Targeted agents in non-small cell lung cancer therapy: What is there on the horizon?

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Abstract
Lung cancer is a heterogeneous group of diseases. There has been much research in lung cancer over the past decade which has advanced our ability to treat these patients with a more personalized approach. The scope of this paper is to review the literature and give a broad understanding of the current molecular targets for which we currently have therapies as well as other targets for which we may soon have therapies. Additionally, we will cover some of the issues of resistance with these targeted therapies. The molecular targets we intend to discuss are epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), anaplastic large-cell lymphoma kinase (ALK), KRAS, C-MET/RON, PIK3CA, ROS-1, RET, fibroblast growth factor receptor (FGFR), Ephrins and their receptors, BRAF, and immunotherapies/vaccines. This manuscript only summarizes the work which has been done to date and in no way is meant to be comprehensive.

Keywords: Cellular mechanism, HGF, MET, oncogene, receptor tyrosine kinase, targeted cancer therapy

INTRODUCTION
Lung cancer is a world-wide problem. In the United States, there are approximately 226,000 new cases annually with an estimated 160,000 deaths.[1] It is the largest cause of cancer deaths in the United States. Survival rates have improved slightly since the 1990’s.[2] However, most patients still present with inoperable disease. Until the last decade, we have treated this disease with a “one size fits all approach.” Early data in the treatment of metastatic non-small lung cancer (NSCLC) suggested that all platinum-containing doublets were equally efficacious in prolonging progression-free survival (PFS) and overall survival (OS).[3] More recent findings have suggested that histology plays a role in the treatment outcome. Scagliotti et al., data were notable for a slight improvement in OS and PFS with platinum and pemetrexed for non-squamous histology whereas, platinum and gemcitabine had a slight advantage in squamous cell histology.[4] Additionally, there has been a small subset of patients who respond to the newer targeted agents as was initially seen with the drug gefitinib, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI).[5] There are many more targets that are being discovered and studied, some which may play a role in the treatment of this dread disease.

To date, there have been multiple driver oncogenes described predominantly in adenocarcinoma of the lung of those who were never or light smokers. These include, EGFR, anaplastic large-cell lymphoma kinase (ALK), v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), MET - is a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor, Recepteur d’Origine
Nantais (RON), ROS1 a newly explored chromosome translocation, Ephrin type-B receptor -4(EPHB4), Ephrin type-A receptor -2(EPHA 2). In squamous cell carcinoma of the lung, Phosphatidylinositol 3-kinases (PI3K) and Fibroblast growth factor receptor (FGFR) alterations have been identified. This is an exciting time in non-small cell lung cancer treatment as the development of targeted therapy has afforded us a more personalized approach. We have clinically effective therapies for patients with NSCLC whose tumors harbor EGFR mutations, ALK rearrangements, and ROS-1 rearrangements which may result in survival prolongation.[6-11]

Despite these advances, we still have a long way to go to better treat our patients. The scope of this paper is to describe the targets for which there are therapies or resistance is an issue. Additionally, we will describe some of the newer targets and immune therapies under investigation.

**EPIDERMAL GROWTH FACTOR RECEPTOR**

Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK), is involved with cell differentiation, proliferation, angiogenesis, and apoptosis. Initial responses to EGFR TKI were seen in a non-selective Japanese population with gefitinib.[12] Following an open access trial where all patients with NSCLC were treated with gefitinib, the clinical characteristics of these patients noted to respond were described as Asian females with adenocarcinoma (bronchoalveolar carcinoma), and were non-smoking or light smokers.[13] It was not until the work of Lynch and Paez, that the EGFR molecular mutations that were targeted were described.[14,15] Currently, patients are routinely tested for EGFR mutations and recent trials have demonstrated that patients harboring activating mutations for EGFR, with metastatic lung cancer, should receive an EGFR-TKI as initial treatment.[9,16,17] We also have evaluated EGFR-TKI’s with the addition of chemotherapy that has not been successful to date.[18,19] Patients harboring EGFR mutations initially respond well to EGFR-TKI’s however, acquired mutations do occur while on therapy which can render the patient resistant to the available drugs. These mutations include EGFR T790M point mutation. The strategies currently in development to overcome resistance include the use of oral irreversible, small molecules or Human Epidermal Growth Factor Receptor and pan-human epidermal receptor (pan-HER) inhibitors. These drugs include afatinib, neratinib, peltinib, (Astra Zeneca) AZD8931, canertinib and (Pfizer) PF299.[20] Studies are ongoing to evaluate these drugs in patients with acquired resistance to EGFR inhibition. MET upregulation can also account for acquired resistance in these patients. Studies directed at inhibiting MET along with continued EGFR-TKI therapy are being carried out to evaluate overcoming acquired resistance. Drugs in development targeting C-met include ARQ197 (ArQule) a TKI and Met MAb (Onartuzumab) a monoclonal antibody.[20]

**ANAPLASTIC LARGE-CELL LYMPHOMA KINASE**

Anaplastic large-cell lymphoma kinase (ALK) is a more recent target of a RTK which is promising in NSCLC. It is in the insulin receptor superfamily and its precise function is not well understood. Activating mutations or translocations of ALK have been found in a few different types of malignancies and most notably, initially found in lymphoma for which it was named. In NSCLC, echinoderm microtubule-associated protein-like 4 EML4-ALK is the most common of a group of aberrant fusion genes occurring in 2-7% of patients typically found in never or light smokers.[17] Patients that harbor a mutation are often susceptible to targeted kinase inhibition with crizotinib.[17] This compound recently received FDA approval for use in patients harboring ALK mutation with NSCLC. Resistance to this compound develops over time and the mechanism is unclear. Currently, heat shock protein-90 (HSP-90) has been identified as a potential target for crizotinib resistance and is being evaluated for patients who become resistant to crizotinib. Ganetespib has demonstrated some activity in EML-4ALK-mutated patients and is currently in study. There is much work to be done to evaluate the mechanisms of resistance to better target this group of patients.

**KIRSTEN RAT SARCOMA VIRAL ONEOGENE HOHOLOG (KRAS)**

In NSCLC, KRAS mutations occur predominately at codon 12 or 13 most often in patients with a history of tobacco use.[21,22] Mutations are responsible for KRAS activation which commonly occurs in NSCLC. This is most common in patients with adenocarcinoma (30%), although approximately, 5% of patients with squamous cell carcinoma may have activation.[23,24] KRAS mutations in NSCLC patients are believed to be a negative prognostic indicator but, this too is controversial.[25,26] Studies which evaluated the use of EGFR inhibition both by monoclonal antibody as well as TKIs failed to demonstrate a difference between KRAS mutants and an unselected population of NSCLC for response rates, overall survival (OS), and progression free survival PFS when EGFR inhibition was used.[27-30] Understanding of the clinical implications and biologic role
of KRAS mutations in NSCLC has remained elusive.[24] It is believed that rat sarcoma RAS proteins function as guanosine diphosphate/guanosine triphosphate-regulated binary on-off switches. RAS mutants tip the regulated switch to on, leading to independent and persistent activation of the signaling pathway Raf-MEK-ERK cascade. This cascade is associated with proliferation, metastasis, and survival of the malignant cell.[31-34] Additionally, recent studies have also demonstrated that RAS uses additional effectors to promote tumorigenesis including BRAF and Phosphatidylinositol 3-kinase catalytic subunit PIK3CA.[35] Currently, there are no targeted agents that have proven to be efficacious in the KRAS mutation population. In NSCLC the response rates to EGFR inhibition with monoclonal antibody is the same with or without KRAS mutations, unlike the findings in colorectal cancer.[36-39] There are no direct inhibitors of KRAS, but, it appears there may be potential targets which function downstream of RAS. These include the RAS/RAF/MEK pathway. This pathway includes many proteins including mitogen-activated protein kinases which was originally called extracellular - signal - regulated kinases (ERK). This pathway acts as an on / off switch by adding phosphate groups to neighboring proteins. Sorafenib is a weak inhibitor of proto-oncogene RAF but, MEK appears more promising.[40-41] The BATTLE trial initially demonstrated a benefit with sorafenib in KRAS-mutated NSCLC patients, however, this did not ultimately prove out.[42] There are however, multiple inhibitors of BRAF GlaxoSmithKline (GSK2118436) and MEK (selumetinib) under investigation in this population.

**C-MET/RON**

MET is part of the RTK family. It is a proto-oncogene which encodes for the protein hepatocyte growth factor receptor. Its natural ligand is hepatocyte growth factor and scatter factor. MET’s role in carcinogenesis is activation of oncogenic pathways such as RAS, PI3K, Signal transducer and activator of transcription 3 (STAT-3), and Beta-catenin, angiogenesis, and metastasis.[29] MET can be activated by mutations, autocrine/paracrine growth, overexpression by gene amplification, or decreased degradation.[43] MET gene mutations and amplification has been reported at low frequency, but as predictors of therapeutic sensitivity.[44] Studies have suggested that approximately 40% of lung cancer tissue overexpresses MET.[45] Amplification have been described in EGFR resistance and studies are ongoing to overcome EGFR resistance with addition of MET inhibition.[46] Clinical studies are ongoing evaluating Foretinib (multikinase Met Inhibitor), MetMaB (single-arm humanized anti-Met antibody), Exelixis compound XL-184 [Kinase inhibitor of MET, vascular endothelial growth factor receptor 2 (VEGFR2) and rearranged during transfection, (RET)], ficlatuzumab, and preclinical studies with MedKoo Biosciences/Pfizer compound PHA665752. All of these clinical trials are evaluating MET inhibition in EGFR-acquired resistance.[45]

RON is a MET-related RTK. Macrophage stimulating protein is its natural ligand. Beta-1-integrins can also activate RON via c-Src-dependant signaling pathways.[47] RON is localized to chromosome band 3p21.3, a region known for tumor suppressor function and loss of heterozygosity.[48,49] Its role is regulation of inflammation and contributes to growth and metastasis. Ron signaling has a major effect on the motility and activation of macrophages. In lung cancer, however, the role is very synergistic or additive with MET which promotes transformation, cell spreading, and motility as well as promotes survival.[50] MET and RON are both implicated in tumor progression and development of metastasis.[52-54] methylene incorporated compound MGCD265 is a multikinase inhibitor directed against c-MET, VEGR1, 2, 3, RON, and Tie-2, and is currently in early clinical trials.

**PIK3CA**

Phosphatidylinositol-3-kinase p110 alpha catalytic subunit isoform (PIK3CA) amplification, and to a lesser extent, mutations are seen in NSCLC.[55-57] PIK3CA mutations and amplification may be involved in EGFR resistance.[58] protein kinase B/mammalian target of rapamycin PI3K/AKT/mTOR pathway is activated in early stages of development of lung cancer.[59] AKT regulates cell survival in tumors and has been implicated in the oncogenesis and progression of lung cancer.[60] PI3K is activated by EGFR stimulation which subsequently activates AKT. Activation of PI3K and AKT signaling occurs with somatic mutations of PIK3CA clustering in exons 9 and 20.[60,61] PIK3CA amplification has been reported in approximately 15% of patients with NSCLC.[36,57,62] These mutations and amplifications appeared to be associated with poor survival and resistance to treatment with EGFR TKIs.[60,62]

Drugs which appear to interfere with this pathway include inhibitors of mTOR, AKT, and PI3K. Currently, many of these targeted agents are under development in the treatment of NSCLC both with and without the use of cytotoxics. PI3K inhibitors include Novartis Pharmaceuticals BKM120, Genentech and Exelixis GDC0941, and XL-147, respectively. AKT inhibitors include Merck MK 2206. mTOR inhibitors include sirolimus, everolimus and temsirolimus. Of note, Novartis BEZ235 is a dual PI3K and mTOR inhibitor which appears promising in early clinical development.[64,65]
ROS1

Ros1 is a newly explored chromosomal translocation and is a member of the RTK of the insulin receptor family in lung cancer although it has been described in other tumors. As this is a new target, little is known about tumors which possess this translocation. A recent study demonstrated approximately 2% of patients possess this translocation and the patients typically have a similar profile to patients with EML4-ALK translocation. The study also demonstrated cell-line sensitivity to crizotinib. Patients with ROS1 translocation were enrolled into an expansion access cohort of an early phase of crizotinib development with promising results.

RET/RET FUSION

RET has been described in multiple endocrine neoplasia type 2 (MEN 2) syndrome and sporadic medullary thyroid cancer. RET is involved with cell proliferation, neuronal navigation, cell migration, and cell differentiation. More recently, a novel gene fusion involving RET tyrosine kinase and either KIF5B or CCDC6 was reported in lung adenocarcinomas which is similar to those translocations found in thyroid cancers. The patients who seemed to have these translocations tended to be younger in age, never-smokers, had early lymph-node metastases, poor differentiation, and a solid-predominant subtype. The RET fusion gene was evaluated in 936 patients with surgically resected NSCLC and found to occur in 1.4% of never-smokers, had early lymph-node metastases, poor differentiation, and has been under investigation. BGK951 is a small molecule inhibitor that inhibits RET tyrosine kinase and either KIF5B or CCDC6. This compound showed some promising results for patients with NSCLC as clinically available TKI's such as sunitinib, sorafenib, and vandetanib are commercially available. Cells expressing Kinesin heavy chain isoform 5A -RET protoncogene KIF5B-RET were noted to be sensitive to multitargeted kinase inhibitors that inhibit RET. Additionally, these drugs have been shown to target RET kinase and have shown activity in patients with thyroid cancer.

FIBROBLAST GROWTH FACTOR RECEPTOR

Fibroblast growth factor receptor (FGFR) is a membrane-bound tyrosine kinase which binds to fibroblast growth factor (VEGF) and EGFR. Gly388ARG polymorphism is associated with a poor prognosis. Mutations of FGFR are rare. FGFR is amplified in approximately 20% and appears to be particularly important in squamous histology NSCLC. It is implicated in epithelial to mesenchymal transition responsible for invasion, metastasis and resistance to EGFR inhibition. FGFR signaling appears to be important in squamous and large cell histology NSCLC where EGFR resistance is common. Currently, Small molecule inhibitors brivanib, dovitinib, Astra-Zeneca compound (AZD4547), and Taibo compound (TSU-68) are in clinical trials. A Soluble fusion protein FGF ligand trap, FP-1039 is in clinical trial as well. Monoclonal antibodies are currently in early development including AV369, AV269b, and AV370.

VASCULAR ENDOTHELIAL GROWTH FACTOR

Angiogenesis is important in the development and maintenance of human tissues including malignancies. Angiogenesis has been studied and found to be promising in cancer since Dr. Folkman’s initial studies. Vascular endothelial growth factor (VEGF) is believed to play a specific and crucial role in the regulation of angiogenesis and has been under investigation. Angiogenesis is an early event in tumor development and is important in tumor growth and metastasis. The ability to feed tumor growth depends on the balance of many molecules released by tumor cells and the surrounding host tissue. There are many different processes involved in angiogenesis and involve many other mediators including multiple VEGFRs, plasminogen activators, matrix metallo-proteinases, transforming growth factor – Betas, and platelet-derived growth factor, inhibitors of matrix metalloproteinase, and many others. As our knowledge has grown so has the number of agents which target angiogenesis. The ECOG 4599 trial was the first study in non-squamous cell lung cancer which showed some promising results for inhibition of angiogenesis with bevacizumab when added to carboplatin and paclitaxel. There are many new small TKIs currently in development.

EPHRINS AND THEIR RECEPTORS

Erythropoietin producing human (Eph hepatocellular carcinoma) is the largest group of RTKs in the genome. There are two classes of receptors A and B based on sequence conservation and mutual interactions or binding affinity. In humans, there are a total of 14 Eph receptors known, in Class A, there are nine and class in B, there are five and eight Ephrin ligands for class A and B respectively.
suggests that Eph promotes tumor growth, invasion, metastasis, and neovascularization. Signaling between the ligands and/or receptors has emerged as likely key mechanism in tumor-suppressor function[101-103] Eph A2 and Eph B4 function as oncogenes however, there is conflicting evidence as they also appear to have tumor-suppressor function.[104,105] Eph – RTKs, particularly within the A class appear to play a role in tumor progression as well as suppression.[101] EphA2 expression may be prognostic in NSCLC-adenocarcinoma for the development of metastatic disease, particularly CNS metastasis and is elevated in patients with a history of tobacco use.[101,102] Conversely, low levels of EphA2 appear to be associated with a good prognosis.[101] Mutations in EphA2 appear to increase activation and promote invasion of the malignancy.[106] Multiple somatic mutations have been identified in Eph A3, frequently, mutations are found in adenocarcinoma of the lung and the role this plays is unclear.[101,107-110] In-vitro studies suggest a possible tumor suppressor role for Eph A3 in NSCLC.[101] Eph B3 correlates with tumor growth and promotion.[111] Cross-talk between Class A and B Eph may play a critical role in tumor regulation and tumor progression.[101] As we go forward, EPH targeting (especially EPHB2 and EPHB4) will likely become very important.

**BRAF**

BRAF mutations have been reported in numerous solid tumors including melanoma, thyroid cancers, colorectal cancer, and some ovarian cancers.[112-115] More recently, BRAF mutations have been described in NSCLC.[116,117] There have been somatic mutations described predominately in females with lung adenocarcinoma which arise independent of smoking history.[118,119] Additionally, BRAF mutations may also be found rarely in squamous cell carcinoma of the lung and may not be mutually exclusive with EGFR mutations.[118] BRAF mutations appear to be associated with a poor prognosis and frequently histologically showed micropapillary features.[118,119] BRAF is believed to be involved in early events of lung cancer tumorigenesis.[120] Preclinical data suggest BRAF mutations might predict sensitivity of NSCLC cells to MEK inhibitors.[121,122] BRAF inhibitors currently under development in NSCLC include Vemurafenib, GSK2118436, and CEP32496. MEK inhibitors under development in BRAF mutated NSCLC include Selumetinib.

**VACCINES**

Vaccines and immunotherapy have fallen out of favor until recently when re-exploration of this technique has revealed some limited responsiveness, although the lung cancer community remains cautiously optimistic. Past exploration with immune therapy has been unsuccessful due to the heterogeneity of lung cancer. Additionally, tumor response rates have been low and efficacy needs enhancement with combination therapy. The primary objective of vaccination is to provoke an adaptive antitumor immune response.[122-124] Numerous vaccines and immunotherapies are currently in clinical studies for NSCLC. These include MAGE-A3 which is a tumor-specific antigen present in 30-50% NSCLC patients. The MAGRIT phase III study for vaccination in NSCLC evaluates patients post-operatively with or without chemotherapy with disease-free survival as the primary endpoint.[125] MUC1 vaccination randomized MUC1-positive patients with advanced NSCLC to chemotherapy with or without vaccination. Initial studies demonstrated an increased OS hence a larger study is ongoing.[125]

**PD-1/PDL-1**

Treatment of cancer by immune response has been tried in many tumor types and has become the standard treatment in some malignancies such as melanoma. The immune system in the past has been pursued in lung cancer but, with only anecdotal success. Lung cancer is considered not to be responsive to immunotherapy.[126] Recently, there has been renewed interest in harnessing the immune response for treatment of lung cancer. Most interestingly, there has been much work with PD-1. Programmed death 1 (PD-1) protein is a T-cell coinhibitory receptor which is similar in structure to cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4).[127] There are two known ligands for PD-1, PD-L1 (B7-H1), and PD-L2 (B7-DC).[128-131] The interaction between PD-1 and PD-L1 has been shown to down-modulate T-cell responses in-vitro and in-vivo.[132-136]

In a recent trial, an objective response was noted in 5 of 49 patients (10%) with advanced NSCLC who received anti-PD-L1.[137] Additionally, in a companion trial evaluating anti-PD-1 antibody, an 18% response rate was seen in patients with NSCLC (14 of 76 patients).[137] Both studies did show durable responses with these therapies across all tumor types.[127,137] Additional work is ongoing.

**CONCLUSION**

This is an exciting time in NSCLC research and treatment. There are numerous molecules which have been identified as potential treatment targets. There is a frenzy of research being carried out which, has begun to demonstrate on a molecular level the amount of histological and molecular heterogeneity which exists in NSCLC cells. Additionally, we now see that patients with some of these molecular
targets may have new treatment options that may result in prolonged survival and improved quality of life. While we have made great advances, we have much more work ahead of us. All of these efforts and knowledge however, bring us closer to a more personalized approach to our patients’ care.

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