Personalised medicine for nonsmall cell lung cancer

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ABSTRACT After years of standard care prescribed to cancer patients without any selection except the primary site and histology of the tumour, the era of precision medicine has revolutionised cancer care. Personalised medicine refers to the selection of patients for specific treatment based on the presence of specific biomarkers which indicate sensitivity to corresponding targeted therapies and/or lower toxicity risk, such that patients will have the greatest chance of deriving benefit from the treatments. Here, we review personalised medicine for nonsmall cell lung cancer.

Introduction

Lung cancer is the leading cause of cancer-related mortality among men and women worldwide. Nonsmall cell lung cancer (NSCLC), the most common subtype (85% of lung cancers), has an overall 5-year survival rate of 16%, which has not improved significantly for several decades [1]. The poor prognosis of lung cancer could be attributed to the diagnosis of the disease at an advanced stage (only 15% of cases are diagnosed at early stages) and the lack of a cure, as well as the very short survival for patients with advanced stages of NSCLC. Until recently, NSCLCs were all treated similarly, based on staging. Surgery alone was the standard of care for patients with stage I–IIIA NSCLC, resulting in survival rates ranging from 23% in stage IIIA, to 33% in stage IIB and up to 89% in stage IA [2]. Adjuvant chemotherapy after surgical resection of localised NSCLC improves survival at 5 years by ∼5% and is recommended in all resected cases except stage IA [3]. Stage IIIB NSCLCs are generally treated by a combination of thoracic radiotherapy and platinum-based chemotherapy [4]. Until very recently, any stage IV NSCLC patients were exclusively treated by chemotherapy without selection of patients for histology or any other biomarker. In the past 10 years, the therapeutic arsenal for NSCLCs has diversified significantly, with the

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emergence of targeted therapies and, more recently, immunotherapies. The concept of personalised medicine has grown with the integration of predictive biomarkers, giving the potential to identify patients who may experience the lowest toxicity and/or derive the greatest benefit from treatments such as chemotherapy and immunotherapy, or personalised treatment based on individual tumour profiling. From the year 2000 onwards, personalised medicine has integrated routine best practice for lung cancer patients with advanced stage disease. Here, we review the current personalised medicine strategies for NSCLC patients.

Personalised medicine based on histology

Historically, the first step towards personalised medicine in NSCLC has been treatment allocation according to histological type. In the early 2000s, the standard of care in patients with advanced NSCLC and good performance status (score 0–1) was chemotherapy combining a platinum compound (cisplatin or carboplatin) with a third-generation drug such as gemcitabine, vinorelbine or a taxane compound (docetaxel or paclitaxel) [5]. When compared head to head in phase III studies, these doublets have shown comparable efficacy, with some differences in toxicity profiles [6–9]. Then, a randomised non-inferiority phase III study compared cisplatin/pemetrexed to cisplatin/gemcitabine in chemotherapy-naive patients with stage IIIB or IV NSCLC and a performance status of 0–1 [10]. For the whole study population, overall survival for cisplatin/pemetrexed was non-inferior to cisplatin/gemcitabine (median survival 10.3 months *versus* 10.3 months, respectively; hazard ratio (HR) 0.94, 95% CI 0.84–1.05). However, when outcome was assessed according to histological types, overall survival was significantly longer with cisplatin/pemetrexed than with cisplatin/gemcitabine in the subgroup of patients with adenocarcinoma (12.6 months *versus* 10.9 months; HR 0.84, 95% CI 0.71–0.99; p=0.03) and in the subgroup of patients with large cell carcinoma (10.4 months *versus* 6.7 months; HR 0.67, 95% CI 0.48–0.96; p=0.03). In contrast, in the subgroup of patients with squamous cell carcinoma, overall survival was significantly shorter with cisplatin/pemetrexed than with cisplatin/gemcitabine (9.4 months *versus* 10.8 months; HR 1.23, 95% CI 1.00–1.51; p=0.05). Despite several methodological issues (no central review of histology, up to 30% of cytological diagnosis, *etc.*) and unanswered questions (lack of difference in terms of progression-free survival (PFS), impact of thymidilate synthase expression on treatment efficacy, *etc.*), these results were confirmed by a treatment-by-histology interaction analysis (p=0.0011), and also by a combined analysis of three phase III trials [11]. The combination of cisplatin and pemetrexed was thus approved for first-line treatment of advanced NSCLC, but restricted to nonsquamous histologies. The same restriction subsequently applied to pemetrexed monotherapy, in the setting of second-line treatment [12, 13] and maintenance therapy [14, 15].

Bevacizumab, an anti-vascular endothelial growth factor (VEGF) monoclonal antibody, was initially assessed in chemotherapy-naive patients with advanced NSCLC in a randomised phase II study [16], comparing carboplatin/paclitaxel alone *versus* the same chemotherapy plus bevacizumab 15 $mg \cdot kg^{-1}$ or 7.5 $mg \cdot kg^{-1}$. The efficacy of the combination was superior to the chemotherapy alone regarding objective response rate (ORR), PFS and overall survival. The tolerance was characterised by new safety issues and especially the development of hypertension, proteinuria and bleeding events, including grade 5 pulmonary haemorrhages, predominantly in squamous NSCLC patients, who were therefore excluded from subsequent trials. Therefore, a first phase III randomised trial (ECOG4599) randomised previously untreated stage IV NSCLC patients between carboplatin/paclitaxel for six cycles *versus* the same chemotherapy regimen plus bevacizumab 15 $mg \cdot kg^{-1}$ until progression [17], the combination being significantly superior to the chemotherapy alone. Another phase III randomised trial (AVAiL) [18], blinded regarding the use of the bevacizumab, compared a "European" chemotherapy regimen (*i.e.* cisplatin/gemcitabine) alone or with bevacizumab (7.5 $mg \cdot kg^{-1}$ and 15 $mg \cdot kg^{-1}$) and again found the combination significantly superior to the chemotherapy alone regarding ORR and PFS, but not overall survival [19]. Altogether, these two phase III studies (summarised in table 1) led to the approval of bevacizumab in combination with first-line platinum-based chemotherapy for stage IV NSCLC patients, but with nonsquamous histologies only.

Necitumumab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, was assessed in two phase III trials with similar designs in patients with NSCLC, according to histological type. First, in the INSPIRE trial, conducted in previously untreated, stage IV nonsquamous NSCLC [20], patients received either the cisplatin/pemetrexed doublet alone, or with necitumumab. Enrolment in this trial was stopped after 633 patients had enrolled, following an independent data monitoring committee recommendation based on an observed imbalance in fatal thromboembolic events between the two arms. There was no significant difference in overall survival (primary end-point), PFS, ORR and disease control rate between treatment groups. The SQUIRE phase III trial, conducted in patients with squamous cell carcinoma using cisplatin/gemcitabine [21], met its primary objective with a significant improvement of

TABLE 1 Main clinical trials of bevacizumab in previously untreated patients with advanced nonsmall cell lung cancer

First author [ref.]	Year(s)	Treatment	Bevacizumab mg·kg^{-1}	ORR %	PFS months	Overall survival months
Johnson [16]	2004	Carboplatin+paclitaxel±bevacizumab (6 cycles)	0	18.8	4.2	14.9
			7.5	28.1	4.3	11.6
			15	31.5	7.4 (p=0.023)	17.7 (p=0.63)
Sandler [17]	2006	Carboplatin+paclitaxel±bevacizumab (6 cycles) ±bevacizumab until progression	0	15	4.5	10.3
			15	35 (p<0.001)	6.2 (p<0.001)	12.3 (p=0.03)
Reck [18, 19]	2009, 2010	Cisplatin+gemcitabine±bevacizumab (6 cycles) ±bevacizumab until progression	Placebo	20.1	6.1	13.1
			7.5	34.1 (p<0.0001)	6.7 (p=0.003)	13.6 (p=0.420)
			15	30.4 (p=0.0023)	6.5 (p=0.03)	13.4 (p=0.761)

ORR: objective response rate; PFS: progression-free survival.

overall survival in the experimental arm (11.5 months *versus* 9.9 months; HR 0.84, 95% CI 0.74–0.96; p=0.01). Based on these results, the approval of necitumumab has been restricted to squamous NSCLC.

Nintedanib is a triple angiokinase inhibitor that simultaneously inhibits signalling pathways activated by VEGF, fibroblast growth factor and platelet-derived growth factor. The LUME-Lung randomised phase III trial [22] showed a significantly improved PFS in the nintedanib/docetaxel group compared with the placebo/docetaxel group (3.4 months *versus* 2.7 months; HR 0.79, 95% CI 0.68–0.92; p=0.0019). Overall survival was significantly improved for patients in the experimental arm in all patients with adenocarcinoma (12.6 months *versus* 10.3 months; HR 0.83, 95% CI 0.70–0.99; p=0.0359) and in a predefined group of patients with adenocarcinoma and poor prognosis (10.9 months *versus* 7.9 months; HR 0.75, 95% CI 0.60–0.92; p=0.0073). Finally, in all patients, regardless of histology, overall survival did not significantly differ between the two arms. These results led to the approval of nintedanib, in combination with docetaxel, as second-line treatment for advanced NSCLC, but only in patients with adenocarcinoma histology.

Personalised medicine based on tumour molecular profile

EGFR mutations

EGFR is a transmembrane receptor protein with tyrosine kinase activity, against which specific tyrosine kinase inhibitors (TKIs) have been developed. First evaluated in previously treated patients with NSCLC [23, 24], EGFR TKIs were then evaluated in combination with chemotherapy [25, 26], but did not improve the outcome compared to chemotherapy alone. In 2005, lung cancer care was revolutionised by the discovery that activating mutations in the tyrosine kinase domain of *EGFR* cause sensitivity to the effect of EGFR TKIs in patients with NSCLC [27]. The *EGFR* mutations are located in exons 18, 19, 20 and 21, but the large majority of sensitising mutations of *EGFR* are deletions of exon 19 (most frequently delE746–A750) and point mutations of exon 21 (L858R). The discovery of *EGFR* as an oncogenic driver and the identification of its mutation as a predictor of the response to EGFR TKIs dramatically changed lung cancer treatment, because it offered the first opportunity for bioguided targeted therapy. The two first EGFR TKIs to be developed were gefitinib and erlotinib. Mok *et al.* [28] published the first data showing an advantage of using biomarker-guided therapy. Gefitinib provided a better outcome than chemotherapy to patients with NSCLC harbouring an EGFR-activating mutation. The ORR to gefitinib was 71% and the PFS was 9.5 months in patients with advanced NSCLC that harboured an EGFR-activating mutation, thus showing a very significantly better outcome (HR 0.48, p<0.001) compared to standard chemotherapy, which had an ORR of 47% and a PFS of 6.3 months. Inversely, chemotherapy was more favourable than gefitinib in the group of patients without activating mutations of *EGFR* (HR 2.85, p<0.001). Subsequently, several other randomised trials with erlotinib or gefitinib confirmed the superiority of first-line treatment with first-generation EGFR TKIs compared with chemotherapy for patients with advanced *EGFR*-mutated NSCLC tumours (table 2) [29–33]. These two drugs were thus approved and recommended for this indication. Subsequently, afatinib, a second-generation EGFR TKI, also showed similar benefit compared to chemotherapy in NSCLC with EGFR-activating mutations in several randomised studies. Gefitinib, erlotinib and afatinib have been approved and are recommended in first-line therapy in advanced NSCLC with activating mutations in the tyrosine kinase domain of *EGFR* (tables 2 and 3) [34, 35].

TABLE 2 Phase III randomised trials comparing EGFR TKIs with chemotherapy in first-line therapy in *EGFR*-mutant nonsmall cell lung cancer

First author [ref.]	Study name	Ethnicity	Patients with *EGFR* mutation n	EGFR TKI	Chemotherapy	ORR# %	PFS# months
Mok [28]	IPASS	Asians	261	Gefitinib (n=132)	Carboplatin/paclitaxel (n=129)	71/47	9.5/6.3 (HR 0.48)
Mitsudomi [29]	WJTOG3405	Asians	117	Gefitinib (n=58)	Cisplatin/docetaxel (n=59)	62/32	98.2/6.3 (HR 0.49)
Maemondo [30]	NEJ002	Asians	228	Gefitinib (n=114)	Carboplatin/paclitaxel (n=114)	74/31	10.8/5.4 (HR 0.30)
Zhou [31]	OPTIMAL	Asians	154	Erlotinib (n=82)	Carboplatin/gemcitabine (n=72)	83/36	13.1/4.6 (HR 0.37)
Rosell [32]	EURTAC	Caucasians	173	Erlotinib (n=86)	Doublets with platinum (n=87)	71/47	9.58/5.5 (HR 0.37)
Wu [33]	ENSURE	Asians	217	Erlotinib (n=110)	Cisplatin/gemcitabine (n=107)	63/34	11.0/5.5 (HR 0.34)
Sequist [34]	LUX-Lung 3	Caucasians	345	Afatinib (n=230)	Cisplatin/pemetrexed (n=115)	56/23	11.1/6.9 (HR 0.58)
Wu [35]	LUX-Lung 6	Asians	364	Afatinib (n=242)	Cisplatin/gemcitabine (n=122)	67/23	11.0/5.6 (HR 0.28)

EGFR: epidermal growth factor receptor; TKI: tyrosine kinase inhibitor; ORR: objective response rate; PFS: progression-free survival; HR: hazard ratio. #: comparisons presented as EGFR TKI/chemotherapy.

Although initial response or disease stabilisation are generally observed for about 11 months with one of these three drugs in first-line therapy, all patients with NSCLC harbouring *EGFR* mutations will eventually become resistant and disease will progress. The mechanism of resistance to first-line EGFR TKIs has been studied and identified. The most frequent acquired mechanism of resistance to first-line EGFR TKIs is the T790M mutation in exon 20 of *EGFR* [36]. It is detected in >50% of cases of secondary resistance to EGFR TKIs, but can also exist *de novo*. Other secondary mechanisms of resistance acquired with first-/second-generation EGFR TKIs have been discovered: the activation of bypass pathways (by *KRAS* mutations, *c-MET* amplification or *HER2* amplification), the mesenchymal–epithelial transition, the high expression of insulin-like growth factor receptor 1 (IGF1R), *BIM* (B-cell lymphoma-2-like 11 gene) polymorphisms or the histological transformation into small cell lung cancer. Osimertinib, a third-generation EGFR TKI, has shown an ORR of 77% and PFS of 10.1 months in patients with NSCLC habouring a T790M mutation and was found to be significantly superior to chemotherapy in these patients (HR 0.30, p<0.001) [37]. Osimertinib is now approved and recommended after first-line EGFR TKIs in the presence of the T790M mutation. For the other mechanisms of resistance, no specific treatment is recommended other than chemotherapy, *i.e.* the combination of cisplatin and pemetrexed for

TABLE 3 Currently approved targeted therapies in nonsmall cell lung cancer along with their target

Drug	Target	Line
Erlotinib	*EGFR* sensitising mutation	1
Gefitinib	*EGFR* sensitising mutation	1
Afatinib	*EGFR* sensitising mutation	1
Osimertinib	*EGFR* T790M mutation	1+
Crizotinib	*ALK/ROS1*	1
Ceritinib	*ALK*	2
Dabrafenib+trametinib	*BRAF*	1

EGFR: epidermal growth factor receptor; ALK: anaplastic lymphoma kinase.

adenocarcinomas and the classical association of cisplatin and etoposide in the case of transformation in small cell lung cancer. However, it is possible to prescribe specific inhibitors of the receptor tyrosine kinase MET, *i.e.* crizotinib, in the context of clinical trials when MET is amplified.

Anaplastic lymphoma kinase rearrangements

Initially discovered as a translocation in anaplastic large-cell lymphomas, gene rearrangement involving the anaplastic lymphoma kinase (ALK) was identified in lung cancer in 2007 and was subsequently found to predict very high sensitivity to crizotinib [38]. As for EGFR TKIs for *EGFR*-mutated NSCLC, crizotinib results are better than chemotherapy when used in second-line therapy after platinum doublet (ORR 65% *versus* 20%, p<0.001; and PFS 7.7 months *versus* 3.0 months, HR 0.49, p<0.001) [39] and in first-line therapy (ORR 74% *versus* 45%, p<0.001; and PFS 10.9 months *versus* 7.0 months, HR 0.45, p<0.001) in patients with *ALK*-rearranged NSCLC [40]. Consequently, crizotinib is recommended in a first-line setting for patients with advanced NSCLC with *ALK* rearrangements.

Cure has not been obtained with crizotinib either, and all patients also ultimately progress after ~10 months of disease control. Resistance mechanisms in patient with *ALK*-rearranged NSCLC treated by crizotinib have been studied and are found to be either ALK-related or not. In the first case, secondary *ALK* mutations that confer acquired resistance to crizotinib, such as L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, G12102R and G1269A, as well as *ALK* amplifications have been reported [41]. Activations of bypass pathways, *i.e.* by *EGFR* and *KRAS* mutations or *MET* amplifications, are also responsible for acquired resistance to crizotinib in patients with *ALK*-rearranged NSCLC. Several second-generation ALK TKIs have been developed. Alectinib and ceritinib, when used in patients previously treated with crizotinib, showed ORRs of 48% and 56%, respectively [42, 43]. Notably, the cumulative PFS of patients with *ALK*-rearranged NSCLC who received crizotinib followed by alectinib or ceritinib at resistance reached ~50 months.

Afterwards, patients also develop resistance to these second-generation ALK inhibitors, *e.g.* by the acquisition of new mutations in *ALK*, some being drug-specific, such as F1174C/V and G1202del with ceritinib or I1171T/N/S and V1180L with alectinib, and other mutations being common to both drugs, such as G1202R [44]. Brigatinib, another second-generation ALK inhibitor, showed results similar to those of alectinib and ceritinib in patients previously treated with crizotinib: ORR was 71% and PFS 12.3 months [45]. Patients treated with brigatinib will also develop the G1202R mutation as well as specific resistance mutations in *ALK*, *e.g.* E1210K+S1206C or E1210K+D1203N [44]. Most recently, lorlatinib has been shown to inhibit ALK and has attracted a great deal of interest as it has activity against the G1202R mutation developed secondarily with all the other drugs. It also has a very high penetration into the central nervous system [44]. Again, new mutations of resistance in *ALK* occur with lorlatinib treatment (C1156Y +L1198F mutations) [44]. Ceritinib and alectinib are currently recommended in guidelines for second-line therapy after progression to crizotinib in advanced NSLCLC with *ALK* rearrangement, independently of the resistance mechanism [46]. Brigatinib may be approved shortly for the same indication. In the future, it may also become indicated to select second- and third-line therapy based on the resistance mechanism, particularly as lorlatinib is the only ALK inhibitor with activity against the G1202R-mutated *ALK* NSCLC.

In addition, second-generation ALK TKIs, initially studied in patients who failed in response to first-line crizotinib, may move to the first-line setting. The ASCEND-4 trial showed an ORR of 73% *versus* 27% and a doubled PFS of 16.6 months *versus* 8.1 months for ceritinib *versus* chemotherapy groups, respectively (HR 0.55, p<0.00001), as first-line therapy too [47]. The ORR and PFS with ceritinib appear superior in first-line compared with crizotinib for *ALK*-rearranged NSCLC; however, this study did not provide a direct comparison between ceritinib and crizotinib. However, the J-ALEX trial, performed in an Asian cohort, showed an ORR of 85.4% *versus* 70.2% with alectinib and crizotinib, respectively, and significantly improved PFS with alectinib (HR 0.34, 95% CI 0.17–0.71) in first-line therapy for patients with *ALK*-rearranged NSCLC [48]. More recently, the ALEX trial showed a very significant benefit of using alectinib in first-line therapy as compared with crizotinib (PFS HR 0.47, 95% CI 0.34–0.65). Interestingly, alectinib also significantly delayed the time to central nervous system progression compared with crizotinib, with an incidence of brain progression at 12 months of 9.4% *versus* 41.4%, respectively (HR 0.16, 95% CI 0.10–0.28) [49]. Thus, second-generation ALK inhibitors, *i.e.* ceritinib and alectinib, may be prescribed in the first-line setting in the future and this may change the profile of resistance of patients with *ALK*-rearranged lung tumours, in terms of delay and type of mechanism. A summary of the main studies testing targeted therapies in NSCLC with *ALK* rearrangement is presented in table 4.

ROS1 rearrangements

First described in glioblastomas and cholangiocarcinomas, ROS1 is a receptor of the insulin receptor family with tyrosine kinase activity. More recently, rearrangements of the *ROS1* gene have occasionally

been identified in NSCLC (1–2%) [42, 50]. ALK and ROS1 tyrosine kinase domains have a high similarity, so crizotinib was tested on NSCLC harbouring *ROS1* rearrangement and showed ORRs of 72% and 80% and PFSs of 19.2 months and 9.1 months, in a cohort from the USA [51] and in a European cohort [50], respectively. In the Asian Open-Label phase II study, ceritinib also benefited heavily pretreated patients with NSCLC harbouring *ROS1* rearrangement. Among the 28 patients that were evaluable for response, the ORR was 62%. Of the eight patients with brain metastases, intracranial disease control was 63%. The median PFS was 9.3 months for all patients and 19.3 months for crizotinib-naive patients [52].

Emerging molecular targets

Other targetable oncogenic drivers have also been identified, including *RET* fusions, *MET* amplification, *MET* exon 14 splice junction mutations, *BRAF* mutations and *HER2* mutations. New targeted therapies have been developed and are currently being evaluated worldwide for these new drivers. In patients with *RET* fusion tumours, studies of small cohorts of patients have been recently reported with ORRs of 28–37%, 18–53% and 22% for cabozantinib, vandetanib and sunitinib, respectively [53–55]. Alectinib is potentially active on NSCLC with *RET* fusions as well, with two responses among four patients treated [56]. Patients with NSCLC with *MET* amplifications may benefit from treatment with crizotinib [57, 58]. More recently, a splice-site mutation of *MET* was identified. This mutation is responsible for the loss of exon 14 of *MET* and Cbl-binding site on the MET receptor protein. Consequently, degradation of the MET receptor is reduced and its expression level is increased. NSCLCs with a *MET* exon 14 splice mutation also experience a dramatic clinical response to crizotinib [59]. For NSCLC patients with V600E mutations of *BRAF*, several therapeutic options exist: dabrafenib in monotherapy (ORR 33%), dabrafenib in combination with trametinib (ORR 63.2%) and verumafinib in monotherapy (ORR 42%) [60–63]. In NSCLC patients with *BRAF* V600E mutations, the US Food and Drug Administration (FDA) and the European Medicines Agency have approved the combination of dabrafenib and trametinib, and it thus has become a standard treatment. Trastuzumab [64] and afatinib [65] may be proposed for patients with NSCLC harbouring *HER2* mutations, although these treatments are less effective than usually observed with targeted therapies for oncogenic drivers.

TABLE 4 Main trials published with targeted therapies in nonsmall cell lung cancer with *ALK* rearrangement

First author [ref.]	Year	Phase	Treatment setting	Previous ALK inhibitor	ALK TKI	Comparison therapy	ORR# %	PFS# months
Solomon [40]	2014	III	First-line	No	Crizotinib (n=172)	Platinum doublet (n=171)	74/45	10.9/7.0 (HR 0.45)
Soria [47] (ASCEND-4)	2017	III	First-line	No	Ceritinib (n=189)	Platinum doublet (n=187)	73/27	16.6/8.1 (HR 0.55)
Hida [48] (J-ALEX)	2017	III	Previous chemotherapy allowed	No	Alectinib (n=103)	Crizotinib (n=104)	92/79	Not reached/ 10.2 (HR 0.34)
Peters [49] (ALEX)	2017	III	First-line	No	Alectinib (n=152)	Crizotinib (n=151)	83/76	Not reached/ 11.1 (HR 0.47)
Shaw [39]	2013	III	Second-line	No	Crizotinib (n=173)	Pemetrexed (n=174)	65/20	7.7/3.0 (HR 0.49)
Shaw [42]	2014	I	Any lines	Accepted	Ceritinib ⩾400 mg (n=114)		58	7.0
			Any lines	Crizotinib	Ceritinib (n=80)		56	6.9
Shaw [43]	2016	II	Any lines	Crizotinib	Alectinib (n=69)		48	8.1
Gettinger [45]	2016	I/II	Any lines	Accepted	Brigatinib (n=52)		77	
			Any lines	No	Brigatinib (n=4)		100	Not reached
			Any lines	Crizotinib	Brigatinib (n=52)		74	14.5
			Any lines	Accepted but brain metastasis	Brigatinib (n=6)		83	No event

ALK: anaplastic lymphoma kinase; TKI: tyrosine kinase inhibitor; ORR: objective response rate; PFS: progression-free survival; HR: hazard ratio. #: comparisons presented as ALK TKI/comparison therapy.

The master enemy: KRAS mutations

KRAS mutations are detected in up to 32% of NSCLCs at diagnosis, mainly in lung adenocarcinoma and in smokers [66]. *KRAS* mutations are associated with a poor prognosis [67] and result more frequently in brain metastasis compared with lung adenocarcinomas without these mutations [68]. Usually, *KRAS* mutations in NSCLC are single amino acid substitutions in codon 12 of exon 2 and less commonly in codon 13 of exon 2 (~10%). Currently, international guidelines recommend treating *KRAS*-mutant lung adenocarcinomas with chemotherapy, *i.e.* a platinum doublet, as efficient targeted therapies against *KRAS* mutations are not available [69]. Many drugs acting at different levels of KRAS pathways have been tested without showing any significant benefit. Drugs tested in *KRAS*-mutant NSCLC have failed to improve the prognosis so far; these include farnesyl transferase inhibitors, several MEK inhibitors, PI3K inhibitors, mTOR inhibitors, MET inhibitors, Hsp90 inhibitors and CDK4/6 inhibitors [69]. In addition, KRAS is difficult to target directly. Specific inhibitors of the G12C *KRAS* mutant are only in development [70]. Clinical benefits have not yet been demonstrated and, even if they are, the large majority of *KRAS* mutations would remain without a direct inhibitor.

Recommendation for molecular testing

First-line setting

As routine practice, testing for *EGFR* mutations, *BRAF* mutations and rearrangements of *ALK* and *ROS1* must be performed at diagnosis to choose the appropriate first-line therapy in all patients with advanced pure lung adenocarcinomas or an adenocarcinoma component in mixed tumours. Pure squamous cell carcinoma patients and those with neuroendocrine tumours are usually not tested for molecular biomarkers. However, pure lung squamous cell carcinomas with clinical features that are associated with high risk for *EGFR/ALK/ROS1* aberrations (*e.g.* never/light smokers) should also be tested. To standardise biomarker testing in lung cancer, the College of American Pathologists, the International Association for Study of Lung Cancer and the Association of Molecular Pathologists have published guidelines [71] that recommend, at diagnosis, that all advanced NSCLC patients with an adenocarcinoma or a tumour with an adenocarcinoma component should be tested for *EGFR* mutations, *ALK* rearrangements and *ROS1* fusions, using the most accessible tissue (primary tumour or metastasis). Similar recommendations have been published by other scientific societies, including the American Society of Clinical Oncology [72], the National Comprehensive Cancer Network (in the USA) [73] and the European Society for Medical Oncology [46], for their own guidelines. Since the publication of these guidelines, the search for *BRAF* mutations should be added as there is an FDA-approved treatment for such patients. In addition, *RET* fusions, *MET* amplifications, *MET* exon 14 splicing mutations and *HER2* mutations should be searched for in all patients with advanced NSCLC, with an adenocarcinoma component or light smoking habits, who were found negative for *EGFR*, *ALK*, *ROS1* and *BRAF* testing. Of note, these secondary tests may be performed in parallel when there is no concern about tissue availability and multiplex panels will be utilised increasingly for *EGFR*, *ALK*, *ROS1*, *RET*, *BRAF*, *HER2* and *MET* testing in patients with NSCLC, as they are more cost–benefit effective than successive single-gene analyses, being cheaper and requiring less time and tissue.

Second-line setting

At resistance after first-line therapy with EGFR TKI for patients with advanced NSLCLC harbouring activating mutations of *EGFR*, repeating molecular testing on a fresh biopsy, ideally sampled at the progressing site when technically feasible, is recommended, and osimertinib should be prescribed for patients if the T790M mutation of *EGFR* is detected. However, tissue rebiopsy may be clinically challenging and results in a 35% failure rate overall, including an inability to obtain tissue and non-contributive biopsies. Obtaining a surrogate sample to detect molecular abnormalities by means of a noninvasive approach is thus required, *e.g.* so-called liquid biopsies, implying genomic testing on cell-free DNA from peripheral blood. T790M detection in the circulating tumour DNA (ctDNA) has shown a sensitivity of 40–80% and specificity of 95–100%, depending on the technique being used [74]. As the sensitivity is relatively low, false-negative results occur frequently. Thus, liquid biopsy is recommended as a first option to detect *EGFR* T790M mutations at the time of acquired resistance to first-line EGFR TKIs [75]. However, because of the low sensitivity, any negative results on the ctDNA testing in the blood should lead to tissue rebiopsy and *EGFR* testing in the tumour sample. Recently, the FDA has approved *EGFR* mutation ctDNA testing at frontline and at relapse for T790M, by using the cobas® EGFR Mutation Test v2 (Roche Molecular Diagnostics, Pleasanton, CA, USA).

Of note, after treatment with first-line crizotinib ALK inhibitors in patients with advanced NSCLC with *ALK* rearrangements, testing for the mechanism of resistance, *i.e. ALK* mutations, is currently not recommended for a treatment decision.

Personalised medicine based on immune profile

Anti-tumour immunity was first described more than 100 years ago. It relies both on innate immunity and adaptive immunity [76]. Adaptive immunity is divided into two phases: the priming phase, where dendritic cells interact with T-cells, and the effector phase, where T-cells interact with cancer cells [77]. These two phases involve activating signals and inhibitory signals. In the priming phase, inhibitory signals rely on CTLA-4 (cytotoxic T lymphocyte antigen 4), expressed by T-cells, whereas in the effector phase, inhibitory signals rely on PD-1 (programmed cell death 1), expressed by T-cells, and PD-L1 (programmed cell death ligand 1), expressed by cancer cells. Lung cancer cells are able to avoid anti-tumour immunity *via* several mechanisms, including activation of these inhibitory signals [78]. A better knowledge of anti-tumour immunity biology and of the mechanisms used by cancer cells to avoid the immune system has led to the development of immunotherapy drugs.

Immunotherapy in lung cancer first started with the development of anti-cancer vaccines. Several vaccines (anti-MAGE-A3, anti-BLP25, belagenpumatucel-L) have been studied in phase III clinical trials, but none of them demonstrated any efficacy in the treatment of NSCLC patients [79–81]. Later, based on adaptive immunity mechanisms, immune checkpoint inhibitors (ICIs) were developed to target anti-cancer immunity inhibitory signals. Although the anti-CTLA-4 monoclonal antibodies ipilimumab and tremelimumab showed promising results in combination with chemotherapy or in the maintenance setting after first-line chemotherapy, they were not approved for the treatment of NSCLC [76, 82]. PD-1 inhibitors were the first ICIs to receive FDA approval for NSCLC. Both nivolumab and pembrolizumab improved overall survival and reduced toxicity in comparison with docetaxel chemotherapy in the second-line treatment of NSCLC in phase III clinical trials [83–85] and are approved in this setting. They were also studied in the first-line setting. While pembrolizumab [86] improved outcomes in comparison with chemotherapy and is FDA-approved, nivolumab [87] failed to show any survival improvement in first-line NSCLC treatment in phase III trials. Furthermore, atezolizumab, a PD-L1 inhibitor, also showed an overall survival improvement in comparison with docetaxel in the second-line setting in a phase III clinical trial [88] and also received FDA approval. The results of phase III clinical trials are reported in table 5.

Consequently, the recent development of ICIs has revolutionised the treatment of advanced NSCLC, offering new treatment options approved in the second-line setting. However, ICIs do not fit to every

TABLE 5 Phase III randomised trials of PD-1 and PD-L1 inhibitors showing efficacy results and the technologies used for PD-L1 testing

	CheckMate 017 [83]		**CheckMate 057 [84]**		**CheckMate 026 [87]**		**Keynote 010 [85]**		**Keynote 024 [86]**		**OAK [88]**	
Population	sNSCLC		nsNSCLC		PD-L1 ⩾1%		PD-L1 ⩾1%		PD-L1 ⩾50%		NSCLC	
Treatment line	⩾Second-line		⩾Second-line		First-line		⩾Second-line		First-line		⩾Second-line	
Drug	Nivo	Doce	Nivo	Doce	Nivo	Chemo	Pembro	Doce	Pembro	Chemo	Atezo	Doce
ORR %	20	9	19	12	26.1	33.5	18	9	44.8	27.8	14	13
PFS months	3.5	2.8	2.3	4.2	4.2	5.9	3.9, 4.0[#]	4.0	10.3	6.0	2.8	4.0
Overall survival months	9.2	6.0	12.2	9.4	14.4	13.2	10.4, 12.7[#]	8.5	80.2%[¶]	72.4%[¶]	13.8	9.6
PD-L1 expression												
Antibody	28-8		28-8		28-8		22C3		22C3		SP142	
Platform manufacturer	Dako		Dako		Dako		Dako		Dako		Ventana	
Analysed cells	Tumour cells		Tumour cells		Tumour cells		Tumour cells		Tumour cells		Tumour and immune cells	
Thresholds	1%, 5%, 10%		1%, 5%, 10%		1%, 5%, 10%		1%, 50%		1%, 50%		Tumour: 1%, 5%, 50%; Immune: 1%, 5%, 10%	
Sampling time	Pretreatment		Pretreatment		Pretreatment		Any		Pretreatment		Pretreatment	
Preanalytic	Archival FFPE		Archival FFPE		Archival FFPE		Archival or new		Archival or new		Archival or new	

ORR: objective response rate; PFS: progression-free survival; PD-L1: programmed cell death ligand 1; NSCLC: nonsmall cell lung cancer; Nivo: nivolumab; Doce: docetaxel; Chemo: chemotherapy; Pembro: pembrolizumab; Atezo: atezolizumab; FFPE: formalin-fixed paraffin-embedded. [#]: two doses of pembrolizumab were tested (2 mg·kg^{-1} and 10 mg·kg^{-1}); [¶]: 6-month overall survival rate.

patient with advanced NSCLC. For example, approximately half of patients with nonsquamous NSCLC receiving second-line nivolumab will have a progressive disease after the first tumour assessment [84]. Moreover, some patients seem to be more likely to develop immune-related adverse events such as pneumonitis [89]. For these reasons, there is a need to identify biomarkers to predict the efficacy and tolerance of ICIs. The first biomarker studied in this field was the expression of PD-L1. PD-L1 is expressed by cancer cells in order to increase anti-cancer immunity inhibitory signals. It is directly targeted by PD-L1 inhibitors such as atezolizumab but is also a ligand of PD-1, targeted by nivolumab and pembrolizumab. Chae *et al.* [90] reported association between ORR and PD-L1 expression data from several clinical trials studying the efficacies of ICIs. In most trials, their efficacy was better for patients with PD-L1-positive tumours. However, phase III studies of anti-PD-1 and anti-PD-L1 reported conflicting results regarding the potential predictive effect of PD-L1 expression (table 5). In the CheckMate 017 study, PD-L1 expression was neither prognostic nor predictive for nivolumab efficacy in squamous NSCLC patients [83], whereas in the CheckMate 057 study nivolumab was associated with a greater efficacy than docetaxel in patients with PD-L1-positive tumours, whatever the threshold (1%, 5% or 10%) [84]. In the pembrolizumab Keynote 010 study, only patients with PD-L1 expression on ⩾1% tumour cells were enrolled. Overall survival was longer with pembrolizumab in the overall population, but the difference between pembrolizumab and docetaxel was even greater in the subgroup of patients with PD-L1 expression on ⩾50% of tumour cells [85]. By contrast, in the OAK study, PD-L1 expression by either tumour cells or immune cells was not predictive of atezolizumab efficacy [88]. In the first-line setting, pembrolizumab as monotherapy was only studied in patients whose tumours had a PD-L1 expression level of >50% [86]. These differences can be explained by the different technologies used in those phase III clinical trials (table 5). The antibodies and platform chosen for PD-L1 immunohistochemistry were different for each drug in phase III trials. Moreover, while for nivolumab and pembrolizumab PD-L1 expression was tested only on the tumour cell membrane, for atezolizumab, PD-L1 expression was also tested on tumour-infiltrative immune cells. Moreover, pathologists experience difficulties with PD-L1 immunohistochemistry interpretation, especially for cases with focal PD-L1 expression or in small biopsy specimens [91]. Ongoing efforts are being made to harmonise PD-L1 testing, for example in the Blueprint study [92] or the German harmonisation study [93].

Because of the pitfalls of using PD-L1 immunohistochemistry as a biomarker test for ICIs, other immunological biomarkers were also studied. PD-L2 expression has been described in some tumours, but its predictive role for the efficacy of PD-1 and PD-L1 inhibitors has been poorly studied in solid tumours [94]. Preclinical models have also demonstrated the role of $CD8^+$ cytotoxic T-lymphocytes (CTL) in the anti-tumour adaptive immune system [94]. More recently, $CD8^+$ CTL density in the tumour has been correlated with melanoma patients' response to PD-1 inhibitors [95]. For this reason, $CD8^+$ CTL tumour infiltration is a promising biomarker to predict the efficacy of ICIs but still has to be assessed.

Furthermore, genetic biomarkers have been studied. Mutation load has been described as a potential biomarker for response to ICIs because genomic alterations can encode neoantigens and change the anti-cancer immune response. A study of large databases of mutation load in solid tumours indeed showed that cancers with the highest mutation load had the highest response to anti-PD-1 and anti-PD-L1 treatments [96]. In addition, specific molecular profiles such as smoking-related signature, high neoantigen burden or DNA repair pathway mutations were found to be related to better responses to anti-PD-1 treatments [97]. In subgroup analyses of the clinical trials, nivolumab had a better efficacy in patients with *KRAS*-mutant NSCLC and was less efficient in patients with *EGFR*-mutant NSCLC in comparison with docetaxel in subgroup analyses [83, 84].

Conclusions and perspectives

In conclusion, various approaches to personalised medicine exist: 1) identification and targeting of a key driver oncogene that will predict for efficacy (*e.g. EGFR* mutations); 2) identification of a biomarker associated with higher efficacy of a specific treatment (*e.g.* PD-L1 immunohistochemistry); 3) identification of a biomarker associated with less toxicity (*e.g.* nonsquamous histology and bevacizumab). Treatment allocation according to histology is now the first level of personalised medicine in NSCLC management. Currently, all patients with advanced adenocarcinoma and nonsmokers with other histologies should be tested for *EGFR* mutations, *ALK* rearrangements and *ROS1* fusion. *BRAF* mutations should be searched for too. When harbouring an *EGFR* sensitising mutation, *ALK* or *ROS1* rearrangements or *BRAF* mutations, patients with advanced NSCLC will receive bioguided therapy as first-line treatment, namely EGFR TKIs (*i.e.* gefinitib, erlotinib or afatinib) or ALK/ROS1 TKI (crizotinib) or BRAF inhibitors (dabrafenib and trametinib), respectively (table 3) [71]. *RET* fusion, *MET* exon14 mutation and *HER2* mutations, but with a lower degree of evidence, have available targeted treatments as well, and should thus be tested for. These last markers can be tested sequentially if the tumour is negative for the three other markers, or simultaneously, through multiplex testing. In the research setting, ongoing molecularly

stratified umbrella clinical trials offer the possibility to perform panel next-generation sequencing (NGS) analyses and to propose bioguided treatment in the context of clinical trials. One example is the SAFIR 02 Lung, a phase II randomised trial utilising high-throughput genomic testing and comparing bioguided treatment *versus* chemotherapy in maintenance in metastatic NSCLC. In the future, the single gene–single treatment approach that we are currently practising may be replaced by approaches considering a broader spectrum of molecular abnormalities as NGS analyses of tumours are generalised.

Recently, ICIs have revolutionised the treatment of advanced NSCLC. They became standard treatment in a second-line setting for NSCLC and their use may be extended in the near future with the results of ongoing studies in the first-line setting or in combination with chemotherapy, other ICIs or targeted therapies. Consequently, PD-L1 expression testing is now recommended before first-line treatment in patients with metastatic NSCLC with negative or unknown test results for *EGFR* mutations and *ALK* and *ROS1* rearrangements. No biomarker is recommended for the use of nivolumab in NSCLC patients, while a minimum level of 1% and 50% PD-L1 tumour proportional score is required for the second- and first-line use of pembrolizumab in NSCLC patients, respectively. Additional biomarkers such as $CD8^+$ CTL tumour infiltration, mutation load or molecular profile are being studied. However, their predictive effect has yet to be confirmed in clinical trials and future biomarkers have to be identified to refine the prediction of ICI efficacy.

Besides these successes and promises, personalised medicine has challenges and limits. When performing personalised medicine, a very important challenge is the need to standardise tests, both for laboratory procedures and their assessment for positivity. Illustrations of biomarker testing issues have been reported previously, with the failure to validate the use of ERCC1 expression to predict sensitivity to cisplatin. Reported initially as a favourable predictive factor for response to the platinum doublet in a first cohort of patients [98], the results could not be reproduced either in the same cohort or in an independent cohort [99]. The reasons for these failures were 1) differences in antibody batches and 2) the lack of specificity of the antibody for the relevant isoform of ERCC1. A more recent illustration of the test standardisation issue arose from the various tests used for PD-L1 expression in different trials testing the drugs against PD-1 and PD-L1 and the ongoing effort to homogenise a validated test to be used across laboratories to assess PD-L1 expression. Similarly, with other techniques, including molecular biology, rigorous assays and thresholds for clinically significant positivity need to be defined, as for *EGFR* mutation detection in ctDNA, for example. This example highlights the necessity of applying several methodological quality assurance steps before translating biomarkers use into clinical use. It is mandatory to standardise techniques and validate them across cohorts and laboratories. To ensure the biological and clinical relevance of biomarkers, it is also crucial to understanding the biology when designing biomarker assays and to define cut-offs for positivity that have clinical impact.

Another limit of personalised medicine lies in the development of specific resistance mechanisms to treatments. The resistance mutations developed after treatment with the different ALK inhibitors illustrate a new paradigm that will be observed with all TKIs: using a specific TKI is always followed by the emergence of resistant clones, and using sequential TKIs leads to an increasingly heterogeneous disease, which is much less amenable to precision medicine. The sequential use of drugs and/or their combinations will have to be carefully managed to keep the tumour sensitive to treatment for longer periods.

Finally, the next challenge for precision may be the need to integrate both biomarkers from the tumour and from the hosts, *i.e.* microbiota. Recent data have revealed that the gut microbiota influences the host response to anti-cancer drugs. Immunomodulations due to microbiota can confer variations in responses to immunotherapy, chemotherapy, radiotherapy and also targeted therapies. This field is currently widely under investigation and it is thus very likely that future precision medicine will combine pharmacogenomics information with custom microbial organisms or their specific metabolites to improve therapeutic responses and/or manage toxicities [100].

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