Genomic aberrations guiding treatment of non-small cell lung cancer patients

Saber, Ali; Wekken, van der, Anthonie J.; Hiltermann, Thijo; Kok, Klaas; van den Berg, Johanna; Groen, Harry J. M.

Published in:
Cancer Treatment Communications

DOI:
10.1016/j.ctrc.2015.03.005

Publication date:
2015

Cancer Treatment Communications (2015) 4, 23–33

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/ctrc

Genomic aberrations guiding treatment of non-small cell lung cancer patients

Ali Saber[1,a], Anthonie van der Wekken[b,1], Thijo J.N. Hiltermann[b], Klaas Kok[c], Anke van den Berg[a], Harry J.M. Groen[b,*]

[a]*Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, The Netherlands*
[b]*Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen, P.O. Box 30.0001, 9700 RB Groningen, The Netherlands*
[c]*Department of Genetics, University of Groningen, University Medical Center Groningen, The Netherlands*

Received 28 January 2015; received in revised form 21 March 2015; accepted 28 March 2015

KEYWORDS
Non-small cell lung cancer;
Next generation sequencing;
Targeted therapy;
Resistance

Abstract
Lung cancer is the main cause of cancer-related death worldwide and conventional treatment strategies must be improved. In addition to mutations in several well-known cancer-associated genes, recent advances in sequencing technology have led to the discovery of numerous novel gene mutations and translocations. Some of these genomic aberrations occur at similar frequencies in all lung cancer subtypes, whereas others appear to be specific for adenocarcinoma or squamous cell lung cancer. High frequency mutations or recurrent translocations support involvement of the affected genes in the pathogenesis of lung cancer. The presence of activating aberrations is indicative for putative driver genes that might be essential for tumor cell growth and survival. These driver genes are potential targets for developing new treatments for lung cancer patients. Indeed, multiple tyrosine kinase inhibitors (TKIs) are currently used to treat lung cancer patients based on the presence of activating mutations, and novel drugs are under investigation. Patients benefit for about one year from current targeted treatments, but progression of disease inevitably occurs and resistance of the tumor to the TKI used can be observed in re-biopsied tumor samples. The aim of this review is to provide an overview of mutated genes in non-small cell lung cancer, an overview of targeted treatment strategies that are currently applied, and the known resistance mechanisms.

1. Lung cancer

Lung cancer is the leading cause of cancer-related deaths worldwide, with over 228,000 new cases and more than 159,000 deaths reported in 2013 in the United States [1,2].

*Corresponding author. Tel.: +31 503616161; fax: +31 503619320.
E-mail address: h.j.m.groen@umcg.nl (H.J.M. Groen).
[1]These authors contributed equally in this manuscript.

http://dx.doi.org/10.1016/j.ctrc.2015.03.005
2213-0896/

Overall, the 5-year survival rate is about 16% and late diagnosis is significantly associated with poor prognosis [1]. Lung cancer can be divided into two main subtypes based on histology: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Approximately 85% of lung cancer patients are diagnosed with NSCLC [3], which can be further subdivided into three main groups, i.e. adenocarcinoma (AC), squamous cell carcinoma (SQCC), and large cell carcinoma [4]. The AC subtype used to be more frequent in women and non-smokers, but nowadays it is more frequent than other histological subtypes in both men and women [3]. The diagnosis of lung cancer is made by histology/cytology of a tumor detected by imaging techniques such as computed tomography (CT) and positron emission tomography (PET) [5]. Treatment of lung cancer patients depends primarily on the performance status, stage of the disease, the presence of oligometastases and on histological type [6]. Surgery is the primary treatment for patients with stage I or II NSCLC [7], although adjuvant chemotherapy is advised by many guidelines to increase survival of the patients. In non-resectable, stage III NSCLC disease, chemoradiation is the preferred treatment [8]. Nowadays, treatment of lung cancer patients with advanced disease is guided by mutation analysis in the case of a documented tumor-driver mutation. The number of different tyrosine kinase inhibitors (TKIs) available for the treatment of non-small cell lung cancer patients is rapidly increasing due to new diagnostic developments.

In this review we give a brief overview of genes mutated in lung cancer, followed by a more in depth overview on potential therapeutic targets identified by next generation sequencing (NGS) technology. We also provide an overview of current targeted treatment approaches and the known resistance mechanisms.

2. Mutational landscape of lung cancer

Lung cancer, like other malignant neoplasms, is a result of an accumulation of different genetic alternations during life [9]. The *TP53* gene, originally described in 1979 [10], was the first tumor suppressor gene to be discovered. *TP53* is mutated in approximately 45% of NSCLC patients [11]. In 1982, a human gene with transforming activity was identified in a lung carcinoma cell line. This gene is homologous to the Kirsten Rat Sarcoma virus [12] and was referred to as *KRAS*. Mutations in *KRAS* are mostly found in codons 12, 13, and 61. They occur more frequently in patients with AC (5–40%) than in other subtypes of lung cancer, and are associated with smoking behavior [9].

Developments in sequencing technologies in recent years and the need to identify novel therapeutic targets have encouraged scientists to sequence large numbers of lung cancer samples. Entire gene families like protein kinases [13,14] or a combination of genes known to be mutated in lung cancer and other cancer types [15] have been analyzed. Analysis of 518 protein kinases in 33 primary lung tumors and cell lines revealed 188 somatic mutations in 141 genes, including genes known to have a role in lung tumorigenesis. For 21 genes, mutations were found in more than two samples. Seven of these genes had mutations in the kinase domain, including *BRAF*, *MAP2K4* and *FGFR2*. In addition, activating mutations were identified in *FGFR1* and *EPHA5* and inactivating mutations in *ATM* [13]. Analysis of 623 genes in 188 lung AC specimens by Ding et al. (2008) revealed 26 frequently mutated genes, including well-known cancer related genes such as *TP53*, *RB1*, *EGFR* and *KRAS* [15]. In addition, they also identified mutations in oncogenes such as *ERBB4* (*HER4*), *ERBB2* (*HER2*) and in multiple ephrin receptors (EPHAs). Altogether they observed a significant excess of mutations and copy number changes in genes involved in the mTOR, MAPK, Wnt, and the p53 signaling pathways [15]. Mutation analysis of the coding regions of more than 1500 genes of 134 primary lung tumors revealed that 18 and 19 genes were mutated at a frequency significantly above the background mutation rate in AC and SQCC, respectively. Five of these genes including *TP53*, *KRAS*, *KEAP1*, *MUC16*, and *BAI3* were shared between AC and SQCC. Differences in the set of mutated genes for various subtypes suggest that different mechanisms are involved in tumorigenesis [16]. Targeted sequencing of 145 cancer-related genes in 24 NSCLC biopsy samples, by Lipson and colleagues in 2012, revealed recurrent mutations in 21 genes, including well-known lung cancer genes together with mutations in druggable lung cancer genes such as *BRAF* and *EGFR* [17].

Together, these initial targeted and high throughput approaches indicated several targets, such as *EGFR*, *KRAS*, *BRAF* and *EML4-ALK*, that are nowadays treated with selected targeted drugs in the clinic. Although only a small proportion of all NSCLC patients (approximately 7%) will profit from these treatments (patients with complete and partial response), several tens of thousands of patients can still benefit worldwide because about 25% of patients with the subtype histology AC are suitable for studies with targeted therapies.

2.1. Potential therapeutic targets identified by next generation sequencing

Whole genome and exome sequencing (WGS and WES, respectively) have enabled researchers to dig even deeper into the mutational landscape of different cancer subtypes. These developments led to increased output of sequencing studies [18]. NGS gives us the opportunity to generate large amount of sequencing data within limited time period in a more cost effective way compared to conventional sequencing. Although, NGS is being improved every day, still we need to be careful in data interpretation and mutation calling. For instance, artifacts that can occur during sample preparation, amplification bias and DNA polymerase error should be always taken into account while working with NGS data [19].

A comprehensive overview of mutation frequencies per gene for all types of cancer is given in the COSMIC database (⟨http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/⟩). For lung cancer, the top-20 most commonly mutated genes are shown in Fig. 1.

The first studies on lung cancer using massively parallel sequencing have been performed on either cell lines or single primary tumors [20-22]. A complete genomic analysis of a single NSCLC primary tumor revealed more than 50,000 somatic variations, including new mutations in genes known

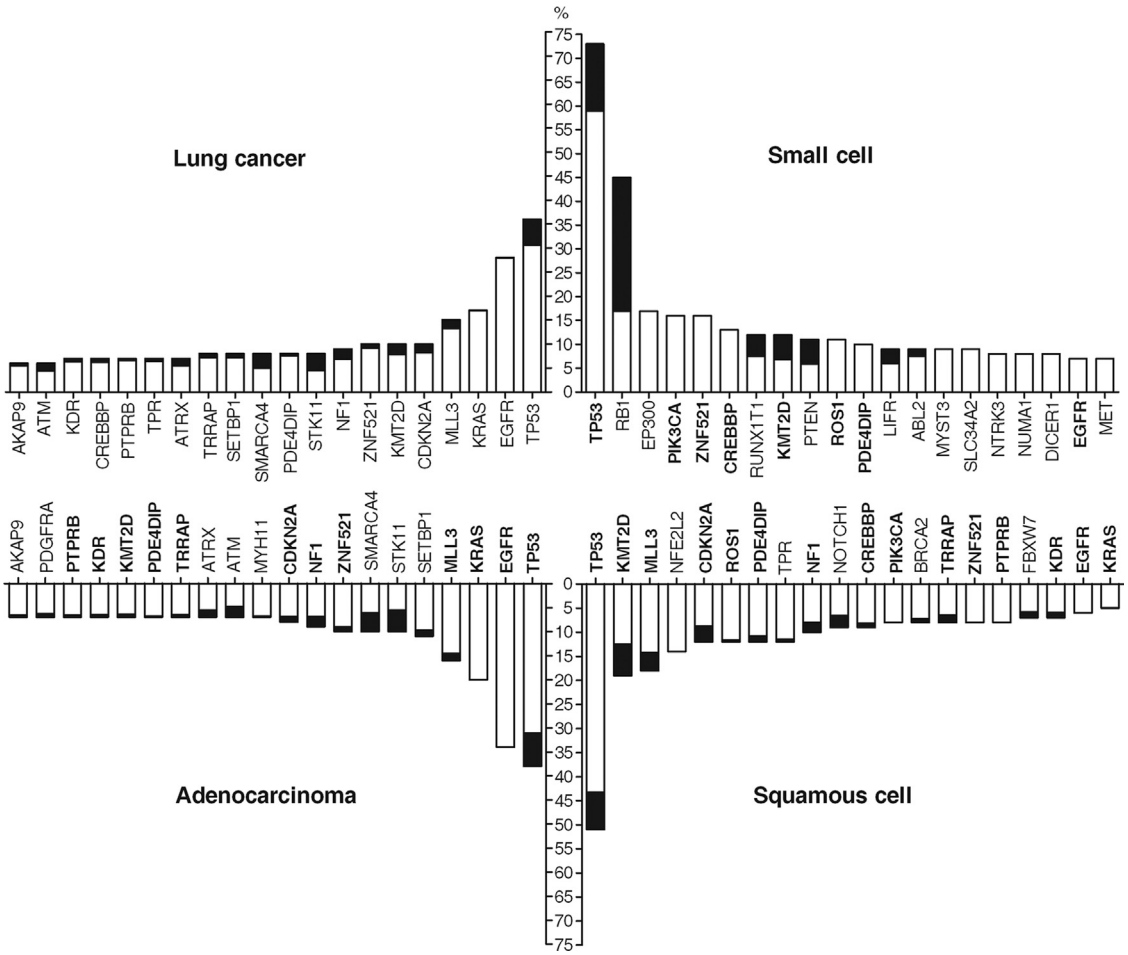

Fig. 1 A schematic representation of the top-20 mutated genes in lung cancer overall and in the three main subtypes based on the COSMIC database (2014). In this database, mutation frequencies were calculated using a weighted average mutation frequency based on sample size across all studies. See the COSMIC website for more information (⟨http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/⟩). Black bars: Nonsense and indel frameshifts; white bars: missense, synonymous, in-frame ins/del, complex and other mutations. Bold genes: These are among the top-20 genes in at least 2 out of 3 subtypes.

to be mutated at a high frequency in lung cancer, such as *NF1* [20]. Mutation analysis using WGS on a lung AC and a liver metastasis of the same patient resulted in the detection of a *KIF5B-RET* fusion gene [23] similar to the previous report [17]. As *KIF5B-RET* is quite common, it might have an important functional role in the pathogenesis of lung cancer.

Liu and colleagues [24] performed WES on a cohort of 31 primary non-small cell lung cancer (NSCLC) tumors and showed somatic mutations in several known lung cancer genes, including some of the targetable ones such as *EGFR* and *ERBB2* (*HER2*). Moreover, mutations were identified in *DCC* and *MML3*, which are targets for treatment in colon cancer and leukemia, respectively. *CSMD3* mutations were detected in 19% of the patients; notably, this gene was the second most frequently mutated gene in their cohort after *TP53*.

WGS in a panel of 17 NSCLC primary tumors revealed nine commonly mutated genes, including several known lung cancer genes and three novel lung cancer genes such as *DACH1*, a tumor suppressor gene in prostate cancer and gliomas, and two fusion genes: *EML4-ALK* and *KDELR2-ROS1* [25]. They also detected mutations in *JAK2* similar to a previous report using a targeted approach [17]. JAK2 inhibitors are used to treat patients with myelofibrosis [26].

One of the largest studies to date performed WES on 178 lung SQCC samples and identified over 48,000 non-silent mutations [27]. As expected, *TP53* was the most commonly mutated gene with a frequency of 81%. Inactivating mutations in *CDKN2A*, *KEAP1*, *CUL3*, *NOTCH1* and *NOTCH2* were identified, as well as activating mutations in and amplification of *NFE2L2*, a key component of the oxidative stress response pathway [27]. Activation of the NFE2L2-KEAP1 signaling pathway through mutations in *NFE2L2* is a characteristic of pediatric liver tumors as well [28]. The Cancer Genome Atlas (TCGA) Network also found *SOX2* and *TP63* to be activated via amplification. These two genes are important components of the squamous cell differentiation pathway. They identified three tyrosine kinase families which are potentially targetable, including ERBB, FGFR and JAK, all of which were mutated and/or amplified [27].

In a study [29] of 183 lung AC samples, 25 genes were significantly mutated, of which 19 had been reported previously and six were novel genes in lung AC, including

CHEK2 and *BRD3*. In this study the authors also found inactivating mutations in *STK11*, *PTEN*, *RB1*, *SETD2* and *CDKN2A* [29] and *CTNNB1* was found to be mutated in 3% of the patients. This gene is highly mutated (70%) in liver cancer patients and it is a component of WNT-CTNNB1 pathway [28,29].

A study [30] on a large cohort of Korean patients ($n=104$) with SQCC showed a good overlap with sequencing data previously published by TCGA Network [27], but also reported some marked differences. *CDKN2A* mutations were less common in Korean patients, while mutations in *RB1* were significantly more frequent. Interestingly, they found activating mutations in *PIK3CA*, which is one of the therapeutic targets of the PI3K/AKT/mTOR pathway. In one of the Korean lung cancer samples, an FGFR3-TACC3 fusion transcript was detected [30]. *FGFR3* has been reported as a potential therapeutic target in glioblastoma and bladder cancer [31,32].

Together, these next generation studies have made significant contributions to the identification of genes that are of interest as novel targets for therapy. To select the most promising target, it is essential to reveal the impact of the mutations and to discriminate between activating driver mutations and non-driver or inactivating mutations. In summary, next generation and targeted sequencing indicate that genes such as *ERBB2* (*HER2*), *ERBB4*, *JAK2*, *RET*, *ROS1*, *DCC*, *MLL3* might be good candidates for targeted treatment in lung cancer patients. In addition, inhibitors of the MEK kinase could be tested in tumors with *NF1* mutations. More importantly, *PIK3CA* seems to be a suitable candidate, together with *FGFR3* and *NFE2L2*, for treatment of patients with SQCC. At the moment, inhibitors for RET and ROS1 fusions are in preclinical and clinical trials and ROS1 inhibitors might also be effective on patients with activating *ROS1* mutations.

3. Targeted therapies currently in use

Specific aberrations in genes or pathways can lead to increased protein levels, and/or pre-active protein kinases that stimulate tumor cell growth. These aberrations can be targeted with small molecules such as TKIs (administered orally) and/or with monoclonal antibodies that are administered intravenously. At the moment, more clinical targets have been discovered in AC than in SQCC. Commonly used therapies in AC target the tyrosine kinase part of *EGFR*, *HER2*, *VEGFR* and ALK protein [33]. In addition, a number of novel drugs against *KRAS* (AC/SQCC), *BRAF* (AC/SQCC), *ROS1* (AC), *RET* (AC), *FGFR1* (SQCC), *PIK3CA* (AC/SQCC) and *DDR2* (SQCC) are being evaluated in clinical trials or soon will be [34]. The known target genes, the kinase inhibitors used, and clinical outcomes in non-SQCC clinical trials phase III are summarized in Table 1. No phase III study has been performed in SQCC yet. Therefore, we only focus on non-SQCC. Frequencies of hotspot mutations, fusion genes and possible resistance mechanisms are summarized in Tables 2 and 3.

3.1. EGFR inhibitors

TKIs, such as erlotinib, gefitinib, and afatinib, are currently registered for treatment of *EGFR* mutant non-SQCC patients. Patients with activating *EGFR* mutations, such as exon 19 insertions/deletions, and nonsynonymous mutations such as G719X in exon 18, or L858R and L861Q in exon 21, are sensitive to erlotinib and gefitinib, with a tumor response of about 75%. Patients with such mutations show an increased progression-free survival (PFS) after targeted treatment compared to treatment with chemotherapy [35]. This effect was observed in both first and second line treatment. Hazard ratios for progression-free survival varied between 0.43 and 0.34. Most pivotal studies of first-line EGFR-TKI were limited to lung cancer patients carrying *EGFR* exon 19 deletions or L858R point mutations. Only patients with *EGFR* exon 19 deletions showed an overall survival benefit with afatinib.

The effect of EGFR-TKI in patients with rare *EGFR* mutations has not been defined but seems less striking [36]. A meta-analysis revealed a significant association between increased *EGFR* copy number and improved survival outcome [37]. In NSCLC, *EGFR* mutation screening is the best method to predict tumor response to TKIs compared to fluorescence in situ hybridization (FISH) and immunohistochemistry [38]. In almost 25% of patients tumor growth is enhanced when *EGFR* treatment is ended, indicating that the tumor remains at least partly dependent on the EGFR signaling [39].

3.2. Mechanisms of resistance to EGFR inhibitors

After 9 to 12 months of treatment with an EGFR-TKI, the tumor appears to become resistant due to a spectrum of mechanisms. In about 50% of cases, an originally low-frequency TKI-resistant mutation, the T790M (gatekeeper) mutation, can increase to detectable and clinically relevant frequencies upon treatment with a TKI. In addition, amplification of *MET* or *HER2*, mutations in *PKI3CA* or *BRAF*, activation of the *AXL* kinase, and transformation to small cell lung cancer are the most prominently induced resistance mechanisms to TKI treatment [34,40]. These last two mechanisms have been proven in cell lines, xenografts or tumors of patients, and can therefore be considered as truly causative in relation to the resistance [41-43]. Some authors proposed that new mutations developing in *EGFR* (like the T790M gatekeeper mutation) are associated with resistance in patients treated with EGFR-TKI [40], but also mutations in other genes such as *GAS6*, *VIM*, *NF-kB*, *ADAM17* and *NOTCH1* have been found in tumors of patients who became resistant to EGFR-TKI [43-45]. Finally, in studies using cell lines, mutations in *COL6A1*, *IGFR1*, *TGFB or mTOR* genes or mutations affecting the Hedgehog, pp.53, Wnt pathways were found to be associated with resistance mechanisms [43]. A true causal relation needs to be proven for these mechanisms.

3.3. BRAF inhibitors

BRAF is a serine/threonine kinase that is activated by RAS and signals its proliferative actions through MEK kinase. *BRAF* mutations are more common in lung ACs but are observed in less than 1% of NSCLC tumors [46]. In a study of 697 lung AC patients, *BRAF* mutations were present in 18 patients (3%), half were V600E (50%), the other most common mutations were G469A (39%) and D594G (11%) [47]. NSCLC patients with

Table 1 Overview of targeted phase III studies in advanced non-SQCC.

Protein/Inhibitors	Mutation % in non-SQCC subtype	Study	Progression-free survival hazard ratio (95% CI)
EGFR	10%		
erlotinib		EURTAC	0.37 (0.25-0.54)
		OPTIMAL	0.16 (0.11-0.26)
gefitinib		IPASS	0.48 (0.36-0.64)
		WJOTG3405	0.52 (0.38-0.72)
		NEJ002	0.32 (0.24-0.44)
afatinib		LuxLung 3	0.58 (0.43-0.78)
		LuxLung 6	0.28 (0.20-0.39)
BRAF	3%		
dabrafenib			N/A
Trametinib			N/A
vemurafenib			N/A
ALK	3-6%		
crizotinib		PROFILE 1007	0.49 (0.37-0.64)
alectinib			N/A
ceritinib			N/A
ROS1	1%		
crizotinib			N/A
RET	1%		
vandetanib			N/A
sunitinib			N/A
sorafenib			N/A

No phase III study in SQCC is available. N/A: Not available

the *BRAF* V600E mutation have a shorter disease-free survival and overall survival after chemotherapy than patients without such mutations. At the moment, vemurafinib and dabrafenib are the two *BRAF* inhibitors that are clinically available. There are two case reports of lung AC patients with a V600E mutation who responded to treatment with vemurafinib [48,49]. The first, still ongoing, experience with dabrafenib in advanced NSCLC showed a tumor response rate of 40% [50]. Preclinical data suggest that non-V600E-mutated BRAF kinases are resistant to vemurafenib. In addition, *BRAF* mutations may predict sensitivity of NSCLC cells to MEK inhibitors [51].

3.4. Mechanisms of resistance to BRAF inhibitors

Different resistance mechanisms have been found in preclinical studies. A switch of full-length *BRAF* to aberrant *BRAF* (p61VE) has been shown in treated cell lines becoming resistant to BRAF inhibitors. Another mechanism that has been described in cell lines is upregulation of the EGFR protein due to loss of the *c-Jun* feedback loop [52]. A single lung cancer patient with a *BRAF* mutation and treated with dabrafenib showed a *KRAS* mutation in a re-biopsy at tumor progression. The hypothesis was therefore put forward that the cause of resistance was due to a G12D *KRAS* mutation [53]. Other mechanisms that have been described mostly in melanoma are MAPK-dependent mechanisms, like *NRAS* or *CRAF* upregulation, *BRAF* upregulation and *MEK* mutations. MAPK-independent mechanisms, like *PTEN* loss and upregulation of PDGFR-β and IGF-1 R, have also been described [54].

3.5. ALK inhibitors

EML4-ALK is a fusion gene generated by an inversion of a segment of chromosome 2. It was the first targetable fusion onco-kinase identified in NSCLC [34]. It is most often observed in young, light- or never smoking patients, occurring equally in males and females [55]. It can be detected in up to 4% of NSCLC patients [34] and 3-6% of patients with lung AC [56]. *ALK* fusion genes rarely coexist with *KRAS* or *EGFR* mutations in lung cancer patients [57]. The EML4-ALK fusion protein results in enhanced ALK kinase activity [55]. Crizotinib was the first registered ALK inhibitor used in clinical practice (Table 1). It was originally designed for inhibition of the c-MET protein but it turned out to have an inhibitory effect on ALK kinase as well [58]. The overall response rate in a FISH-based ALK-positive NSCLC group treated with crizotinib was 65% (95% confidence interval [CI]; 58-72) versus 20% (95% CI; 14-26) in the chemotherapy group. In a phase III study, the median PFS for crizotinib was 7.7 months. In the chemotherapy group PFS was 3.0 months [59].

Ceritinib and alectinib are also two potent second generation ALK-TKIs [60] that can be used in crizotinib resistant patients [61,62]. In a phase I clinical trial, Shaw and colleagues treated ALK-positive patients with ceritinib. Majority of the patients had been pretreated with crizotinib. The overall response rate with ceritinib was 58% (95% CI; 48-67), median PFS was 7 months in those who received >400 mg daily. Moreover, they observed tumor responses in patients with different *ALK* resistance mutations (L1196M, G1269A and S1206Y) as well as the patients without any detectable *ALK* mutation [61]. Treatment of an AC patient with alectinib

Table 2 Frequencies of mutation and fusion partners of therapeutic targets in non-SQCC.

Target	Mutation	Fusion partner	Frequency	References
EGFR				[91]
Exon 18	G719C/S/A		7%	
	other		4%	
Exon 19	E746_A750del		37%	
Exon 20	V769M		2%	
	D770G/Y		5%	
	T790M		2%	
	other		5%	
Exon 21	L833F		5%	
	A840T		5%	
	L858R		26%	
	other		2%	
BRAF	V600E		50%	[47]
	G469A		39%	
	D594G		11%	
ALK		*EML4*	89%	[34]
		KIF5B	4%	
		KLC1	4%	
		TFG	3%	
		PTPN3	N/A	
ROS1		*SLC34A2*	40%	[69,70]
		CD74	40%	
		SDC4	20%	
		EZR	N/A	
RET		*KIF5B*	69%	[77,78]
		CCDC6	15%	
		NCOA4	8%	
		TRIM33	N/A	

(300 mg twice daily) resulted in complete response [62]. Both drugs are also effective for brain metastasis.

3.6. Mechanisms of resistance to ALK inhibitors

ALK-positive patients develop tumor resistance to targeted therapy. This resistance can be due to gatekeeper mutations in the kinase domain of the *ALK* gene (L1196M and G1269A), copy number gain of the EML4-ALK fusion gene, and mutations in *EGFR* and *KRAS* [63]. Other reported *ALK* mutations linked to resistance are V1135E, L1152R, C1156Y, F1174L, L1198P, G1202R, D1203N, S1206Y, G1269S, G1269A and L1318M. Targeted NGS showed an association between the development of resistance to crizotinib in two patients and new nonsynonymous mutations outside the ALK kinase domain [15,63-65]. The observation of different nonsynonymous mutations in *MET* could be important because crizotinib also is a potent MET inhibitor [66]. And lastly, mutations in pathways of *AXL* and the development of epithelial-mesenchymal transition (EMT) have been described as factors that contribute to ALK-TKI resistance [67].

Table 3 Proposed resistance mechanisms in targetable mutations and translocations in non-SQCC.

Protein	Resistance mechanisms	References
EGFR	AXL/GAS6 axis activation	[43]
	Small cell transformation	[41,42]
	T790M mutation	[40]
	KRAS mutation	[40]
	MET/*HER2* amplification	[40]
	Mutations in *NF-kB*/*ADAM17*/ *NOTCH1*/*VIM*	[43-45]
	Mutations in COL6A1/ IGFR1/ Hedgehog/ TGFB/ p53/ Wnt/mTOR pathways	[40,43]
BRAF	NRAS/BRAF upregulation	[54]
	MEK mutations	[54]
	PTEN loss	[54]
	EGFR upregulation	[52,54]
	IGF-1R upregulation	[54]
	PDGFR-β upregulation	[54]
ALK	*ALK* mutation	[15,63-64]
	ALK copy number gain	[63]
	KRAS/*EGFR* mutations	[64]
	KIT amplification	[92]
	AXL overexpression	[67]
	EMT	[67]
ROS1	ROS1 mutations	[73,75]
	Signaling switch to EGFR	[74]
	KRAS/NRAS mutations	[76]
	KRAS overexpression	[76]

3.7. ROS1 inhibitors

In about 1-2% of patients with NSCLC, a translocation of *ROS1*, which is located on chromosomal region 6q22, has been found with different fusion partners (*SLC34A2*, *SDC4*, *CD74*, *EZR*). Preclinical studies and case reports show that *ROS1* kinase activity is inhibited by crizotinib. *ROS1* fusions occur more often in younger patients and in the AC subtype [68,69]. Crizotinib is a potent inhibitor of cell growth in cell lines as well as in patients with a *ROS1* fusion [70]. A phase I clinical trial by Shaw and colleagues on fifty ROS1-positive patients with AC subtype treated with crizotinib showed an overall response rate of 72% (95% CI; 58-84) and the median PFS of 19.2 months [71]. Another study with thirty two AC patients with ROS rearrangement treated with crizotinib showed an overall response rate of 80% and median PFS was 9.1 months [72]. No randomized studies have been published yet.

3.8. Mechanisms of resistance to ROS1 inhibitors

A G2032R mutation of *ROS1* was found in a crizotinib-resistant patient. Foretinib (a MET and VEGFR inhibitor) seems to be a potent compound to overcome this resistance [73]. In the search for resistance mechanisms in cell lines, a switch in signaling from ROS1 to EGFR was observed, when ROS1 was inhibited by crizotinib [74]. The same study

also reported that treatment with an EGFR inhibitor in combination with a ROS1 inhibitor was effective in cell lines resistant to ROS1 inhibitors. In another study, a new mutation, L2155S, was found in a ROS1-positive NSCLC cell line resistant to crizotinib. Authors also showed that L2155S and G2032R mutations can induce resistance to crizotinib in Ba/F3 cells [75]. KRAS/NRAS mutations or KRAS overexpression have been shown as other possible resistance mechanisms to crizotinib in HCC78-crizotinib resistant cell line [76].

3.9. RET inhibitors

A translocation of the rearranged during transfection (RET) gene located at chromosome 10 can be identified in about 1% of non-smoking patients with lung ACs. *KIF5B*, *CCDC6*, *TRIM33* and *NCOA4* serve as fusion partners [77,78]. As a result of this fusion, the normally low expression level of RET is increased in lung AC cells [79]. Over 12 drugs have been described wit RET inhibitory properties [80]. Most potent were cabozantinib (IC_{50} 4 nM), alectinib (IC_{50} 4.8 nM) and ponatinib (IC_{50} 7 nM) [80,81]. Vandetanib, sunitinib, sorafenib and cabozantinib are all multikinase inhibitors, and the first three show *in vitro* activity against RET expressing NIH3T3 and Ba/F3 cell lines [17]. It has been shown that alectinib can inhibit cell growth by suppressing phosphorylated RET both in cell line and RET-positive mouse model [81]. A case report confirmed the anti-tumor effects of vandetanib in a NSCLC patient [82]. In a phase II clinical trial, cabozantinib was tested on three RET-positive patients. Two of the patients showed partial response, while the other one remained with stable disease [78].

3.10. HER2 and cMET

HER2 is mutated (mostly exon 20) in approximately 2% of NSCLC patients [83]. Activating mutations in *HER2* will result in activation of downstream signaling pathways (AKT and MEK) leading to cell proliferation and survival [84]. Blocking HER2 in *HER2*-mutated cell line resulted in cell cycle arrest and cell death [85]. Treatment of sixteen NSCLC patients with different combination of HER2 inhibitors (showed a median PFS of 5.1 months [83].

cMET is a tyrosine kinase receptor (TKR) which is coded by *MET* proto-oncogene and it is widely expressed by cells with epithelial-endothelial origin [86]. Different mechanisms can lead to aberrant activation of MET such as *MET* gene mutation or amplification and cMET/HGF overxpression [87]. Preclinical studies revealed inhibition of cell growth by crizotinib in MET dependent lung cancer cell lines, while tivantinib is independent of MET signaling and results is apoptosis [88]. A phase I clinical trial showed that tivantinib in combination with erlotinib was well tolerated in advanced NSCLC patients based on CYP2C19 genotype [89]. A phase II study showed a median PFS of 3.8 months in erlotinib plus tivantinib group compared to erlotinib or tivantinib plus placebo group [90]. Several clinical trials are still ongoing.

4. Conclusion

Nowadays, more and more somatic mutations and fusion genes are being identified using NGS approaches. The affected genes can be considered as potential targets for treatment of NSCLC. At the moment, we only have a few gene mutations and fusion genes that can be targeted with TKIs, although many other specific TKIs are under investigation. A striking and common feature is that tumor resistance develops after about one year of targeted treatment. Therefore, the search for different resistance mechanisms is important so that treatment regimens can be adapted at an early enough stage. At the moment, most resistance mechanisms are described on the basis of their associations with newly detected mutations observed at disease progression. We need to put more effort into functional studies to discover the role of mutations in new and known cancer genes and to define novel therapeutic drivers, which may even be genes with mutations at low frequencies. This is important, because even a gene with a low mutation frequency can save a considerable number of patients with NSCLC.

In the future, we need to integrate the NGS results with epigenetic, transcriptome, copy number and proteomic analyses. This should preferably be done in primary tumors, metastases and the subsequent relapses with developing resistance to gain a good insight into the tumor cell evolution and to help design strategies to treat lung cancer patients optimally. Moreover, complete overviews of the mutational landscape of each patient's tumor will aid providing personalized therapy to patients and allow a timely switch to drugs that attack or work round resistance.

Combination therapies of different targeting drugs that are based on this mutational landscape will probably be more effective in prolonging the survival of patients and increasing their quality of live. Nowadays, the treatment of cancer patients should be based more on their genetic profiles and less on traditional organ- or cancer subtype-based strategies.

Three kinds of lung cancer patient groups may emerge in the future. In the first group are younger patients – mainly past, light or non-smokers – with limited somatic genomic instabilities that have one or two driver genomic aberration (*s*) that can be targeted with small molecules or combinations of these drugs. In the second group, smoking patients with SQCC usually have many somatic genomic alterations. These genomic changes might result in many abnormal peptides or proteins that can be recognized by the immune system and may induce an immune response. These patients will profit from immunotherapy. The third group still needs chemotherapy.

Conflict of interest

None.

Disclosure of funding

None.

References

[1] S.P. Nana-Sinkam, C.A. Powell, 3rd ed,Molecular Biology of Lung Cancer: Diagnosis and Management of Lung Cancer, 143, American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Cheste30S–e339.

[2] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (2013) 11–30.
[3] H. Francis, B. Solomon, The current status of targeted therapy for non-small cell lung cancer, Intern. Med. J. 40 (2010) 611–618.
[4] W.D. Travis, Pathology of lung cancer, Clin. Chest Med. 32 (2011) 669–692.
[5] B. Fischer, U. Lassen, J. Mortensen, S. Larsen, A. Loft, A. Bertelsen, J. Ravn, P. Clementsen, A. Hogholm, K. Larsen, T. Rasmussen, S. Keiding, A. Dirksen, O. Gerke, B. Skov, I. Steffensen, H. Hansen, P. Vilmann, G. Jacobsen, V. Backer, N. Maltbaek, J. Pedersen, H. Madsen, H. Nielsen, L. Hojgaard, Preoperative staging of lung cancer with combined PET-CT, N. Engl. J. Med. 361 (2009) 32–39.
[6] M.A. Socinski, T. Evans, S. Gettinger, T.A. Hensing, L.V. Sequist, B. Ireland, T.E. Stinchcombe, Treatment of stage IV non-small cell lung cancer: diagnosis and management of lung cancer, 3rd ed, American College of Chest Physicians evidence-based clinical practice guidelines. Cheste341S–e3368.
[7] M.A. van der Drift, H.E. Karim-Kos, S. Siesling, H.J. Groen, M.W. Wouters, J.W. Coebergh, E. de Vries, Janssen-Heijnen ML. Progress in standard of care therapy and modest survival benefits in the treatment of non-small cell lung cancer patients in the Netherlands in the last 20 years, J. Thorac. Oncol. 7 (2012) 291–298.
[8] E.E. Vokes, J.E. Herndon 2nd, M.J. Kelley, M.G. Cicchetti, N. Ramnath, H. Neill, J.N. Atkins, D.M. Watson, W. Akerley, M.R. Green, Induction chemotherapy followed by chemoradiotherapy compared with chemoradiotherapy alone for regionally advanced unresectable stage III Non-small-cell lung cancer: cancer and leukemia Group B, J. Clin. Oncol. 25 (2007) 1698–1704.
[9] K. Okudela, T. Woo, H. Kitamura, KRAS gene mutations in lung cancer: particulars established and issues unresolved, Pathol. Int. 60 (2010) 651–660.
[10] D.I. Linzer, A.J. Levine, Characterization of a 54 K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells, Cell 17 (1979) 43–52.
[11] T. Takahashi, H. Suzuki, T. Hida, Y. Sekido, Y. Ariyoshi, R. Ueda, The p53 gene is very frequently mutated in small-cell lung cancer with a distinct nucleotide substitution pattern, Oncogene 6 (1991) 1775–1778.
[12] C.J. Der, T.G. Krontiris, G.M. Cooper, Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses, Proc. Natl. Acad. Sci. USA 79 (1982) 3637–3640.
[13] H. Davies, C. Hunter, R. Smith, P. Stephens, C. Greenman, G. Bignell, J. Teague, A. Butler, S. Edkins, C. Stevens, A. Parker, S. O'Meara, T. Avis, S. Barthorpe, L. Brackenbury, G. Buck, J. Clements, J. Cole, E. Dicks, K. Edwards, S. Forbes, M. Gorton, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, D. Jones, V. Kosmidou, R. Laman, R. Lugg, A. Menzies, J. Perry, R. Petty, K. Raine, R. Shepherd, A. Small, H. Solomon, Y. Stephens, C. Tofts, J. Varian, A. Webb, S. West, S. Widaa, A. Yates, F. Brasseur, C.S. Cooper, A.M. Flanagan, A. Green, M. Knowles, S. Y. Leung, L.H. Looijenga, B. Malkowicz, M.A. Pierotti, B.T. Teh, S.T. Yuen, S.R. Lakhani, D.F. Easton, B.L. Weber, P. Goldstraw, A.G. Nicholson, R. Wooster, M.R. Stratton, P.A. Futreal, Somatic mutations of the protein kinase gene family in human lung cancer, Cancer Res. 65 (2005) 7591–7595.
[14] C. Greenman, P. Stephens, R. Smith, G.L. Dalgliesh, C. Hunter, G. Bignell, H. Davies, J. Teague, A. Butler, C. Stevens, S. Edkins, S. O'Meara, I. Vastrik, E.E. Schmidt, T. Avis, S. Barthorpe, G. Bhamra, G. Buck, B. Choudhury, J. Clements, J. Cole, E. Dicks, S. Forbes, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, A. Jenkinson, D. Jones, A. Menzies, T. Mironenko, J. Perry, K. Raine, D. Richardson, R. Shepherd, A. Small, C. Tofts, J. Varian, T. Webb, S. West, S. Widaa, A. Yates, D.P. Cahill, D.N. Louis, P. Goldstraw, A.G. Nicholson, F. Brasseur, L. Looijenga, B.L. Weber, Y.E. Chiew, A. DeFazio, M.F. Greaves, A.R. Green, P. Campbell, E. Birney, D.F. Easton, G. Chenevix-Trench, M.H. Tan, S.K. Khoo, B.T. Teh, S.T. Yuen, S.Y. Leung, R. Wooster, P.A. Futreal, M.R. Stratton, Patterns of somatic mutation in human cancer genomes, Nature 446 (2007) 153–158.
[15] L. Ding, G. Getz, D.A. Wheeler, E.R. Mardis, M.D. McLellan, K. Cibulskis, C. Sougnez, H. Greulich, D.M. Muzny, M.B. Morgan, L. Fulton, R.S. Fulton, Q. Zhang, M.C. Wendl, M.S. Lawrence, D.E. Larson, K. Chen, D.J. Dooling, A. Sabo, A.C. Hawes, H. Shen, S.N. Jhangiani, L.R. Lewis, O. Hall, Y. Zhu, T. Mathew, Y. Ren, J. Yao, S.E. Scherer, K. Clerc, G.A. Metcalf, B. Ng, A. Milosavljevic, M.L. Gonzalez-Garay, J.R. Osborne, R. Meyer, X. Shi, Y. Tang, D.C. Koboldt, L. Lin, R. Abbott, T.L. Miner, C. Pohl, G. Fewell, C. Haipek, H. Schmidt, B.H. Dunford-Shore, A. Kraja, S.D. Crosby, C.S. Sawyer, T. Vickery, S. Sander, J. Robinson, W. Winckler, J. Baldwin, L.R. Chirieac, A. Dutt, T. Fennell, M. Hanna, B.E. Johnson, R.C. Onofrio, R.K. Thomas, G. Tonon, B.A. Weir, X. Zhao, L. Ziaugra, M.C. Zody, T. Giordano, M.B. Orringer, J.A. Roth, M.R. Spitz, I.I. Wistuba, B. Ozenberger, P.J. Good, A.C. Chang, D.G. Beer, M.A. Watson, M. Ladanyi, S. Broderick, A. Yoshizawa, W.D. Travis, W. Pao, M.A. Province, G.M. Weinstock, H.E. Varmus, S.B. Gabriel, E.S. Lander, R.A. Gibbs, M. Meyerson, R.K. Wilson, Somatic mutations affect key pathways in lung adenocarcinoma, Nature 455 (2008) 1069–1075.
[16] Z. Kan, B.S. Jaiswal, J. Stinson, V. Janakiraman, D. Bhatt, H.M. Stern, P. Yue, P.M. Haverty, R. Bourgon, J. Zheng, M. Moorhead, S. Chaudhuri, L.P. Tomsho, B.A. Peters, K. Pujara, S. Cordes, D.P. Davis, V.E. Carlton, W. Yuan, L. Li, W. Wang, C. Eigenbrot, J.S. Kaminker, D.A. Eberhard, P. Waring, S.C. Schuster, Z. Modrusan, Z. Zhang, D. Stokoe, F.J. de Sauvage, M. Faham, S. Seshagiri, Diverse somatic mutation patterns and pathway alterations in human cancers, Nature 466 (2010) 869–873.
[17] D. Lipson, M. Capelletti, R. Yelensky, G. Otto, A. Parker, M. Jarosz, J.A. Curran, S. Balasubramanian, T. Bloom, K.W. Brennan, A. Donahue, S.R. Downing, G.M. Frampton, L. Garcia, F. Juhn, K.C. Mitchell, E. White, J. White, Z. Zwirko, T. Peretz, H. Nechushtan, L. Soussan-Gutman, J. Kim, H. Sasaki, H.R. Kim, S.I. Park, D. Ercan, C.E. Sheehan, J.S. Ross, M.T. Cronin, P.A. Janne, P.J. Stephens, Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies, Nat Med 18 (2012) 382–384.
[18] D. Bick, D. Dimmock, Whole exome and whole genome sequencing, Curr. Opin. Pediatr. 23 (2011) 594–600.
[19] E.J. Fox, K.S. Reid-Bayliss, M.J. Emond, L.A. Loeb, Accuracy of Next Generation Sequencing Platforms, Next Gen. Seq. Appl. (2014) 1.
[20] W. Lee, Z. Jiang, J. Liu, P.M. Haverty, Y. Guan, J. Stinson, P. Yue, Y. Zhang, K.P. Pant, D. Bhatt, C. Ha, S. Johnson, M.I. Kennemer, S. Mohan, I. Nazarenko, C. Watanabe, A.B. Sparks, D.S. Shames, R. Gentleman, F.J. de Sauvage, H. Stern, A. Pandita, D.G. Ballinger, R. Drmanac, Z. Modrusan, S. Seshagiri, Z. Zhang, The mutation spectrum revealed by paired genome sequences from a lung cancer patient, Nature 465 (2010) 473–477.
[21] E.D. Pleasance, P.J. Stephens, S. O'Meara, D.J. McBride, A. Meynert, D. Jones, M.L. Lin, D. Beare, K.W. Lau, C. Greenman, I. Varela, S. Nik-Zainal, H.R. Davies, G.R. Ordonez, L.J. Mudie, C. Latimer, S. Edkins, L. Stebbings, L. Chen, M. Jia, C. Leroy, J. Marshall, A. Menzies, A. Butler, J.W. Teague, J. Mangion, Y.A. Sun, S.F. McLaughlin, H.E. Peckham, E.F. Tsung, G.L. Costa,

C.C. Lee, J.D. Minna, A. Gazdar, E. Birney, M.D. Rhodes, K.J. McKernan, M.R. Stratton, P.A. Futreal, P.J. Campbell, A small-cell lung cancer genome with complex signatures of tobacco exposure, Nature 463 (2010) 184–190.

[22] P.J. Campbell, P.J. Stephens, E.D. Pleasance, S. O'Meara, H. Li, T. Santarius, L.A. Stebbings, C. Leroy, S. Edkins, C. Hardy, J.W. Teague, A. Menzies, I. Goodhead, D.J. Turner, C.M. Clee, M.A. Quail, A. Cox, C. Brown, R. Durbin, M.E. Hurles, P.A. Edwards, G.R. Bignell, M.R. Stratton, P.A. Futreal, Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing, Nat. Genet. 40 (2008) 722–729.

[23] Y.S. Ju, W.C. Lee, J.Y. Shin, S. Lee, T. Bleazard, J.K. Won, Y.T. Kim, J.I. Kim, J.H. Kang, J.S. Seo, A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing, Genome Res. 22 (2012) 436–445.

[24] P. Liu, C. Morrison, L. Wang, D. Xiong, P. Vedell, P. Cui, X. Hua, F. Ding, Y. Lu, M. James, J.D. Ebben, H. Xu, A.A. Adjei, K. Head, J.W. Andrae, M.R. Tschannen, H. Jacob, J. Pan, Q. Zhang, F. Van den Bergh, H. Xiao, K.C. Lo, J. Patel, T. Richmond, M.A. Watt, T. Albert, R. Selzer, M. Anderson, J. Wang, Y. Wang, S. Starnes, P. Yang, M. You, Identification of somatic mutations in non-small cell lung carcinomas using whole-exome sequencing, Carcinogenesis 33 (2012) 1270–1276.

[25] R. Govindan, L. Ding, M. Griffith, J. Subramanian, N.D. Dees, K.L. Kanchi, C.A. Maher, R. Fulton, L. Fulton, J. Wallis, K. Chen, J. Walker, S. McDonald, R. Bose, D. Ornitz, D. Xiong, M. You, D.J. Dooling, M. Watson, E.R. Mardis, R.K. Wilson, Genomic landscape of non-small cell lung cancer in smokers and never-smokers, Cell 150 (2012) 1121–1134.

[26] K. Vaddi, N.J. Sarlis, Gupta V. Ruxolitinib, an oral JAK1 and JAK2 inhibitor, in myelofibrosis, Expert Opin. Pharmacother. 13 (2012) 2397–2407.

[27] TCGA, Comprehensive genomic characterization of squamous cell lung cancers, Nature 489 (2012) 519–525.

[28] M. Eichenmuller, F. Trippel, M. Kreuder, A. Beck, T. Schwarzmayr, B. Haberle, S. Cairo, I. Leuschner, D. von Schweinitz, T.M. Strom, R. Kappler, The genomic landscape of hepatoblastoma and their progenies with HCC-like features, J. Hepatol. (2014).

[29] M. Imielinski, A.H. Berger, P.S. Hammerman, B. Hernandez, T.J. Pugh, E. Hodis, J. Cho, J. Suh, M. Capelletti, A. Sivachenko, C. Sougnez, D. Auclair, M.S. Lawrence, P. Stojanov, K. Cibulskis, K. Choi, L. de Waal, T. Sharifnia, A. Brooks, H. Greulich, S. Banerji, T. Zander, D. Seidel, F. Leenders, S. Ansen, C. Ludwig, W. Engel-Riedel, E. Stoelben, J. Wolf, C. Goparju, K. Thompson, W. Winckler, D. Kwiatkowski, B.E. Johnson, P.A. Janne, V.A. Miller, W. Pao, W.D. Travis, H.I. Pass, S.B. Gabriel, E.S. Lander, R.K. Thomas, L.A. Garraway, G. Getz, M. Meyerson, Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing, Cell 150 (2012) 1107–1120.

[30] Y. Kim, P.S. Hammerman, J. Kim, J.A. Yoon, Y. Lee, J.M. Sun, M.D. Wilkerson, C.S. Pedamallu, K. Cibulskis, Y.K. Yoo, M.S. Lawrence, P. Stojanov, S.L. Carter, A. McKenna, C. Stewart, A.Y. Sivachenko, I.J. Oh, H.K. Kim, Y.S. Choi, K. Kim, Y.M. Shim, K.S. Kim, S.Y. Song, K.J. Na, Y.L. Choi, D. N. Hayes, S. Cho, Y.C. Kim, J.S. Ahn, M.J. Ahn, G. Getz, M. Meyerson, K. Park, Integrative and comparative genomic analysis of lung squamous cell carcinomas in East asian patients, J. Clin. Oncol. 32 (2014) 121–128.

[31] D. Singh, J.M. Chan, P. Zoppoli, F. Niola, R. Sullivan, A. Castano, E.M. Liu, J. Reichel, P. Porrati, S. Pellegatta, K. Qiu, Z. Gao, M. Ceccarelli, R. Riccardi, D.J. Brat, A. Guha, K. Aldape, J.G. Golfinos, D. Zagzag, T. Mikkelsen, G. Finocchiaro, A. Lasorella, R. Rabadan, A. Iavarone, Transforming fusions of FGFR and TACC genes in human glioblastoma, Science 337 (2012) 1231–1235.

[32] S.V. Williams, C.D. Hurst, M.A. Knowles, Oncogenic FGFR3 gene fusions in bladder cancer, Hum. Mol. Genet. 22 (2013) 795–803.

[33] F. Janku, I. Garrido-Laguna, L.B. Petruzelka, D.J. Stewart, R. Kurzrock, Novel therapeutic targets in non-small cell lung cancer, J. Thorac. Oncol. 6 (2011) 1601–1612.

[34] P. Savas, B. Hughes, B. Solomon, Targeted therapy in lung cancer: IPASS and beyond, keeping abreast of the explosion of targeted therapies for lung cancer, J. Thorac. Dis. 5 (2013) S579–S592.

[35] C.K. Lee, C. Brown, R.J. Gralla, V. Hirsh, S. Thongprasert, C.M. Tsai, E.H. Tan, J.C. Ho, T. Chu da, A. Zaatar, J.A. Osorio Sanchez, V.V. Vu, J.S. Au, A. Inoue, S.M. Lee, V. Gebski, J.C. Yang, Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: a meta-analysis, J. Natl. Cancer Inst. 105 (2013) 595–605.

[36] M. Beau-Faller, N. Prim, A.M. Ruppert, I. Nanni-MetellusR. Lacave, L. Lacroix, F. Escande, S. Lizard, J. L. Pretet, I. Rouquette, P. de Cremoux, J. Solassol, F. de Fraipont, I. Bieche, A. Cayre, E. Favre-Guillevin, P. Tomasini, M. Wislez, B. Besse, M. Legrain, A.C. Voegeli, L. Baudrin, F. Morin, G. Zalcman, E. Quoix, H. Blons, J. Cadranel, Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10,117 patients: a multicentre observational study by the French ERMETIC-IFCT network, Ann. Oncol. 25 (2014) 126–131.

[37] I.J. Dahabreh, H. Linardou, P. Kosmidis, D. Bafaloukos, S. Murray, EGFR gene copy number as a predictive biomarker for patients receiving tyrosine kinase inhibitor treatment: a systematic review and meta-analysis in non-small-cell lung cancer, Ann. Oncol. 22 (2011) 545–552.

[38] L.M. Sholl, Y. Xiao, V. Joshi, B.Y. Yeap, L.A. Cioffredi, D.M. Jackman, C. Lee, P.A. Janne, N.I. Lindeman, EGFR mutation is a better predictor of response to tyrosine kinase inhibitors in non-small cell lung carcinoma than FISH, CISH, and immunohistochemistry, Am. J. Clin. Pathol. 133 (2010) 922–934.

[39] J.E. Chaft, G.R. Oxnard, C.S. Sima, M.G. Kris, V.A. Miller, G. J. Riely, Disease flare after tyrosine kinase inhibitor discontinuation in patients with EGFR-mutant lung cancer and acquired resistance to erlotinib or gefitinib: implications for clinical trial design, Clin. Cancer Res. 17 (2011) 6298–6303.

[40] R. Rosell, T.G. Bivona, N. Karachaliou, Genetics and biomarkers in personalisation of lung cancer treatment, Lancet 382 (2013) 720–731.

[41] R. Morinaga, I. Okamoto, K. Furuta, Y. Kawano, M. Sekijima, K. Dote, T. Satou, K. Nishio, M. Fukuoka, K. Nakagawa, Sequential occurrence of non-small cell and small cell lung cancer with the same EGFR mutation, Lung Cancer 58 (2007) 411–413.

[42] M.F. Zakowski, M. Ladanyi, M.G. Kris, EGFR mutations in small-cell lung cancers in patients who have never smoked, N. Engl. J. Med. 355 (2006) 213–215.

[43] Z. Zhang, J.C. Lee, L. Lin, V. Olivas, V. Au, T. LaFramboise, M. Abdel-Rahman, X. Wang, A.D. Levine, J.K. Rho, Y.J. Choi, C.M. Choi, S.W. Kim, S.J. Jang, Y.S. Park, W.S. Kim, D.H. Lee, J.S. Lee, V.A. Miller, M. Arcila, M. Ladanyi, P. Moonsamy, C. Sawyers, T.J. Boggon, P.C. Ma, C. Costa, M. Taron, R. Rosell, B. Halmos, T.G. Bivona, Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer, Nat. Genet. 44 (2012) 852–860.

[44] T.G. Bivona, H. Hieronymus, J. Parker, K. Chang, M. Taron, R. Rosell, P. Moonsamy, K. Dahlman, V.A. Miller, C. Costa, G. Hannon, C.L. Sawyers, FAS and NF-kappaB signalling modulate dependence of lung cancers on mutant EGFR, Nature 471 (2011) 523–526.

[45] A. Baumgart, S. Seidl, P. Vlachou, L. Michel, N. Mitova, N. Schatz, K. Specht, I. Koch, T. Schuster, R. Grundler, M. Kremer, F. Fend, J.T. Siveke, C. Peschel, J. Duyster, T. Dechow, ADAM17 regulates epidermal growth factor receptor expression through the activation of Notch1 in non-small cell lung cancer, Cancer Res. 70 (2010) 5368–5378.
[46] M. Serizawa, Y. Koh, H. Kenmotsu, M. Isaka, H. Murakami, H. Akamatsu, K. Mori, M. Abe, I. Hayashi, T. Taira, T. Maniwa, T. Takahashi, M. Endo, T. Nakajima, Y. Ohde, N. Yamamoto, Assessment of mutational profile of Japanese lung adenocarcinoma patients by multitarget assays: a prospective, single-institute study, Cancer 120 (2014) 1471–1481.
[47] P.K. Paik, M.E. Arcila, M. Fara, C.S. Sima, V.A. Miller, M.G. Kris, M. Ladanyi, G.J. Riely, Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations, J. Clin. Oncol. 29 (2011) 2046–2051.
[48] O. Gautschi, C. Pauli, K. Strobel, A. Hirschmann, G. Printzen, S. Aebi, J. Diebold, A patient with BRAF V600E lung adenocarcinoma responding to vemurafenib, J. Thorac. Oncol. 7 (2012) e23–e24.
[49] S. Peters, O. Michielin, S. Zimmermann, Dramatic response induced by vemurafenib in a BRAF V600E-mutated lung adenocarcinoma, J. Clin. Oncol. 31 (2013) e341–e344.
[50] D. Planchard, J. Mazieres, G.J. Riely, al. e. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients, J. Clin. Oncol. (2013) 31.
[51] A. Marchetti, L. Felicioni, S. Malatesta, M. Grazia Sciarrotta, L. Guetti, A. Chella, P. Viola, C. Pullara, F. Mucilli, F. Buttitta, Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations, J. Clin. Oncol. 29 (2011) 3574–3579.
[52] L. Lin, S. Asthana, E. Chan, S. Bandyopadhyay, M.M. Martins, V. Olivas, J.J. Yan, L. Pham, M.M. Wang, G. Bollag, D.B. Solit, E.A. Collisson, C.M. Rudin, B.S. Taylor, T.G. Bivona, Mapping the molecular determinants of BRAF oncogene dependence in human lung cancer, Proc. Natl. Acad. Sci. USA 111 (2014) E748–E757.
[53] C.M. Rudin, K. Hong, M. Streit, Molecular characterization of acquired resistance to the BRAF inhibitor dabrafenib in a patient with BRAF-mutant non-small-cell lung cancer, J. Thorac. Oncol. 8 (2013) e41–e42.
[54] S. Giroux, Overcoming acquired resistance to kinase inhibition: the cases of EGFR, ALK and BRAF, Bioorg. Med. Chem. Lett. 23 (2013) 394–401.
[55] E.L. Kwak, Y.J. Bang, D.R. Camidge, A.T. Shaw, B. Solomon, R. G. Maki, S.H. Ou, B.J. Dezube, P.A. Janne, D.B. Costa, M. Varella-Garcia, W.H. Kim, T.J. Lynch, P. Fidias, H. Stubbs, J.A. Engelman, L.V. Sequist, W. Tan, L. Gandhi, M. Mino-Kenudson, G.C. Wei, S.M. Shreeve, M.J. Ratain, J. Settleman, J.G. Christensen, D.A. Haber, K. Wilner, R. Salgia, G.I. Shapiro, J.W. Clark, A.J. Iafrate, Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer, N. Engl. J. Med. 363 (2010) 1693–1703.
[56] E. Thunnissen, L. Bubendorf, M. Dietel, G. Elmberger, K. Kerr, F. Lopez-Rios, H. Moch, W. Olszewski, P. Pauwels, F. Penault-Llorca, G. Rossi, EML4-ALK testing in non-small cell carcinomas of the lung: a review with recommendations, Virchows Arch. 461 (2012) 245–257.
[57] D.W. Wong, E.L. Leung, K.K. So, I.Y. Tam, A.D. Sihoe, L.C. Cheng, K.K. Ho, J.S. Au, L.P. Chung, M. Pik Wong, The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS, Cancer 115 (2009) 1723–1733.
[58] J.J. Cui, M. Tran-Dube, H. Shen, M. Nambu, P.P. Kung, M. Pairish, L. Jia, J. Meng, L. Funk, I. Botrous, M. McTigue, N. Grodsky, K. Ryan, E. Padrique, G. Alton, S. Timofeevski, S. Yamazaki, Q. Li, H. Zou, J. Christensen, B. Mroczkowski, S. Bender, R.S. Kania, M.P. Edwards, Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK), J. Med. Chem. 54 (2011) 6342–6363.
[59] A.T. Shaw, D.W. Kim, K. Nakagawa, T. Seto, L. Crino, M.J. Ahn, T. De Pas, B. Besse, B.J. Solomon, F. Blackhall, Y.L. Wu, M. Thomas, K.J. O'Byrne, D. Moro-Sibilot, D.R. Camidge, T. Mok, V. Hirsh, G.J. Riely, S. Iyer, V. Tassell, A. Polli, K.D. Wilner, P.A. Janne, Crizotinib versus chemotherapy in advanced ALK-positive lung cancer, N. Engl. J. Med. 368 (2013) 2385–2394.
[60] Z. Song, M. Wang, A. Zhang, Alectinib: a novel second generation anaplastic lymphoma kinase (ALK) inhibitor for overcoming clinically-acquired resistance, Acta Pharmaceutica Sin. B 5 (2015) 34–37.
[61] A.T. Shaw, J.A. Engelman, Ceritinib in ALK-rearranged non-small-cell lung cancer, N. Engl. J. Med. 370 (2014) 2537–2539.
[62] H. Ajimizu, Y.H. Kim, M. Mishima, Rapid response of brain metastases to alectinib in a patient with non-small-cell lung cancer resistant to crizotinib, Med. Oncol. 32 (2015) 477.
[63] R.C. Doebele, A.B. Pilling, D.L. Aisner, T.G. Kutateladze, A.T. Le, A.J. Weickhardt, K.L. Kondo, D.J. Linderman, L.E. Heasley, W.A. Franklin, M. Varella-Garcia, D. R. Camidge, Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer, Clin. Cancer Res. 18 (2012) 1472–1482.
[64] D. Huang, D.W. Kim, A. Kotsakis, S. Deng, P. Lira, S.N. Ho, N.V. Lee, P. Vizcarra, J.Q. Cao, J.G. Christensen, T.M. Kim, J.M. Sun, J.S. Ahn, M.J. Ahn, K. Park, M. Mao, Multiplexed deep sequencing analysis of ALK kinase domain identifies resistance mutations in relapsed patients following crizotinib treatment, Genomics 102 (2013) 157–162.
[65] T. Sasaki, J. Koivunen, A. Ogino, M. Yanagita, S. Nikiforow, W. Zheng, C. Lathan, J.P. Marcoux, J. Du, K. Okuda, M. Capelletti, T. Shimamura, D. Ercan, M. Stumpfova, Y. Xiao, S. Weremowicz, M. Butaney, S. Heon, K. Wilner, J.G. Christensen, M.J. Eck, K.K. Wong, N. Lindeman, N. S. Gray, S.J. Rodig, P.A. Janne, A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors, Cancer Res. 71 (2011) 6051–6060.
[66] L. Jiang, J. Huang, C. Morehouse, W. Zhu, S. Korolevich, D. Sui, X. Ge, K. Lehmann, Z. Liu, C. Kiefer, M. Czapiga, X. Su, P. Brohawn, Y. Gu, B.W. Higgs, Y. Yao, Low frequency KRAS mutations in colorectal cancer patients and the presence of multiple mutations in oncogenic drivers in non-small cell lung cancer patients, Cancer Genet. 206 (2013) 330–339.
[67] H.R. Kim, W.S. Kim, Y.J. Choi, C.M. Choi, J.K. Rho, J.C. Lee, Epithelial-mesenchymal transition leads to crizotinib resistance in H2228 lung cancer cells with EML4-ALK translocation, Mol. Oncol. 7 (2013) 1093–1102.
[68] K. Bergethon, A.T. Shaw, S.H. Ou, R. Katayama, C.M. Lovly, N.T. McDonald, P.P. Massion, C. Siwak-Tapp, A. Gonzalez, R. Fang, E.J. Mark, J.M. Batten, H. Chen, K.D. Wilner, E.L. Kwak, J.W. Clark, D.P. Carbone, H. Ji, J.A. Engelman, M. Mino-Kenudson, W. Pao, A.J. Iafrate, ROS1 rearrangements define a unique molecular class of lung cancers, J. Clin. Oncol. 30 (2012) 863–870.
[69] H. Yasuda, L.L. de Figueiredo-Pontes, S. Kobayashi, D.B. Costa, Preclinical rationale for use of the clinically available multitargeted tyrosine kinase inhibitor crizotinib in ROS1-translocated lung cancer, J. Thorac. Oncol. 7 (2012) 1086–1090.
[70] K.D. Davies, A.T. Le, M.F. Theodoro, M.C. Skokan, D.L. Aisner, E.M. Berge, L.M. Terracciano, F. Cappuzzo, M. Incarbone, M. Roncalli, M. Alloisio, A. Santoro, D.R. Camidge, M. Varella-Garcia, R.C. Doebele, Identifying and targeting

ROS1 gene fusions in non-small cell lung cancer, Clin. Cancer Res. 18 (2012) 4570–4579.
[71] A.T. Shaw, S.H. Ou, Y.J. Bang, D.R. Camidge, B.J. Solomon, R. Salgia, G.J. Riely, M. Varella-Garcia, G.I. Shapiro, D. B. Costa, R.C. Doebele, L.P. Le, Z. Zheng, W. Tan, P. Stephenson, S.M. Shreeve, L.M. Tye, J.G. Christensen, K. D. Wilner, J.W. Clark, A.J. Iafrate, Crizotinib in ROS1-rearranged non-small-cell lung cancer, N. Engl. J. Med. 371 (2014) 1963–1971.
[72] J. Mazieres, G. Zalcman, L. Crino, P. Biondani, F. Barlesi, T. Filleron, A.M. Dingemans, H. Lena, I. Monnet, S.I. Rothschild, F. Cappuzzo, B. Besse, L. Thiberville, D. Rouviere, R. Dziadziuszko, E.F. Smit, J. Wolf, C. Spirig, N. Pecuchet, F. Leenders, J.M. Heuckmann, J. Diebold, J.D. Milia, R.K. Thomas, O. Gautschi, Crizotinib Therapy for Advanced Lung Adenocarcinoma and a ROS1 Rearrangement: Results From the EUROS1 Cohort, J. Clin. Oncol. (2015).
[73] M.A. Davare, A. Saborowski, C.A. Eide, C. Tognon, R.L. Smith, J. Elferich, A. Agarwal, J.W. Tyner, U.P. Shinde, S.W. Lowe, B. J. Druker, Foretinib is a potent inhibitor of oncogenic ROS1 fusion proteins, Proc. Natl. Acad. Sci. USA 110 (2013) 19519–19524.
[74] K.D. Davies, S. Mahale, D.P. Astling, D.L. Aisner, A.T. Le, T.K. Hinz, A. Vaishnavi, P.A. Bunn Jr., L.E. Heasley, A.C. Tan, D.R. Camidge, M. Varella-Garcia, R.C. Doebele, Resistance to ROS1 inhibition mediated by EGFR pathway activation in non-small cell lung cancer, PLoS One 8 (2013) e82236.
[75] A. Song, T.M. Kim, D.W. Kim, S. Kim, B. Keam, S.H. Lee, D.S. Heo, Molecular changes associated with acquired resistance to crizotinib in ROS1-rearranged non-small cell lung cancer (NSCLC), Clin. Cancer Res. (2015).
[76] M. Cargnelutti, S. Corso, M. Pergolizzi, L. Mevellec, D.L. Aisner, R. Dziadziuszko, M. Varella-Garcia, P. M. Comoglio, R.C. Doebele, J. Vialard, S. Giordano, Activation of RAS family members confers resistance to ROS1 targeting drugs, Oncotarget (2014).
[77] R. Wang, H. Hu, Y. Pan, Y. Li, T. Ye, C. Li, X. Luo, L. Wang, H. Li, Y. Zhang, F. Li, Y. Lu, Q. Lu, J. Xu, D. Garfield, L. Shen, H. Ji, W. Pao, Y. Sun, H. Chen, RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer, J. Clin. Oncol. 30 (2012) 4352–4359.
[78] A. Drilon, L. Wang, A. Hasanovic, Y. Suehara, D. Lipson, P. Stephens, J. Ross, V. Miller, M. Ginsberg, M.F. Zakowski, M.G. Kris, M. Ladanyi, N. Rizvi, Response to Cabozantinib in patients with RET fusion-positive lung adenocarcinomas, Cancer Discov 3 (2013) 630–635.
[79] B.H. Chao, R. Briesewitz, M.A. Villalona-Calero, RET fusion genes in non-small-cell lung cancer, J. Clin. Oncol. 30 (2012) 4439–4441.
[80] J.P. Alao, S. Michlikova, P. Diner, M. Grotli, P. Sunnerhagen, Selective inhibition of RET mediated cell proliferation in vitro by the kinase inhibitor SPP86, BMC Cancer 14 (2014) 853.
[81] T. Kodama, T. Tsukaguchi, Y. Satoh, M. Yoshida, Y. Watanabe, O. Kondoh, H. Sakamoto, Alectinib shows potent antitumor activity against RET-rearranged non-small cell lung cancer, Mol. Cancer Ther. 13 (2014) 2910–2918.
[82] O. Gautschi, T. Zander, F.A. Keller, K. Strobel, A. Hirschmann, S. Aebi, J. Diebold, A patient with lung adenocarcinoma and RET fusion treated with vandetanib, J. Thorac. Oncol. 8 (2013) e43–e44.
[83] J. Mazieres, S. Peters, B. Lepage, A.B. Cortot, F. Barlesi, M. Beau-Faller, B. Besse, H. Blons, A. Mansuet-Lupo, T. Urban, D. Moro-Sibilot, E. Dansin, C. Chouaid, M. Wislez, J. Diebold, E. Felip, I. Rouquette, J.D. Milia, O. Gautschi, Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives, J. Clin. Oncol. 31 (2013) 1997–2003.
[84] S.A. Perera, D. Li, T. Shimamura, M.G. Raso, H. Ji, L. Chen, C.L. Borgman, S. Zaghlul, K.A. Brandstetter, S. Kubo, M. Takahashi, L.R. Chirieac, R.F. Padera, R.T. Bronson, G. I. Shapiro, H. Greulich, M. Meyerson, U. Guertler, P.G. Chesa, F. Solca, I.I. Wistuba, K.K. Wong, HER2YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy, Proc. Natl. Acad. Sci. USA 106 (2009) 474–479.
[85] T. Shimamura, H. Ji, Y. Minami, R.K. Thomas, A.M. Lowell, K. Shah, H. Greulich, K.A. Glatt, M. Meyerson, G.I. Shapiro, K. K. Wong, Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV_G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272, Cancer Res. 66 (2006) 6487–6491.
[86] J. Rafael Sierra, M.S. Tsao, c-MET as a potential therapeutic target and biomarker in cancer, Ther. Adv. Med. Oncol. 3 (2011) 21–35.
[87] L. Landi, G. Minuti, A. D'Incecco, F. Cappuzzo, Targeting c-MET in the battle against advanced nonsmall-cell lung cancer, Curr. Opin. Oncol. 25 (2013) 130–136.
[88] A. Calles, N. Kwiatkowski, B.K. Cammarata, D. Ercan, N.S. Gray, P.A. Janne, Tivantinib (ARQ 197) efficacy is independent of MET inhibition in non-small-cell lung cancer cell lines, Mol. Oncol. 9 (2015) 260–269.
[89] N. Yamamoto, H. Murakami, H. Hayashi, Y. Fujisaka, T. Hirashima, K. Takeda, M. Satouchi, K. Miyoshi, S. Akinaga, T. Takahashi, K. Nakagawa, CYP2C19 genotype-based phase I studies of a c-Met inhibitor tivantinib in combination with erlotinib, in advanced/metastatic non-small cell lung cancer, Br. J. Cancer 109 (2013) 2803–2809.
[90] L.V. Sequist, J. von Pawel, E.G. Garmey, W.L. Akerley, W. Brugger, D. Ferrari, Y. Chen, D.B. Costa, D.E. Gerber, S. Orlov, R. Ramlau, S. Arthur, I. Gorbachevsky, B. Schwartz, J.H. Schiller, Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer, J. Clin. Oncol. 29 (2011) 3307–3315.
[91] G.S. Kerner, E. Schuuring, J. Sietsma, T.J. Hiltermann, R.M. Pieterman, G.P. de Leede, J.W. van Putten, J. Liesker, T.E. Renkema, P. van Hengel, I. Platteel, W. Timens, H.J. Groen, Common and rare EGFR and KRAS mutations in a Dutch non-small-cell lung cancer population and their clinical outcome, PLoS One 8 (2013) e70346.
[92] R. Katayama, A.T. Shaw, T.M. Khan, M. Mino-Kenudson, B.J. Solomon, B. Halmos, N.A. Jessop, J.C. Wain, A.T. Yeo, C. Benes, L. Drew, J.C. Saeh, K. Crosby, L.V. Sequist, A.J. Iafrate, J.A. Engelman, Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers, Sci. Transl. Med. 4 (2012) 120ra117.