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Updates in Pharmacogenetics of Non-Small Cell Lung Cancer

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Abstract

Though significant clinical advances have been made, lung cancer remains the most lethal, with a low 5-year survival rate. The variability in patient response towards therapy is substantial and is associated with lung cancer's genomic landscape. Pharmacogenetic studies have deciphered many clinically relevant associations between tumor genetic alterations and their influences on drug efficacy, toxicity sensitivity and overall outcomes of cancer treatment. Biomarkers are tools in the arsenal that can help in the prediction, prognosis, diagnosis and follow-up of cancer treatment. Bulk and single-cell next-generation sequencing of large patient cohorts have generated a better understanding of the genetic underpinnings of lung cancer, and opening up personalized therapeutic opportunities. Immunotherapy and personalized medicine are providing hope for lung cancer patients. This review highlights the genetic alterations and important lung cancer biomarkers. The pharmacogenetic associations, personalized immunotherapy and challenges associated with effective therapy are also discussed. Pharmacogenetics and pharmacogenomics can open up new vistas for optimized, personalized NSCLC treatment.

Keywords: Lung cancer, NSCLC, Pharmacogenetics, Biomarkers, Personalized medicine, Tyrosine kinase inhibitors, Immunotherapy, Checkpoint inhibitor, Challenges

1. Introduction

Lung cancer is the principal cause of cancer-related death worldwide and affects both smokers and non-smokers [1]. Men have the highest incidence and mortality related to lung cancer, while in women, it is third by incidence and second by mortality. With exceptions, the five-year survival rate of lung cancer patients is between 10 and 20%, the lowest among most cancer types [2]. Histologically 80–85% of lung cancers are classified as non-small cell lung cancer (NSCLC), while the remaining is small cell lung cancer (SCLC). Adenocarcinoma (LUAD; ~ 65%), squamous cell carcinoma (LUSC; ~ 30%), and large cell carcinoma (LCLC) are the major subtypes of NSCLC and originates from different types of lung cells [1, 3, 4]. While SCLC is less frequent but more aggressive as compared to LUAD and LUSC. NSCLC is mainly treated by surgery, chemotherapy, radiation, or targeted therapy but with dismal lung cancer survival outcomes. There has been extensive progress

in aiming targeted drug delivery towards cancer cells; the accuracy, efficacy, and success are often limited by resistance developed by tumor cells and inter-subject variability. Low therapeutic indices, differences in health effects, and toxicity from chemotherapeutic agents are some of the drawbacks of current NSCLC treatment. This has prompted researchers to explore other cancer treatment options, keeping in mind individual patient's genetic responses and adverse drug reactions (ADR) to chemotherapeutic agents as 'pharmacogenetics' studies [5].

Pharmacogenetics is an evolving branch of pharmacology that examines the genetic variation between individuals and its correlation with their response to drugs/pharmaceuticals and other xenobiotics. In comparison, pharmacogenomics encompasses all genes in the genome that modulates drug response. The awareness of the genetic heterogeneity in oncology is of significant importance, owing to the limited therapeutic index of cancer therapies and the possibility of ADR-associated life-threatening complications. Within an individual and comparison among NSCLC patients, genomic alterations are major reasons for variations in chemotherapeutic drug response and related toxicity [6]. Studies on NSCLC genetic and molecular alterations provide new targets for treatment, help in the identification of biomarkers for early diagnosis, and helps to predict patient prognosis and progression [7]. NSCLC genetic polymorphism can act as either predictive or prognostic markers [8]. Single-nucleotide polymorphisms (SNPs) or a single nucleotide substitution can affect the expression or functionality of essential enzymes and/or targets in the metabolism and activity of anticancer drugs. Genetic polymorphism is extensively investigated as a prognostic or predictive factor in various tumor types, including NSCLC [9].

Further, pharmacogenetics also helps to prevent cancer-related mortalities by forecasting pre-symptomatic diagnosis, designing customized or tailor-made dosage regimens for individuals, and optimization of a therapeutic window of antineoplastic drugs on a personalized basis. However, pharmacogenetics is an evolving arena in cancer treatment and has obvious underlying limitations that need to be investigated. Determination of genetic variants has primarily relied on SNP, although lately, haplotypes of SNPs and non-genetic factors (like age, lifestyle, diet, profession, and intestinal microflora) are included in studies. Nonetheless, an additional challenge in pharmacogenetics for treating NSCLC deals with validation and standardization of genotyping procedures that are a major deciding factor in the personalization of cancer therapy. Many therapeutic interventions can be utilized for pharmacogenetics-associated NSCLC treatment. The examples include, but not restricted to chemotherapeutic agents (cisplatin, gemcitabine, pemetrexed, taxanes, etc.), immune checkpoint inhibitors [programmed cell death 1 (PD-1), cytotoxic T-lymphocyte protein 4 (CTLA-4)], and a combination of immunotherapy with chemotherapy [10]. This chapter throws light on the current status of pharmacogenetics-based therapeutics in NSCLC with a focus on genetic alterations by gene mutations, exploration of possible treatment modalities, challenges involved, and prospects of pharmacogenetics in treating NSCLC.

2. Gene mutation in NSCLC and Pharmacogenetics

Human Genome Project (HGP) has revealed that the genetic composition of humans is 99% similar, with only 1% variations leading to individual differences. This clarifies why individuals show a difference in response to anticancer drugs concerning drug pharmacology, toxicity, and controlling proliferation, invasion,

and metastasis of tumor cells in NSCLC [11, 12]. Simultaneously, several studies have suggested a personalized medicine approach to achieving maximum efficacy and minimum toxicity of anticancer drugs using pharmacogenetics to target NSCLC [12]. Thus, NSCLC patients' categorization based on underlying genetic and molecular alterations can help in personalizing anticancer drugs and dosage regimens. Therapy tuned to individual patient's genotypic and phenotypic landscape can achieve the highest therapeutic benefits [13]. The complete knowledge of driver mutation pathways and biomarkers can explain NSCLC heterogeneity for identifying personalizing therapies. Information about driver mutation frequency and associated functional changes can help decipher actionable, personalized molecular targets. Therefore, a brief description of the critical driver genes that frequently undergo mutations-associated with lung cancer (**Figure 1**).

NSCLC is a heterogeneous disease, and recent sequencing studies have revealed the genomic landscape (**Figure 1**). Common genomic alterations in LUAD include KRAS, EGFR, HER2, MET, RET, ALK, and ROS1, while the important alteration in tumor suppressor includes TP53, KEAP1, LKB1, and NF1 (**Figure 1**) [1, 14]. Interestingly the major genomic alterations in LUSC include TP53, PIK3CA, CDKN2A, NFE2L2, KEAP1, SOX2, PTEN, CDKN2A, RB1, CCND1, NOTCH1, MLL2, and HLA-A (**Figure 1**) [1, 15] (**Figure 1**). Inhibitors of these genes are primarily used as a treatment procedure and as one of the targeted therapies. Several driver oncogenes involved in NSCLC have been identified to be targeted with prior information of molecular testing and individual patient's pharmacogenetics towards drugs employed. Examples of such targets include EGFR, ALK, KRAS, BRAF, ROS 1, PTEN, HER 2, MET, and FGFR, identified in some patient subsets of NSCLC as potential treatment targets [16].

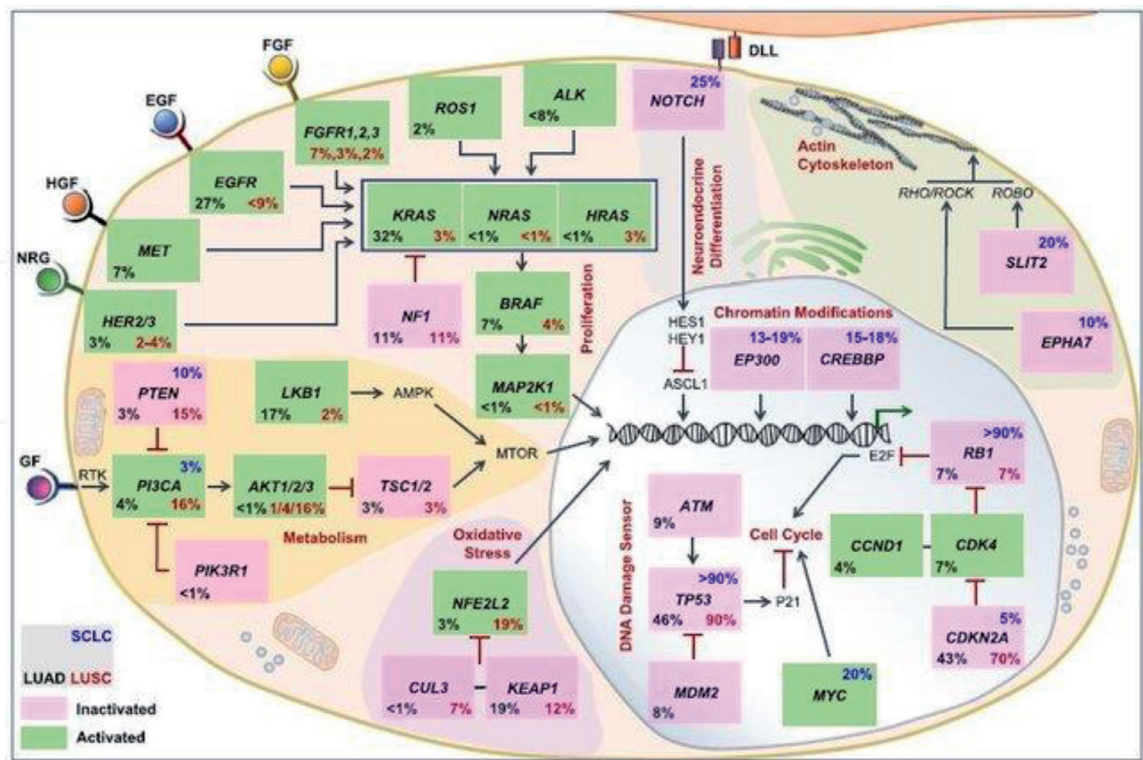


Figure 1. Comparison of genetic changes in major oncogenic pathways in individuals with LUAD, LUSC, and SCLC and the frequencies are shown in the specific boxes. The genetic alterations data is a sum of somatic defects, homozygous deletions, focal amplifications, and major changes of gene expression. Reproduced from Salehi-Rad et al. [1].

2.1 Epidermal growth factor receptor (EGFR)

EGFR (HER1 in humans) is part of the ErbB family of receptor tyrosine kinases (RTKs). Intracellular signaling occurs when RTKs are bound extracellularly to form homo or heterodimers [17]. Clinically significant mutations occur within the tyrosine kinase domain and are associated with drug sensitivity. The genetic alteration, including mutation or amplification in EGFR, results in increased tumoral metastasis, angiogenesis, and proliferation. Multiple mutations in the EGFR tyrosine kinase domain (deletion exon 19, L858R in exon 21) are associated with NSCLC. ATP-competitive TKIs bind to EGFR and yield promising clinical outcomes. Though targeted therapy for most EGFR mutations has produced better clinical outcomes, T790M mutation inhibition in EGFR resulted in resistance to targeted therapy [18]. Three generations of TKI have been reported for personalized NSCLC precision therapy based on patient pharmacogenetics; first-generation examples being gefitinib and erlotinib, second-generation Afatinib, and Neratinib while the third generation includes osimertinib [12]. Afatinib is an irreversible TKI, which has a unique property that, unlike other TKIs it does not require CYP3A4 activity in the liver for its targeted anticancer action. Thus, NSCLC patients with pharmacogenetics of deficient or abnormal CYP3A4 activity can be treated with afatinib over other established TKIs [19].

2.2 Anaplastic lymphoma kinase (ALK) and ROS Proto-Oncogene 1 (ROS 1)

A fusion gene of anaplastic lymphoma kinase (ALK) with echinoderm microtubule-associated protein-like 4 (EML4) (EML4-ALK) is prevalent in 3–5% NSCLC patients. EML4-ALK variants act as driver mutations and modulate the JAK/STAT, PI3K/AKT, and MAPK pathways, thereby provide proliferative and survival advantages to the cancer cells. Crizotinib blocks the kinase activity of the EML4-ALK and induces cancer cell apoptosis. ALK fusion is also familiar with other genes, including HIP1, KIF5B, KLC1, DCTN1, PTPN3, STRN, and show association with NSCLC. Individuals with NSCLC having significant ALK rearrangements can be genetically identified with advanced techniques like comprehensive next-generation sequencing (NGS), immunohistochemistry, and in-situ fluorescence hybridization (FISH). In advanced NSCLC stages, ALK TKIs have confirmed the progression-free survival of patients with better prognoses. Several second-generation ALK inhibitors can help target ALK-positive NSCLC, such as alectinib, ceritinib, and AP26133 developed and are currently under evaluation in clinical trials [20]. ROS1 rearrangements are observed in 1–2% of NSCLC patients. ROS 1 is a receptor tyrosine kinase and is structurally homologous to ALK protein and serves as the basis of using ALK inhibitors to target ROS1+ NSCLCs. Crizotinib and entrectinib are FDA approved and show a quick positive response by slowing cancer progression in ROS-1+ NSCLC [21].

2.3 BRAF

BRAF encodes a threonine/serine protein kinase that is an effector protein of KRAS. BRAF activates the MAPK signal transduction, which regulates cell proliferation and survival. Mutations in BRAF are about 1 to 3% in NSCLC, with a predominance of V600E (50%), G469A (39%), D594G (11%), and K601E, G469S, G596R, G466R, and T599dup [21]. Dabrafenib is a BRAF inhibitor combined with trametinib (MEK inhibitor), is FDA approved for BRAF V600E+ metastatic NSCLC. Vemurafenib, another BRAF inhibitor, showed a 42% overall response rate for BRAF V600E+ NSCLC in a basket trial.

2.4 Kristen Rat Sarcoma Viral oncogene (KRAS)

KRAS encodes a G protein and is a member of the RAS proto-oncogene family. KRAS-GTP complex activates the RAS/MAPK, PI3K/mTOR, and RalGDS-RalA/B signaling pathways and regulates cell proliferation, differentiation, and survival. Mutations in KRAS are recurrent in NSCLC (25–40%), especially LUAD. Ras gene and three forms are present H-Ras, N-Ras and K-Ras. In general, KRAS mutations have a poor prognosis. Out of KRAS mutations, G12C, G12V, G12D, and G12A are common and more frequent in male smokers. Common mutation among smokers is G12C (about 41%), while in nonsmokers, it is G12D (56%) and G12V. Though compounds are discovered that target the GDP-binding pocket (ARS-853, SML-8-73-1) but the efficacy and toxicity have been a hurdle. Currently, there is no specific developed FDA-approved anticancer agent that uniquely targets KRAS; however, MEK and PI3K/mTOR/MEK inhibitors are thought to be selective in the inhibiting downstream targets of KRAS mutant cases [22]. KRAS gain-of-function mutations serve as predictive markers for NSCLC chemotherapy, but recent studies present a geographical bias. KRAS mutations are frequent amongst westerns (30%) compared to Asian (10%) LUAD patients [23]. Moreover, the prognostic and predictive response is more efficient in LUAD than other NSCLC subtypes, and proper clinical pharmacogenetic evaluation and implementation is needed.

2.5 Receptor 2 of the human epidermal growth factor (HER2)

HER2 (ERBB2) is a proto-oncogene, encodes for tyrosine kinase receptors, and relies on heterodimerization for activation with receptors from the EGFR family. Upon activation, HER2, in turn, triggers downstream signaling like PI3K/mTOR, RAF/MEK/ERK, and the MEK/JNK pathways and regulates cell proliferation, differentiation, and migration. HER2 genetic alteration not only drives several tumors (breast and gastric cancer) but also plays a crucial role in NSCLC formation. HER2 amplification and overexpression are associated with 7–34.9% of NSCLCs and are related to poor prognosis. Activating mutations (like HER exon 20) are observed in 2–4% of NSCLCs cases, especially in LUAD [24]. The mutations in the tyrosine kinase domain are investigated as attractive therapeutic targets in NSCLC. Targeted therapy against HER2 (TKIs and antibody) is under clinical investigation, and the value of HER2 for such patient screening is gaining precedence. HER2-targeted TKIs, include afatinib, ipatinib, neratinib, and pyrotinib, while trastuzumab and T-DM1 conjugate are antibody-based [25].

2.6 REarranged during Transfection (RET)

RET proto-oncogene encodes for an RTK, localized to chromosome 10, and activates replication, cell proliferation, motility and differentiation. The GDFN family ligand (GFL) interaction with GDFN-family receptor α (GFR α) initiates RET receptor protein dimerization and formation GFL-GFR α -RET heteromeric complex. The complex formation results in RET activation and downstream signaling via RAS/RAF/MEK/ERK or PI3K/AKT1/mTOR pathways. Abnormal RET signaling may lead to cancerous growth and have a driver potential. RET Rearrangements are common in lung cancers (1–2%). RET fusions are common in NSCLC with partner genes like kinesin family member 5B (KIF5B), coiled-coil domain containing 6 (CCDC6), tripartite motif-containing 33 (TRIM33), Cut like homeobox 1 (CUX1), nuclear receptor coactivator 4 (NCOA4), and KIAA1468 [26]. Selpercatinib, a TKI that is FDA approved for use in RET+ NSCLC [27]. Other TKIs like vandetanib, cabozantinib, sorafenib and sunitinib can inhibit activated RET signaling and tumorigenic

Target gene (% mutation frequency)	Genetic alterations	Mutation effect	Drugs employed	References
EGFR [10–25]	Mutation	↑ angiogenesis, proliferation and metastasis	Gefitinib, Erlotinib, Afatinib, Neratinib, Osimertinib	[14]
EML4-ALK [2–4]	Fusion	↑ proliferation, migration and survival	Alectinib, Crizotinib, Ceritinib,	[30, 31]
ROS 1 [2, 3]	Fusion, rearrangement	↑ cell survival and resistance	Crizotinib and Foretinib	[32]
KRAS [2–5]	Mutation	↑ Chemotherapy resistance, survival and proliferation	Sorafenib, Ridaforolimus, Selumetinib + Docetaxel	[33]
MET [1, 2]	Amplification, exon 14 skipping	↑ proliferation and metastasis	Cabozantinib, Crizotinib Ornatuzumab, Tivantinib	[34]
BRAF [2, 3]	Mutation	↑ Proliferation, and survival, ↑ Resistance to EGFR inhibitors	Sorafenib, Debrafenib	[35]
RET	Fusion, rearrangement	↑ Proliferation	Carbozantinib, Ponatinib, Vandetanib	[13]
FGFR 1	Amplification	↑ chemo resistance proliferation and survival	Dovitinib Nintedanib, Ponatinib	[36]
PTEN	Deletion and mutation	↑PI3K signaling, survival and proliferation	PI3K inhibitors	[37]
PIK3CA [1–3]	Mutation	↑ Metastasis and survival	Buparlisib, inhibitors for AKT	[38]
TP53 [30–50]	Mutation	↓ apoptosis, ↑ Growth	—	[39]
DDR2	Mutation	↑ invasion and Cell migration,, proliferation and survival	Dasatinib	[40]
CDKN2A	Deletions	↑ Cell growth	—	[41]
HER2 [5–10]	Mutation	↑ Amplification	Afatinib, Dacomitinib, neratinib, Trastuzumab	[16]

Table 1.
Mutating genes associated with NSCLC, mechanism of mutation with its effects, and targeted therapy.

transformation. Activating RET mutations like M918T and acquired RET mutations (G810R, G810S, and G810C) in response to selpercatinib treatment is also reported in NSCLC [28]. Thus, RET is an interactive target and a biomarker for NSCLC.

Other actionable biomarkers in the lung include MET, PI3KCA, NTRK1, FGFR2, and DDR2 and are explained in detail elsewhere [29]. As driver mutations affect specific and exclusive cellular pathways to cause cancer, opportunities are being

explored for targeted drug therapies towards various mutating genes associated with NSCLC, as mentioned in **Table 1**.

3. Genetic alterations and lung cancer treatment response

NSCLC is a widely prevalent and challenging health problem for the human race. Despite rapid advances in lung cancer treatment, it is still one of the leading causes of death worldwide. Traditional chemotherapeutic approaches failed to yield satisfactory results in terms of treatment outcome. Interestingly, the association of genetic variations with treatment outcome of some of the most commonly used chemotherapeutic drugs has opened new vistas in the domain of lung cancer treatment [12]. The relatively new area of Pharmacogenetics aims to correlate the association between genetic variations and drug effects and formulate a rational personalized drug treatment offering minimum side effects and maximum efficacy [42]. The inherited genetic variations such as single-nucleotide polymorphisms (SNPs) have been primarily studied, most commonly focusing on the candidate gene approach. These genetic changes can either lead to the altered expression or function of drug-metabolizing enzymes or their targets, thereby modulating the activity of chemotherapeutics [43].

Pemetrexed is a commonly used folate antimetabolite, a multi-targeted anti-cancer drug used in NSCLC treatment. Pemetrexed causes inhibition of critical enzymes in the folate pathway including, thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase leading to a reduction in folate depletion resulting in altered purines and pyrimidines synthesis [44]. Thymidylate synthase (TS) expression is associated with the treatment outcome, especially in nonsquamous carcinoma patients treated with pemetrexed-based chemotherapy [45]. Studies conducted on the role of polymorphisms in TS, such as polymorphic tandem repeats located in the TS enhancer region (TSER), provide conflicting results. While some studies have observed that increased expression of TS with three copies (TSER*3) of the R than with two copies (TSER*2) is associated with treatment outcome in lung cancers, other studies did not observe such an association [46]. However, the homozygous variant T677 T of methylenetetrahydrofolate reductase was associated with prolonged progression-free survival compared to the wild-type or heterozygous genotype. The observation could be due to increased TS inhibition by pemetrexed due to the polymorphic variant since methylenetetrahydrofolate reductase is an essential regulator of folate homeostasis.

The entry of pemetrexed into the cells is mediated by the reduced folate carrier (RFC). A study investigating the combined action of pemetrexed and bevacizumab suggested the role of polymorphisms in RFC exon6 and progression-free survival. A similar association was also observed with IVS7 (1478) polymorphism in glutamyl hydrolase (GGH) while GGH IVS2 (1307) CC genotype was associated with significantly longer overall survival [47]. On the contrary, no association was observed with the outcome for GGH IVS7 (1478) and IVS2 (1307) in a randomized phase II trial involving fifty four patients for treatment with pemetrexed and gemcitabine [48]. The study also reported an association of RFC-exon6-SNP with outcome following treatment with pemetrexed.

The tyrosine kinase inhibitor (TKI) family has been clinically successful as an anti-cancer strategy. An enhanced expression of EGFR leads to the activation of pathways and proto-oncogenes that can lead to lung cancer development. For the EGFR gene, most of the studies have focused on polymorphisms present in regions regulating the expression, such as those present in the 5'-flanking region and intron-1. Two important SNPs located in the transcriptional start

site of the promoter region of EGFR are –191C/A and –216G/T. The –191C/A polymorphism causes enhanced EGFR expression and activity, while the –216G/T genotype, located at the binding site for the transcription factor Sp1, increases mRNA expression. However, the A-G variant, causing substitution of an arginine with a lysine at codon 497 (R497K), leads to the reduction of EGFR activity [49]. All three polymorphisms were evaluated for association with gefitinib treatment response in advanced NSCLC patients. Out of the three polymorphisms, the –216G/T variant showed a significant association with prolonged progression-free survival, high rates of stable disease/partial response, and treatment-related side effects such as rash and diarrhea [50].

Another EGFR polymorphism present in intron one was also reported to play an important role in the treatment outcome of gefitinib for NSCLC in different ethnic groups. The dinucleotide polymorphism is associated with a variable number of CA repeats in NSCLC. Upon gefitinib-treatment, it was observed that a smaller number of CA repeats was associated with increased EGFR transcription and better survival. This was observed in both Asian and Caucasian populations. For instance, studies conducted in Chinese patients treated with gefitinib reported better responses in NSCLC patients with shorter CA repeats (less than 16 repeats) [51]. However, the results were inconsistent in Caucasian patients as no association was observed for CA repeats and clinical outcomes in patients treated with gefitinib [52]. Similar observations were also made in a study involving advanced NSCLC patients treated with erlotinib [53].

Genetic polymorphisms in Protein kinase B (AKT1), DNA repair pathway genes like ATP-binding cassette superfamily G member 2 (ABCG2) also play an essential role in determining the treatment outcome in NSCLC patients. In studies involving the Caucasian populations, lower Akt protein levels were observed associated with haplotype having two polymorphisms (SNP3 and SNP4). The same haplotype was also found associated with lower rates of apoptosis-induction by radiation in EBV-transformed lymphoblastoid. In another Caucasian study involving NSCLC patients treated with gefitinib, AKT1-SNP4 A/A genotype was associated with shorter overall survival while AKT1-rs2498804 GT and GG alleles resulted in metastases in the brain [54]. Similar observations were also made in a Korean study where it was observed that in NSCLC patients, several genetic variations in the PI3K/AKT pathway served as a useful marker in response to various chemotherapeutic drugs [55].

ABCG2 is a member of the ATP-binding cassette (ABC) transporter family and plays a crucial role in the absorption and elimination of gefitinib. ABCG2 binds gefitinib and is expressed at higher levels in the gastrointestinal tract. Polymorphisms in ABCG2 could affect the metabolism of gefitinib due to variations in expression, function, and localization of ABCG2. One such polymorphism, ABCG2 421C/A (Q141K), has been found to be associated with a decreased protein expression and associated activity of ABCG2, resulting in the accumulation of both gefitinib and erlotinib [56] though conflicting reports are also available.

Investigators have also explored the association of selected genetic variations with toxicity caused by EGFR-TKIs, such as rash and diarrhea. In a study involving 52 NSCLC patients undergoing treatment with gefitinib, different intron-1 CA repeat variants were found to be associated with varying grades of skin rash [57]. Similarly, studies have also reported the association of genetic variations in EGFR and ABCG2 with diarrhea in patients undergoing treatment with gefitinib. Examples of such variants are EGFR 191C/A and A/A, EGFR 216G/G, R497K A/A, and ABCG2 421C/A variant [50, 58]. However, another study failed to find such an association with ABCG2 421C/A, though moderate–severe diarrhea was found to be associated with ABCG2 15622C/T polymorphism and the ABCG2

(1143C/T, -15622C/T) haplotype [59]. A category of enzymes that play an essential role in the metabolism of chemotherapeutics is cytochrome P450s. Commonly used anti-cancer drugs used in the treatment of NSCLC such as gefitinib and erlotinib are metabolized by the CYP3A4, CYP3A5, and CYP1A isozymes. However, erlotinib but not gefitinib is metabolized by CYP1A2. CYPs also exhibit a large number of genetic variations, which results in different pharmacokinetics of TKIs in NSCLC patients. In cases undergoing treatment with erlotinib and having skin rash due to A/A variant of CYP3A4 resulting in lower CYP3A4 expression [60]. The same study also reported the association of CYP3A5*3 G polymorphism with grade ≥ 2 rash and diarrhea.

The ALK gene, encoding a TK receptor, gets fused with echinoderm microtubule associated protein like 4 (EML4), leading to the development of lung cancer. The fusion gene EML4-ALK encodes a fusion protein that leads to the constitutive activation of ALK kinase as a result of oligomerization of ALK in absence of the ligand. Crizotinib is a commonly used ALK-inhibitor drug that targets lung cancer caused as a result of the EML4-ALK fusion protein. It acts as a ATP-competitive inhibitor and binds to the ATP binding pocket necessary for kinase activity leading to carcinogenesis [61]. The role of ALK gene mutations in determining the treatment outcome in lung cancer patients receiving Crizotinib was brought to light when it was observed that a male patient of lung cancer developed resistance to the drug after an excellent initial response [62].

Further investigations revealed that the cause of resistance was two mutations in the ALK gene (C1156Y and L1196M). The observations were validated by an *in vitro* study in which the mutated gene, when transfected into mouse cells, resulted in reduced drug sensitivity and enhanced cellular growth when exposed to different concentrations of ALK inhibitors. In another study involving 14 ALK-positive patients, the same pattern of treatment response was observed. After promising initial response to the drug, the patients experienced tumor progression. In this study also, the reason for drug resistance was identified as mutations on the ALK gene (L1196M and G1269A) along with two more gains of copy number [63]. A study by 3D modeling into the insights of mechanisms by which the mutations alter crizotinib activity revealed that L1196M, G1202R, S1206Y and 1151insT mutants are near the crizotinib-interacting ATP-binding pocket. L1196M worked as a gatekeeper mutation as it prevents the interaction between crizotinib and the ATP-binding pocket while G1202R and S1206Y decrease affinity to crizotinib by changing the solvent-exposed region [64].

4. Antibodies and immune checkpoint inhibitors in non-small cell lung cancer

Apart from the conventional chemotherapeutic agents used for the treatment of NSCLC, immune checkpoint inhibitors (ICIs) have gained much attention in recent times. Though our immune system can target the cancer cells, yet cancer cells escape this immunosurveillance and destruction. The main hallmark of anti-tumor immune response is T cell-mediated identification of tumor-specific antigens. Tumor cell often activates immune checkpoints to effect an immune escape. Programmed cell death protein 1 (PD-1/CD279) and cytotoxic T-lymphocyte protein 4 (CTLA4) are the best-studied checkpoint inhibitors. Programmed cell death ligand-1 (PD-L1) expression, especially by tumor cells, can inhibit the response of PD-1 expressing effector T cells and induce T cell exhaustion. Treatment using anti-PD-1 or anti-PD-L1 antibody causes checkpoint blockade and thereby releases the inhibitory brake on anti-tumor effector T cell function [65, 66]. The approved

monoclonal antibodies for targeting PD-1 are nivolumab and pembrolizumab while the anti-PDL1 antibodies are atezolizumab and durvalumab for lung cancer treatment [67]. The effects of pembrolizumab may be influenced by two possibilities: change in its binding site on the receptor or genetic changes that may reduce the immune system's capability to target cancer cells. A study conducted on cases showing resistance against pembrolizumab did reveal mutations that inactivated Janus kinase1 (JAK1), Janus kinase2 (JAK2), and β 2 microglobulin (B2M). The data indicated that the immunological pathways were affected by the mutations [68].

CTLA-4, also known as CD152, is a receptor expressed on the surface of lymphocytes and fibroblasts. This receptor on the surface of T lymphocytes competes with CD28 (co-stimulatory receptor) to bind to the B7 ligands CD80 and CD86, expressed on the surface of antigen-presenting cells. Since the CTLA4 receptor has a higher affinity for binding to the B7 ligands, it inhibits the binding of CD28, which leads to the decreased production of the cytokine IL-2 and ultimately prevents the activity of the Cancer-Immunity Cycle (CIC) [69]. Thus, inhibition of CTLA4 checkpoint can lead to the suppression of binding between CTLA4 receptor and ligand B7. This will boost the clearance of cancer cells by activating the innate and adaptive components of the immune system. US FDA has already approved ipilimumab and tremelimumab as immune checkpoint inhibitors targeting the CTLA4 for patients with metastatic melanoma. Moreover, studies are going on with immune checkpoint inhibitors targeting the CTLA4 for NSCLC and may deliver promising results [70].

The field of immunotherapy has shown significant advancements in the treatment of several cancers, including NSCLC. However, the success is also accompanied by serious challenges, particularly in NSCLC. Some NSCLC patients show primary resistance and are unresponsive to ICIs, while others develop secondary resistance during/after the treatment. Moreover, a unique spectrum of immune-related adverse events (IRAEs) also limits the use of ICIs. The mechanism governing both the primary and secondary resistance needs further investigation. Immunopharmacogenomics can explain these resistance mechanisms. The current phase III studies of PD-1 and PD-L1 inhibitors, either alone or in combination with conventional approaches in different stages of NSCLC, will serve to improve the treatment outcome significantly [1]. However, there are still many challenges ahead though immunotherapy with checkpoint inhibitor has already raised new hopes of novel treatment modality with better and more effective treatment outcomes for NSCLC patients.

5. Challenges in pharmacogenetics in lung cancer

Lung cancer is one of the leading causes of cancer-related death worldwide, with a 5-year survival rate of approximately 15%, suggesting a comprehensive genomic alteration map may help. The lack of an early diagnosis and inefficiency in conventional therapies causes poor prognosis and lung cancer patients' overall survival. Moreover, pharmacogenetic trials ended in conflicting and inconclusive data because of non-standardized methodologies, sample heterogeneity, clinical sample processing techniques, and the inadequate number of enrolled individuals. Clinical sample preparation protocols are varied and challenging to follow in a clinical setting. Collection of needle biopsy of lung tumor is a challenge in itself. The tumor cores are usually retained as Formalin-Fixed Paraffin-Embedded (FFPE) tissue specimens. Defining the normal tissue needs more attention than we think. Recent findings suggest that normal-looking tissue adjacent to the

tumor may be existing in an intermediate state. Considering tumor variability, it is unclear whether core biopsies are indicative of the oligoclonal nature of NSCLC? Data generated from specific experiments suggest histological markers can vary significantly and therefore contributing to sequence data heterogeneity within and amongst various studies. Another aspect related to biomarkers is robustness, sensitivity, and false-positive assessment of the molecular diagnostic, especially regarding immune checkpoint therapy.

Lack of pharmacogenetics biomarkers is another challenge for NSCLC pharmacogenetics. Biomarkers are significant in drug development and are used to measure the investigational drug effects on people. Cancer biomarkers are essential for diagnosis, risk assessment, the staging of cancer, screening, patient stratification, prognosis, and predict the impact of the therapy [71]. The selection of cytostatic drugs is based on the estimated responsiveness as per the predictive molecular biomarkers. In NSCLC, the predictive biomarkers that are providing for targeted therapy include EGFR and ALK. As an example, FDA-approved drugs like afatinib are associated with biomarker EGFR, and ceritinib is related to the biomarker ALK. Other essential genomic alterations in key genes like KRAS, ROS1, MET, NTRK1, FGFR, BRAF, PI3KCA, RET, PTEN, and DDR2 provide valuable information (**Figure 2**). The REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) guidelines provides criterion and suggestions for designing prognostic and tumor biomarker studies [72]. Several NSCLC studies still do not follow and comply with the standardization protocols of REMARK, thus obfuscating the clinical use scenario of biomarkers. Many trials do not include biomarker analysis as a criterion for including patients, especially in NSCLC, and serves as a significant challenge by creating selection bias. Tumor prognostic biomarker staining (for TUBB3) and scoring was done in a fraction of NSCLC in the N + IFCT-0002 trial [73].

Immune checkpoint therapy relies on monoclonal antibodies and may mediate a variety of adverse hypersensitivities, including anaphylaxis (type I), cytotoxic (type II), immune (type III), and T cell-mediated (type IV) reactions [74]. The Discovery of predictive biomarkers for immune-associated adverse reactions are essential pharmacogenetic needs for personalized cancer therapy. Genetic polymorphism, especially in the genes associated with antibody recognition, presentation, and immune response, may affect the efficacy. The role of polymorphism

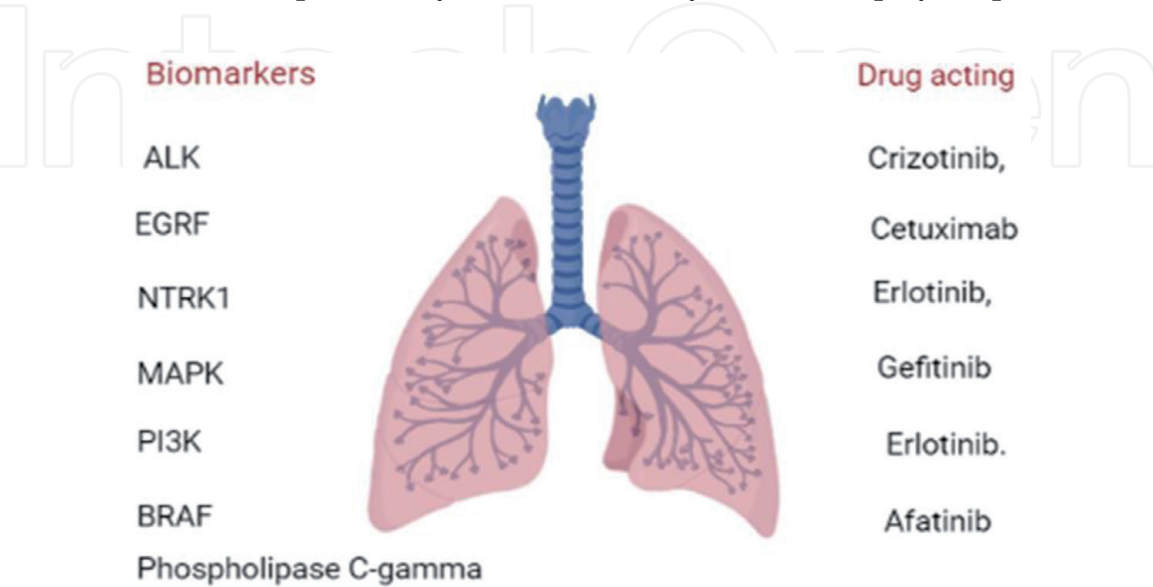


Figure 2.
Representation of biomarkers and targeting drug acting on the same.

concerning the metabolism of therapeutic antibodies can alter antibody half-life and therapeutic response. Genotypic variation in the PD-L1 (rs4143815 C/C and C/G genotype in comparison to G/G phenotype) show higher progression-free survival upon treatment with nivolumab in NSCLC [75]. A study conducted by Rizvi et al. showed a correlation of pembrolizumab efficacy with an increased nonsynonymous somatic mutational burden. A higher mutational burden was associated with an expanded neo-antigen repertoire and effective T cell-specific response [76, 77]. The Discovery of personalized biomarkers for risk assessment, detection, diagnostic, prognostic, and monitoring can be crucial in tailored NSCLC therapy. Pharmacogenetic studies correlating genetic alterations regarding immunotherapy are yet to be correctly established.

6. Future direction and conclusion

In this modern world of fast-growing medicine and research, treatment is not just about curing an ailment but also providing a better standard of life and living. With new social standards, smoking habits, and environmental pollution, NSCLC diagnoses are projected at approximately 116,660 women and 119,100 men in 2021. To treat lung cancer, it is essential to identify the disease at the earliest. The Discovery of NSCLC biomarkers can help identify disease susceptibility and aid in disease screening, diagnosis, prognosis, prediction of response, and monitoring disease recurrence. Recent advances in novel detection techniques like high throughput omics technology, multiplexed immunofluorescence microscopy, bioluminescence resonance energy transfer (BRET), CRISPR-based biosensors, surface-enhanced Raman spectroscopy have generated hope for better treatment. Bulk and single-cell next-generation sequencing (NGS), circulating cell-free DNA (cfDNA), single-cell proteomics can help in biomarker discovery and push modern pharmacogenetics and personalized medicine. Discovery strategies including hotspot panels (frequently observed gene mutations), Actionable gene panels (targeted gene exons), disease-focused panel (genes involved in a disease), comprehensive panels (correlative genes), and validated panels (tested genes) NGS applications can reduce biomarker discovery time. Machine learning-based data analyses platforms and algorithms may help undertake candidate polymorphism search; candidate pathway searches better predict correlations between gene alterations and therapeutic response.

Exploring new molecular signature-based personalized medicine can open up future potential healthcare environments. Considering the massive expansion in NGS-based NSCLC molecular data generation, integrating pharmacogenetics and genomic knowledge with the potential of theranostics can lead to effective therapy. Theranostics, the fusion of therapeutics and diagnostics, using a nanotechnology-based delivery platform can pave the way to precision and personalized medicine [78]. Nanotechnology is a quickly evolving biomedical research area and has been used to address several biological issues, including therapeutics and diagnostics [79]. Nanoscale-based delivery platforms like liposomes, polymeric nanoparticles, metal nanoparticles, and bio-nano particles can be efficiently used for theranostic applications for targeting cancer. Nanoparticle offers a benefit over standard medicinal therapies regarding biocompatibility, enhanced permeability retention, higher drug loading, targeting precision, a significant degree of versatility, and real-time monitoring of the disease [80]. Nanoparticle-based nanotheranostics can provide multifunctional benefits including, imaging, prognostic, diagnostics, and monitoring therapeutic outcome in NSCLC patients. Mukherjee et al. presented a detailed analysis of lung cancer theranostics [4].

Liquid biopsy and microfluidic technology can help in early disease detection. NGS has already helped identify new cancer-driving mutations, and this has encouraged scientists for drug repurposing. Scientists are deciphering synthetic lethality interactions, where two or more gene simultaneous alteration in the presence of a therapeutic may lead to lethality. Efficacy of immune checkpoint therapies is associated with genotypic variance, and immune-based biomarkers may provide a clear understanding of immunopharmacogenetics. Big data analyses of the growing pharmacogenetic or pharmacogenomic dataset can soon lead us to personalized NSCLC therapeutics.

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Conflicts of interest

The authors declare no conflict of interest. The authors have no other pertinent affiliations or financial connection with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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