Targetable “Driver” Mutations in Non Small Cell Lung Cancer

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Abstract Lung cancer remains the leading cause of cancer-related mortality in the world despite advances in the field of cancer therapeutics. Traditional treatment with empirically chosen cytotoxic chemotherapeutic agents, have given small, but real survival benefits. Recent advances and insights into molecular pathogenesis of lung cancers have provided some novel molecular targets, offering newer strategies and agents that are tumor specific. Studies have identified mutations in specific genes that are involved in driving the development of lung cancer and so it is important to subsequently target them with specific drugs thus changing paradigms of management of this type of cancer. Recently, Lung Cancer Mutation Consortium (LCMC) has identified at least one of the many recognized “driver mutations” in nearly two thirds of the patients with advanced cancer. This study suggests that identification of driver mutations can help in molecular targeted therapeutics and in addition supplant tumor histology in guiding treatment decisions, identifying subset of patients who may benefit therapy. This review focuses on these mutations identified in specific genes serving as “drivers” of lung tumorigenesis and suggests that clear promise for the future of lung cancer treatment is indeed personalized therapy with drugs chosen according to the patient mutation profile. Most clinically relevant translational advances made in genes involved in lung tumorigenesis namely EML4-ALK fusions, HER2, PIK3CA, AKT, BRAF, MAP2K1, MET mutations and amplifications along with the well established EGFR and KRAS mutations are discussed in the context of NSCLCs. These studies emphasize the need for treatment management based on mutation profile along with routine histology based

classification of these tumors in future for a directed therapy and thus a better therapeutic outcome.

Keywords Driver mutations · Molecular profiling based on mutations · Personalized therapy

Background

Lung cancer remains the leading cause of cancer-related mortality in the world despite advances in the field of cancer therapeutics. Lung cancers can be broadly divided into two histological groups: Non Small Cell lung Cancers (NSCLC) and Small Cell Lung Cancer (SCLC). NSCLCs are further subdivided into mainly adenocarcinoma, squamous cell carcinoma and large cell carcinoma, roughly accounting for almost 80% of the lung tumors.

Surgical resection remains the most beneficial curative strategy for approximately 25% of the patients presenting with early stage disease (Stage IA–IIIA), but up to 65% of these cases relapse within 2 years [1]. However, majority of the lung cancers present at an advanced stage (Stage IIIB–IV) wherein systemic therapy is the mainstay of management. In the 1970s, patients with advanced lung cancers were only offered best supportive care and were not considered for chemotherapy. Platinum chemotherapy came into being in the 1980s. During that decade 1990–2000 Vinorelbine, Paclitaxel, Gemcitabine, and Docetaxel were all approved for the treatment of NSCLC. Platinum based doublet chemotherapy remained the standard systemic option from the 1990s and beyond, wherein morphologic pathological appearance of lung cancer guided treatment decisions.

In the recent years several newer agents have been tried in armamentarium against NSCLC, including Gefitinib and

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Erlotinib (tyrosine kinase inhibitors) and Pemetrexed (novel antifolate) and Bevacizumab (monoclonal antibody against vascular endothelial growth factor). The response to chemotherapy with the above mentioned regimens have been variable, with patients crossing over from one regimen to another in clinical trials showing responses, which are suggestive of the need of optimizing treatment based on the evidence-based personalized medicine.

The recent years has also seen the focus shift to targeted therapy and histology-directed therapy. The distinction between squamous and non-squamous histology was the first step in personalized treatment of patients with advanced NSCLCs. This has been shown to have importance in potential efficacy of selected agents as well as toxicity. Studies have shown adenocarcinoma histology as a predictor of response to Pemetrexed and squamous histology as a predictor of risk due to bleeding complications, after treatment using Bevacizumab [2]. There are however, several caveats to treating based on histology of the tumor alone. There can be difficulties in establishing the histological subtype based on cytology due to insufficient tumor material often resulting in NSCLC NOS (not otherwise specified). In NSCLCs, NOS represents a significant proportion of (15–30%) and may include poorly differentiated or undifferentiated tumors that have the poorest survival benefit among major NSCLC histologies from chemotherapy regimens. Moreover, distinction between squamous and non-squamous histology by hematoxyline and eosin staining alone may not always be straightforward, with studies showing inter observer variability, thus emphasizing the need for additional molecular markers for diagnostic accuracy [3].

Despite these advances, the overall 5 year survival rate for patients with NSCLC has remained at less than 15%. The median survival of patients with advanced NSCLC ranges from 9–12 months and median progression free survival (PFS) being 4–6 months. While the progress has been slow, it has been continuous. From a few months' median survival in the 1970s and 1980s, survival has slowly inched up so that most patients with good performance status diagnosed with advanced-stage lung cancer can expect to live a year or beyond. The need of the hour is a new classification system that incorporates markers, prognostic and predictive as well as molecular signatures along with the traditional patient and tumor characteristics. Classification of the lung carcinoma based on morphology as well as molecular alterations involved is the recently evolving concept influencing treatment decisions.

Introduction

The major subtypes of NSCLC have been traditionally grouped as single entity for therapy in clinical trials. Recent

molecular studies however indicate that adenocarcinomas have distinct genomic alterations allowing classification into clinically relevant molecular subsets. These molecular subsets with specific genomic changes however look morphologically similar. Such specific molecular level alterations are sometimes important for initiation and maintenance of the tumor serving as “drivers” in lung tumorigenesis.

The objective of this review is to catalogue the well characterized therapeutically relevant molecular events in NSCLCs pertaining to alterations that have been found important for “driving” lung tumorigenesis and suggesting an integrated molecular analysis to aid in treatment planning as well as planning for clinical trials. It focuses on the alterations of genes that are found to be important for tumor maintenance serving as “drivers” and causing the “oncogene addiction” in lung tumors. Driver mutations are causally implicated in oncogenesis, conferring growth advantage to cancer cell and get positively selected in the microenvironment of the cancer tissue. They are different from passenger mutations that are also found within the cancer genomes but exist as by-product of cancer cell development. Passenger mutations are often not for growth advantage, not clonally selected and occur without functional consequences occurring during cell division.

Apart from the well-known genetic alterations involving EGFR and KRAS mutations in 10–30% of lung adenocarcinomas [4, 5], there are other events such as translocations, (EML4ALK) loss/gain of function mutation/deletions (BRAF, PIK3CA AKT1, MEK), protein over expression as a result of gene amplification such as Myc (2.5–10%), Cyclin D1 (5%) [6] and HER2 over expression reported in 25% of the cases [7].

Research over the past 40 years has uncovered some of the crucial players in the EGFR signal transduction pathway which can be roughly divided into two categories: the pro-survival arm with PI3K-mTOR-AKT cascade and the proliferative arm with Ras-Raf-Mek-Erk cascade. This is suggestive of the fact that inhibitors that target different key components of this network (in combination with EGFR-TKIs) might provide greater therapeutic efficacy. This can be particularly useful in a setting where EGFR-TKI monotherapy has a consistent pattern of diminishing returns and eventually becomes ineffective. Figure 1 depicts the signal transduction pathway with the so-called “drivers” illustrating this above mentioned phenomenon. Figure 2 shows the flow chart that could aid in making therapeutic decision prior to treatment of advanced NSCLCs.

EGFR

EGFR has been previously shown to be dysregulated in NSCLCs by protein over expression, gene amplification,

Fig. 1 Signal Transduction pathways downstream of activated HER family of receptors. Two kinds of cell survival strategies that operate downstream of activated HER family of tyrosine kinases (represented as homo and hetero dimers) with the cascade of signaling events with some of the key signaling components are shown. The Ras-Raf-MEK-

ERK pathway is shown in the left and phosphatidyl inositol 3 kinase PI3K-AKT pathway is shown in the right. The key points along the pathway that can have targeted inhibition are depicted with a "star". ERK- Extracellular Regulated kinase, GRB2- Growth Factor receptor bound protein 2, mTOR- mammalian target of Rapamycin

or mutations [8]. In unselected NSCLCs, EGFR mutations are found in 10% of cases from North America, western Europe, but are reported in 30–50% of patients of East Asian descent and mostly (> 50%) associated with adenocarcinomas with bronchoalveolar features that arise among non-smokers [9–13].

EGFR as a Predictive Marker

Mutations identified in EGFR serve as the best illustration of the therapeutic relevance of molecular testing of lung tumors influencing treatment decisions. Gefitinib (Iressa; AstraZeneca) and Erlotinib (Tarceva; OSI Pharmaceuticals, Genentech) are two small-molecule drugs that specifically target the tyrosine kinase activity of EGFR-tyrosine kinase. Both drugs are reversible inhibitors of the EGFR kinase, designed to act as competitive inhibitors of ATP-binding at the active site of the EGFR kinase [14]. Clinical and

pathological features, including Asian ethnicity, female sex, adenocarcinoma histology and light/never smokers have been identified as predictors of response to an EGFR TKI. These epidemiologic and clinical factors select for a population with a molecularly distinct subset with sensitizing mutations in the tyrosine kinase domain.

The most prevalent EGFR kinase domain mutations in NSCLCs account for about 45% inframe deletions of exon 19 nested around the LREA string of amino acids located between residues 747–750 of EGFR polypeptide [15]. Another recurrent mutation is the L858R substitution in exon 21 within the activation loop of EGFR comprising of another 40–45% of EGFR mutations. Exon –18 nucleotide substitutions and exon 20 inframe insertions account for another 5% of EGFR mutations. Most noteworthy, clinically relevant mutation in exon 20 is T790M, detected in 50% of cases as a second site mutation associated with acquired resistance to Gefitinib and Erlotinib [16–18]. The incidence of EGFR mutations

Fig. 2 Schematic representation of suggested steps aiding in a therapeutic decision

in NSCLCs among clinical responders to Gefitinib or Erlotinib is 77% compared to 7% in NSCLCs that are refractory to these EGFR-TKIs [19–22]. The most notable features of these findings are that EGFR mutations strongly predict the efficacy of inhibitors of EGFR with response rates higher than 70% as shown in multiple studies [23, 24].

Phase III randomized controlled trials of EGFR TKIs in the first line setting have shown a benefit in response and progression-free survival but not in overall survival for patients with EGFR mutated NSCLC receiving EGFR TKI as the first line treatment versus platinum doublets Carboplatin and Paclitaxel [25]. On the basis of the IPASS study data and further supported by similar observations in other studies [26–28] consideration for EGFR-TKI treatment can be done only in patients with confirmed EGFR mutation by EGFR mutational testing, however, assessment of EGFR gene copy number, EGFR expression need not be routinely incorporated in management decisions. Unlike EGFR gene mutations, EGFR gene amplifications as measured by qPCR (quantitative Polymerase chain reaction) seems to be common in smoking associated tumours and does not show the same predilection towards distinct ethnicity or tumour histology [29].

New EGFR TKIs

Recent studies have evaluated a new group of irreversible EGFR TKIs namely BIBW2992 (oral, irreversible, dual

EGFR and HER2 TKI) with 94% of patients showing disease control and PFS of 12 months [30] and PF299804 (oral, irreversible, EGFR/HER2/HER3/HER4 TKI), showing a significantly higher response compared to Erlotinib [31].

KRAS

KRAS mutations in lung adenocarcinomas were shown in several studies; however, the identification of these mutations as mutually exclusive to activating EGFR mutations revitalized the interest in the signaling mechanisms of the EGFR pathway. Overall, KRAS mutations have been reported in 15–20% of all patients with NSCLC and approximately in 30–50% of patients with adenocarcinoma histology [32].

Mutant EGFR and KRAS might have overlapping and/or redundant signaling roles in NSCLC etiology [9, 33], thus explaining conspicuous absence of KRAS mutations in EGFR-TKI- responsive tumors [17]. About 15–30% of NSCLCs harbor activating mutations in codons 12 and 13 of the KRAS gene [34]. By and large, KRAS and EGFR mutations seem to be mutually exclusive in NSCLC, defining distinct subsets of tumours, with EGFR mutations being characteristic of tumors that arise in non-smokers [9], whereas KRAS mutations are more common in smoking-associated cancers and chance of having a KRAS mutation is not affected by degree of exposure to tobacco. This

mutation is known to be a persistent risk and implies that it is an early event in the process of carcinogenesis [35], however now it has recently been shown that smoking history need not always be correlated to KRAS status [36].

KRAS Mutations and Prognosis

Several studies have shown that KRAS mutations depict decreased survival and impact on prognosis is negative [37, 38]. As for the predictive aspect, KRAS mutations in NSCLC are associated with decreased response to EGFR-TKIs. KRAS mutants are shown to be associated with a larger tumor size and no median survival differences between the KRAS wild type versus the mutant. Unlike EGFR mutations, tumors with KRAS mutations scarcely respond to EGFR TKIs [39], thus proposing a mechanism of primary resistance to Gefitinib and Erlotinib. KRAS mutations are almost always found in NSCLCs with wild-type EGFR making it difficult to unequivocally decipher whether insensitivity to EGFR TKIs is due to the presence of mutated KRAS or the absence of mutated EGFR. KRAS mutated adenocarcinomas can be divided into a Bronchioalveolar carcinoma (BAC)/adenocarcinoma with bronchoalveolar features and a non-BAC group. The mutations are reported in 28%–86% of the former. The non-BAC group is the so-called classic KRAS mutated adenocarcinoma is related to smoking history and has a poor prognosis. Conversely, the BAC/adenocarcinoma with bronchioloalveolar features group has a more favorable prognosis, and the mucinous BAC/adenocarcinoma with bronchioloalveolar features group show little relationship with smoking exposure [40].

While, EGFR TKIs have no activity in almost all patients with KRAS mutation, the response of EGFR monoclonal antibody Cetuximab is less clearly impacted in NSCLC, unlike the colorectal cancer with KRAS mutation. Previous studies have shown patients treated with Vinorelbine and Cisplatin along with Cetuximab had an improved overall survival [41].

EML4-ALK Fusion

The EML4-ALK fusion protein is the one of the newest molecular targets in NSCLC [42]. ALK is a receptor tyrosine kinase, not normally expressed in the lung. It was originally identified in anaplastic large-cell lymphoma as a chimeric protein encoded by an open reading frame spanning the breakpoint of a (2;5)(p23;q35) chromosomal rearrangement. When the ALK gene fuses with another gene, it promotes lung cancer cell growth by encoding the production of a tumor-specific protein called anaplastic lymphoma kinase (ALK), an enzyme that is instrumental to cancer cell growth and development. EML4-ALK fusions result from

diverse small inversions within the short arm of chromosome 2, with at least nine different variants that have been identified so far [43–45].

Clinico Pathological Features of EML4-ALK

The frequency of EML4-ALK translocation ranges from 3%–7% in unselected NSCLC. Despite this low frequency, EML4-ALK translocated NSCLC appears to represent a distinct subset of patients. EML4-ALK and EGFR mutations are mutually exclusive. Similar to EGFR mutations, the frequency of EML4-ALK fusions is increased in people with adenocarcinomas, in young adult patients, and in people who have never smoked (<100 cigarettes in a lifetime) or who are ever smokers (≤ 15 pack-years). EML4-ALK translocations are generally found in tumors with wild type EGFR and KRAS [45].

EML4-ALK as a Therapeutic Target

Previous Studies [46] have shown a targeted inhibitor of ALK effective against advanced NSCLCs carrying activated ALK kinase. Tumors that harbored EML4-ALK fusions seemed responsive to pharmacological inhibitor of ALK-PF-02341066 (Pfizer, New York, NY, USA) a small molecule tyrosine kinase inhibitor with submicromolar activity against ALK. In this study, majority of patients had received multiple previous therapies, an overall response rate was found to be 57% (confirmed partial and complete responses) and a rate of stable disease of 33% (stable disease plus unconfirmed partial responses) in contrast to the 10% response rate in such cancers that were treated with second-line chemotherapy [47]. The rate and speed of clinical response has been claimed to be similar to those shown by EGFR tyrosine kinase inhibitors in EGFR-mutant NSCLCs suggesting the ALK-positive tumors indeed constitute a second genetically defined subgroup of oncogene-driven lung cancers that are found to be highly susceptible to targeted therapy.

Another phase I trial using Crizotinib (PF-02341066) showed high response rate in patients with adenocarcinoma with EML4-ALK translocations. Crizotinib, which is taken orally, inhibits the ALK enzyme. This study showed more than 90% of patients responding with shrinkage of their tumors [48]. Based on these results, Phase III trials comparing Crizotinib to chemotherapy is ongoing. It is noteworthy that ALK fusion positive lung tumours are resistant to treatment with the EGFR TKIs Gefitinib and Erlotinib.

Another study has described a mechanism for acquired resistance to Crizotinib in a patient with lung cancer harboring EML4-ALK fusion protein with mutations. Analysis of the patient's pleural fluid at the time of progression revealed two new mutations (C1156Y and L1196M) that were not

present in the diagnostic sample. The mutations were mutually exclusive and were both found to confer decreased sensitivity to Crizotinib in cell culture models. The L1196M mutation coincides with the ALK ‘gatekeeper’ site within the kinase domain and is analogous to the EGFR T790M mutation that occurs in approximately 50% of patients who develop acquired resistance to EGFR TKIs. This so-called “gatekeeper mutation” is critical for binding of competitive inhibitors to the ATP binding pocket of various kinases. The exact function of the C1156Y mutation in mediating drug resistance remains to be determined [49].

ERBB2 (HER2)

The frequency of HER2 mutations is increased in NSCLC patients who are women, never smokers and in patients of Asian origin, and are more frequent in adenocarcinoma than in other subtypes of NSCLC. They are reported in 2% of NSCLC [50, 51]. ERBB2/HER2 mutations involve inframe insertions in exon 20, mostly involving the amino acid sequence Tyr-Val-Met-Ala at codon 776. Inframe insertions in HER2 lead to constitutive activation of the receptor. HER2 mutations are mutually exclusive to EGFR or KRAS mutations harboring tumors.

HER2 as a Therapeutic Target

Previous studies [51] have shown cells harboring HER2 mutations undergoing constitutive phosphorylation and activation of HER2 and show resistance to EGFR TKIs. Treatment with small-molecule tyrosine kinase inhibitors that target the kinase activity of both EGFR and HER2 eg Lapatinib, tested as HKI-272, have been found to be effective. Transgenic mice models expressing the HER2 Tyr-Val-Met-Ala mutation developed lung adenosquamous carcinomas; and in these models, substantial tumor shrinkage was observed when BIBW 2992 (Boehringer Ingelheim, Germany), a tyrosine kinase inhibitor inhibiting both EGFR and HER2 was combined with inhibitor of the downstream effector protein mTOR (Sirolimus) [52]. Consistent with this finding, BIBW 2992 was shown to have promising activity in patients with lung adenocarcinoma positive for HER2 mutations [53].

B-RAF

B-RAF is a serine threonine kinase enzyme that links the cell signaling mechanism between RAS GTPases and the enzymes of the MAPK family which are known to be involved in the control of cell proliferation. Somatic B-RAF mutations have been well documented in melanomas,

with 80% mutations affecting the exon 15 (Val600Glu). In contrast to melanoma, where only one specific mutation is found (V600E), in lung cancer, there are multiple different mutations that can occur in BRAF. In NSCLC, 1–3% of adenocarcinoma shows BRAF mutations which are mostly the non Val600Glu mutations [54–56] with kinase domain mutation Leu596Val and activation domain - G loop mutation Gly468Ala. The number of patients studied to date has been small, and it appears that BRAF mutations are most likely to occur in adenocarcinomas and in former or current smokers. It also appears that BRAF mutations are not found in patients who have KRAS or EGFR mutations. In laboratory studies, cells with BRAF mutations were resistant to EGFR inhibitors. Previous studies had reported two novel alterations in BRAF namely an inframe deletion in exon 15 and K601L missense mutation in their study group [57].

BRAF as a Therapeutic Target

Some of the BRAF inhibitors are under trial like PLX4032. Sorafenib, which was originally considered as a BRAF inhibitor is in fact a multikinase inhibitor of both RAF1 and VEGF. The ESCAPE trial with Sorafenib along with Carboplatin and Paclitaxel as a first line of treatment was closed early since a superior survival outcome for Sorafenib arm could not be achieved and there was a deleterious effect of Sorafenib on the squamous histological types [58].

mTOR Kinase

Though mTOR (Mammalian target of rapamycin) is not reported or considered as a “driver” in NSCLCs, mTOR kinase is an important mediator of tumor cell growth and proliferation. In the presence of stimulation at the EGFR receptor in combination with sufficient nutrients and energy, the mTOR pathway is activated, and cell growth is initiated. It is located downstream, along the PI3K-AKT pathway where it serves as a central sensor for nutrient/energy availability [59]. mTOR is reported to be activated in >50% of lung carcinomas [60].

mTOR Kinase as a Therapeutic Target

The evaluation of the antiproliferative effects of rapamycin and its most recently discovered derivatives, cell cycle inhibitor (CCI)-779 (Temozolimus), RAD001 (Everolimus), and AP23573, in malignant neoplasms has been reported [61]. Preliminary studies done on 50 patients who were previously untreated for NSCLC, enrolled in a phase II trial of CCI-779, and reported a partial response in 4 patients (PR rate of 8%), and 15 patients with stable disease (SD rate of 30%). The median PFS time was 2.3 months and the median OS

time was 6.6 months [62]. RAD001 was evaluated in a phase II trial of patients with an ECOG performance status of two or higher who failed ≤ 2 cycles of platinum-based therapy (arm 1) vs. those who failed ≤ 2 cycles of platinum-based therapy as well as an EGFR antagonist. In this study of 74 evaluable patients, the median PFS was 11.3 weeks in arm 1 and 9.7 weeks in arm 2 [63]. An exciting phase II trial is currently underway combining mTOR and EGFR inhibition in NSCLC. There is some preclinical data suggesting synergy between Gefitinib and RAD 001 (Everolimus) [64].

PIK3CA

The region of chromosome 3q (3q25-27) where PIK3CA (3q26) is located is frequently amplified in lung cancers especially the squamous cell carcinoma. Most of the mutations have been studied in exon 9 (S541F, E542K, Q546K) and exon 20 (M1043L, H1047R, H1047L). The mutational status of PIK3CA is usually not mutually exclusive to EGFR and KRAS. Adenocarcinomas with PIK3CA gains have other gene mutations too suggesting that PIK3CA gains may not be enough for pathogenesis, however in most of the squamous cell carcinomas with PIK3CA gains, no other alterations in the genes studied indicates that PIK3CA gains may play a pivotal role in pathogenesis of lung squamous cell carcinoma.

PIK3CA as a therapeutic target

As these alterations confer a growth advantage to the cancer cells, targeting PI3K/AKT pathway is a potential therapeutic option for squamous cell carcinomas. Studies show lung cancer cell lines harboring PIK3CA activating mutations have shown sensitivity to the dual PIK3CA/mTOR inhibitor, PI-103 [65].

AKT Mutations

The AKT1 gene encodes the protein kinase B; a serine threonine kinase activated by PI3K- α and mediates the PI3K signaling. The frequency of AKT1 mutations is only 1% reported in NSCLC but they have been identified only in squamous cell carcinomas [66, 67].

AKT as a Therapeutic Target

The mutation Glu17Lys reported, occurs in the pleckstrin homology domain of AKT1 leading to PI3K independent protein kinase B activation. ATP – competitive inhibitors to protein kinase B MK2206 is being tested in the phase I trials currently.

MAP2K1 (MEK) Mutations

MAPKK1 (MEK) is a serine threonine kinase that activates MAPK2 and MAPK3 downstream of B-RAF [68]. There are three mutations reported in the non-kinase portion of the protein namely Glu56Pro, Lys57Asn, and Asp67Asn. About 1% of the NSCLCs have been reported to have MEK mutations which are mutually exclusive to EGFR, KRAS, HER2, PIK3CA and BRAF mutations.

MEK as a Therapeutic Target

The gain of function mutations displays sensitivity to the small molecule non-ATP competitive MEK inhibitor, AZD6244. This inhibitor has studied in a NSCLC trial comparing with pemetrexed when used as a second line treatment in unselected patients with a response rate of 5%.

MET Amplifications

MET gene is located on chromosome 7q21-q31 and it encodes the hepatocyte growth factor receptor (HGFR), a receptor tyrosine kinase. MET amplification is found in both squamous cell carcinoma and adenocarcinoma [69] and is believed to be independent of KRAS mutations and EGFR amplification [70, 71].

MET amplification is in fact another mechanism that contributes to the resistance to EGFR TKIs either in the presence or absence of the T790M mutation. A mechanism called “kinase switch” confers the secondary resistance to

Table 1 List of targets and their respective inhibitors and current phase of clinical trial

Target	Inhibitor	Clinical trial
EGFR	Gefitinib, Erlotinib	Approved
ErbB Family (EGFR, HER2)	Lapatinib	Phase – III
ALK	PF-02341066	Phase I, II, III
HER2	HKI – 272, BIB 2992	Phase I, II Phase I,II, III
PI3K	BEZ-2235	Phase I
AKT	MK2206	Phase I
B-RAF	Sorafenib	Phase I, II
MET	PF-02341066, GSK-1363089 XL184	Phase I,II Phase I Phase I, II
MEK	PD-325901 CI- 1040 AZD-6244	Phase I, II Phase I, II Phase I, II
Mtor	Sirolimus	Phase II

EGFR TKI by amplification of MET gene [72, 73]. MET amplification has been reported in about 20% of tumors from patients with acquired resistance to EGFR TKIs and in 21% of patients not previously treated with EGFR specific TKIs. Mutations in MET are rare in NSCLCs unlike the renal and gastric cancers. MET amplification is associated with poorer prognosis in surgically resected NSCLC [74].

MET as Therapeutic Target

Small-molecule inhibitors of HGFR developed, and have shown promise as anti-cancer therapy in phase I trials [75]. PF-02341066 (Pfizer) being developed as an ALK inhibitor also inhibits HGFR kinase activity in mutant MET cells [76]. Phase I trials are in progress with inhibitors targeted towards specific and multiple kinases that are active against HGFR like PF-02341066 [Pfizer], GSK1363089 [formerly known as XL880], and XL184 [Exelixis, San Francisco, CA, USA]. A phase II trial using AMG 102, (Amgen, Thousand Oaks, CA, USA) a fully humanized IgG2 monoclonal antibody that binds to and neutralizes HGF thus preventing its binding to HGFR is being tested in combination with chemotherapy is ongoing [77, 78].

ARQ197 (ArQule) is a non-ATP competitive agent which was highly selective of c-MET receptor after biochemical assessment in a panel of 230 kinases. This has been found promising in pre-clinical experiments, inhibiting HGF stimulation and showed decreased phosphorylation of several c-MET downstream effectors including AKT, MAPK and STAT-3. Recent studies have shown a randomized phase II trial, Erlotinib with ARQ197 compared to Erlotinib with placebo in patients with advanced NSCLC patients who had received at least one prior regimen with a primary endpoint being PFS. The combination arm showed better PFS 16.1 weeks versus the 9.7 weeks in patients who received Erlotinib alone [79]. Table 1 shows the list of above mentioned targets along with its respective inhibitors and details of the trial.

Conclusion

Molecularly targeted therapeutics can help in identifying subsets of patients likely to benefit therapy and aid in better therapeutic outcomes. Treatment of lung cancer however still remains complex and challenging. Of late genotyping for driving mutations suggests an increasingly central strategy for oncology care. Oncogenes addicted to activated kinases are highly sensitive to drugs that selectively inhibit the kinase involved. This therefore increases the motivation to develop technologies that can simultaneously determine the mutational status of several genes.

The heterogeneity of the NSCLCs has compelled the belief that mutation profiling of lung tumors, particularly beyond morphology and histopathology might have implications in lung cancer management. Along with histology predictive of the malignant behavior, molecular testing of above mentioned driver mutations can additionally help understand the downstream molecular signaling events and in turn predict the behavior of individual tumors which would ultimately aid in clinical management.

Landmark studies have shown benefits in targeting single or small number of mutations. We suggest a systematic prospective genotyping of all the lung tumors with a suitable platform to assay multiple genetic lesions at the same time. There are already many academic centers as a part of Lung Cancer Mutation Consortium which are developing and performing multiplex mutation profiling assays to aid in prospective genotyping of tumors. Following the same, concrete collaborative steps are necessary to standardize methods across institutions, to identify appropriate candidates, design appropriate trials, and execute them with adequate numbers to achieve the necessary end points. This kind of implementation would facilitate realization of the promise of personalized cancer care, with fewer side effects and better outcomes.

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