HER-3 Expression and MEK Activation in Non-small Cell Lung Cancer

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Abstract

Background

HER-3 has a role in pathogenesis and development of resistance to targeted therapies in non-small cell lung cancer (NSCLC).

Methods

We analysed tumour samples from 45 patients with adenocarcinoma of the lung. HER-3 and HER-2 expression was identified using immunohistochemistry as well as bioinformatic interrogation of The Cancer Genome Atlas (TCGA).

Results

HER-3 was highly expressed in 42.2% of cases and observed more frequently than HER-2 overexpression. *ERBB3* copy number alone did not account for HER-3 overexpression. Bioinformatic analysis of TCGA demonstrated that MEK activity score (a score of downstream pathway activity and surrogate of functional HER-3 signalling) did not correlate with HER-3 ligands. EGFR pathway signalling was predominant with a significantly positive correlation of *EGFR* and *AREG* expression with MEK activity score. In the *ERBB3* expressed group, *ERBB3* RNA expression levels were significantly correlated with MEK activity score. *ERBB3* expression level remained highly positively correlated with MEK activity after adjusting for *EGFR* expression.

Conclusion

HER-3 expression is common and it is not associated with gene amplification nor is it ligand dependent. HER-3 is an alternative pathway to EGFR activating MEK. HER-3 is a potential therapeutic target in NSCLC by virtue of frequent overexpression and functional downstream signalling.

Background

Non-small cell lung cancer

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 cancer (NSCLC), of 33% and 6% respectively (1). Systemic treatment with chemotherapy and immunotherapy is standard of care, while a small subset of cancers demonstrate oncogene-addiction with effective targeted treatments. These include EGFR mutated, ALK rearranged and newer targets such as ROS, RET and NTRK.

HER-3

HER-3 (receptor tyrosine-protein kinase erbB-3) is a member of the epidermal growth factor receptor (EGFR) family of receptors. Investigation of HER-3 as a therapeutic target has not been previously prioritised due to its impaired kinase activity (2). HER-3 acts as a heterodimeric partner for other members of the EGFR family, namely EGFR/HER-1 and ERB2/HER-2/Neu (3) (4) (5) (6). It has also been demonstrated that HER-3 can form heterodimers with MET, AXL and IGF1R (7) (8) (9). HER-3 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). EGFR has a number of ligands, including amphiregulin, epidermal growth factor and transforming growth factor, while HER-2 has no known ligand and remains in an 'open,' active conformation (10) (11). However, HER-2/HER-3 dimerisation can occur independently of ligand binding (12). More lately it has been appreciated that HER-3 can also homodimerise on binding to the heregulin ligands, NRG1 and NRG2, so having intrinsic, non-EGFR or HER-2 mediated activity (13) (14). Downstream signalling of HER-3 is mainly via the PI3K/AKT pathway, with MAPK/ERK and JAK/STAT pathways also activated (15) (16).

ERBB3, (Erb-B Receptor Tyrosine Kinase 3) the HER-3 gene, is under the regulation of several miRNAs including miR125a, miR125b and miR205 (17) (18) (19). Studies have shown that the ubiquitin-proteasome pathway plays a significant role in cancer initiation and progression by regulating protein levels (20) (21) (22) (23). E3 ubiquitin ligases regulate EGFR family receptors. NRDP 1 mediates HER-3 degradation (24) (23). NRG1 stabilises USP8 which in turn stabilises NRDP1 (20) NEDD4 is a novel interaction partner and ubiquitin E3 ligase of HER-3 (21).

Evidence for the role of HER-3 in lung cancer pathogenesis is largely drawn from retrospective data sets correlating HER-3 expression with poor prognosis and metastatic development (25) (26) (27). Accumulating evidence supports the role of HER-3 in the development of resistance to EGFR targeted therapies (28) (29) (30) (31). From preclinical data, dual targeting of EGFR and HER-3 is capable of overcoming acquired resistance to EGFR inhibition (29) (31). MET amplification or overexpression is a mechanism of resistance to EGFR-TKI occurring in 5% of cases (32). HER-3 phosphorylation is observed in EGFR mutant tumours with MET amplification, indicating that MET activates PI3K/AKT pathway via HER-3 phosphorylation (33). MEK inhibition causes a transcriptional upregulation of both HER-2 and HER-3 and the formation of heterodimeric complexes in KRAS mutant non-small cell lung cancer (NSCLC) and colon cancer and combined MEK and dual EGFR-HER-2 inhibition has a synergistic effect (30). Mutations of HER-3 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 HER-3 receptor as well as the dimerization affinity with HER-2 and EGFR. (34) (35) (36).

Here, we explore HER-3 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 HER-3 expression and propose that HER-3 could be an alternate pathway for MEK pathway activation.

Methods

Patients and tissue samples

The Drug Development Unit at the Royal Marsden (RMH) recruits patients with advanced solid malignancies to early phase clinical trials. Patient with adeno-NSCLC attending for consultation in our phase I clinic provided written informed consent and were enrolled into a tissue for research consent. Approval was gained from the local research and ethics committee for the use of the clinical data. Archival formalin fixed paraffin embedded tumour blocks were analysed for HER-2 and HER-3 protein expression and genomic analysis for HER-3 copy number was performed. Patient clinical data were retrospectively collected from the RMH electronic patient record system.

Immunohistochemistry and scoring system

Tissue blocks were freshly sectioned and only considered for immunohistochemistry (IHC) analyses of HER-2 and HER-3 if adequate material was present. Protein expression on 3µm thick FFPE sections was assessed by a Pathologist blinded to the clinical data.

HER-3 and HER-2 antibody validation by siRNA and immunohistochemical assays were performed as previously described.

Briefly, HER-3 immunoreactivity was investigated using the rabbit monoclonal anti-HER-3 antibody clone D22C5 (#12708, Cell Signaling Technology, London, UK). HER-2 immunoreactivity was investigated using the mouse monoclonal anti-HER-2 antibody clone CB11 (#NCL-L-CB11, Leica Biosystems, Newcastle, UK). Rabbit and Mouse IgGs (#I-1000 and I-2000, Vector, Burlingame, CA, USA) were used as negative controls. For HER-3 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). HER-3 immunoreactivity was detected using Dako EnVision Flex high pH kit (#K800021-2, Agilent; Glostrup; Denmark). For HER-2 immunohistochemistry automated antigen retrieval and detection were performed using Leica Bond RX (Leica Biosystems; Newcastle; UK). HER-2 immunoreactivity was detected using the Bond Polymer Refine Detection system (#DS9800, Leica Biosystems; Newcastle; UK).

For HER-2, membranous expression was scored according to College of American Pathologists (CAP) HER-2 scoring guidelines (37). No staining or membrane staining that is incomplete and is faint/barely perceptible and in $\leq 10\%$ of tumour cells (Score 0); incomplete membrane staining that is faint/ barely perceptible and in $>10\%$ of tumour cells (Score +1); weak to moderate complete membrane staining observed in $>10\%$ of tumour cells (Score 2+) and circumferential membrane staining that is complete, intense and in $>10\%$ of tumour cells (Score +3). For HER-3 staining, the same guidelines were used to generate an ad-hoc HER-2-like score for membranous staining. Additionally, HER-2 and HER-3 cytoplasmic staining was visually assessed, percentage of cells staining weakly, moderately or strongly were noted and H score calculated.

Digital PCR

Digital polymerase chain reaction (PCR) was performed on a QX100 droplet digital PCR system (Bio-Rad). Copy Number Assays were performed with a FAM-ERBB3 validated Bio-Rad assay, reference ID: dHsaCP2500395 (67bp amplicon) and a recommended reference copy number Hex-assay for AP3B1, assay ID: dHsaCP2500348 (60bp 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 HER-3 expression and other binary data was analysed using two sided Fishers 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 (RSEM) for 507 adeno-NSCLC was downloaded from cBioportal for analysis (38). MEK activity score is an accumulation measurement of MEK pathway activity based on a previously validated 18-gene transcript signature of MEK signalling (39). The associations between gene expression levels (and with MEK signalling) were assessed by linear regression. 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 tumour 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 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 9 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 1 phase 1 trial drug.

HER-2 and HER-3 protein expression

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

Table 1
Membranous HER-3 and HER-2 protein
expression by immunohistochemistry.

HER-3 staining	% (n)	
0	35.5% (16)	57.8% (26)
1	22.2% (10)	
2	15.5% (7)	42.2% (19)
3	26.7% (12)	
HER-2 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 HER-3 positive and HER-3 negative patients

Clinical Characteristics	HER-3 positive % (n)	HER-3 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 1 Clinical Trial	17% (10)	26% (15)
TKI: tyrosine kinase inhibitor		

Correlation of HER-3 protein expression by IHC with clinical characteristics

The median overall survival from diagnosis for HER-3 positive patients was 40.5 months, and for HER-3 negative patients 30.8 months. There was no statistical difference in survival between HER-3 positive and HER-3 negative cases (Gehan-Breslow-Wilcoxon; $p = 0.4596$) (Fig. 1B). There was no difference in median survival between HER-3 positive and negative cases in those patients with EGFR mutations (median OS 42.6 months HER-3 positive, median OS 43.9 months HER-3 negative; Gehan-Breslow-Wilcoxon; $p = 0.4927$).

HER-3 gene amplification

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

EGFR and KRAS mutations and correlation with HER-3

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% (8 cases). HER-3 protein overexpression was present in 8.9% (4 cases) of *EGFR* mutation and in 14.2% (4 cases) of *KRAS* mutation. There was no correlation between HER-3 overexpression and *EGFR* positivity (Fishers exact test $p = 0.7043$) or HER-3 and *KRAS* positivity (Fishers exact test; $p = 0.6908$) (Fig. 2).

Baseline genomic data in TCGA

Interrogation of the PanCancer TCGA cancer genome atlas 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 (Fig. 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 ($r = 0.26$; $p = 8 \times 10^{-6}$) (Fig. 3B); amplification cases had 1.7 times higher expression of *ERBB3* RNA than diploid cases ($p = 0.004$).

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* was highly correlated with *ERBB2* expression levels ($r = 0.09$ and $p = 2.5 \times 10^{-23}$) (Figure S1). *ERBB3* expression also positively correlates with *ERBB4* expression ($r = 0.1$ and $p = 0.001$) but there was no correlation with the expression levels of *EGFR* or ($r = -0.12$ and $p = 0.5$).

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 (Fig. 3C). We measured absolute gene expression level of *ERBB* family genes and derived MEK pathway activity using a validated 18-gene MEK signature (39). *EGFR* RNA expression levels correlated with MEK activity ($r = 0.16$ and $p = 3.0 \times 10^{-5}$), *ERBB3* RNA expression did not correlate with MEK activity ($r = 0.09$ and $p = 0.78$) (Fig. 3D). We further characterised MEK activation score with gene expression levels of ERBB ligand and proteins of ubiquitination (Table 3). AREG (*EGFR* ligand) and *RNF41* (an ubiquitin ligase) had a significant positive correlation with MEK activity score.

Table 3
Correlation of MEK activity score with expression of genes of interest.

Gene	r-value of correlation	p-value for correlation
<i>EGFR</i>	0.16031096	3.04E-05
<i>ERBB2</i>	-0.0453439	0.73555119
<i>ERBB4</i>	0.08977428	0.21373994
<i>AREG</i>	0.27539042	1.68E-23
<i>NRG2</i>	-0.1101635	0.96120646
<i>NRG1</i>	-0.0513447	0.8346032
<i>HRG</i>	-0.0578008	0.08479387
<i>NEDD1</i>	-0.0384113	0.36325262
<i>RNF41</i>	0.13750166	0.0009244
<i>USP8</i>	0.03146702	0.52317997
EGFR; epidermal growth factor, AREG: amphiregulin, NRG2: neuregulin 2, NRG1: neuregulin 1, HRG: heregulin, NEDD1: neural precursor expressed, developmentally down-regulated protein1, RNF41: ring finger 41, USP8: ubiquitin-specific protease 8		

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 ($p = 0.12$) (Fig. 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

HER-3 protein levels in adeno-NSCLC and mechanism of overexpression

Reports of the incidence of HER-3 protein overexpression in NSCLC vary greatly according to the cohort. Previous groups have reported rates of 6.5% (NSCLC) (40), 15.9% (advanced adeno-NSCLC) (27), 35% (EGFR mutant NSCLC) (41), 64% (stage 1 NSCLC) (42), 85.1% (resected stage I-IIIa adeno-NSCLC) (43)

and 86.1% (resected stage 0 and 1A1 adeno-NSCLC) (44). IHC methods and scoring have not been previously standardised. Our data uses a validated and robust scoring system based on optical density and H-score. Although our patient cohort is small, we demonstrate that overexpression of HER-3 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 (45). The cBioportal and GENIE data sets report amplification in just over 1% frequency (38). A smaller series of 71 cases reported a 1% amplification rate in lung adenocarcinoma (34). In a series of 82 NSCLC patients HER-3 amplification was identified in 26.8% of patients who were treated with the EGFR TKI gefitinib. *ERBB3* amplification was more common in those with *ERBB2* gain ($p = 0.055$) and *EGFR* gain ($p = 0.06$) but not with *EGFR* mutations ($p = 0.17$) (46). It was not possible to perform *ERBB3* PCR on all cases in our cohort as tumour tissue was limited, but a sample of 27 cases had sufficient tumour sample to pass quality control. There appeared to be clustering of HER-3 overexpressing cases in those with *ERBB3* copy number gain but this does not account for the majority of HER-3 overexpression.

Bioinformatic analysis of large, publicly available datasets allows testing of hypotheses. We interrogated the PanCancer TCGA data set of adeno-NSCLC samples. This dataset includes transcriptome (RNA) and genomic (DNA) data. The genomic data supports our finding of low levels of *ERBB3* amplification, which was the most common cause of *ERBB3* genetic alteration. It also provided the confirmation that *ERBB3* copy number alteration highly associates with its gene expression.

The MEK activity score is an 18 gene signature derived from an experiment to test a mixed-tumour response to the MEK inhibitor, selumetinib. The MEK activity score is a surrogate marker of MEK pathway activation. It is not specific for activation through the HER-3 channel as the MEK pathway is also initiated upon HER-2, EGFR and other receptor activation. It also does not take into account HER-3 downstream activation via the PIK3A/AKT/mTOR pathway. However, as HER-3 activity is predominantly via HER-2/HER-3 and EGFR/HER-3 hetero-dimerisation, we inferred that the MEK activity score could be used as a surrogate of, but not specific to, HER-3 protein expression. If a key node in the HER-3 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. *RNF41* also had a significant positive correlation with MEK activity score. This is difficult to interpret given the fact that an increase in a degradation protein would be expected to correlate with a lower target protein level.

In conclusion, HER-3 protein is common in adeno-NSCLC, amplification levels of *ERBB3* are low and on TCGA analysis the mechanism of HER-3 protein overexpression does not appear to be *ERBB3* ligand dependent. Further investigation is required to elucidate the mechanism of HER-3 protein overexpression in adeno-NSCLC.

HER-3 expression as a prognostic marker

Yi *et al*²⁶ demonstrated that HER-3 overexpression is associated with a poor prognosis in a cohort of 443 patients with advanced NSCLC (27). The median overall survival in our study was not significantly different between the HER-3 negative and positive patient group. This phase 1 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 co-morbidities.

HER-3 in relation to oncogenic drivers in adeno-NSCLC

HER-2 protein overexpression is reported in 6–30% of patients with lung cancer, which in keeping with our results (47) (48) (49). HER-2 expression is less frequently observed in NSCLC than HER-3 expression. There was some overlap, though no statistically significant association between, HER-3 expression with HER-2 expression or *KRAS* and *EGFR* mutations. Previous data have demonstrated that HER-3 plays a role in EGFR TKI resistance. Half of the cases in this cohort with EGFR mutation also co-expressed HER-3. No difference in overall survival was seen between HER-3 expressing and non-expressing groups in the EGFR-mutant cases in this cohort though patient numbers were small.

We therefore conclude that HER-3 represents a common target in NSCLC independent of *KRAS* and *EGFR* mutations. We have a simple tool in the form of IHC that can be used to “pre-screen” and stratify patients to receive such HER-3 targeting compounds. Furthermore, there are some patients in which HER-3 expression occurs concurrently with driver mutations and the role of HER-3 in these groups, including in drug resistance, requires further investigation. The relationship between HER-3 expression and other drivers in NSCLC (such as ROS, ALK, NTRK and RET) is yet to be determined.

HER-3 and functional activity in adeno-NSCLC

In the TCGA dataset MEK activity score was associated with *EGFR* and *AREG* (EGFR ligand) gene expression. This suggests that signalling via EGFR is key to the oncogenic behaviour in lung cancer. EGFR has been previously reported as a key driver of oncogenesis in lung (and other) cancers (50). Although our initial analysis did not show correlation of *ERBB3* with MEK signature, we stratified *ERBB3* expression into two groups; “expression low” and “expression high”; and analysed 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. This supports the hypothesis that HER-3 is an alternative pathway for MEK activation in the HER-3 expression high group. This analysis implies a degree of functional downstream pathway signalling via HER-3. In effect, this suggests that when EGFR drives the cancer, the effect of HER-3 is minimal as EGFR drives the downstream signalling. However, as HER-3 expression increases this becomes the main driver of pathway activation. This finding has relevance in terms of proposing HER-3 as a viable drug target in adeno-NSCLC. As a membrane target HER-3 can be potentially targeted with drugs such as HER-3 target antibody drug conjugates in which expression on the surface is key to activity. However, a degree of functional MEK pathway activation

suggests that in some patients drugs that rely on abrogation of signalling (such as HER-3/ HER-2 dimerisation drugs) can also be attempted. The challenge in this patient cohort is to predict patients in which this approach will be effective, our data relies on gene expression but transcriptome analysis is complex in a clinical trial setting.

Conclusion

In conclusion, HER-3 protein is commonly overexpressed in adeno-NSCLC though gene amplification levels are low. In the search for non-genomic causes of HER-3 protein overexpression, the MEK activity score was used as a surrogate of HER-3 overexpression; however no correlation with ligands of HER-3 were seen. Further investigation is therefore needed to continue to elucidate the mechanism of HER-3 overexpression. In adeno-NSCLC, signalling via the EGFR pathway is predominant but in the HER-3 expression high group HER-3 is an alternative pathway, with downstream signalling via the MEK pathway. Since HER-3 overexpression is more common than previously thought and appears to be functional in non-EGFR driven adeno-NSCLC, its utility as a therapeutic target in adeno-NSCLC should be a drug development priority.

List Of Abbreviations

Adeno-NSCLC	Adenocarcinoma of the lung
AKT	Protein kinase B
ALK	Anaplastic lymphoma kinase
AREG	Amphiregulin
AXL	Tyrosine-protein kinase receptor UFO
CAP	College of American Pathologists
CNV	Copy number variant
EGFR	Epidermal growth factor receptor
ERBB3	Erb-B Receptor Tyrosine Kinase 3
ERBB4	Erb-B Receptor Tyrosine Kinase 4
ERK	Extracellular Receptor Kinase
FFPE	Formalin fixed paraffin embedded
HER-1	Receptor tyrosine- protein kinase erbB-1
HER-2	Receptor tyrosine- protein kinase erbB-2
HER-3	Receptor tyrosine- protein kinase erbB-3
IGF-1	Insulin- like growth factor 1
IGF1R	Insulin-like growth factor 1 receptor
IHC	Immunohistochemistry
JAK	Janus Kinase
KRAS	Kirsten rat sarcoma
MAPK	Mitogen activated protein kinase
MEK	Mitogen-activated protein kinase kinase 1
mTOR	Mammalian target of rapamycin
NEDD4	Neural precursor expressed 4
NRDP1	Neuregulin receptor degradation protein-1
NRG-1	Neuregulin 1
NRG-2	Neuregulin 2
NSCLC	Non small cell lung cancer
NTRK	Neurotrophic tropomyosin-related kinase

PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol 3-kinase
RET	Rearranged during Transfection tyrosine kinase
RMH	Royal Marsden Hospital
RNF41	Ring Finger Protein 41
ROS	C-ros proto-oncogene
siRNA	Small interfering RNA
STAT	Signal Transducer and Activator of Transcription.
TGCA	The Cancer Genome Atlas
TKI	Tyrosine kinase inhibitors
USP8	Ubiquitin-specific protease 8

Declarations

Ethical approval and Consent to participate

Approval was obtained from the Royal Marsden Committee for Clinical Research (ref SE808). Written informed consent was obtained from participants prior to obtaining their archival tissue for research.

Consent for publication

Not applicable

Availability of supporting data

The datasets supporting the conclusions of this article are available in the within the article and its supplementary materials. Some of these data were derived from the following resources available in the public domain: The Cancer Genome Atlas <https://portal.gdc.cancer.gov/projects>.

Competing interests

T Manickavasagar	None declared
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J de Bono	Has served on advisory boards and received fees from many companies including Astra Zeneca, Astellas, Bayer, Boehringer Ingelheim, Cellcentric, Daiichi, Genentech/Roche, Genmab, GSK, Janssen, Merck Serono, Merck Sharp & Dohme, Menarini/Silicon Biosystems, Orion, Pfizer, Qiagen, Sanofi Aventis, Sierra Oncology, Taiho, Vertex Pharmaceuticals. He is an employee of The ICR, which have received funding or other support for his research work from AZ, Astellas, Bayer, Cellcentric, Daiichi, Genentech, Genmab, GSK, Janssen, Merck Serono, MSD, Menarini/Silicon Biosystems, Orion, Sanofi Aventis, Sierra Oncology, Taiho, , Pfizer, Vertex, and which has a commercial interest in abiraterone, PARP inhibition in DNA repair defective cancers and PI3K/AKT pathway inhibitors (no personal income). He was named as an inventor, with no financial interest, for patent 8,822,438. He has been the CI/PI of many industry sponsored clinical trials. JDB is a National Institute for Health Research (NIHR) Senior Investigator.

All unrelated to this work

A Minchom

Has served on advisory boards and received fees from Merck, FARON, Novartis, Bayer, Janssen

All unrelated to this work

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Authors' contribution

All authors read and approved the final manuscript.

TM	Data interpretation, drafted work
WY	Data analysis, data interpretation, drafted work
SC	Data analysis, data interpretation, revised work
BG	Data analysis, data interpretation, revised work
SM	Data analysis, data interpretation, revised work
AF	Data analysis, revised work
MC	Data analysis, revised work
RR	Data analysis, revised work
CB	Data analysis, revised work
MERO	Revised work
JB	Revised work
SP	Revised work
UB	Revised work
JL	Revised work
JdB	Revised work
AM	Data interpretation, drafted work

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Nil

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Figures

A **B** **C** **Figure 1**

Adeno-NSCLC patient samples. Panel of HER-3 negative cases (0-1+) and HER-3 positive cases (2-3+) (A), Kaplan-Meier curve of HER-3 positive versus HER-3 negative cases (B), ERBB3 gene copy number and HER-3 IHC (C) AP3B1; Adaptor Related Protein Complex 3 Subunit Beta 1, ERBB3: Erb-B2 Receptor Tyrosine Kinase 3, HER-3: receptor tyrosine-protein kinase erbB-3, IHC: immunohistochemistry, NSCLC: non-small cell lung cancer

Figure 2

Frequency of aberrations in NSCLC as percentage of total population EGFR: epidermal growth factor receptor, ERBB3: Erb-B2 Receptor Tyrosine Kinase 3, HER-3: receptor tyrosine-protein kinase erbB-3; NSCLC: non-small cell lung cancer

A **B** **C** **D** **Figure 3**

Adeno-NSCLC TCGA. Frequency of HER family genetic alterations (A); ERBB3 RNA expression with ERBB3 CNA (B), Gene expression of EGFR, ERBB2, ERBB3, and ERBB4 (C); Correlation of ERBB3 expression level with MEK activity (D). CNA: copy number aberration, EGFR: epidermal growth factor receptor, ERBB3: ErbB2 Receptor Tyrosine Kinase 3, HER-3: receptor tyrosine-protein kinase erbB-3; NSCLC: non-small cell lung cancer, TCGA: The Cancer Genome Atlas

Figure 4

Correlation of gene expression with MEK activity in adeno-NSCLC TCGA. Correlation of ERBB3 expression level with MEK activity in ERBB3 expression high cancers (A), Correlation of ERBB3 expression level with MEK activity in ERBB3 expression low cancers (C). Correlation of EGFR expression with MEK activity in ERBB3 expression high cancers (B), correlation of EGFR expression with MEK activity in ERBB3 expression low cancers (D) HER-3: receptor tyrosine-protein kinase erbB-3, MEK: Mitogen-activated protein kinase kinase, NSCLC: non-small cell lung cancer, RSEM: RNA-Seq by Expectation Maximization

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