Focus Area:
RECENT ADVANCES IN LUNG CANCER

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From the Desk of Director Research

Lung cancer is a deadly scourge with a dismal prognosis and is the biggest cause of cancer deaths in many countries. The events of past decade have revolutionized the diagnosis and treatment of human cancers and have put the era of personalized medicine on even keel. The lung cancer has been in the vanguard of such efforts. The better understanding of tumor biology through the efforts of “The cancer Genome Atlas” coupled with rapid advances in platform development for identification of lung biomarkers and the rapid drug development programs to make the driver mutations actionable has made Lung Cancer archetype of personalized medicine.

This issue; dedicated to advances in lung cancer, deals with details of actionable genetic alterations beyond EGFR mutations and Alk rearrangement. ROS1 rearrangement, MET amplification, exon 14 skipping mutations in Met gene, RET rearrangement, NTRK1 fusion are all druggable in different phases of clinical trials. The acquired resistance to TKI has been a damper to the resounding success of TKIs and is actively being tackled by newest generation of TKIs. The topic has been discussed in detail by Dr Mohit Agarwal. Immunotherapy is the new and effective armamentarium available to oncologist. Several new checkpoint inhibitors engaging PD1 and PDL1 are available or in offing. Dr Sahoo will detail the role of checkpoint inhibitors in lung cancer.

The current issue features the regular articles, such as Special Feature, Guest Article, Perspective and In Focus. We are grateful to Dr P S Dattatreya, Medical Oncologist, Omega Hospitals, Hyderabad, for the ‘Special Feature’, Dr Purvesh Parikh, Executive Director of ICON Truss and Director of Precision Oncology, Asian Cancer Institute, Somaiya Hospital, Mumbai for the ‘Guest Article’ and Dr Tarini Prasad Sahoo, Director, Cancer and Haematology, Chirayu Medical College and Hospital, Medical Oncology, Bhopal, MP for the ‘Outlook’.

I hope this issue of cancer news will be a delightful study and update the reader to recent advances.

Suggestions/comments from the readers are welcome. Wishing our readers a Happy, Prosperous and Healthy New Year 2017!

Dr Anurag Mehta

CONTENTS

• Special Feature: Beyond EGFR and ALK: Molecular Heterogeneity of NSCLC [3-5]
• Guest Article: Immuno-Oncology in Lung Cancer [6]
• Outlook: Clinical Implications of Immune Checkpoint Inhibitors in NSCLC [7-10]
• Cancer Control: Lifestyle Programs to Reduce Cancer; Silica and Lung Cancer [11]
• Globe Scan: Diesel Exhaust Exposure and Mining Industry; Carcinoid Tumors of the Lung [11]
• Research & Development: High Expression of PHGDH; New Molecule for SCLC; Significance of p63; XALKORI for NSCLC [12]
• Perspective: Molecular Diagnostics in Lung Cancer [11-15]
• In Focus: The EGFR Enigma: overcoming Drug Resistance [16-19]
Introduction

Lung cancer is the leading cause of cancer deaths in males and females across the globe. Traditionally lung cancer has been classified based on the histological characteristics. Advancements in the understanding of the molecular pathways that drive the tumor growth, have led to the molecular sub-classification of lung cancer. The Lung Cancer Mutation Consortium identified presence of a driver alteration in 62% of evaluated patients e.g. KRAS (25%), sensitizing EGFR (15%), ALK rearrangements (8%), RAF (2%), HER2 (2%), PIK3CA (1%), MET amplification (1%) etc (Figure 1). Lung cancer thus is a heterogeneous disease with several biological events driving tumour growth and progression.

Figure 1

The identification of epidermal growth factor receptor (EGFR) mutations or rearrangements of the anaplastic lymphoma kinase (ALK) gene, has led to a paradigm shift and the development of specific molecular treatments for patients. Several randomized phase III trials have shown that gefitinib, erlotinib and afatinib are more effective in terms of response rate (RR) and progression free survival (PFS) and better tolerated than standard platinum-based doublet chemotherapy when given to untreated advanced NSCLC patients with tumors harbouring an activating EGFR mutation. In ALK-positive advanced NSCLC patients, crizotinib significantly improved PFS and RR and had an acceptable safety profile. In addition to EGFR and ALK there are various other molecular targets for which molecules are either available or in clinical development and will be reviewed in this article.

Novel Targets Beyond EGFR and ALK

RAS

The RAS family of oncogenes was originally identified through the study of rat sarcoma(ras), inducing retrovirus, with KRAS, NRAS, and HRAS, each representing different human gene homologs. The RAS family of proteins is a central mediator of the mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT), and phosphoinositide 3-kinase (PI3K) signaling pathways, which together control cell proliferation and apoptosis. KRAS mutations are predominantly found in non-squamous histology and in smokers and are usually mutually exclusive with EGFR mutations and ALK gene rearrangements.

The current focus of targeted therapeutics for patients with KRAS-mutated lung cancer is against downstream effectors of activated KRAS. Recently, agents targeting the Ras/Raf/MEK/ERK pathway, such as MEK inhibitors have been evaluated as a therapeutic strategy against KRAS-mutant NSCLC. In a phase II trial, 87 previously treated patients with KRAS mutant NSCLC, the addition of an oral MEK inhibitor, selumetinib, to docetaxel significantly improved progression-free survival (median 5.3 mo versus 2.1 mo, HR 0.58, 80% CI 0.42-0.79), with a trend toward increased overall survival (median 9.4 mo versus 5.2 mo, HR 0.80, 80% CI 0.56-1.14). Trametinib, another MEK1/2 inhibitor has also shown an interesting activity in KRAS-mutant patients in combination with docetaxel or pemetrexed.

ROS1

ROS1 is a receptor tyrosine kinase of the insulin receptor family that acts as a driver oncogene in 1 to 2 percent of NSCLC. Histologic and clinical features that are associated with ROS1 translocations include adenocarcinoma histology, younger patients, and never-smokers. ROS1 translocations are identified by a FISH break-apart assay similar to that used for ALK translocations. The ROS1 tyrosine kinase is highly sensitive to crizotinib. In an open label, study of crizotinib conducted in 50 patients, with ROS1 translocation, the objective response rate was 72 percent with median duration of response of 17.6 months, and the median progression-free survival of 19.2 months.
HER 2 Mutation

Mutations in HER2 have been detected in approximately 1 to 2 percent of NSCLC tumors, usually involving small in-frame insertions in exon 20. These tumors are predominantly adenocarcinomas, are more prevalent among never-smokers, and a majority of these patients are women. Current evidence suggests that patients with tumors harboring HER2 insertions respond to trastuzumab or afatinib.

BRAF Mutation

BRAF is a downstream signaling mediator of KRAS which activates the MAP kinase pathway. BRAF mutations have been observed in 1 to 3 percent of NSCLC and are usually associated with a history of smoking. Activating BRAF mutations can occur either at the V600 position of exon 15 or outside this domain. BRAF inhibition with vemurafenib or dabrafenib appears to be an effective strategy in the treatment of progressive BRAF V600-mutant NSCLC. In phase II trials of dabrafenib and vemurafenib done in previously treated patients, the objective response rates were 32% and 42% respectively. Combination therapies with BRAF inhibitors are also a promising approach. In a recent study of 59 patients with previously treated, advanced NSCLC with the V600E mutation, the combination of dabrafenib plus trametinib was associated with an objective response rate of 63 percent in 57 evaluable patients, and the disease control rate was 79 percent.

MET Abnormalities

MET is a tyrosine kinase receptor for hepatocyte growth factor (HGF). Abnormalities associated with MET include overexpression due to gene amplification and exon 14 skipping mutations. MET gene amplification identified by FISH, occurs in approximately 6 percent of patients with NSCLC and appears to be smoking related. Crizotinib is a potent MET inhibitor. In a highly selected group of 12 patients with intermediate or high MET gene amplification crizotinib demonstrated responses in 5 patients and stable disease in 5 additional, which were unusually prolonged in MET-high patients. Since MET amplification can also contribute to acquired resistance to EGFR TKI therapy, combinations of MET inhibitors are being investigated in this patient population as well.

RET Translocation

The RET gene encodes a cell surface tyrosine kinase receptor that is frequently altered in medullary thyroid cancer. Recurrent translocations between RET and the various fusion partners (CCDC6, KIF5B, NCOA4) have been identified in 1 to 2 percent of patients with adenocarcinoma or adenosquamous carcinoma of the lung. These patients are usually young and nonsmokers. In an interim analysis of a phase II trial of cabozantinib in 16 patients with RET translocation, the ORR was 38 percent with median PFS of seven months and the median OS of 10 months.

PIK3CA Alterations

PIK3CA encodes the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), which is an intracellular central mediator of cell survival signals. AKT1 acts immediately downstream of PI3K. PTEN inhibits AKT by dephosphorylation. Oncogenic alterations in this pathway include gain-of-function mutations in PIK3CA and AKT1, and loss of PTEN function. Alterations in the PI3K signaling pathway appear more frequently in tumors of squamous histology and smokers. PIK3CA mutations also may promote resistance to EGFR TKIs in EGFR-mutant NSCLC. Currently PIK3CA inhibitors are under clinical development.

FGFR1 Amplification

Fibroblast growth factor receptor-1 (FGFR1) is a cell surface tyrosine kinase receptor that mediates cell survival and proliferation. Gene amplification of FGFR1 has been detected in 13 to 25 percent of squamous tumors and is associated with smoking and worst survival. Small molecule inhibitors of FGFR1 are in clinical development. In a phase I study of the FGFR small molecule TKI BGJ398 which included patients with FGFR1-amplified squamous cell carcinoma of the lung 4 partial responses were seen.

Conclusions and Future Directions

Identification of novel molecular targets has improved our understanding of the biology of lung cancer and highlight the importance of an individualized therapeutic approach based on the molecular profile of each tumor. Comprehensive genomic analysis of the tumor specimen to identify the druggable targets should be carried out in order to identify the patients who are amenable to targeted therapy. For patients without access to a targeted clinical trial, some approved therapies may be used “off label” with anticipated benefit for some patients, e.g. crizotinib for ROS1 and MET, afatinib and trastuzumab for Her2 mutations etc. Another challenge with the growing number of targeted therapies is acquired resistance. The concept of repeated biopsies and use of liquid biopsies at the time of tumor progression to investigate the specific resistance mechanism offers a promising approach.
References


IMMUNO-ONCOLOGY IN LUNG CANCER

Lung cancer is the commonest cancer in India as well as globally. Earlier patients had to face a challenging situation and mortality was high, as 90% of patients present at a stage when only palliative (non-curative) treatment was possible.

Over the last decade the outcome has changed dramatically, thanks to three advances. The first was a better delineation of the molecular changes that were driving the lung cancer. The second was the availability of targeted agents that specifically acted on patients populations enriched by use of specific biomarkers. And the third (and most recent) advance was immuno-oncology. No wonder it has been called as the pathbreaking advance for the year 2015 and beyond.

The immune system is supposed to protect our body from external threats as well as changes (mutations) that lead to significant damage to normal cells from within. Cancer is a manifestation of the failure of this immune system.

Better understanding of the changes that lead to this deficiency as well as insights into tumor-T cell interaction paved the way for the immuno-oncology revolution. Focus on PD-1, PD-L1 and CTLA3 pathways resulted in several new therapeutic agents being developed, evaluated in clinical trials, and given regulatory approval.

In fact the benefit in lung cancer was so dramatic that fast-track approval was awarded on promising phase II data. The reason for this was manifold. There was a new class of agents that worked in heavily pretreated patients. They resulted in a response in a significant number of patients, and often sustained much after the treatment administration was discontinued. The quality of life was also significantly better than the control group (with chemotherapy). No wonder, patients and oncologists were eagerly waiting for their availability in India.

In the palliative setting, what is the ultimate benefit that patients with lung cancer should expect? A quarter of patients can expect to be alive with good quality of life, two years after the initiation of immunotherapy! And with the survival curve showing a pleateau, there is potential for survival beyond 5 years as well, a hitherto imposibility in metastatic lung cancer.

Today about 100+ patients have already been treated with Nivolumab, and the other molecules are not far behind. Benefit of immuno-therapy has now become a reality for our patients in India.

While we remain enthusiastic, a few words of caution are necessary.

A new class of molecules always carries the baggage of new problems. This is especially true when the immune system is being tweaked. Caution is to be advocated regarding auto-immunity as well as infections. Patients need to be extra vigilant about following the instructions of their doctors sincerely and diligently. Any change in their health should not be taken lightly. Rather, they should promptly report to the hospital at the slightest of cough, diarhoea or breathlessness. Precautionary measures recommended must also be followed with care.

So far we do not understand why only a small fraction of patients benefit from immuno-oncology. Nor do we know why the benefit is evident only after a delay/gap-the survival curves separating only after the median survival is reached. Currently available biomarker, PD-L1 testing provides conflicting results. A better way of identifying patient most likely to respond is sorely required.

The manner in which response is evaluated also needs modification. As the immune system is mobilized and lymphocytes start infiltrating the tumor, there could actually be an increase in size as measured on imaging (CT or PET scan - pseudo-progression).

As more data is accumulated and we gain firsthand experience in the use of immuno-oncology molecules, lung cancer patients stand to get increasing benefit. Such insights will become increasingly available in the following months and we look forward to the same.

(Dr Purvesh Parikh, Executive Director of ICON Truss and Director of Precision Oncology, Asian Cancer Institute, Somaiya Hospital, Mumbai)
CLINICAL IMPLICATIONS OF IMMUNE CHECKPOINT INHIBITORS IN NSCLC

Abstract

Historically, lung cancer was long considered a poorly immunogenic malignancy. In recent years, however, immune checkpoint inhibitors have emerged as promising therapeutic agents in non-small cell lung cancer (NSCLC). To date, the best characterized and most therapeutically relevant immune checkpoints have been cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), and the programmed cell death protein-1 (PD-1) pathway. In early studies, PD-1/programmed cell death ligand-1 (PD-L1) inhibitors demonstrated promising antitumor activity and durable clinical responses in a subset of patients. Based on these encouraging results, multiple different PD-1/PD-L1 inhibitors have entered clinical development, and two agents (nivolumab and pembrolizumab) have gained regulatory approval in the United States for the treatment of NSCLC. In several large, randomized studies, PD-1/PD-L1 inhibitors have produced significant improvements in overall survival compared with single-agent docetaxel delivered in the second-line setting, effectively establishing a new standard of care in NSCLC.

Introduction

The earliest attempts at cancer immunotherapy can be dated back to late 19th century, widely credited to William Coley, a New York surgeon. Inspired by reports of rare spontaneous tumour regressions in sarcoma patients developing erysipelas, Coley began performing intratumoral injections of live or inactivated Streptococcus pyogenes and Serratia marcescens in patients with inoperable malignancies. They were intended to stimulate the body’s “resisting powers” and kill bystander tumour cells. However, as our collective understanding of cancer immunology has evolved, more promising forms of immunotherapy have emerged. In particular, strategies targeting negative regulators (i.e., checkpoints) of the immune system have demonstrated significant antitumor activity across a range of solid tumours, including non-small cell lung cancer (NSCLC). In recent years, checkpoint inhibitors targeting the programmed cell death protein-1 (PD-1)/programmed cell death ligand-1 (PD-L1) axis have shown significant antitumor activity in NSCLC. This article intends to provide an overview of the rationale for checkpoint inhibitors in cancer immunotherapy with a focus on NSCLC. It also details the recent landmark studies that led to regulatory approval of the PD-1 inhibitors nivolumab and pembrolizumab.

Immune Checkpoints in Cancer

The immune system has long been thought to play an important role in the surveillance and rejection of malignancies. Cancer cells commonly possess genetic and/or epigenetic alterations that can lead to the generation of neoantigens, which can be recognized as “non-self” by the host immune system. However, such responses can be limited by multiple mechanisms of immune suppression that render antitumor immunity ineffective. To date, various mechanisms have been proposed, including:

(a) Down regulation of antigen-presenting machinery;
(b) Immunoediting (i.e. T-cell recognition of tumour specific antigens leads to outgrowth of clones lacking immuno dominant antigens);
(c) Induction of self-tolerance (i.e., tumour specific T cells are unable to kill antigen-expressing tumour cells), and
(d) Upregulation of immune checkpoints in the tumour microenvironment.

Recent cancer immunotherapy efforts have focused on immune checkpoints. T-cell activation is a tightly regulated process that involves a balance between co-stimulatory and co-inhibitory signals. Co-inhibitory signals (i.e., immune checkpoints) serve to maintain self-tolerance and avoid destruction of normal host tissue. However, such signaling interactions can be co-opted by tumours, facilitating immune escape. This vulnerability has formed the basis for the development of therapeutic monoclonal antibodies targeting immune checkpoints. Ultimately, immune checkpoint inhibitors target the “brakes” on the immune system, with the goal of inducing immune cell proliferation and activation against cancer cells. To date, the best characterized and most therapeutically relevant immune checkpoints are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein-1.

CTLA-4

Under normal conditions, two immunologic signals are required for T-cell activation:

(a) Engagement of major histocompatibility complex bound antigen on antigen-presenting cells (APCs) by the T-cell receptor (TCR), and
(b) Co-stimulation via B7-CD28 interactions.
The first signal generates specificity, and the latter amplifies TCR signaling, leading to T-cell activation. T-cell activation also induces a parallel, inhibitory pathway mediated by CTLA-4 that can attenuate and terminate such responses. CTLA-4 is a CD28 homolog that is expressed exclusively on T cells. CTLA-4 leads to downregulation of T-cell responses through several mechanisms. The importance of CTLA-4 as a negative regulator of T-cell responses is highlighted by CTLA-4 knockout mice, which display a fatal phenotype of widespread lymphoproliferation and immune hyperactivation. Preclinical studies demonstrated that anti-CTLA-4 antibodies had a therapeutic window.

**PD-1/PD-L1 AXIS**

PD-1, like CTLA-4, is an immune checkpoint that has emerged as an important therapeutic target. PD-1 is expressed on the surface of activated T cells, B cells, and natural killer cells. Interaction of PD-1 with one of its two known ligands, PD-L1 and PD-L2, leads to disruption of intracellular signaling and downregulation of effector T-cell function. PD-L2 is predominantly expressed on APCs, and PD-L1 can be expressed on various cell types, including T cells, epithelial cells, and endothelial cells. PD-L1 expression can also be upregulated on tumor cells and other cells in the local tumor environment. PD-L1 expression has been reported across a range of malignancies, including NSCLC. Although CTLA-4 predominantly functions more proximally at the stage of initial T-cell activation, PD-1 is thought to regulate effector T-cell function within tissues and tumors.

**Therapeutic Targeting of Immune Checkpoints in Lung Cancer**

**CTLA-4 BLOCKADE**

CTLA-4 was the first immune checkpoint targeted therapeutically. In advanced melanoma, the CTLA-4 antagonist ipilimumab produced improvements in overall survival (OS) in two large phase III trials, culminating in regulatory approval by the US Food and Drug Administration (FDA) in 2011. Despite this activity in melanoma, CTLA-4 antagonists have shown minimal single-agent activity in NSCLC. More recently, however, ipilimumab has shown more promising results when combined with cytotoxic chemotherapy in NSCLC. In a phase II trial conducted by Lynch et al., patients with treatment-naive, advanced NSCLC were randomized to receive carboplatin/paclitaxel with or without ipilimumab. Two different dosing schemes of ipilimumab concurrent ipilimumab or phased ipilimumab were used. Patients receiving concurrent ipilimumab were treated with four cycles of ipilimumab and carboplatin/paclitaxel, followed by two cycles of carboplatin/paclitaxel alone. In contrast, patients in the phased arm received two cycles of carboplatin/paclitaxel alone, followed by four cycles of carboplatin/paclitaxel and ipilimumab. Using a primary endpoint of immune-related progression free survival (irPFS), Lynch et al. observed no difference in irPFS between the concurrent ipilimumab and control arms (hazard ratio [HR], 0.81; p = .13); however, phased ipilimumab significantly improved irPFS compared with the control (median, 5.7 months vs. 4.6 months, respectively; HR, 0.72). These results prompted the initiation of a phase III trial evaluating the combination of carboplatin/paclitaxel plus phased ipilimumab in previously untreated patients with squamous histology. In addition to this approach, significantly more enthusiasm has surrounded the use of CTLA-4 antagonists (e.g., ipilimumab, tremelimumab) in combination with other immunotherapies, most notably PD-1/PD-L1 inhibitors.

**PD-1 Inhibitors**

Two classes of antibodies targeting the PD-1/PD-L1 axis have entered clinical development: PD-1 inhibitors and PD-L1 inhibitors. The former agents target the PD-1 receptor on activated immune cells, blocking its interaction with two ligands, PD-L1 and PD-L2. In contrast, PD-L1 inhibitors block the interaction between PD-L1 and PD-1 and the interaction between PD-L1 and B7.1 (an inhibitory receptor on T cells).

**Nivolumab**: Nivolumab is a fully human immunoglobulin G4 (IgG4) PD-1 inhibitor. In a pivotal phase I study of nivolumab, 122 patients with heavily pretreated advanced NSCLC (more than 55% having received three or more lines of previous therapy) received nivolumab at doses of 1–10 mg/kg once every 2 weeks. Common adverse events (AEs) included fatigue, decreased appetite, and diarrhea. The objective response rate (ORR) was 17% across all dose levels. Moreover, the median duration of response (DOR) with nivolumab was impressive at 17 months, suggesting that PD-1 inhibition might generate more durable responses compared with those seen with conventional therapies. More recently, two large phase III trials of nivolumab in NSCLC have reshaped the therapeutic landscape of the disease: CheckMate 017 and CheckMate 057. CheckMate 017 was a phase III, randomized trial for patients with advanced, squamous NSCLC and disease progression during or after first-line, platinum-based chemotherapy. The study enrolled 272 patients, randomizing subjects to treatment with either nivolumab or docetaxel. (The primary endpoint being overall survival).
In January 2015, an independent data and safety monitoring committee (DSMC) recommended early termination of the study because a prespecified interim analysis demonstrated that the primary endpoint had been met. Specifically, nivolumab produced a significant improvement in OS compared with docetaxel (median, 9.2 vs. 6.0 months, respectively; HR, 0.59; 95% confidence interval [CI], 0.44–0.79; p < .001). Secondary endpoints included ORR and PFS, both of which favored the nivolumab arm. The ORR was 20% among patients receiving nivolumab versus 9% for those receiving docetaxel. The corresponding median PFS was 3.5 months versus 2.8 months (HR, 0.62; 95% CI, 0.47–0.81; p < .001). Also, treatment-related AEs occurred less frequently in the nivolumab arm than in the docetaxel arm. Grade 3/4 AEs were seen in only 7% of patients in the nivolumab group compared with 55% in the docetaxel group. Nonetheless, collectively, these data helped form the basis for the regulatory approval of nivolumab for previously treated squamous NSCLC.

Shortly after the report of the CheckMate 017 study, findings from a companion study, CheckMate 057, were presented. The latter was a randomized, international phase III study that enrolled patients with nonsquamous NSCLC with progression during or after platinum-based chemotherapy. In total, 582 patients were randomized to receive either nivolumab or docetaxel and again the primary endpoint being OS. Based on an interim analysis (minimum OS follow-up of 13.2 months), an independent DSMC declared that OS among patients receiving nivolumab was superior to that of patients receiving docetaxel. Specifically, among patients receiving nivolumab, the median OS was 12.2 months (95% CI, 9.7–15.0) versus 9.4 months (95% CI, 8.0–10.7) in the docetaxel group (HR, 0.73; 95% CI, 0.59–0.89). Based on these data, the US FDA expanded the approved use of nivolumab in October 2015 to include patients with advanced NSCLC whose disease had progressed during or after platinum-based chemotherapy.

In the CheckMate study, PD-L1 biomarker analyses were incorporated into the study design; however, these assessments were performed retrospectively and did not factor in study eligibility. As use of PD-L1 testing has expanded, it has become clear that PD-L1 immunohistochemistry is imperfect, because responses have been observed among both PD-L1-positive and -negative patients. In CheckMate 017, nivolumab produced a survival benefit independent of PD-L1 expression. Recently, the FDA approved the Dako28-8 PD-L1 assay as a complementary diagnostic test for nivolumab for patients with nonsquamous NSCLC. Of note, complementary biomarkers are distinct from companion diagnostics. Complementary biomarkers provide additional information regarding who is most likely to benefit from a given drug, but they are not required for use. In contrast, companion diagnostics are considered essential for the safe and effective use of a drug.

**Pembrolizumab**

Pembrolizumab is a humanized, IgG4 monoclonal antibody directed against PD-1. The safety and activity of pembrolizumab were initially evaluated in KEYNOTE-001, a large phase I study that enrolled 495 subjects with previously treated and untreated NSCLC. Patients received pembrolizumab at doses of either 2 or 10 mg/kg every 3 weeks or 10 mg/kg every 2 weeks. Common treatment-related AEs were fatigue (19.4%), pruritus (10.7%), and decreased appetite (10.5%). In general, immune-mediated events were relatively infrequent. For all NSCLC patients in that study, the ORR was 19.4%. No difference was found in efficacy according to the dose, schedule, or histologic type.

Moreover, just as with other PD-1/PD-L1 inhibitors, the responses were often durable. At the time of reporting, 84.4% of responders had no evidence of disease progression (median DOR, 12.5 months; range, 1.0–23.3). In contrast to CheckMate 017 and 057, KEYNOTE-001 sought to prospectively define and validate PD-L1 expression as a predictive biomarker for pembrolizumab. On the basis of the analysis of the biopsy samples, membranous PD-L1 expression on 50% or more tumor cells (proportion score, >50%) was selected as the PD-L1 cut off for the remainder of the trial. In total, 23.2% of patients had a proportion score (PS) of >50%. At that cut point, the ORRs with pembrolizumab were 36.6% and 45.2% in the training and validation cohorts, respectively. The median PFS among all patients with a PS of at least 50% was 6.3 months (95% CI, 2.9–12.5). Given this encouraging activity, pembrolizumab was granted accelerated approval by the US FDA in October 2015. This was accompanied by approval of the 22C3 monoclonal antibody as a companion diagnostic for determining PD-L1 expression. More recently, pembrolizumab was evaluated in an international phase II/III trial (KEYNOTE-010) that enrolled patients with previously treated NSCLC. Altogether, 1,034 subjects were enrolled and randomized to receive pembrolizumab 2 or 10 mg/kg or docetaxel 75 mg/m² every 3 weeks. The study had coprimary endpoints of OS and PFS in the total study population and those with high PD-L1 expression (PS >50%). In the total study population, both pembrolizumab arms had significantly improved OS compared with the
docetaxel arm. The median OS was 10.4 months for patients receiving pembrolizumab 2 mg/kg (HR, 0.71 vs docetaxel; 95%CI, 0.58-0.88), 12.7 months for those receiving pembrolizumab 10mg/kg (HR, 0.61 vs docetaxel; 95% CI, 0.49–0.75; p <0.001), and 8.5 months for patients receiving docetaxel. Among the patients with high PD-L1 expression treated with either dose of pembrolizumab, OS was also significantly improved compared with docetaxel. Ultimately, the KEYNOTE studies, together with CheckMate 017 and 057, have established PD-1 pathway blockade as a new standard of care in the management of previously treated, advanced NSCLC.

**Other PD-L1 Inhibitors**

Encouraging data have emerged from atezolizumab and Durvalumab among few others. The most promising data has come from atezolizumab, in the form of a phase II randomized study in NSCLC. The POPLAR study enrolled 287 patients with previously treated NSCLC, randomizing patients to receive either atezolizumab or docetaxel. Patients were stratified by PD-L1 status, histologic type, and previous lines of therapy. The primary endpoint was OS in the intention-to-treat population and PD-L1 subgroups. In the intention-to-treat population, atezolizumab significantly improved OS compared with chemotherapy (median, 12.6 vs.9.7 months; HR, 0.73). PD-L1 expression on tumor cells or tumor-infiltrating immune cells was associated with an OS benefit. A phase III trial of atezolizumab (OAK study) is now ongoing in a similar patient population. Furthermore, atezolizumab was recently granted break through therapy designation by the FDA for the management of previously treated, advanced NSCLC patients whose tumors expressed PD-L1.

**Checkpoint Inhibitor Combinations**

Although PD-1/PD-L1 inhibitors have dramatically transformed the management of NSCLC, most patients do not respond to therapy. Consequently, focus has been placed on identifying novel treatment combinations that might increase the ORRs, generally using PD-1 inhibitors as a therapeutic foundation. Currently, various strategies are being pursued, including PD-1/PD-L1 inhibitors combined with other checkpoint inhibitors (e.g., CTLA-4, LAG-3, TIM-3), costimulatory checkpoints (e.g.,OX40, GITR,4-1BB), other immunomodulatory molecules (e.g., indoleamine 2,3-dioxygenase [IDO]), chemotherapy, vaccines, and radiation.

**Predictive Biomarkers of Response**

Despite the activity of PD-1/PD-L1 inhibitors in NSCLC, only <20% of patients ultimately respond to therapy, underscoring the critical need for predictive biomarkers. As detailed, immunohistochemical assessments of PD-L1 expression have been the most thoroughly studied to date. In general, PD-L1 expression has been associated with higher ORRs (range, 23%-83%) to PD-1/PD-L1 inhibitors, but responses have also been observed among PD-L1-negative patients (ORRs, 9%-20%). PD-L1 assays have been further complicated by a lack of standardization in testing methods across agents. Each PD-1/PD-L1 inhibitor in clinical development has used different anti-PD-L1 antibodies, different scoring cutoffs, and various scoring algorithms. Given this lack of a reference standard for PD-L1 testing, efforts are now ongoing to harmonize various PD-L1 assays. Some early studies have shown that tobacco exposure was associated with higher ORRs to PD-1/ PD-L1 inhibitors. In addition, the presence of EGFR (epidermal growth factor receptor) mutations and ALK (anaplastic lymphoma kinase) rearrangements (alterations typically associated with a lack of tobacco exposure) have been associated with lower ORRs to PD-1 inhibitors.

**Conclusions**

Agents targeting the PD-1/PD-L1 axis have transformed the management of NSCLC and emerged as a new standard of care for previously treated, advanced NSCLC. Nonetheless, a number of challenges remain. Currently, PD-1/PD-L1 inhibitors are being explored in several other lung cancer settings. Clinical trials evaluating PD-1 pathway blockade as neoadjuvant/adjuvant therapy, consolidation therapy after definitive chemoradiation, and in small cell lung cancer are now ongoing and/or planned. However, the greatest focus to date has been in transitioning PD-1/PD-L1 inhibitors to the first-line setting. Furthermore, given the sheer number of possible immunotherapy combinations in this space, novel trial designs will be necessary to identify the most promising combinations in a timely manner.

(Dr Tarini Prasad Sahoo, Director, Cancer and Haematology, Chirayu Medical College and Hospital, Medical Oncology, Bhopal, MP)
Diesel Exhaust Exposure and Mining Industry

EC exposure has been monitored in Western Australian miners since 2003. On the basis of published risk functions, the authors estimated excess lifetime risk of lung cancer mortality for several employment scenarios. Personal EC measurements (n=8614) were available for 146 different jobs at 124 mine sites. The mean estimated EC exposure level for surface occupations in 2011 was 14 µg/m3 for 12 hour shifts. Levels for underground occupation groups ranged from 18 to 44 µg/m3. Underground diesel loader operators had the highest job-specific exposed: 59 µg/m3. A lifetime career (45 years) as a surface worker or underground miner, experiencing exposure levels as estimated for 2011 (14 and 44 µg/m3 EC), was associated with 5.5 and 38 extra lung cancer deaths per 1000 males, respectively. Hence, EC exposure levels in the contemporary Australian mining industry are still substantial, particularly for underground workers. The estimated excess numbers of lung cancer deaths associated with these exposures support the need for implementation of stringent occupational exposure limits for diesel exhaust.


Carcinoid Tumors of the Lung

Carcinoid tumors of the lung are the tumors originating from the neuroendocrine cells. Surgical excision remains the gold standard for the treatment. Treatment with interventional bronchoscopic excision has also been reported as an alternative option in typical carcinoid tumors of the lung. Data of 14 patients, who had undergone bronchoscopic excision due to typical carcinoid tumor of the lung between April 2008 and July 2015, were retrospectively evaluated. Bronchoscopic excision procedures were performed under general anesthesia, while control bronchoscopies were carried out with flexible bronchoscopy. Time between the first and last bronchoscopies was accepted as the follow-up duration. A total of 14 patients was evaluated with eight (57.1%) males. The most common symptoms were shortness of breath and coughing. Mean of $5.69\pm3.35$ (2-12) bronchoscopy procedures were performed in the patients during the diagnosis, treatment and follow-up. Mean follow-up duration was $32.0\pm19.22$ months. None of the patients developed recurrence during the mean.

Turkey: Med Glas (Zenica), Feb 1, 2016

Silica and Lung Cancer

A new report, highlights the link between silica (one of the commonest minerals on earth and a major ingredient in sand, granite, soil and glass) and lung cancer. They reported found a “strong and consistent evidence that silica exposure increases lung cancer risk.” They have shown, through this meta-analysis that the risk of lung cancer is higher in workers exposed to crystalline silica dust or workers working in industry. First, exposure to crystalline silica impairs alveolar-macrophage-mediated particle clearance, thereby increasing persistence of silica in the lungs, which results in macrophage activation, and the sustained release of chemokines and cytokines. Second, extracellular generation of free radicals by crystalline silica depletes antioxidants in the lung-lining fluid. Third, crystalline silica particles are taken up by epithelial cells followed by intracellular generation of free radicals that directly induce genotoxicity.

BMC Public Health, Nov 4, 2016
High Expression of PHGDH

Tumors have exceptionally high demands for energy and anabolism because of their rapid growth. The de novo serine synthesis pathway initiated by phosphoglycerate dehydrogenase (PHGDH) has been recognized as a hallmark of metabolic adaptation in carcinogenesis. Due to the importance of PHGDH in cancer, the authors attempted to determine the clinical significance of PHGDH in 319 patients with non-small cell lung cancer (NSCLC). They evaluated the mRNA and protein expression levels of PHGDH gene, using quantitative reverse transcriptase polymerase chain reaction and tissue array-based immunohistochemistry, respectively. Significantly increased PHGDH expression in mRNA and protein levels was identified in tumor tissues versus matched adjacent nontumor tissues. More interestingly, immunohistochemical expression of PHGDH was significantly associated with lymph node metastasis ($P=.021$) and TNM stage ($P=.016$). Multivariate survival analysis using Cox regression model demonstrated that high PHGDH levels and advanced TNM stage (III+IV) were independent predictors of prognosis in NSCLC. In conclusion, this study suggested the clinical implication of PHGDH in NSCLC. PHGDH may be a promising therapeutic target in NSCLC.

Transl Oncol, Dec 9, 2016

New Molecule for SCLC

As a major cause of treatment failure, therapeutic tumor resistance, whether innate or acquired, is mediated by multifactorial compensatory and adaptive events, and following the known principle of “fighting fire with fire”, multifactorial inactivation or inhibition is likely required to bypass or reverse it. These multiple events, too numerous to list in full here, lead to an insidious drug-resistant state, not only to one particular therapeutic regimen per se but also to related ones in a phenomenon known as cross- or pan-resistance, resulting in an ever-increasing uphill battle for the treating oncologist. Such pleiotropy is an argument in favor of the use of combination therapy. An alternative to combination therapy is a multitargeted single agent, such as RRx-001. RRx-001 is a systemically nontoxic reactive oxygen species-mediated epi-immunotherapeutic agent, acting on DNA methyltransferases and histone deacetlylases with vascular normalizing properties that multifactorially reverse the tumor cell resistance and chemo-immuno-radiosensitize the cancer cells. Two of the key RRx-001 antiresistance mechanisms are epigenetic inhibition and immune stimulation; however, a hallmark of any successful sensitizer is target promiscuity, and RRx-001, which is no exception, also possesses prooxidant, apoptotic, antiangiogenic, and P-gp inhibitory properties.

Clin Med Insights Oncol, Nov 6, 2016

Significance of p63

Genomic abnormalities and specifically the amplification of chromosomal region 3q26-3qter in lung cancer represent a major signature of neoplastic transformation. Here, the authors address the significance of p53 homologue p63 mapping to 3q27 in lung tumorigenesis. They analyzed p63 gene copy number (CN) by fluorescence in situ hybridization and expression by immunohistochemistry in tissue microarrays of 217 non-small cell lung cancers (NSCLCs) and correlated them with survival. The p63 genomic sequence was amplified in 88% of squamous carcinomas, in 42% of large cell carcinomas, and in 11% of adenocarcinomas of the lung. Furthermore, p63 genomic amplification and protein staining intensity associated with better survival. These observations suggest that p63 genomic amplification has an early role in lung tumorigenesis and deserves additional evaluation as a biomarker for lung cancer progression.

BMC Cancer, Sept, 2016

XALKORI for NSCLC

XALKORI (Erlotinib) is indicated for the treatment of patients with metastatic NSCLC whose tumors are ROS1-positive as detected by an FDA-approved test. About 4% of patients with non-small cell lung carcinoma have a chromosomal rearrangement that generates a fusion gene between EML4 (‘echinoderm microtubule-associated protein-like 4’) and ALK (‘anaplastic lymphoma kinase’), which results in constitutive kinase activity that contributes to carcinogenesis and seems to drive the malignant phenotype. The kinase activity of the fusion protein is inhibited by crizotinib. Crizotinib inhibits the c-Met/Hepatocyte growth factor receptor (HGFR) tyrosine kinase, which is involved in the onogenesis of a number of other histological forms of malignant neoplasms. Crizotinib caused tumors to shrink or stabilize in 90% of 82 patients carrying the ALK fusion gene. The recommended dose of XALKORI is 250 mg orally, twice daily until disease progression or no longer tolerated by the patient.
MOLECULAR DIAGNOSTICS IN LUNG CANCER

Ir RECIST: Ready for Prime Time?

There has been a paradigm shift in oncology drug development in recent years with the increased use of immunotherapeutic agents for cancer treatment these days. The basic problem accompanying such paradigm shifts is how to build research strategies to incorporate action of these newer compounds. The patterns of response created by immunotherapy being different from those of chemotherapy are not captured by the traditional World Health Organisation (WHO) tumour response criteria or the RECIST. Hence, developing immunotherapy in oncology requires us to address the unique characteristics of immunotherapeutic agents and to provide adequate tools for their evaluation, including the adjustment of clinical trial endpoints.

The Immune-Related Response Criteria (irRC) is a set of published rules that define when tumors in cancer patients improve (respond), stay the same (stabilize), or worsen (progress) during treatment, where the compound being evaluated is an immuno-oncology drug.

Cytotoxic Vs Immunotherapeutic Agents

RECIST for determining tumor response is applicable to cytotoxic agents. These agents directly kill a tumor cell or prevent tumor cells from dividing (e.g. chemotherapy). Therefore, response of cytotoxic agents can be easily measured from the start of therapy. Early increase in tumor burden and/or an early increase in tumor size signifies progressive disease and once progression is detected, drug cessation is recommended. So response after initial treatment of a cytotoxic agent can often predict remission and survival. Immuno-oncology agents differ from cytotoxic agents in that they stimulate an innate immune response against the tumor:

- Vaccines: trigger the immune system to initiate an anti-tumor response against an existing cancer
- Monoclonal Antibodies: antibodies directed against tumor cells; they can block signaling pathways needed for tumor growth and trigger an immune-mediated cytotoxic response
- Checkpoint inhibitors: tumors escape detection by the immune system through expression of “checkpoint” proteins on their cell surface. CTLA-4 and PD-1 receptors are examples of “checkpoint” receptors; targeted inhibition towards these receptors enhances T cell response towards the tumor
- Cytokines: stimulates a broad-based immune response (e.g. interleukin-2 and interferon-α) [1]

Need of Newer Response Criteria for Immunotherapeutic Agents

In 1962, a physicist introduced a concept that changed the way we perceive science. In his book 'The Structure of Scientific Revolutions', Thomas Kuhn postulated that science does not progress in a continuous linear way but instead undergoes periodic revolutions, which he named ‘paradigm shifts’ [2]. In recent years, with the rise of immunotherapeutic agents for cancer treatment, we have observed a paradigm shift in oncology drug development.

One common problem accompanying such paradigm shifts is how to build research strategies to fit the mechanism of action of the newer compounds. Standard drug development models and evaluation scales were created for, and well suited to, cytotoxic drugs. These drugs act by killing cancerous cells, and if effective, can cause fast reduction in tumour size. The problem is that this is not the mechanism of action of many innovative drugs currently in development, including immunologic agents [2]. As stated by Hoos and colleagues, developing immunotherapy in oncology requires us to address the unique characteristics of immunotherapeutic agents and to provide adequate tools for their evaluation, including the adjustment of clinical trial endpoints [3].

The in vivo effects of immunotherapies can be divided conceptually into three phases: after a drug administration, T-cell proliferation and immune activation is observed. There upon over a period of weeks or months, the clinical effect of a drug can be measured by reduction in tumour size and improvement in patient’s performance. Finally, this can give rise to delayed effect on a patient's survival, i.e. several months following the drug administration. Immunotherapy creates patterns of response different from those of chemotherapy [3], and thus they are not captured by the traditional World Health Organisation (WHO) tumour response criteria or the RECIST (Response evaluation criteria in solid tumours) [4].
To address the need for new tools to evaluate the clinical activity of immunotherapeutic agents, a consortium of approximately 200 oncologists, immunotherapists, and regulatory experts joined forces in 2004 and 2005. This led to the development of the immune-related response criteria (irRC) [5] aiming at capturing patterns of tumour response beyond those seen with cytotoxic agents. These assess tumour burden as a continuous variable, allowing the evaluation of percentage changes in several target lesions overtime. Hence by doing so, they capture the growth kinetics of the total measurable tumour burden.

<table>
<thead>
<tr>
<th>New, measurable lesions (i.e. ≥5 × 5 mm)</th>
<th>RECIST</th>
<th>WHO</th>
<th>irRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always represent PD</td>
<td>Always represent PD</td>
<td>Incorporated into tumour burden</td>
<td></td>
</tr>
<tr>
<td>New, non-measurable lesions (i.e.)</td>
<td>Changes contribute to defining BOR of CR, PR, SD, and PD</td>
<td>Changes contribute to defining BOR of CR, PR, SD, and PD</td>
<td>Do not define irRC (but preclude irRC)</td>
</tr>
<tr>
<td>Non-index lesions</td>
<td>Disappearance of all lesions in one observation in randomized studies. Confirmation is needed for non-randomized studies, according to study protocol</td>
<td>Disappearance of all lesions in two consecutive observations not less than four weeks apart</td>
<td>Disappearance of all lesions in two consecutive observations not less than four weeks apart</td>
</tr>
<tr>
<td>Complete response (CR)</td>
<td>At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters, in the absence of new lesions or unequivocal progression of non-index lesions</td>
<td>A ≥50% decrease in SPD of all index lesions compared with baseline in two observations at least four weeks apart, in the absence of new lesions or unequivocal progression of non-index lesions</td>
<td>A ≥50% decrease in tumour burden compared with baseline in two observations at least four weeks apart</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>A 50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions</td>
<td>A 50% decrease in tumour burden compared with baseline cannot be established nor 25% increase compared with nadir</td>
<td></td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study. The sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression</td>
<td>At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)</td>
<td>At least 25% increase in tumour burden compared with nadir (at any single time point) in two consecutive observations at least four weeks apart</td>
</tr>
<tr>
<td>Progressive disease (PD)</td>
<td>At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)</td>
<td>A ≥25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least four weeks apart</td>
<td></td>
</tr>
</tbody>
</table>
Table 1 compares the RECIST, the WHO, and the irRC criteria. One of the most important differences is the concept of tumour burden and not only that of target lesions. It incorporates changes in all lesions to define the response pattern. The appearance of new lesions are evaluated in the context of all disease and not considered progressive disease per se. The irCR has also higher thresholds to determine progression or response, 25% increase and 50% decrease respectively; in comparison to RECIST that uses 20% increase and 30% decrease respectively. Clinical response to immune therapies can manifest after conventional progressive disease (PD) – “pseudoprogression”. Discontinuation of immune therapy may not be appropriate in some cases, unless PD is confirmed.

Allowance for “clinically insignificant” PD (e.g., small new lesions in the presence of other responsive lesions) is recommended. Durable stable disease may represent antitumor activity.

In a recent study performed by Hamid and colleagues, two doses of pembrolizumab were administered to a total of 276 patients with melanoma in two cohorts, ipilimumab-pretreated or ipilimumab-naïve. Pembrolizumab (formerly lambrolizumab) is monoclonal IgG4 antibody targeting the PD-1 receptor. Linking the T-cell PD-1 receptor with PD-L1, expressed by many tumour cells leads to a negative regulation of immune response. Pembrolizumab acts by avoiding this interaction, thus increasing T-cell mediated antitumour activity. The study found that the two doses of pembrolizumab (2 mg/kg or 10 mg/kg) had similar efficacy and safety profiles in both cohorts [6].

Interesting results came out in a study measuring efficacy of ipilimumab, using the RECIST and irRC criteria. Using RECIST, the overall response rates at 2 mg/kg and 10 mg/kg were 33% and 40% respectively among the ipilimumab-naïve patients, and 26% for both doses in the ipilimumab-pretreated group. Analysing the results using the irRC criteria, these figures were 39% and 40% in the naïve group and 27% and 32% in the pretreated group respectively. Similar results occur in the analysis of progression-free survival (PFS): no difference was seen between the two dose groups. However, the analysis by irRC detected a higher number of responders: the 24-week PFS rate in the naïve cohort was 48% and 50% for the two dose levels respectively according to RECIST, and 50% and 60% for irRC. In the pretreated cohort, these figures were 37% and 44% for the two dose levels respectively according to RECIST, and 57% for both dose levels by irRC [7].

This study provides another opportunity to evaluate the efficacy of irRC as response criteria. Also it can provide new insights into the real effect of pembrolizumab in patients with melanoma and can help to consolidate these criteria as the gold standard for evaluating the clinical response of immunologic agents in oncology.

References
THE EGFR ENIGMA: OVERCOMING DRUG RESISTANCE

The discovery of epidermal growth factor receptor (EGFR) mutations in nonsmall cell lung cancer (NSCLC) has allowed the identification of a subset of patients whose tumors are exquisitely sensitive to EGFR tyrosine kinase inhibitors (TKIs). Among patients with EGFR-mutant tumors, a 75% RR is observed, indicating that approximately 25% of cases do not respond to a TKI (compared with 90% of unselected patients with NSCLC). Despite the efficacy and superiority of EGFR TKIs over chemotherapy as first-line therapy, all patients will ultimately develop progressive disease, with a median of 9–13 months progression-free survival. A better understanding of the molecular mechanisms underlying resistance to EGFR TKIs can help design new drugs and therapeutic strategies to overcome resistance. This has been illustrated by the new generation TKIs that are effective on the T790M mutation, which is the most frequent mechanism of acquired resistance to EGFR TKIs. Hence, it is prudent to understand molecular basis of both the primary and secondary resistance to EGFR TKIs.

Definition of resistance

The study of resistance to EGFR TKIs in EGFR-mutant NSCLC patients can be divided into primary and acquired resistances, which have different origins. Primary resistance refers to patients who had progressive disease or stable disease as the best response to EGFR TKIs, whereas acquired, or secondary resistance refers to patients who had progressive disease following an initial objective response or prolonged stable disease. JACKMAN et al. [1] have proposed a detailed definition of acquired resistance to EGFR TKIs, which relies on: (1) the presence of a known activating EGFR mutation associated with sensitivity to EGFR TKI or a prolonged response or stable disease to EGFR TKI (>6 months); (2) treatment with an EGFR TKI as a monotherapy; (3) disease progression upon uninterrupted exposure to EGFR TKI; and (4) no additional systemic therapy since discontinuation of EGFR TKI. The occurrence of osteoblastic reactions during treatment with TKIs, while the primary tumor and metastases are stable or in response, should not be considered as disease progression [2].

Primary Resistance to EGFR TKIs

Depending on the mutation present in EGFR, tumors exhibit differential TKI sensitivities. While the most common EGFR-activating mutations, L858R and exon 19 deletion, typically confer sensitivity to EGFR TKIs, other primary EGFR mutations can confer resistance. Exon 20 insertions or duplications, which account for approximately 4-9% of EGFR mutations, appear to be resistant to EGFR inhibitors in vivo. Other much less frequent, primary EGFR mutations such as G719X and L861X, have been reported.

Although recognized mainly as a mechanism for AR, another EGFR exon 20 mutation, T790M, has also been associated with primary resistance. Minor clones with the T790M mutation have been identified in treatment-naive tumors that contain classic sensitizing mutations. While this mutation has low allelic frequencies in treatment-naive tumors, pressure from TKIs may select for enriched growth of these T790M clones, leading to overall AR.

<table>
<thead>
<tr>
<th>Rare mutations</th>
<th>Frequency among EGFR rare mutations</th>
<th>ORR %</th>
<th>DCR %</th>
<th>PFS =6 months on EGFR TKI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 18 mutations</td>
<td>38</td>
<td>7</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>Exon 20 mutations</td>
<td>51</td>
<td>8</td>
<td>44</td>
<td>20</td>
</tr>
<tr>
<td>Complex exon 18 and exon 20 mutations</td>
<td>11</td>
<td>57</td>
<td>86</td>
<td>43</td>
</tr>
</tbody>
</table>
An important cause for acquired resistance of NSCLC to gefitinib or erlotinib. As the ligand for MET receptor, hepatocyte growth factor (HGF, also known as scatter factor), once binding to MET receptor, will promote the phosphorylation of MET tyrosine kinase and subsequently trigger the activation of downstream PI3K/AKT/mTOR pathway, which is the key signaling pathway for cell proliferation, survival and anti-apoptosis. About 22% of the EGFR-TKI acquired resistant specimens have been demonstrated to possess MET gene amplification.

**GF-1 Receptor:** Growing evidences have emerged for the involvement of the IGF-1 Receptor (IGF-1R) pathway in the acquisition of resistance to EGFR-TKIs. Constitutive activation of IGF-1R pathway has been detected in multiple gefitinib or erlotinib resistant lung cancer lines.

**HER2:** HER2 mutation occurred at about 2% of patients with NSCLC, significantly more frequent in never smokers, adenocarcinoma histology, oriental ethnicity and female gender. Almost all HER2 mutations locate in exon 20, encoding the kinase domain of HER2 protein. NSCLC cells holding the mutant HER2 are more potent in activating downstream signal transducers and exert resistance to EGFR-TKIs and knockdown of the mutant HER2 succeed in restoring sensitivity to EGFR-TKIs.

The persistent activation of HER3 was detected in human cancer cells and essential for the binding activated EGFR, HER2, MET to PI3K/AKT signaling. HER3 lies upstream the PI3K signaling pathway and functions as an accessory partner of EGFR and HER2. Continuous EGFR-TKI exposure often triggers the overexpression of HER3 as a result of the loss of AKT-mediated negative feedback signaling. The over expressed HER3 promotes the forward shift in the equilibrium of the HER3 phosphorylation-dephosphorylation reactions and results in superphosphorylated state of HER3 and AKT, which requires much higher concentration of RTK-TKIs to fully disassociate the heterodimers or much more potent HER3-targeted drugs to completely dephosphorylate HER3.

**Activation of Compensatory Signaling Pathways**

**PI3K/AKT/mTOR signaling**

**JAK2/STAT3 pathway**

SCLC phenotypic transforming-No exact mechanism underlying this phenomenon has been launched. Probably, SCLC cells originate from the minor pre-existent cells under the selection pressure of EGFR-TKIs, or trans-
differentiate from the adenocarcinoma cells, or arise from the multi-potent stem cells.

**EMT Phenotypic Transforming**

Epithelial to mesenchymal transition (EMT) refers to a complex program by which close-connected and polar-ranged epithelial cells turn into spindle-shape mesenchymal cells with significantly increased motility, invasiveness, and resistance to apoptosis. Clinically, EMT has been proven to contribute about 5% to EGFR-TKI resistance via biopsy paired specimens achieved pre- and post-resistance [6]. However, how EMT promotes TKI resistance remains unknown. Nevertheless, some common mechanisms, such as EGFR T790m Mutation or MET amplification are unlikely the culprit.

**Targeting EGFR T790M**

*Secondary Generation EGFR-TKIs:* In view of the fact that resistant tumor cells are still addicted to the EGFR signaling pathway, new drugs which can irreversibly block EGFR-TK via the formation of covalent bonds in the pocket of the catalytic site should be able to increase the potency of EGFR-TK inhibition. One such inhibitor, the second generation EGFR-TKI afatinib (BIBW2992), designed to bind covalently with Cys-797 at the gatekeeper pocket, can potently and selectively block both wild-type and mutant forms of ErbB family receptors (7). The pooled analysis of those two large open-label phase III studies announced in the 2014 ASCO Annual Meeting proved the favorable antitumor activity of afatinib. Median OS was prolonged from 24.3 months in chemotherapy group to 27.3 months in the afatinib group (HR=0.81; CI 0.66 to 0.99; p=0.037). Especially in the Del19 subgroup, the HR was 0.59 (CI 0.45 to 0.77; p<0.001), preferable for the afatinib group. Despite of the benefits on the T790M-positive NSCLC patients achieved from the utility of second generation EGFR-TKIs, the improvement seems to be rather limited. It is largely caused by the insufficient drug concentrations as the toxicity of this drug limits the blood concentrations under the level required to overcome the EGFR T790M mutation.

*Third Generation of EGFR-TKIs*

In view of this, the third generation EGFR-TKIs that selectively target the mutant EGFR, in particular the T790M mutation, but exhibit minimal potency toward the wild-type receptor emerged in quick succession. CO-1686 is one of them and exhibits potent inhibition of EGFR T790M but circumvents wild-type EGFR.

Osimertinib(8) (Tagrisso™, AZD9291) is an oral, third-generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) that is being developed by AstraZeneca for the treatment of advanced non-small cell lung cancer (NSCLC). Osimertinib has been designed to target the EGFR T790M mutation that is often present in NSCLC patients with acquired EGFR TKI resistance, while sparing wild-type EGFR. In November 2015, the tablet formulation of osimertinib was granted accelerated approval in the USA for the treatment of patients with metastatic EGFR T790M mutation-positive NSCLC (as detected by an FDA-approved test) who have progressed on or after EGFR TKI therapy.

**Targeting HGF-MET Pathway**

*Anti-HGF Neutralizing Antibody:* TAK-701 is a potent humanized monoclonal antibody to HGF. It works by suppressing the HGF binding to MET receptor and thus restrains the proliferation effects of MET pathway.

**MET Tyrosine Kinase Inhibitors**

Tivantinib is a non-ATP-competitive small molecule MET inhibitor. It works by stabilizing the inactive conformation of MET, and thus hinders the activation of downstream signaling pathway. Given the well-tolerance and potential ability of tivantinib both as single-agent therapy and in combination with erlotinib announced in several preclinical and phase I clinical trials, a series of work have and are being carried out to evaluate its antitumor efficacy.

**MET Monoclonal Antibody:** Onartuzumab (MetMAb) is a newly developed humanized monoclonal antibody targeting MET. It blocks the HGF binding to MET, and thus attenuates the activation of its downstream transducers and effectors [9]. It was evaluated in a randomized phase II trial comparing erlotinib with and without onartuzumab in advanced NSCLC patients. Despite there being no statistically significant differences between the two arms in the overall population, the combinational arm showed 47% reduction in the risk of disease progression and significant prolongation in median PFS (2.9 months versus 1.5 months; HR 0.53, 95% CI 0.283-0.99, p=0.04) and median OS (12.6 months versus 3.8 months; HR 0.37, 95% CI 0.19-0.72, p=0.002) in the subset of MET-positive, which was confirmed to be associated with bad prognosis.
Other Strategies Under Trial

**Targeting HER3 pathway: combination of pertuzumab and erlotinib**

**Targeting PI3K/AKT/mTOR pathway**
1. PI3K inhibitors-BAY 80-6946
2. mTORC1 inhibitors- Everolimus
3. Dual PI3K /mTORC1 /mTORC2 inhibitor
4. AKT inhibitors

**Inhibiting epithelial-mesenchymal transition-Histone deacetylase (HDAC) inhibitors**

**Newly emerged multi-targeted agents-HSP90 inhibitors**

**Targeting miRNAs**

miRNAs are a class of 18-24 nt small noncoding RNAs that negatively regulate the target gene expression either by inhibiting mRNA translation or by promoting mRNA degradation [10]. Emerging evidences have suggested their master regulatory roles in oncogenesis either as oncogenes or as tumor suppressor genes [11]. In NSCLC, upregulated miRNA30b, miRNA30c, miRNA221, miRNA222 are associated with resistance to gefitinib treatment through the regulation of PTEN and APAF-1 expression, while miRNA103 and miRNA203 induce apoptosis in gefitinib resistant cells and promote mesenchymal to epithelial transformation via the down-regulation of PKC-å, SRC and Dicer.

**Conclusions and Perspectives**

EGFR activating mutations have been over optimistically recognized as the Achilles’ heel of NSCLC after some clinical successes have been achieved. Unfortunately, almost all patients initially responding to gefitinib or erlotinib would inevitably progress to develop acquired resistance. To our relief, the constantly updating knowledge of the mechanisms underlying has already been translated to the development of novel targeted agents, rational combination regimens and improved survival benefits to NSCLC patients.

**References**


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Conference Highlights

- Conference Theme: Breast Cancer in the Era of Precision Medicine

- Live Surgical Workshops on
  - Robotic Nipple Sparing Mastectomy + Implant Reconstruction / Roll
  - Open Nipple Sparing Mastectomy + Implant
  - Robotic Nipple Sparing Mastectomy + Implant by Dr. Golshan Mehra, USA

- Master Videos in Breast Cancer Surgery
- Brachytherapy Workshop
- Breast Imaging & Intervention Workshop
- Hands-on Workshop - VABB
- Oncology Nursing Workshop
- Breast Pathology and Genetics & Genomics Workshop

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