Beyond EGFR and ALK: targeting rare mutations in advanced non-small cell lung cancer

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Abstract: Lung cancer remains the leading cause of cancer-related death in men and women, despite its constantly declining rates in incidence and mortality in the developed world. The past decade has witnessed an unprecedented rise in the development of molecular targeted therapies in various types of tumors. In non-small cell lung cancer (NSCLC), the greatest paradigm shift is the implementation of EGFR and ALK tyrosine kinase inhibitors in the first line and subsequent lines of therapy, with impressive results. Though less frequent than the molecular alterations in the aforementioned genes, a number of aberrations in potential oncogenic drivers has been discovered, namely mutations in the genes KRAS, BRAF, HER2, PI3KCA and DDR2, ROS1 and RET rearrangements and MET, HER2 and FGFR1 gene amplifications. A great number of clinical trials are currently underway, evaluating agents specifically designed to target these alterations, with mixed results so far. The greatest cumulative benefit offered by these trials is that, despite their success or failure in their objective goals, they have provided us with a better understanding of the complexity of the molecular intracellular processes, necessitating thus the accurate interpretation of the preclinical data in order to appropriately select the patients that may derive benefit from targeted treatment strategies.

Keywords: Non-small cell lung cancer (NSCLC); oncogenic drivers; KRAS; BRAF; MET; HER2; PI3KCA; DDR2; RET; ROS1; FGFR1

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Introduction

Lung cancer remains the leading cause of cancer-related death in both sexes, although its incidence and mortality rates, at least in the developed world, are steadily declining, mostly as a result of the anti-smoking campaign (1). The recent advances in molecular biology of cancer, made possible by the implementation of techniques such as next-generation sequencing, have deciphered the oncogenic processes in many types of malignant diseases, including lung cancer. Initiatives such as The Lung Cancer Mutation Consortium have elucidated the molecular heterogeneity of non-small cell lung cancer (NSCLC), discovering molecular alterations in key regulatory pathways that spearhead the malignant process, which in turn made possible their effective targeting and the subsequent impairment of the cancer growth (2). The clinical benefit from targeting the epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) translocations has already been established through pivotal clinical trials, and a number of effective targeted agents for these alterations comprise a vital part of our armamentarium against
NSCLC harboring the relevant aberrations. Although these two molecular alterations occur most frequently among oncogene-addicted NSCLC, others have been described as well and attempts to target them in clinical trials have already been taken, with varying levels of success. Herein, we present the most frequent genetic alterations that may function as oncogenic drivers in NSCLC, apart from EGFR and ALK, and we analyze some of the attempts that have been made so far in order to discover a specific and efficient targeted agent for each of them.

**BRAF mutations**

BRAF (B-Raf proto-oncogene, serine/threonine kinase) inhibition was recently officially added to the armamentarium of targeted therapies for oncogene-driven NSCLC, via the implementation of the combination of BRAF inhibitor, namely Dabrafenib, with a MEK inhibitor, namely Trametinib. A BRAF mutation, which in at least half the cases represents the V600E mutation, constitutes the driving force of oncogenesis in approximately 1–2% of NSCLC (3), and deregulates the mitogen-activated protein kinase (MAPK) pathway, thus affecting major cell processes such as cell proliferation, differentiation, angiogenesis, senescence and cell death.

In the two larger relevant studies, nearly all the tumors harboring a BRAF mutation were adenocarcinomas of poor differentiation and manifested with a more aggressive pattern. Also, in both studies, most of the patients carrying the mutation were current or former smokers (4,5).

Dabrafenib showed clinical activity as a single agent in this setting in a phase II, multicentre, non-randomised, open-label study in previously treated and untreated patients with stage IV metastatic BRAFV600E-positive NSCLC (NCT01336634). The dual inhibition of BRAF and MEK via the combination of Dabrafenib-Trametinib has produced impressive results, both in first line and in subsequent lines of therapy. The combination was first compared to Dabrafenib monotherapy in previously treated patients in a phase II study, where it produced an impressive overall survival (OS) of 18 months over 12.7 months of single agent Dabrafenib (6). Similarly, in the phase II study in treatment-naïve patients, it showed an overall response rate (ORR) of 64%, progression-free survival (PFS) of 11 months and preliminary OS of 25 months (7). Based on these results, the regulatory authorities of USA and European Union granted approval to the combination for patients carrying the V600E mutation, irrespective of the line of therapy.

**KRAS mutations**

KRAS (KRAS proto-oncogene, GTPase) constitutes the most frequently mutated oncogene in NSCLC, at least in Caucasian populations, with reported frequencies reaching up to 26% (8), while its presence signals the absence of driver mutations in EGFR and ALK (9). The most common alteration is a missense mutation in codon 12, followed by mutations in codons 13 and 61 (10,11). These mutations result in a constitutively active, GTP-bound protein product, which constantly produces anti-apoptotic and pro-proliferation signals, mainly through the MAPK pathway, thus promoting the oncogenic process.

KRAS mutations mostly characterize non-squamous NSCLC, with varying levels of differentiation. Although initially believed that KRAS mutations were found predominately in smokers, recent reports provided evidence of the mutation appearing with a frequency of 15% in non-smokers (12). Among the different human races, it appears to be more prevalent in African-Americans as compared to Caucasians, with the lower prevalence found in Asians (13).

Ironically, although appearing in such a high frequency, KRAS mutated-NSCLC seems to be the hardest one to target effectively. The most promising novel agent was Selumetinib, a MEK 1/2 inhibitor, targeting a molecule downstream the constitutively active MAPK pathway. This small molecule demonstrated superior response rates and PFS in combination with Docetaxel over placebo, in a recent phase II trial, thus paving the way for the phase III SELECT-1 (14). This trial was set in the second line, in patients harboring a KRAS mutation, with the same drug combination (Selumetinib and Docetaxel vs. Placebo and Docetaxel), with PFS being the primary endpoint. Unfortunately, no improvement was noted either in PFS or in OS and ORR in the experimental arm (15).

Another approach implemented Abemaciclib, a CDK4/6 inhibitor currently employed in the treatment of hormone receptor-positive metastatic breast cancer, which produced a disease control rate of 55.2% in heavily pretreated NSCLC patients, in an early trial (NCT01394016). The phase III JUPINER trial, that compared Abemaciclib with Erlotinib in a pretreated population harboring KRAS mutations, failed to show superiority in the experimental arm (16).

The reasons behind these failed attempts at targeting...
KRAS-mutant NSCLC are still unclear. A possible explanation might be the reactive upregulation of other intracellular molecules or molecular pathways, as it has proven to happen with AKT, which function as an escape mechanism (17). If that is indeed the resistance mechanism, dual inhibition strategies may provide benefit in this difficult to approach therapeutically clinical setting.

**MET deregulation**

The MET (MET proto-oncogene, receptor tyrosine kinase) gene encodes for a transmembrane receptor that is normally activated by the binding of its ligand, the hepatocyte growth factor (HGF). Aberrant or excessive activation of the receptor, through gene amplification or mutation, has an established role in various oncogenic processes such as cell survival, proliferation and metastasis (18). MET overexpression in NSCLC has been found in frequencies between 25–75% and has been associated with inferior outcomes (19,20).

An important association between EGFR and MET has been established. Firstly, MET has been found to upregulate EGF, thus impairing the effect of EGFR-targeting TKIs (21). Furthermore, amplification of the MET gene is a mechanism of acquired resistance to first-generation EGFR-TKIs (22,23), while it has also been recently involved in resistance to the third generation EGFR inhibitor Osimertinib in EGFR-mutated NSCLC (24). Moreover, the exon 14 skip mutation, which is found in approximately 3% of NSCLC, is an established resistance mutation that confers inferior prognosis, and which may be targeted with TKIs (25).

Based on these data, numerous clinical trials have been designed, targeting the MET gene product or its ligand, which tested novel small molecules or monoclonal antibodies, alone or in combination with EGFR-TKIs. Unfortunately, the majority of the trials did not produce positive results.

One of the first novel molecules tested, Tivantinib, a novel MET-TKI inhibitor, was combined with Erlotinib in a randomized, phase II trial that, although not achieving statistical significance in PFS (median PFS was 3.8 and 2.3 months in the Tivantinib and placebo group, respectively, with HR 0.81; 95% CI, 0.57 to 1.16; P=0.24) (26), prompted the design of the phase III MARQUÈE trial, that compared the combination Tivantinib-Erlotinib vs. Erlotinib-placebo in pretreated, non-squamous NSCLC patients. The study population included 1,048 patients, one third of whom were heavily pretreated. In the interim analysis no difference was shown between the two groups in the primary endpoint of OS, and the trial was terminated for futility, though a difference was noted in the secondary endpoints of PFS and RR (27).

Onartuzumab, a monoclonal antibody targeting the extracellular domain of the MET receptor, was also combined with Erlotinib in a phase II trial in previously treated NSCLC patients who were unselected for MET expression. Although in the intention to treat (ITT) population no difference in OS and PFS was noted, in the subgroup that expressed MET (as a score 2+ or 3+ as per the MET IHC scoring system) a longer PFS and OS was noted (PFS 2.9 vs. 1.5 months, HR 0.53; P=0.04 and OS 12.6 vs 3.8 months, HR 0.37; P=0.002) (28). These results prompted the evaluation of Onartuzumab with Erlotinib versus placebo and Erlotinib in a large, randomized phase III trial in a preselected for MET expression patient population with pretreated advanced NSCLC. Unfortunately, no difference was observed between the two arms in terms of OS, PFS and RR (29). A potential explanation for this may be that patients were selected on the basis of positivity as estimated by IHC and not by MET gene amplification.

Recently, another randomized phase II trial attempted to introduce emibetuzumab, a MET-targeting monoclonal antibody, in combination with Erlotinib in treatment-naïve, EGFR-mutated NSCLC patients. This trial also failed to produce positive results, though an exploratory analysis suggested a potential PFS benefit in the subgroup of high MET expressors (30).

Although targeting NSCLC patients with MET inhibitors based on gene amplification and/or overexpression of the protein product has failed to produce the required results so far, it seems that targeting the exon 14 skip mutation (METdel14) may prove much more promising. In a recent multicenter retrospective analysis of 148 patients with METdel14 mutant NSCLC, a vast difference in survival was noted between those who were never treated with MET inhibition and those who did. More specifically, in the first subgroup the OS was 8.1 months, which is consistent with the dismal prognosis conferred by this specific mutation. However, in the patient subgroup that received at least one MET inhibitor (including Crizotinib, Glesatinib, Capmatinib, and ABBV-399), the OS reached 24.6 months. These encouraging
results may offer insight in the proper way to select patients for future clinical trials testing MET inhibitors in NSCLC patients (31).

**PI3KCA mutations**

Phosphatidylinositol-3 kinases (PI3K) belong to a family of heterodimeric kinases, whose physiologic role is the conversion of phosphatidylinositol-3,4-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate, which subsequently activates its downstream pathway, namely AKT/mTOR, to regulate growth, survival, and motility of the cell. The PI3K family has been divided in three classes, of which PI3KCA (Class I) is mostly implicated in human carcinogenesis, through its constitutively active catalytic subunit (p110), that can be aberrantly activated either through mutation or through gene amplification (32).

PI3KCA gene amplification characterizes mostly the squamous subtype of NSCLC, where it has been reported in frequencies ranging between 33% and 37%, as compared to only 5% to 6% in adenocarcinoma (33,34). Mutations in the PIK3CA gene occur more rarely, in about 2–5%, being prevalent again in squamous NSCLC (33–35), while they seem to confer inferior prognosis in adenocarcinoma (36). Interestingly, these mutations have been reported to occur in parallel with other oncogenic driver mutations and they have also been discovered in EGFR-mutant NSCLC that has developed acquired resistance to EGFR-TKIs (37,38). This may provide a hint toward the function of these mutations, since they most probably develop later in the multistep oncogenic process, possibly representing escape mechanisms from the TKI inhibition.

In that context, several small molecules targeting PI3KCA are in development. Buparlisib (BKM120) is a pan-PI3K inhibitor that has demonstrated activity in NSCLC in preclinical models combined with the downstream mTOR inhibitor Everolimus (38). This was confirmed in a phase I dose-escalation study (39), but a subsequent phase II basket trial (NCT01501604) that investigated efficacy of single-agent Buparlisib in a variety of cancers harboring PIK3CA mutations (lung, breast, colorectal, cholangiocarcinoma and other solid tumors), was prematurely terminated due to poor accrual. Another phase II study, BASALT-I (NCT01820325), evaluating Buparlisib in patients with relapsed squamous or non-squamous NSCLC, molecularly selected for PI3KCA mutation, failed to demonstrate sufficient clinical activity of the drug (40). The investigators concluded that PI3K mutations may not be the main oncogenic driver in NSCLC, suggesting instead that combination strategies with other targeted agents may provide better results. Interestingly, this combination approach provided positive results in a phase II study of Buparlisib combined with Paclitaxel in pretreated Head-Neck squamous cell carcinoma patients (41).

Pictilisib (GDC-0941) is another PI3K inhibitor that is being studied in a phase Ib trial in advanced NSCLC in unselected patients, in combination with cytotoxic chemotherapy, with the addition of Bevacizumab in non-squamous NSCLC (NCT00974584). The drug is also being studied in a large phase II, placebo-controlled trial in previously untreated advanced or recurrent NSCLC, where it is combined with Carboplatin/Paclitaxel, with the addition of Bevacizumab in non-squamous NSCLC (NCT01493843). The study has completed accrual and results are awaited.

Pilaralisib (SAR245408, XL147) is a pan-class I PI3K inhibitor that has shown dose-dependent activity in inhibiting growth in human cancer cell lines with constitutive PI3K activation (42) in combination with Erlotinib in patients with solid malignancies. In a phase I dose-escalation study in combination with Erlotinib in unselected patients with solid tumors, it produced limited antitumor activity, irrespective of the PI3K mutation status. In yet another phase Ib study including a subset of NSCLC, where it was combined with Carboplatin/Paclitaxel, it did not offer any further enhancement in the antitumor activity of cytotoxics (NCT00756847).

The information provided from these trials, which may be useful to incorporate in future efforts to implement PI3K inhibition in clinical practice, is that single-agent PI3K inhibitors offer limited, if any, activity, while combination with other targeted agents and/or cytotoxic chemotherapy, may prove to be more efficacious. On the other hand, we also need to establish the optimal setting in which PI3K inhibition will be incorporated, since it seems that PI3K is most active as an oncogenic force in advanced, pretreated cancer, where it functions as an escape mechanism to other targeted agents.

**RET rearrangement**

The RET fusion oncogene was initially discovered in thyroid carcinoma (43,44), and since then it has been implicated as an oncogenic driver in a variety of solid tumors. In NSCLC, at least 12 different gene partners of RET have been described (45–47), leading to various forms
of rearrangement, with perhaps the most frequent and best characterized of them occurring between the exons 1–15 of KIF5B and the exons 12–20 of RET proto-oncogene, which encodes the tyrosine kinase portion. The downstream pathways activated are the JAK/STAT3 and RAS/RAF/MEK/ERK, which promote cell proliferation and survival (48).

The frequency of the rearrangement in NSCLC is estimated between 1% and 2% (45), and the definitive standard for its detection is FISH, regardless of the fusion partner (49). A retrospective analysis revealed that RET-driven oncogenesis produces poorly differentiated tumors, featuring a solid, lepidic or papillary predominant morphologic pattern, often including signet-ring cells. In that same study, most of the patients were never-smokers and younger than 60 years (82% and 73%, respectively). Furthermore, although all patients had small primary tumors, even smaller than 3 cm, they were characterized by extensive lymph node involvement, (at least N2 at presentation) (49), a correlation also noted in another large retrospective study (50).

Several multitarget agents have exhibited activity against RET-rearranged tumors, such as Vandetanib, Cabozantinib, Lenvatinib, Alectinib, and Sunitinib, with response rates ranging between 16% and 47%, and median PFS from 2.3 to 7.3 months (51-54). Sunitinib and Sorafenib have failed in their respective clinical trials (55,56). A retrospective worldwide analysis (GLORY) including patients receiving a variety of TKIs (vandetanib, cabozantinib, lenvatinib, sunitinib, sorafenib, alectinib, ponatinib, and regorafenib) also produced disappointing results, with ORR, PFS and OS reaching 26%, 2.3 and 6.8 months, respectively (50). The most promising agent so far is Vandetanib, which was evaluated in 2 non-randomized phase II trials in 2017, in Japanese (LURET) and Korean populations. The ORR, PFS and OS in these trials were 53%, 5 and 11 months and 18%, 4.5 and 11.6 months, respectively. An interesting finding in both studies was the negative predictive role of the KIF5B-RET fusion variant as compared to other fusion types (51,52).

A possible explanation for the moderate success of targeting RET in NSCLC may be the early onset of acquired resistance, mainly as a result of the presence of concomitant molecular alterations in a clonally diverse cell population (57-59). In that regard, a dual or triple inhibition could prove to be an effective strategy. Indeed, a hint of the potential value of this approach has been offered by a recent phase I study, where the combination of Vandetanib with Everolimus produced a preliminary ORR of 83% in a RET-rearranged population with advanced NSCLC (60). Finally, the future may hold promise for a novel, selective RET inhibitor, potent against the KIF5B-RET fusion, currently designated LOXO-292 or BLU-667, that has demonstrated significant in vitro and in vivo activity (61).

**ROS rearrangement**

The ROS gene rearrangement promotes carcinogenesis in a unique yet still unclear way, since most of the fusion partners of the ROS1 proto-oncogene lack dimerization domains. Nevertheless, the receptor encoded by the rearranged ROS gene regulates downstream pro-survival and antiapoptotic signalling pathways, such as the MAPK, PI3K/AKT, and STAT3. Nine different ROS1 fusion partners have been identified so far, with CD74 being the most common (62).

In NSCLC, ROS rearrangements are found in approximately 1%, with FISH being the definitive diagnostic method, irrespective of the fusion partner (63). Similarly to other driver oncopgenes, ROS1 rearrangements are mutually exclusive with other mutations, such as ALK, EGFR or KRAS. Clinically, the patients harboring that specific rearrangement have the same characteristics as those with the ALK one: they are diagnosed at a younger age, they have a light or no smoking history, and their tumors are usually adenocarcinomas (64).

Since the ATP-binding sites of ROS and ALK share a 77% homology, molecules initially developed against ALK have been found to demonstrate activity, both in vitro and in vivo, against ROS as well (65,66). Based on this fact and on data from preclinical models that demonstrated activity of Crizotinib against ROS-rearranged NSCLC (62), an expansion cohort of ROS1-positive patients, previously TKI-untreated, was included in a phase I trial evaluating the activity of Crizotinib in ALK-rearranged NSCLC patients. The results indicated a clinically meaningful benefit of this drug to this subset of patients, which showed a 72% ORR and a median PFS of 19.2 months, thus establishing Crizotinib as the standard of care for ROS1-rearranged NSCLC (63). Other ALK-targeting agents have been tested in this setting: Ceritinib has produced similar results in a TKI-naïve population, producing an ORR of 62% a median PFS of 19 months (67), while Entrectinib, a ROS- and NTRK-TKI, showed an ORR of 78%, intracranial responses of 83% and a PFS of 29.6 months in a similar patient population (68).

An important issue with ROS inhibition, as is with
ALK- and EGFR-inhibition, is that after a certain time of treatment, most of the patients will eventually develop resistance, which, in a considerable number, is attributed to novel mutations within the targeted molecule. Second-line TKIs are being developed: Lorlatinib, an ALK-inhibitor developed to target Crizotinib-resistant ALK-rearranged NSCLC, has demonstrated activity in this setting; in a phase II trial of ROS1-positive patients, 70% of which were Crizotinib-resistant, Lorlatinib produced a 36% ORR, an intracranial ORR of 56% and a median PFS of 9.6 months (69). Another problem with ROS1 targeting is the presence of de novo resistance to currently employed agents, conferred by mutations such as CD74-ROS1G2032R, for which Ceritinib, Lorlatinib and Entrectinib have performed poorly in preclinical models (70-72). Cabozantinib, however, has produced more encouraging results in preclinical studies, and a phase II trial is currently ongoing (73).

**HER2 alterations**

HER2 is a unique member of the ErbB family of transmembrane receptors, that lacks an activating ligand. Instead, activation results from either homodimerization of the molecule, or the heterodimerization with other members of the family, namely EGFR and HER3. Heterodimerization leads to activation of transduction pathways including PI3K, MAPK and JAK/STAT, which augment proliferation and survival (74). HER2 has been found altered in a variety of solid neoplasms, and specific anti-HER2 agents have been incorporated in breast and gastric cancers where the HER2 protein product is amplified. In NSCLC, HER2 protein expression and gene amplification are present in 2–6% and in 1–5% of NSCLC respectively (75,76). HER2 is also mutated in approximately 2–4% of NSCLC, with the most frequent mutation being the YVMA 776–779 insertion in exon 20, which results in a constantly active Kinase domain that phosphorylates downstream signals such as AKT and MEK (77).

HER2 mutations in NSCLC have been correlated with adenocarcinoma histology, they are mutually exclusive with EGFR and KRAS mutations, while in certain reports they have been associated with female gender, Asian ethnicity and never-smoking status (77). The prognostic significance of these alterations has yet to be elucidated, with some reports attributing poor prognosis in cases where the protein product has been found amplified by IHC, while the amplification of the gene itself, as detected by FISH, is of indeterminate significance (76).

Although the current armamentarium of anti-HER2 agents features various drugs with different mechanisms of action that have a proven efficacy in a similar setting in breast and gastric cancer, and despite the encouraging preclinical and early clinical results, the targeting of HER2 in NSCLC has so far failed to provide positive results in larger trials. To begin with, Dacomitinib, an irreversible pan-HER inhibitor, has demonstrated a poor ORR of only 12% in a phase II clinical trial of patients with advanced (stage III or IV) NSCLC with HER2 mutations or amplifications (78). Similarly low ORR, in an equivalent setting, was produced by Neratinib, another irreversible pan-HER inhibitor, either as a single agent or in combination with the mTOR inhibitor Temsirolimus [Neratinib (0%), and Neratinib plus Temsirolimus (19%)] (79). Furthermore, Afatinib, a dual HER2 and EGFR inhibitor with first-line approval for EGFR-mutant NSCLC, when employed for the treatment of HER2-mutant NSCLC patients in a phase II clinical trial, produced no responses at all (80), while in a multicentric, retrospective study, it showed an ORR of only 15% (81). Finally, TDM-1 also failed to show adequate benefit in NSCLC patients with HER2 overexpressing tumors, producing responses of 0% and 20% in HER2 IHC2+ or 3+, respectively (82).

No clear explanation has been offered yet for these negative results that, up to some extent, reflect the negative results produced in gastric cancer trials where the incorporation of HER2 agents, other than trastuzumab, in various lines of therapy, failed to show any clinical benefit. A potential explanation and a hint for future trials may be derived from a basket TDM-1 trial, where, in a subset of HER2-mutant NSCLC patients, the ORR was 44% and the median PFS was 5 months and did not correlate with IHC positivity. Instead, IHC positivity differed widely in responders and was not predictive of clinical benefit (83). These data may point to the need of implementing a scoring system for evaluating HER2 positivity consisting of more than 3 (ICH) or 2 (FISH) values.

**FGFR1 amplification**

FGFR1 (fibroblast growth factor receptor 1) belongs to a family of transmembrane tyrosine kinases consisting of four
members (FGFR1-4), that are active mainly in squamous epithelia, where they regulate proliferation via the RAS/RAF/MAPK and the PI3K/AKT signaling pathways (84). In NSCLC, especially in the squamous subtype, FGFR1, and possibly FGFR2 and FGFR3, have been found to promote carcinogenesis in a variety of ways (85,86). An interaction has been discovered between FGFR1 and EGFR in the progression of the malignant phenotype (87), and overexpression of the receptor has been associated with inferior survival. More specifically, the FGFR1 gene is amplified in about 20% of squamous-NSCLC, and 3.5-fold amplification has been recognized as the cut-off point for distributing patients in different survival groups. That cut-off limit may function as a stratification factor for clinical trials (88-90).

Several small molecules have been tested in this setting. Brivanib, or BMS-540215, a multitargeted TKI that also targets FGFR1, has failed to produce responses in a randomized discontinuation study in previously treated, unselected NSCLC patients (91). Dovitinib (TKI258) inhibits FGFR1-3 and is being tested in an ongoing phase II trial in a population of pretreated patients with squamous NSCLC, selected for FGFR1 amplification (NCT01861197). Another phase II trial of Nintedanib in the same setting, in patients with FGFR1-amplified squamous-NSCLC, has been completed and results are awaited (NCT01948141).

**DDR2 mutations**

Discoidin domain receptor 2 (DDR2) is a receptor tyrosine kinase which, along with DDR1, is implicated in cell processes crucial for tissue homeostasis and repair, such as cell adhesion, migration and proliferation (92,93). Gain-of-function mutations in DDR2 have been discovered mainly in squamous NSCLC in a frequency of around 4%, where they appear to promote carcinogenesis via cell migration and proliferation, thus rendering this receptor a promising targetable molecule (94,95). Dasatinib initially appeared effective for targeting DDR2, since it had demonstrated activity in preclinical models of SCC cell-lines that harbored activating DDR2 mutations, while a rapid response has also been noted in a patient carrying a relevant mutation, when received treatment with the combination of Dasatinib plus Erlotinib (94).

Based on these positive early results, a phase II trial was developed, in which patients with advanced squamous NSCLC that harbored DDR2 mutation or inactivating BRAF mutation and have failed standard chemotherapy, received daily 140 mg of Dasatinib. Unfortunately, this trial was terminated prematurely, after the enrollment of only five patients, due to the development of extreme toxicity in the test subjects. More specifically, 3 of 5 (60%) patients experienced ≥ grade 3 toxicities (dyspnea, fatigue, AST elevation, anorexia, nausea), while intolerable grade 2 pleural effusions were noted in 2 of 5 patients (96). Another phase II trial in a similar setting also terminated prematurely due to lack of efficacy and slow accrual (NCT01514864). The negative outcome of these trials can be primarily attributed to excessive toxicity, which appears to hinder the potential benefit of this drug in a specific, molecularly selected patient population. Consequently, a phase II trial is currently active, attempting to discover the highest tolerated dose of the combination of Dasatinib and Crizotinib that can be safely administered to patients with advanced cancer (NCT01744652).

**Conclusions**

Even though the majority of the clinical trials investigating targeted agents for NSCLC have not produced the desired results so far, their failures might be attributed mostly to poor patient selection. Indeed, as manifested better in the various trials targeting MET, strict selection criteria need to be applied when evaluating positivity for a specific genetic alteration. In that last case, it has been made evident that gene amplification rather than simple protein expression characterize true positivity and reflect an active role in the oncogenic process of a specific gene. Furthermore, while learning more about the molecular cellular biology, we realize the vast complexity of intracellular pathways and the importance of their interaction. In many of the above mentioned trials there have been hinds about the importance of implementing dual inhibition, combining two agents that inhibit different pathways, or even the combination of cytotoxics with targeted agents. Also, it is of paramount importance to decide the time frame where we offer targeted therapy, taking into account the potential escape mechanisms and the unavoidable clonal selection our treatment is bound to eventually produce in the diverse tumor cell population.

To conclude, it is clear that NSCLC is a heterogeneous disease driven by a spectrum of molecular alterations. Consequently, it is necessary to carefully translate the
results of basic science into clinical trials, not only by using the right set of biomarkers, but also by learning how to best interpret them. In this way we may achieve rationalization in our decisions when managing patients with this specific tumor type, tailoring and individualizing our treatment approach in order to provide optimal care to our patients.

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Footnote

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