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The Impact of Genomic Profiling for Novel Cancer Therapy–Recent Progrss in Non-Small Cell Lung Cancer

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Abstract

There is high expectation for significant improvements in cancer patient care after completion of the human genome project in 2003. Through pains-taking analyses of genomic profiles in cancer patients, a number of targetable gene alterations have been discovered, with some leading to novel therapies, such as activating mutations of *EGFR, BRAF* and *ALK* gene fusions. As a result, clinical management of cancer through targeted therapy has finally become a reality for a subset of cancer, such as lung adenocarcinomas and melanomas. In this review, we will summarize how gene mutation discovery leads to new treatment strategies using non-small cell lung cancer (NSCLC) as an example. We will also discuss possible future implications of cancer genome analyses.

Keywords

Lung cancer; targeted therapy; EGFR; ALK

1. INTRODUCTION

Targeted therapy is a special type of chemotherapy that targets the specific difference between cancer and normal cells. The concept of targeted therapy has been evolving over the years. In early years, targeted therapy means to selectively kill cancer cells based on a high cell proliferation rate of the cancer cells. In the last 10 years, significant progress in cancer treatments has been made through identification of novel genetic alterations in the cancer genome. Strategies in targeted therapy are largely based on gene mutation, pathway activation and alterations in the immune system. The most significant progress is the use of inhibitors for mutant kinases.

Discovery of BCR-ABL tyrosine kinase inhibitor STI571 (other names include imatinib mesylate and Gleevec) by Novartis scientists and successful clinical trials in CML patients by Drs. Druker and Sawyer (Druker et al., 2001a, 2001b) established a major milestone for targeted therapy, and promoted imatinib mesylate as the first-line drug for treatment of CML

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This article is dedicated to the memory of Wei Wang, who died of NSCLC five years ago.

with BCR-ABL gene fusion. Because imatinib mesylate also targets several other kinases, such as c-kit and PDGFRA, it has been shown to be effective for treatment of GI stromal tumor (GIST) (Demetri et al., 2002), NF1 (Robertson et al., 2012) as well as a subset of melanoma with c kit expression (Carvajal et al., 2011; Guo et al., 2011).

2. MAJOR GENETIC ALTERATIONS IN non-small cell lung cancer (NSCLC)

Lung cancer is the leading cause of cancer-related death, claiming ~158,000 American lives every year (which exceeds the combined mortality from breast, prostate and colorectal cancers) (Siegel et al., 2015). However, research in lung cancer is still under-funded, with $1442 per lung cancer death in comparison of $6849, $13419 and $26398 for colon, prostate and breast cancer respectively (Foundation, 2015). Currently, the 5-year survival rate for all types of lung cancer is ~17%. The majority of all newly diagnosed cases are patients with advanced lung cancer, which have a median survival of ~12 months following the first-line chemotherapy.

Common genetic alterations in lung cancer include *p53* mutations/deletion, *p16* gene silencing through methylation, *LKB1* loss-of-function mutations and activating *KRAS* gene mutations. Overall, three major signaling pathways are affected in lung cancer: p53 signaling, the RB/p16 signaling axis and the RAS signaling. Mutations or deletions of *p53* occur in 50% of NSCLC (Robles et al., 2002; Cooper et al., 2013). Although there are several strategies to target p53 signaling for cancer therapies, no drugs are now available for cancer treatment. P53 is regarded as the guardian of the genome, and *p53* gene mutations result in many changes in the cancer genome (Lane, 1992; Khoo et al., 2014).

Inactivation in *p16*, via CpG island methylation, chromosomal deletion or point mutation, commonly occurs (~50%) in NSCLC although RB mutations are not common in NSCLCs (Otterson et al., 1994; Liggett and Sidransky, 1998; Sanchez-Cespedes et al., 1999). The p16 protein inhibits phosphorylation of Rb through CDK4 (hence *in*hibitor of *k*inase *4*, or INK4) (Liggett and Sidransky, 1998). It is known that *p16* hypermethylation is associated with poor prognosis (Jin et al., 2001; Kim et al., 2001; Ng et al., 2002). In addition, Cyclin D1 is highly expressed in 47% of NSCLCs, which is also associated with a poor prognosis (Jin et al., 2001). Cyclin D1 inhibits RB function by enhancing RB phosphorylation by Cdk4. Furthermore, a second protein p14ARF that is encoded by the p16 locus, is transcribed from an alternate reading frame but results in a totally unrelated protein (Sanchez-Cespedes et al., 1999). The p14ARF protein prevents MDM2-mediated p53 degradation, resulting in p53 activation. The *p14ARF* gene inactivation is found in 19–37% of NSCLCs (Sanchez-Cespedes et al., 1999; Sherr, 2001; Sherr and McCormick, 2002).

The RAS signaling pathway is frequently activated in lung cancer through mutations of several genes, including activated gene mutations in several growth factor receptors (see more below), KRAS and PIK3CA as well as loss-of-function gene mutations in *PTEN* and *LKB1*. While PTEN loss increases PIK3CA activity, LKB1 loss-of-function promotes mTOR signaling. Taken all together, almost all cancer cells have elevated RAS signaling (Cooper et al., 2013). Furthermore, in tumors with *LKB1* inactivation, metabolism

inhibitors, such as phenformin, are predicted to be more effective in NSCLC treatment (Liu et al., 2013; Shackelford et al., 2013).

In the last few years, several targetable oncogenic mutations have been discovered in lung adenocarcinomas, including EGFR, HER2, FGFR1 and c-MET (reviewed in Thomas et al., 2015). Additionally, several gene fusions involving *ALK, RET*, and *ROS-1* have been reported. Other gene mutations include activating mutations in the PI3K/AKT pathway (PIK3CA and AKT) and the BRAF/MEK signaling (BRAF and MEK1/2).

EGFR gene mutation is often mutually exclusive from KRAS gene mutation. The same is true for ALK fusion and KRAS gene mutation, indicating that these are the driving mutations for NSCLC. While the specific inhibitors for KRAS are not clinically available, several specific small molecule inhibitors have been developed to target RAS downstream molecules, and have been approved for cancer treatment.

It is worth noting that the frequency of gene mutation varies among different patient population (Couraud et al. 2012). For example, *EGFR* gene mutation occurs only in 5% of American cancer patients who are current smokers, in 28% of never-smoking American patients, but ~50% of never-smoking Asian women. Similarly, *ALK* fusion occurs more frequently in never-smoking Asian women than in current smoking American men. The exact molecular mechanisms underlying the gene mutation for *EGFR* and *ALK* are still elusive. It is known that *p53* gene mutations are often associated with smoking history, particularly G to T transversions. Furthermore, squamous cell carcinomas are different from adenocarcinomas in gene mutations. The frequency of *p53* gene mutation is more common in squamous cell carcinomas (~90%) (*vs.* <50% in adenocarcinomas), while KRAS mutations occur in ~36% of lung adenocarcinomas but rarely in squamous cell carcinomas. Silencing of *p16* is common in squamous cell carcinomas (~45%) but rare in adenocarcinomas. Mutations of *EGFR, ALK, cMET* and *ROS-1* are rare in squamous cell carcinomas but commonly found in lung adenocarcinomas (8%–50% depending on smoking history, gene type and gender). Below we will focus on specific clinical drugs used to target specific gene alterations.

3. INHIBITORS FOR MUTANT KINASES

3.1 Mutant tyrosine kinase inhibitors

3.1.1 EGFR inhibitors—Identifying novel gene mutation has revolutionized treatment of NSCLC. The best example is EGFR. Initial studies using EGFR inhibitor gefitnib (Iressa) had tumor-inhibitory effects in only 10%–19% of patients with NSCLC (Fukuoka et al., 2003). Later analyses indicate that most patients with activating EGFR mutations had better responses to gefitnib than those without such mutations (Lynch et al., 2004; Paez et al., 2004). Initial observation indicates that treatment with the EGFR kinase inhibitor gefitinib causes tumor regression in some patients with NSCLC, more frequently in Asian population. *EGFR* activating gene mutations occur in 14% of lung adenocarcinomas. However, lung cancers from Asian women without smoking history have much higher percentage of *EGFR* gene mutations (~50%), twice of the rate in cancer patients from the US and Europe.

Following FDA approval of Gefitinib in 2003, a similar drug, Erlotinib (Tarceva ®) was also approved in 2004 (Fig. 1 for details).

Although EGFR inhibitors have been approved for treatment of NSCLC, most of the patients develop drug resistance within 15 months. In nearly 60% of treatment-resistant NSCLC, at least a secondary *EGFR* gene mutation is detectable. There are also other genetic changes, such as gene mutations in *MET, BRAF, AXL, MAPK1* or *PI3KCA*. For example, T790 to M790 mutation (T790M) is the most common *EGFR* gene mutation, responsible for resistance to gefitinib treatment. Although it is difficult to role out possible acquired gene mutation in the *EGFR* gene, increasing evidence indicates pre-existence of *EGFR* mutation in a small number of cells of the original tumor and expansion of these cells with T790M EGFR during treatment (from 1 in 100,000 cells to 1 in 2 cells). Despite a lot of efforts in identifying an EGFR inhibitor that can block T790M *EGFR* functions, only recent studies identified AZD9291 as an irreversible inhibitor capable of inhibiting tumors with both T790M *EGFR* and other sensitive mutant *EGFR* (Finlay et al., 2014). Patients with T790M *EGFR* mutation treated with AZD9291 have ~13 months of progression-free survival (Janne et al., 2015), higher than the placebo group (~7 months) (Thress et al., 2015). Similarly, rociletinib is another well-tolerated drug that has a high response rate in T790M EGFR positive NSCLC tumors (Sequist et al., 2015). However, new resistance to these inhibitors eventually develops, and additional gene alterations are identified in the tumor (Piotrowska et al., 2015; Thress et al., 2015).

In addition, large scale screening of effective cell proliferation suppression leads to some novel findings. In patients-derived specimens, screening of effective inhibitors indicates that combined inhibition of *EGFR* and *FGFR* can suppress cell proliferation in *EGFR* mutant cell lines with a mutation in *FGFR3* gene (Crystal et al., 2014). Hopefully, these studies will generate additional strategies to mitigate drug resistance to EGFR-based targeted therapy. During targeted therapy, in addition to genetic alterations, some NSCLC can transform into SCLC morphology, making metastasis more quickly.

3.1.2 ALK inhibitors—ALK caused by chromosome rearrangements is another important drug target in NSCLC (Fig. 1). About 8% of NSCLC contains *ALK* mutations, with ELM4-ALK variant 1 as the most common alteration (Friboulet et al., 2014; Katayama et al., 2014; Shaw and Engelman, 2014). The most effective first-generation ALK inhibitor is crizotinib, but patients often relapse after treatment for 1–2 years. About one third of relapsed patients have additional *ALK* mutations whereas other patients have mutations in genes like *EGFR* and *IGF1R*, or bypass gene mutations downstream of ALK (such as *MAP2K1* K57N and *PIK3CA* H1047R). Second- generation ALK inhibitors have shown effective activity against resistant ALK mutants, such as ceritinib, aletinib and PF-06463922. Currently, crizotinib is regarded as the first-line drug for ALK mutation-containing NSCLC, and ceritinib and alectinib are used for the second-line treatment of NSCLC (Gainor et al., 2015).

3.1.3. Additional tyrosine kinase inhibitors—Other mutant kinase genes in NSCLC include *ROS-1* and *RET* (Fig. 1), and ALK inhibitor crizotinib has shown effectiveness through suppressing ROS-1 activity in NSCLC patients (Shaw et al., 2014). Because crizotinib is also effective in suppressing cMET, it is highly possible that crizotinib can be

used to treat NSCLC patients with *cMET* amplification. There are at least two multi-kinase inhibitors (vandetinib and cabozantinib) with RET tyrosine kinase inhibitor activity. Current clinical trials with these two drugs in the treatment of lung adenocarcinomas have shown some promising results (Gautschi et al., 2013; Mukhopadhyay et al., 2014).

Based on the experience in these tyrosine kinase inhibitors for lung cancer treatment, it has been recommended that it will be more effective to use the most potent tyrosine kinase inhibitor early in disease progression. Furthermore, it will beneficial to combine the targeted therapy with surgery and immunotherapy early on. Nevertheless, for all successful clinical trials or effective clinical care of cancer patients with NSCLC, identifying the genetic makeup of the tumor is the essential first step.

3.2 Serine/threonine kinase inhibitors

Many signal transducers are regulated by serine/threonine kinases, and thus inhibitors against serine/threonine kinases are known cancer drug targets for decades. However, only in the last 10 years, successful clinical trials using this type of inhibitors have been achieved. For example, B-RAF inhibitors can effectively shrink a subset of melanomas.

3.2.1 B-RAF inhibitors—*BRAF* mutations are common in melanomas, and successful clinical trials with Vemurafenib and Dabrafenib have led to FDA approval of these BRAF inhibitors for melanoma treatment in the clinic (Chapman et al., 2011; Poulikakos et al., 2011). Only a small subset of NSCLC (2%) contains activating *BRAF* gene mutations (Kris et al., 2014). Due to the low incidence of *BRAF* mutations in NSCLC, most clinical trials using *BRAF* inhibitors were done together with other cancer types (Falchook et al., 2012) or as case reports (Rudin et al., 2013; Robinson et al., 2014; Schmid et al., 2015). In some studies, the response was short-lived, with relapse a few months later, and tumor recurrence was associated with other somatic gene mutations such as *KRAS*.

3.2.2. MEK inhibitors—As downstream signaling effectors, MEK1/2 have been important candidate targets for cancer therapy. A specific MEK1/2 inhibitor trametinib has been approved for treatment of melanomas with *BRAF* gene mutation after successful clinical trials (Flaherty et al., 2012a, 2012b). Because *MEK1* mutation has been a major driver for resistance to BRAF or EGFR inhibitors, combination of BRAF inhibitor dabrafenib with trametinib has proven to be beneficial to the melanoma patients, with significant improvements in patient survival (Flaherty et al., 2012a, 2012b; Larkin et al., 2014; Long et al., 2014; Robert et al., 2015).

In NSCLC, *MAPK2K1* (encoding MEK1) mutation is one of the driving factors responsible for drug resistance to EGFR inhibition (Ercan et al. 2012). Combination of EGFR inhibitor WZ4002 with MEK1/2 inhibitor trametinib prevents the appearance of EGFR resistance in *EGFR* mutant lung cancer in preclinical models (Tricker et al., 2015). Thus, it is anticipated that combined inhibition of EGFR and MEK1/2 will be more effective in NSCLC with EGFR mutations. Other studies also indicate more than additive effects with combination of two types of inhibitors (Heppt et al. 2015). Although *PIK3CA* and *AKT* gene mutations occur frequently in EGFR inhibitor resistant tumors, there are no FDA approved specific inhibitors to target *PIK3CA* and *AKT*.

4. IMMUNOTHERAPIES OF NSCLC

One of the major features of cancer cells is to escape the immune surveillance system of the host by up-regulation of immune checkpoint proteins and immunosuppressive cytokines. As shown in Fig. 2, an increase in PD-L1/2 expression in the tumor cells and high expression of PD-1 in the T cells can trigger suppressive effects on T cell-mediated immune surveillance system, allowing the tumor cells to survive.

Recent clinical trials indicate that modulating immune checkpoint emerges as a promising strategy to overcome cancer cell-mediated immune suppression. In early 2012, specific neutralizing antibodies against PD-1 and PD-L1 already showed good activities in tumor shrinkage in NSCLC through targeting the PD-1/PD-L1/2 signaling axis. These antibodies-based therapies are well tolerated. Although long-term survival is reported in treated patients, the response rate is generally low (around 20%) (Rizvi et al., 2015b). Patient selection is critical for immune therapy. Earlier studies showed that none of the PD-L1 negative tumors respond to the treatment whereas 36% of PD-L1 positive tumors respond to the treatment (Topalian et al., 2012).

It is not known why only a subset of PD-L1 positive tumors responds to the treatment. Recent whole-exome sequencing of NSCLC treated with PD-L1 antibody pembrolizumab revealed that the genomic landscape of the tumor, not a single gene alteration, determines the response to PD-1 inhibition (Rizvi et al., 2015a). Other studies indicated that *EGFR* mutation can stimulate expression of PD-1 and PD-L1 in NSCLC (Akbay et al., 2013). The data from CTLA-4 neutralizing antibodies are impressive in melanomas (Weber et al., 2015) but more works remain to be done in NSCLC.

5. CURRENT CLINICAL PRACTICE GUIDELINES FOR NSCLC PATIENTS

5.1 Diagnosis

Based on the progress, both EU and the US medical oncology organizations have issued recommendation on new guidelines for clinical practice for NSCLC patients. In the guidelines, detection of *EGFR* mutations and *ALK* gene fusions is recommended for lung adenocarcinomas (Leighl et al., 2014; Reck et al., 2014) (Fig. 3). Mutation testing is recommended in all patients with advanced NSCLC as long as a component of adenocarcinoma is involved. Testing is not recommended in patients with a definite squamous cell carcinoma diagnosis. Testing is recommended for patients who are never/former light smokers.

In contrast, routine use of serum markers such as carcinoembryonic antigen (CEA) is not recommended now. At present, contrast-enhanced computed tomography (CT) scan of the chest and upper abdomen is recommended for precise diagnosis of cancer. Disease stages should be determined during diagnosis to facilitate therapy strategies.

5.2 Treatment

Treatment options will vary depending on genetic alterations in the tumor (Fig. 3). For squamous cell carcinomas and *EGFR*/*ALK* negative adenocarcinomas, the first-line

treatment is platinum-based chemotherapy, and the second-line treatment for these tumors is docetaxel, and doctaxel plus ramucirumab (or nivolumab). For *EGFR* mutation positive adenocarcinomas, the first-line treatment is afatinib or erlotinib followed by chemotherapy (second line). For ALK translocation positive adenocarcinomas, the first-line treatment is crizotinib, and the second-line is certinib.

Because most targeted therapies will eventually end up with cancer relapse, subsequent lines of treatment options will be determined by the specific alterations in the cancer genome. For example, if *EGFR* T790M mutation occurs, newer generations of EGFR threonine kinase inhibitor (such as AZD9291) will be used. In comparison with the guideline 10 years ago, the new guidelines highlight the individual differences in different tumors, and the targeted therapy is aimed at a small proportion of the patient pool.

6. PERSPECTIVES

Targeted therapies in subsets of NSCLC have already extended the patient's lifespan over one year (3.5 years for patients with targeted therapies *vs.* 2.4 years for patients without genotype-directed therapies) (Kris et al., 2014), highlighting significance of cancer genetic profiling and targeted therapy for clinical cancer care. Testing of *EGFR* mutations and *ALK* rearrangements is now recommended for all lung adenocarcinomas and lung cancer patients from never-smokers. However, NSCLC is still the number one cause of cancer-related death. Several major challenges are emerging in our efforts to reduce the mortality rate.

First of all, in the clinical setting, identifying the suitable patient population to perform targeted therapy or immune therapy at the earliest possible time point is critically important for a good outcome. A comprehensive care strategy (combined targeted therapies and immune therapy) is needed for all patients. With an increased number of rare genetic mutations in NSCLC (Cancer Genome Atlas Research, 2014) and increasing number of targeted drugs and immune therapy strategies, it will be feasible to perform genomic profiling or next-generation sequencing for each patient. It remains an issue as to cost coverage for these tests (afforded by medical insurance companies or out-of-pocket expense from the patients).

Second, it becomes clear that NSCLC consists of many small subsets of rare genotypes, and developing targeted therapies towards ever growing rare genotypes is a daunting task. Inter-institutional collaborative efforts among clinicians and basic scientists, and more integration of tumor genotyping and clinical information are more needed than ever to make significant progress in the treatment of NSCLC.

Third, while the targeted therapy significantly improves the quality of life in the patients, most targeted therapies are short-lived (a few months to 2 years). This creates a major challenge in prolonging the lifespan of the cancer patients. Thus, it is critical to identify resistance mechanisms quickly in order to select additional strategies to militate against drug resistance.

In addition, despite all the clinical success in subsets of NSCLC patients, basic understanding of the mechanisms underlying tumor development in different genotypes has

lagged behind the clinical care of NSCLC. For example, it is still not clear why patients with *EGFR* mutations respond better to EGFR inhibitors than patients with EGFR overexpression. Similarly, it remains elusive why downstream signaling inhibitors (BRAF and MEK inhibitors) are not effective in patients with *EGFR* mutations or *KRAS* mutations. More funding for lung cancer-related basic research is urgently needed.

Looking forward, we anticipate much more progress in reducing NSCLC-related mortality in the next ten years through integration of technology with clinical care, through more collaboration among physicians and scientists, and through more research investment in lung cancer biology.

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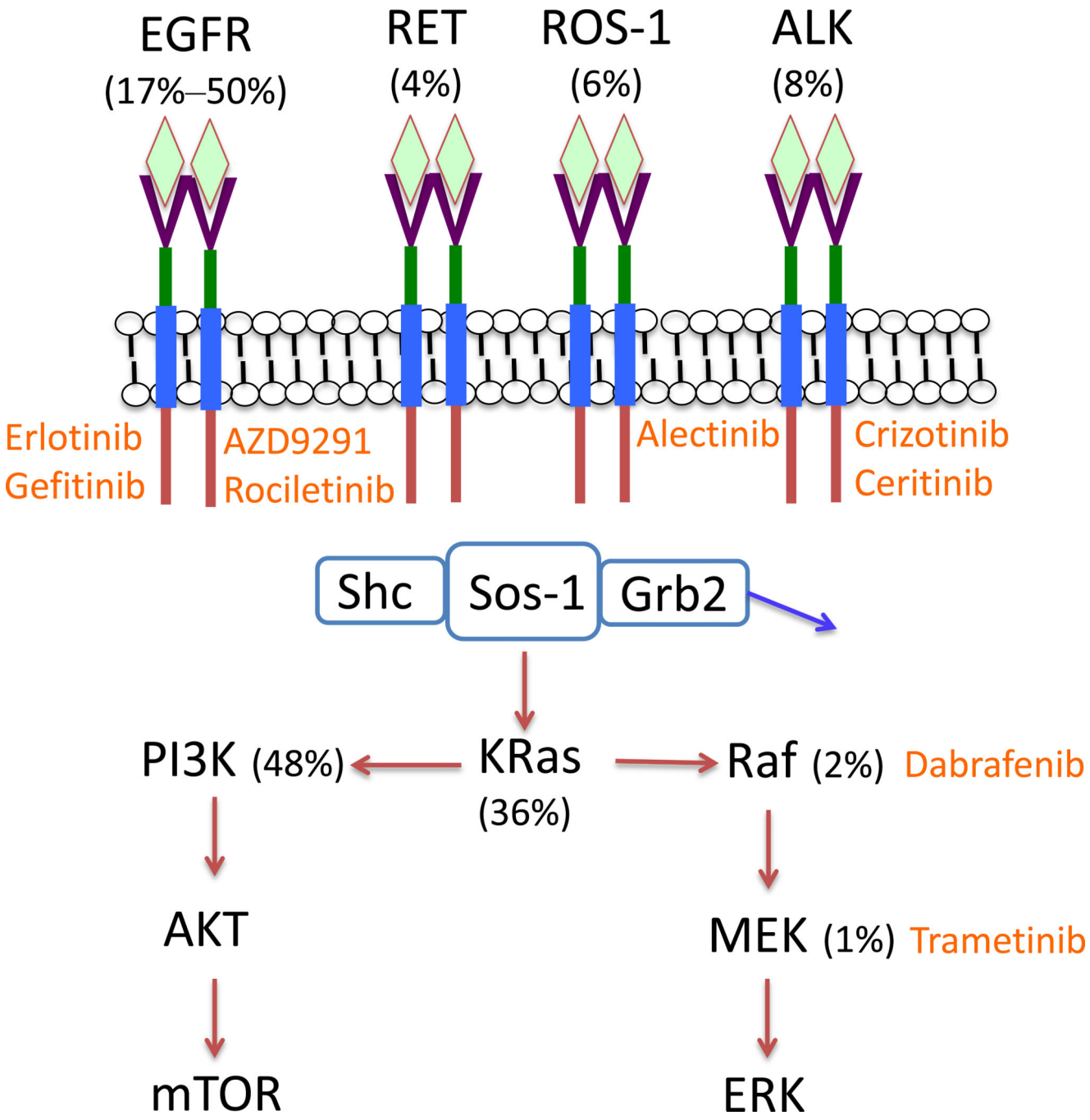

Figure 1. Mutations of the genes in the growth factor/KRAS signaling axis in NSCLC

The frequency of gene mutations is shown in the bracket (mostly from the data of lung adenocarcinomas except for data of PI3K which is from the data from lung squamous cell carcinomas). Currently approved targeted drugs are shown in orange next to the target molecules. There are four drugs targeting EGFR and three drugs for ALK. ALK inhibitors are also active in suppressing ROS1 activity.

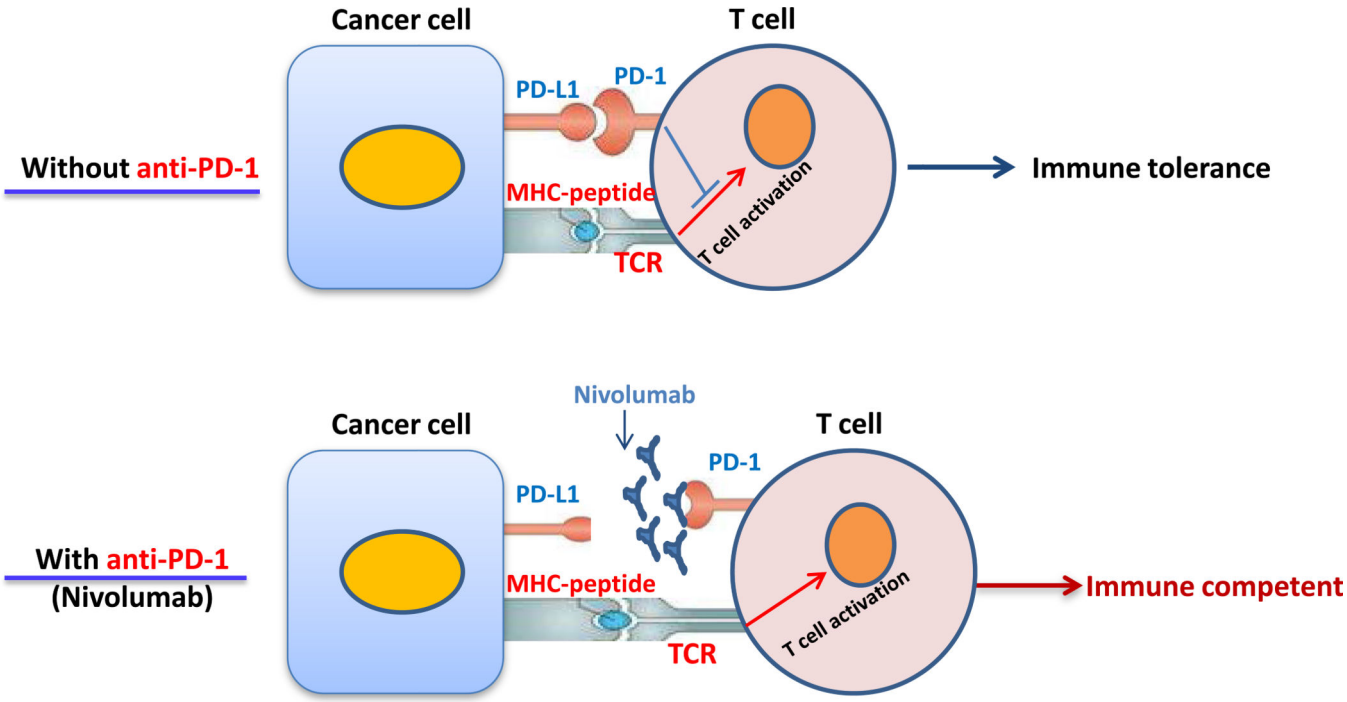

Figure 2. The effect of anti-PD-1 on T cell function

In the absence of anti-PD-1 antibodies (nivolumab), oncogenic signaling induces expression of PD-L1 that can be recognized by PD-1 expressing T cells. PD-1 and PD-L1 interaction triggers signaling events leading to suppression of T cell activation and consequently immune tolerance of cancer cells. When PD-1 neutralizing antibodies nivolumaba are used, PD-1 and PD-L1 interaction is interrupted, allowing T cell activation to occur and resulting in immune detection of cancer cells and consequently elimination of cancer cells. PD-L1 neutralizing antibodies have simiarl effects as PD-1 neutralizing antibodies. PD-L1, programmed death-ligand 1; PD-1, programmed death protein 1.

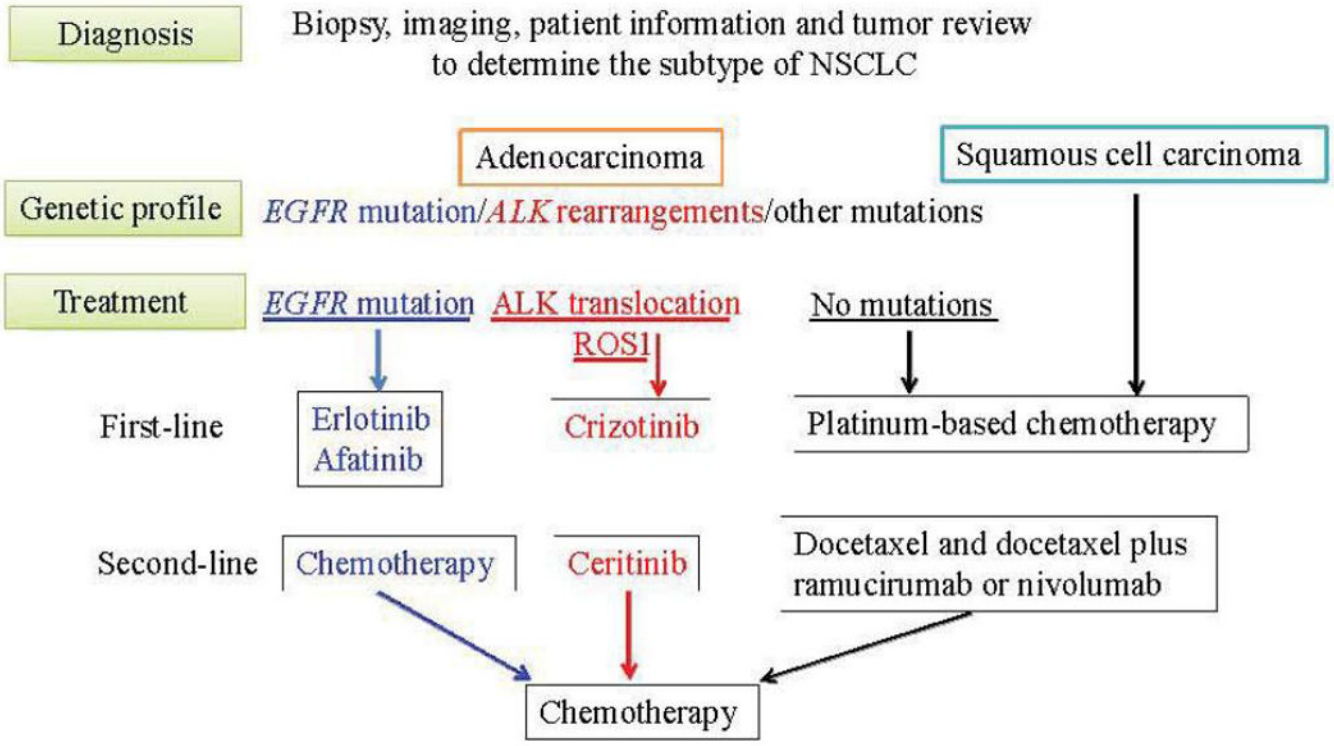

Figure 3. A flow chart of the current standard care for NSCLC (particularly adenocarcinoma and squamous cell carcinoma)

This flow chart is the most recent guidelines for treatment of NSCLC patients. The treatment options depend o the genotype of the tumor. For example, tumors with *EGFR* gene mutations will be first treated with w specific EGFR inhibitor (highlighted in blue). IN contrast, tumors with *ALK* gene rearrangements will be treated with an ALK inhibitor (highlighted in red). For tumors without these somatic gene mutations, standard chemotherapy will be used (highlighted in black).

Although currently not approved yet, AZD9291 and rociletinib have shown effectiveness in $EGFR^{T790M}$ mutation positive adenocarcinomas, and may be approved soon. EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma receptor tyrosine kinase; NSCLC non-small cell lung cancer.