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overview

Therapeutic implication of molecular profiling in patients with non – small cell lung cancer

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Abstract

Traditional, 'one size fits all' approach to chemotherapy has reached efficacy plateau. The development of molecular biomarkers is crucial for improving efficacy of therapy in patients with NSCLC. We review prognostic and predictive value of molecular biomarkers with special overview on biomarker-driven personalized therapy of NSCLC. Main focus is this review is on significance of EGFR mutations and ALK – EML4 alterations on due to availability of targeted drugs against these specific aberrations.

INTRODUCTION

Lung cancer is the second most common cancer and a leading cause of cancer-related deaths in both males and females worlwide, with non-small cell lung cancer (NSCLC) representing around 85% of all cases *(1)*. The majority of patients present at the time of diagnosis with advanced disease which still remains incurable. Standard first line treatment for advanced disease remain platinum – based doublets, with median survival of around 10 months, andless than two percent of patients still alive after two years *(2, 3)*. Despite poor overall results, some patients benefit from chemotherspy, so selection of patients which would benefit from chemotherapy is crucial to maximase efficacy and limit toxic effects. In the last five years, we have learnt that personalized therapy, with tailoring chemotherapy regimen according to histology can improve efficacy *(4)*. Furhter progress has been done with improved understanding of the molecular mechanisms involved in development, growth and spread of cancer. These advances made it possible to focus on specific pathways active in NSCLC which enabled development of a new drugs targeting these cancer cell specific alterations. Since clinical characteristics alone have proven insufficient to predict effect, targeted drugs showed maximum effect in patients whose cancer cells harbor specific molecular alteration, so detection of those alterations became crucial for choice of specific drug *(5)*. These molecular changes appear to be both predictive and prognostic in patients with NSCLC *(6)*. In this paper we will review the most important molecular biomarkers for targeted drugs in patients with NSCLC.

Nucleotide excision repair (NER) pathway

The NER pathway is involved in repairing DNA damage and its components have been assessed in different types of cancer including lung cancer. Three biomarkers from this group have been studied

extensively in lung cancer: excision repair cross-complementary group 1 (ERCC1), ribonucleotide reductase messenger 1 (RRM1) and breast cancer gene 1 (BRCA1).

Excision repair cross-complementary group 1 (ERCC1)

ERCC1 is protein involved in NER and interstrand cross-link repair (ICL-R) pathways. ERCC1 recognizes and removes platinum adducts from DNA and simultaneously repairing interstrand DNA cross-link *(7)*. In vitro studies showed that lung cancer cell lines with high expression of ERCC1 are resistant to platinum *(8)*. Simon and colleagues assessed prognostic value of ERRC1 in surgically treated, chemotherapy – naive patients with NSCLC *(9)*. Expression of ERRC1 was measured by quantitative reverse transcriptase – polymerase chain reaction (qRT-PCR). They showed almost three times greater overall survival in patients with high ERCC1 expression compared to those with low expression (94.6 months versus 35.5 months, p = 0.01).

The International Adjuvant Lung Cancer Trial (IALT) demonstrated an absolute benefit of 4.1% in 5-year overall survival among 1867 patients who were treated with adjuvant cisplatin-based chemotherapy *(10)*. Ollausen and colleagues retrospectively analyzed tissue samples from IALT in a study named IALT-Bio. ERCC1 from paraffin-embedded formalin-fixed tissue samples from 867 out of 1867 patients enrolled in IALT trial, was assessed by immunohistochemistry. In the control group, which did not receive adjuvant chemotherapy, survival was significantly longer in patients with high ERCC1 expression than in those with low ERCC1 expression (adjusted hazard ratio for death, 0.66; 95% CI, 0.49 to 0.90; P=0.009). This finding was in concordance with study conducted by Simon *et al. (9)*. Inversely, adjuvant chemotherapy, as compared with observation, significantly prolonged survival among patients with ERCC1-negative tumors (adjusted hazard ratio for death, 0.65; 95% confidence interval ŠCIĆ, 0.50 to 0.86; P=0.002) but not among patients with ERCC1-positive tumors (adjusted hazard ratio for death, 1.14; 95% CI, 0.84 to 1.55; P=0.40). Thus, IALT – Bio study confirmed predictive role of ERCC1 for platinum-based chemotherapy.

One small, uncontrolled study conducted on 56 patients with advanced NSCLC (stage IIIB and IV) treated with cisplatin and gemcitabine, showed that low ERRC1 expression is associated with longer survival comparing with high ERCC1 expression (HR 0.32, 95% CI 0,14–0,71; p = 0,005) *(12)*. Again, ERCC1 level were assessed with qRT-PCR.

Larger, prospective studies in patients with advance lung cancer are needed to confirm prognostic and predictive value of ERCC1.

Ribonucleotide reductase messenger 1 (RRM1)

RRM1 is one of the regulatory components of ribonucleotide reductase involved in the formation of deoxyribonucleotides from ribonucleotides, one of the crucial steps in DNA synthesis and repair. Additionally, RRM1 mediates suppression of cell migration and tumor metastasis by inducing tumor-suppressor gene PTEN, responsible for attenuation of different growth-factor pathway signaling *(6)*. RRM1 is the predominant target of the nucleoside analogue gemcitabine.

In 187only surgically treated patients, Zheng and colleagues investigated RRM1, ERCC1 and PTEN expression by use of immunofluoroscence and automated quantitative analysis (AQUA) *(13)*. Patients with low RRM1 expression had significantly lower disease-free survival in comparison to those with high RRM1 expression (54.5 months versus 120 months, HR 0.46; p= 0.004).

Rosell and colleagues conducted a trial on 100 patients with advanced lung cancer.[14] Patients were treated with gemcitabine and cisplatin or gemcitabine, cisplatin and vinorelbine or gemcitabine and vinorelbine followed by vinorelbine/ifosfamide. RRM1 and ERCC1 mRNA expression was analyzed from paraffin-embedded samples obtained during bronchoscopy by real-time quantitative reverse transcription-PCR. In the gemcitabine/cisplatin arm, patients with low RRM1 mRNA expression levels had significantly longer median survival compared to with high levels (13.7 versus 3.6 months; 95% CI, 9.6–17.8 months; P = 0.009=. Median survival was also significantly longer among patients with low mRNA expression levels of both RRM1 and ERCC1 (not reached), than among those with high expression of both genes (6.8 months; 95% CI, 2.6–11.1 months; P = 0.016).

A prospective phase II trial was conducted by Simon and colleagues trying to assess therapeutic decision making based on RRM1 and ERCC1 expression levels in patients with advanced lung cancer *(15)*. Patients were allocated to one of four treatment arms according to RRM1 and ERCC1 expression: gemcitabin and carboplatin (low RRM1 and low ERCC1); gemcitabin and docetaxel (low RRM1 and high ERCC1); docetaxel and carboplatin (high RRM1 and low ERCC1); docetaxel and vinorelbine (high RRM1 and high ERCC1). Disease response rate was seen in 44% of patients (23 of 53), with median survival of 13.3 months and 1-year survival of 59%. The study raised an issue of importance of tissue acquisition in tailoring the treatment in patients with lung cancer. Namely, already diagnosed patients had to undergo another biopsy for molecular analysis which resulted in high drop-out rate (eligible were 53 out of 85 patients). Never the less, repeated biopsy was well tolerable.

On the other hand, Danish study did not confirm correlation between RRM1 and gemcitabine *(16)*. Vilmar and colleagues included a total of 443 patients that were randomly assigned to either paclitaxel, cisplatin with gemcitabine or cisplatin and vinorelbine arm. Immunohistochemical evaluation of RRM1 was correlated to clinical end-points. Disease control rate, progression-free survival (PFS) and overall survival (OS) were substantially improved in patients with RRM-negative tumors receiving cisplatin snd vinorelbine when compared to patients with RRM-positive tumors (68.8% versus 31.2%, P = 0.046, 6.90 months versus 3.93 months, P = 0.000

and 11.57 months versus 7.4 months, P = 0.002, respectively). Surprisingly, RRM1 protein expression was without any predictive impact in patients treated with cisplatin, paclitaxel and gemcitabine.

ITACA study an ongoing, which is prospective, randomized trial with half of patients treated with standard chemotherapy regimen, and other half with tailored chemotherapy according to molecular profiling (ERRC1, RRM1 and thymidilate synthase) will give us more insight in the prognostic and predictive value of mentioned biomarkers.

Breast cancer gene 1 (BRCA1)

BRCA1 is involved in transcription-coupled nucleotide excision repair. Additionally, BRCA1 and beta-tubulin co localise to the microtubules of the mitotic spindle, therefore, BRCA1 may be involved in regulation of mitotic spindle assembly. BRCA1 is a component of the BRCA1-associated genome surveillance complex, which contains several mismatch proteins, suggesting its role in mismatch repairs *(6)*. BRCA1 is involved, like ERCC1, in platinum resistance. BRCA1 is more extensively studied in breast cancer, bust several studies have been done in patients with lung cancer.

Prognostic value of BRCA1 was evaluated in 126 surgically treated, chemotherapy-naive, patients with NSCLC. Increased expression was associated with decrease overal survivall (HR 1.98; 95% CI 1.11–6.00; p = 0.02) *(17)*.

Spanish Lung Cancer Group conducted a pilot trial of molecular analysis of BRCA1 for selection of adjuvant treatment in completely resected patients with stage II and III NSCLC *(18)*. Adjuvant tretament was selected according to BRCA1 levels: patients with highe BRCA 1 expression received single-agent docetaxel; patients with intermediate levels of BRCA1 received docetaxel plus cisplatin; and patients with low BRCA1 expresione received cisplatin and gemcitabin. The conclusion of the study was that single agent docetaxel in adjuvant setting had no detrimental effects on survival when compared to combination treatment. The Spanish Lung Cancer Group has opened a phase III study of customized adjuvant chemotherapy based on BRCA1 mRNA levels.

Cell-cyle regulators

Precise regulation of the cell cycle is a fundamental requirement for the homeostasis of eukaryotic cells, and uncontrolled cell proliferation is an invariable characteristic of human cancers. Indeed, the proliferation of cancer cells is sustained in the absence of growth factors and is insensitive to growth-inhibitory signals *(19)*. Among the many pathways altered in lung cancer malignancy, the most important involve disruption of the normal cell cycle regulation.

p53

The mutations in tumor-suppressor gene TP53 are the most common molecular alterations in lung cancer. The p53 binds to DNA and has three main physiological functions: cell-cycle regulation, induction of apoptosis and stabilization of the genome. The p53 has been studied extensively in lung cancer, both at DNA and protein level. It has been reported that somatic mutations and increased expression of TP53 were frequently found in ~23% and ~65% of NSCLC, respectively *(20)*. TP53 is the most extensively investigated prognostic marker in NSCLC, and the meta-analyses have suggested a weak to modest negative prognostic role for TP53. Steels and colleagues identified aberrant TP53 as a prognostic factor in NCSCL at any stage (localized or advanced disease) and any histology (adenocarcinoma and squamous cell carcinoma). Moreover, no significant differences were found when comparing the different antibodies used in the Immunohistochemical assays *(21)*. Mitsodumi and colleagues conluded that the presence of an aberrant TP53 status could identify a disease with more aggressive features and, thus, a potentially shorter survival *(22)*. Several studies indicate that TP53 mutations confer chemoresistance to lung cancer cells in vivo and in vitro. Determining TP53 status may be of great value in the choice of chemo/radiation therapy. For instance, it is well known that tumors containing the mutant TP53 are more resistant to ionizing radiation than those with the wild-type TP53. The frequent inactivation of TP53 in human tumors suggests that the reconstruction of the TP53 mediated pathway in tumor cells might be an attractive tumor cell-specific strategy for treating cancers *(20)*. Tsao and colleagues have evaluated the prognostic and predictive value of p53 gene and/or protein aberrations using tumor samples from JBR.10 *(23),* a North American phase III intergroup trial that randomly assigned 482 patients with completely resected stage IB and II non-small-cell lung cancer (NSCLC) to receive four cycles of adjuvant cisplatin plus vinorelbine or observation alone. Untreated p53-positive patients had significantly shorter overall survival than did patients with p53-negative tumors (hazard ratio [HR] = 1.89; 95% CI, 1.07 to 3.34; p =0.03). However, these p53-positive patients also had a significantly greater survival benefit from adjuvant chemotherapy (HR = 0.54; p = 0.02) compared with patients with p53-negative tumors (HR = 1.40; p = 0.26; interaction p = 0.02) *(24)*.

The results of these studies suggest that expression of p53 is a prognostic and predictive factor of benefit ffrom adjuvant chemotherapy in patients with NSCLC *(6)*.

KRAS

KRAS is a member of the RAS family of oncogenes, which encode small GTPases that activate various signalling pathways that regulate cell proliferation, differentiation, cell shape and cell survival, and other activities. RAS proteins function as molecular switches that cycle between a GDP-bound inactive state and GTP-bound active state.Mutation in KRAS gene are usually found in codons 12 and 13 and have been found in up to 30% of lung adenocarcinomas. These mutations are usually found in smokers and are much more common in adenocarcinoma than in other subtypes of lung cancer *(25)*.

A meta-analysis involving 881 cases of NSCLC done by Huncharek and colleagues showed that patients which harbor KRAS mutations have shortened overall survival, with RR of 2.35 (95% CI = 1.61–3.22) *(26)*. A previously mentioned molecular analysis form JBR.10 *(24)*, also looked for mutations in codons 12, 13 and 61 in HRAS, KRAS and RAS genes. Mutations were identified in 26% of patients and were more common in patients with large cell and adenocarcinoma compared to other subtypes. RAS mutation was not significant prognostic marker for survival (HR 1.23, 95% CI 0.76–1.97; p = 0.40). In patients with wild type RAS, adjuvant chemotherapy significantly prolonged survival in comparison to observation only (HR 0.69; p = 0.03). On the contrary, there was no benefit in patients harboring RAS mutations with adjuvant chemotherapy (HR 0.95; p = 0.87).

In registration erlotinib trial, BR.21 KRAS mutation status was found in 15% of patients (30 out of 206). In patients with KRAS mutations treated with erlotinib poorer outcome was reported versus observation (HR 1.67; 95% CI 0.82–4.50, p = 0.31). On the other hand, improved outcome was seen in patients with wild-type KRAS treated with erlotinib as compared to observation group (HR 0.69; 95% CI 0.49–0.97; p = 0.03) *(27)*.

Jänne and colleagues just recently published results of a randomized, double-blind, phase II clinical trial of oral MEK inhibitor selumetinib in combination with docetaxel compared with docetaxel alone in patients with KRAS-mutant NSCLC that had progressed after first--line therapy for advanced disease *(28)*. This was the first clinical trial to prospectively enroll patients with KRAS--mutant NSCLC to assess benefit with a targeted compound: selumetinib targets MEK, a downstream effector molecule in the RAS signaling pathway. Although this small multicentre trial did not achieve its primary aim of significant improvement in overall survival, there was a numerical improvement in overall survival with selumetinib plus docetaxel compared with placebo plus docetaxel, with median overall survival of 9.4 and 5.2 months, respectively (HR 0.80; 80% CI 0·56–1·14; one-sided p = 0.21). Median progression-free survival was 5.3 months in the selumetinib plus docetaxel group compared to 2.1 months in the placebo plus docetaxel group (HR 0.58; 80% CI 0·42–0·79; one-sided p=0·014). Furthermore, response rate was 37% in the combination group compared with 0% in the docetaxel alone group (p<0·0001) *(29)*. However, to evaluate the clinical benefit of sulemetinib plus docetaxel in KRAS – mutant NSCLC, additional larger are warranted.

Protein kinases

Protein kinases play a crucial role in signal transduction, cellular proliferation, differentiation, and other regulatory mechanisms. The identification of growth-related protein kinases, especially tyrosine kinases, as a therapeutic target for cancer and adenosine triphosphate (ATP)-binding domain of tyrosine kinases as an attractive target for drug design have led to clinical development of an a specturm of tyrosine kinase inhibitors (TKIs) in various malignancies, including lung cancer.[30]

The most extensively studied tyrosine kinases in the recent past are epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK).

Inhibitors of epidermal growth factor receptor

The signaling pathway of the epidermal growth factor receptor (EGFR), a cell-surface receptor, is activated in more than half of patients with NSCLC, and this activation can be the result of protein overexpression, increased gene copy number, or genetic mutations. The ERBB receptor family consists of four receptor tyrosine kinases: EGFR (also called ERBB1 or HER1), ERBB2 (HER2/neu), ERBB3 (HER3), and ERBB4 (HER4). All of these except HER3 have tyrosine kinase activity *(31)*. Binding of secreted growth factors, such as the epidermal growth factor (EGF) and other EGF-like growth factors, including transforming growth factor α and epiregulin, induces receptor dimerization, resulting in the phosphorylation of tyrosine residues in the kinase domain. These phosphotyrosines recruit partner proteins that trigger intracellular signaling cascades, chiefly through the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, which are involved in the induction of cell proliferation, protection from apoptosis, activation of angiogenesis, and development of metastasis *(31, 32, 33)*.

Erlotinib and gefitinib were developed as reversible and highly specific small-molecule tyrosine kinase inhibitors that competitively block the binding of adenosine triphosphate to its binding site in the tyrosine kinase domain of EGFR, thereby inhibiting autophosphorylation and blocking downstream signaling. Erlotinib and gefitinib appear to be especially effective in tumors with gene mutations that activate EGFR. Such tumors may become dependent on the activity of the EGFR pathway for their survival *(34)*.

In previously untreated patients with advanced NSCLC of all histologies, randomized trials of combination of EGFR-TKIs with conventional chemotherapy showed no improvements in OS over chemotherapy alone.[30] Combination of gefitinib with chemotherapy in INTACT 1 and 2 trials, as well as erlotinib with chemotherapy in TALENT and TRIBUTE trials demonstrated no survival benefit of concurrent administration of EGFR TKI and platinum – based chemotherapy *(35, 36, 37, 38)*.

In two phase 3 studies, erlotinib and gefitinib were compared with placebo in patients with advanced NSCLC after failure of standard chemotherapy. In BR.21 trial, 731 patients with stage IIIB or IV NSCLC and ECOG 0 – 3, and failure of first or second line chemotherapy, were randomly assigned to receive either erlotinib (at a daily dose of 150 mg) or placebo *(39)*. Erlotinib was superior to placebo in prolongation of overall survival (6.7 vs. 4.7 months, p = 0.001), progression-free survival (2.2 vs. 1.8 months, p < 0.001), and quality of life. Similarly designed ISEL trial, randomly enrolled 1692 patients with advance disease (stage IIIB and IV) after

failure of one or two lines of chemotherapy *(40)*. Patients were receiving either gefitinib (at daily dose of 250 mg) or placebo. Gefitinib was better than placebo in time to treatment failure (3.0 vs. 2.6 months, $p < 0.001$). However, no significant differences were observed in overall survival.

In a noninferiority INTEREST trial, gefitinib was compared with docetaxel as second-line therapy in unselected patients with NSCLC *(41)*. Study enrolled 1433 patients who had received at least one platinum-based regimen and were randomly assigned to receive either gefitinib or docetaxel. Gefitinib therapy resulted in noninferior overall survival compared with docetaxel (7.6 vs. 8.0 months, HR 1.02, 95% CI 0.91 – 1.15), with better improvement in quality of life.

Subgroup analyses from above mentioned clinical trials showed that patients with certain clinical and histologic characteristics (females, East Asians, never or light smokers, adenocarcinomas histology) who received erlotinib or gefitinib had higher rates of response and overall survival. Furthermore, the presence of activating EGFR mutations leading to intrinsic activation of the receptor has been consistently found to be strongly associated with a better response *(31)*.

In selected chemo-naive patients with advanced NSCLC, 4 phase III open-label, randomized trials in East Asian patients demonstrated superior ORR and progression-free survival (PFS) when gefitinib was compared with standard first-line platinum-based chemotherapy *(42, 43, 44, 45)*. Patients in IPASS and First-SIGNAL trials conducted in East Asia were selected according to clinical characteristics to increase probability of response: never-smokers/former light smokers and adenocarcinoma histology, while in trials WJTOG3405 and NEJ002 populations were defined based on the presence of EGFR-activating mutations. IPASS, the largest of these trials, was designed with PFS as the primary end point to assess the noninferiority of gefitinib compared with carboplatin/paclitaxel in clinically selected 1217 patients. The study showed superiority of gefitinib for PFS (HR 0.74, 95% CI 0.65–0.85; $p < 0.001$), ORR (43% vs 32.2%; $P < 0.001$), and quality of life in the overall study population.[42] Recently reported survival results showed no significant difference in OS between the treatment arms (HR 0.90, 95% CI 0.79–1.02; P 5.109), both in the mutation – positive and mutation – negative subgroups *(46)*. All of the three other trials First – Signal *(45)*, NEJ002 *(43)*. and WJTOG3405 *(44)*. showed superiority of gefitinib in improving PFS compared to chemotherapy in patients with activating EGFR mutations.

Similarly designed trials were conducted with erlotinib. OPTIMAL trial, was a trial conducted in China in patients with advanced NSCLC harboring EGFR activating mutation (exon 19 deletion or exon 21 L858R point mutation) receiving either oral erlotinib (150 mg/day) until disease progression or unacceptable toxic effects, or up to four cycles of gemcitabine plus carboplatin *(47)*. In total, 162 patients were enrolled. Median PFS was significantly longer in erlotinib-treated patients than in those on chemotherapy (13.1 vs 4.6 months; HR 0.16, 95% CI 0.10-0.26; $p < 0.0001$). Tolerability of erlotinib was much better compared to chemotherapy.

EURTAC, a phase III randomized study conducted in Europe that compared erlotinib with platinum-based chemotherapy in 174 chemo-naýve Caucasian patients with EGFR-activating mutations (exon 19 deletion or L858R mutation in exon 21) with no history of chemotherapy for metastatic disease.[48] Median PFS was 9.7 months (95% CI 8.4 – 12.3) in the erlotinib group, compared to 5.2 months (4.5 – 5.8) in the standard chemotherapy group (HR 0.37, 95% CI 0·25-0·54; $p < 0.0001$). However, there was no benefit in overall survival.

In the maintenance setting, several phase III trials have demonstrated only slight improvements in PFS, but no OS significant advantage with either gefitinib *(49, 50)* or erlotinib *(51, 52)*. after chemotherapy in unselected patients with advanced NSCLC. In the SATURN trial, small group of patients (16 out of 24) with activating EGFR mutations were treated with erlotinib in the maintenance setting and showed significant improvement in PFS (HR 0.10; 95% CI 0.04 – 0.25; $p < 0.0001$).

Next – generation EGFR TKIs include irreversible inhibitors that simultaneously target multiple members of the EGFR family. An irreversible EGFR TKIs may overcome resistance to gefitinib or erlotinib through covalently binding to EGFR. Most extensively studied was afatinib. Afatinib 40–50 mg/day was evaluated in a phase II trial (LUX-Lung 2) in patients with advanced lung adenocarcinomas harboring activating EGFR mutations *(53)*. Study enrolled 129 patients and in the overall population, DCR was 86%, confirmed objective RR was 60%, median PFS was 14 months, and median OS was 24 months. Surprisingly, LUX – Lung 1, phase Iib/III trial of afatinib didn't improved survival comparing with placebo in patients with advanced lung adenocarcinoma who had failed 1 or 2 lines of chemotherapy and progressed after =12 weeks of therapy with first line EGFR TKIs (gefitinib or erlotinib) *(54)*. Afatinib in 345 EGFR positive, chemo – naive patients was tested against pemetrexed/cisplatin combination *(55)*. Afatinib in molecularly selected population significantly prolonged PFS comparing with chemotherapy (11.1 vs 6.9 months; HR 0.58; 95 CI 0.43 – 0.78; p=0.0004), and delay in time to deterioration of cancer – related symptoms of cough and dyspnea.

Anaplastic lyphoma kinase inhibitors

EML4 – ALK translocation was identified as a driver mutation of lung carcinogenesis just a few years ago, in 2007 *(56)*. Fusion of different genes is described, but the most common one is fusion of N-terminal portion of the protein encoded by the echinoderm microtubule-associated protein-like 4 (EML4) gene with the intracellular signaling portion of the receptor tyrosine kinase encoded by the ALK gene. Such chimeric protein, EML4-ALK, has strong oncogenic activity both in vitro and in vivo *(57)*. Inhibition of tyrosine kinase domain of ALK lead to antitumor effects in vitro and in transgenic mice *(58)*.

Depending on study cohort reported, the frequency of EML4-ALK in patients with NSCLC varies between 1% and 13%, with median around 4% *(30)*. A typical patient with EML4-ALK translocation is a young, never or light smoker. EML4-ALK–positive tumors are more likely to be advanced-stage adenocarcinomas at presentation *(59)*. Patients with EGFR mutations and ALK aberrations share some similar clinical features. However, EML4 – ALK translocation and EGFR mutation are usually exclussive in the same tumor *(59)*. Crizotinib is an orally bioavailable, selective small-molecule inhibitor of the catalytic activity of c-Met kinase and the ALK fusion protein. Phase I study, conducted by Kwak and co-workers has led to accelerated FDA approval of the drug in patients with NSCLC harboring ALK – EML4 translocation *(60)*. They have screened almost 1500 patients and ALK – EML4 aberration was present in 82 patients. In heavily pretreated patients, after a mean treatment duration of 6.4 months, the overall response rate was 57% including one confirmed complete response, and 33% percent of patients had stable diasease, with clinical effect (CR+PR+SD) in 90% of patients. Update results on 149 ALK positive patients, demonstrated objective response in 60.8% of patients including three complete responses *(61)*. Median PFS was 9.7 months (95% CI 7.7 – 12.8). Median OS data are not yet mature, but estimated OS at 6 and 12 months was 87.9% (95% CI 81.3-92.3) and 74.8% (66.4 – 81.5), respectively.

The response to treatment was exclusive to patients with ALK translocations. In preliminary results of a multicener, randomized, phase III study, comparing second – line crizotinib to chemotherapy (pemetrexed or docetaxel) a response rate of 53% was seen in 76 patients available for measurement of tumor response *(62)*. Despite the excellent initial responses, the median PFS of patients who received crizotinib in the phase I trial was limited to 10 months. *(60, 61)*.

Squamous cell lung cancer

Our understanding of the molecular mechanisms that underlie the development of NSCLC has improved significantly during the last decade, but the benefit of these discoveries to patients was limited those with adenocarcinoma subtype. Squamous – cell lung cancer (SQCLC) has for a long time been considered as tumor without targetable molecular abnormalities *(63)*. Recent discoveries reveled a potential targeted molecular abnormalities in patients with SQCLC *(64, 65)*.

The PI3K pathway

The PI3K pathway is central to cell survival, metabolism, motility, and angiogenesis. Several researchers have shown that abnormalities of PI3K/PTEN/AKT/mTOR pathway are more common in SQCLC than in adenocarcinoma *(63)*. The mutation rate for PIK3CA in SQCLC ranges from 3.6% to 6.5%. Downstream of PI3K, the E17K mutation of AKT1 results in constitutive activation of AKT1. This mutation has been reported in up to 7% of SQCLC and has not been described in adenocarcinomas *(63)*. PTEN is a tumor suppressor gene that negatively regulates the PI3K-AKT-mTOR pathway and PTEN loss results in increased pathway activity. The frequency of PTEN mutations is much more common in SCCLC than in adenocarcinomas *(67)*. Several inhibitors of PIK3CA are in different stages of development, which include inhibitors of the various isoforms of PI3K, AKT1 and mTOR, and dual inhibitors of PI3K/mTOR.

FGFR1 amplification

The fibroblast growth factor receptor 1 (FGFR1) is a transmembrane receptor tyrosine kinase that participates in the regulation of embryonal development, cell proliferation, differentiation, and angiogenesis. Activation of FGFR1 leads to downstream signaling via PI3K/AKT, RAS/MAPK pathways that are central to growth, survival, migration, and angiogenesis in many cancers. FGFR1 amplification was first characterised as an oncogenic event in SQCLC in 2010 *(68)*. The frequency of FGFR1 amplification in SQCLC is around 20% *(69)*. Several selective FGFR1 tyrosine kinase inhibitors (AZD4547, BGJ398) are currently in early phases of clinical testing.

DDR2 mutation

DDR2 is a receptor tyrosine kinase that binds collagen and has been shown to promote cell migration, proliferation, and survival *(70)*. Mutations in DDR2 have been reported in lung cancer, with frequency in SQCLC between 3 and 4% *(71)*. Dasatinib, nilotinib and imatinib, inhibitors of DDR1, are drugs that are registered for treatment of chronic myelogenous leukaemia. Molecular driven treatment studies are planned for patients with SQCLC.

Insulin – like growth factor 1 receptor (IGF1R)

The insulin – like growth factor pathway is important in embryonic development, growth, and metabolism, and dysregulation of the insulin-like growth factor pathway has been described in multiple tumor types *(72)*. IGF1R activation triggers downstream pathways, including the RAS/RAF/MAPK pathway and the PI3K pathway, leading to cell proliferation and inhibition of programmed cell death. IGF1R overexpression is much more common in SQCLC than in other subtypes of lung cancer *(73)*. Figitumumab, an antibody against IGF1R, showed promising results in combination with chemotherapy, especially in SQCLC with overexpression of IGF1R *(74)*. Disappointingly, two Phase-III studies with figitumumab in combination with either chemotherapy or erlotinib were recently closed because of futility and increased toxicity *(70)*.

Tissue acquisition

After decades with only modest improvements in the therapy of NSCLC, we have recently showed significant improvements with targeted agents directed against specific molecular aberrations. We have also learned about the importance of molecular testing for proper selection

of patients with advanced NSCLC who will benefit from targted treatment. In the molecular oncology era the tissue has become an issue *(75)*. For example, in IALT *(10)* and IALT – Bio *(11)*. trials of adjuvant chemotherapy after complete resection or tumor, only 41% of patients (867 out of 1867) had available tissue for immunohistochemistry staining for ERCC1. So, one of the greatest challenges in thoracic oncology nowadays, is to obtain adequate tissue for morphologic confirmation and molecular analyses in a minimally invasive manner. Most commonly used methods of tumor acquisition in patients with advance disease include bronchoscopic and image-guided (usually CT – guided) percutaneous transthoracic needle biopsy (TTNB). Bronchoscopy is suitable for obtaining biopsy of central lesions, with a help of endobronchial ultrasound for obtaining needle-aspirates from mediastinal and hilar lymph nodes *(75)*. Reported successful rates of molecular profiling with tissue obtained by TTNB varies across the studies *(76, 77)*. Because of the need of minimizing complications of invasive procedures for obtaining tissue (e.g. surgery, minimally invasive surgery, VATS, TTNB) less invasive methods are needed. In recent years, molecular profiling from cytology samples (needle –aspirates, bronchial aspirates, pleural fluids or cerebrospinal fluid) *(78, 79, 80, 81)* has shown significant improvement, even comparable with surgically obtained tissue *(82)*. Molecular profiling from circulating tumor cells (CTC) and free serum DNA will offer even less invasive methods for obtaining adequate material *(83, 84)*.

CONCLUSION

One – size fits all treatment in patients with – non small cell lung cancer reached efficacy plateau *(3)* In the last few years we have learnt about significance of individualized treatment. First, the significance of histology for selection of chemotherapy regimen *(4)*, and then importance of molecular profiling for tailoring treatment *(75)* Several studies have confirmed prognostic and predictive role of NER pathway in patients with NSCLC *(12, 13, 14)*. The most significant improvement in treatment was done with the discovery of drugs directed against tyrosine – kinases proteins, especially drugs against EGFR (gefitinib, erlotinib) *(39, 41, 42, 43, 44, 45)* and ALK – EML4 (crizotinib) *(59, 60, 61)*. Consequently, the importance of adequate tissue for molecular profiling has nowadays become an important issue. Minimally invasive procedures, such as core biopsies (with tissue obtained by bronchoscopy or trans – thoracic biopsy) are required to obtain the tissue. Nowadays, even less invasive methods for obtaining tissue for molecular profiling from cytology samples, circulating tumor cells or free plasma DNA are available *(76, 77, 78, 79, 80, 81)*. Still, there are many more biomarkers that are potential targets, and even though a lot of early phase research was done, there is still a long way to validate those markers and find drugs which will successfully target specific molecular alterations.

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