Mutations and Other Biomarkers in Advanced Non-small Cell Lung Carcinoma with Implications in the Philippine Setting

Joven Q. Tanchuco, M.D, MHA, FPCP*

Summary

Lung cancer remains a top cause of new cases and deaths from malignancies globally and locally. The development of targeted therapy for advanced non-small cell lung cancer (NSCLC), particularly adenocarcinoma, promises to improve survival significantly among suitable patients as compared to chemotherapy. About 50% of NSCLC patients have some driver mutations that can be treated by targeted therapy. The most common mutation is that involving EGFR which is found in as much as 90% of patients with driver mutations, most especially in those with adenocarcinoma, in women and never-smokers and those of East Asian ancestry. This is followed by patients with ALK or ROS1 rearrangements in another 5% each. Proper molecular profiling is, however necessary at the outset to identify patients who are suitable for targeted treatment. Fortunately, in the Philippines, testing for EGFR, ALK and ROS1 mutations are possible with several of the tyrosine kinase inhibitor drugs (TKIs) that target these mutations also available. A smaller proportion of patients have BRAF mutations (<5%) but the drug needed to treat this is not available commercially in our country.

There are other mutations in advanced NSCLC which are considered potential drug targets for treatment. However, developing a clinically acceptable drug for use in lung cancer has been less successful. KRAS mutations, for example, can be as common as EGFR mutations (and sometimes more so) but no suitable drug for lung cancer has been identified yet. This is also true for METex14, HER2, VEGF, and others that are less common. Clinical studies continue to be done involving these target molecules. These biomarkers have sometimes found usefulness as indicators of poor prognosis and/or likelihood of developing drug resistance but for the most part, have remained in the realm of research.

Immunotherapy was not included as a topic in this article.

The search continues for new molecules to be used in targeted therapy for lung cancer. Development of drug resistance to TKIs, often inevitable and just a matter of time, continue to drive these development efforts. The remaining approximately 50% of NSCLC with no driver mutations also push efforts to search for appropriate drugs that will be good for them – including immunotherapy. Studies are also being done to look at various combinations of targeted therapy with chemotherapy and even immunotherapy. It will not be an overstatement to say that the future of lung cancer, especially NSCLC is rapidly evolving and will be creating data that may be very different from what we know at present. Clinicians who encounter and/or treat lung cancer should keep abreast of this rapidly changing information in properly advise their patients on suitable therapies. This is particularly true in financially constrained settings such as the Philippines where even just the cost of testing for these mutations can already be a significant barrier to whether or not to use targeted therapy.

Keywords: non-small cell lung cancer, targeted therapy, mutations

Introduction

Lung cancer of all types remains a major global and local health problem. According to the WHO, there were more than 18 million new cases of cancer globally in 2018.[1,2] Of these, new cases of lung cancer ranked number one comprising 11.6% of the total. Lung cancer also accounted for the greatest number of deaths due to malignancy with 1.8 million persons (18.6%) dying with lung cancer. Among males, lung cancer remains number one in incidence (31.5 per 100,000 age-standardized incidence rate) and as a cause of death (22%). Among females, breast cancer accounted for more new cases (46.3 per 100,000 ASIR), but lung cancer is second (14.6 per 100,000). Lung cancer, however, is the second most common cause of cancer deaths (13.8%) among females. More importantly, using the global data for 2012, a study reported that 19 out of the 38 countries included have increased incidence of lung cancer among women.[3]

** Associate Professor, Department of Biochemistry and Molecular Biology; Clinical Associate Professor, Section of Pulmonary Medicine, Department of Internal Medicine, University of the Philippines-Philippine General Hospital*

Corresponding author: Joven Q. Tanchuco, M.D., University of the Philippines-Philippine General Hospital, Manila City, Philippines
Email: joven.tanchuco@gmail.com

In the Philippines, according to a 2015 local report, it is estimated that there were 109,280 new cases of cancer with 66,151 cancer deaths.[4] Lung cancer remains the most common cause of new cases (12.5%) and deaths (17.8%) in both sexes combined. According to this report, many patients with lung cancer do not survive for a year following diagnosis, with almost a 1:1 ratio of new cases to mortality, a trend which is also observed globally.[5] For lung cancers (both sexes) diagnosed between 1993-2002 and using population-specific life tables, the five-year relative survival rate of Metro Manila patients with lung cancer were reported at 12%, which is lower than the average globally at that time.

More recently, based on the Globocan 2018 estimates, there were 141,021 new cases of cancer with 86,337 cancer deaths in the Philippines.[6] Lung cancer accounts for the second most common cause of new cases of cancer in both sexes combined for all ages, accounting for 12.2% vs breast which accounted for 17.6%. However, lung cancer still ranks number one as the cause for new cases among males and is the most common cause of cancer deaths (17.9%) combined for both sexes and all ages. Globally, the five-year survival rate of lung cancer is 17.8% and is much lower than that of other leading cancers.[7]

With such a big impact on cancer morbidity and mortality globally and locally, it is of urgent importance to find solutions to address this. Lung cancer comprises several histologic types and subtypes, categorized at the outset as small cell (20% of all lung cancers) and non-small cell (80%) because of their differential response to conventional chemotherapy and radiotherapy. Non-small cell lung cancer (NSCLC) is less responsive to these interventions as compared to small cell cancer. NSCLC, which we will cover in this article, is further subdivided into squamous cell (25% of lung cancers), adenocarcinoma (40%), large cell (10%) as well as many other subtypes and combinations that are less common.[8,9] Most patients with advanced NSCLC present with metastatic disease and, if left untreated, have a median survival after diagnosis of four to five months, a one-year survival of < 10% and five-year survival of <5.0%.[10,11]

Similar to other cancers, survival in lung cancer depends on the histologic sub-type and the stage of the cancer. Because the overall survival of these patients has remained dismal, attention has been given to preventive measures. Among these, screening for lung cancer has been advocated as key to catching it early and performing potentially curable surgery for early-stage disease. There has been substantial progress in defining the suitable patients who should undergo screening for lung cancer, but unfortunately widespread implementation even in rich western countries remain a challenge.[12,13] In lower-income countries, such as the Philippines where lung cancer screening is not widely covered by public or private health insurance, the barriers to such an approach are even much greater for the majority of the population at high risk.

Another key preventive measure is eliminating cigarette smoking, a top reversible risk factor for most types of lung cancer. Unfortunately, cigarette smoking is not a risk factor for adenocarcinoma which is the single largest group of lung cancers. The Philippines has made progress in curtailing cigarette smoking including Sin Tax legislation, graphic warnings and prohibiting smoking in public places, among others.[14] But these may not make much difference in those lung cancers where smoking is not a risk factor.[15] As we will see later on, most of the patients who may benefit from currently available targeted therapy for lung cancer driver mutations are non-smokers.

Interestingly, in some countries where smoking cessation have helped to improve lung cancer outcomes, it has been noted that the relative incidence of lung cancer among non-smokers has been increasing.[16] Air pollution, both outdoor and indoor has been cited as an emerging significant risk factor for the development of lung cancer.[17-19] The evolving importance of air pollution might need to be investigated further even now in relation to the pathogenesis and treatment of lung cancer.[20] There may be different pathways involved, with different molecular mediators; and thus potentially different treatment targets. In the Philippines, for example, vehicle emissions contribute the majority of outdoor air pollution.[21] With its more varied causes, and often significant economic impact, controlling air pollution can turn out to be an even greater challenge than controlling cigarette smoking. As part of the Global Burden of Disease studies, fine particle air pollution has been identified as the largest environmental risk factor worldwide, responsible for a substantially larger number of attributable deaths than other more well-known behavioral risk factors such as alcohol use, physical inactivity, or high sodium intake.[22] Although still lower than cigarette smoking, it is estimated that about 29% of deaths from lung cancer are due to exposure to air pollution.

Oncogenesis

With challenges still being faced in lung cancer prevention such as screening and smoking cessation, with the latter not being relevant in a significant number of lung cancer cases; there remains a need to better understand how lung cancer develops in the hopes of finding better ways to control it. There are multiple possible pathways for the cancerous transformation of a normal cell. Human cancers arise via a multistep mutagenic process reflective of genetic and epigenetic changes that drive progressive transformation of normal cells into malignant counterparts. Many types of human cancers have an age-dependent incidence implicating potentially four to seven rate-limiting stochastic events.[23] This observation mirrors pathologic analyses of tumors that reveal the existence of premalignant lesions that progressively evolve from normal to invasive to metastatic cancer.

Many human tumor types with distinct genotypes have six essential alterations in cell physiology that appear to

collectively dictate the malignant phenotype. These cellular processes are: 1) self-sufficiency in growth signals (oncogene addiction), 2) insensitivity to growth-inhibitory signals (loss of tumor suppressors), 3) evading programmed cell death (anti-apoptosis), 4) limitless replication potential (aberrant cell cycle), 5) sustained angiogenesis, and 6) invasion/metastasis.[24] Two additional hallmarks have also been proposed recently: evasion of immune surveillance and the cancer cell stress response phenotypes.[25,26] Understanding the mechanisms involved can help identify potential targets for treatment.[27,28] Almost all of these can include mutations resulting in altered signaling that causes the malignant transformation of cells.

However, not all of the general mechanisms mentioned above are seen as currently relevant to NSCLC and/or could be translated into clinical intervention targets. This article focuses its discussion on those mutations that have more immediate clinical application in the Philippine setting, i.e., help to understand those mutations that pave the way for understanding currently available targeted therapy for NSCLC in the local setting. It is not the purpose of this article to discuss comparative clinical response rates of targeted therapeutic interventions, other than to help understand the importance of the mutations involved.

Mutations in NSCLC

Numerous signaling pathways exist in and between cells to coordinate the numerous processes needed for the whole organism to survive. As a safeguard, and most likely as a product of successful evolution, such signaling pathways are commonly redundant, subject to complex regulation and duplicate or overlap similar processes intracellularly and across different cells. Thus, single mutations do not necessarily mean the inevitable malignant transformation of cells. Our current understanding of oncogenesis dictates that a handful of "rogue" mutated cellular genes – and not just one – designated as oncogenes, are required to result in cancer. These aberrant processes hijack the cell, causing it to survive indefinitely and proliferate abundantly.[22] The second unifying theme is that the genesis and progression of a tumor is governed by the activation of specific oncogenes and inactivation of tumor suppressor (counter-regulatory) genes. The random accumulation of these genetic alterations progressively drive the evolution of cancer from a benign expansion of cells to an invasive and metastatic tumor.

Up to this point, one can think that because of the random occurrences of multiple mutations that drive oncogenesis, it may be impossible to counter cancers by targeting these multiple mutations simultaneously. However, a third concept, that of "oncogenic addiction" has emerged.[28] In its simplest form, oncogene addiction refers to the observation that a tumor cell, despite its plethora of genetic alterations, can seemingly exhibit dependence on a single oncogenic pathway or protein for its sustained proliferation and/or survival. Because normal cells would have overlapping or even corrective and counterregulatory mechanisms, inactivating the normal counterpart of such oncogenic proteins in normal tissues through the use of modifier molecules are often tolerated without obvious unintended consequence. A profound final implication of all this is that switching off this crucial pathway upon which cancer cells have become dependent should have devastating effects on the cancer cell while sparing normal cells that are not similarly addicted.[29] This, of course, is the discriminating activity required for any effectively targeted cancer therapeutic.

Compared with other cancers, lung cancer is often described as one with the highest rates of genetic alterations.[30] Presently, mutations involving genes encoding for components of the epidermal growth factor receptor (EGFR) and downstream mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinases (PI3K) signaling pathways have been described in NSCLC, particularly adenocarcinoma. These mutations can also define mechanisms of drug sensitivity and primary or acquired resistance to kinase inhibitors. Other mutations of potential relevance to treatment decisions in NSCLC include: Kirsten rat sarcoma viral oncogene (KRAS), anaplastic lymphoma kinase (ALK) and oncogene product of an avian sarcoma RNA tumor virus (ROS1) receptor rearrangements (used synonymously with the terms fusions, translocations, or cross-overs), V-raf murine sarcoma viral oncogene homolog B1 (BRAF), human epidermal growth factor receptor 2 (HER2), and MET, which encodes the hepatocyte growth factor receptor (HGFR).[31] Mutations involving PI3K catalytic protein alpha (PI3KCA), AKT1, MAPK kinase 1 (MAP2K1 or MEK1) have also been reported but their final roles in NSCLC oncogenesis remains to be defined. These mutations are mutually exclusive, except for those involving PI3KCA and BRAF mutations, EGFR mutations, or ALK rearrangements that can co-exist in the same cell.[32-33]

The mutations occur in varying frequencies across patients with NSCLC. In a study of 18,679 molecular analyses involving 17,664 patients with advanced NSCLC, six genes were profiled for any alteration: EGFR, HER2, KRAS, BRAF, PIK3CA and ALK.[34] Overall, a genetic alteration was recorded in about 50% of the analyses. KRAS mutations were reported in 4,894 of 17,001 (29%) analyses for which data were available, EGFR mutations in 1947 of 17,706 (11%), BRAF mutations in 262 of 13,906 (2%), PIK3CA mutations in 252 of 10,678 (2%), and HER2 mutations in 98 of 11,723 (1%). ALK rearrangements were reported in 388 (5%) of 8134 analyses. This distribution reflects the relative prevalence of these more common mutations in the general populations of advanced NSCLC. We will be discussing alterations in EGFR, ALK, ROS1, and BRAF for which targeted therapy is available. We will also be briefly touching on the others which may have current implications in prognosis and drug resistance and in the future may have available drugs for use in lung cancer. The

mutations and other biomarkers to be covered in this article are summarized in Table I. Immunotherapy was not covered in this article due to article length considerations.

Interestingly, different sub-cohorts showed different profiles of the mutation patterns. For example, EGFR mutations were seen in 21% among women and 44% among never smokers as compared to 12% in the overall population. On this basis, women and never smokers appear to be better candidates for tyrosine kinase inhibitors (TKI). We will be going back to this insight later. In financially-challenged locations such as in the Philippines where testing might present a barrier, being able to better predict the relative success of identifying mutations based on certain demographic profile could be an advantage.

EGFR Mutations

Oncogenesis. In a study involving the largest EGFR mutation profiling data among Filipino patients with NSCLC (n=626), an overall 49.4% EGFR mutation rate was reported.[35] In this study, the mutation rates according to histologic types, were as follows: adenocarcinoma=49.9% (n=287/575), squamous cell carcinoma=3.5% (n=9/26), NSCLC not otherwise specified=50% (n=10/20), adenosquamous cell carcinoma=66.7% (n=2/3), and adenocarcinoma with neuroendocrine features=50% (n=1/2). Consistent with the literature, a significantly higher incidence of EGFR mutation among women than men (60.2% vs 39.8%) was also found. With regards to individual mutation types, the most common mutations detected were deletions in exon 19 (54.7%, n=168), followed by L858R point mutation in exon 21 (27.4%, n=84).

Epidermal growth factor receptor (EGFR) is a 170 kDA long type 1 transmembrane glycoprotein with an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain that regulates signaling pathways to control cellular proliferation.[36] The gene that codes for EGFR is located on the short arm of chromosome 7 (7p11). EGFR belongs to the receptor tyrosine kinase (RTK) superfamily of cell-surface receptors that serve as mediators of cell signaling by extra-cellular growth factors. Members of the ErbB (taken from the erythroblastic leukemia viral oncogene to which the receptors are homologous) family of RTKs, such as EGFR (also known as ERBB1 or HER1), also include ERBB2 (also known as HER2), ERBB3 (also known as HER3) and ERBB4 (also known as HER4).

There are 11 growth factor ligands that activate ErbB receptors, seven of which are capable of stimulating EGFR.[37] Once activated, EGFR, through a series of dimerization steps is capable of acting as a tyrosine kinase which eventually leads to a whole range of downstream signaling pathways. Among the different molecules are mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K), AKT (also known as protein kinase B), Ras (rat sarcoma) protein and many others. These are known to affect cell survival, growth, cell proliferation and metabolism, cell migration, angiogenesis and a host of other effects. Since these are processes needed by normal cells, regulatory and counter-regulatory mechanisms are in place to ensure that they are controlled appropriately. However, the increase in the activating signals and/or loss of suppression can lead to the proliferation of these downstream molecules. Such

Table I. Mutations and other biomarkers in non-small cell lung carcinoma

Mutation	Prevalence in NSCLC	Treatment[b]	Diagnostic test[b]
EGFR[a]	49.4% (Filipinos) [35] 30% (Filipinos) [81] 12% (Unselected) [34] 21% (Women) [34] 44% (Never smokers) [34]	gefitinib, erlotinib afatinib osimertinib	Cobas® [215] NGS Use of circulating tumor DNA (ctDNA) for testing to identify EGFR mutations in lung cancer has no recommendation [68]
ALK[a]	5% (Unselected) [34] 3.8% (Unselected) [47]	crizotinib	FISH, IHC NGS [68]
ROS1[a]	3% (Unselected) [118,119]	crizotinib	FISH, NGS RT-PCR [68]
BRAF	2-4% [127]	none	Not recommended unless EGFR, ALK and ROS1 testing is negative [68]
KRAS	29% (Unselected) [34] 15-25% (Unselected) [101]	none	Not routinely recommended [68]
MET	3% (Unselected) [141]	none	Not routinely recommended [68]
HER2	1% (Unselected) [34] 2-6% (Unselected) [181]		
RET	1-2% (Unselected) [173]	none	Not routinely recommended
PIK3CA	2.9% [201]	none	Not routinely recommended
No mutation	50% (Unselected) [34]		By elimination
VEGF	Varies according to stage [231]	bevacizumab (in combination with other therapies)	ELISA

[a] *Varies across different populations. Highest among Asians, women, and never smokers*
[b] *Available in the Philippines.*

events have been closely linked to malignancy. Many of these individual downstream molecules are also being investigated to identify potential therapeutic targets.[38-39]

In NSCLC, overactivation of downstream signal molecules can be due to overexpression or amplification of EGFR.[40-41] Several studies have shown that EGFR overexpression in NSCLC is associated with reduced patient survival, frequent lymph node metastasis and poor chemosensitivity.[42-46] Amplification of EGFR expression by itself, however, is not a common occurrence; being reported in only about one percent of NSCLC patients.[47] Increased levels of EGFR gene expression has also been observed in cancers of the head and neck, ovary, cervix, bladder, esophagus, stomach, brain, breast, endometrium, and colon; and frequently seem to confer an adverse prognosis.[48,49]

On the other hand, mutations that occur within the EGFR gene resulting in altered regions of the extracellular and intracellular portions of EGFR have been more widely described. Together, overexpression and mutations of EGFR was observed in 43–89% of cases of NSCLC.[50] These two events are not independent of each other: studies have reported that one-quarter of NSCLC had mutations in the EGFR tyrosine kinase domain and these were associated with increased receptor expression in 75% of cases.[51,52] It is currently thought that mutations occur as early events in the carcinogenesis of some lung cancers and gene overexpression occurring later.[36] Thus, both EGFR mutation and gene amplification status may be important in determining which tumors will respond to TKIs.

Of the known EGFR tyrosine kinase domain mutations, > 90% occur as short in-frame deletions of the 747 to 750 amino acids leucine, arginine, glutamic acid, and alanine or (now commonly referred to as LREA using the one letter abbreviations for the amino acids) in exon 19 or as point mutations in exon 21, the latter resulting in arginine replacing leucine at codon 858 (L858R).[53] Both of these mutations are together labeled as "classic" mutations. Between 50% and 60% of classic EGFR mutations are exon 19 LREA frame deletions with the rest due to exon 21 L858R replacements.

Uncommon mutations comprise approximately 10% of EGFR mutations and are defined as all mutations excluding LREA deletion and L858R. Two examples of less common mutations occur at exons 18 and 21. The most frequently detected exon 18 mutation is G719X, followed by the E709X mutation.[54,55] Studies in which patients with uncommon EGFR mutations have been analyzed as a single group have often shown that the response to EGFR TKIs is lower among this group than among patients with either of the common mutations alone. However, when analyses are performed on individual mutations or smaller selective subsets, substantial clinical heterogeneity exists.[56-58]

Both the classic and uncommon mutations can result in continuous activation of signal transduction pathways, leading to cell proliferation or anti-apoptosis, regardless of the presence of extracellular ligand. Of note, EGFR and KRAS mutations appear to be mutually exclusive.[59] In the Philippines, it is possible to distinguish between classic and some of the less common mutations through commercially available tests offered in the bigger hospitals.

Targeted therapy involving EGFR. If mutations of the EGFR pathway molecules are responsible for the transformation to malignant cells, then inhibiting the activation of EGFR and/or any of the downstream molecules could prove a meaningful strategy to treat NSCLC. For EGFR, its tyrosine kinase activity seems to be an obvious area for intervention. More specifically, since Adenosine triphosphate (ATP) is the source of the phosphate for the tyrosine kinase activity, most of the early TKIs resembled ATP. By binding to the ATP binding site, the kinase activity is inhibited. Two oral small molecules, erlotinib, and gefitinib, were the first of a group of tyrosine kinase inhibitors (TKIs) that were used to target EGFR stimulation. Initial trials with gefitinib among unselected lung cancer patients, however, showed no survival benefit. But a significant survival benefit was seen when a subset of non-smoking Asian women with adenocarcinoma was looked into.[60] We have mentioned previously that adenocarcinoma, women, and non-smokers tend to have the highest presence of EGFR mutations.[34] Subsequent trials of gefitinib and erlotinib this time among patients with demonstrated EGFR mutations showed significant responses.[61-63] Thus, was born the era of pre-treatment molecular profiling in NSCLC to determine suitability for and selection of appropriate targeted therapy. Before this, particularly in the era of chemotherapy, patients were uniformly subjected to the same treatment without consideration of their molecular profile.

Second-generation TKIs, afatinib, and dacomitinib and third-generation TKI, osimertinib have since been made commercially available. First and second-generation TKIs share structural similarities with the second-generation TKIs possessing higher affinity for the TKI binding sites. Second-generation TKIs were initially intended to overcome the acquired resistance to first-generation TKIs that were already being seen. They are capable of forming covalent bonds with EGFR binding sites and are thus irreversible inhibitors, unlike the first generation. They were also capable of targeting other members of the ErbB family of receptors which were hoped to lead to higher efficacy.[64] In the end, however, first- and second-generation TKIs had comparable clinical outcomes.[65]

Third generation TKIs, on the other hand, were intended from the outset to binding sites on EGFR with T790M mutation as a way to address acquired TKI resistance (see below). Its structure is therefore different compared to the first and second-generation TKIs. Osimertinib has about 200 times more affinity for EGFR with T790M mutations compared to

normal EGFR, thus preserving its targeted therapy status.[66] Although initially intended for use after previous EGFR TKIs failed, a study showed that osimertinib also has superior efficacy over that of standard EGFR-TKIs in the first-line treatment of EGFR mutation–positive advanced NSCLC, with a similar safety profile and lower rates of serious adverse events.[67] In the Philippines, osimertinib is at present approved for use as first-line therapy for NSCLC patients with EGFR mutations. However, some preliminary studies are now starting to describe resistance to even osimertinib.[68-73]

For reasons that are still being studied, patients with LREA mutations have had consistently better outcomes with EGFR TKIs than patients with L858R single-point substitutions.74-78 TKI treatment benefit was 50% greater (HR: 0.24; 95% CI: 0.20–0.29) for patients with LREA deletions than for those with L858R substitution (HR: 0.48; 95% CI: 0.39–0.58; *p*=0.001). Patients with LREA deletions also had a greater overall survival than those with L858R substitution after gefitinib or erlotinib therapy. In a pooled analysis focusing on the two randomized trials comparing afatinib with chemotherapy—LUX-Lung 3 and LUX Lung-6 trials – it was suggested that afatinib could improve overall survival compared with chemotherapy among patients with LREA deletions but not for patients with L858R substituted disease.[78]

Interestingly, L858R substitutions, rather than LREA deletions have been associated with longer overall survival for TKI treatment-naïve patients.[79] Whereas patients with LREA mutations seem to respond better to targeted therapy, the opposite appears to be true when looking at those who received chemotherapy. Chemotherapy resulted in significantly greater progression-free survival for patients with L858R substitutions than for patients harboring LREA deletions (median progression-free survival, 6.1 vs. 5.1 months; *p*=0.003).

The cause of this difference in response to EGFR TKIs, or possibly chemotherapy, by EGFR mutation subtype is also not known. However, in the randomized trial in which afatinib was compared with gefitinib (LUX-Lung 7), the overall survival did not differ significantly in the two treatment arms among patients with LREA deletion.[65]

Molecular profiling for EGFR mutations in NSCLC. Since deciding on targeted therapy depends on being able to identify EGFR mutations, the availability of the appropriate tests to accurately identify this is important. Being able to identify both EGFR gene mutations and overexpression are desirable to identify appropriate patients who can benefit from TKIs. In East Asian countries, the prevalence of EGFR mutations can be as high as 50–60%.[80] A study also reported that in the Philippines, >30% of samples showed the presence of EGFR mutation.[81] Women and never smokers are also identified as having a higher prevalence of this mutation regardless of their geographic location. Therefore, women with confirmed advanced NSCLC in the Philippines who are never-smokers should be offered EGFR profiling to determine their suitability for targeted therapy.

The College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology have published molecular testing guidelines in lung cancer, including information about testing of EGFR gene mutations.[68,82] These were later endorsed by the American Society of Clinical Oncology.[83] The use of immunohistochemistry for EGFR testing is NOT being recommended in these guidelines. In addition, there is now recommendation to include testing for mutations of ErBB2 or HER2 and use of five percent sensitivity assays for EGFR T790M mutations in patients with secondary resistance to EGFR inhibitors. Either cell blocks or other cytologic preparations are suitable. The use of circulating cell-free DNA (ctDNA) for testing to identify EGFR mutations in lung cancer has no recommendation yet except in some clinical settings in which tissue is limited and/or insufficient. In the Philippines, commercial EGFR testing is available.

It is considered inevitable that patients on EGFR TKIs develop acquired resistance and disease can progress (see below). Therefore, it is highly recommended that, at the time of disease progression, a repeat biopsy is performed to confirm the presence of the EGFR T790M mutation and any driver mutations (e.g. exon 19 deletions and L858R mutations) prior to further treatment.[84] Following disease progression, it can be more challenging to obtain a tissue biopsy than at primary diagnosis. Blood containing circulating tumor DNA (ctDNA) otherwise also referred to as liquid biopsy offers a less invasive and potentially quicker alternative for EGFR mutation testing and follow up.[85] However, ctDNA testing is less sensitive for EGFR T790M than exon 19 deletion and L858R. When using ctDNA testing, it should be noted that false negatives have been reported (30–40%+).86,87 Therefore, if a plasma-based ctDNA test is used and the result is negative, it is recommended that this be followed up with a tissue-based test. A tissue biopsy is highly recommended if the patient is well enough to tolerate the procedure, as this is the primary source of the mutations. When ordering a ctDNA test, it is suggested that a tissue biopsy also be requested, which can subsequently be used if the ctDNA test is negative.

Resistance mechanisms in TKI targeted therapy. Almost all patients who had a response to first- or second-generation EGFR TKIs eventually develop resistance to these drugs. The most common mechanism of resistance, accounting for approximately 50% of all cases, is the acquired T790M mutation in exon 20.[84,88,89] The mutation substitutes a threonine (T) with a methionine (M) at position 790 of exon 20 affecting the ATP binding pocket of the EGFR kinase domain. Current guidelines recommend testing for T790M mutation in lung adenocarcinoma patients who have progressed after treatment with an EGFR-targeted tyrosine kinase inhibitor other than osimertinib.[68] Upon the occurrence of T790M mutation, the first-generation EGFR TKI can no longer bind

to the ATP binding pocket at the receptor, thus losing efficacy on inhibition of downstream signaling. Pre-clinical studies showed that more potent second generation TKIs can overcome the inhibition but only at higher doses. Unfortunately, the higher doses also affected the normal EGFRs and caused severe toxicities. In clinically acceptable doses, second-generation TKIs are deemed unable to overcome the loss of inhibition arising from acquired T790M mutations.[90,91]

The exact mechanism of acquiring T790M mutation remains largely unknown and needs to be further looked into. A study seeking to understand this showed that although a big driver of acquired mutation is TKI exposure, some patients without prior TKI exposure can have the T790M mutation.[92] It would seem therefore that selection pressure, similar to the emergence of resistance to antibiotics, may not be the only mechanism. Interestingly, one of the significant findings was that exon 19 LREA deletions are more likely to develop T790M mutation than L858R substitutions. It is not clear how this difference in mutation status contributes to the greater eventual development of T790M mutations.

Other mechanisms of resistance to TKIs are relatively heterogeneous, and these may include bypass mechanisms such as HER2 and/or MET amplification and HGF or AXL overexpression; as well as downstream alterations such as those involving PTEN loss and PIK3CA and/or BRAF mutation, and transformation to small cell lung cancer.[54,93] Mutations conferring TKI resistance with the disappearance of the T790M mutation have also been described.[94] Using the next genome sequencing (NGS) to better elucidate mechanisms of TKI resistance is being advocated to better understand these events.[95] In any case, the incidence of each of these mechanisms of resistance are much lower than that of the T790M mutation, and only rarely do these mutations or amplifications occur concurrently with T790M mutation. An interesting possibility being mentioned is the use at the outset of combinations with several drugs that have specificity for these downstream resistance pathways such as that for MET, BRAF, HER2, PIK3CA, etc. Because of the small numbers involved, it is not yet possible to draw any firm conclusions about this.

With emergence of TKI resistance, development of third-generation EGFR TKIs designed to target the T790M mutation while sparing wild-type (non-malignant) EGFR became an imperative. The structural differences between the first and second generation TKIs as compared to the third-generation ones highlights this innovation. At this time, osimertinib is the only commercially available third generation TKI in the Philippines. Unfortunately, resistance to osimertinib and possible mechanisms including bypass downstream processes and further mutations of EGFR are now also being described.[68-72] Data from the AURA3 trial showed that the most frequent mutations detected in the patient plasma after progression with second-line osimertinib included EGFR C797 mutations (15%; C797S n=10 and C797G n=1), MET-amplification (19%), HER2-amplification (5%) and the PIK3CA mutation (5%).[96] In a subgroup analysis of the FLAURA trial for first-line osimertinib, the most frequent acquired resistance mechanisms detected also in the patient plasma were MET-amplification (15%) and the EGFR C797S mutation (7%), followed by HER2-amplification and the PIK3CA and RAS mutations (two to seven percent).[97] A study is being planned to investigate further events related to secondary resistance to osimertinib (Phase 2 Platform Study in Patients With Advanced Non-Small Lung Cancer Who Progressed on First-Line Osimertinib Therapy - ORCHARD).[98]

Understanding the emergence of TKI resistance in the Philippines needs to be undertaken in order to better understand the likelihood of developing resistance to currently available EGFR TKIs.[99]

ALK rearrangement

Anaplastic lymphoma kinase (ALK) rearrangement is relatively an uncommon occurrence in NSCLC, with about 3.8% of all cases identifiable with this driver mutation.[47] ALK is a transmembrane receptor tyrosine kinase and is a member of the insulin receptor superfamily. It is coded by the ALK gene on chromosome 2p and has a role in cell division and survival via different downstream pathways. The 3′ end of the ALK gene is juxtaposed with the 5′ end of the echinoderm microtubule-associated protein-like 4 (EML4) gene forming the EML4-ALK fusion protein. Other fusion proteins can also be formed with the juxtaposition of ALK with TFG and KIF5B genes.

The formation of dimers by the amino-terminal portion of the ALK fusion proteins result in the constitutive activation of the ALK protein kinase domain independent of any ligand. This leads to the dysregulation of cell proliferation and plays a key role in the tumorigenic process. Downstream signaling from ALK fusion proteins involves the Ras/Raf/MEK/ERK1/2 cell proliferation module and the JAK/STAT cell survival pathway. ALK fusion proteins are usually – but not always – found in young, never (or light) smokers with NSCLC.[100] For the most part, with rare exceptions, EML4-ALK rearrangements and EGFR mutations are mutually exclusive.

Targeted therapy involving ALK rearrangements. Just like in EGFR mutations, inhibiting the tyrosine kinase activity of the fusion protein seems an obvious way to go. When EGFR TKIs are given to patients with EML4-ALK rearrangements, their response rate is similar to those patients without EGFR mutations.112 TKIs specific for ALK fusion proteins therefore have to be used. Such a molecule is crizotinib which is commercially available in the Philippines for the treatment of ALK-positive NSCLC. Crizotinib is also found to be effective against downstream molecules ROS kinase and MET kinase (see below).

Similar to the EGFR TKIs, the emergence of crizotinib drug resistance with a median occurrence at approximately

10 months after the initiation of therapy has driven the development of second-generation drugs for the treatment of NSCLC with ALK rearrangements. About 28% of the cases of crizotinib resistance are related to nearly a dozen different mutations of ALK in the EML4-ALK fusion protein; the other cases of resistance are related to the upregulation of alternative signaling pathways (e.g., KRAS and EGFR), gene amplifications (ALK and KIT), or to as yet other undefined mechanisms.[101] Newer TKIs such as alectinib and brigatinib have been shown as superior to crizotinib.[102,103] Ceritinib, alectinib and brigatinib also have better penetration to the brain compared to crizotinib.[104] Current guidelines allow for the use of crizotinib, alectinib, brigatinib and ceritinib as first line treatment of NSCLC with ALK rearrangements.[105] A third line ALK TKI, lorlatinib has been recently approved by the US FDA for second- or third-line treatment of ALK-positive metastatic NSCLC. Lorlatinib is described as better able to penetrate the blood-brain barrier and less prone to develop TKI resistance.[106] Unfortunately, aside from crizotinib, none of the other ALK TKIs are currently commercially available in the Philippines.

Molecular Profiling in ALK rearrangement NSCLC. A fluorescence in situ hybridization (FISH) kit and Immunohistochemistry (IHC) used for the diagnosis of the disease are available. NGS is being cited as useful as well but is not yet available for patient use. NGS has been particularly relevant in identifying EML4-ALK protein variants as well as in patients who have progressed after first line treatment with ALK TKI. Reverse transcription polymerase chain reaction (RT-PCR) is no longer recommended for testing to detect ALK rearrangements.[107]

ROS1 rearrangements

The ROS1 locus is located on chromosome 6 and encodes for an orphan tyrosine kinase receptor (i.e., with no known ligand and biologic function in humans) related to ALK, along with members of the insulin-receptor superfamily.[124,108] First discovered as the oncogene product of an avian sarcoma RNA tumor virus, ROS1 (ROS1 proto-oncogene receptor tyrosine kinase) is activated by chromosomal rearrangement in a variety of human cancers, including NSCLC, cholangiocarcinoma, gastric cancer, ovarian cancer, and glioblastoma multiforme.[109-113] Rearrangement leads to fusion of a portion of ROS1 that includes the entire tyrosine kinase domain with one of 14 different partner proteins.[114,115] The resulting ROS1 fusion kinases are constitutively activated and drive cellular transformation, although the exact mechanisms are not very clear.[116] Downstream signaling via SHP-1/SHP-2, JAK/STAT, PI3K/AKT/mTOR and MAPK/ERK pathways have been described.[117] Whether the various ROS1 fusion kinases have different oncogenic properties are not known.

ROS1 rearrangements occur in up to about three percent of patients with NSCLC. As with ALK rearrangements, ROS1 rearrangements are more commonly found in patients who have never smoked or have a history of light smoking and who have histologic features of adenocarcinoma.[118,119] However, at the genetic level, ALK and ROS1 rearrangements rarely occur in the same tumor, with each defining a unique molecular subgroup of NSCLC.

Targeted therapy in ROS1 rearrangements. Crizotinib and other ALK TKIs are the recommended therapy for ROS1 rearrangements.[120] However, only crizotinib has received US FDA approval for treating ROS1 mutant NSCLC patients.[121] The kinase domains of ALK and ROS1 share 77% of their amino acid identity within the ATP-binding sites. In preclinical cell-based assays for inhibition of autophosphorylation of different kinase targets, both ALK and ROS1 are sensitive to crizotinib, with a half-maximal inhibitory concentration of 40 to 60 nM. Also, in cell lines expressing ROS1 rearrangements, crizotinib potently inhibits ROS1 signaling and cell viability. Finally, previous case reports have described marked responses to crizotinib in patients with ROS1-rearranged NSCLC.[119,122]

One apparent difference between ALK and ROS1 rearrangements in NSCLC patients may lie in the durability of the response to crizotinib. In the ALK expansion cohort of 143 patients, the median duration of response was 49.1 weeks, and the median progression-free survival was 9.7 months.123 In comparison, the preliminary estimated median duration of response in the ROS1 cohort was longer, at 17.6 months (75.9 weeks), and the median progression-free survival was 19.2 months.[124] Although the exact reason for this better response is as yet unknown, the more potent inhibition of crizotinib of the ROS1 tyrosine kinase activity can lead to more effective target inhibition and more durable responses. However, it could also be that ALK rearrangement by itself confers a more malignant behavior among the cells.

As in patients with ALK-rearranged NSCLC, resistance to crizotinib eventually develops in NSCLC ROS1 fusion patients. Two distinct mechanisms of resistance to crizotinib in ROS1 fusion NSCLC have been described: a secondary mutation in ROS1 gene that hinders drug binding and activation of EGFR, which enables cancer cells to bypass crizotinib-mediated inhibition of ROS1 signaling. As mentioned previously, more potent, structurally distinct, next-generation ALK inhibitors have been shown to effectively overcome crizotinib resistance in ALK-rearranged NSCLC. Some, but not all, of these new ALK inhibitors also target ROS1. Whether ROS1-rearranged NSCLC will also be susceptible to sequential therapy with increasingly potent inhibitors remains to be determined.[125,126]

Molecular profiling in ROS1 NSCLC. Testing for ROS1 rearrangements are recommended only in NSCLC adenocarcinoma patients.68 ROS1 immunohisto-chemistry (IHC) may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic

method. Alternative testing methods include FISH, NGS, and reverse transcription-polymerase chain reaction (RT-PCR).

BRAF Mutations

Mutations in V-raf murine sarcoma viral oncogene homolog B (BRAF) are identified in two to four of lung adenocarcinomas.[127] BRAF mutation results in activation of the MAPK pathway that promotes cell growth, proliferation, and survival. In a study which looked into the presence of BRAF mutation in 1,046 samples from patients that underwent radical surgery of primary NSCLC, BRAF mutation was detected in 37 tumors (3.5%): 36 in adenocarcinomas and one in squamous NSCLC.[128] About 56.7% of BRAF mutation was V660E. Although this study suggested the association of BRAF mutation with female sex, later studies did not confirm this data.[129,130] Currently, therefore there are no clinical features that can help to identify which patients with NSCLC are likely to present with a BRAF mutation.[131]

Although some reports have correlated BRAF mutation in NSCLC with a poorer outcome and reduced efficacy of platinum doublets, the prognostic implication of BRAF V600E mutated NSCLC remains generally unclear.[132] Additionally, BRAF mutation is one of the mechanisms of resistance to EGFR-TKI that has been reported in one to two percent of cases.[54,93]

Molecular profiling for BRAF mutations. BRAF molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include BRAF as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative.[68] There are several ongoing clinical studies looking at different molecules that can act through the BRAF pathways.[120] Dabrafenib and trametinib are available commercially in other countries for the treatment of NSCLC patients with BRAF mutations. Dabrafenib is a BRAF inhibitor, which attacks the BRAF protein directly while trametinib is a MEK inhibitor and attacks the related MEK proteins. However, these are not commercially available in the Philippines.

Other Mutations

The following discussions are intended to complete this article. Routine profiling for these mutations is not recommended.[68] At this time, these biomarkers may be determined only for the purpose to better predict potential responsiveness to commercially available targeted therapy and/or potentially anticipate and understand TKI resistance, most likely in the research setting. There is no definite targeted therapeutic approach recommended as a result of identifying these mutations outside of an investigative purpose. However, because several studies are ongoing looking for effective targeted therapy selective for these alterations, it may be worthwhile to know about them as the treatment landscape could easily change. In addition, targeted therapy may be available for these alterations in malignancies other than lung cancer, so that the potential of finding suitable drugs to be used for lung cancer may just be a matter of time.

KRAS mutations

A significant proportion of lung cancer patients carry the Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation. Approximately 15–25% of patients with lung adenocarcinoma have tumor associated KRAS mutations.[34,47] In the majority of cases, these mutations are missense mutations which introduce an amino acid substitution at position 12, 13, or 61. The result of these mutations is constitutive activation of KRAS signaling pathways.[133-135]

KRAS is one of three different human rat sarcoma genes (RAS): KRAS, HRAS (homologous to the oncogene from the Harvey rat sarcoma virus), and NRAS (first isolated from a human neuroblastoma). RAS proteins are small GTPases which cycle between inactive guanosine diphosphate (GDP)-bound and active guanosine triphosphate (GTP)-bound forms. RAS proteins are central mediators downstream of growth factor receptor signaling (EGFR pathway) and therefore are critical for cell proliferation, survival, and differentiation. RAS can activate several downstream effectors, including the PI3K-AKT-mTOR pathway, which is involved in cell survival, and the RAS-RAF-MEK-ERK pathway, which is involved in cell proliferation.[136-138]

RAS has been implicated in the pathogenesis of several cancers. Activating mutations within the RAS gene result in constitutive activation of the RAS GTPase, even in the absence of growth factor signaling. The result is a sustained proliferation signal within the cell. Specific RAS genes are recurrently mutated in different malignancies. KRAS mutations are particularly common in colon cancer, lung cancer, and pancreatic cancer.

In the vast majority of NSCLC cases, KRAS mutations are found in tumors with no mutations for EGFR or ALK. Therefore, KRAS mutation defines a distinct molecular subset of the disease. In contrast to the situation with EGFR mutations, KRAS mutations are found in tumors from both former/current smokers and never smokers. They are rarer in never smokers and are less common in East Asian as compared to US or European patients.[139-140]

Even though KRAS mutations are very common in NSCLC and were identified in NSCLC tumors as far back as three decades ago, there is still no clinically successful drug for this mutation in NSCLC.[141] The search for usable drugs continue.[115,142]

The exact role of KRAS as either a prognostic or predictive factor in NSCLC is unknown at this time. KRAS mutations are negative predictors of radiographic response to the EGFR tyrosine kinase inhibitors, erlotinib, and gefitinib.[141,143] Unlike in colon cancer, KRAS mutations have not yet been shown in NSCLC to be negative predictors

of benefit to anti-EGFR antibodies. There have also been recent suggestions that KRAS mutations may predict positive response to immunotherapy.[144] In general, very few prospective randomized trials have been completed using KRAS as a biomarker to stratify therapeutic options in the metastatic setting. Current guidelines state that KRAS molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy in lung cancer. It is appropriate to include KRAS as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative.[68]

MET Mutations

MET, also known as the N-methyl-N7-nitroso-guanidine human osteosarcoma transforming gene is a proto-oncogene encoding a receptor tyrosine kinase c-MET for hepatocyte growth factor (HGF). The natural ligand HGF is secreted by mesodermal cells during development.[145] Upon binding to its receptor, c-MET dimerization and autophosphorylation occurs, which in turn activates downstream effects including proliferation, motility, mitogenesis, protection from apoptosis and morphogenesis. Several intracellular pathways participate in c-Met signaling, including growth factor receptor-bound protein 2 (Grb2), MAP kinase, PI3K, signal transducer and activator of transcription (STAT) and phospholipase C – gamma (PLC-γ).

The c-MET pathway exhibits significant cross-talk with other signaling pathways. Interactions between MET and HER2 family members have emerged as a major mechanism of tumor progression and treatment resistance in EGFR mutated NSCLC.[146] Given the significance of bypass mechanisms involving the MET pathway in EGFR TKI resistance, emerging data suggest that MET inhibitors in combination with EGFR TKIs may have a role in therapy for both EGFR TKI resistant as well as EGFR TKI naïve patients.[147] MET signaling has also been shown to interact with the vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) pathways.[148]

In some cases, the kinases are constitutively active due to mutations in the gene. MET mutations occur at a prevalence of around three percent in adenocarcinomas and around two percent in other lung neoplasms, making them also suitable targets for the treatment of lung cancer. Mechanisms of activation of MET may also be through a variety of alterations, such as protein overexpression, disrupted ubiquitin-mediated degradation causing prolonged stability of the MET protein, gene amplification and MET exon 14 skip mutation.[147] Aberrant MET expression is widely observed in various other malignancies, including gastrointestinal and hepatocellular carcinoma.[149,150]

Several MET-targeted agents (small molecular TKIs and antibodies against HGF or MET) have been investigated.[151] Disappointingly, despite the wide spectrum of MET alterations in NSCLC, randomized trials with MET inhibitors have not resulted in clinical benefit.[152-154] These disappointing results have led to pessimism about the role of MET in the pathogenesis of NSCLC and the validity of MET as a targetable driver in NSCLC.

Recently, re-emergence of MET exon 14 (METex14) splicing alterations in NSCLC has led to renewed optimism of METex14 alteration as a targetable mutation that may lead to the approval of MET-specific inhibitors in NSCLC.[155] At least five MET-targeted TKIs, including crizotinib, cabozantinib, capmatinib, tepotinib, and glesatinib are being investigated clinically for patients with MET exon 14 altered-NSCLC.[156]

At this time, MET molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include MET as part of a larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative.[68] Next-generation sequencing is the most frequently used tool for diagnostic testing of METex14 alterations.[157,158]

RET Rearrangements

Rearranged during transfection (RET) rearrangements are more recently identified oncogenic driver alterations. RET rearrangements occur in one to two percent of non-small cell lung cancer (NSCLC) cases. The RET gene is a receptor tyrosine kinase protooncogene that can acquire oncogenic activity through mutation or rearrangement.[159] RET is located in the pericentromeric region of chromosome 10p11.22–q11.21 and is normally expressed in neurons, sympathetic and parasympathetic ganglia, testis germ cells, urogenital tract cells, adrenal medullary cells, and thyroid C cells.[160] RET ligands are members of the glial cell line-derived neurotrophic factor family. Ligand binding with RET results in autophosphorylation and activation of downstream cellular proliferation, cell migration, and differentiation pathways including RAS/MAPK/ERK, PI3K/AKT, and PLC-γ. While some RET alterations can result in tyrosine kinase inactivity as seen in Hirschsprung disease, RET overactivity is the one seen in a variety of human malignancies, including NSCLC. RET overactivity due to point mutations are associated with medullary thyroid carcinoma, but chromosomal rearrangements are the ones seen in NSCLC.[159,161-167]

The most common fusion partner for RET rearrangements in NSCLC patients is kinesin family member 5B protein (KIF5B) seen in about 50-70% of RET fusion-positive cases of NSCLC.[162] RET fusion with more than 10 other proteins have been described, including coiled-coil domain-containing 6 (CCDC6), nuclear receptor coactivator 4 (NCOA4), tripartite-motif containing 33 (TRIM33), cut like homeobox (CUX1), KIAA1217, FRMD4A, and KIAA1468.[15,159,161] When a fusion partner is present in combination with RET, this results inactivation of the oncogenic tyrosine kinase domain of RET. Subsequently, this leads to autophosphorylation of RET and downstream cell signaling through intracellular pathways such as the mitogen-activated protein kinase (MAPK), PI3K/AKT and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways. RET fusions behaving

in this manner result in oncogenic activation promoting unchecked cellular proliferation.

Since RET rearrangements typically do not co-occur with other well-established oncogenic mutations in NSCLC such as EGFR, KRAS, ALK, HER2, and BRAF, they are believed to harbor independent oncogenic driver potential.[168] They were a little more common in patients with adenocarcinoma than in those with squamous carcinoma (1.73% vs 0.84%). Also, they are more commonly associated with younger age, female gender, non-smokers, and Asian ethnicity.

Currently, an inhibitor specific only for RET is not available, but trials of TKIs with anti-RET activity have been conducted in NSCLC. These include vandetanib, which also has activity against VEGF and EGFR; sorafenib, which has activity against VEGF and BRAF; sunitinib, which has activity against VEGF; and cabozantinib, which has activity against VEGF and MET. The 2019 National Comprehensive Cancer Center Network (NCCN) guidelines recommend the use of cabozantinib and vadentanib targeted therapy for RET rearrangements.1[69] More selective RET inhibitors are being looked into.[159,168] Since RET rearrangement can sometimes accompany EGFR resistance, the use of multikinase inhibitors can improve the survival of the right patients.[166]

RET molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include RET as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative.[68] Moreover, RET oncogenes cannot be adequately detected by immunohistochemistry. FISH, RT-PCR, and NGS are acceptable methods to detect RET rearrangements; with a combination of at least two of these tests being recommended.[170]

ERBB2 (HER2) Alterations

ERBB2 is a member of the ErbB family of RTKs mentioned previously in this article under EGFR mutations. Brief mention was made of ERBB2 as the second most important mechanism for TKI resistance when targeting EGFR mutations. However, aberrations involving ERBB2 is now emerging as a separate oncogenic driver that merits consideration in targeted therapy for NSCLC. It is estimated that as much as two to six percent of lung adenocarcinomas involve ERBB2 and includes both amplifications or overexpressions (one to two percent) and mutations (two to four percent).[171] These numbers are reported to be rising.[172,173] ERBB2 amplification and mutations are typically not associated with each other and are proposed to be clinically distinct driver alterations.[174,175]

As mentioned previously, the ERBB2 receptor does not have a known endogenous ligand for its extracellular domain. It is a proto-oncogene located on the long arm of human chromosome 17 (17q21). ERBB2 forms a heterodimer with other ErB family receptors and causes activation of downstream signaling through the PI3K/AKT and RAS/MAP/MEK pathways. ERBB2 is a preferred dimerization partner of other HER receptors, and ERBB2-containing heterodimers can exert a potent oncogenic signal. ERBB2 amplification and overexpression is known to drive oncogenesis in several other cancer types, such as breast, ovarian, and gastric tumors.[176] In breast cancers, targeted therapies such as trastuzumab and lapatinib are effective in-clinic treatment.[177] However, ERBB2 aberrations in lung cancer showed resistance to these treatments, likely through tissue-specific mechanisms.[178,179] Another study suggested the emergence of de novo PIK3CA mutations and compensatory increases in ERBB2 and ERBB3 copy numbers.[180]

The majority of ERBB2 mutations involve exon 20 insertions, most commonly as a recurrent 12–base pair insertion causing duplication of amino acids tyrosine, valine, methionine, and alanine at codon 775.[181] Most patients with the mutation were never-smokers, female and presented with advanced disease at the time of diagnosis.[172] Unlike EGFR mutations, the prevalence of ERBB2 mutations appears to be similar between Asian and white patient populations. There are a number of studies currently looking into targeted therapy for NSCLC driven by ERBB2 alterations, including monotherapy with TKIs of various generations or in combination with conventional chemotherapy and monoclonal antibodies like trastuzumab emtansine and newer antibodies.[179,182]

Based on expert consensus opinion, ERBB2 molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include ERBB2 mutation analysis as part of a larger testing panel performed either initially or when routine EGFR, ALK, and ROS1 testing are negative.[68] ERBB2 status is also useful as a prognostic factor, with patients found to have overexpression indicating a worse or unfavorable outcome.[173]

VEGF

Generally, tumors cannot grow beyond approximately 2.0 mm in diameter without developing a vascular supply.[183] Not only does neovascularization permit further growth of the primary tumor, but it also provides a pathway for migrating tumor cells to gain access to the systemic circulation and establish distant metastases. Angiogenesis, whether physiological or pathological, is governed by a host of proangiogenic and antiangiogenic factors.[184] Among these, vascular endothelial growth factor (VEGF) is the most potent and specific of the endothelial cell mitogens, acting both as an endothelial cell survival factor and as a key factor in mobilizing circulating endothelial cell precursors to nascent blood vessels. Not only does VEGF promote the vascularization and growth of the primary tumor, but it also appears to play a key role in the early establishment of new metastatic foci. VEGF mRNA is upregulated in the majority of human tumors and this tends to correlate with poor prognosis.

The mechanisms causing increased expression of VEGF remains largely unknown. In NSCLC, EGFR activation has been shown to be associated with the stimulation of tumor angiogenesis.[185] Current data also suggest that EGFR signaling pathway modulates angiogenesis by way of upregulation of VEGF or other key angiogenic factors. This certainly appears to be the case in NSCLC where high serum VEGF level is associated with increasing intra-tumoral angiogenesis and poor prognosis.[186]

Given the role that VEGF appears to play in NSCLC carcinogenesis, inhibition of VEGF provides a particularly attractive strategy for antiangiogenic therapy in cancer.[187] In fact, the potential use of anti-angiogenesis agents in lung cancer is one of the earliest attempts to apply targeted therapy. However, clinical trials failed to attend the promising expectations deriving from preclinical studies with anti-VEGF agents in NSCLC. The occurrence of resistance mechanisms limits the use of anti-VEGF drugs in lung cancer therapy. Such resistance may arise because of the ability of mutant EGFR to activate alternative angiogenesis pathways such as the gp130/JAK/STAT3 signaling pathway by means of IL-6 upregulation.[188] Other resistance mechanisms have also been proposed.[189,190] It has been stated that strategies to overcome resistance to VEGF inhibition via dual targeting and to identify predictive biomarkers should be priorities on the research agenda for antiangiogenic therapy.[191,192]

Potential strategies to improve the efficacy of anti-VEGF therapies in lung cancer might be the employment of combinatory therapies with chemotherapy, immunotherapy or agents that inhibit signaling pathways and proangiogenic factors activated in response to VEGF blockade.[193] The interim results of an ongoing study on EGFR positive NSCLC patients showed that bevacizumab (an anti-VEGF monoclonal antibody) plus erlotinib combination therapy improves progression-free survival compared with erlotinib alone.[194] Bevacizumab is also approved for use in combination with atezolizumab (an immune checkpoint inhibitor), paclitaxel and carboplatin in metastatic NSCLC who are EGFR and ALK negative. Bevacizumab is available in the Philippines. It is however associated with significant side-effects.

PIK3CA Mutations

Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) is a 110 kDa catalytic subunit which along with an 85 kDa regulatory subunit comprises the complete enzyme phosphatidylinositol 3-kinase. The catalytic subunit p110α is encoded by the PIK3CA gene on chromosome 3q26.3. The phosphatidylinositol 3-kinases (PI3K) play a pivotal role in cell metabolism, proliferation, cell survival, degranulation, vesicular trafficking, and cell migration.[195]

Mutations in the PIK3CA gene are commonly found in a variety of cancers.[196] In NSCLC, mutations within PIK3CA usually affecting the helical binding domain (exon 9, E545K or E542K) or the catalytic subunit (exon 20, H1047R or H1047L), are considered oncogenic and targetable. However, in contrast to classical oncogenic driver mutations like activating EGFR mutations, PIK3CA mutations in lung adenocarcinomas have not been described to be mutually exclusive. Rather, co-occurrence with aberrations in EGFR, BRAF, ALK and, most frequently, KRAS was found. These observations raise the question of whether the PIK3CA mutation alone is a sufficient oncogenic driver in NSCLC tumor formation.[197-199]

A small series of four patients with squamous cell NSCLC (SQCC) and PIK3CA mutations have been identified resulting in a frequency of 4.2% in the described cohort.[200] Another study involving 1,144 patients reported the frequency of PIK3CA mutations overall as 3.7%. Among patients with squamous cell carcinoma, it was reported as 8.9% (16/179) and in those with adenocarcinoma as 2.9% (25/859) with the difference being statistically significant.201 The most common mutations occurred in Exon 9, particularly E545K (57.1%) and E542K (14.3%) as well as in Exon 20, H1047R (16.7%). KRAS, BRAF, and EGFR mutations were also noted. Unexpectedly, among those with PI3KCA mutations, 18 patients (42.9%) suffered from NSCLC occurring as a secondary malignancy. The authors state the need to further look into this. Another study reported a greater occurrence of PIK3CA mutations among NSCLC patients who also had COPD.

Accordingly, specific PI3K inhibition in solid tumors did not lead to impressive response rates in mutated patients yet. Up to now, PI3K-inhibitors have not yet proven to be clinically effective, at least in lung cancer, in the majority of patients with PIK3CA mutation.[202,203] It has been suggested that PIK3CA mutated NSCLC is a clinically and genetically heterogeneous group and do not define a distinct lung cancer subgroup amendable to specific therapy.[201]

In addition, although previously described to explain resistance to EGFR TKIs, a study looking into this showed that among the 344 EGFR TKI-treated EGFR mutant patients, there was no significant difference in treatment response (p=0.476) and progression-free survival (*p*=0.401) between PIK3CA mutation-positive and negative patients.[202] In contrast, among patients with early-stage squamous cell carcinoma, PIK3CA mutations appear to predict a survival advantage.[203-205]

The exact role and significance of PIK3CA mutations in NSCLC therefore remain unclear as of this time.

Other Considerations

Costs. In the Philippines, where treatment for lung cancer is not fully covered by health insurance the primary consideration is costs related to diagnosis and treatment. Malignancies are particularly impacted because of the unexpected nature of the disease as well as the urgency involved in its treatment. This is more so true for targeted

therapies for lung cancer as not only are the drugs very expensive; one must also add the costs of diagnostic and other follow up tests that are also very expensive.

To be fair, these issues of costs related to care of patients with lung cancer using targeted therapies are also true in other countries.[206] However, these are somehow mitigated because most of these countries have national health services which fund therapy – although possibly not all of it. In the EU, it has been estimated that cancer cost amounts to €126 billion in 2009 with lung cancer having the highest economic cost amounting to €18.8 billion or 15% of overall cancer costs.[207] Perhaps new in this cost matrix is the significantly high costs of diagnostic and monitoring tests.[208,209] Again, this is particularly significant in this era of targeted therapy because of the need to profile at the outset specific mutations as well as continue to look for new mutations as treatment progresses. A study, for example, reported that at current costs, by WHO cost-effectiveness threshold criteria, osimertinib is not cost-effective as a first-line therapy of EGFR-mutated NSCLC.[210]

In the Philippines, recourse through public funding for treatment of lung cancer is very limited. At this time, Philhealth's Z benefit packages are available only for cancers of the breast, colorectal, prostate, cervix and acute lymphocytic leukemia.[211-213] The Philippine Charity Sweepstakes Office (PCSO) through its Individual Medical Assistance Program (IMAP) specifically mentions targeted therapy as one of the allowable services which patients can avail of.[214] The amount provided however is not standard nor guaranteed continuously and is determined on a case to case basis.

Availability of molecular profiling. At this time, EGFR and ALK testing are commercially available in select private tertiary medical centers. Of mention is the Cobas® EGFR Mutation Test v2 which is a real-time PCR test that identifies 42 mutations in exons 18, 19, 20 and 21 of the epidermal growth factor receptor (EGFR) gene, including the T790M resistant mutation.[215] It is designed to enable testing of both tissue and plasma specimens with a single kit, and allows labs to run tissue and plasma on the same plate simultaneously. Included is a cell-free DNA (cfDNA) sample preparation kit to optimize extraction of DNA from plasma. The costs would depend on the panels to be selected as well as the individual pricing of the hospitals.

The Philippine Genome Center has also been put up with among its listed projects the "Development of Mutation Detection Assay for Identifying likely Responders to EGFR-targeted Therapy" as well as the determination of KRAS, PIK3CA, BRAF, PTEN and AKT1 biomarkers which are also useful in NSCLC.[99] At this time, however, the Center does not offer testing of individual patients for diagnostic purposes.

Availability of the drugs. If a patient can afford the targeted therapy, it is another question whether or not it is available commercially in the Philippines. At this time, the following TKIs can be bought through the respective drug distributors: gefitinib, afatinib, ceritinib, osimertinib, and crizotinib.

Alternatively, patients can be enrolled in ongoing researches locally. These have the benefit of free treatment. Physicians can consult websites where some of these ongoing clinical trials in the Philippines can be listed.[216,217] Of course, patients will be screened for possible inclusion and in most cases will not be able to choose which particular treatment arm they will be assigned. The clinical trial sites are usually located in larger tertiary medical centers, usually in the National Capital Region or bigger regional cities, and may not be conveniently accessible for those coming from farther away.

Lastly, drugs that are commercially available in other countries can be potentially brought into the Philippines through the compassionate use permit being issued by the local FDA.[218] Certain requirements have to be fulfilled by the attending physician to avail of drugs through this route.

Adverse effects. The term targeted therapy suggests the absence of or at least minimal undesirable adverse events such as those commonly seen in the use of systemic chemotherapy. Although this may be true relatively speaking, and TKIs are generally safe, their use is not necessarily innocuous and not without its dangers. Although the TKIs share the ability to inhibit the activity of the mutated tyrosine kinase, the list of adverse effects varies across individual drugs. Many of these side effects may not even be explainable by their known mechanism of action. We are not able to list these down in detail but most of these could be grouped into events affecting the skin, the heart, blood, lungs and gastrointestinal system.[219] Although adverse events severe enough to require the discontinuation of the drug are quite rare (usually about five percent of the subjects in most of the clinical trials involving the TKIs) they can occur which can significantly affect the patient's well-being, treatment compliance and quality of life. Adjusting the dose and/or treating the adverse event prophylactically as needed, as well as patient education in anticipation of the events are recommended approaches.[85,220-223]

Conclusion

The treatment of patients with lung cancer, particularly advanced NSCLC is now in an era of significant improvement in outcomes with the use of targeted therapy in select patients. Understanding the various relevant mutations and alterations are key in selecting the appropriate molecular profiling targeted therapy and follow up tests needed to manage such patients. In the Philippines, a suitable selection of drugs are available but other considerations, most especially costs of the diagnostic tests and the drugs for targeted therapies with a lack of financial support mechanisms can be serious barriers for most patients.

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