Chapter 17

Achievements in targeted therapies

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Cytotoxic chemotherapy has historically been the cornerstone of advanced lung cancer treatment, but in recent years, new insights into the molecular pathways of this tumour have led to important therapeutic advances. The definition of different molecular profiles characterise some subpopulations that potentially will benefit from each target agent in terms of efficacy and quality of life. This landscape is evolving quickly as new oncogenic drivers are becoming the target for specific drugs. In this chapter, the state of the art will be presented together with perspectives on targeted therapies in lung cancer.

Targeted therapies are the perfect example of precision medicine and their role is rapidly emerging throughout different oncological diseases, including lung cancer. As opposed to traditional cytotoxic chemotherapy, which unselectively addresses rapidly dividing cells, targeted therapies are specific inhibitors of different molecules involved in cancer cell growth, survival or neoangiogenesis.

For many years, standard first-line systemic treatment for metastatic NSCLC has consisted of doublet chemotherapy (carboplatin or cisplatin, combined with a non-platinum-derived cytotoxic agent, such as taxanes, gemcitabine, vinorelbine or, more recently, pemetrexed). The introduction of targeted agents in the last few years has deeply changed the treatment paradigm in this setting and markedly modified the natural history of this disease.

The better understanding of molecular mechanisms underpinning oncogene addiction [1] has allowed, in the last 10 years, for the identification of different molecular subtypes of NSCLC, each dependent on a specific molecular driver, ultimately resulting in a constitutively active mutant signalling protein.

As depicted in figure 1, the most frequent molecular drivers described in NSCLC are *EGFR* mutations, *ALK* rearrangements, *FGFR* and Kirsten rat sarcoma viral oncogene homologue (*KRAS*) mutations.

However, to date, known and confirmed "druggable" mutations in everyday clinical practice are limited to lung ADCs (about 50% of all NSCLC). Outside these alterations, treatment

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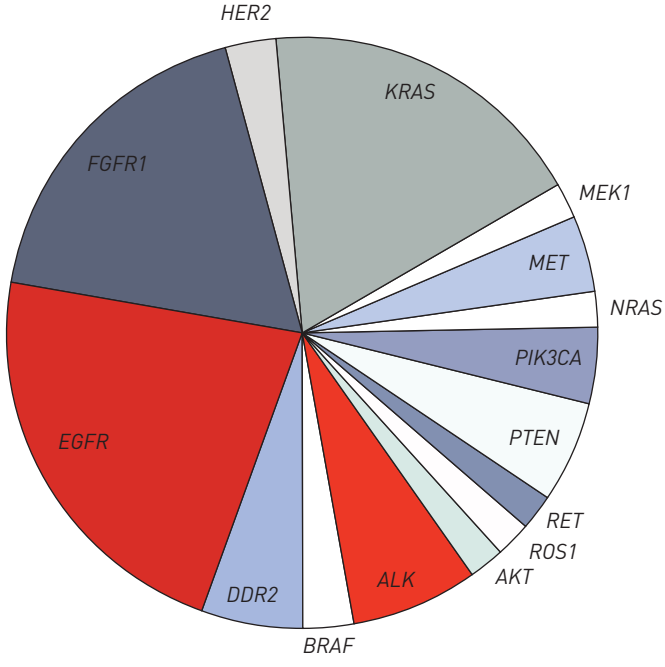

Figure 1. Frequency of known driver mutations in NSCLC. *KRAS*: Kirsten rat sarcoma viral oncogene homologue; *MEK1*: mitogen-activated protein kinase kinase 1; *MET*: Met; *NRAS*: neuroblastoma Ras viral oncogene homologue; *PIK3CA*: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α; *PTEN*: phosphatase and tensin homologue; *RET*: Ret; *ROS1*: c-ros; *BRAF*: B-Raf; *DDR2*: discoidin domain receptor 2. Data from [2].

choice is still dependent on the histological subtype or clinical characteristics, and mainly limited to cytotoxic drugs.

The following sections will describe those targeted agents already approved by regulatory agencies in lung ADCs and some new ones that will enter clinical practice in the near future.

EGFR and EGFR-directed agents

The *EGFR* gene is located on chromosome 7p12–13 and encodes a 170-kDa receptor tyrosine kinase [3]. EGFR (also known as HER1) is a transmembrane receptor, belonging to the ERBB family of cell-surface receptor tyrosine kinases together with HER2, HER3 and HER4, having an extracellular ligand-binding region, a single membrane-spanning region and a cytoplasmic tyrosine kinase-containing domain. Signalling through EGFR activation is pivotal to cell proliferation, evasion of apoptosis, angiogenesis and metastasis in various neoplasms, including NSCLC [4].

EGFR binding to EGF (its main ligand) triggers receptor homo- or heterodimerisation with other ERBB members on the cell surface, leading to activation of the intrinsic kinase domain and phosphorylation of tyrosine residues within the cytoplasmic tail. This process activates downstream effectors such as Ras/Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT/mTOR, with consequent cell

survival and proliferation. Other EGFR ligands include TGF-α, amphiregulin, epigen, betacellulin, heparin-binding EGF and epiregulin [4, 5].

Under normal circumstances, EGFR signalling relies on ligand-dependent activation of the receptor. However, *EGFR* mutations can independently sustain cell growth and survival.

EGFR mutations are localised in exon 19, mainly consisting of an in-frame deletion (45–50%), and in exon 21, consisting of the L858R point mutation (40–45%); many other less common mutations have been also identified [6]. As far as clinical characteristics are concerned, *EGFR* mutations are known to be more commonly observed in never-smokers, ADC, women and Asian people [7]. In unselected NSCLC, this genetic alteration is found in about 10% of the Caucasian population, as opposed to 30% of the Asian population [8–10].

EGFR-directed agents can be divided into different subgroups presented in table 1. EGFR TKIs are the key drugs in the management of EGFR mutant NSCLC patients. The selection of cases cannot rely on clinical characteristics and mutation testing in this subset of patients drives treatment decisions. *EGFR* mutation analysis must be performed according to evidence-based recommendations [11].

EGFR-activating mutations cluster in the catalytic kinase domain; although over 100 mutations in the kinase domain have been identified in lung ADCs, most patients harbour one of seven major mutations (exon 19 deletion (del-19), L858R in exon 21, exon 20 insertions, G719X, L861X, exon 19 insertions and T790M [12], exon 19 deletion and L858R accounting for about 85% of EGFR mutations [13–15]).

EGFR mutation detection

EGFR mutation testing is recommended in nonsquamous histology and never-smokers (independently of histotype) by a validated mutation test and it can be performed on the primary tumour or at a metastatic site. Several methods are currently available for this purpose, including direct sequencing and PCR [16].

These tests could be classified in two main groups: screening methods, *i.e.* those detecting all mutations in exons 18–21 including novel variants, and targeted methods, *i.e.* those identifying only already described mutations. IHC using mutation-specific antibodies has also been evaluated [17–22], but is not widely adopted due to concerns about its lower sensitivity and specificity compared with DNA-based molecular techniques. According to European Society for Medical Oncology guidelines, a wide coverage of mutations in exons 18–21 is strongly recommended, together with the identification of drug resistance-conferring mutations (such as exon 20 insertions or T790M substitution). *EGFR* mutation detection in peripheral blood has recently been shown to be highly predictive of the corresponding mutational status of the primary tumour [23].

First-generation EGFR TKIs: gefitinib and erlotinib

First-generation TKIs were initially investigated in early phase II trials in previously treated, unselected patients [24, 25]: clinical benefit was observed within 3–4 weeks of treatment and responses (response rates ranging from 12% to 18%) were found to be higher in ADC histology, never-smokers and female patients.

Table 1. EGFR-directed agents

Subgroup	Agents	Description
First-generation, reversible TKIs	Gefitinib Erlotinib Icotinib	Reversibly compete with ATP binding to tyrosine kinase domain of EGFR, inhibiting ligand-dependent receptor activation
Second-generation, irreversible TKIs	Afatinib Dacomitinib Neratinib Canertinib	Pan-HER inhibitors that irreversibly bind the ATP-binding pocket of EGFR *via* covalent bonds, inducing permanent inhibition
Third-generation TKIs	Mutant-selective inhibitors (CO1686, AZD9291, HM61723)	Specifically target mutant forms of EGFR, exhibiting minimal activity toward the wild-type receptor
Monoclonal antibodies	Cetuximab Panitumumab Nimotuzumab Necitumumab	Competitively inhibit ligand binding to EGFR, preventing ligand-induced activation and downstream signalling

Subsequent phase III trials comparing platinum-based chemotherapy in association with a first-generation TKI *versus* chemotherapy alone in unselected patients did not show any significant difference in terms of survival (both in patients treated with gefitinib or erlotinib) [26–30]. A similar lack of success was reported in a large phase III trial (Iressa Survival Evaluation in Lung Cancer) comparing gefitinib with placebo as a second- or third-line treatment for locally advanced or metastatic NSCLC [31].

However, a trial by Shepherd *et al.* [32] demonstrated a survival benefit in unselected patients treated with erlotinib after failure of first- and second-line chemotherapy, and this led to the approval of erlotinib in NSCLC as a second- or third-line treatment (regardless of the mutational status).

As opposed to the early trials, different results were drawn from later studies in which patients were treated with EGFR-directed agents as a first-line treatment after being selected according to their clinical characteristics and, further on, according to specific molecular characteristics (*EGFR* mutational status).

In the Iressa Pan-Asia Study, Asian patients were selected according to their smoking history (never- or light smokers) and histology (ADC), and randomised to carboplatin–paclitaxel *versus* gefitinib as a first-line treatment. The primary end-point was PFS. Activating *EGFR* mutations were found in 60% of tested patients, and both overall response rate (ORR) and PFS favoured single-agent gefitinib in this subgroup. No significant difference in overall survival was observed and this was attributed to patient crossover from chemotherapy to gefitinib at the time of relapse [28]. In pointing out the role of *EGFR* mutations as predictive biomarkers in patients treated with gefitinib, this pivotal trial changed clinical practice. The beneficial role of a first-generation TKI in the Caucasian population was defined by the European Randomised Trial of Tarceva *versus* Chemotherapy, which compared erlotinib *versus* chemotherapy (cisplatin–gemcitabine or cisplatin–docetaxel) as a first-line treatment

[33]. As shown in table 2, other phase III trials on NSCLC patients selected on the basis of *EGFR* mutational status demonstrated a significant increase of PFS and led to a fundamental change in the approach to advanced NSCLC patients [34–37].

While evidence of an activating *EGFR* mutation is mandatory for first-line EGFR-TKI treatment, erlotinib is still registered and reimbursed, as previously reported, even in second- and third-line therapy, irrespectively of molecular characteristics, on the basis of the results of BR.21 trial [25]. Although rarely, even *EGFR* wild-type patients (or those supposed as such) respond to EGFR-TKI administration [40] and the mechanisms underlying this activity are still not completely clear. While in some cases such activity is due to rare mutations not assessed by commonly used targeted methods or could depend on false-negative results [41, 42], in other patients, TKI activity could rely on EGFR expression, amplification or phosphorylation, EGFR ligand expression, or other still unknown mechanisms [43–45].

Table 2. First-line TKIs in *EGFR*-mutated patients

First author [ref.]	Study	Treatment	Patients n	Median PFS months	Median OS months
Mok [28]	IPASS	Gefitinib *versus* Carbo/Pacli	261	9.8 *versus* 6.4 (HR 0.48, p<0.001)	21.6 *versus* 21.9 (HR 1.00, p=0.99)
Han [34]	First-SIGNAL	Gefitinib *versus* Cis/Gem	42	8.4 *versus* 6.7 (HR 0.61, p=0.084)	30.6 *versus* 26.5 (HR 0.823, p=0.65)
Mitsudomi [35]	WJTOG	Gefitinib *versus* Cis/docetaxel	172	9.2 *versus* 6.3 (HR 0.489, p<0.0001)	35.5 *versus* 38.8 (HR 1.18)
Maemondo [36]	NEJ02	Gefitinib *versus* Carbo/Pacli	228	10.8 *versus* 5.4 (HR 0.32, p<0.001)	27.7 *versus* 26.6 (HR 0.88, p=0.31)
Zhou [37]	OPTIMAL	Erlotinib *versus* Carbo/Gem	154	13.1 *versus* 4.6 (HR 0.16, p<0.0001)	22.7 *versus* 28.9 (HR 1.04)
Rosell [33]	EURTAC	Erlotinib *versus* platinum-based chemo	173	9.7 *versus* 5.2 (HR 0.37, p<0.0001)	19.3 *versus* 19.5 (HR 1.04, p=0.87)
Sequist [38]	LUX-Lung 3	Afatinib *versus* Cis/Pem	345	11.1 *versus* 6.9 (HR 0.59, p=0.0004)	Not reported
Wu [39]	LUX-Lung 6	Afatinib *versus* Cis/Gem	364	11.0 *versus* 5.6 (HR 0.28, p<0.0001)	22.1 *versus* 22.2 (HR 0.95, p=0.76; immature data)

OS: overall survival; IPASS: Iressa Pan-Asia Study; First-SIGNAL: First-Line Single-Agent Iressa *versus* Gemcitabine and Cisplatin Trial in Never-Smokers with Adenocarcinoma of the Lung; WJTOG: West Japan Thoracic Oncology Group; OPTIMAL: Erlotinib *versus* Standard Chemotherapy in the First-Line Treatment of Patients with Advanced *EGFR* Mutation-Positive NSCLC; EURTAC: European Randomised Trial of Tarceva *versus* Chemotherapy; Carbo: carboplatin; Pacli: paclitaxel; Cis: cisplatin; Gem: gemcitabine; chemo: chemotherapy; Pem: pemetrexed; HR: hazard ratio.

Second-generation EGFR-TKIs: afatinib and dacomitinib

The LUX-Lung 3 phase III trial investigated the role of afatinib in the first-line setting of advanced NSCLC patients harbouring *EGFR* mutation. This trial was designed for 345 patients to compare the TKI with cisplatin–pemetrexed and a significant improvement in PFS was shown (11.1 *versus* 6.9 months). This effect was found to be even stronger (PFS 13.6 *versus* 6.9 months) in patients with common *EGFR* mutations (Del-19 and L858R) [38]. LUX-Lung 6 was conducted on Asian patients only, using cisplatin–gemcitabine as the standard arm and demonstrating similar results [39]. These trials led to afatinib approval in TKI-naïve patients with locally advanced or metastatic NSCLC harbouring *EGFR* mutations. The ongoing LUX-Lung 7 trial will provide data on afatinib *versus* gefitinib in first-line, *EGFR* mutation-positive patients (www.clinicaltrials.gov identifier number NCT01466660).

Dacomitinib did not show any significant improvement in PFS when used as a second- or third-line treatment in unselected patients [46]. Its role in *EGFR*-mutated patients is currently under evaluation in phase III trials comparing dacomitinib *versus* gefitinib in the first-line setting (ARCHER 1050 trial; NCT01774721).

EGFR monoclonal antibodies

EGFR-directed monoclonal antibodies block receptor signalling through specific competitive binding to EGFR on the extracellular surface of cells. Their action on tumour cells is also enhanced by antibody-dependent cellular cytotoxicity. EGFR-directed monoclonal antibodies evaluated in NSCLC include cetuximab, panitumumab, nimotuzumab and necitumumab.

Cetuximab is a chimaeric monoclonal antibody (mAb) that selectively binds the extracellular domain of EGFR. The role of cetuximab in combination with first-line chemotherapy has been investigated in phase II and III trials [47–50] and within a subsequent meta-analysis [51], and a modest benefit in all efficacy end-points from the addition of cetuximab to standard platinum-based treatment in NSCLC was reported (table 3). Moreover, the addition of cetuximab to chemotherapy was characterised by an increased toxicity in terms of rash, diarrhoea, neutropenia and infusion reaction, and this is the main reason why (together with modest efficacy advantage) the First-Line Erbitux in Lung Cancer trial did not led to treatment approval by regulatory agencies.

Table 3. Combination of chemotherapy and cetuximab in advanced NSCLC

First author [ref.]	Study	Treatment	Patients n	Median PFS months	Median OS months
Butts [49]		Cis or Carbo/Gem ± Cet	65	5.09 *versus* 4.21	11.99 *versus* 9.26
Rosell [50]		Cis/Vnb ± Cet	86	5.0 *versus* 4.6	8.3 *versus* 7.3
Pirker [47]	FLEX	Cis/Vnb ± Cet	1125	4.8 *versus* 4.8	11.3 *versus* 10.1
Lynch [48]	BMS099	Taxane/Carbo ± Cet	676	4.4 *versus* 4.24	9.69 *versus* 8.38

OS: overall survival; FLEX: First-Line Erbitux in Lung Cancer; Cis: cisplatin; Carbo: carboplatin; Gem: gemcitabine; Cet: cetuximab; Vnb: vinorelbine.

Necitumumab, a fully human mAb, has been evaluated in phase III trials in combination with cisplatin–pemetrexed in advanced nonsquamous NSCLC (INSPIRE trial, stopped early for unexpected toxicity) and with cisplatin–gemcitabine in squamous histology (SQUIRE trial). In the SQUIRE trial, the addition of necitumumab to cisplatin–gemcitabine statistically significantly improved overall survival (11.5 *versus* 9.9 months) and PFS (5.7 *versus* 5.5 months) [52].

Nimotuzumab (95% human antibody) is under investigation in phase II trials (NCT00983047 and NCT01393080).

None of the EGFR-directed monoclonal antibodies is currently part of the therapeutic armamentarium for NSCLC treatment, in any clinical setting.

Toxicity

Despite the high selectivity of targeted therapies, a range of previously unknown and sometimes unpredictable side-effects have been described. Toxicity related to the aforementioned EGFR-directed agents mainly reflects their off-target effects, *i.e.* targeting wild-type EGFR in normal tissues. The most common toxicities are rash/dermatitis acneiform, diarrhoea, stomatitis, dry skin and pruritus [53, 54].

Skin disorders are generally mild or moderate in severity and can be managed by appropriate topical or systemic interventions, or by reducing the TKI dose [55]. As for other treatments, there is possibly an underestimation of toxicity by clinicians when compared with patients and this fact must be taken into account, especially if dealing with long-term therapies, which is the aim of these targeted drugs [56].

Drug resistance

Despite the benefit that targeted agents brought to NSCLC treatment, not all *EGFR*-mutated patients exhibit durable responses, the median PFS is still less than 1 year and almost all patients develop resistance [33, 37, 38].

Primary resistance may occur in some patients not responding to TKIs from the very beginning and acquired resistance may also develop over time, due to several mechanisms. Secondary, acquired gatekeeper mutations in the EGFR kinase domain (such as T790M, L747S, D761Y and T854A) and PI3K mutations alter the binding kinetics of the receptor and its downstream effectors, representing a form of oncogenic drift [13, 14]. Other mechanisms of resistance include activation of second oncogenic drivers through different mechanisms, such as *MET* amplification, *EGFR* amplification, *HER2* upregulation and *ALK* amplification [57]. In addition, a shift towards SCLC histology and epithelial–mesenchymal transition have been described as additional mechanisms of acquired resistance [57, 58].

Clinically, TKI resistance can be classified into two categories: oligoprogression and systemic progression. Even without strong prospective data, in oligometastatic patients, local therapies (radiotherapy, surgery and local ablation) are feasible options together with continuation of the oral TKI beyond Response Evaluation Criteria In Solid Tumors (RECIST)-defined disease progression [59, 60]. The rationale of such approach is avoiding the flare-up phenomenon (rapid recurrence of symptoms and symptomatic decline) [61], taking advantage of the remaining drug sensitivity. Recent trials evaluated the role of TKI beyond progression. In the phase II ASPIRATION trial, Asian patients with advanced *EGFR* mutation-positive NSCLC

received erlotinib beyond progression. The difference between PFS1 (calculated until RECIST progression) and PFS2 (calculated until progression assessed by doctor discretion) was 3.7 months [62]. The phase III Iressa Treatment Beyond Progression in Addition to Chemotherapy *versus* Chemotherapy Alone study explored the role of gefitinib beyond progression in addition to chemotherapy *versus* chemotherapy alone in *EGFR*-mutated NSCLC, but the study did not meet the primary end-point (PFS) and no benefit was seen in terms of survival or response rate from continuing the TKI with cytotoxic drugs [63].

The main therapeutic strategy to overcome EGFR-TKI resistance has been the development and introduction of third-line EGFR-TKIs. Combinatorial targeting of the EGFR pathway has been also investigated.

A single-arm, phase Ib trial investigated afatinib and cetuximab, in patients who developed acquired resistance to erlotinib or gefitinib, described a PFS of 4.7 months, but with some issues concerning the toxicity profile [64]. By contrast, a similar phase I/II trial of erlotinib and cetuximab did not demonstrate efficacy [65].

The identification of molecular drivers and the rapidly increasing development of corresponding new targeted agents led to an approach of cotargeting EGFR and further intracellular pathways as an alternative strategy to overcome EGFR-TKI resistance. In this setting, Met targeting has been thoroughly investigated (given its pivotal role both in *de novo* and acquired resistance) but combinatorial EFGR–Met targeting was discouraged by the results of phase III trials [66, 67]. No targeted-agent combination is currently recommended in EGFR-TKI-resistant NSCLC patients outside clinical trials and further data are warranted.

Third-generation TKIs

Third-generation TKIs (CO1686, AZD9291 and HM61713) are oral, irreversible EGFR inhibitors that block mutated EGFR, including drug resistance mutations such as T790M. At therapeutic dose, they do not inhibit wild-type EGFR and such binding specificity deeply limits the observed toxicity [68, 69]. AZD9291 and CO1686 already showed very promising results in phase I/II trials reporting remarkable overall disease control rates (89% for CO1686 and 96% for AZD9291) [69, 70]. Phase II and III studies exploring these drugs in TKI-naïve and in TKI-resistant patients are currently ongoing. A recent phase I trial evaluated the role of HM61713 in EGFR-TKI-pre-treated patients, divided into two arms according to the time since prior EGFR-TKI treatment (<4 *versus* ⩾4 weeks), showing a good safety profile together with a disease control rate of 76.5% and 73.1% in the two arms, respectively [71].

ALK rearrangements

The *EML4–ALK* fusion gene was first described as an oncogenic driver in lung ADC in 2007 [72]. This rearrangement is generated by an inversion in chromosome 2p that juxtaposes the 5′-end of *EML4* with the 3′-kinase domain of *ALK*, leading to constitutive ALK kinase activation.

Many variants of this fusion protein have been described to date [73] and other *ALK* partners emerged, including TRL-fused gene (*TFG*) [74], kinesin family member 5B (*KIF5B*) [75, 76], kinesin light chain 1 (*KLC1*) [77] and translocated promoter region (*TPR*) [78].

This genetic alteration is found in 2–7% of NSCLC patients, a percentage depending on the detection method used and on the screened population [79–82]. Typically, *EML4–ALK*-positive tumours are ADCs, mainly young nonsmoking patients, but not exclusively; the genetic alteration seems to be mutually exclusive to *KRAS* and *EGFR* mutations [83–87].

ALK rearrangement detection

Currently, the gold standard and US Food and Drug Administration (FDA)-approved diagnostic test for *ALK* gene rearrangement detection is the break-apart FISH test [88]. However, this method is expensive, not always reproducible and not widely available.

For such reasons, clinical and preclinical research is evaluating alternative methods, such as real-time PCR, IHC with dedicated antibodies, and NGS techniques [89–92]. In Europe, IHC has recently been approved as one of the standard diagnostic tests to detect *ALK* rearrangements: in a paper by MARCHETTI *et al.* [93], an algorithm was proposed where ALK IHC is the first diagnostic step in EGFR- and KRAS-negative NSCLC, whereas FISH is reserved for IHC-positive patients only.

As for *EGFR* mutations, *ALK* rearrangement testing is recommended in all patients with advanced nonsquamous NSCLC at diagnosis and should be carried out in parallel with *EGFR* mutation analysis; moreover, *ALK* rearrangement testing should be considered even in SCC from patients with minimal or remote smoking history [23, 94].

ALK-rearranged tumours: treatment

Crizotinib

In August 2011, crizotinib, an oral, small-molecule inhibitor of the Met, ALK and ROS1 tyrosine kinases, was approved by the FDA under an accelerated procedure for *ALK*-rearranged locally advanced and metastatic NSCLC patients on the basis of the results of phase I and II trials (PROFILE 1001 and PROFILE 1005) showing high response rates and a good tolerability profile [79, 95].

A phase III trial in previously treated patients [96] showed a statistically significant improvement in PFS (7.7 *versus* 3.3 months; hazard ratio (HR) 0.49 (95% CI 0.37–0.64), p<0.001) and response rate (65% *versus* 20%) with crizotinib, as compared with single-agent chemotherapy (pemetrexed or docetaxel) in advanced NSCLC, *ALK*-rearranged patients. More recently, preliminary data from a phase III, open-label, randomised trial comparing first-line platinum (either cisplatin or carboplatin)/pemetrexed chemotherapy *versus* crizotinib were presented, showing significant improvements in PFS (median 10.9 *versus* 7 months; HR 0.454 (95% CI 0.346–0.596), p<0.0001) and ORR (74% *versus* 45%; p<0.0001) with the TKI [97].

Novel ALK inhibitors

Ceritinib (LDK378), a novel ALK inhibitor with some activity on ROS1, insulin-like growth factor-1 receptor and insulin receptor, was evaluated in a phase I/II, open-label, randomised trial that enrolled 163 *ALK*-positive metastatic NSCLC patients progressing on or intolerant to crizotinib [98, 99]. Ceritinib led to an ORR of 58% (95% CI 48–67%) and, notably, an ORR of 56% in patients previously treated with crizotinib (95% CI 45–67%). Based on these data, on April 2014, the FDA granted accelerated approval of ceritinib for the treatment of metastatic, *ALK*-positive NSCLC previously treated with or intolerant to crizotinib.

Alectinib (RO5424802/CH5424802), another second-generation ALK inhibitor, demonstrated activity in both crizotinib-naïve [100] and pre-treated patients in phase I/II trials [101, 102]. The drug is currently approved in Japan for the treatment of *ALK*-positive, advanced NSCLC patients.

These novel ALK inhibitors can effectively cross the blood–brain barrier, also leading to good responses in patients harbouring central nervous system metastasis.

ASP3026 is also a novel ALK inhibitor showing activity in cases with the crizotinib-resistant gatekeeper mutation L1196M; preliminary results from a phase I trial demonstrated clinical activity in *ALK*-positive NSCLC that progressed on prior crizotinib [103]. AP26113 is a potent inhibitor of wild-type ALK that maintains activity against several crizotinib-resistant ALK mutants, as shown in a phase I/II, single-arm trial conducted by Gettinger *et al.* [104]. Due to these results, both agents, along with other compounds currently under active investigation (X-396, X-376, PF-06463922, TSR-011, RXDX-101, CEP-28122 and CEP-37440) could lead to further progress in *ALK*-positive NSCLC treatment [105].

Toxicity

Crizotinib is usually well tolerated. The most common toxicities are neutropenia, diarrhoea, nausea, abdominal pain, transaminase elevation (especially alanine transaminase) and visual disorders; rare but potentially fatal drug-related pneumonia and hepatic insufficiency could also occur. Male patients could develop symptomatic hypogonadism [106].

Ceritinib could induce gastrointestinal toxicity, fatigue and transaminase alteration [107], while alectinib was reported to be associated with fluid retention and fatigue [108]. The most frequent ASP3026 adverse events are fatigue, vomiting, nausea and constipation, while AP26113, in addition, induced cough, headache and pulmonary symptoms such as dyspnoea and hypoxia.

Drug resistance

Similarly to the *EGFR* population, in *ALK*-rearranged patients, after an initial response, progression occurs: the three main mechanisms of ALK resistance are mutation of the ALK tyrosine kinase domain (accounting for approximately 25% of cases), amplification of the *EML4–ALK* gene and activation of alternative signalling pathways [109–113].

Clinical research is currently focussing on evaluating second-generation ALK inhibitors in this context (see earlier). Moreover, interesting results come from heat shock protein (Hsp) inhibition [114]. Drugs inhibiting this target have been investigated in *ALK*-rearranged patients with low response rates, when used alone and compared with ALK inhibitors, but other trials are currently combining an ALK-TKI with and Hsp90 inhibitor, in order to prevent resistance development or overcome it [115] (NCT01579994 and NCT01772797).

An intriguing aspect, although still debated, is the activity of pemetrexed in *ALK*-rearranged patients, as observed in subgroup analyses and retrospective trials [96, 116].

Angiogenesis inhibition

Neoangiogenesis, which promotes tumour growth and metastasis, is characterised by the formation of abnormal and chaotic vessels leading to an altered tumour microenvironment, which increases VEGF production, causing an autonomous proangiogenic and

promitogenic loop. Currently available antiangiogenic drugs are directed against circulating VEGF or VEGFR.

Bevacizumab is a humanised mAb that targets circulating VEGF, approved for nonsquamous, advanced NSCLC first-line treatment, in addition to platinum doublet chemotherapy.

The registration of this drug was based on two phase III trials, which evaluated platinum-based doublet chemotherapy *versus* the same doublet plus bevacizumab. The Eastern Cooperative Oncology Group E4599 trial enrolled 878 patients with stage IIIB/IV nonsquamous NSCLC randomised to six cycles of carboplatin and paclitaxel with or without bevacizumab 15 $mg \cdot kg^{-1}$. Overall survival was significantly greater in the experimental arm (12.3 *versus* 10.3 months, p=0.003), as well as PFS (6.2 *versus* 4.5 months, p<0.001) and response rate (35% *versus* 15%, p<0.001) [117]. The Avastin in Lung Cancer trial randomised the same population to cisplatin and gemcitabine chemotherapy plus bevacizumab at two different doses (7.5 or 15 $mg \cdot kg^{-1}$) or placebo. Compared with the placebo group, the risk of progression or death at any time was reduced by 25% in the evacizumab 7.5 $mg \cdot kg^{-1}$ group (HR 0.75, 95% CI 0.64–0.87; p=0.0003) and by 15% in the bevacizumab 15 $mg \cdot kg^{-1}$ group (HR 0.85, 95% CI 0.73–1.00; p=0.0456), while no significant improvement was shown for overall survival [118]. The main toxicities of bevacizumab are arterial hypertension, haemorrhagic events, proteinuria and neutropenia [119].

Nintedanib is an oral inhibitor of VEGFR, platelet-derived growth factor and FGFR. This agent was added to docetaxel in second-line setting in a phase III randomised placebo-controlled trial (LUME-Lung 1). The combination significantly prolonged PFS in the whole population (median 3.4 *versus* 2.7 months, p=0.0019); moreover, overall survival was increased at a pre-planned subgroup analysis in ADC patients, especially in those who progressed within 9 months after starting first-line chemotherapy (10.9 *versus* 7.9 months, p=0.0073) [120].

A second study investigated nintedanib plus pemetrexed *versus* placebo plus pemetrexed in advanced nonsquamous NSCLC as second-line therapy. The trial was stopped after an interim analysis that suggested lack of improvement from the addition of nintedanib. The final analysis of the intention-to-treat population showed a significant although modest improvement of PFS with the experimental combination (median PFS 4.4 *versus* 3.6 months, p=0.04) [121].

Ramucirumab, an IgG1 mAb against the extracellular domain of VEGFR2, has been evaluated in a double-blind, placebo-controlled, phase III trial in association with docetaxel in second-line treatment of stage IV NSCLC. The addition of the mAb significantly improved ORR (22.9% *versus* 13.6%, p<0.001), PFS (median PFS 4.5 *versus* 3.0 months; HR 0.762, p<0.0001) and overall survival (median overall survival 10.5 *versus* 9.1 months; HR 0.857 (95% CI 0.751–0.98), p=0.0235), with only a slight increase of grade 3–4 neutropenia, fatigue and hypertension, while grade 5 adverse events were comparable between the two arms [122].

Promising new targets and agents

BRAF somatic mutations occur in 1–2% of NSCLC, mainly in ADC and former/current smokers. V600E point mutation represents the most common, even if other sites of

mutations are reported in lung cancer. Dabrafenib, a B-Raf inhibitor, showed clinical activity in a phase II study in 78 *BRAF* V600E-mutated NSCLC patients, with an ORR of 32% and a disease control rate of 56% after 12 weeks of treatment [123].

PI3K inhibitors are of interest in SCC where *PIK3CA*, *PTEN* (a phosphatase and tensin homologue) and *AKT* mutations are more frequent [124]. Currently, buparlisib (BKM-120) and pictilisib (GCD-0941) are under investigation in association with chemotherapy, and data are awaited (NCT01911325 and NCT01493843).

Met protein overexpression is found in approximately 25–75% of early-stage NSCLC and such alteration, along with gene amplification, is associated with poor prognosis [125–127]. While only 4–7% of untreated NSCLCs harbour *MET* amplification, 20% of patients previously treated with EGFR-TKIs show this alteration, possibly mediating EGFR-directed agent resistance [128, 129]. Moreover, elevated serum levels of HGF, which binds the Met tyrosine kinase receptor, have been associated with poor prognosis and aggressiveness in both NSCLC and SCLC, along with primary and secondary resistance to EGFR-TKIs [130–133].

mAbs binding HGF are currently being tested. Of these, ficlatuzumab seems to confer some benefit in *EGFR*-mutated patients with low c-Met expression when associated with gefinitib, possibly by delaying resistance onset [134].

In contrast, onartuzumab, a mAb against Met, after promising results in a phase II trial [135], was deemed ineffective, with the early closure of the phase III trial (MetLung) in Met-positive (IHC detection), advanced NSCLC patients [136]. Small molecules inhibiting Met include tivantinib (ARQ-197), cabozantinib (XL-184) and crizotinib. Two phase III trials investigating tivantinib, a non-ATP-competitive Met inhibitor, in association with erlotinib were both prematurely stopped because of either futility (MARQUEE trial) [66] or toxicity (ATTENTION trial) [67]. Cabozantinib and crizotinib are currently under investigation as Met inhibitors. Interestingly, cabozantinib is also a potent inhibitor of VEGFR2, AXL and Ret, showing activity in tumours positive for the *RET* fusion gene [137], which account for approximately 1.7% of lung ADCs [138].

ROS1 rearrangement, which is detected in about 1% of ADCs [139], is actively targeted by crizotinib, with an ORR of 72% (95% CI 58–84%) and a median PFS of 19.2 months (95% CI 14.5 months–not reached) [140]; second-generation ALK inhibitors are also being investigated in clinical trials in this subset of patients.

A unique class of patients is represented by those carrying *HER2* mutations or amplification. While some data suggest activity of HER2-targeted agents such as trastuzumab and dacomitinib [141, 142], further studies are warranted to explore treatment opportunities actively in this subpopulation.

KRAS is the most common mutated gene in NSCLC, and is frequently detected in patients with smoking history (25% *versus* 6% in former/current smokers and nonsmokers, respectively) [143] and ADC (34%) [144]. Selumetinib inhibits MEK1/MEK2, downstream kinases of the Ras/Raf/MEK/ERK signalling pathway. This small molecule has been investigated with docetaxel in a phase II trial in patients who had progressed to first-line therapy, showing a statistically significant improvement in ORR (37% *versus* 0%, $p<0.0001$) and PFS (HR 0.58, 80% CI 0.42–0.79; one-sided $p=0.0014$), and numerically superior, although

not statistically significant, overall survival (9.4 *versus* 5.2 months). However, the combination regimen was more toxic: higher rates of neutropenia, febrile neutropenia and serious adverse events leading to hospitalisation (48% *versus* 19%) [145]. Currently, a phase III trial is ongoing testing docetaxel plus selumetinib *versus* docetaxel plus placebo (NCT01933932).

Conclusion

The introduction of targeted drugs in the clinical management of lung cancer patients is undoubtedly a step forward in tailored therapy. The current treatment choice relies on a careful assessment of histological and molecular features (*EGFR* mutations and *ALK* translocation are already part of routine diagnostic work-up), in order to identify those patients who may benefit more from a certain therapeutic approach.

This assessment is important both at diagnosis and at relapse, since cancer cells may dynamically change their features over time. Such modifications can occur for intrinsic cancer changes due to progression or they can be enhanced by selective pressure superimposed by treatment, ultimately leading to drug resistance (*i.e.* T790M mutation in patients who received EGFR-TKIs and *ALK* amplification in crizotinib treated patients).

One of the main hurdles underpinning disease progression and drug resistance is tumour heterogeneity and drug adaptation. Heterogeneous subclonal events caused by genetic drift may account for drug resistance and different clinical behaviour of the disease at different sites or diverse biological responses within the same lesion.

Liquid biopsies have recently been introduced as a tool to overcome limitations in collecting tissue samples, by genotyping circulating cell-free DNA or circulating cell DNA.

In current clinical practice, a single-gene testing approach is mainly used to identify variants (*e.g. EGFR* mutation or *ALK* rearrangement) to guide treatment decisions, and such serial testing takes time and depletes tumour tissue. In addition, the cost of single-gene methods scales linearly with the number of genes interrogated and targeted NGS of cancer-related genes could be a future method to detect commonly altered genes on a single platform.

The introduction of targeted drugs in the therapeutic armamentarium has also revolutionised the way in which clinical trials are designed in thoracic oncology. As opposed to previous clinical trials exploring the role of chemotherapy in wide, unselected groups of patients, those on targeted therapies are run on selected patient populations and, consequently, on small sample sizes. The so-called basket trials are designed to explore treatment efficacy in a quick and safe way: patients with multiple diseases and one or more targets are enrolled in small cohorts, according to their biomolecular features. Cohorts with good responses can be expanded, whereas cohorts with poor responses can be closed rapidly, allowing patients to shift to a new drug.

Finally, even though the identification of new active molecules is rapidly speeding up, the actual introduction of these compounds to clinical practice is not straightforward. This is due to the need for approval by regulatory agencies, which often lead to different scenarios across the world, depending on local approvals and regulations, which is inevitably stressful for both the patients and the physicians taking care of them.

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