

HER3 expression and MEK activation in non-small-cell lung carcinoma

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Aim: We explore HER3 expression in lung adenocarcinoma (adeno-NSCLC) and identify potential mechanisms of HER3 expression. **Materials & methods:** Tumor samples from 45 patients with adeno-NSCLC were analyzed. HER3 and HER2 expression were identified using immunohistochemistry and bioinformatic interrogation of The Cancer Genome Atlas (TCGA). **Results:** HER3 was highly expressed in 42.2% of cases. *ERBB3* copy number did not account for HER3 overexpression. Bioinformatic analysis of TCGA demonstrated that MEK activity score (a surrogate of functional signaling) did not correlate with HER3 ligands. *ERBB3* RNA expression levels were significantly correlated with MEK activity after adjusting for *EGFR* expression. **Conclusion:** HER3 expression is common and is a potential therapeutic target by virtue of frequent overexpression and functional downstream signaling.

Tweetable abstract: HER3 expression is common in adeno-NSCLC and is a potential therapeutic target by virtue of frequent overexpression and functional downstream signaling.

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Background

Non-small-cell lung carcinoma

Lung cancer is the leading cause of cancer mortality worldwide. There is a poor 5-year survival rate in patients with regional and distant disease in non-small-cell lung carcinoma (NSCLC), of 33 and 6%, respectively [1]. Systemic treatment with chemotherapy and immunotherapy is standard of care, while a small subset of cancers demonstrates oncogene-addiction with effective targeted treatments. These include EGFR mutated, ALK rearranged and newer targets such as ROS, RET and NTRK.

HER3

HER3 is a member of the EGFR family of receptors. Investigation of HER3 as a therapeutic target has not been previously prioritized due to its impaired kinase activity [2]. HER3 acts as a heterodimeric partner for other members of the EGFR family, namely EGFR/HER-1 and ERB2/HER2/Neu [3–6]. HER3 possesses weak tyrosine kinase activity and so preferentially forms heterodimers with kinase-proficient receptor tyrosine kinases in the presence of its ligands neuregulin (NRG 1 and NRG 2) [2]. It has also been demonstrated that HER3 can form heterodimers with MET, AXL and IGF1R [7–9]. EGFR has a number of ligands, including amphiregulin, epidermal growth factor and transforming growth factor, while HER2 has no known ligand and remains in an 'open', active conformation [10–12]. However, HER2/HER3 dimerization can occur independently of ligand binding [13]. More recently it has been appreciated that HER3 can also homodimerize on binding to the heregulin ligands, NRG1

and NRG2, so having intrinsic, non-EGFR or HER2 mediated activity [14,15]. Downstream signaling of HER3 is mainly via the PI3K/AKT pathway, with MAPK/ERK and JAK/STAT pathways also activated [16,17].

ERBB3, the HER3 gene, is under the regulation of several miRNAs including miR125a, miR125b and miR205 [18–20]. Studies have shown that the ubiquitin-proteasome pathway plays a significant role in cancer initiation and progression by regulating protein levels [9,21–25]. E3 ubiquitin ligases regulate EGFR family receptors. NRDP1 mediates HER3 degradation [9,22]. NRG1 stabilizes USP8 which in turn stabilizes NRDP1 [21] NEDD4 is a novel interaction partner and ubiquitin E3 ligase of HER3 [23].

Evidence for the role of HER3 in lung cancer pathogenesis is largely drawn from retrospective data sets correlating HER3 expression with poor prognosis and metastatic development [26–29]. Accumulating evidence supports the role of HER3 in the development of resistance to EGFR targeted therapies [30–35]. From preclinical data, dual targeting of EGFR and HER3 is capable of overcoming acquired resistance to EGFR inhibition [32,35]. MET amplification or overexpression is a mechanism of resistance to EGFR-TKI occurring in 5% of cases [36]. HER3 phosphorylation is observed in EGFR mutant tumors with MET amplification, indicating that MET activates PI3K/AKT pathway via HER3 phosphorylation [37]. MEK inhibition causes a transcriptional upregulation of both HER2 and HER3 and the formation of heterodimeric complexes in KRAS mutant NSCLC and colon cancer and combined MEK and dual EGFR-HER2 inhibition has a synergistic effect [34]. Mutations of HER3 have been described in the extra-cellular domain or tyrosine kinase domain as a rare event in lung cancer and can influence the activity of HER3 receptor as well as the dimerization affinity with HER2 and EGFR [38–40].

Here, we explore HER3 expression levels in adenocarcinoma of the lung (adeno-NSCLC) patients and correlate it with clinical parameters. We also interrogate large genomic datasets to identify the potential mechanism of HER3 expression and propose that HER3 could be an alternate pathway for MEK pathway activation.

Materials & methods

Patients & tissue samples

The Drug Development Unit at the Royal Marsden recruits patients with advanced solid malignancies to early phase clinical trials. Patients with adeno-NSCLC attending consultations in our Phase I clinic provided informed written consent. The archival or fresh tissue from biopsies were used in the study. Approval was gained from the local research and ethics committee for the use of the clinical data. Archival formalin fixed paraffin embedded tumor blocks were analyzed for HER2 and HER3 protein expression and genomic analysis for HER3 copy number was performed. Patient clinical data were retrospectively collected from the Royal Marsden hospital electronic patient record system.

Immunohistochemistry & scoring system

Tissue blocks were freshly sectioned and only considered for immunohistochemistry (IHC) analyses of HER2 and HER3 if adequate material was present. Protein expression on 3 μ m thick formalin-fixed paraffin-embedded (FFPE) sections was assessed by a pathologist blinded to the clinical data.

HER3 and HER2 antibody validation by siRNA and immunohistochemical assays were performed as previously described. Briefly, HER3 immunoreactivity was investigated using the rabbit monoclonal anti-HER3 antibody clone D22C5 (#12708, Cell Signaling Technology, London, UK). HER2 immunoreactivity was investigated using the mouse monoclonal anti-HER2 antibody clone CB11 (#NCL-L-CB11, Leica Biosystems, Newcastle, UK). Rabbit and Mouse IgGs (#I-1000 and I-2000, Vector, CA, USA) were used as negative controls. For HER3 IHC, antigen retrieval was conducted by heating slides in high pH buffer using a microwave, staining was subsequently performed using BioGenex i6000 autostainer (Launch Diagnostics, Kent, UK). HER3 immunoreactivity was detected using Dako EnVision Flex high pH kit (#K800021-2, Agilent, Glostrup, Denmark). For HER2 IHC automated antigen retrieval and detection were performed using Leica Bond RX (Leica Biosystems). HER2 immunoreactivity was detected using the Bond Polymer Refine Detection system (#DS9800, Leica Biosystems).

For HER2, membranous expression was scored according to College of American Pathologists HER2 scoring guidelines [41]. No staining or membrane staining that is incomplete and is faint/barely perceptible and in $\leq 10\%$ of tumor cells (score 0); incomplete membrane staining that is faint/barely perceptible and in $>10\%$ of tumor cells (score 1+); weak-to-moderate complete membrane staining observed in $>10\%$ of tumor cells (score 2+) and circumferential membrane staining that is complete, intense and in $>10\%$ of tumor cells (score 3+). For HER3 staining, the same guidelines were used to generate an *ad hoc* HER2-like score for membranous staining.

Additionally, HER2 and HER3 cytoplasmic staining was visually assessed, percentage of cells staining weakly, moderately or strongly were noted and H score calculated.

Digital PCR

Digital PCR was performed on a Q \times 100 droplet digital PCR system (Bio-Rad, CA, USA). Copy number assays were performed with a FAM-ERBB3 validated Bio-Rad assay, reference ID: dHsaCP2500395 (67 bp amplicon) and a recommended reference copy number Hex-assay for AP3B1, assay ID: dHsaCP2500348 (60 bp amplicon). Digital PCR analysis was performed using QuantaSoft v1.3.2.0 software from Bio-Rad to assess the number of positive droplets. At least two negative control wells with no DNA were included in every run and each assay was run at least in duplicate (see [Supplementary methods](#)).

Statistical methods

Statistical analysis of IHC and clinical data was performed on Graphpad Prism v7.0d. The association between HER3 expression and other binary data was analyzed using two sided Fisher's exact test. For survival Kaplan–Meier curves were constructed and Gehan–Breslow–Wilcoxon test compared populations. Survival was taken from diagnosis to death and those alive at follow-up were censored.

Bioinformatic methods

Genomic data and gene expression (RNA-Seq by expectation maximization) for 507 adeno-NSCLC was downloaded from cBioportal for analysis [42]. The RNA-Seq by expectation maximization value represents the normalized expression level of each gene in each tumor. It was calculated by: estimated number of reads that aligned to a gene and; the scaled version of the raw counts using a standard 75th-percentile normalization approach [43]. This approach followed the The Cancer Genome Atlas (TCGA) Research Network recommendations and TCGA lung adenocarcinoma publication [43]. We interrogated the TCGA to determine the relationship between *ERBB3* expression and particular oncogenes of interest, namely *EGFR* and *KRAS* mutations.

The MEK activity score is an accumulation measurement of MEK pathway activity based on a previously validated 18-gene transcript signature of MEK signaling [44]. Linear regression was used to assess the associations between gene expression levels (and with MEK signaling). All statistical test was performed using R (v1.1.43).

Results

Clinical characteristics

From December 2010 to December 2018, 45 patients with adeno-NSCLC were referred to the Drug Development Unit, consented to tissue analysis and had available archival tumor specimens. The majority of samples were from biopsies (41), the rest being from surgical resection (4). There were relatively fewer males (19) than females (26) in the cohort. The median age at diagnosis was 58 (range: 38–72) and the median overall survival (OS) was 38 months. The median number of lines of treatment for advanced disease was 3 (range: 0–7): 43 patients received chemotherapy (median 2: range 1–4), 22 patients received EGFR tyrosine kinase inhibitor (TKI) (median 1: range 1–3) and nine patients received immunotherapy. All patients who had an EGFR mutation received an EGFR TKI and 25 patients were enrolled in a Phase I trial and received at least one Phase I trial drug.

HER2 & HER3 protein expression

HER3 protein expression showed a membranous distribution with no nuclear expression ([Figure 1A](#)). High expression of HER3 (2+ or 3+) was seen in 42.2% of cases ([Table 1](#)). HER2 expression was positive in 31.1% of cases, while HER2 and HER3 co-expression was seen in 11.1% (5/45). No correlation between HER2 and HER3 expression was demonstrated (Fisher's exact test; $p > 0.9999$). Patient characteristics according to HER3 status are described ([Table 2](#)).

Correlation of HER3 protein expression by IHC with clinical characteristics

The median OS from diagnosis for HER3 positive patients was 40.5 months, and for HER3 negative patients 30.8 months. There was no statistical difference in survival between HER3 positive and HER3 negative cases (Gehan–Breslow–Wilcoxon; $p = 0.4596$) ([Figure 1B](#)). There was no difference in median survival between HER3 positive and negative cases in those patients with EGFR mutations (median OS: 42.6 months HER3 positive, median OS: 43.9 months HER3 negative; Gehan–Breslow–Wilcoxon; $p = 0.4927$).

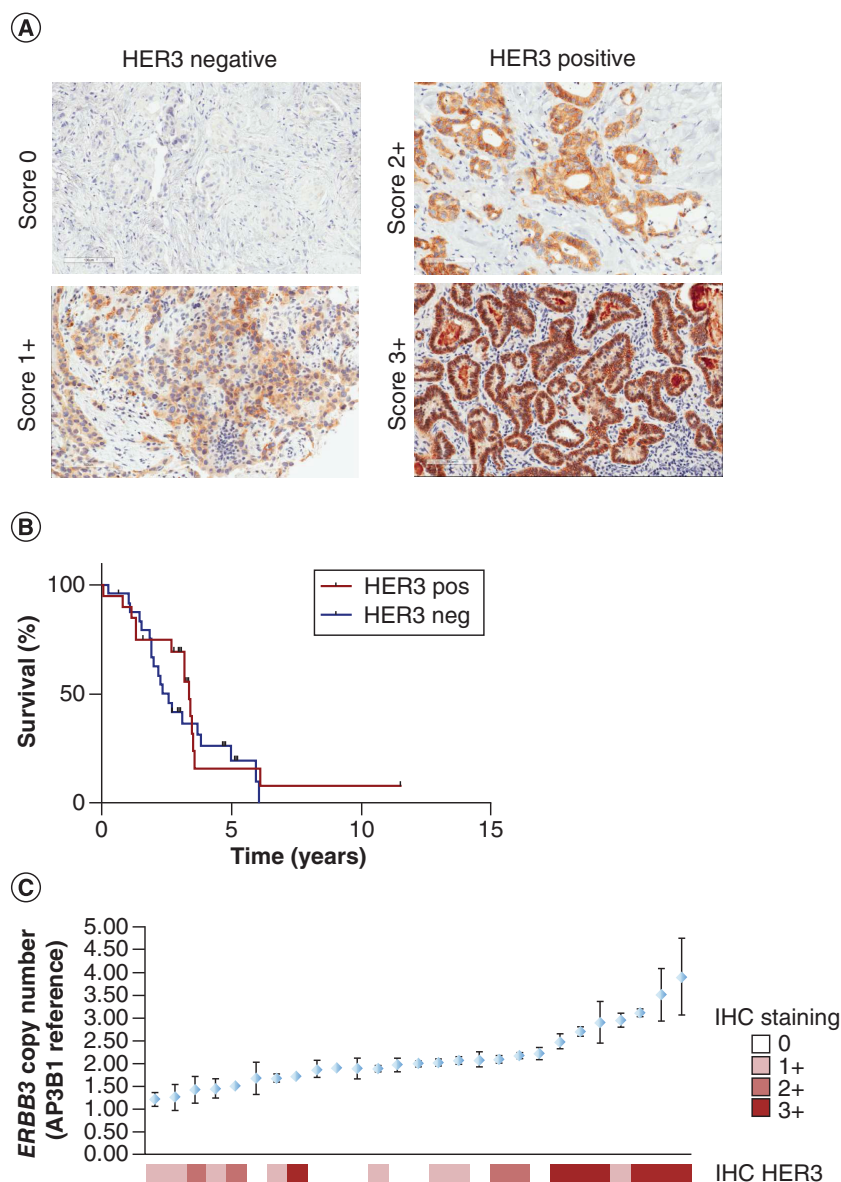


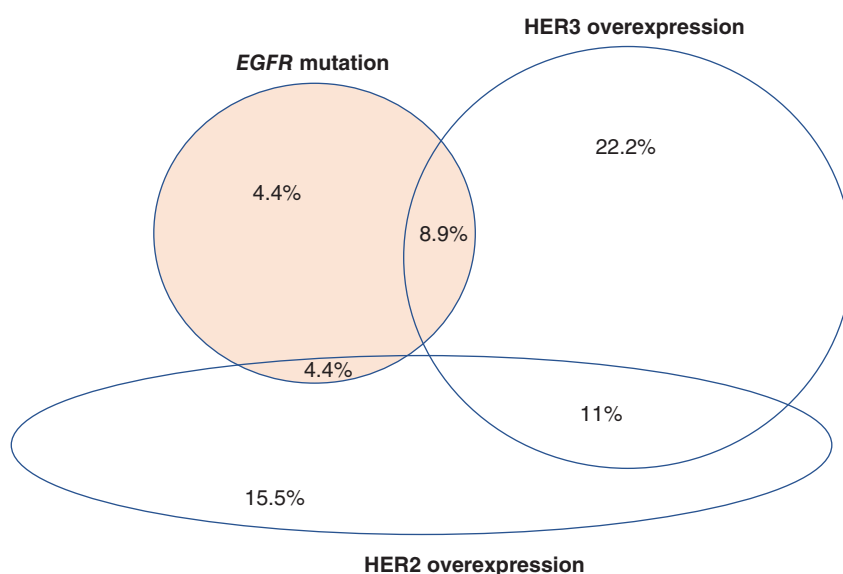
Figure 1. Adeno-non-small-cell lung carcinoma patient samples. (A) Panel of HER3 negative cases (0–1+) and HER3 positive cases (2–3+). For HER3 staining, the guidelines from CAP HER2 scoring guidelines were used to generate an ad-hoc HER2 like score. **(B)** Kaplan–Meier curve of HER3 positive versus HER3 negative cases. **(C)** *ERBB3* gene copy number and HER3 immunohistochemistry.

Table 1. Membranous HER3 and HER2 protein expression by immunohistochemistry.		
HER3 staining	% (n)	
0	35.5% (16)	57.8% (26)
1	22.2% (10)	
2	15.5% (7)	42.2% (19)
3	26.7% (12)	
HER2 staining	% (n)	
0	33.3% (15)	68.9% (31)
1	35.5% (16)	
2	22.2% (10)	31.1% (14)
3	8.8% (4)	

Table 2. Clinical characteristics for HER3 positive and HER3 negative patients.

Clinical characteristics	HER3 positive, % (n)	HER3 negative, % (n)
Male	26% (5)	74% (14)
Female	54% (14)	46% (12)
Median age (years)	58	57.5
Smoking status		
Non smoker	16% (3)	12% (3)
Current smoker	5% (1)	12% (3)
Ex-smoker	47% (9)	35% (9)
Unknown	32% (6)	42% (11)
Prior therapy		
No prior treatment	2% (1)	2% (1)
Prior chemotherapy	35% (17)	43% (25)
Prior immunotherapy	10% (5)	7% (4)
Prior TKI	23% (11)	22% (13)
Phase I clinical trial	17% (10)	26% (15)

TKI: Tyrosine kinase inhibitor.

**Figure 2. Adeno-non-small-cell lung carcinoma patient samples.** Frequency of aberrations in adeno-non-small-cell lung carcinoma as percentage of total population.

HER3 gene amplification

Digital PCR of *ERBB3* was performed in 27 cases. There was low frequency of small copy number gain in HER3 with 11% (3/27) having copy number variant of 3–4 (Figure 1C). Six out of the seven samples with this highest copy number variant scored 3+ on HER3 IHC.

EGFR & KRAS mutations & correlation with HER3

As standard of care, *EGFR* mutational analysis had been performed in all 45 patients and *KRAS* mutational analysis in 28 patients. *EGFR* mutation was present in 17.8% (eight cases). HER3 protein overexpression was present in 8.9% (four cases) of *EGFR* mutation and in 14.2% (four cases) of *KRAS* mutation. There was no correlation between HER3 overexpression and *EGFR* positivity (Fisher's exact test; $p = 0.7043$) or HER3 and *KRAS* positivity (Fisher's exact test; $p = 0.6908$) (Figure 2).

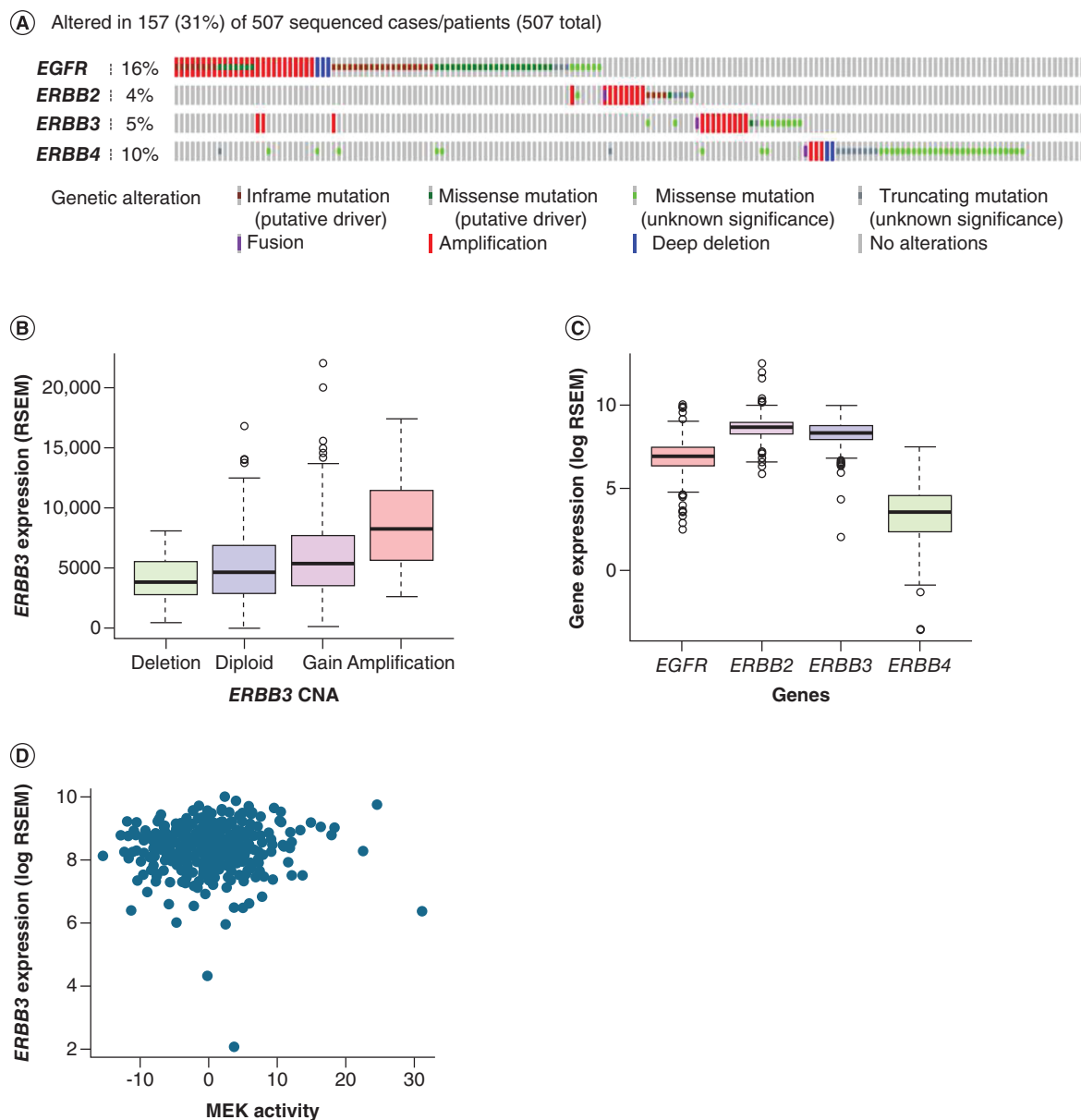


Figure 3. The Cancer Genome Atlas adeno-non-small-cell lung carcinoma. (A) Frequency of HER family genetic alterations. **(B)** *ERBB3* RNA expression with *ERBB3* CNA. **(C)** Gene expression of *EGFR*, *ERBB2*, *ERBB3* and *ERBB4*. **(D)** Correlation of *ERBB3* expression level with MEK activity. RSEM: RNA-Seq by expectation maximization.

Baseline genomic data in TCGA

Interrogation of the PanCancer TCGA of 507 patients with adeno-NSCLC showed a genetic alteration rate of 31% in the ERBB family with the most common genetic alteration seen in *EGFR* and *ERBB4*, which was evident in 16% (79 cases) and 10% (50 cases). In *ERBB2* and *ERBB3*, genetic alterations were less frequent, seen in 4% (19 cases) and 5% (25 cases), respectively (Figure 3A). Among the different types of genetic alteration, amplification was the most common alteration in *ERBB3*, observed in 48% out of all alterations (12 cases). *ERBB3* copy number change demonstrated a positive association with *ERBB3* RNA expression ($r = 0.26$; $p = 8 \times 10^{-6}$) (Figure 3B); amplification cases had 1.7-times higher expression of *ERBB3* RNA than diploid cases ($p = 0.004$).

In the TCGA dataset *ERBB3* amplification co-occurred with *EGFR* amplification; the amplification of *ERBB3* was four-times more likely to co-exist with *EGFR* amplification than not, though this did not reach statistical significance ($p = 0.1$). There was no co-amplification between *ERBB3* and *ERBB2*, but the expression of *ERBB3*

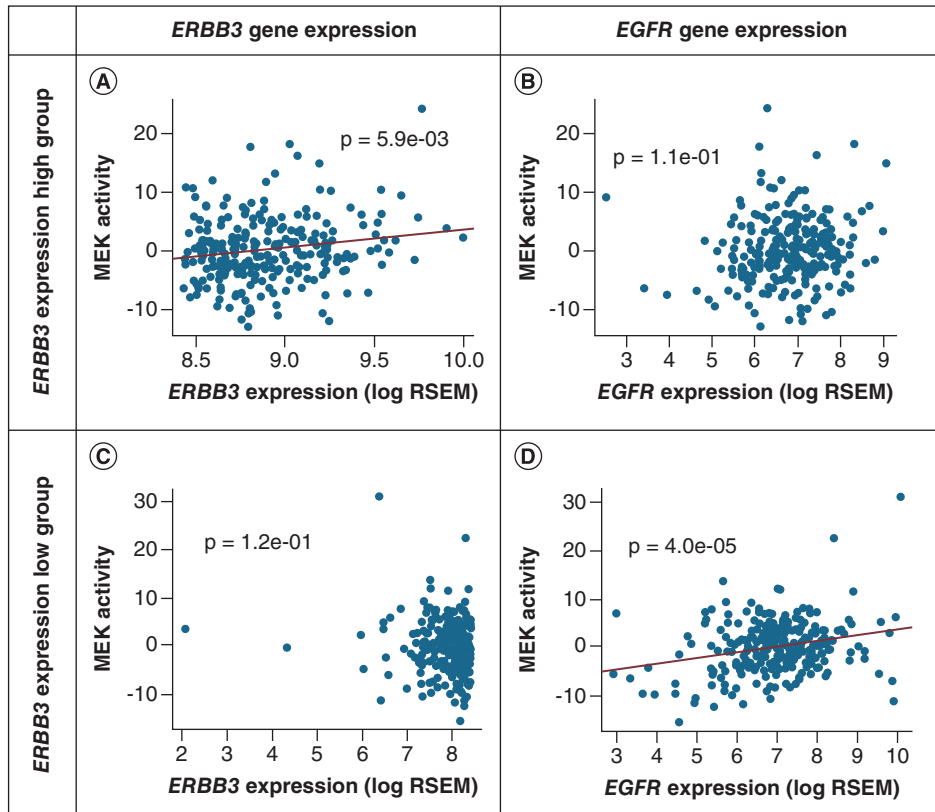


Figure 4. Correlation of gene expression with MEK activity in The Cancer Genome Atlas adeno-non-small-cell lung carcinoma. (A) Correlation of *ERBB3* expression level with MEK activity in *ERBB3* expression high cancers. (B) Correlation of *EGFR* expression with MEK activity in *ERBB3* expression high cancers. (C) Correlation of *ERBB3* expression level with MEK activity in *ERBB3* expression low cancers. (D) Correlation of *EGFR* expression with MEK activity in *ERBB3* expression low cancers. RSEM: RNA-Seq by expectation maximization.

was highly correlated with *ERBB2* expression levels ($r = 0.09$; $p = 2.5 \times 10^{-23}$) (Supplementary Figure 1A). *ERBB3* expression also positively correlated with *ERBB4* expression ($r = 0.1$; $p = 0.001$) but there was no correlation with the expression levels of *EGFR* ($r = -0.12$; $p = 0.5$). *EGFR* and *ERBB2* gene copy number also increased with gene expression (*EGFR*: $r = 0.4$; $p < 2 \times 10^{-16}$; and *ERBB3*: $r = 0.35$; $p = 4 \times 10^{-16}$) (Supplementary Figure 1B). *ERBB3* gene copy number decreased with *ERBB2* gene expression ($r = -0.1$; $p = 0.09$) (Supplementary Figure 1C).

MEK activity score correlation with key nodes in *ERBB* pathway in TCGA data

We performed further analyses of RNA sequencing data from TCGA. We observed *ERBB2* and *ERBB3* RNA expression levels were higher than *EGFR* and *ERBB4* in adeno-NSCLC (Figure 3C). We measured absolute gene expression level of *ERBB* family genes and derived MEK pathway activity using a validated 18-gene MEK signature [44]. *EGFR* RNA expression levels correlated with MEK activity ($r = 0.16$; $p = 3.0 \times 10^{-5}$), *ERBB3* RNA expression did not correlate with MEK activity ($r = 0.09$; $p = 0.78$) (Figure 3D). We further characterized MEK activation score with gene expression levels of *ERBB* ligand and proteins of ubiquitination (Supplementary Table 1). *AREG* (*EGFR* ligand) and *RNF41* (an ubiquitin ligase) expression had a significant positive correlation with MEK activity score.

In order to stratify *ERBB3* RNA expression effects on MEK activity, we grouped patients into *ERBB3* 'expression high' (higher 50% of *ERBB3* RNA expression cases) and *ERBB3* 'expression low' (lower 50% of *ERBB3* RNA expression cases). In the *ERBB3* expression high group, *ERBB3* RNA expression levels were significant and positively correlated with MEK activity score ($r = 0.19$; $p = 0.006$), whereas no correlation was observed in the *ERBB3* expression low group ($p = 0.12$) (Figure 4). The expression of *EGFR* did not correlate with MEK activity score in the *ERBB3* expression high group ($p = 0.11$), though did correlate with MEK activity score in the *ERBB3*

expression low group ($r = 0.2$; $p = 4 \times 10^{-5}$). In the *ERBB3* expression high group, *ERBB3* expression levels still highly positively correlated with MEK activity even after adjusting for *EGFR* expression effects ($p = 0.007$).

Discussion

HER3 protein levels in adeno-NSCLC & mechanism of overexpression

Reports of the incidence of HER3 protein overexpression in NSCLC vary greatly according to the cohort. Previous groups have reported rates ranging from 6.5 to 86.1% [28,45–49]. IHC methods and scoring have not been previously standardized. Our data use a validated and robust scoring system based on optical density and H-score. Although our patient cohort is small, we demonstrate that overexpression of HER3 protein is common in advanced adeno-NSCLC, occurring in over 40% of patients.

The rate of *ERBB3* gene amplification in NSCLC is low. The Cancer Genome Atlas Research Group sequenced 230 cases of lung adenocarcinoma and did not identify any *ERBB3* amplification [43]. The cBioportal and GENIE data sets report amplification in just over 1% frequency [42]. It was not possible to perform *ERBB3* PCR on all cases in our cohort as tumor tissue was limited, but a sample of 27 cases had sufficient tumor sample to pass quality control. There appeared to be clustering of HER3 overexpressing cases in those with *ERBB3* copy number gain but this does not account for the majority of HER3 overexpression. We interrogated the PanCancer TCGA dataset of adeno-NSCLC samples. This dataset includes transcriptome (RNA) and genomic (DNA) data. The genomic data support our finding of low levels of *ERBB3* amplification. It also provided the confirmation that *ERBB3* copy number alteration highly associates with its gene and HER3 protein expression.

HER3 expression as a prognostic marker

Yi *et al.* demonstrated that HER3 overexpression is associated with a poor prognosis in a cohort of 443 patients with advanced NSCLC [28]. The median overall survival in our study was not significantly different between the HER3 negative and positive patient group. This Phase I patient cohort may not be truly reflective of the broader population of NSCLC patients, as these patients tend to be fitter with fewer or no comorbidities.

HER3 in relation to oncogenic drivers in adeno-NSCLC

HER2 protein overexpression is reported in 6–30% of patients with lung cancer, which in keeping with our results [50–52]. HER2 expression is less frequently observed in NSCLC than HER3 expression. There was some overlap, though no statistically significant association between, HER3 expression with HER2 expression or *KRAS* and *EGFR* mutations though this is somewhat limited by small sample size. TCGA data demonstrated correlation of *ERBB3* and *ERBB2* expression. Previous data have demonstrated that HER3 plays a role in EGFR TKI resistance. Half of the cases in this cohort with EGFR mutation also co-expressed HER3. No difference in overall survival was seen between HER3 expressing and nonexpressing groups in the EGFR-mutant cases in this cohort though patient numbers were small.

HER3 & functional activity in adeno-NSCLC

The MEK activity score is an 18 gene signature derived from an experiment to test a mixed-tumor response to the MEK inhibitor, selumetinib [44]. The MEK activity score is a surrogate marker of MEK pathway activation though not specific for activation through the HER3 channel as the MEK pathway is also initiated upon other receptor activation. As HER3 activity is predominantly via HER2/HER3 and EGFR/HER3 hetero-dimerization, we inferred that the MEK activity score could be used as a surrogate of, but not specific to, HER3 protein expression. If a key node in the HER3 pathway is found to correlate with MEK activity score, it can be concluded that, in that cohort, the node may play a key part in pathway. The lack of correlation with *ERBB3* ligands (*NRG 1* and *2*) expression is an important negative finding (i.e., HER3 protein expression does not appear to be ligand dependent).

In the TCGA dataset MEK activity score was associated with *EGFR* and *AREG* (EGFR ligand) gene expression. This suggests that signaling via EGFR is key to the oncogenic behavior in lung cancer. EGFR has been previously reported as a key driver of oncogenesis in lung (and other) cancers [53]. Although our initial analysis did not show correlation of *ERBB3* with MEK signature, we stratified *ERBB3* expression into two groups; 'expression low', where *ERBB3* expression was less than 50% of the median expression and 'expression high', where *ERBB3* expression was 50% or more than the median expression levels; and analyzed this along with *EGFR* gene expression. In the *ERBB3* expression high group, *ERBB3* RNA expression levels positively correlated with MEK activity score but not in the expression low group. The expression of *EGFR* correlated with MEK activity score in the *ERBB3* expression low

group. HER3 may be an alternative pathway for MEK activation in the HER3 expression high group. This analysis implies a degree of functional downstream pathway signaling via HER3. In effect, this suggests that when EGFR drives the cancer, the effect of HER3 is minimal as EGFR drives the downstream signaling. However, as HER3 expression increases this becomes the main driver of pathway activation.

Conclusion

We conclude that HER3 is commonly over-expressed in NSCLC and co-expresses with other HER family receptors. HER3 over-expression was not fully attributable to gene copy-number change. In the search for nongenomic causes of HER3 protein overexpression, the MEK activity score was used as a surrogate of HER3 overexpression; however no correlation with ligands of HER3 were seen. Further investigation, therefore, is needed to continue to elucidate the mechanism of HER3 overexpression in adeno-NSCLC. We have a simple tool in the form of IHC that can be used to 'prescreen' and stratify patients to receive HER3 targeting compounds. HER3 targeting compounds are in development such as the HER3 antibody drug conjugate patritumab deruxtecan [54], and the monoclonal antibodies lumretuzumab [55] and seribantumab [56]. There are some patients where HER3 expression occurs concurrently with driver mutations (EGFR). The role of HER3 in these groups, including in drug resistance, is the subject of ongoing investigation. Additionally, we propose that HER3 may signal via the MEK pathway (oncogenic role, for further functional validation). The 'clinical reality' of targeting HER3 may be more complex with heterogeneity of response depending on other genomic and as yet, undefined, features.

Future perspective

We foresee remarkable change in lung cancer care, with increasingly more targeted therapies, stratifying patients according to novel targetable aberrations, and to overcome resistance. Antibody drug conjugates as well as bispecific antibodies use will be routine practice and enable precise targeting and delivery of cytotoxics.

Summary points

- In a cohort of 45 patients with adeno-non-small-cell lung carcinoma (NSCLC), HER3 protein overexpression occurred in 42.2% of cases and was more frequently seen than HER2 overexpression. There was no statistically significant association between HER3 expression and HER2 expression, EGFR or KRAS mutations.
- The median overall survival in our study was not significantly different between the HER3 negative and positive patient groups.
- On The Cancer Genome Atlas analysis, the mechanism of HER3 overexpression was not fully attributable to *ERBB3* copy number nor was *ERBB3* ligand dependent. Further investigation is required to elucidate the mechanism of HER3 overexpression.
- MEK activity score was used as a functional surrogate marker for HER3 overexpression. In the *ERBB3* expression high group, *ERBB3* RNA expression levels positively correlated with MEK activity score but not in the expression low group. The expression of *EGFR* correlated with MEK activity score in the *ERBB3* expression low group. This supports the hypothesis that HER3 is an alternative pathway for MEK activation in the HER3 expression high group.
- HER3 overexpression is common, and has functional downstream signaling, particularly in non-EGFR driven adeno-NSCLC.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/Imt-2020-0031

Author contributions

Interpretation of data has been contributed by T Manickavasagar and A Minchom. Preparation of manuscript has been contributed by A Minchom, T Manickavasagar and W Yuan. Data analysis has been contributed by A Minchom, W Yuan, S Carreira, B Gurel, S Miranda, A Ferreira, M Crespo, R Riisnaes and C Baker. Review of manuscript has been contributed by S Carreira, B Gurel, S Miranda, A Ferreira, M Crespo, R Riisnaes, C Baker, M O'Brien, J Bhosle, S Popat, U Banerji, J Lopez and JD Bono.

Financial & competing interests disclosure

M O'Brien sits on advisory boards for MSD, Abbvie, BMS, BI and Pierre Fabre. S Popat has received honoraria from BMS, Roche, Takeda, AstraZeneca, Pfizer, MSD, EMD Serono, Guardant Health, AbbVie, Boehringer Ingelheim, Medscape, Tesaro, Paradox, In-

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Ethical conduct of research

Informed written consent was gained from patients to use the archival of fresh tumor samples. Approval was gained from the local research and ethics committee for the use of the clinical data.

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