Non-small cell lung cancer - genetic predictors

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Background. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer that is the leading cause of cancer-related mortality worldwide. Several predictive markers have been found in NSCLC patients to date but only a few are currently used for tailored therapy.
Methods and Results. PubMed and Web of Science online databases were used to search review and original articles on the most important predictive markers in NSCLC.
Conclusion. EGFR activating mutations (exons 18 to 21) and EML4-ALK rearrangement are clinically important markers able to select NSCLC patients which benefit from EGFR or ALK tyrosine kinase inhibitors (gefitinib, erlotinib, crizotinib). Other markers, such as KRAS mutation, EGFR T790M mutation and C-MET amplification, are responsible for resistance to these inhibitors. Overcoming of this resistance as well as discovery of new potential markers and inhibitors is the main goal of ongoing research and clinical trials in NSCLC.

Key words: NSCLC, EGFR, KRAS, ALK, C-MET, ROS1, tyrosine kinase inhibitors, resistance

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INTRODUCTION

Lung cancer is the most frequent cause of cancer-related deaths worldwide and it is responsible for more than 1 million deaths annually[1,2]. The main reason is high tumor aggressivity and high metastasis potential. Non-small cell lung cancer (NSCLC) is diagnosed in approximately 85% of lung cancer cases and includes the adenocarcinoma, squamous cell carcinoma and large cell carcinoma subtypes[3]. The intensive research has been made in the past few years on genetic, transcriptional, translational and epigenetic levels and the remarkable discoveries have been found. At least nine important driver mutations causing NSCLC have been described, mainly in adenocarcinoma subtype. Several markers are already used for best treatment strategy selection. Developing new drugs targeting the markers, clarification of predictive value of these markers as well as new markers discovering is still the subject of intensive research[4]. In this review, the clinically most important genetic alterations in NSCLC, such as EGFR, KRAS, C-MET, EML4-ALK and ROS1 are summarized.

EGFR

The epidermal growth factor receptor (EGFR) gene is located on 7p11 and encodes a tyrosine-kinase receptor from the HER family which is involved in development, progression, angiogenesis and metastasis of various cancer types. After ligand binding (EGF, TGF-α, amphiregulin), the receptor hetero-/homodimerizes, autophosphorylates tyrosine residues and activates two main downstream signaling pathways – RAS/MAPK and PI3K/AKT (ref.[5]). Three mechanisms of EGFR activation in tumor cells have been described, including EGFR mutations, amplification/gene copy number gain (CNG) and overexpression.

Amplification/overexpression

EGFR overexpression is found in up to 80% of NSCLC cases and EGFR CNG/amplification is found in almost 60% of them, while these events often occur concurrently[6-12]. Increased EGFR expression was considered to be a poor prognostic factor in NSCLC patients[12,13] but a meta-analysis combining 18 studies of 2972 patients did not confirm the prognostic significance of EGFR expression (HR=1.14; 95% CI 0.97-1.34; P=0.103) (ref.[14]).

The predictive value of EGFR amplification/overexpression for responsiveness to EGFR tyrosine kinase inhibitors (EGFR TKIs) was tested in several studies. Initial studies, including the large trials BR.21 and ISEL, found clear association between increased EGFR copy number and good response to EGFR TKIs(ref.[9,15-17]). Other studies have not confirmed this finding[18,19]. In a recent meta-analysis[20] which combined 22 independent studies (2005-2009) including 1821 NSCLC patients treated with EGFR TKIs monotherapy, EGFR CNG was significantly associated with increased overall survival (OS) (HR=0.77; 95% CI 0.66-0.89; P=0.001), progression-free survival (PFS) (HR=0.60; 95% CI 0.46-0.79; P<0.001) and time-to-progression (TTP) (HR=0.50; 95% CI 0.28-0.91; P=0.02). The following studies published by Brugger et al.[21] and Hirsch et al.[22] did not confirm the predictive significance of EGFR FISH positivity to erlotinib. The clinical relevance of EGFR amplifications is difficult to decipher

because about 50% of EGFR-mutated cases show the co-existence of increased EGFR copy number. The predictive value of EGFR copy number could be therefore affected by the occurrence of simultaneous EGFR mutation[8,15,23]. At the present, EGFR copy number testing is not recommended in the selection of treatment in NSCLC.

Activating mutations

In 2004, two independent research groups[24,25] sequenced EGFR in advanced NSCLC patient samples. The aim was to evaluate the possible predictive value of EGFR mutations for EGFR TKIs therapy. In 14 out of 15 patients who were good responders to gefitinib therapy, small in-frame deletions or amino acid substitutions were identified. No EGFR mutations were found in gefitinib non-responders. In these studies, EGFR activating mutations were identified[24,25].

Activating mutations of EGFR, occurring in exon 18 to 21 in the ATP-binding pocket part of the tyrosine-kinase domain, have been reported in 5 to 30% NSCLC cases depending on study population (app. 15% incidence in Caucasians compared to 30% in Asians). These mutations lead to a ligand-independent EGFR activation and are preferentially found in never/former smokers, women, East Asians and patients with adenocarcinoma histology. More than 3000 somatic EGFR mutations have been described to date[26]. Deletions in exon 19 (including residues 746 to 753) and arginine to leucine substitution (L858R) in exon 21 constitute about 90% of them[6,8,27]. Substitution of glycine to serine, alanine or cysteine in codon 719 (G719X) of exon 18 occurs in an additional 4% of cases and other missense mutations and small in-frame duplications/insertions in exon 20 account for the rest[6].

Targeted therapy

The most effective inhibitors of EGFR tyrosine kinase signalization are the small anilinoquinazoline derivatives, that act as reversible ATP-competitive inhibitors, erlotinib (Tarceva®, Genentech) and gefitinib (Iressa®, AstraZeneca).

After successful preclinical[28] and phase I clinical studies[29,30], gefitinib progressed to phase II studies. Objective response rates between 10 and 20% were reported in two double-blind, randomized phase II trials (IDEAL 1 and 2) which enrolled 210 and 221 NSCLC patients previously treated with one or two regimes[31,32]. Based on these results, gefitinib was FDA approved for advanced NSCLC patient treatment in May 2003. Based on results from unsuccessful ISEL study, in June 2005, FDA limited the use of gefitinib. However, the IPASS trial confirmed the benefit of patients with EGFR mutations of gefitinib therapy and European Medicines Agency (EMA) approved gefitinib for the treatment of locally advanced or metastatic NSCLC patients with EGFR activating mutation in June 2009 (ref.[33,34]).

The low-molecular weight inhibitor, erlotinib, showed antitumor activity in preclinical and phase I clinical studies[35]. Erlotinib was FDA approved in November 2004 based on the results of phase III randomized trial BR.21 which included 731 NSCLC patients treated by erlotinib or placebo in second or third line setting. The OS of the treated group was 2 months longer than the placebo group (6.7 months vs. 4.7 months). The 1-year OS was 31% for the erlotinib group compared to 22% for the control group[36].

The predictive role of EGFR mutations to EGFR TKIs therapy sensitivity was revealed by different studies and confirmed by large meta-analysis including 59 studies of 3101 NSCLC patients. EGFR mutations were predictive of response to single agent EGFR TKIs with sensitivity and specificity of 0.78, resp. 0.86 (ref.[37]). Many other studies elucidating EGFR TKIs efficiency in different settings and biomarker-selected populations were recently reviewed[38,39]. In general, EGFR TKIs treatment significantly improves the survival of NSCLC patients with EGFR mutations compared to chemotherapy.

Several clinical trials, clearly reviewed by Patil et al.[40], are evaluating the efficacy of cetuximab (Erbitux®, Merck KGaA) in combination with various types of treatment and assessing the predictive role of EGFR, KRAS and other potential biomarkers. Predictive value of EGFR mutations, amplification or overexpression and KRAS mutations for cetuximab therapy was not confirmed to date[41,42].

EGFR TKIs and *de novo* resistance

The best described mechanism of *de novo* resistance to EGFR TKIs is mutation in the KRAS oncogene which is present in 20 to 30% of lung cancer patients. KRAS and its importance for NSCLC therapy management is discussed below. Another cause of *de novo* resistance is the occurrence of insertion mutations in exon 20 of EGFR. *In vitro* studies have demonstrated that insertion in EGFR exon 20 causes both oncogenic transformation and resistance to EGFR TKIs (ref.[43]). Experiences with patients harboring EGFR exon 20 insertions corresponds with preclinical data. Clinical data showed very few responses to EGFR TKIs in these patients[15,44,45]. Substitution of methionine to threonin at position 790 (T790M) of the EGFR exon 20 was reported in 2.7-40% of TKI-naïve cases[44,46,47]. Patients with this mutation were found to have poorer outcome on EGFR TKIs therapy[47-49]. A secondary T790M mutation is more frequent and is associated with acquired resistance (described below). *De novo* resistance to EGFR TKIs therapy was also found in NSCLC patients with HER2 exon 20 insertions. Cancer cells presenting this mutation remain sensitive to HER2 targeted therapies but show resistance to EGFR TKIs (ref.[50,51]).

TKIs and acquired resistance

Acquired resistance to EGFR TKIs is a serious problem because the majority of initially responsive, EGFR TKIs-treated patients develop resistance within 12 months[52]. Resistance to EGFR TKIs may be caused by presence of cancer stem cell-like cells which are selected during EGFR TKIs therapy[53]. Generally, two crucial mechanisms of acquired resistance have been described, secondary T790M EGFR mutation and C-MET amplification. T790M mutation was described as the first mechanism of EGFR TKIs acquired resistance in 2005 by Kobayashi and Pao et al.[54,55]. Both groups studied NSCLC

patients with EGFR activating mutation (L858R or exon 19 deletion), who progressed on the gefitinib or erlotinib therapy. The T790M mutation was identified by comparison of pre- and post-progression samples and confirmed on NSCLC cell lines *in vitro*. A secondary T790M mutation is localized in the ATP-binding pocket of the kinase domain and is present in approximately 50% of NSCLC patients with acquired resistance[27,56-58]. Substitution in codon 790 increases ATP binding affinity of EGFR tyrosine kinase domain and EGFR TKIs are not able to bind. T790M mutated cells lose sensitivity to gefitinib and erlotinib but not to irreversible TKIs (e.g. pan-HER inhibitor PF0299804) (ref.[59]). A second mechanism of acquired resistance, C-MET amplification, is discussed below.

KRAS

KRAS (Kirsten rat sarcoma viral oncogene homolog) gene localized on 12p12 encodes membrane-bound GTPase protein which, as well as other members of the RAS protein family (NRAS and HRAS), plays an important role in EGFR-mediated signal transduction. EGFR activates KRAS through the adaptor protein Grb-2 (growth factor receptor-bound protein 2) and guanine nucleotide-exchange factor (GEF) molecules which are responsible for exchange of GDP to GTP. GTP-KRAS binds target proteins (e.g. RAF), activates them and GTPase activating proteins (GAP) stimulate GTP hydrolysis. KRAS-mediated signaling regulates several cellular processes, such as proliferation, differentiation and survival.

Activating mutations

Pathologic KRAS activation resulting from mutations in the KRAS gene has been found in many cancer types including NSCLC. KRAS mutations occur in approximately 20% of lung cancer cases[60,61]. The majority (about 90%) of found point mutations occur in exon 2 (codon 12 and 13), less frequent are mutations in exon 13 (codon 61) (ref.[26,61]). Point mutation leads to amino acid substitution and GAP insensitivity resulting in constitutively active GTP-binding KRAS signal transduction. KRAS mutations are more frequently found in Caucasian population, adenocarcinomas, males and current smokers[60,62,63]. In never smoking patients with adenocarcinoma, KRAS mutation is probably associated with transition mutation (G to A) compared to transversion (G to T or G to C) in current smokers[60]. Recent meta-analysis has shown KRAS mutations occurring in 26% of former or current smokers vs. 6% in never smokers[64]. The majority of studies have shown that KRAS and EGFR mutations are mutually exclusive[63,65-68]. Co-existence of both mutations was reported by Han et al. only[69].

Prognostic role

Several studies have evaluated the importance of KRAS mutations for survival, recurrence and metastasis. In 2005, Mascaux et al.[70] published the results of meta-analysis comparing KRAS prognostic significance in 28 independent retrospective studies with a total number of 3620 patients included. This meta-analysis showed a worse survival of KRAS mutated patients with HRs of 1.30 (95% CI, 1.20-1.49; P=0.01). In subgroup analysis, KRAS was a statistically significant prognostic factor in adenocarcinomas (HR=1.52; 95% CI, 1.30 to 1.78; P=0.02) but not in squamous cell carcinomas. Following studies did not confirm KRAS mutations as an independent prognostic factor[67,68]. The prognostic importance of KRAS mutations in NSCLC remains controversial and needs to be confirmed on prospective well-defined NSCLC patient cohorts.

Resistance to EGFR TKIs

Although the prognostic role of KRAS mutations is not clearly described, the predictive significance of EGFR TKIs therapy response was confirmed in several studies. KRAS mutations have been reported to be associated with *de novo* resistance to EGFR inhibitors in NSCLC patients in several studies[15,16,19,58,65,69]. Recently Mao et al. published meta-analysis of 22 studies analyzing 1470 NSCLC patients, KRAS mutation was detected in 16% (231/1470). Objective response rate (ORR) of KRAS mutated patients was 3% compared to 26% ORR in patients with wt-KRAS. This analysis confirmed that KRAS mutations are negative predictors of tumor responsiveness to EGFR TKIs therapy in NSCLC (ref.[64]). However, due to the mutual exclusivity of EGFR and KRAS mutations, the clinical importance of KRAS assessment in NSCLC remains low.

C-MET

The C-MET protooncogene is localized on chromosome region 7q31 (ref.[71]) and codes a tyrosine kinase receptor - hepatocyte growth factor receptor (HGFR). HGF/SF (hepatocyte growth factor/ scatter factor) is the only known ligand of this receptor. HGF binding results in phoshorylation of C-MET tyrosine residues[72], recruitment of adaptor proteins Grb2, Gab1, SHC and activation of downstream MAPK, PI3K-Akt and STAT signaling pathways[73-75]. C-MET and HGF are required for normal tissue development and therefore they are widely expressed in a various cell types. C-MET/HGF dysregulation and pathogenic activation is described in almost all cancer types[76-78] and has been identified as a promising therapeutic target. The first reported oncogenic C-MET activation resulting from translocation of chromosome 1 and 7 was found in an osteosarcoma cell line. Fusion TRP-MET protein has constitutive tyrosine kinase transforming activity[76]. C-MET can be activated by many other mechanisms, such as amplification, overexpression of receptor or ligand and point mutation[57,79,80].

Amplification/overexpression

C-MET amplification leads to receptor overexpression and constitutive HGF-independent activation[81]. C-MET amplification has been reported in range from 3 to 21% of EGFR TKI-naïve NSCLC patients and is associated with poor prognosis, increased proliferation, tumor invasiveness and angiogenesis[82-87]. The greatest percentage of

reported C-MET FISH-positive cases results from chromosome 7 polysomy. True C-MET amplification is rare event in NSCLC, occurs in 3 to 7% cases[84-86,88,89]. Some studies have reported association between C-MET and EGFR amplification[83,84]. Chromosome 7 polysomy is probably responsible for significant correlation between EGFR and C-MET FISH positivity. Higher copy number/overexpression of C-MET was found in brain metastasis compared to primary lung tumor tissues. C-MET-activated tumor cells have probably higher potential to migrate and create metastasis[90,91].

Resistance to EGFR TKIs

The importance of C-MET copy number evaluation rapidly increased when Engelman et al. found that the cause of acquired resistance to gefitinib in an NSCLC cell line (HCC827) is amplification of chromosomal region 7q31.1–7q33.3 where C-MET is localized. Consequently, C-MET-driven EGFR TKIs resistance was confirmed on 18 NSCLC patient samples[57]. C-MET amplification has been described in approximately 20% of NSCLC patients with acquired resistance[57,88,92-94]; in some cases T790M mutation of EGFR occurs simultaneously. Engelman et al. found that the bypass mechanism of C-MET signaling activation in resistant cells is through ERBB3-mediated PI3K-Akt signaling pathway[57].

Turke et al. theorized that NSCLC cells become C-MET amplified and therefore resistant during EGFR TKIs treatment by selection of a preexisting small C-MET amplified clone[95]. This study was performed on EGFR TKIs-sensitive NSCLC cell line HCC827 and 27 paired NSCLC patient samples (pre- and post-therapy). In the cell line study, a small subpopulation of C-MET amplified cells increased 300x over 19-days EGFR TKIs exposure. In tumor samples, C-MET-driven resistance was observed in 4 out of 27 cases, rare subpopulation (< 1%) of C-MET amplified cells was found in pre-treatment specimens in all 4 cases. These data suggest that acquired C-MET-driven resistance can be suppressed by dual EGFR and C-MET inhibition.

Targeted therapy

Several strategies of C-MET inhibition based on the mechanism of HGF/C-MET activation have been reported. In C-MET amplified/overexpressed tumors, selective blockade of active receptor by small-molecule inhibitors or monoclonal antibodies seem to be effective. Several C-MET TKIs such as PHA665752 (ref.[81,96]), PF-02341066 (crizotinib, Xalkori®, Pfizer), SGX523 (ref.[97,98]), ARQ197 (tivantinib, ArQule) (ref.[99,100]) and XL184 (cabozantinib, Exelixis) (ref.[101]) as well as monoclonal antibody MetMAb (onartuzumab, Genentech) (ref.[102]) were tested in a preclinical setting on NSCLC cell lines and xenograft models.

Cabozantinib, dual inhibitor of VEGFR2 and C-MET, has reached clinical testing in several cancer types. In NSCLC, cabozantinib is investigated in combination with erlotinib compared to erlotinib alone in phase I/II clinical study (NCT00596648) (ref.[103]). This inhibitor seems to be an effective inhibitor of tumor angiogenesis and metastasis in C-MET-deregulated NSCLC cases[101].

One of the most promising molecules is the non-ATP-competitive selective C-MET inhibitor tivantinib which passed phase I and II clinical trials. Sequist et al.[104] reported results of double-blind randomized phase II trial (NCT00777309) including 167 randomly assigned previously treated, EGFR TKI-naïve NSCLC patients. Patients who obtained erlotinib combined with tivantinib (ET) were compared to patients obtaining erlotinib with placebo (EP). Median PFS was 3.8 months for ET compared to 2.3 months for EP (HR=0.81; 95% CI, 0.57-1.16; P=0.24). ET-treated patients had significantly longer time to development of new metastasis (7.3 vs. 3.6 months, P<0.01). Significantly better response to ET therapy was observed in KRAS mutated patients compared to KRAS mutated in the EP regime (HR=0.18; 95% CI, 0.05 to 0.70; P=0.006). In this study, only 2 patients had true C-MET amplification, increased copy number (≥ 4 copies/cell) was found in 37 patients. C-MET positive patients tend to benefit from the ET regime and this benefit rises with increasing cut-off of C-MET copy number. Tivantinib in combination with erlotinib can prolong PFS, OS and time to metastasis in NSCLC patients compared to erlotinib alone. Ongoing clinical trials combining tivantinib and erlotinib in different setting are summarized in Table 1.

Crizotinib, a dual inhibitor of ALK and C-MET kinases is approved for treatment of NSCLC patients with ALK rearrangement. Nevertheless, response to crizotinib was shown in non-ALK rearranged NSCLC cell lines, xenograft model[105] as well as patient with *de novo* amplification of C-MET (ref.[106]). Anti-tumor activity of crizotinib is studied in randomized phase I/II trial (NCT00965731) in NSCLC patients treated by erlotinib alone versus erlotinib in combination with crizotinib[103]. The results from this study could clarify the inhibitory effect of crizotinib in C-MET amplified cases as it was shown on xenograft models[107].

MetMAb (onartuzumab) in combination with erlotinib have been evaluted in randomized, double-blind, phase II trial (NCT00854308). PFS was 2.2 vs. 2.6 months for patients obtained erlotinib + MetMAb (EM) vs. erlotinib + placebo (EP). In subgroup of C-MET positive NSCLC patients, PFS was 2.9 for EM vs. 1.5 months for EP. Efficiency of MetMAb in NSCLC should be confirmed by ongoing clinical trials combining MetMAb with erlotinib (NCT01456325), bevacizumab/pemetrexed (NCT01496742) and paclitaxel + platinum (NCT01519804) (ref.[103]).

ALK

The ALK (anaplastic lymphoma kinase) protein is a transmembrane tyrosine kinase receptor normally expressed only in the small intestine, testis and brain[108] but not in normal lung tissue[109]. Translocation of the ALK gene t(2;5) leading to NPM1-ALK fusion was firstly reported by Morris et al.[108] in anaplastic large cell lym-

Table 1. Trials are listed on the US National Institute of Health database[103].

Study	Phase	Treatment schedule	Stage	Biomarker selection	EGFR TKIs pretreated	Outcome	Estimated enrollement
NCT01251796	Phase I	erlotinib + tivantinib	IIIB,IV	CYP2C19 poor metabolism	possible	Primary: DLT Secondary: T and E farmacokinetics, antitumor activity	ND
NCT01580735	Phase II	erlotinib + tivantinib	IIIB,IV	EGFR mutation	yes	Primary: ORR Secondary: PFS, OS, DCR; safety profile	40
NCT01395758	Phase II randomised	Arm 1: erlotinib + tivantinib Arm 2: pemetrexed, docetaxel, or gemcitabine	IVA,IVB	KRAS mutation	no	Primary: PFS Secondary: OS, ORR, safety of T+E combination	98
NCT01244191	Phase III randomised	Arm 1: erlotinib + tivantinib Arm 2: erlotinib + placebo	IIIB,IV	no	no	Primary: OS Secondary: PFS, OS in wt-EGFR pts.	988
NCT01377376	Phase III randomised	Arm 1: erlotinib + tivantinib Arm 2: erlotinib + placebo	IIIB,IV	wt-EGFR	no	Primary: OS Secondary: PFS, ORR, adverse events	ND

CYP2C19-cytochrome P450 2C19; DLT-dose-limiting toxicity; PFS-progression free survival; OS-overall survival; ORR-objective response rate, T-tivantinib; E-erlotinib; pts.-patients; DCR-dissease control rate; wt-wild type; ND-not defined

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phoma (ALCL). Alterations of ALK gene were also identified in neuroblastomas[110] and inflammatory myofibroblastic tumors[111]. In 2007, Soda and colleagues identified a small inversion in the short arm of chromosome 2, inv(2)(p21p23) in NSCLC patients. This inversion leads to fusion of the N-terminal part of the echinoderm microtubule associated protein like-4 (EML4) with kinase domain of ALK (ref.[112]).

EML4-ALK fusion

EML4-ALK rearrangement is being found in approximately 2-7% NSCLC cases[113-115]. The fusion leads to protein redistribution to cytoplasm[112] and protein dimerization via coiled-coil domains of EML4 resulting in phosphorylation and highly oncogenic ALK kinase activation[116,117]. More than 13 variants of EML4-ALK have been identified to date containing different parts of EML4; the coiled-coil domain is preserved in all variants. Exon 13 (variant 1), resp. exon 6a/b (variant 3a/b) of EML4 fused to the ALK exon 20 are the two most frequent variants which are present in more than 50% cases[117-120]. Tree other rare fusion partners of ALK are known in NSCLC, KIF5B (ref.[121]), TFG (ref.[122]) and KLC1 (ref.[123]). The incidence of these fusion partners is less than 1% (ref.[121,122,124]). Heuckemann et al. showed that protein stability and sensitivity to treatment depend on EML4-ALK variant and fusion partner type[125].

Except for ALK rearrangement, ALK amplification/CNG have been reported[120,126]. Increased ALK copy number was associated with EGFR FISH positivity but no association with prognosis was found[126]. The significance, if any, of ALK CNG for response to therapy, prognosis or histopathologic features, needs to be analyzed.

A subgroup of EML4-ALK patients has typical clinical and histological features. ALK rearrangement is typically found in adenocarcinoma with signet ring cell subtype, younger patients[113,114,116,117] with never or light (10 packs per year) smoking history[114,127]. No other association with gender or ethnicity has been found. ALK fusion is mutually exclusive in most NSCLC cases[114,128,129], concurrent EGFR and KRAS mutations were described in only few cases[130-134].

Targeted therapy

EML4-ALK fusion is a therapeutic target for the ATP-competitive TKI crizotinib. In preclinical analyses, the inhibitory effect of crizotinib was confirmed on ALK rearranged cell lines derived from a variety of human cancers[135,136]. Based on these studies, crizotinib entered multicenter, open-label phase I trial (NCT00585195). In this study, crizotinib showed significant antitumor activity in enrolled 82 advanced, ALK-positive NSCLC patients. The ORR to crizotinib was 57% at mean treatment duration of 6.4 months. The estimated probability of 6 month progression-free survival was 74% (ref.[113]). In the retrospective data analysis from this study, reported by Shaw et al.[115], the 1-year OS was 74% and 2-year OS was 54%. ALK-positive patients treated by crizotonib had similar OS compared to EGFR TKIs-treated EGFR-mutant patients (P=0.786) but significantly better OS than ALK-positive crizotinib-untreated group. Moreover, ALK-positive crizotinib-treated patients had significantly better OS (P=0.020) than controls (wt-EGFR, ALK-negative) treated by conventional chemotherapy. Based on the results of phase I study and ongoing phase II studies (255 patients; NCT00932451), crizotinib (Xalkori®, Phizer) was FDA approved in August 2011 and EMA approved in October 2012 for treatment of locally advanced or metastatic ALK-positive NSCLC patients. Ongoing clinical trials evaluating efficiency of crizotinib in different setting are summarized in Table 2.

Resistance to crizotinib

Similar to other TKIs therapies, *de novo* as well as acquired resistance to crizotinib have already been reported. Two mutations in ALK kinase domain, C1156Y and L1196M, were identified as potential mechanisms of resistance to crizotinib therapy in 28-years old NSCLC patient[137]. Both mutations as cause of acquired resistance to crizotinib were confirmed in following studies[134,138] and other resistance-related mutations, L1152R, G1269A/S and S1206R, have been described[134,139,140]. Some other potential mechanisms of resistance, such as EML4-ALK CNG, KRAS and EGFR concurrent mutations, were described by Doebele et al.[134].

Several treatment strategies overcoming crizotinib resistance are tested on cell lines and xenografts models[141,142]. The Hsp90 inhibitors which show the most promising results are tested in number of clinical trials. Inhibitors IPI-504 (Phase II; NCT01228435), AP26113 (Phase I/II; NCT01449461), CH5424802 (Phase I/II; NCT01588028), X396 (Phase I; NCT01625234) are tested in advanced lung cancer patients in monotherapy[125,143] whereas STA-9090 (Phase I/II; NCT01579994) and AT13387 (Phase I/II; NCT01712217) inhibitors are tested in combination with crizotinib (detailed in Table 2) (ref.[103]).

OTHER CLINICALLY IMPORTANT BIOMARKERS

HER-2 (17q) overexpression has been described in approximately 20% NSCLC cases, whereas insertion in HER-2 exon 20 is the rare event (2%). These mutations occur mainly in adenocarcinoma, non-smokers and Asians and are associated with resistance to EGFR TKIs (ref.[50]). This resistance can be overcome by dual TKIs inhibition by lapatinib or BIBW 2292 (ref.[51,144]).

Translocation of ROS1 gene (6q) was identified as potential driver mutation in NSCLC cell lines[122]. ROS1 gene rearrangement has been described in approximately 2% NSCLC cases and tree fusion partners, CD74, SLC34A2 and FIG, have been identified to date[145,146]. Patients with ROS1 rearrangement have similar features as patients harboring EGFR mutation or ALK rearrangement, ROS1 rearranged patients are more likely Asian, younger and never smokers with adenocarcinoma histology[145]. ROS1 rearrangement leads to constitutive kinase activity and sensitivity to TKIs *in vitro*[135]. Bergethon et al.

Table 2. Trials are listed on the US National Institute of Health database[103].

Study	Phase	Treatment schedule	Stage	Biomarker selection	Pretreatment	Outcome	Est. enrollement
NCT01637597	Exploratory	crizotinib	IIIB,IV	ALK positive	yes (Pt-CHT)	Primary: ORR (comparing different methods of ALK evaluation) Secondary: PFS, OS	42
NCT01712217	Phase I	crizotinib + AT13387	IIIB,IV	ALK positive	yes (crizotinib)	Primary: DLT Secondary: pharmacokinetics, antitumor activity, CTCs, PFS, OS	228
	Phase II randomised	Arm 1: crizotinib Arm 2: crizotinib + AT13387	IIIB,IV	ALK positive	yes (crizotinib)	Primary: ORR Secondary: safety, PFS, OS, overall RR	
	Phase II randomised	Arm 1: AT13387 Arm 2: crizotinib + AT13387	IIIB,IV	ALK positive	yes (crizotinib)	Primary: objective overall RR Secondary: safety, PFS, OS	
NCT01441128	Phase I	Cohort 1: crizotinib + PF-00299804 Cohort 2: PF-00299804 untill progression; then crizotinib + PF-00299804	IIIB,IV	no	yes (CHT or targeted)	Primary: overall safety profile Secondary: pharmacokinetic parametrs, ORR; predictive biomarkers	22
NCT01579994	Phase I/II	ganetespib (STA-9090) + crizotinib	IIIB,IV	ALK positive	no (possible CHT)	Primary: MTD, efficacy Secondary: OS, overall RR, safety profile	55
NCT01500824	Phase II	crizotinib	IIIB,IV	ALK positive	yes (CHT)	Primary: ORR Secondary: PFS, OS, DCR, DR, TTR	50
NCT00932451	Phase II	crizotinib	IIIB,IV	ALK positive	yes (CHT)	Primary: ORR Secondary: DR, DCR, OS, HRQoL, plasma concetration of crizotinib, type of EML4-ALK variant, protein expression, PFS, TTR, QTc	1100
NCT01639001	Phase III randomised	Arm 1: crizotinib Arm 2: pemetrexed+cisplatin/carboplatin	IIIB,IV	ALK positive	no	Primary: PFS Secondary: ORR, DR, OS, TTD, HRQoL	200
NCT00932893	Phase III randomised	Arm 1: crizotinib Arm 2: docetaxel Arm 3: pemetrexed	IIIB,IV	ALK positive	yes (1x Pt-CHT)	Primary: PFS Secondary: ORR, AE, DR, DCR, OS, HRQoL, plasma concetration of crizotinib and ph.biomarkers, type of EML4-ALK variant, TTR, QTc	318
NCT01154140	Phase III randomised	Arm 1: crizotinib Arm 2: pemetrexed+cisplatin/carboplatin	IIIB,IV	ALK positive	no	Primary: PFS Secondary: ORR, AE, plasma concetration of crizotinib, type of EML4-ALK variant, patients reported outcome, OS	334
NCT01597258	Phase IV	(all patients treated by crizotinib)	ND	ND	ND	Primary: incidence of adverse drug reactions Secondary: ORR	2000

DLT-dose-limiting toxicity; CTCs-circulating tumor cells; PFS-progression free survival; OS-overall survival; ORR-objective response rate; overall RR-overall response rate; CHT-chemotherapy; Pt-CHT-platinum-based chemotherapy
DCR-disease control rate; DR-duration of response; TTR-time to response; TTD-time to deterioration; HRQoL-Health Related Quality of Life; QTc-corrected QC; ph.-pharmacodynamics; MTD-maximum tolerated dose; AE-adverse events; ND-not defined
AT13387 - Hsp90 inhibitor; PF-00299804 - panHER inhibitor; STA-9090 - Hsp90 inhibitor

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2013 Jun; 157(2):125-136.

showed promising antitumor activity of crizotinib in one patient with ROS1 rearrangement treated in clinical trial NCT00585195 (ref.[145]).

Mutations in PIK3CA, BRAF and AKT genes were reported in up to 3% of lung cancer cases. Large scale of BRAF, MEK, AKT and mTOR inhibitors are tested in ongoing clinical trials and are a promise for new personalized treatment opportunities in NSCLC patients[147,148].

CONCLUSION

Personalized medicine requires molecular genetic testing prior to decision about which therapeutic regimen is appropriate for an individual patient. Several predictive markers have been identified in NSCLC patients but only the minority of them is clinically used for therapy individualization. Nevertheless, personalized therapeutic opportunities of NSCLC are expected to increase in the following years. Number of clinical trials is currently evaluating efficiency of inhibitors directed against various genetic markers and ongoing intensive research is focused on identification of new therapeutic targets as well as testing new therapeutics. In future, clear algorithm reflecting clinically importance of each marker will be required for the routine diagnostics in NSCLC because of limited sample material. New methodologies combining currently using methods able to evaluate several markers simultaneously will be needed for appropriate NSCLC patient care management.

In conclusion, EGFR mutations and EML4-ALK rearrangement are currently the strongest predictive markers and only clinically applicable markers for patient selection to targeted therapy in NSCLC.

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CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure. The authors stated that there are no conflicts of interest regarding the publication of this article.

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