GENETIC SIGNATURES IN LUNG CANCER TREATMENT- A PROMISE OF PRECISION MEDICINE

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REVIEW

HIGHLIGHTS

This review article provides a detail account of molecular markers involved in decision making process of lung cancer treatment for better clinical outcome for individual patient or for selecting precision medicine. Molecular markers and their role in deciding the prognosis is discussed elaborately.

ABSTRACT

Lung cancer is one of the most common malignant diseases, causing a significant health burden worldwide. Numerous genetic alterations in several genes have been established, to be potential markers in lung cancer diagnosis and prognosis. Last decade has transformed the treatment of lung cancer with the discovery of biomarkers such as EGFR mutations conferring sensitivity to tyrosine kinase inhibitors. Novel and improved targeted drugs are available in the clinical setting for lung cancer treatment; patients receive molecular profiling to identify the type of tumor, and also to overcome resistance to targeted therapies. This article gives an account of most important markers and their potentials towards precision medicine in lung cancer.

KEY WORDS *Biomarkers; lung cancer; mutations; treatment; molecular profiling*

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INTRODUCTION

Lung cancer is a heterogeneous, complex and challenging disease which necessitates a thorough knowledge of underlying pathological and molecular markers to precise the therapeutic outcome. Lung cancer involves genetic and epigenetic alterations. It is one of the leading causes of mortality worldwide [1]. Lung cancer accounted for, approximately, 28% of total cancer deaths in United State in year 2010. In India, it is most the most common cancer in Indian metro cities [2]. The reason for associated high mortality is the frequent presence of regional and distant metastasis at diagnosis (78% of cases), with 5-year survival rate of 25% (regional) and 4% (distant). Also increased incidences of relapse after treatment and therapeutic resistance equally contribute towards high mortality [3].

The two major histological categories of lung cancer are, Non-Small Cell Lung Cancer (NSCLC) representing 80-85% cases and Small Cell Lung Cancer (SCLC) accounting for 15-20% of cases. NSCLC and SCLC differ at the molecular level at exhibit subtype specificity [4]. Histologically, NSCLC is further subdivided into lung adenocarcinoma (LAC), arising in the cells lining the alveoli, squamous cell carcinoma (SCC), large cell carcinoma (LCC), bronchoalveolar lung cancer and adenosquamous carcinoma. These subtypes of cancer differ in terms of their molecular drivers, pathogenesis, disease progression and requirement of differential treatment strategies [5].

The diagnosis and treatment of lung cancer are based on accurate and predictive immunohistochemistry, pathological findings and assessment/identification of relevant prognostic molecular markers using genomic profiling. The pathologists and oncologists play important role in the decision making process of treatment of lung cancer, via identifying the molecular and morphological characterization refined by IHC and genomic profiling. Current treatment rarely cures the disease due to the high relapse rate coupled with delayed diagnosis resulting into poor prognosis and poor overall survival rate. Pan genomic analyses, expression array analysis of numerous genes, proteomics, microinhibitory RNA analysis and Next Generation Sequencing (NGS) of entire lung cancer genomes have been initiated, however these have yet to impact routine clinical practice. These technological advances in the development of novel targeted therapies have paved the way for personalized medicine/ precise medicine in cancer disease.

The present review highlights the genetic alterations in several genes such as EGFR (Epidermal Growth Factor receptor gene), ERBB2 (Erb-B2 Receptor Tyrosine Kinase 2, ALK (Anaplastic Lymphoma Kinase), KRAS (Kirsten Ras oncogene homolog from the mammalian ras gene family), MET 1 (Methyltransferase 1 gene), ROS 1 (Receptor Tyrosine Kinase (RTK) of the insulin receptor family), in relation to the pathogenesis of lung cancer and altered response to drugs used in the treatment or better known as personalized or precise medicine which helps in rationalization and optimization of treatment in lung cancer. Genetic biomarkers in the NSCLC practice of prognostic relevance and prediction of therapeutic response have been mentioned in Table 1.

Molecular landscape of NSCLC

Inherited predisposition to lung cancer has already been well established in familial linkage studies [6, 7]. Several Single Nucleotide Polymorphisms (SNPs) at 15q24-q25.1, have been identified in association with increased risk of nicotine dependence and developing lung cancer, in many Genome Wide Association Studies (GWAS) [4]. Another genome-wide linkage study of pedigrees of multiple generations of lung cancer from the Genetic Epidemiology of Lung Cancer Consortium (GELCC) has mapped a familial susceptibility locus to 6q23-25 [4]. Shiraishi et al. (2016), have summarized GWAS related to lung cancer and the putative roles of various SNPs at susceptible loci in different genes in relation to lung cancer [8].
A meta-analysis also revealed that genetic variants at 15q25.1 (consists of six genes, the three genes CHRNA5, CHRNA3 and CHNB4 nicotinic receptor subunits, fourth gene in this region is proteasome alpha 4 subunit isoform 1 (PSMA4), plays a role in cancer cell proliferation and apoptosis, 5p15.33, gene encoding for telomerase subunits (telomerase reverse transcriptase -Cleft lip and palate transmembrane protein 1-like protein or TERT-CLPTM1L, rs401681), and 6p21.33 (a part of the HLA region highly polymorphic) have significant association with lung cancer risk [9]. The Cancer Genome Atlas (TCGA) has identified several mutations in key oncogenes in association with lung adenocarcinoma, and squamous cell carcinoma.

The extensive review of literature suggests that genetic alterations in lung cancer include p53 mutations/deletion, p16 gene silencing via methylation, LKB1 (gene encoding for a serine–threonine kinase and is involved directly into phosphorylation and activation of AMPK, a central metabolic sensor) loss-of-function mutations and activating KRAS gene mutations.

The three major signaling pathways included in the pathogenesis and progressions of lung cancer are: p53 signaling, the RB/p16 signaling axis and the RAS signaling. Mutations or deletions of p53 occur in approximately 50% of NSCLC patients [10, 11]. There are several methods to target p53 signaling for

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cancer therapies. The p53 gene has been regarded as the guardian of the genome, and gene mutations result in many alterations in the cancer genome [12, 13].

With the help of Next Generation Sequencing (NGS), the molecular landscapes of many tumor types has been explored and has increased our understanding of genetic alterations, responsible for uncontrolled apoptosis in cancer cells and also help in predicting the drug response as well as resistance to many drugs used in the treatment at an individual patient's level. Genomic profiling based treatment selection has provided a standard of care in LAC patients, with mutant epidermal growth factor receptor (EGFR) and anaplastic lymphoma receptor tyrosine kinase (ALK) gene rearrangements dictating therapies (gefitinib/erlotinib and crizotinib, respectively) and has shown improved response rates over conventional chemotherapy [14, 15]. Sequenom or SNaPShot platform provides the information regarding therapeutically actionable genomic alterations in lung cancer. As the cost of NGS and time to results is rapidly reducing, personalized medicine has become an imminent possibility [3]. There has been a major paradigm shift in the treatment of lung cancer with the availability of treatment options in prevalent and previously undruggable mutations in genes e.g. Kirsten rat sarcoma viral oncogene homolog (KRAS), serine/threonine kinase 11 (STK11/LKB1), and tumor p53 (TP53), and the prediction of combination therapies have significantly improved the treatment by reducing the associated mortality [3].

Biomarkers	Tumor Molecular Target	Effect of Mutation	Drugs/ Pharmacological Agent
EGFR Mutation	EGFR tyrosine kinase	Reduced sensitivity to EGFR tyrosine kinase Inhibitors	Inhibitors of EGFR tyrosine kinase e.g. gefitinib, erlotinib, afatinib
ALK protein expression/Translocation	ALK Fusion proteins	Increased sensitivity to Crizotinib	ALK inhibitors e.g. crizotinib
ROS1 fusion protein	ROS1	Increased sensitivity to Crizotinib Reduced sensitivity to EGFR TKIs	Crizotinib
BRAF Mutation	BRAF Serine Threonine Kinase		Vemurafenib
MET Protein expression/ Copy number	MET receptor tyrosine Kinase	Increased sensitivity to Crizotinib, Decreased sensitivity to EGFR TKIs	MetMAb Monoclonal Antibodies (Unsuccessful in trials)
PI3K, KRAS, MEK, BRAF status: mutations,	PI3K lipid kinase complex mTOR in some cases	Altered response to PI3K Inhibitors	PI3K Inhibitors
KIF5B–RET fusion (translocation)	PI3K lipid kinase complex mTOR in some cases	Altered response to PI3K Inhibitors	PI3K inhibitors
KRAS mutation		Reduced sensitivity to EGFR TKIs	

Table 1. Genomic markers affecting the efficacy of treatment in lung cancer

Defined molecular targets in LAC

Several studies have established biomarkers and specific mutations leading to divergent phenotypes in lung carcinogenesis, which varies across different ethnicities.

Epidermal growth factor receptor (EGFR)

EGFR, is an Epidermal growth factor receptor belong to the ErbB family of tyrosine kinase receptors, transmembrane receptor proteins involved in cell proliferation and consists of four members – EGFR, ErbB-2 (HER2), ErbB-3, and ErbB-4. EGFR receptor is activated only after ligand binding. EGFR shows over-expression or aberrant activation in 50–90% cases of NSCLCs responsible for poor survival and resistance to chemotherapeutic agents; therefore, the focus was on the development of targeted inhibitors for this molecule [4].

There are three main mechanisms for EGFR activation: overexpression of EGFR (40-80% in case of NSCLC) in malignant cells; enhanced production of ligand and activated mutations in EGFR in malignant cells, [16]. The deletion in exon 19 downstream of the lysine residue at 745 position, and point mutations in exon 21

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L858R, L861Q and L861R, in exon 18, G719A/C/S and in exon 20 insNPG, insSVQ, ins G and point mutations in exon 20, V769A in EGFR gene are the most common mutations associated with disease progression and TKI resistance, and their prevalence have been reported to be significantly higher in Japanese, Korean, and Chinese patients as compared to white patients from the United States or Europe [17]. The diagnostic tests approved by The USA Food and Drug Administration (FDA) include the Cobas EGFR mutation test for common activating mutations or Cobas EGFR mutation test v2 for the T790M mutation. However, for which include exon 19 deletions and L858R missense substitutions at position 858 (Leucine replaced by arginine) making therapeutic decision, any Clinical Laboratory Improvement Amendments (CLIA) certified-laboratory, EGFR mutation result is used by clinician [18]. The treatment of choice against EGFR receptor mutations includes tyrosine kinase inhibitors (TKIs), which have become the first-line therapy in lung cancer. This frontline targeted therapy improves the response rate as well as progression-free survival as compared to chemotherapy in patients with metastatic EGFR mutant lung cancer disease [19]. TKIs include gefitinib and erlotinib or monoclonal antibodies, e.g., cetuximab. The pharmacological mechanism of action of these drugs involves reversible competitive inhibition of adenosine triphosphate (ATP) for the tyrosine kinase domain of EGFR resulting in the blockade of downstream pathways. The mutations of EGFR varies with ethnicity and geography across the globe, with Asian populations having up to 50% of adenocarcinomas driven by activating EGFR mutations compared to only 10% to 15% in Caucasians [20].

One of the landmark clinical trial, Iressa Pan-Asia Study (IPASS) was carried out recruiting 1,217 patients from several East Asian countries with untreated stage IIIB or IV adenocarcinoma on gefitinib or carboplatin and paclitaxel chemotherapy to investigate the response of treatment and EGFR mutations [21]. Recruited subjects had advanced pulmonary adenocarcinoma and who were nonsmokers or former light smokers to receive gefitinib (250 mg per day) (609 patients) or carboplatin (at a dose calculated to produce an area under the curve of 5 or 6 mg per milliliter per minute) plus paclitaxel (200 mg per square meter of body-surface area) (608 patients). and EGFR was analysed as a potential biomarker. The primary endpoint met by this study was with a 12-month progression-free survival (PFS) of 24.9% with gefitinib versus 6.7% with chemotherapy [21]. EGFR status was found in approximately a third of patients, and 60% were found to harbour an activating mutation. It was observed that in these patients, PFS was significantly prolonged with gefitinib treatment as compared to chemotherapy [16]. Gefitinib was found to be superior to carboplatin-paclitaxel as an initial treatment for pulmonary adenocarcinoma among nonsmokers or former light smokers in East Asia. The presence of the tumor with a mutation in the EGFR gene was reported as a strong predictor of a better outcome with gefitinib [21, 22].

Further, confirmatory trials compared gefitinib, erlotinib or afatinib to chemotherapy specifically in EGFR-mutated NSCLC cases and it was found that first-line EGFR TKIs afforded superior Objctive Response Rate, PFS and quality of life compared to chemotherapy. Thus it was established that in case of EGFR activating mutation, EGFR TKIs should be given as first-line therapy [16, 23- 28]. The second TKI drug afatinib is indicated specifically in the treatment of patients with advanced NSCLC and harboring exon 19 or exon 21 (i.e., L858R) EGFR mutations. The cytotoxic chemotherapy has been found to be a preferred therapeutic option in NSCLC without targetable driver mutations in EGFR or with wild type or homozygous normal status [29].

Resistance to EGFR targeted therapy

Although the treatment has been revolutionized using EGFR TKIs in EGFR-mutant NSCLC, a significant number of patients' responses have not proved to be optimum with many of these progressing after 7-12 months. Resistance can occur primarily or can develop after exposure to targeted agents, and can exist as resistant clones within a tumor or in different tumors within the same patient. They may develop 'acquired resistance', either via secondary EGFR mutations or activation of EGFR independent pathways. Therefore, clinicians consider re-biopsy at progression of disease in order to assess tumor biology. In 50% cases, it is the acquisition of a mutation in exon 20 of EGFR gene, encoding for T790M, where, threonine is replaced by methionine, altering the configuration of the kinase domain and enhancing its affinity (over wild-type) for ATP, with a corresponding decreased affinity for first-generation reversible TKIs [16]. Insertion mutation at 790 in exon 20 makes EGFR 100 fold sensitive to TKIs in comparison with other mutations. The second mechanism (in 5-20%) involves amplification of MET to circumvent EGFR inhibition via PI3K-AKT-mTOR (Phosphatidylinositol 3-kinase (PI3K), Akt (a serine/threonine kinase also known as protein kinase B or PKB), and mammalian target of rapamycin or mTOR) signaling [27, 30, 31]. The other possible resistance mechanisms include mutations in PIK3CA, HER2, BRAF, amplification and in 5%, the unexpected transformation into small cell lung cancer [27, 31- 35].

The T790M mutation in EGFR gene alters the conformational structure of the receptor protein so that the drugs (e.g. erlotinib, afatinib, gefitinib) are not able to penetrate and bind to the receptor tyrosine kinase binding pocket in order to produce the desired therapeutic effect/result. As a result of this, the EGFR is

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reactivated and cancer cells grow in a continuous manner. These are treated with osimertinib. Osimertinib failed due to the presence of C797S mutation in EGFR gene which confers resistance to this drug. The C797S mutation impairs the covalent binding between the cysteine residue at position 797 of EGFR and osimertinib mesylate, thereby inducing the resistance to osimertinib and confers the resistance to third generation TKIs [36]. However, there is no effective therapeutic strategies against C797S/T790M/activating-mutation (triple-mutation)-mediated EGFR–TKIs resistance [37]. Brigatinib was found to be effective against triple-mutation-harbouring cells in vitro and in vivo studies. The computational simulation suggested that brigatinib fits into the ATP-binding pocket of triple-mutant EGFR. The structure activity relationship analysis revealed that the key component in brigatinib inhibited the triple-mutant EGFR. Thus, the combination therapy of brigatinib with anti-EGFR antibody is a powerful candidate to overcome triple-mutant EGFR.

Although there are significant advances in the treatment of lung cancer, up to 30% of drug resistance is of unkown cause hence empirical cytotoxic chemotherapy remains the treatment of choice [27]. When compared to chemotherapy, resistance to targeted therapy can be optimized rationally after the identification of underlying aberrant pathways. It has been observed in LUX-Lung1 clinical trial that second-generation irreversible ErbB-family TKIs e.g. afatinib, which acts by covalently binding to EGFR/HER1 and HER2, can significantly overcome the T790M mutation with 7% ORR and PFS improved from 1.1 months with placebo for 3.3 months [37].

It has been observed that the third generation EGFR TKIs e.g. CO-1868 and AP26113 which specifically target T790M mutations were evidenced of efficacy in acquired resistance but with reasonable toxicity [38]. Although addressing the drug resistance to targeted therapy appears the challenge for the rationally choosing combinations of drugs as more effective than first-line single-agents whilst balancing toxicity and costs [4].

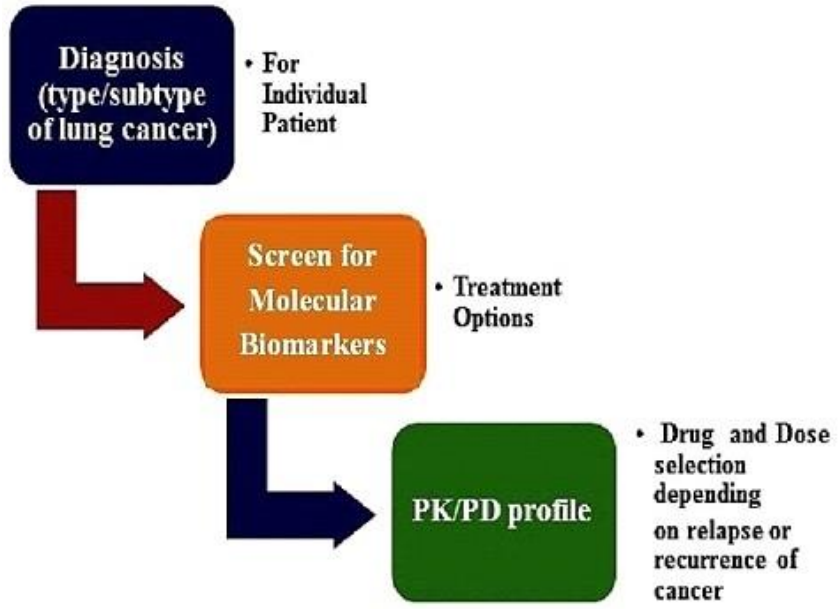

Fig: 1. Precision medicine for the treatment. PK- Pharmacokinetic, PD- Pharmacodynamic

Anaplastic lymphoma kinase (ALK)

ALK is a receptor tyrosine kinase. Activating mutations and transforming rearrangements in this receptor have been observed in anaplastic lymphomas, colorectal cancer, and ovarian cancer. ALK fusions have also been found in NSCLC in 3-7% cases of lung tumors, more commonly observed in never smokers or light smokers, in patients with younger age and adenocarcinomas particularly with acinar or singlet ring histology [39]. Many ALK rearrangements are observed in NSCLC, the commonest being Echinoderm microtubule-associated protein like-4 or EML4-ALK fusion gene. The EML4-ALK fusion gene is the result of inversion within the short arm of chromosome 2 where ALK joins and is a chimeric protein with constitutive ALK activity. This fusion gene encodes for a protein with a ligand independent, constitutively active kinase domain which leads to persistant mitogenic signaling through downstream activation of the MAPK (Mitogen-activated protein kinases), JAK-STAT (Janus kinase - Signal Transducer and Activator of

ISSN: 2456-2262
PRECISION MEDICINE IN ONCOLOGY

Transcription), and PI3K/AKT pathways and malignant transformation thereby exhibiting dramatic clinical phenotypes to ALK targeted therapy [3]. The standard method of detecting ALK positivity in NSCLC is by using fluororscence in situ hybridization (FISH) technique. However, in advanced stage NSCLC, sometimes, it is not possible to collect tissue, therefore, micro RNA expression can be evaluated in lung cancer patients for ALK mutations as a noninvasive biomarker from blood. Recently, Li et al. (2017), have identified, a potential panel of microRNAs for distinguishing between patients with ALK-positive and ALK-negative NSCLC. The miR-660-5p and miR-362-5p were found to have a potential role as predictors of response to crizotinib treatment in ALK positive patients [40].

The targeted drug-crizotinib has made possible to achieve good progression free survival of 72 % in patients with ALK fusion positivity. Crizotinib is a dual kinase inhibitor and it acts via inhibiting ALK, MET and ROS 1 kinases and has already become a part of the routine armamentarium for NSCLC [39]. The patients harbouring ALK fusions donot respond well to EGFRTKIs [27, 39].

Kwak et al (2010) carried out a clinical study involving 82 patients with ALK-positive NSCLC and evaluated the effectiveness of ALK inhibition with crizotinib. In this study, 57% of patients were found with a complete or partial response (1 out of 46 complete; 45 out of 46 partial) [15]. Further, in a Phase I trial recruiting 149 ALK translocation-positive NSCLC patients, a reduction in tumor size by 90 %,, with 61% displaying an objective response was observed [41]. Crizotinib inhibits multiple tyrosine kinases; therefore, it has also been used clinically to treat adenocarcinomas.

Numerous second generation small molecule inhibitors of ALK fusion gene have been developed and Ceritinib, has been clinically approved in 2014, as ALK inhibitor, which can overcome crizotinib resistance in preclinical and Phase I clinical trials of NSCLC patients harboring ALK rearrangements [41]. The ongoing trials and preliminary studies have shown that heat shock protein Hsp90, a chaperone protein involved in ALK synthesis has shown promise in reducing ALK protein levels in NSCLC. Ganetespib (STA-9090), a Hsp-90 inhibitor has been found to be effective in a Phase IIb/III study in combination with docetaxel [3, 41]. Brigatinib has shown promise in treating ALK-positive metastatic non-small cell lung cancer (NSCLC) patients who either progressed with or could not tolerate treatment with Xalkori (crizotinib) in phase II trial.

The reported overall prevalence of EML4-ALK translocation among patients with NSCLC is 3% to 5%, and has been mainly observed among patients, never or light smokers, adenocarcinoma, younger age of onset, and wild-type EGFR/K-Ras [42]. However, no strong evidence to suggest an ethnic difference of EML4-ALK translocation among patients with NSCLC were found. Because Eastern Asian patients with NSCLC have a higher proportion of never-smokers and younger age of onset, it is likely that a higher prevalence of ALKpositive may be observed among Eastern Asian patients than Caucasian patients.

Kirsten rat sarcoma 2 gene (K-RAS)

K-RAS gene (Kirsten Rat Sarcoma 2 viral oncogene homolog) belongs to a family of GTPases which transduces growth signals from multiple tyrosine kinases including EGFR and MET [3]. Mutations in KRAS results into constitutive signalling in around 30% of adenocarcinoma cases and 4% of SCC cases [43]. The frequency of KRAS mutations are more likely to be observed in Caucasians, former or current smokers and only 6% Asians carry these mutations [43]. K-Ras mutation has been predominantly reported among Caucasians with adenocarcinoma (approximately 26% versus 16% among patients with NSCLC of other cell types, and very uncommon among patients with squamous cell carcinoma) and ever-smokers (25% versus. 6% among never-smokers) [44].

These mutations have been found to be associated with prediction of poor prognosis as well as resistance to chemotherapy and EGFR TKIs [45, 46]. A few prospective randomized trials have been carried out to identify KRAS mutations as biomarker to stratify therapeutic options in the metastatic setting. KRAS mutations were reported as the negative predictors of radiographic response to the EGFR tyrosine kinase inhibitors, erlotinib and gefitinib [47]. Tao et al (2016) observed the potential roles that p16 (CDKN2A) or cyclin dependent kinase inhibitor 2A and retinoblastoma gene activation in sensitization to MEK pathway inhibitor in resistant KRAS-mutant NSCLC in vitro as well as in vivo [48]. The researchers suggested that MEK inhibitor in combination with CDK4/6 inhibitor has significant anti-KRAS-mutant NSCLC activity and radiosensitizing effect in preclinical models, potentially providing a novel therapeutic strategy for patients with advanced KRAS-mutant.

Recently in a clinical study, Park et al. (2017), investigated the predictive value of KRAS mutations and its mutation types to pemetrexed and gemcitabine based treatment in NSCLC patients [49]. In ths study, KRAS mutations were observed in 45 subjects with mutation type as followed: G12C (transversion mutation) (n = 13), G12D (transition mutation) (n = 12), G12V (transversion mutation) (n = 12), other (n =

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PRECISION MEDICINE IN ONCOLOGY

8). In this study, G12C KRAS mutation showed reduced PFS to pemetrexed singlet treatment and the transversion KRAS mutation were found as a good predictive marker of gemcitabine based chemotherapy. The authors concluded that Non-small cell lung cancer subpopulations with different KRAS mutation showed different response and sensitivity to drug.

The direct RAS inhibition with salirasib was a failure; therefore, novel approaches are under development in order to inhibit downstream molecules in the RAS/RAF/MEK/ERK and PI3K/AKT/ mTOR pathways [41, 51].

Alternative approaches include targeting the HSP90 [50, 51]. Selumetinib (AZD6244; ARRY-142866) a MEK1/MEK2 inhibitor showed a PFS advantage in combination with docetaxel in a phase II trial in advanced KRAS-mutant NSCLC, also being investigated in a confirmatory phase III study, SELECT-1 in addition to preclinical combinations with AKT inhibitors [52, 53].

Mesenchymal-epithelial transition (MET) gene

MET gene is located on chromosome 7, and is a site for encoding receptor tyrosine kinase, involved in downstream signaling pathway. Numerous studies have established that amplification of mesenchymal-epithelial transition (MET) factor found in about 5% of lung adenocarcinoma and results in overexpression of its gene product-hepatocyte growth factor receptor (HGFR) is involved in cell proliferation, migration, invasion and metastasis [54]. The inhibition of MET/HGFR mediated growth is carried out by HGF antagonists, anti-HGFR mAb, anti-MET mAb and MET TKIs such as tivantinib (ARQ197), cabozantinib (XL184) and by crizotinib [55]. It has been found that MET amplification is the second most common cause of acquired EGFR TKI resistance. Dual EGFR and MET inhibition, with erlotinib and tivantinib respectively, were investigated in NSCLC in phase III trial MARQUEE, after phase II data suggested improved PFS for KRAS-mutants [55]. The monoclonal antibody against MET i.e. Onartuzumab has also shown promise in a phase II trial and phase III in the MetLung study where it was combined with erlotinib for MET-positive NSCLC [56]. Despite these early promising results, confirmatory studies using MET TKIs and MET mAb (monoclonal antibodies) have yielded unsatisfactory results and early trial closures for both phase III trials. MARQUEE trial was stopped in year 2012, due to lack of efficacy found in MetLung trial was also terminated early. There are many TKIs available against MET.

c-ROS oncogene 1 (ROS1)

ROS1 ROS1 is a orphan receptor tyrosine kinase receptor protein belonging to insulin family and this gene is located on 6q22. Nearly 2% of patients have been found to carry ROS1 fusions or chromosomal rearrangements with CD74, EZR, SLC24A2, and FIG genes found in NSCLC patients. The reported incidence is higher among light and no smokers, in young people and among Asians. CD74-ROS1 is the most frequently found ROS1 fusion in NSCLC patients. The techniques used for detection of ROS1 fusions involve a break reak apart FISH assay or immunohistochemistry. The reported frequency of ROS1 rearrangements ranges from 0.9% to 1.7% in an unselected NSCLC population. However, the frequency increases from 3.9% to 7.4% in lung adenocarcinoma patients with wild-type EGFR/KRAS/ALK [57]. Crizotinib, is highly effective and potent inhibitor of ROS1 kinase activity, and has been approved by FDA for the treatment of patients with advanced ROS1-positive NSCLC. The ROS1 investigation is considered only after tests for EGFR mutation and ALK gene rearrangement are negative.

In a study carried out by Scheffler et al. (2015), 1137 patients with lung adenocarcinoma were investigated for ROS1 status, in positive cases, next-generation sequencing (NGS) technique was used and overall survival (OS) was compared with genetically defined subgroups of ROS1-negative patients [58]. It was found that 19 patients had rearrangements, stage IV patients had best OS among all subgroups and response to chemotherapy was remarkably high and overall survival was significantly better compared to other subgroups including EGFR-mutated and ALK-fusion-positive NSCLC. The response to chemotherapy was found to be remarkably high and overall survival was improved as compared to other subgroups, including EGFR-mutated and ALK-fusion-positive NSCLC [58].

Rearranged during transfection (RET) gene

RET, the proto-oncogene belongs to cadherin superfamily, is located on chromosome 10q11.2, encodes a receptor tyrosine kinase involved in neural crest development, growth, and differentiation. RET mutations have been found to be implicated in several different diseases including multiple endocrine neoplasia thyroid carcinoma, and NSCLC. In a study involving 1,876 patients with lung carcinomas, FISH and RT-PCR were used to detect RET gene rearrangements. Around 1.2% of LAC cases were reported positive for RET rearrangement in this study. RET rearrangement were found to be correlated with younger patients, adenocarcinomas with no other known oncogenic drivers, small primary tumors, and non-smokers. In NSCLC, chromosomal rearrangements result in the fusion of RET's C-terminal region to the N-terminal of several proteins (KIF5B, CCD6, NCOA4, TRIM33), leading to constitutive activation of the RET kinase domain and oncogenic activity [3].

ISSN: 2456-2262
PRECISION MEDICINE IN ONCOLOGY

The treatment options are limited for patients harboring RET rearrangements and are under clinical trials. Carbozantinib (XL-184), a multi-TKI and RET inhibitor, in Phase II clinical trials to determine its efficacy in NSCLC patients with RET fusion-positive advanced NSCLC [58]. The available clinical data for patients treated with carbozantinib found that there was a partial response among two of the three patients, and one had prolonged Carbozantinib (XL-184), a multi-TKI and RET inhibitor, was under Phase II clinical trials to determine its efficacy in NSCLC patients advanced NSCLC [59, 60,61]. Other small-molecule inhibitors currently being investigated for efficacy in RET-positive LAC patients include ponatinib (Phase II), levatinib/E7080 (Phase II), MGCD516 (Phase I/Ib), and sunitinib (Phase II).

The biomarkers related to physiogenomic alterations leading to lung cancer has been reviewed in detail by Mackinnon et al. [62].

FUTURE PERSPECTIVES

Recent progresses into the elucidation of genomic aberrations or mutations involved in lung cancer in combination with other pathological findings have paved the way for precision medicine. Drug resistance and high relapse rate of disease are main problems which limit the therapeutic benefit in a specific subset of patients with lung cancer. Characterization of mechanisms involved in drug resistance will help in optimizing and rationalizing the treatment of lung cancer. Further, validation of predictive biomarkers and tumor genetic signatures has made it possible to specifically tailor the treatment decisions in individual lung cancer patients. Several research studies and clinical trials are on-going to investigate the clinical relevance and therapeutic effectiveness of novel and targeted drugs. The major challenge is to harness the knowledge of molecular and pathophysiogenomic biomarkers into the development of cost effective novel targeted therapeutic strategies based on specific genomic alterations, molecular profiling is crucial for regulating the proliferation and metastasis of lung cancer leading to improved patient survival with the disease and is a part of clinical routine. Genomic screening in lung cancer patients will facilitate identification of underlying cause of acquired resistance to targeted therapies and will improve the clinical outcome of the disease on individualized basis.

CONFLICT OF INTEREST

The authors declare no competing interests.

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