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MINI-REVIEW

Current Drugs and Drug Targets in Non-Small Cell Lung Cancer: Limitations and Opportunities

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Abstract

Lung cancer is a serious health problem and leading cause of death worldwide due to its high incidence and mortality. More than 80% of lung cancers feature a non-small cell histology. Over few decades, systemic chemotherapy and surgery are the only treatment options in this type of tumor but due to their limited efficacy and overall poor survival of patients, there is an urge to develop newer therapeutic strategies which circumvent the problems. Enhanced knowledge of translational science and molecular biology have revealed that lung tumors carry diverse driver gene mutations and adopt different intracellular pathways leading to carcinogenesis. Hence, the development of targeted agents against molecular subgroups harboring critical mutations is an attractive approach for therapeutic treatment. Targeted therapies are clearly more preferred nowadays over systemic therapies because they target tumor specific molecules resulting with enhanced activity and reduced toxicity to normal tissues. Thus, this review encompasses comprehensive updates on targeted therapies for the driver mutations in non-small cell lung cancer (NSCLC) and the potential challenges of acquired drug resistance faced in the field of targeted therapy along with the imminent newer treatment modalities against lung cancer.

Keywords: Lung cancer - driver mutation - translocation - drug target - targeted therapy - acquired resistance - NSCLC

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Introduction

Lung cancer is undisputedly the most commonly diagnosed cancer with its annual death rate being over 1.3 million globally (Molina et al., 2008; Jemal et al., 2010). More than 85% of carcinomas of lung are because of tobacco & smoking while non-smokers approximately accounts for 12-15% of cases which are often due to exposure to chemical like asbestos, radon gas or by genetic factors. Depending upon cellular morphology, lung cancer is primarily classified as Small Cell Lung Carcinoma (SCLC) & Non-Small Cell Lung Carcinoma (NSCLC) and the later comprises of about 80%. NSCLC is further divided into adenocarcinoma (highest rate of occurrence 40%), squamous carcinoma and large-cell carcinoma due to differentiated histological subtypes (Travis et al., 2011). The systemic treatments for lung cancer consist of classical surgery, standard chemotherapy and radiotherapy either individually or in combination (Natukula et al., 2013; Wang and Cai, 2013; Cao et al., 2014; Di et al., 2014; Kiziltan et al., 2014). Regardless of these conventional therapies, the 5 year survival rate has remained disappointingly low at only 15% for more than four decades (Spira and Ettinger, 2004).

Amongst the different types of lung cancer, NSCLC is a heterogeneous disease which typically harbors numerous "oncogenic driver mutations" occurring at varied frequency of 2 to 25% (Kris et al., 2011). Improved efforts to delineate and elucidate the molecular mechanisms in lung carcinogenesis clearly states that there is functional association between the pathways that drives lung tumourigenesis and key oncogenes having mutations. These oncogenic driver mutations activate signaling cascades in constitutive manner leading to uncontrolled cell growth and proliferation (Pao and Girard, 2011; Cooper et al., 2013; Shtivelman et al., 2014). As a result, novel treatment modalities which directly target the driver mutations responsible for the process of tumourigenesis were discovered and termed as *"Targeted Therapies"*. Targeted Therapy is a highly specific therapeutic approach that precisely targets tumor cells and increases the overall survival of the patient. Hence the obvious priorities to better understand the complexity of biological networks underlying lung cancer pathogenesis gave exemplary shift in tailoring therapies that will efficiently improve outcomes for patients.

To date, several oncogenic driver mutations have been recognized in adenocarcinoma, including genes encoding for epidermal growth factor receptor *(EGFR)*, anaplastic lymphoma kinase *(ALK)*, kirsten rat sarcoma

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viral homolog (kras), v-raf murine sarcoma viral oncogene homolog b (braf), met, her2, ret, ros1, etc (D'Arcangelo et al., 2013; Oxnard et al., 2013). All these vital mutations which play a crucial role in NSCLC disease progression are found to be strongly associated with each other either by their functional similarities or by literature/ experimental evidences as depicted in the Figure 1 (Cardarella and Johnson 2013).

EGFR is implicated in diverse fundamental functioning of tumor cells including cell growth & development, proliferation, apoptosis regulation, angiogenesis, and metastatic invasion hence making it a prime target in lung cancer against which TKIs such as erlotinib and gefitinib have been developed (Scagliotti et al., 2004; Bethune et al., 2010; da Cunha Santos et al., 2011; Antonicelli et al., 2013). Another agent Crizotinib was approved recently which inhibits anaplastic lymphoma kinase *(ALK)* oncogene (Husain and Rudin 2011, Shaw and Solomon 2011). Once the oncogenic mutation is diagnosed, these therapies are administered as first line of treatment which offers prolonged response that often last for a year and sometimes much longer as compared with conventional chemotherapy. However, patients eventually demonstrate disease progression due to acquired resistance to the previous highly effective targeted therapy (Katayama et al., 2012; Gainor et al., 2013). Lack of better understanding of molecular mechanisms leads to intrinsic or acquired resistance resulting in limited efficacy of potential targeted therapy. Moreover, due to molecular heterogeneous nature of tumors it is very difficult to find any common mechanism of drug resistance for lung cancer. With the optimism that identification of new mechanisms and targets that contribute to drug resistance will provide opportunities for development of novel therapies in overcoming drug resistance. This review demarcates, contemporary curative role of EGFR inhibitors, ALK inhibitor collectively with other molecular aberration in lung cancer, including K-RAS, B-RAF, etc in adenocarcinoma and also discusses drug acquired resistance along with the development of new therapeutic strategies to overcome these resistance.

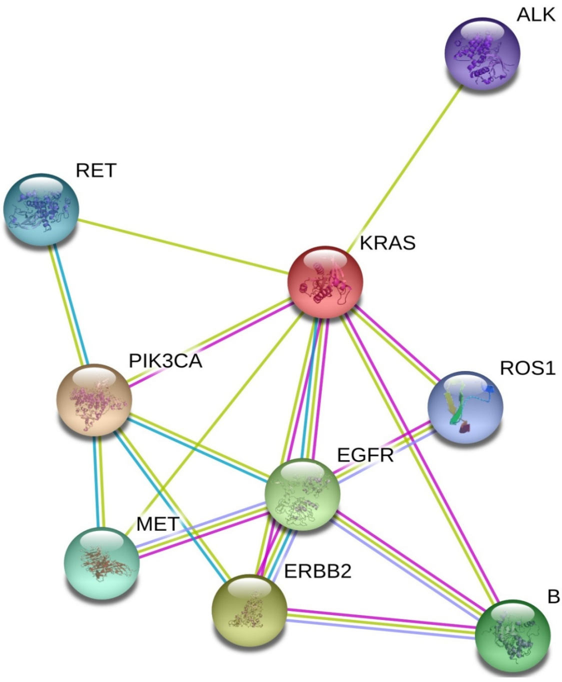

Figure 1. 1 String Output Showing Interaction of Most Common Gene Targets in Lung Cancer

Current Drug Targets in NSCLC

Extensive manual curation of literature pertaining to current drug & drug targets in NSCLC was carried out from PUBMED data base. The major drug targets and their targeted therapy for translocations and mutations associated with drug responsiveness and resistance is reviewed here.

Epidermal growth factor receptor (EGFR)

EGFR, an ERBB family tyrosine kinase receptor, is a transmembrane glycoprotein localized at p12 cytoband of chromosome 7. Dimerization upon ligand binding activates protein kinase which initiates various downstream signaling pathways chiefly RAS-RAF-MEK-MAPK and PI3K-Akt-mTOR cascades (Scagliotti et al., 2004; Bethune et al., 2010). EGFR mutations are most frequently diagnosed in non- smoker Asian females with adenocarcinoma histology (Usuda et al., 2014).

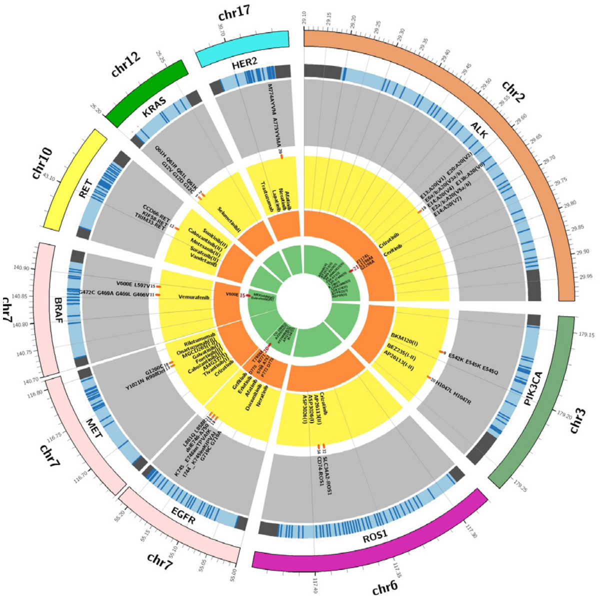

Figure 2. Circos Plot of Oncogenic Driver Mutations Occurring at Various Frequencies in NSCLC. Outer most track indicates the total length of the chromosomal coordinates of the respective gene (names shown inside) in the clockwise direction (scale 1 = 1000000bps). The second track is denoting the complete gene including intronic portion in light blue colour and the exons shown in dark blue bands. The next (grey track) shows the numbers of primary mutations (either insertion, deletions, point mutations) present within particular exon of the gene denoted by red colour and the consecutive track (yellow colour) depicts either the FDA approved inhibitors or small molecules that are currently under clinical trials against these primary mutations. The second last track of orange colour shows the locations of the drug acquired resistance mutations particularly for EGFR, ALK and BRAF oncogenes. And the innermost green circle gives the detail of the compounds that are in early clinical development process (trials) against these acquired resistant mutations

The most common mutations in EGFR Tyrosine kinase domain (Exon 18-21) that lead to its constitutive activation consists of point mutations at position 858 from leucine-to-arginine & deletion (delE746-A750) within exon 21 & exon 19 respectively (Da Cunha et al., 2011; Siegelin and Borczuk 2014). Further, increased expression and high copy numbers of EGFR were observed in more than 75% and 60% of advanced NSCLC respectively, thus portraying its potential as a target of prime importance for early diagnosis and developing newer therapeutic approaches (Antonicelli et al., 2013). Scorpion Amplified Refractory Mutation System (SARMS), Fluorescence in situ hybridization (FISH), Polymerase Chain Reaction (PCR), Immuno-histochemistry with mutation-specific antibodies and various other methodologies have been established to analyze the mutational profile of EGFR tyrosine kinase domain (Ellison et al., 2013). Inspite of this, direct DNA Sequencing undisputedly remains the unsurpassed option till date in order to study the mutational landscape of EGFR because of its ability to check multiple mutations at the same time (Roengvoraphoj et al., 2013).

EGFR inhibition can be achieved either by the conventional chemotherapy, or by monoclonal antibody (Cexuximab) or by small molecule Tyrosine Kinase Inhibitors (TKIs) such as Gefitinib (Iressa) and Erlotinib (Tarceva) in combinations with cohemotherapies (Alimujiang et al., 2013; Roengvoraphoj et al., 2013; Fang et al., 2014). These TKIs approved by FDA, targets EGFR specific mutation, hence establishing it as potential biomarker for therapeutic response. Patients harboring EGFR somatic mutations responded to gefitinib/erlotinib in much better way in terms of increased sensitivity and progression free survival as compared to chemotherapy which proved to be more effective in patient having wild type EGFR or non-mutants (Mok et al., 2009; Zhang et al., 2010; Song et al., 2014).

Anaplastic lymphoma kinase (ALK)

ALK rearrangements are observed in about 7% of the NSCLCs patients majority of which comprises of young never smoker females with adenocarcinoma histology (Shaw et al., 2009). The intracellular domain of Anaplastic Kinase (ALK) is fused in opposite direction with N-terminus of Echinoderm Microtubule-associated Protein like 4 Gene *(EML4)* owing to short inversion in p arm of chromosome 2 (inv(2)(p21p23) resulting into constitutive tyrosine kinase activity followed by stimulation of PI3K-Akt and the MAPK signaling pathways for cell proliferation and apoptosis inhibition making the cell an "oncogenic addict". This fusion leads to formation of seven different variants of ALK-EML4 (Choi et al., 2008; Shaw and Solomon 2011). It also forms some of rare fusion proteins by joining with the partners like KLC1, TRK-fused gene (TFG) and kinesin family member 5B (KIF5B) that eventually leads to ALK kinase activation (Rikova et al., 2007; Takeuchi et al., 2009). *ALK* rearrangements are diagnosed using various molecular biology techniques such as RT-PCR, posterior sequencing, using IHC-antibody of higher sensitivity (Shaw et al., 2011). However, FISH analysis using break-apart probes to ALK still remains the golden standard method as compared to the other assays (Kim et al., 2011). ALK mutations are also considered as probable therapeutic target in EGFR wild-type as these rearrangements are never commonly found to co-exist with EGFR or KRAS mutations (Shaw et al., 2011).

Inactivation of EML4-ALK fusion can be achieved by a c-MET inhibitor under the trade name of Crizotinib (PF02341066) which is FDA approved drug manufactured by Pfizer (Gandhi and Janne 2012; Ou et al., 2012). Patients treated with Crizotinib not only showed a good response rate (>58%) with 9 months median progression free survival in early phase I trials but also demonstrated almost negligible toxicity in patients as compared to other drugs in clinical trials. Further, collective outcome of all major phase II and Phase III studies helped in deciding the effective dosage of drug as 250mg twice per day (Kwak et al., 2010; Ou et al., 2012). As a result, crizotinib exemplified the huge potential of targeted therapy in treating ALK positive NSCLC patients by successfully completing the usually long process of drug discovery in very short span of 4 years.

Kirsten rat sarcoma viral homolog (KRAS)

KRAS is an established G-protein encoding proto oncogene which is significantly associated with RAF/MAPK/MEK/ERK signaling cascade. Mutant KRAS hydrolyses the RAS bound GTP to GDP resulting into stimulus independent constitutive activation RAS/RAF/MAPK downstream pathway. KRAS mutation comprises of approximately 30% of NSCLC patients with substitution of single amino acid mostly at codon 12, 13 and 61 within exon 2 and 3 being the most common mutations. Predominant association with adenocarcinoma histology over squamous cell subtype and an increased prevalence in patients having smoking history and tobacco exposure makes KRAS the most common driver mutation in lung cancer patients. KRAS mutations are generally not found to co-exist along with EGFR mutations or ALK fusions in same NSCLC tumor apart from some exceptions (Riely et al., 2009; Suda et al., 2010; Karachaliou et al., 2013; Peter et al., 2013)

V-Raf murine sarcoma viral oncogene homolog B (BRAF)

BRAF is a serine/Threonine protein kinase which acts as a downstream molecule of KRAS that is activated upon phosphorylation in a GTP dependent manner, thus mediating essential functions of cell including survival and proliferation by stimulating MEK/MAPK cascade (Robinson and Cobb 1997). These mutations approximately account for about 3-5% in smoking habituated adenocarcinoma patients and amongst them papillary phenotypic histology is commonly reported as compared to lepidichistotype in NSCLC. Majority of BRAF mutations are found in exon 15 (V600E:50%, D594G:11%) and exon 11(G469A:39%) occurring in a mutually exclusive manner with KRAS mutations excluding some exceptions (Paik et al., 2011; Marchetti et al., 2011).

These driver mutations were targeted with cetuximab in colon cancer patients which demonstrated deteriorated response rates while dabrafenib treatment in randomized phase II trials responded in partial manner in lung adenocarcinoma patients. Recently, BRAF mutants in melanoma were treated with vemurafenib or dabrefenib depicting a relatively good response rate as compared to other drugs in clinical trials (Jose et al., 2013).

Other oncogenes in adenocarcinoma (NSCLC)

As the occurrence rate of several other driver oncogenic mutations like MET, HER2, RET, ROS1, PIK3CA is relatively low ranging between 1-3%, there are only few study reports and data exist till date. These alterations are characterized by means of advanced technology of Next Generation Sequencing (NGS). Even though these modifications are rare events, they do have clinical relevance and also targeted therapies are already available for some of them pertaining to other malignancies (D'Arcangelo et al., 2013). The rest of alterations are under the early phase trials with the aim of finding the proper treatment options.

Met Amplification: MET proto-oncogene located on chromosome 7q31, encodes a protein known as hepatocyte growth factor receptor (HGFR) which possesses tyrosine kinase activity. Activation of MET may occurs by several ways including reduced degradation, mutations, over expression, etc which consequently affects cell proliferation and survival mechanisms in such a manner that cell becomes malignant (Ma et al., 2003; Cipriani et al., 2009). Possibly the mechanism called "Kinase Switching" leads to amplification of this gene. Alteration in MET gene and its amplifications have been reported to be predictors of response to treatment in several malignancies (Okuda et al., 2008; Cappuzzo et al., 2009). Expression profiles of MET and phosphor-HGFR (MET) were studied in Patients with Adenocarcinoma of lung that often ended up in diagnosis of MET over-expression with frequency of about 40% (Ma et al., 2008) Investigational analysis of biopsy samples from patients with acquired resistance against anti- EGFR TKIs confirms the clinical significant role of MET amplification in development of this acquired resistance (Arcila et al., 2011; Sequist et al., 2011).

This identification of MET amplification has led to the discovery of many anti-MET/HGF antibodies and small molecule TKI inhibitors. Combined treatment of MetmAB (onartuzumab) with Gefitinib/erlotinib was given to NSCLC patients with secondary resistance in randomized phase II trial, which showed good response to therapy with increased overall survival rate as compared to erlotinib/gefitinib when given alone. Phase III clinical trials conducted for MET inhibitor- Tivantinib, were discontinued because of the ineffectiveness of drug. Numerous other MET inhibitors are in various phases of Clinical testing with expectation of providing improved treatment alternative in EGFR TKI non-responders (Jessica et al., 2006; Robinson and Sandler 2013).

Her2 Amplification and Mutations: HER2 is a receptor tyrosine kinase belonging to the epidermal growth factor receptor (EGFR/ERBB) family. It undergoes hetero-dimerization with any of other family members causing auto-phosphorylation of tyrosine residues thus resulting in initiation of a variety of downstream signaling pathways. HER2 mutations occur at frequency of around 2-3% in East Asian female adenocarcinoma patients with non-smoking status. It mainly consists of the insertions in exon 20 of TK domain within protein and appears to be associated with drug resistance, increased metastatic potential, increased production of VEGF, and poor prognosis (Swanton et al., 2006; Swanton et al., 2013).

HER2 positive NSCLC patients when treated with Trastuzumab (Anti-HER2 mAB) in phase II trials either alone or in combination with chemotherapy showed better response (> 35%) to treatment. Currently, clinical testing is also ongoing with several other small molecules including afatinib or dacomitinib indicating partial response to treatment in early phases (Ise et al., 2011; Ana and Enriqueta 2013).

ROS1 Translocation: The incidence of fusion gene formation of ROS1which is receptor tyrosine kinase found to be 1-2% in adenocarcinoma patients generally having non-smoking history. These rearrangements show resemblance with ALK mutant subclass of adenocarcinoma patients and thus are inhibited by crizotinib (Bergethon et al., 2012; Davies et al., 2012; Shaw et al., 2012).

RET Translocations: RET translocations occur at frequency of less than 2% and are recognized in never smoker patients of lung adenocarcinoma. This is again a receptor tyrosine kinase which is probably oncogenic in NSCLC. Vandetenib, a Ret kinase inhibitor which is previously known to treat Medullary thyroid carcinoma is under the randomized phase II testing for patients with RET positive NCSLC which may be a successful treatment option (Lipson et al., 2012; Sasaki et al., 2012; Takeuchi et al., 2012).

PIK3CA Mutation: PIK3CA mutations are present in lung adenocarcinoma at overall rate of 1-2%. In many malignancies the phosphatidylinositide3-kinase (PI3K) pathway is found to be deregulated, mainly because of the alterations in PIK3CA gene which is central to this cascade. Currently, extensive research and early phase trials are conducted in order to find targeted treatment either effective alone or in combination, for PIK3CA positive lung cancer (Yamamoto et al., 2008; Samuels and Waldman 2010; Sarris et al., 2012; Wang et al., 2012).

Limitations of Targeted Therapeutic Drugs

Although patients treated with these TKIs initially showed good efficacy but eventually ended up with progression of disease due to its prolonged administration. This drug acquired resistance was attributed to the development of EGFR mutation T790M in exon 20 of TK domain which can be found in more than half of the patients with TKI resistance (Kobayashi et al., 2005; Pao et al., 2005). It is presumed that the substitution of Methionine instead of Threonine at 790 position within the ATP binding pocket of TK Domain results in a conformational change. This structural change makes Gefitinib/Erlotinib lose specificity to its binding site, resulting in a decrease in their effectiveness which leads to disease progression (Balak et al., 2006). Early detection of this mutation is only possible by repetition of biopsy at regular intervals after the initiation of anti-EGFR therapy. Various other clinical trial investigations emphasize that there are numerous other mechanisms such as mesenchymal-epithelial transition c-MET-oncogene amplification, KRAS mutation, EGFR amplification or polysomy of chromosome 7, activation of autocrine loop, over expression of ERBB3, activation of various EGFR downstream cascades including PIK3CA ,v-raf murine sarcoma viral oncogene homolog B1, etc that are associated with development of TKIs resistance (Engelman and Janne 2008; Sequist et al., 2011).

Several attempts are being made at clinical and preclinical level in order to discover new generation drugs against the developed secondary resistance for EGFR TKIs. Significant improvement in progression free survival and enhanced efficacy against wild-type EGFR and mutated forms of KRAS was demonstrated by a small molecule inhibitor of c-MET, ARQ197 during phase II trials (Sequist et al., 2011; Scagliotti et al., 2012). Another second generation drug in phase II of clinical trials, HKI-272/neratinib specifically targets HER2 and ErbB receptors which demonstrate reduced overall activity, thus depicting a very disappointing outcome as drug against acquired resistance (Wong et al., 2009; Sequist et al., 2010). An oral TKI for EGFR and HER2 named Afatinib (BIBW9229) demonstrated excellent activity particularly against L858R mutation of EGFR along with wild-type EGFR & exon 19 deletion but showed diminished activity for secondary TKI mutation T790M conferring the resistance(Li et al., 2008; Yang et al., 2008).

While there were drugs which did not show significant outcomes for this resistant mutation, on the other side there were some potential lead compounds which demonstrated a relatively better outcome, proving to be a ray hope in entangling this problem. AZD9291 and CO-1686, the third generation EGFR TKIs specifically designed for mutants, confirms better efficacy during phase I trial with the patient's refractory to TKI therapy including patients with T790M secondary mutation. Considerable improvements were seen in progression free survival during Phase II trial of PF299804 as compared to erlotinib in treating the patients with the acquired mutation (Ercan et al., 2010; Walter et al., 2011; Cross et al., 2014). Apart from this, an antibody IMC-11F8 for lung cancer and inhibitor XL647 against VEGFR, EGFR and HER2 are giving assuring results but are currently under clinical investigation (Miller et al., 2008; Pietanza et al., 2012). Thus, finding new potent drug for inhibition of *EGFR* in T790M mutant NSCLC is presently a challenging area of scientific research.

Acquired Crizotinib resistance is another major budding problem correlated with continuous medication however its mechanism of resistance is unclear in one third of patients. It is proposed that this resistance can either be *(i)* a resultant effect of numerous mutations in ALK-TK domain incorporating mutants like L1152R, C1156Y, L1196M, G1202R, G1269A, etc or *(ii)* by activation of EGFR & KIT pathways, gene amplification etc or *(iii)* by concurrent existence of multiple mechanisms in the same patient (Katayama et al., 2012). Hence, there is an urge to find new ALK inhibitors with superior effectiveness and ability to revert these resistant mutations. CH5424802, showed excellent overall response rate of more than 90% during phase I and phase II trials of wild type ALK positive patients. Similarly, LDK378 also showed 75% of response rate and was found to be effective for both naïve and mutants forms of ALK. Many other clinical studies and in-vitro experiments are going on with AUY922inhibitor against Heat Shock Protein 90 (HSP90), demonstrating partial treatment response; however efficacy against resistant ALK needs to be demonstrated (Seto et al., 2013; Shaw et al., 2013; Rolfo et al., 2014). With the same approach, many other ALK inhibitors are under development with the hope to target the resistant mutation of kinase domain more specifically and with increased sensitivity.

Although with lot of advancement in field of targeted therapeutic research, till date there are no direct anti-KRAS therapies available for cancer associated with KRAS mutations. Since EGFR is present in the upstream of KRAS, there have been attempts to target these mutations with anti-EGFR TKIs and mAbs. Various clinical investigations conducted so far have confirmed that patients with positive KRAS mutation confer resistance to EGFR inhibitors such as gefitinib, erlotinib and/or chemotherapy, resulting in faster disease progression rate and reduced overall survival (Pao et al., 2005; Massarelli et al., 2007). In Phase II trials, NSCLC Patients with wild type KRAS when treated with erlotinib showed about 30% response rate in contrast to merely 5% in KRAS mutated patients (Linardou et al., 2008; Mao et al.,2010). Further, anti-EGFR monoclonal antibodies, cetuximab or panitumumab also failed to curb these mutations (Osumi et al., 2013). Thus, collectively these studies indicate that somatic mutations present in KRAS oncogene results in poor efficacy and are negative predictor of response of these anti EGFR-TKIs & MAbs.

Various other approaches have been adopted to indirectly inhibit KRAS by targeting molecules of pathway downstream to KRAS. One of these includes MEK inhibitor selumetinib when given along with docetaxel in Phase II randomized trial, showed good efficacy with higher response rate and progression free survival. Another way of targeting KRAS mutant which is in clinical trials is by utilizing PIK3CA/mTOR/AKT pathway inhibitors combined with selumetinib to obstruct the KRAS downstream signaling (Simmons et al., 2012; Zou et al., 2012).

Presence of characteristic V600E mutation in NSCLC is directly co-related with poor prognosis, lack of response and development of resistance for EGFR-TKIs, thus limiting utilization of BRAF inhibitors (Ohashi et al., 2012). Apparently several lead compounds such as MEK inhibitors are under experimental trial, demonstrating good clinical response initially but their approval for commercial use is still awaited (Jose et al., 2013).

Hence, even though with the successful initial treatment in NSCLC many tumors often relapse in a more aggressive manner after receiving first line of therapy. Development of drug resistance has been the major hindrance to this therapeutic progress. It is therefore necessary to extensively study the molecular mechanism behind this drug resistance and think of innovative therapeutic strategies with the hope of discovering compounds that in particular inhibit target molecules within drug-resistant cells.

Opportunities: New Treatment Modalities

Established treatment alternative have limited therapeutic success in non-small cell lung cancer (NSCLC), as it becomes resistant to therapy. Hence, to develop better therapeutic modalities for lung cancer has become a pre-requisite. Presently various data suggest that (a) Immunotherapy and (b) Natural Compounds either alone or in combinations with chemotherapy are in prime focus and being tested for their treatment efficacy against lung cancer.

Immunotherapies

The progress and advancement in understanding the tumor immunology have directed towards the new treatment approach called *immunotherapies* which are under early investigation trials for NSCLC and other cancers. In contrast to chemotherapy and targeted therapy, it works by stimulating and reinstating patient's own immune system to eliminate tumor. Immunotherapeutic Strategies incorporates *antigen based or cell based vaccination approaches* to stimulate T-cell responses against tumor cells and *immune checkpoint monoclonal antibodies inhibitors* to restart T-cell mediated responses to NSCLC cells.

Various antigenic vaccines including MAGE-A3 for melanoma-associated antigen 3, L-BLP25 for MUC1 and EGF for epidermal growth are under early phase trial studies for NSCLC. On other hand, whole tumor cell vaccine Belagenpumatucel against transforming growth factor beta-2(TGFβ2) have also shown prolonged survival in adenocarcinoma patients during phase II trials. Monoclonal antibodies against immune checkpoints includes (a) Ipilimumab- an inhibitor of cytotoxic T-lymphocyte-associated antigen 4 pathway,(b) Nivolumab, Pembrolizumab [MK-3475] compounds targeting the programmed cell death protein 1 (PD-1) pathway and (c) BMS-936559, MPDL3280A against ligand for PD-1 have produced objective responses by enhanced T-cell-mediated antitumor activity during clinical development for lung cancer(). Preliminary evidences demonstrate the potential of immunotherapeutics as compared with the current treatment modalities in NSCLC patients (Anish and Hassan 2012; Hall et al., 2013; Sarah and Johan 2014). Moreover, dendritic cell-based auto vaccines are also developed where antigen presenting dendritic cells (DC) when injected in patients enhances the tumor specific immune response thereby preventing metastasis and recurrence consequently shows reduction in tumor (Hirschowitz et al., 2004). Preliminary evidences demonstrate the potential of immunotherapeutics to increase overall survival rate as compared with the current treatment modalities (Winter et al., 2011).

Natural compounds as adjuvant therapy

Natural compounds have traditionally been a basic source of anticancer drugs, but pharmaceutical companies announced their obsolescence due to the emergence of targeted therapies. Although targeted therapies significantly enhanced the treatments of few malignancies, the overall gain remained unsatisfactory which rejuvenated the attention for natural products. In contrast with targeted therapeutics, natural products have distinct advantages such as their non-toxic nature, abundant availability, cost effective and the major fact that they generally target multiple pathways simultaneously, which might contribute to lung carcinogenesis (Ancuceanu and Istudor, 2004). From various experimental studies it is evident that, some of novel natural products show their probable implication during preclinical and clinical investigation against lung cancer.

Epigallocatechin-3-gallate, EGCG can hinders TGF-β-induced EMT by down-regulation of phosphorylated Smad2 and Erk1/2 in A549 cells (Laurie et al., 2005; Ma et al., 2014). Curcumin blocks proliferation of NSCLC cell lines by arresting cell cycle at G0/G1 phase thus resulting in tumor shrinkage. Additionally, it appreciably augment the cytotoxicity of erlotinib/gefitinib, downregulates the expressions of EGFR and its phosphorylation , stimulates apoptosis, inhibits invasion by MTA-1 mediated inactivation of Wnt/β-catenin signaling pathway represses the NF-κB activation in erlotinib/gefitinib-resistant NSCLC cells. These findings indicate that curcumin is a potential adjuvant during TKIs treatment in adenocarcinoma patients (Lee et al., 2011; Datta et al., 2013; Li et al., 2013; Lu et al., 2014). An oral, water soluble silibinin (milk thistle) extract considerably abrogates the tumor volume by preventing the loss of EMT markers and repressing the synthesis of mesenchymal markers leading to a inhibition of invasive potentials (Singh et al., 2006; Cufi et al., 2013; Mateen et al., 2013). Similarly, Withaferin A which repressed the proliferation and apoptosis of NSCLC cells by deactivating PI3K/Akt pathways (Cai et al., 2014). Another compound called apigenin inhibited HIF-1 and VEGF expression suggesting suppression of angiogenesis in lung cancer cell lines (Liu et al., 2005). Other natural compounds including flavanoids, Luteolin, thymoquinone, resveratrol, harmol, Emodin, berbamine, Trilinolein and many more proven to be promising chemopreventive agent against NSCLC cells (Ancuceanu and Istudor 2004, Chou et al., 2011; Ulasli et al., 2013; Hou et al., 2014). Various traditional Chinese medicines like Scutellaria barbata, Saikosaponin D, Bupleurum scorzonerifolium, Marsdenia tenacissima extract, etc have been shown to inhibit A549 cell growth by inducing apoptosis and blocking the cell cycle progression (Chen et al., 2010).

Conclusion

Recent advances in biological understanding of cancer along with the development of various targeted therapies have directed oncology toward a targeted, approach to diagnosis and treatment. Personalized targeted treatment holds foremost significance for NSCLC patients as they are diagnosed with specific oncogenic mutations. Thus molecular-profile-driven identification of such patients who receive maximum advantage from the targeted therapies is of supreme importance. From various molecular targets only few (EGFR, ALK) in NSCLC demonstrated clinical benefit while rest of others has not proven useful. For the identification of predictive biomarkers to make appropriate therapeutic decision, oncologists have to depend on standardized and authenticate methods of molecular assessment. But sample collection & processing, tumor heterogeneity, molecular diagnostic tests and clinical assessment of biomarkers & drugs are major challenges faced during clinical application of molecular characterization. Thus, there is need for improvisation of diagnostic methods and of optimizing the accuracy and sensitivity of mutational testing that may be introduced into routine clinical practice.

Despite the fact that EGFR and ALK TKIs when given to respective molecular subgroup NSCLC patients showed prolonged responses to treatment, their continuous medication eventually results into development of acquired resistance thus limiting their applicability. Currently, finding novel therapeutic strategies to circumvent the problem of acquired resistance is the subject of ongoing research. To overcome this inevitable challenge, not only better understanding of molecular mechanisms underlying development of resistance is required but also the identification of all possible molecular alteration needs to be explored. Understanding these mechanisms will be advantageous in terms of patient selection, discovery of new targets and drug class that surmount acquired resistance. Besides this a multi-agent therapy including both synthetic and natural compound may prove to be beneficial than only single agent. However, this needs to be validated in-vitro using combinatorial approach.

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