ratio is of importance in terms of defining an inferior therapeutic response to single agent trastuzumab.[25–27]

The current study highlights the relative high occurrence of *HER2* exon 20 mutations as a primary oncogenic driver in NSCLC especially LUAD. Our cohort has demonstrated p.(E770_A771insAYVM) as the strikingly dominant insertion mutation against the most often globally reported p.(A771_Y772insYVMA). There is no overlap between *HER2* gene amplification and existence of *HER2* exon 20 mutations and hence these tests cannot be used interchangeably. Since only three insertion mutations p.(E770_A771insAYVM), p.(A771_Y772insYVMA) and p.(V777_G778insGSP) made up the entire gamut of *HER2* exon 20 insertion mutations, a multiplexed reverse transcriptase PCR or pyrosequencing can be used as the identification strategy in institutions lacking NGS facility.

Take home messages

- Amplification of human epidermal growth factor receptor 2 (HER2) is so far not actionable; however, its missense and in-frame insertions may be actionable in lung adenocarcinoma (LUAD).
- There is a relatively higher occurrence of *HER2* exon 20 mutations as primary oncogenic driver in non-small cell lung cancer especially LUAD.
- Our cohort has demonstrated (p.E770_A771insAYVM) as the strikingly dominant insertion mutation against the most often globally reported (p.A771_Y772insYVMA).

Handling editor Runjan Chetty.

Acknowledgements The authors acknowledge the efforts of doctors, residents and technicians of Departments of Medical Oncology and Pathology of Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India.

Contributors On behalf of all the contributors, AM will act and guarantor and will correspond with the journal this point onward. This manuscript is original research work, and all authors have seen and agreed to the final version of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by the Institutional Review Board of the Hospital (vide letter no. 748/RE/AMH-36).

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iD
Anurag Mehta http://orcid.org/0000-0001-6517-3664

REFERENCES

1 Gutierrez C, Schiff R. Her2: biology, detection, and clinical implications. *Arch Pathol Lab Med* 2011;135:55–62.
2 Wolff AC, Hammond MEH, Allison KH, *et al*. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of clinical Oncology/College of American pathologists clinical practice guideline focused update. *Arch Pathol Lab Med* 2018;142:1364–82.
3 Bartley AN, Washington MK, Ventura CB, *et al*. Her2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American pathologists, American Society for clinical pathology, and American Society of clinical oncology. *Arch Pathol Lab Med* 2016;140:1345–63.
4 Connell CM, Doherty GJ. Activating HER2 mutations as emerging targets in multiple solid cancers. *ESMO Open* 2017;2:1–11.
5 Collisson EA, Campbell JD, Brooks AN, *et al*. Comprehensive molecular profiling of lung adenocarcinoma: the cancer genome atlas research network. *Nature* 2014;511:543–50.
6 Pillai RN, Behera M, Berry LD, *et al*. Her2 mutations in lung adenocarcinomas: a report from the lung cancer mutation Consortium. *Cancer* 2017;123:4099–105.
7 Li C, Sun Y, Fang R, *et al*. Lung adenocarcinomas with HER2-activating mutations are associated with distinct clinical features and HER2/EGFR copy number gains. *J Thorac Oncol* 2012;7:85–9.
8 Network NCC. Non-Small cell lung cancer (Version3.2020), 2020. Available: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf [Accessed 10 Apr 2020].
9 Chakravarty D, Gao J, Phillips S, *et al*. OncoKB : A Precision Oncology Knowledge Base. *JCO Precis Oncol* 2017;1:1–16 https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
10 Li BT, Ross DS, Aisner DL, *et al*. Her2 amplification and HER2 mutation are distinct molecular targets in lung cancers. *J Thorac Oncol* 2016;11:414–9.
11 Li BT, Shen R, Buonocore D, *et al*. Ado-trastuzumab emtansine for patients with HER2-mutant lung cancers: results from a phase II basket trial. *J Clin Oncol* 2018;36:2532–7.
12 Arcila ME, Chaft JE, Nafa K, *et al*. Prevalence, clinicopathologic associations, and molecular spectrum of ErbB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. *Clin Cancer Res* 2012;18:4910–8.
13 Tate JG, Bamford S, Jubb HC, *et al*. Cosmic: the Catalogue of somatic mutations in cancer. *Nucleic Acids Res* 2019;47:D941–7.
14 Travis WD, Brambilla E, Nicholson AG, *et al*. The 2015 World Health organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. *J Thorac Oncol* 2015;10:1243–60.
15 Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015;17:405–23.
16 Lih C-J, Harrington RD, Sims DJ, *et al*. Analytical validation of the next-generation sequencing assay for a nationwide Signal-Finding clinical trial: molecular analysis for therapy choice clinical trial. *J Mol Diagn* 2017;19:313–27.
17 Kopanos C, Tsiolkas V, Kouris A, *et al*. VarSome: the human genomic variant search engine. *Bioinformatics* 2019;35:1978–80.
18 Landrum MJ, Lee JM, Benson M, *et al*. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res* 2018;46:D1062–7.
19 Prabhash K, Advani SH, Batra U, *et al*. Biomarkers in non-small cell lung cancers: Indian consensus guidelines for molecular testing. *Adv Ther* 2019;36:766–85.
20 Kim EK, Kim KA, Lee CY, *et al*. The frequency and clinical impact of HER2 alterations in lung adenocarcinoma. *PLoS One* 2017;12:1–13.
21 Shigematsu H, Takahashi T, Nomura M, *et al*. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* 2005;65:1642–6.
22 Tomizawa K, Suda K, Onozato R, *et al*. Prognostic and predictive implications of HER2/ERBB2/neu gene mutations in lung cancers. *Lung Cancer* 2011;74:139–44.
23 Barlesi F, Mazieres J, Merlio J-P, *et al*. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French cooperative thoracic intergroup (IFCT). *Lancet* 2016;387:1415–26.
24 Buttitta F, Barassi F, Fresu G, *et al*. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer* 2006;119:2586–91.
25 Yoshizawa A, Sumiyoshi S, Sonobe M, *et al*. Her2 status in lung adenocarcinoma: a comparison of immunohistochemistry, fluorescence in situ hybridization (fish), dual-ISH, and gene mutations. *Lung Cancer* 2014;85:373–8.
26 Rüschoff J, Dietel M, Baretton G, *et al*. Her2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch* 2010;457:299–307.
27 Gow C-H, Liao W-Y, Liu Y-N, *et al*. Discordant HER2 exon 20 mutation status determines a differential sensitivity to afatinib. *J Thorac Oncol* 2015;10:e58–60.

J Clin Pathol: first published as 10.1136/jclinpath-2020-206730 on 11 June 2020. Downloaded from http://jcp.bmj.com/ on March 11, 2021 at Seoul National University Medical Library and Hospital. Protected by copyright.