Chapter 10

Screening of Gene Mutations in Lung Cancer for Qualification to Molecularly Targeted Therapies

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Additional information is available at the end of the chapter

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1. Introduction

In many developed countries non-small-cell lung cancer (NSCLC), which accounts for approximately 85% of lung cancers, is the first cause of death in patients with malignant neoplasms. Depending on patients' medical status, surgical resection is possible in early stages of NSCLC. Regrettably, only 15-30% of newly diagnosed NSCLC cases can be qualified for operation. Therefore, chemotherapy and radiotherapy plays the dominant role in the multidisciplinary treatment of patients with NSCLC and small-cell lung cancer (SCLC). Unfortunately, both options of treatment in locally advanced and metastatic lung cancer have limited efficacy [1]. Molecularly targeted therapies offer new possibilities of lung cancer treatment in genetically predisposed patients. Within the next few years, personalised therapy of whole lung cancer population based on screening of different gene mutations will become a fact.

The development of cancer usually depends on strong carcinogenic effect of substances found in cigarette smoke on bronchial epithelial cells. Those carcinogens lead to genetic disorders that cause appearance of preinvasive changes: squamous dysplasia preceding carcinoma *in situ* and squamous cell carcinoma as well as atypical adenomatous hyperplasia (AAH) preceding development of adenocarcinoma. The preinvasive cells as well as cancer cells are characterised with large genome changes. Comparative genomic hybridisation (CGH) studies have identified chromosomal aberrations, particularly amplifications and deletions, in lung cancer cells. Cancer cells exhibit deletions of chromosome 17 short arm, with loss of *p53* gene (deletion of 17(p12-13) and chromosome 9 short arm, with loss of *p16* gene (*CDKN2A*) (deletion of 9(p21-22). Both mentioned genes are suppressor genes and lack of their protein products allows aneuploid cancer cells to survive and accumulate serious chromosomal aberrations like deletions 3(p14-21), 8(p21-23), 13(q14), 13(q22-24) and allelic losses at 9(p21), 13(q24) as well as gains at 1(q21-31), 3(q21-22), 3(q25-27), 5(p13-14), 8(q23-

24), 7(p12). The presence of deletions generates abnormal expression or impaired function of tumour suppressor genes such as *RB1*, *FHIT*, *RASSF1A*, *SEMA3B* and *PTEN*. However, the gain of chromosomal region including oncogenes is associated with overexpression or increased activity of *MYC*, *KRAS*, *EGFR*, *CCDN1*, *MCM2*, *RUVBL1*, *SOX2* and *BCL2* genes [2, 3]. Moreover, cell subclones with new genetic abnormalities may become dominant within metastases or within persistent or recurrent cancer deposits through selective pressures exerted by chemotherapy or molecularly targeted therapy [4].

Deletion of chromosome 17 short arm, with loss of *p53* gene, is the most frequent disturbance in lung cancer (50-70%). Squamous cell carcinoma (SCC) of lung exhibits higher frequencies of deletions at chromosomal regions 3(p14-21), 8(p21-23), 17(p13) (*p53* gene), 13(q14) (*RB1* gene), 9(p21) (*CDKN2A* gene) and amplification of 3(q21-22) (*SOX2* gene) when compared with adenocarcinoma (AC). Amplification of 7(p11) and 14(q13) causing increased gene dosage and protein expression of thyroid transcriptional factor-1/NK2 homeobox-1 (TITF-1/NKX2-1) and of epidermal growth factor receptor (EGFR) are prevalent in lung adenocarcinoma [2, 3].

2. Genetic mutations in lung cancer cells

Apart of chromosomal aberrations single gene mutations can appear in lung cancer cells. These mutations can be revealed with molecular biology techniques. Mentioned mutations do not often appear simultaneously in one cancer cell (less than 3% of tumour cells). They concern genes important for correct proliferation, differentiation and cell growth such as oncogenes and genes for signal proteins involved in a complicated network of intracellular signal transmission (predominantly genes for tyrosine and threonine-serine kinases).

The most important kind of genetic disturbances observed in NSCLC cells are point mutations (single nucleotide substitutions), small (few to a few dozen base pairs) deletions or insertions and formation of fusion genes as a result of translocation of gene fragments, usually within a single chromosome. Some of these alterations change the structure of proteins (sense mutations) which play an important role in oncogenesis, others shift the expression of oncogenes and suppressor genes, while some remain silent. Such processes lead to protein malfunction: they can increase or decrease protein expression or cause differences in normal enzyme activity.

Accumulation of driver mutations in different genes is detected depending on history of tumour exposure to carcinogens. Failure of DNA repair and progressive genetic instability leads to appearance of mutation that drives cancer development, its growth and metastases [4]. Molecular type of lung cancer is partially consistent with histological type of tumour. Although frequency of occurrence of some driver mutations is extremely rare, in only 20% of NSCLC tumours important mutations are not detected. Small cell lung cancer is less characterised in terms of incidence of genetic mutations. Until 2011, 1738 mutated genes and tens of thousands of different types of mutations were identified in NSCLC [2, 5, 6, 7]. Figure 1 shows the percentage of tumours with identified mutations in all histological types of NSCLC.

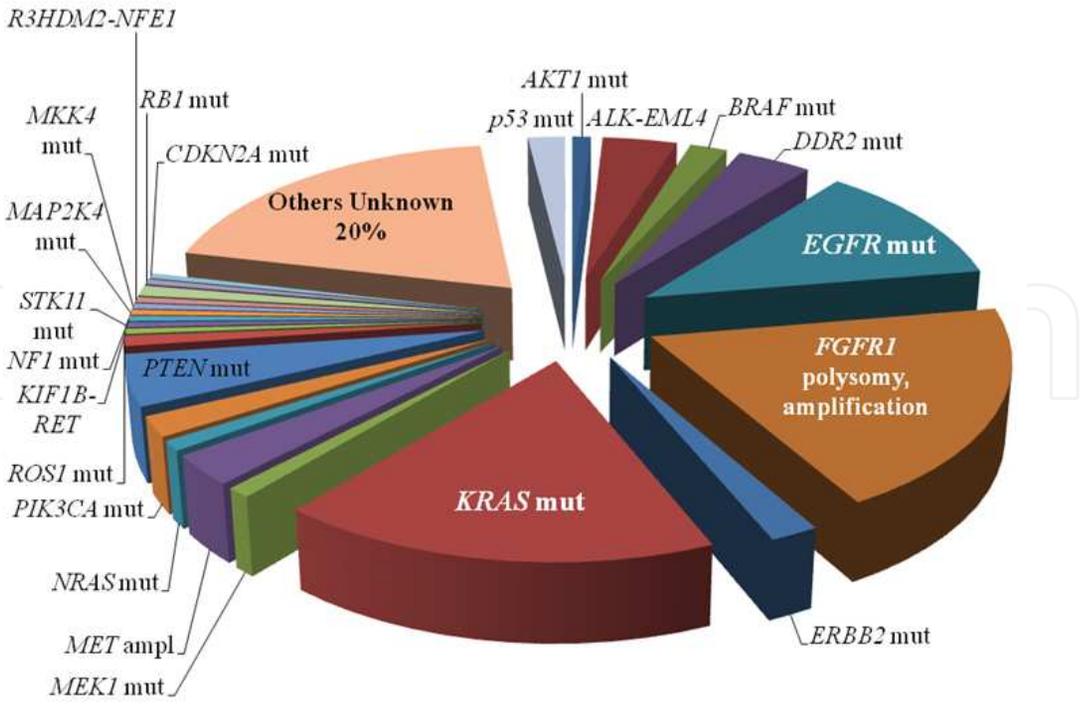

Figure 1. The percentage of NSCLC tumours with identified mutations in different genes (*EGFR* gene amplification and polysomy, as well as *p53* gene abnormalities which are common in NSCLC tumours have not been included on the graph).

NSCLC is a heterogeneous aggregate of histological subtypes, which traditionally have been grouped together because of similarities of treatment outcome. Ideally, a tumour classification system should include morphologic and genetic distinctions between tumour types, which will help to define specific subset of patients responsive to certain molecularly targeted treatment. In terms of genetic mutations squamous cell carcinomas are the least described. Mutations have not been detected in over 50% of already screened tumours (Fig. 2). On the other hand adenocarcinoma cases are definitely better described and in only 20% of tumours screening fails to describe any mutations. Among 10-15% of non-smokers (but also light-smokers and former smokers) adenocarcinoma might develop regardless of tobacco smoking. In these cases in almost all tumours different genetic mutations have been found, mostly in epidermal growth factor receptor (*EGFR*) and *KRAS* genes as well as presence of *EML4-ALK* fusion gene (Fig. 3). Accumulated evidence suggested that lung cancer in ever smokers and never smokers follow distinct molecular pathways and may therefore respond to distinct therapy. One could speculate than non-small cell lung cancer in ever and never smokers are two distinct disorders regarding their molecular level and the manner of treatment planning [5, 6, 7, 8, 9, 10, 11, 12].

The most frequent irregularity found among squamous cell carcinoma patients is an amplification of gene for fibroblast growth factor receptor type 1 (FGFR) and *p53* gene abnormalities. These disturbances could overlap with other mutations. In SCC it is extremely rare to detect *EGFR*, *PTEN*, *ERBB2 (HER2)*, *PIK3CA*, *DDR2* or *BRAF* mutations, which are more typical for adenocarcinoma [5, 6, 7].

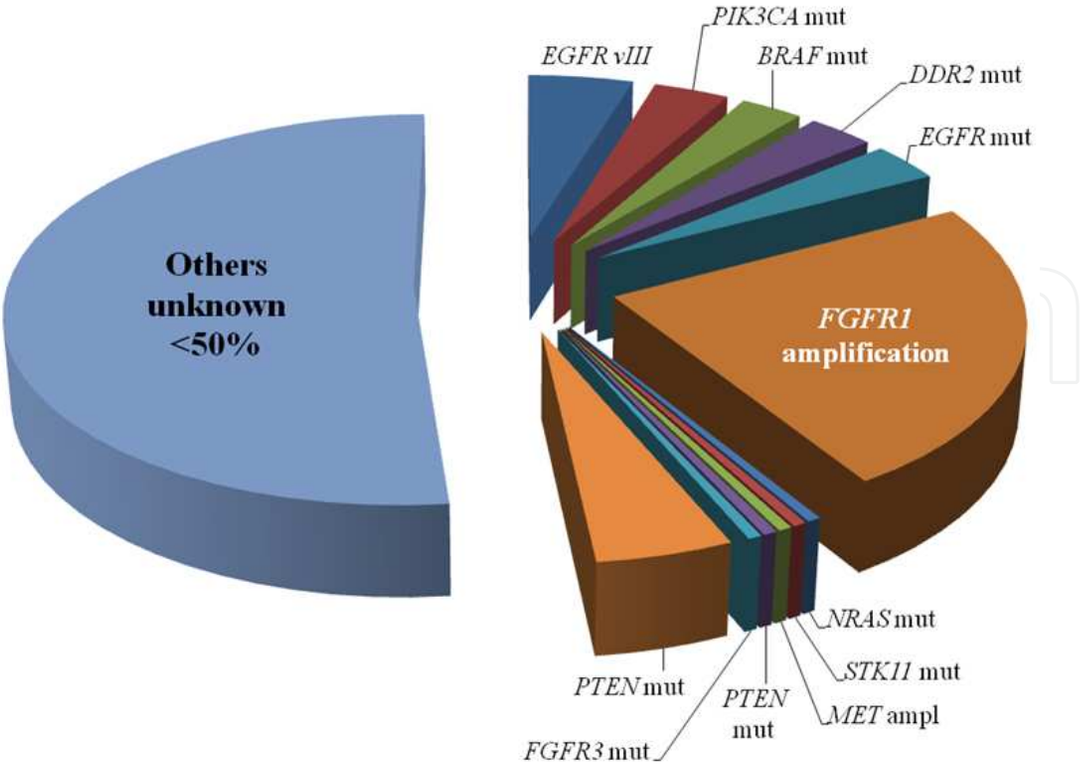

Figure 2. The percentage of SCC tumours with identified mutations in different genes (*p53* gene abnormalities which are common in SCC tumours have not been included on the graph)

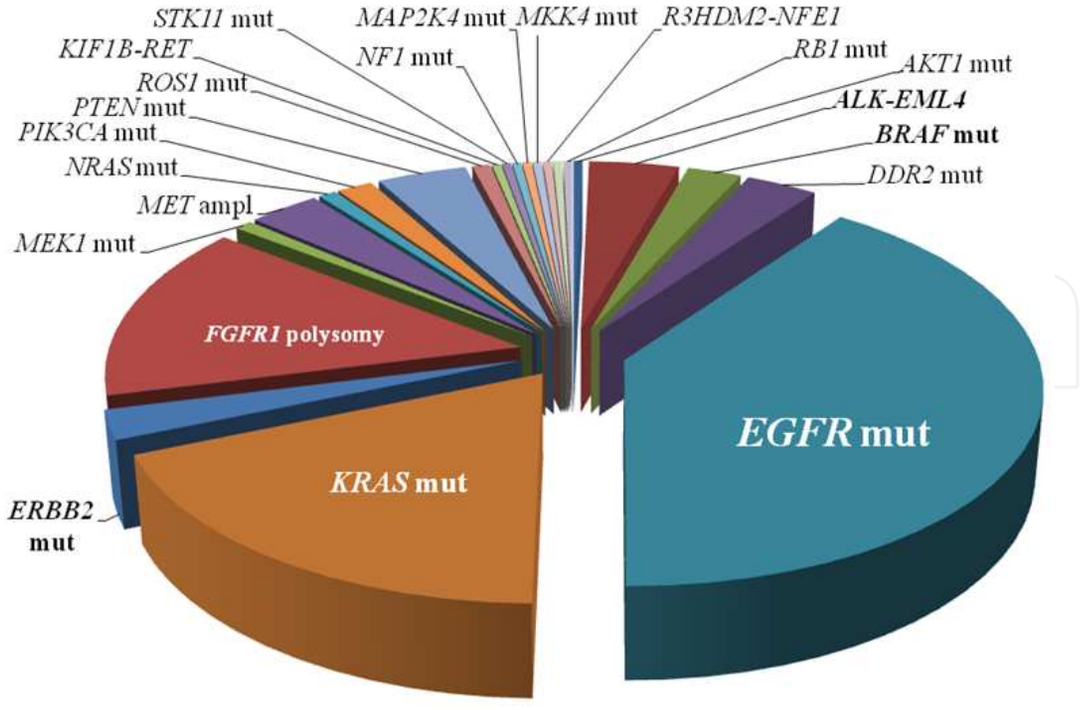

Figure 3. The percentage of AC tumours with identified mutations in different genes (*EGFR* gene amplification and polysomy have not been included on the graph).

Among patients with adenocarcinoma the most often detected irregularities are in *EGFR* gene, Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene and *p53* gene. In non-smoking Caucasian population activating mutations in *EGFR* gene appear with frequency of over 50%. The most common mutations in this gene are: small (9-21 base pair) deletions in exon 19 (48% of detected mutations) and missense mutations in exon 21 (L858R, 41% of detected mutations). Substitutions in exon 18-21 or insertions and duplications in exon 20 are rare but they also appear [2, 9, 13].

Mutations in exon 18-21 of *EGFR* gene concern tyrosine kinase domain of the EGF receptor. Overexpression of EGFRs' tyrosine kinase function leads to hyperphosphorylation of intracellular signalling proteins of Pi3K/Akt or RAS/RAF/MAPK pathways without having to activate the receptor with its specific ligand – the EGF. The activation of Pi3K/Akt pathway results in stimulation of transcription factors such as STAT or excessive proliferation of cancer cells. Mutations in *EGFR* gene are most common in papillary AC, less frequent in adenocarcinoma with „lepidic predominant" growth and least frequent in solid AC [2, 13].

Mutations in *KRAS* gene are also common and are detected in 15-25% of adenocarcinoma cases. *KRAS* gene, which is coding a low molecular weight guanosine triphosphatase (GTPase) is considered to be the most frequently mutated oncogene in lung AC arising in patients with history of smoking. Most *KRAS* mutations involve replacing glycine with other amino acids such as valine, aspartic acid and glutamic acid in codon 12. Less frequent mutations consider codon 13 and 61. The emergence of the mutation causes the reduction of GDPase activity with subsequent potent activation of mitogenic and proliferative signalling through the RAF/MEK/ERK/MAPK cascade. Mutations in *KRAS* gene are most common in solid mucinous adenocarcinoma and in acinar adenocarcinoma [2, 5, 6, 7].

Among other mutations detected in more than 2% of adenocarcinomas are *EML4-ALK* fusion gene, substitution V600E in *BRAF* oncogene, substitutions in codon 542, 545 and 1047 of *PIK3CA* oncogene, insertion in exon 20 of *ERBB2* gene, polysomy of *FGFR1* gene and amplification of *cMET* gene. Both anaplastic large cell lymphoma kinase (*ALK*) and echinoderm microtubule associated protein 4 (*EML4*) genes are located in chromosome 2p and fusion of both involves small inversions within this region. *EML4-ALK* fusion results in constitutive activation of ALK kinase. *EML4-ALK* fusion gene is prevalent in lung adenocarcinoma (2-4%), especially in signet ring cell carcinoma (<15%), in younger patients and in never- or light smokers. *EML4-ALK* fusion gene is mutually exclusive with *EGFR* and *KRAS* gene mutations. Recently, new fusion genes have been discovered in lung adenocarcinomas, including fusion of kinesis family member 5B (*KIF5B*) with ret proto-oncogene (*RET*) and fusion of coiled-coil domain containing protein 6 (*CCDC6*) with *RET* as well as fusions of *ALK* with c-ros oncogene 1 receptor tyrosine kinase (*ROS1*) [2, 5, 6, 7, 14, 15].

Information about the mutations mentioned above come from large databases such as *Catalogue of Somatic Mutations in Cancer* (COSMIC), *My Cancer Genome*, *The Cancer Genome*

Atlas and the results obtained by the American *Lung Cancer Mutation Consortium* (LCMC) [5, 6, 7, 16].

3. Molecular biology methods in lung cancer diagnostics

Mutation testing has become an essential determinant in clinical practice in decision of treatment options for patients with non-small-cell lung carcinomas. Unfortunately NSCLC tumours, in which the molecular diagnostics is carried out, are highly heterogeneous and the cytological and histological material is often insufficient to complete the analysis (small percentage of cancer cells or DNA fragmentation in the process of paraffin embedding). Direct sequencing is still a frequently used method despite having low sensitivity and being time-consuming and labour-intensive. However, direct sequencing and particularly next generation sequencing (technology based on reversible dye terminators, sequencing by ligation and pirosequencing) are the methods of high-throughput screening for unknown mutations. Microarrays containing oligonucleotide mutation probes are emerging as useful platforms for the diagnosis of multiple genetic abnormalities in cancer cells [17, 18].

The multiplex SNaPshot PCR (minisequencing) technique is a PCR (polymerase chain reaction)-based assay for detection of known mutations. Specific primer which anneals immediately adjacent to the mutated region is extended by one base using a fluorescently labeled ddNTPs, which are detected in capillary electrophoresis. No further extension is possible because of the ddNTP binding. This kind of reaction is being used more and more frequently because of its fast and sensitive detection of many known mutations in a single assay [19].

Recent advances in molecular techniques have enabled the design of sensitive detection assays based on quantitative real-time PCR, but usually with limited degree of mutation coverage. Allele-specific PCR (ASP-PCR), amplification refractory mutation system PCR (ARMS-PCR), clamp PCR and mutant-enriched PCR (ME-PCR) are among these techniques. The most frequently used is the ARMS–PCR method that can detect a known SNP (single nucleotide polymorphism). It consists of two complementary reactions: one containing an ARMS primer specific for the normal DNA sequence that cannot amplify mutant DNA at a given locus and the other one containing a mutant-specific primer that cannot amplify normal DNA. High resolution melting (HRM) real-time PCR is also a technique that might allow fast screening for mutations. The real-time PCR technology itself is highly flexible and many alternative instruments and fluorescent probe systems have been developed recently [17, 18].

For detecting polysomy, gene amplifications and the presence of fusion genes molecular probes labelled with different fluorochromes and fluorescence *in situ* hybridisation (FISH) technique are being used. Techniques related to FISH, but allowing to label only one gene fragment, are silver *in situ* hybridisation (SISH) and chromogenic *in situ* hybridisation (CISH). The FISH technique requires an assessment of signal quantity from labelled genes and chromosome fragments with fluorescence microscopy whereas SISH or CISH staining can be analysed in light microscope [17, 18].

Routine genetic testing for somatic mutations in lung cancer biopsies is becoming the standard for providing optimal patients care. However, it is unclear whether this testing should be routine for all lung cancer patients, because the prevalence of the most common mutations is very low especially in heavy smokers with squamous cell carcinoma. Moreover, great number of molecular biology methods and variety of biological material acquired from patients create a critical need for robust, well-validated diagnostic tests and equipment that are both sensitive and specific for mutations. An *In Vitro* Diagnostic Medical Device (IVD) is defined in Directive 98/79/EC of European Parliament and of the Council. IVD is described as any medical device which is a reagent, calibrator, control material, kit, equipment or system, whether used alone or in combination, intended by the manufacturer to be used *in vitro* for the examination of specimens, including blood and tissue donations, derived from the human body for the purpose of providing information concerning pathological state and congenital abnormalities of patients as well as to monitor therapeutic effect. IVD equipment is labelled by CE marking according to European Product Safety Regulations [20].

4. Molecularly targeted therapies in lung cancer

Molecularly targeted drugs are directed against abnormal proteins and other molecules, specific for cancer cells, participating in metabolic pathways. Excess activation of those pathways is essential for growth and unrestrained proliferation of cancer cells. Blocking these pathways results in inhibition of cell division and in cell apoptosis. Therefore, molecularly targeted drugs show high efficacy in two groups of patients:

1. if the mutation of the gene encoding a signalling pathway protein results in excessive activity while changing its structure, what allows more effective binding of the drug (e.g. activating mutations in *EGFR* gene and the efficacy of tyrosine kinase inhibitors of EGFR),
2. if the mutation of the gene encoding a signalling pathway protein results in excessive activity of the pathway and its blocking, regardless of the matching of the drug to the target protein, impairs tumour cell proliferation, which can be achieved at two levels:
   a. direct blocking of abnormal protein
   b. blocking of subsequent signalling pathway proteins stimulated by the abnormal protein [21].

Therefore, many of the therapies currently under development target several signalling proteins, especially tyrosine kinase receptors (e.g. EGFR, HER2, HER3, IGF-1R, cMET) or proteins in downstream signalling pathway (RAS/RAF/MAPK/mTOR and Pi3K/AKT) [19, 21].

Excessive stimulation of epidermal growth factor receptor increases proliferation of cancer cells in different kinds of tumours, i.a. in non-small-cell lung cancer. Cell growth signal is transmitted from EGFR (HER1), after its heterodimerisation with other member of HER family (ERBB2 – HER2, HER3 or HER4), through phosphorylation of Pi3K/AKT and

RAS/RAF/MAPK/mTOR pathway. The phosphorylation takes place due to EGFR tyrosine kinase activity, which performs hydrolysis of ATP to ADP and free phosphate. Tyrosine kinases are a part of EGFR but also other cell receptors and signalling proteins. Phosphorylation disorder initiated by EGFR tyrosine kinase is associated with the development of NSCLC that is independent from tobacco smoke carcinogens. Blocking of EGFR function may be achieved by using small molecule tyrosine kinase inhibitors (TKI) or monoclonal antibodies (such as cetuximab), which bind to extracellular domain of EGFR. Inhibition of tyrosine kinase function by TKI-EGFR is much more effective if the amino acid structure of the enzyme is disrupted by activating mutations in *EGFR* gene (described in the previous section). Cetuximab on the other hand demonstrates better effectiveness when high expression of EGFR is present on cancer cell surface [2, 9, 11, 12, 21, 22].

At the moment, two reversible EGFR TKIs are in use: gefitinib and erlotinib. Phase III study IPASS, carried out among Asian patients (up to 40% of *EGFR* gene mutation NSCLC carriers), has proven higher efficacy of gefitinib (71,2% response rate, longer progression free survival (PFS) up to 12 months and significant improvement in quality of life, but without overall survival (OS) prolongation) in compare to chemotherapy consisting of carboplatin and paclitaxel in patients with activating *EGFR* gene mutations. However, among patients with wild type *EGFR* gene, first line chemotherapy of advanced NSCLC with gefitinib was ineffective. The study included more than 1 200 adenocarcinoma patients, with a retrospective biomarker analysis performed on specimens from 437 tumour samples with evaluable *EGFR* gene mutation data. Mutations in *EGFR* gene were identified in 261 (59,7%) of these patients. Later studies comparing efficacy of erlotinib or gefitinib and standard chemotherapy had proven that EGFR TKIs are effective in first line of treatment (NEJ 002, WJTOG 3405, OPTIMAL, EURTAC studies) but only in patients with activating mutations in *EGFR* gene (Table 1). Moreover, OPTIMAL study showed that patients with deletion in exon 19 had longer median PFS than those with substitution L858R in exon 21 of *EGFR* gene. However, IPASS and WJTOG 3405 studies have not proven these observations [2, 11, 12, 21, 22, 23].

The BR.21 study concerned the effectiveness of erlotinib monotherapy in second or third line therapy in patients with advanced NSCLC. Erlotinib has prolonged PFS and improved quality of life when compared to best supportive care in the whole patients group, but an objective response was achieved in only 10% of patients. Patients with *EGFR* gene amplification, detected with FISH technique, responded more frequently to therapy with erlotinib. 61 (38,4%) of 159 tumours analysed in BR.21 study were positive for an increased *EGFR* gene copy number. Response rates were 21% and 5% in patients who were FISH-positive and FISH-negative, respectively. This benefit seemed to extend to survival (HR=0,43; p=0,004). It is not certain, if this result was related with underestimation of *EGFR* gene mutations in FISH-positive patients due to the use of sequencing method for *EGFR* gene mutation analysis. The INTEREST study confirmed this suggestion, demonstrating the superiority of gefitinib over docetaxel in second line of treatment in patients with activating mutation of *EGFR* gene. Application of reversible TKI-EGFR in II and III line of treatment in patients without activating mutations in *EGFR* gene is controversial [2, 11, 12, 21, 22, 23].

Study	Patients with mutation	Treatment arms	Response rate	Median PFS	PFS (favouring TKI-EGFR)
IPASS	216 Asian patients	gefitinib vs. paclitaxel/carboplatin	71% vs. 47%	9,5 vs. 6,3 months	HR=0,48 (95% CI: 0,36-0,64)
JP 0056 (NEJ 002)	200 North-East Japan patients	gefitinib vs. paclitaxel/carboplatin	74% vs. 31%	10,8 vs. 5,4 months	HR=0,31 (95% CI: 0,22-0,41)
WJTOG 3405	177 Asian patients	gefitinib vs. docetaxel/carboplatin	62% vs. 32%	9,2 vs. 6,3 months	HR=0,49 (95% CI: 0,34-0,71)
OPTIMAL	165 Asian patients	erlotinib vs. gemcitabine/carboplatin	83% vs. 36%	13,1 vs. 4,6 months	HR=0,16 (95% CI: 0,10-0,26)
EURTAC	170 Caucasian patients	erlotinib vs. platinum doublet	58% vs. 15%	9,7 vs. 5,2 months	HR=0,42 (95% CI: 0,27-0,64

Table 1. Prospective, randomised studies of efficacy of first-line TKI-EGFR and standard chemotherapy in patients with *EGFR* gene mutations [12].

The phase III SATURN study was designed to examine the effect of erlotinib in maintenance therapy dedicated to patients who had clinical benefit after 4 cycles of standard chemotherapy. PFS was significantly prolonged (HR=0,71; p<0,0001) and response rate (11,9% vs. 5,4%) was improved with erlotinib compared to best supportive care in all patients. However, significantly prolonged PFS was observed with erlotinib mainly in group of patients whose tumours had *EGFR* mutation (HR=0,10; p<0,0001) [2, 11, 12, 23].

Although controversial clinical trial results, National Comprehensive Cancer Network (NCCN) recognises that the presence of EGFR-activating mutations represents a "critical" biomarker for appropriate patients selection for TKI-EGFR therapy [24].

Some genetic irregularities may be responsible for occurrence of primary or secondary resistance to reversible TKI-EGFR and disease progression even after more than ten months of therapy. *EGFR* wild-type gene and *KRAS* gene mutations are associated with intrinsic TKI-EGFR resistance. Moreover mutations in *KRAS* and *EGFR* genes do not occur simultaneously in the same cancer cell. Patients with mutated *KRAS* gene experience better PFS with standard chemotherapy than with TKI-EGFR therapy. However, a subgroup of 90 patients from SATURN study who had *KRAS* mutation showed no significant difference in PFS in erlotinib-arm and placebo-arm. Although KRAS mutation has been associated with clinical outcomes with cetuximab in colorectal cancer, no association was reported from

analyses of clinical studies of cetuximab in combination with chemotherapy in patients with NSCLC. Currently, *KRAS* mutation testing is not recommended in molecular diagnosis of NSCLC patients [11, 12].

The secondary resistance to reversible TKI-EGFR is connected with the inability to extend overall survival with erlotinib or gefitinib therapy. Underlying mechanism of resistance to reversible EGFR TKIs is an amplification of *IGF1R* and *MET* gene, but also mutations in exon 20 of *EGFR* and *HER2* genes. The presence of such abnormalities may have a pivotal role in qualification to novel therapies, currently in their last phase of clinical trials. Inhibitors of insulin-like growth factor receptor 1 (IGF1-R), both small molecule as well as monoclonal antibodies, and inhibitors of receptor for hepatocyte growth factor (cMET) (e.g. tivantinib – ARQ-197 or MetMab) may be used in some patients treated with reversible TKI-EGFR among whom a resistance for the therapy has occurred as an alternative way of Pi3K/AKT pathway stimulation created through overexpression of IGF1R and cMET (Figure 4) [25, 26, 27].

The occurrence of T790M mutation in exon 20 of *EGFR* gene and mutations in exon 20 of *HER2* gene may be important for the proper qualifications for the treatment with irreversible EGFR TKIs. Drugs like afatinib (BIBW-2992), PF-00299804 or neratinib (HKI-272) may be effective in case of resistance to reversible TKI-EGFR when a secondary mutation is present (e.g. T790M). The action of afatinib remains until the EGFR protein is removed from the cancer cell surface. Furthermore, afatinib also blocks HER2 and HER4 proteins which are preferential heterodimerisation partners for EGFR during stimulation by EGF. In LUX-Lung 1 study, afatinib efficacy (prolongation of PFS) was proven as a rescue treatment after failure of erlotinib or gefitinib if duration of second-line TKI-EGFR treatment exceeded 24 weeks (HR=0,38, p<0,0001). Irreversible TKI-EGFR may also be more effective than reversible TKI-EGFR in first-line of treatment of patients with activating mutations of *EGFR* gene. In the LUX-Lung 2 study, 129 patients with activating *EGFR* mutations and no previous TKI-EGFR treatment received afatinib as a single agent. Overall response rate was 60% with a promising PFS of 14 months. LUX-Lung 3 and LUX-Lung 6 studies are designed to compare effectiveness of afatinib and chemotherapy based on pemetrexed and cisplatin or gemcitabine and cisplatin in patients with *EGFR* mutations. As first-line treatment of patients with known *EGFR* mutation, PF-00299804 showed encouraging efficacy, which exceeded the erlotinib effectiveness. In patients with T790M and T854A mutations in *EGFR* gene, the combination of irreversible TKI-EGFR therapy with application of monoclonal antibody against EGFR (cetuximab) may be also reasonable [11, 12, 25, 26, 27].

Big hopes for the development of lung adenocarcinoma therapy are related to phase III studies over a novel, small molecule, molecularly targeted drug – crizotinib, an inhibitor of ALK, ROS1 and cMET. Crizotinib is particularly active in patients with *EML4-ALK* fusion gene, inducing disease control in up to 90% of such patients and prolonging their overall survival. In patients with *EML4-ALK* fusion gene, 64% of patients treated with crizotinib

survived more than 2 years and 77% of patients survived more than 1 year. Newly defined kinase fusions (KIF5B with RET and ROS1 with ALK and with other fusion partners) may be also promising targets for molecular therapies [11, 12, 14, 15, 26, 27].

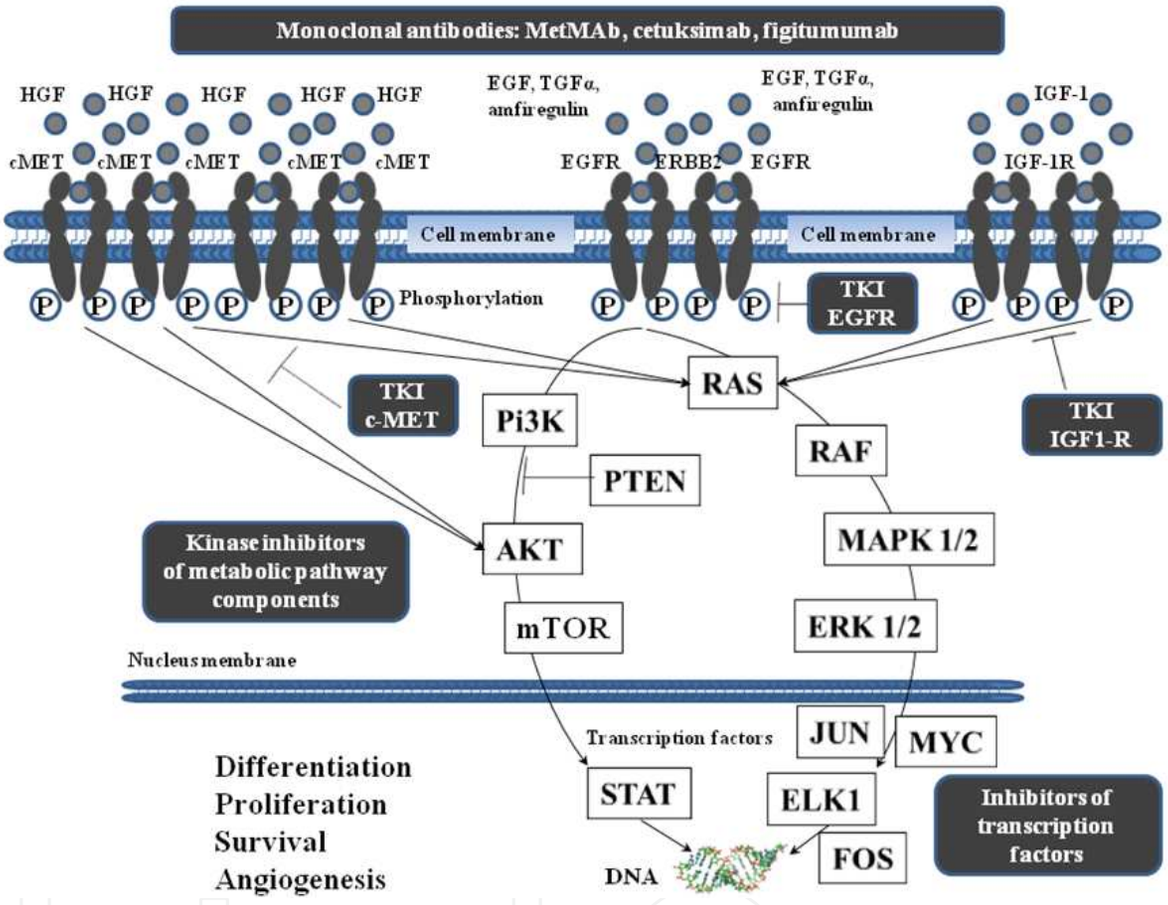

Figure 4. *EGFR* pathway components and possibility of new molecularly targeted therapies application in resistance to reversible TKI-EGFR.

Drugs inhibiting neoangiogenesis within the tumour have also found an application in molecularly targeted therapy of patients with NSCLC. These drugs are bevacizumab – a monoclonal antibody directed against vascular endothelial growth factor (VEGF) and small molecule drugs, inhibiting tyrosine kinase functions of VEGFR, PDGFR, FGFR, RET and c-Kit (vargatef, sunitinib) [26, 27]

American *Lung Cancer Mutation Consortium* (LCMC) had screened NSCLC tumour samples not only for *EGFR* and *ALK* mutations, but also for other known mutations such as *KRAS*, *EGFR*, *EML4-ALK*, *BRAF*, *HER2*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1* and *MET* gene irregularities.

Mutations were found in 54% (280/516) of completely tested tumours, in 15 certified genetic laboratories. Mutation screening is not only for research purposes, but is also designed to determine patients who might benefit from molecularly targeted therapies. Molecular testing could definitely identify the mutations associated with response or resistance to targeted therapies [16]. Nowadays, we have an opportunity to match molecularly targeted therapies with the structure of proteins that are taking part in signalling pathways of neoplasm cells. The efficiency of tyrosine kinase inhibitors of EGFR (erlotinib, gefitinib) and ALK (crizotinib) in NSCLC patients bearing *EGFR* or *ALK* activating mutations is the example of such relationship. These observations create new possibilities for personalisation of known molecularly targeted therapies (registered and tested in clinical trails) in large population of NSCLC patients [16]. LCMC idea was used to describe potential capability of therapy of NSCLC patients, based on presence of mutations in cancer cells. Similarly, the BATTLE program at the M.D. Anderson Cancer Centre in Houston assessed biomarker-guided treatment in patients with previously treated, advanced NSCLC and biopsy-amenable disease. For this purpose, cancer gene databases should be created to determine what is known about germline and somatic gene variants as well as treatment options and their outcomes. According to recent cancer genomic knowledge, clinical trials of novel molecularly targeted drugs, could be offered to cancer patients who are unlikely to benefit from a standard therapy, with relatively poor prognosis and to patients who are more likely to benefit from a novel therapy due to the presence of tumour genetic abnormalities that predict sensitivity, lack of resistance or toxicity of a treatment (Table 2) [4, 16, 19, 26, 27].

Genetic abnormality	**Treatment**	**Mechanism of action**
activating mutation of *EGFR*	erlotinib or gefitinib	small molecule, reversible TKI-EGFR
activating mutation of *EGFR*	erlotinib + OSI-906 or MM-121 or MK-0646	small molecule, reversible TKI-EGFR + small molecule TKI IGF-1R or fully human monoclonal antibody against ErbB3
KRAS mutation; *MET* amplification	erlotinib + tivantinib (ARQ-197) or onartuzumab (MetMAb); JTP-74057 (GSK1120212);	small molecule TKI-EGFR + small molecule TKI cMET or monovalent (one-armed) monoclonal antibody against cMET; small molecule inhibitor of MEK 1/2 serine/threonine kinase;
fusion gene *EML4-ALK* and fusion genes with *ROS1* gene component; *ROS1* mutation	crizotinib, AP-26113, LDK-378, AF-802	small molecule TKI of ALK, ROS1 and cMET; small molecule TKI of ALK and EGFR; small molecule TKI of ALK

NRAS, MEK1 or *BRAF* mutation	GSK-1120212	small molecule inhibitor of MEK 1/2 serine/threonine kinase
BRAF, NRAS mutation	GSK-2118436; vemurafenib (PLX-4032)	small molecule inhibitor of BRAF serine/threonine kinase
mutation in exon 20 of *EGFR* (e.g. T790M); *HER2* mutation	Afatinib (BIBW2992), neratinib, PF299804, CI-1033, EKB-569, AV-412/MP-412, lapatinib	small molecule, irreversible TKI of pan-HER; small molecule, irreversible TKI of EGFR and HER2
PIK3CA mutation	BEZ-235, GDC-0491, SAR-245409, BKM-120, BYL-716, OSI-027, PX-866, MK-8669	small molecule inhibitor of mTOR and PI3K kinases; small molecule inhibitor of pan-PI3K; small molecule selective inhibitor of PI3Kα
MEK1 mutation	JTP-74057 (GSK-1120212); selumetinib (AZD-6244), GDC-0973, MEK-162, MSC-1936369B	small molecule inhibitor of MEK 1/2 serine/threonine kinase (MAPK/ERK kinase1/2 kinases);
DDR2 mutation (S768R)	erlotinib + dazatinib or nilotynib	small molecule inhibitor of BCR-ABL, SRC, c-Kit, EPH and PDGFRβ
FGFR amplification	PD-173074, ponatinib (AP24534), BGJ-398, FP-1039	small molecule TKI of FGFR and VEGFR; small molecule kinase inhibitor of native and mutated BCR-ABL, VEGFR2, FGFR1, PDGFRα, mutated FLT3 and LYN; small molecule TKI of FGFRs; monoclonal antibody against FGFR1
PDGFR amplification, *PDGFR* mutation, *c-Kit* mutation	MEDI-575, IMC-3G3, sunitinib, sorafenib, OSI-930, pazopanib (votrient)	Monoclonal antibody against PDGFR α; small molecule inhibitors of kinases of VEGFR1-3, RET, c-Kit, PDGFR α and β
FGFR and/or *PDGFR* amplification	intedanib (BIBF-1120), dovitinib (TKI258)	small molecule inhibitor of angiokinase (FGFR, PDGFR, VEGFR)
BRCA1 deficiency	olaparib + cisplatin	small molecule inhibitor of poly(ADP-ribose) polymerase (PARP)
AKT1 mutation	MK-2206, GSK-2110183	AKT inhibitors

Table 2. An example of qualification possibilities for molecularly targeted therapies based on NSCLC cell molecular signature (in most countries gefitinib, erlotinib and crizotinib are the only registered drugs in NSCLC therapy; other indications for therapy are hypothetical and are based only on the results of early clinical trials).

5. Summary

It is worth remembering that the presence of mutations may overlap with much more severe genetic abnormalities of lung cancer cells. These irregularities result in profound changes in cancer cells ability to proliferate and in effect it becoming invulnerable to selective molecularly targeted therapies. Therefore, at present only few above mentioned drugs may be used in lung cancer patients instead of standard chemotherapy. In most cases, molecularly targeted therapies will find an application in patients who have already exhausted all standard chemotherapy forms.

Multiple genetic alterations in lung cancer tumours and different targeted therapies based on appropriate molecular status of patients are still under investigation. However, the problems with proper obtaining and storage of tumour tissue for molecular testing as well as choosing adequate molecular methods for gene mutation screening is still open for discussion.

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