Biological therapies in nonsmall cell lung cancer

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ABSTRACT Biological therapies have improved survival outcomes of advanced-stage nonsmall cell lung cancer (NSCLC). Genotype-directed therapies have changed treatment paradigms of patients with *EGFR*-mutant and *ALK/ROS1*-rearranged lung adenocarcinomas, and the list of druggable targets with demonstrated clinical actionability (*BRAF, MET, RET, NTRK1* and *HER2*) continues to expand. Furthermore, we have incrementally understood the mechanisms of cancer immune evasion and foresee ways to effectively circumvent them, particularly at the immune checkpoint level. Drugs targeting the tumour immune-evasive PD-1 pathway have demonstrated remarkable treatment benefits in this disease, with a non-negligible fraction of patients potentially receiving long-term survival benefits. Herein, we briefly discuss the role of various medical disciplines in the management of advanced-stage NSCLC and review the most relevant biological therapies for this disease, with particular emphasis in genotype-directed therapies and immune checkpoint inhibitors.

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, with an approximate 5-year survival rate of 18% [1]. According to pathological characteristics, ~80% of the cases are classified as nonsmall cell lung cancers (NSCLCs). These are further divided in three predominant histological subtypes: adenocarcinoma (50%), squamous cell carcinoma (40%) and large cell carcinoma. Small cell lung carcinoma accounts for the remaining 15–20% of all lung cancers [2]. The vast majority of lung cancer patients are diagnosed with advanced-stage disease.

In parallel to new technology acquisition (*e.g.* molecular profiling and chemistry plasticity), the growing knowledge of cancer biology has enabled the development of novel biological therapies that target several components of the tumour. These treatments have already impacted the natural history of some specific molecular subsets of lung cancers and this trend is expected to expand in the coming years. In particular, oncogene-targeted therapies for biomarker-selected patients and immune checkpoint inhibitors have changed treatment paradigms and transformed the expected outcomes of a substantial fraction of advanced-stage NSCLC patients. Here, we briefly describe some clinically relevant discoveries related to molecular taxonomy and the role of the immune system in lung cancer, and review the most promising and currently available biological therapies in the field. In this context, we also illustrate the importance of a multidisciplinary management of advanced-stage NSCLC patients.

Multidisciplinary management of advanced-stage NSCLC

Tumour pathological and molecular subtyping is paramount for advanced-stage NSCLC therapy guidance. In fact, much of the success of systemic treatments for this disease profoundly relies on obtaining sufficient amounts of tumour, and on appropriate sample flow and tissue priorisitation [3], which cannot be optimally accomplished without a multidisciplinary management of these patients. Pulmonologists and radiologists are crucial to rapid diagnosis and performing the safest effective and efficient biopsy procedures. Notably, as we will comment upon later, tumour rebiopsies are evoked as a new standard of care in the management of patients with epidermal growth factor receptor (*EGFR*)-mutant and anaplastic lymphoma kinase (*ALK*)-rearranged, tyrosine kinase inhibitor (TKI)-resistant tumours, which implies that both disciplines will be involved not only in the initial patient diagnosis but also in later stages of progressive disease and subsequent lines of therapy. In selected patients whose tumours are not accessible for endoscopic or computed tomography-guided core biopsies, the possibility to perform surgical resections from metastatic sites should be discussed in a multidisciplinary team with thoracic or general surgeons. After specimen collection, a lung cancer pathologist must be trained in proper diagnostic, pathological subtyping and molecular subtyping protocols to optimise the use of tissue. This is particularly relevant for small tumour biopsies, in which adequate tissue-sparing protocols must be implemented in each institution. In general, expert panels recommend limiting the use of markers for histological subtyping (*e.g.* restrict immune staining to thyroid transcription factor 1 and p40) in order to avoid tissue waste for further genomic characterisation or immune-profiling (*e.g.* programmed death ligand 1 (PD-L1) expression) of the tumours [4, 5], which will ultimately dictate the selection of most of the biological therapies. Importantly, in order for the patients to receive the most appropriate systemic treatment, thoracic oncologists must integrate this pathologic and molecular information into the appropriate clinical context. Finally, during the course of therapy, the help and close collaboration of other various medical specialists (dermatologists, endocrinologists, gastroenterologists, nephrologists, immunologists, *etc.*) will be needed for early recognition and treatment of toxic effects and therapy-related complications.

Biological therapies for NSCLC

Oncogene-tailored therapies for molecularly selected patients

NSCLC is a paradigmatic example illustrating the success of genotype-driven precision oncology. Many molecular aberrations defining particular subsets of lung cancers can be specifically targeted with oncogene-tailored therapies. High-resolution and high-throughtput molecular profiling of NSCLCs has revealed that the majority of the molecular alterations, and particularly those that are therapeutically vulnerable, are mostly histology specific [3]. Successful identification of biomarkers with therapeutic purposes has been mainly made in lung adenocarcinoma. Different types of genomic alterations involving multiple driver kinase genes, such as *EGFR*, *KRAS*, *ALK, ROS1*, *BRAF*, *MET*, *RET*, neurotrophic receptor tyrosin kinase (*NTRK*) and human EGFR2 (*HER2*), represent specific molecular subtypes of pulmonary adenocarcinomas, with distinct biology, epidemiology, prognosis and therapeutic susceptibilities [3, 6] (tables 1–3). In contrast, the genomic portrait of squamous cell lung carcinoma is mostly composed of inactivating mutations in tumour suppressor genes or mutations in oncogenes not amenable to direct therapeutic targeting [58, 59]. The few potentially actionable alterations commonly found in squamous cell lung cancers, such as fibroblast growth factor receptor 1 (*FGFR1*) amplification, or phosphoinositol

TABLE 1 Genomic features, targeted drugs and efficacy of epidermal growth factor receptor (*EGFR*)-mutant nonsmall cell lung cancers

Genomic features	First- and second-generation EGFR TKIs[#]	RR	mPFS months	Selected third-generation EGFR TKIs overcoming target-mediated resistance	Clinical trial phase (study acronym)	Total/ T790M$^+$ n/n	Context	RR in T790M$^+$	mPFS in T790M$^+$ months
Sensitising kinase domain activating mutations [7]	Gefitinib Erlotinib Afatinib	56–82% [7]	9.2–13.1 [7]	Osimertinib[#]	I+II [8] (AURA1/2)	411/411	TKI resistant	66%	9.7–11
Common (90%) del19, exon 21 L858R					I [9] (AURA1)	60/–	TKI naive	77%	>19
Uncommon (10%) *e.g.* exon 18 G719X, exon 21 L861Q				Rociletinib[¶]	I/II [10] (TIGERX)	130/51	TKI resistant	~30%	6.1
				HM61713[¶]	I/II [11]	173/76	TKI resistant	56%	7
				EGF816	I [12]	152/152	TKI resistant	46.9%	9.7 m
				ASP8273	I/II [13] (NA cohort)	60/40	TKI resistant	65%	6.7 m
					I [14] (Japan cohort)	45/15	TKI resistant	80%	

TKI: tyrosine-kinase inhibitor; RR: response rate; mPFS: median progression-free survival; NA: North American. [#]: European Medicines Agency and US Food and Drug Administration approved; [¶]: no longer in clinical development.

3-kinase (*PI3K*) and discoidin domain-containing receptor 2 (*DDR2*) mutations are challenging targets, and no specific inhibitors have proven clinical efficacy as yet [59].

Approved targets

Sensitising *EGFR* activating mutations

Approximately 10–15% of Caucasian lung adenocarcinoma patients harbour *EGFR* activating mutations (exons 18–21). The most common alterations include exon 19 deletions and a point mutation at position 858 in exon 21 (L858R), accounting for up to 85–90% of *EGFR* mutations in the clinic. Both are sensitive to EGFR TKIs and are known as *EGFR* "sensitising mutations". The remaining 10–15% of the cases are *EGFR* "uncommon mutations" and show variable TKI sensitivity [7]. Mutant receptors have constitutive, ligand-independent tyrosine kinase activity. This activation stimulates several intracellular signalling cascades, such as the PI3K/Akt, RAS/RAF/ERK, phospholipase Cγ, Src kinase and STAT signalling pathways, and has profound consequences in tumour growth, survival and progression potential [60].

Following the results of the landmark IPASS trial, six molecularly selected randomised controlled trials consistently demonstrated significantly higher overall response rates (56–82% *versus* 15–47%) and longer progression-free survival (PFS) (9.2–13.1 *versus* 4.6–6.9 months) in favour of first-generation (gefitinib and erlotinib) or second-generation (afatinib) EGFR TKIs compared to standard platinum-based chemotherapy (table 1). None of the studies demonstrated overall survival (OS) benefits, at least partially due to the high treatment crossover at disease progression [7]. These three drugs are currently approved without distinctions for *EGFR*-mutant NSCLCs. Various meta-analyses have suggested a comparable clinical activity among them (particularly between first-generation TKIs), with some differences in adverse event profile [7, 61, 62]. However, the question of whether the choice between first-and second-generation inhibitors impacts treatment outcomes has not been fully answered to date. Two randomised trials comparing gefitinib with afatinib (Lux-Lung 7) and gefitinib with dacomitinib (ARCHER 1050 (www.clinicaltrials.gov identifier NCT01774721) were designed to address this issue. Data from the LUX-Lung 7 trial have recently been reported. Afatinib significantly increased response rates (70% *versus* 56%, p=0.0083), median PFS (11 *versus* 10.9 months; hazard ratio (HR) 0.73, 95% CI 0.57–0.95; p=0.0195) and median time to treatment failure (13.7 *versus* 11.5 months; HR 0.73, 95% CI 0.58–0.92; p=0.0073) over gefinitib. However, there were no OS differences among treatment arms in this phase IIb trial (n=319). Overall, treatment-related adverse events (mainly skin rash and diarrhoea) and serious adverse

events (10.6% (6.3% diarrhoea) *versus* 4.4% (2.5% interstitial lung disease)) were more common with afatinib [63]. Therefore, this trial suggests that the emergence of acquired resistance might be delayed with second-generation, compared to first-generation, TKIs, but whether these modest differences are clinically relevant for patients is arguable for many physicians.

Despite initial clinical benefit, all patients ultimately progress on first/second-generation EGFR TKI treatment. The acquisition of a secondary mutation in exon 20 (T790M) is the most common *EGFR*-dependent acquired resistant mechanism, observed in up to 50–60% of these patients [64]. Third-generation EGFR TKIs are *EGFR* mutant-selective inhibitors with a particularly potent activity against the T790M mutant kinase, preserving activity against the activating mutation but sparing the wild-type receptor. In turn, they show significantly less toxicity attributable to wild-type EGFR inhibition in normal tissues (skin rash and diarrhoea). Osimertinib has succeeded in overcoming a major proportion of *EGFR* T790M-driven acquired resistance. Pooled data from phase I and phase II trials validated an overall response rate of 66% in a total of 411 resistant, T790M-mutant NSCLCs (table 1) [8]. In marked contrast, its activity dropped to 20–35% of overall response rates and <3 months of PFS in patients with T790M-negative tumours [65]. These results largely exceed the expected benefits for standard chemotherapy and have allowed the recent approval of osimertinib by the main regulatory authorities (the US Food and Drug Administration (FDA) and European Medicines Agency (EMA)) for patients with $T790M^{+}$ tumours progressing on first/second-generation EGFR TKIs. Results from confirmatory phase III trials are pending (NCT02151981). Although similar efficacy outcomes were initially reported for rociletinib [66], the updated response rate data (~30%) [10] suggest a lower activity of this drug compared to osimertinib and it is not currently in clinical development. There are other novel third-generation TKIs in early phases of development, including HM61713, ASP8273, EGF816, AZD3759 and HMPL-813 (table 1).

Nevertheless, as with first- or second-generation inhibitors, acquired resistance to third-generation EGFR TKIs will inevitably develop. Loss of T790M has been described in a significant proportion of cases treated both with osimertinib and rociletinib [67, 68]. In addition, a tertiary C797S mutation is detectable in about 30% of patients treated with osimertinib [67]. Novel allosteric inhibitors selectively targeting *EGFR* C797S mutants have been already discovered but have not yet been clinically tested [69].

On the other hand, non-*EGFR*-dependent mechanisms of acquired resistance have been also identified in patients treated with first/second-generation (40%) and third-generation (less known, probably 50–60%) TKIs [7]. These mechanisms rely on activation of alternative pathways ("by-pass tracks") reinstating cell survival and proliferation. Targeting those oncogenic events are rational combinations to overcome resistance. For instance, *MET* oncogene dysregulation is found in ~10% of the acquired resistant cases. In a molecularly selected phase Ib/II trial, combined treatment of gefitinib plus the c-MET-selective TKI inhibitor capmatinib showed partial responses in 50% of the patients harbouring highly *MET*-amplified tumours, with manageable toxicities [70].

With the aim to demonstrate whether front-line third-generation EGFR TKIs delay the development of *EGFR*-dependent acquired resistance, these drugs are being actively tested in treatment-naïve patients (NCT02296125). Osimertinib treatment has shown a response rate of 77% and an encouraging median PFS of nearly 20 months in 60 previously untreated *EGFR* mutant NSCLCs included in the AURA-1 study (table 1) [9]. In order to delay aggressive forms of heterogeneous resistance, upfront combinatorial strategies targeting alternative pathways are also being tested in clinical trials (*e.g.* third-generation TKIs plus MEK inhibitors; NCT02143466).

Finally, antiangiogenics have demonstrated synergistic clinical activity as first-line combination therapies with EGFR TKIs. Erlotinib plus bevacizumab showed a 6-month PFS improvement over erlotinib alone in a phase II Japanese trial in treatment-naïve patients (16 *versus* 9.6 months; HR 0.54, 95% CI 0.36–0.79; p=0.0015). No unexpected toxicities were reported [71]. The recently presented European, single-arm, phase II BELIEF study confirmed the clinical activity of the front-line front-line combination, particularly for those patients harbouring pre-treatment double-mutant (sensitising mutation plus T790M mutation) tumours (16 months median PFS, 1-year PFS rate 72%) [72]. Following these results, bevacizumab has gained EMA approval as front-line combination treatment with erlotinib for patients with common sensitising *EGFR*-mutant tumours, and represents an alternative to first- or second-generation TKI monotherapy in this setting.

ALK rearrangements

Chromosomal *ALK* rearrangements are found in approximately 3–7% of NSCLCs. To date, >27 fusion variants have been described in human cancer, *EML4* being the most frequent 5′ fusion partner. As is the case with other gene fusions in lung cancer (*e.g. ROS1*, *RET* and *NTRK1*), the resulting gene fusion proteins typically induce ligand-independent dimerisation and transphosphorylation of the tyrosine kinase

domain, resulting in constitutive activation of downstream signalling pathways including PI3K/AKT, RAS/RAF/ERK and STAT [15].

Crizotinib, a first-generation ALK inhibitor, is an orally bioavailable small molecule that targets ALK, ROS1 and MET tyrosine kinases, and represents the cornestorne of treatment of *ALK* rearranged lung cancer. Initial phase I (PROFILE 1001) and phase II trials (PROFILE 1005) in molecularly selected and mostly pretreated cohorts demonstrated a significant 60% response rate and 10–11-months PFS [16]. Subsequently, the randomised phase II PROFILE 1007 study showed better response rate (65% *versus* 20%, p<0.001) and PFS (7.7 *versus* 3.3 months; HR 0.49, 95% CI 0.37–0.64; p<0.001) than second-line chemotherapy [17]. Finally, crizotinib conferred significant improvements in response rates (74% *versus* 45%, p<0.001) and PFS (10.9 *versus* 7 months; HR 0.45, 95% CI 0.35–0.60; p<0.001) compared to standard front-line platinum–pemetrexed-based chemotherapy in the PROFILE 1014 trial (table 2) [18].

Similarly to *EGFR*-mutant cancers, *ALK* TKI resistance involves ALK-dominant (~40%) and *ALK*-nondominant (~60%) mechanisms. Among the former, ~30% of *ALK*-positive NSCLCs treated with crizotinib develop mutations within the ALK tyrosine kinase domain. The *ALK* L1196M mutation is analogous to the T790M mutation in *EGFR*-resistant disease [64]. Next-generation ALK inhibitors are not ALK-selective inhibitors, and they variably target other kinases including ROS1, Trk, MET or insulin-like growth factor 1 receptor, among others. Ceritinib and alectinib accumulate the vast majority of the clinical data, but there are other many in different phases of development, including brigatinib, ensartinib, lorlatinib, entrectinib, TSR-011 and X-376. In general, these compounds have been optimised to overcome some of the limitations associated with crizotinib: they show higher potency against wild-type *ALK*, increased but variable

TABLE 2 Clinical characteristics, targeted drugs and efficacy of anaplastic lymphoma kinase (*ALK*) rearranged nonsmall cell lung cancers

Genomic features	First-generation ALK TKI	RR	mPFS months	Selected next-generation ALK TKIs overcoming crizotinib resistance	Clinical trial phase (study acronym)	Subjects	Context	Trial RR	Trial mPFS months
>27 fusion variants [15] ***EML4* (3′)–*ALK* (5′) most frequent**	Crizotinib	60–74% [16–18]	7.7–10.9 [16–18]	Ceritinib	I (ASCEND-1) [19]	163	TKI resistant	56%	6.9
					II (ASCEND-2) [20]	140	TKI resistant	39%	5.7
					I (ASCEND-1) [17]	83	TKI naïve	72%	18.4
					II (ASCEND-3) [21]	124	TKI naïve	63.7%	18.4
					III (ASCEND-5) [22]	231	TKI resistant	39.1%	5.4
				Alectinib	II (NP28673) [23]	138	TKI resistant	50.8%	8.9
					II (NP28761) [24]	87	TKI resistant	52.2%	8.1
					I (AF-001JP)[#] [25]	46	TKI naive	93.5%	27.7
					III (J-ALEX)[#] [26]	104	TKI naive	92%	
				Brigatinib	I/II [27]	71	TKI resistant	72%	12.9
					II (ALTA) [28]	110[¶]	TKI resistant	54%[¶]	12.9[¶]
					I/II [27]	8	TKI naive	100%	
				Lorlatinib	I/II [29]	41[+]	TKI resistant[+]	46%[+]	
				Ensartinib	I/II [30]	25	TKI resistant	64%	
					I/II [30]	14	TKI naive	71%	

TKI: tyrosine kinase inhibitor; RR: response rate; mPFS: median progression-free survival. [#]: Japanese study; [¶]: data correspond to brigatinib at 180 mg daily dose; [+]: including patients resistant to more than one ALK TKI.

TABLE 3 Clinical characteristics, targeted drugs and efficacy of other actionable oncogenic drivers in nonsmall cell lung cancers

Gene	Genomic alteration	Prevalence in Caucasians	Clinical features	Selected drugs with clinical data available	RR	Selected next-generation drugs overcoming acquired resistance	RR of next-generation drug
ROS1	Rearrangements [31] >9 fusion variants *CD74* (3′)–*ROS1* (5′) most frequent	1–2% [31]	AD (rarely SCC) [31] Never or light smokers Younger age Females>males	Crizotinib	72–80% [32, 33]	Lorlatinib, cabozantinib, ceritinib, entrectinib, brigatinib, foretinib	33%[#] [29]
BRAF	Sensitising kinase domain activating mutations [34] V600E (50% of all cases)	2–4% [34]	Mainly AD [34] Smokers>nonsmokers Irrespective of age/sex	Vemurafenib, dabrafenib, trametinib	32–63% [35, 36]		
MET	Amplification (ratio ⩾5) [37]	~3–4% [37]	Mostly AD [37] Smokers~nonsmokers Older age Females~males	Crizotinib, cabozantinib, capmatinib	67% [38]		
	Exon 14 mutations [39, 40]	2–3% [37, 39, 40]	AD~SCC [41] Smokers~nonsmokers Older age Females~males	Crizotinib, cabozantinib, capmatinib	44% [42]		
RET	Rearrangements [43, 44] >4 fusion variants *KIF5B* (3′)–*RET* (5′) most frequent	~1% [44]	AD (rarely SCC) [43, 44] Never or light smokers Younger age Females>males	Cabozantinib, vandetanib, lenvatinib, sorafenib, sunitinib, alectinib, ponatinib	16–53% [45–48]		
NTRK	Rearrangements [49] 2 fusion variants *MPRIP* (3′)–*NTRK1* (5′) *CD74* (3′)–*NTRK1* (5′)	1% [50, 51]	Mainly AD [50, 51] Smokers>nonsmokers Irrespective of age/sex	Entrectinib, LOXO-01	Strong responses in small cohorts and isolated case reports		
HER2	Kinase domain activating mutations: [52, 53] Exon 20 insertions	1–3% [52, 53]	AD [52, 53] Never or light smokers Younger age Females>males	Afatinib, dacomitinib, neratinib, trastuzumab, TDM1	10–20% [54, 55, 56, 57]		

RR: response rate; AD: adenocarcinomal; SCC: squamous cell carcinoma.[#]: lorlatinib data from a small cohort (n=6) of crizotinib-resistant, *ROS1*$^{+}$ lung cancers.

specificity for the different *ALK* mutant variants and higher central nervous system penetration [73]. Consequently, solid phase II clinical evidence has already shown that second-generation ALK TKIs (ceritinib, alectinib and brigatinib) induce a response in 39–62% of patients with proven crizotinib resistance, achieving a median PFS of 5.7–12.9 months (table 2) [19, 20, 23, 24, 28]. Confirmatory phase III data in previously treated patients have been already reported for ceritinib, which shows superior response rates (39.1% *versus* 6.9%) and PFS (5.4 *versus* 1.6 months; HR 0.49, p<0.001) compared to docetaxel- or pemetrexed-based chemotherapy [22]. In addition, these drugs consistently demonstrate robust and durable central nervous system responses even in patients without prior brain radiotherapy, mirroring the systemic activity [19, 28, 74, 75]. Toxicities differ between drugs and might be relevant in some cases. For instance, overall drug-related adverse events were more frequent in the ceritinib arm (mainly substantial gastrointestinal side-effects and alanine transaminase/aspartate transaminase increase) than in the chemotherapy arm (mainly haematological toxicities) in the ASCEND-5 trial [22]. In contrast, gastrointestinal toxicities are less common with alectinib, and low- to mild-grade myalgia, increased creatine phosphokinase and elevated liver function tests are most commonly observed with this drug [23]. Gastrointestinal events are also relatively common with brigatinib and it is worth noting that early pulmonary events (including dyspnoea, hypoxia and lung opacities) were reported in 6% of the patients treated with this compound [28]. Approval has been recently granted for ceritinib (FDA and EMA) and alectinib (FDA) for patients progressing on crizotinib.

A recent study has unravelled major clinical mechanisms of acquired resistance to second-generation ALK inhibitors. *ALK* kinase domain mutations are more frequently detected upon second-generation TKI progression (~50%) than upon crizotinib resistance (~30%), which is consistent with their higher potency against ALK. Remarkably, each inhibitor is associated with its own spectrum and sensitivity to *ALK* mutations, and all *ALK* mutations acquired upon first- and second-generation ALK TKIs seem to predict sensitivity to the third-generation inhibitor lorlatinib [76]. Clinically, lorlatinib has recently shown encouraging activity in a phase I/II study of 41 patients with ALK^+NSCLCs, mostly refractory to at least one prior TKI, with an objective response rate and median PFS of 46% and 11.4 months, respectively. Remarkably, a response rate of 42% and median PFS of 9.2 months were reported for patients refractory to two or more lines of previous ALK TKIs (table 2). Moreover, durable responses were described in patients harbouring G1202R-mutant tumours, an *ALK* kinase domain mutation conferring clinical resistance to crizotinib, alectinib and ceritinib. Robust central nervous system responses (including leptomeningeal disease) were equally observed. Hypercholesterolaemia and peripheral edoema were the most frequent treatment-related adverse events reported at the recommended phase II dose [29]. Therefore, as next-generation ALK TKIs show distinct sensitivity to *ALK* mutations and also target different kinases potentially involved in bypass track mediated acquired resistance (*e.g.* MET), sequential therapy with next-generation ALK TKIs in progressive disease is a viable and clinically feasible treatment option [29, 77].

Novel ALK TKIs are also being actively tested in ALK TKI-naïve patients. Single-cohort data with ceritinib, alectinib, brigatinib or ensartinib indicate that crizotinib-naïve patients achieve higher response rates (71–93.5%) [19, 21, 25, 27, 30] and longer median PFS (18.4–27.7 months) [19, 78] than crizotinib resistant patients (table 2). Recently, important findings from the phase III J-ALEX trial have been reported. In this study, ALK TKI-naïve Japanese NSCLC patients with ALK^+ tumours were randomised to receive front-line alectinib *versus* crizotinib. Median PFS was significantly longer with alectinib (not reached) than crizotinib (10.2 months) (HR 0.34, 95% CI 0.17–0.71; p<0.0001) (table 2). Of note, the small subgroup of patients with baseline brain metastases also strongly benefited with front-line alectinib in terms of PFS (HR 0.08). The overall toxicity rates were lower with alectinib [26]. These impressive results await confirmation in the ALEX phase III trial, of larger sample size and including Caucasian patients (NCT02075840). In addition, studies comparing ceritinib to platinum-based chemotherapy are fully recruited and results are expected soon (NCT01828099).

In any case, it should be emphasised here the incremental impact of all available therapies, including chemotherapy, in the overall outcome of patients whose current survival frequently exceeds 4 years. It is likely that in the future, in a context of expanding TKI alternatives with differing activity against ALK kinase variants, regardless the initial treatment given, subsequent therapies will be guided by the changing genotype of the tumour as a consequence of the pressure of prior treatments.

ROS1 rearrangements

ROS1 rearrangements are found in 1–2% of NSCLCs. As in the case of *EGFR* mutations or *ALK* fusions, they are very rarely found in squamous cell carcinomas or smokers, and are more prevalent among the young and females. 11 *ROS1* fusion variants have been described to date, *CD71* being the most frequent 5′ fusion partner [31]. ROS1 and ALK kinases are pylogenetically close, and their rearrangements in lung cancer share common carcinogenic properties, clinical characteristics, certain therapeutic vulnerabilities and probably acquired resistance mechanisms.

In line with this premise, the PROFILE 1001 trial was amended early to include an expansion cohort of *ROS1*-rearranged NSCLC patients (n=50). Crizotinib achieved an overall response rate of 72% and median 19.2-month PFS, with 85% OS at 12 months in this 50 patient cohort [31]. A lower median PFS (9.1 months) was reported in a small retrospective observational study (response rate 80%), but again compared favourably with the results obtained with standard platinum-pemetrexed based chemotherapy (table 3) [33]. Crizotinib has been recently approved by FDA for these patients.

Clinical development of next-generation dual ALK and ROS1 inhibitors (lorlatinib, ceritinib, brigatinib and entrectinib) and other ROS1 inhibitors (cabozantinib and foretinib) is currently ongoing [31]. Among them, cabozantinib and lorlatinib have already demonstrated the ability to overcome crizotinib resistance in the clinic [29, 79]. Of note, among the 12 patients with *ROS1*$^{+}$ tumours included in an ongoing phase I/II trial, lorlatinib achieved an objective response rate of 33% and 66% in crizotinib-pretreated and crizotinib-naïve patients, respectively (table 3) [29]. Similarly to that described in *ALK*-driven disease, the fact that *ROS1* mutations confer nonoverlapping resistance to dual ALK and ROS1 inhibitors or ROS1-specific inhibitors opens the possibility to sequence different TKIs upon subsequent disease progressions [80].

Emerging targets

BRAF activating mutations

The prevalence of *BRAF* kinase domain mutations in NSCLC is roughly 2–4% and half of the cases harbour non-V600E mutations, the drug sensitivity and biological properties of which are much less well known than those of V600E. There seems to be no distinct distribution according to smoking status, sex or age [34]. *BRAF* activating mutations induce constitutive phosphorylation of downstream proteins of the RAS/RAF/MEK/ERK mitogen-activated protein kinase pathway, promoting aberrant cell proliferation and survival.

Type I BRAF inhibitors (vemurafenib and dabrafenib) have demonstrated robust clinical activity in *BRAF* V600E mutant NSCLCs in isolated case reports [81–84], retrospective series [85] and prospective basket [86] or histology-specific trials [35]. In the VE-BASKET trial, vemurafenib showed a response rate of 42% in previously treated NSCLC subjects (n=20). The median PFS was 7.3 months and a preliminary 12-month OS rate of 66% was reported [86]. Similar results have recently been published with dabrafenib monotherapy in the NSCLC histology-specific BRF113928 phase II trial (n=84, overall response rate 33%) [35]. Importantly, as already seen in melanoma, the clinical efficacy with the combination of dabrafenib and the allosteric MEK1/2 inhibitor trametinib is higher as compared to dafrafenib monotherapy. Thus, in a total of 57 pre-treated patients included in an independent cohort of the BRF113928 phase II study, the overall response rate was 63% (disease control rate 79%) and the median PFS achieved was 9.7 months (table 3) [36]. The safety profile of these drugs was manageable and consistent with that in melanoma patients.

Evolving targets

De novo MET amplification and *MET* exon 14 alterations

Whereas the co-occurrence of *MET* amplification and *MET* mutations within exon 14 is relatively common in NSCLC (15–20%), these are distinct oncogenic drivers [37, 39, 40]. Separately, they represent approximately 3–4% of NSCLCs. Importantly, *MET* exon 14 alterations have been found in up to 20–30% of sarcomatoid lung carcinomas [37, 39, 40]. Of note, both alterations seem more frequent among older lung cancer patients, with no apparent major differences according to smoking status or between adenocarcinomas *versus* squamous cell carcinomas [37, 41]. *MET* exon 14 alterations are not activating. Instead, they lead to decreased MET degradation (commonly but not exclusively through splice site mutations causing exon 14 skipping), resulting in sustained and constitutive MET signalling [39, 40].

Crizotinib has shown encouraging antitumor activity either in high *MET*-amplified or *MET* exon 14-altered NSCLCs within the subsequently expanded independent cohorts of the still ongoing PROFILE 1001 trial. The level of *MET* amplification seems critical to the benefit of MET TKIs. Thus, crizotinib showed objective responses in 67% of previously treated high *MET* (*i.e. MET*/*CEP7* ratio $\geqslant$5) amplified patients (n=14), but this activity dropped to 0% and 17% in low and intermediate *MET* amplified patients, respectively [38]. In addition, a 44% response rate (disease control rate >90%) has been reported in the cohort of *MET* exon 14-altered tumours (table 3) [42]. Other MET TKIs, such as cabozantinib and capmatinib, had also shown evidence of strong activity in small series of patients harbouring *MET* exon 14 altered-tumours [39, 40, 41, 87]. These promising results have prompted the initiation of prospective trials testing several MET TKI inhibitors for *MET*-amplified and/or *METex14*$^{+}$ patients (*e.g.* NCT02414139).

RET rearrangements

The prevalence of *RET* rearrangements in NSCLCs is roughly 1%, increasing up to 2–3% in *EGFR, ALK* and *KRAS* wild-type tumours [43, 44] and 16% in pan-negative, never-smoker lung adenocarcinomas [43].

Many RET TKIs are multikinase inhibitors already in clinical use, including vandetanib, cabozantinib, lenvatinib, sunitinib, sorafenib and alectinib. Cabozantinib, vandetanib and lenvatinib have shown objective response rates of 16–53% in small molecularly selected phase II studies including mostly highly pretreated patients (table 3). Disease stabilisation was a common feature with both drugs, and immature median PFS estimates ranged 4.7–7.3 months [45–48]. Interestingly, higher clinical activity was seen in vandetaninb-treated patients harbouring the *CCDC6–RET* fusion variant (n=6; response rate 83%, median PFS of 8.3 months) [46]. Similarly remarkable efficacy has been reported in retrospective series or isolated case reports treated with a variety of off-label RET-targeting drugs [44, 88, 89]. Overall, the efficacy data are thus still too immature to select the RET inhibitor of preference, but as off-target toxicities are relatively common and vary among the different agents, this may be a relevant factor to consider the selected TKI for an individual patient.

NTRK rearrangements

NTRK genes encode the TrkA (*NTRK1*), TrkB (*NTRK2*) and TrkC (*NTRK3*) receptor tyrosine kinase proteins. *NTRK1* fusions (*e.g. NTRK1–MPRIP* and *NTRK1–CD74*) [49] have been reported in 1% of unselected NSCLC cohorts [50, 51], rising up to 3–4% in patients who screened negative for other molecular alterations [50, 51]. They are enriched in the adenocarcinoma population and are apparently more frequent among former or current smokers [50, 51].

Among the at least nine potent pan-Trk inhibitors in development [90], entrectinib is at a more advanced stage of clinical testing in NSCLC [91, 92]. All NSCLC patients harbouring *NTRK1*-fused tumours included in two parallel phase I trials (three out of three) showed major objective responses [92]. Notably, central nervous system responses in heavily pre-treated patients have been reported [51]. An ongoing phase II basket trial is actively recruiting patients (NCT02568267).

HER2 aberrations

HER2 gene amplifications and mainly kinase domain mutations represent potential but distinct molecular targets in lung adenocarcinomas. Little or no overlap between both alterations has been published [52, 53]. *HER2*-mutant NSCLCs share common clinical–pathological features with those of *EGFR* mutant cancers and are found in ~2% of lung adenocarcinomas [52, 53]. The most frequent mutations consist of in-frame insertions in exon 20 [52, 53].

Combination therapies with trastuzumab and chemotherapy have shown activity in retrospective European cohorts [54, 55], and isolated clinical cases harbouring *HER2* kinase domain mutations [93–95]. The efficacy of single-agent pan-HER TKIs including afatinib, dacomitinib or neratinib in these patients is modest at best [52, 55–57]. Objective responses were reported in 12% of patients with *HER2*-mutant tumours, but none in *HER2*-amplified cancers, treated with afatinib within a small phase II trial (n=26), with modest 3- and 9-month median PFS and OS, respectively (table 3) [56]. What specific molecular context predicts responsiveness and the optimal treatment strategy (single agent TKIs *versus* trastuzumab with or without chemotherapy) for these patients is yet to be defined.

Potential targets

KRAS has been considered as a clinically difficult to inhibit, if not undruggable, target. More recently, direct inhibition of KRAS with allele-specific (G12C) allosteric covalent inhibitors interacting with the inactive GDP-RAS state have shown potent RAS signalling inhibition *in vitro* [96, 97]. Inhibition of "downstream" proteins and synthetic lethality approaches are being actively pursued in clinical trials. The combination of docetaxel plus selumetinib, an allosteric MEK inhibitor, showed a significant improvement in response rates (37% *versus* 0%) and PFS (5.3 *versus* 2.1 months) when compared to docetaxel alone in a small phase II study [98], but this treatment finally failed to improve outcomes in a recently reported phase III trial [99]. Furthermore, it was associated with substantial toxicity, mainly haematological. However, CDK4 inhibition has been shown to be synthetically lethal in *KRAS*-mutant lung adenocarcinoma mouse models [100]. Disease control rate appeared superior in *KRAS*-mutant (55.2%) compared to *KRAS* wild-type (37.5%) NSCLCs treated with abemaciclib (CDK4/6 inhibitor) in a phase I trial [101]. A phase III trial of abemaciclib compared to erlotinib as second/third-line therapy is currently ongoing (NCT02152631).

The attempts to target aberrant FGFR [102], DDR2 [103] or PI3K [104] pathway activation have been clinically disappointing. More preclinical and clinical research is needed before they can be considered reliable predictive targets in this disease.

Biological therapies for mostly molecularly unselected patients

Antiangiogenics

Bevacizumab, ramucirumab and nintedanib prolong survival in clinically selected candidates for antiangiogenic therapies when combined with chemotherapy. Data from a large meta-analysis confirmed the benefits of

adding bevacizumab to first-line platinum based chemotherapy (HR for OS 0.90, 95% CI 0.81–0.99; p=0.03) [105]. However, the combination of bevacizumab plus weekly paclitaxel is superior to standard second-line docetaxel in terms of response rates (22.5% *versus* 5.5%, p=0.006) and PFS (HR 0.62, p=0.005), and thus may constitute another treatment option in this setting [106]. Its use is restricted to nonsquamous histology due to safety concerns (pulmonary haemorrhage in squamous cell cancers). Both ramucirumab and nintedanib numerically increased median PFS and OS by about 1 month and 1.5–2 months respectively when added to second-line docetaxel in phase III studies [107, 108]. Nintedanib's OS benefit was restricted to nonsquamous histology (HR 0.83, 95% CI 0.70–0.99; p=0.03) and seemed more profound in platinum-refractory subsets [107]. Nintedanib is EMA, but not FDA, approved. The benefit of ramucirumab was independent of histology (HR 0.86, 95% CI 0.75–0.98; p=0.02) and it is the only antiangiogenic agent approved for squamous cell lung cancer patients [108]. An excess of predominantly low-grade toxicities related to antiangiogenic therapy (hypertension, proteinuria, and haemorrhagic or thrombotic events) were noted with bevacizumab and ramucirumab [108, 109]. Bevacizumab and ramucirumab but not nintedanib (predominantly gastrointestinal and hepatic toxicities) increased the rates of neutropenia and febrile neutropenia [107–109].

Anti-EGFR monoclonal antibodies

Cetuximab and necitumumab are the two monoclonal antibodies targeting the extracellular domain of EGFR that have been most extensively studied in NSCLC. The addition of necitumumab to cisplatin and gemcitabine modestly improved OS, as compared to chemotherapy alone, in squamous cell cancers (11.5 *versus* 9.9 months; HR 0.84, 95% CI 0.74–0.96; p=0.012), results that are consistent with those observed with cetuximab [110]. No benefit was observed in a nonsquamous NSCLC trial in combination with cisplatin and pemetrexed [111]. Skin rash, diarrhoea and hypomagnesaemia were significantly increased in the combination arm, with no excess of febrile neutropenia or thromboembolic events [110]. The drug is approved for unselected squamous cell cancers by the FDA, but only *EGFR*$^+$ cases according to EMA.

Immunotherapy

Innate and adaptive immune responses can recognise and kill cancer cells. Dendritic cells, macrophages, neutrophils and natural killer cells are critical to innate immunity, mediating early antigen-nonspecific immune responses by a limited number of receptors (*e.g.* Toll-like receptors). By contrast, adaptive immunity is mediated by T-cells ($CD4^+$ and $CD8^+$) and B-cells, and induces a robust, antigen-specific immune response [112]. There is solid evidence that demonstrates the existence of anti-tumour adaptive T-cell mediated immunity activation in established lung tumours, indicating that lung cancers are immunogenic [113, 114]. As an example supporting this concept, increased levels of clonal tumour infiltrating $CD8^+$ lymphocytes are independent predictors for favorable survival in lung cancer [115]. The reason why these tumours still evolve is precisely because cancer cells develop immune escape mechanisms and proliferate even in the presence of competent immune systems.

Immunotherapy against cancer can be classified as active or passive. Active immunotherapies rely on the activation of the host's own T-cell based anti-tumour responses. Contrary, passive immunotherapies retain intrinsic anti-tumour immunity. Two active immune-based therapies accumulate the majority of the clinical evidence in lung cancer: cancer vaccines and checkpoint inhibitors.

Cancer vaccines

Monovalent vaccines have failed to demonstrate robust survival benefits in randomised phase III trials [116–118], probably due to an insufficient T-cell immunity activation to overcome the immunosuppressive tumour microenvironment [119]. Treatment combinations with drugs reversing these immunosuppressive networks (*e.g.* checkpoint inhibitors) and/or novel potent formulations based on polyvalent vaccines might be promising strategies for the success of tumour vaccines in the future [120].

Immune checkpoint inhibitors: PD-1/PD-L1 pathway blockade

Among the multiple immune suppressive mechanisms that are generated within the tumour microenvironment, tumour cell induced dysregulation of immune checkpoint proteins has revealed as a major mechanism of anti-tumour T-cell immunity inhibition [121]. Upon activation, T-cells up-regulate a number or surface proteins that, in the presence of their ligands, modulate their activity either in an inhibitory (*e.g.* B- and T-lymphocyte attenuator (BTLA), inducible T-cell co-stimualtor, programmed death (PD)-1 and cytotoxic T-lymphocyte-associated 4 (CTLA4)) or stimulatory (*e.g.* OX40, CD40 and CD137) fashion [122]. Among them, CTLA4 and PD-1 are the two main regulators of T-cell activity, at least with respect to their clinical relevance in cancer. PD-1 is up-regulated on a great proportion of tumour-infiltrating lymphocytes, and its two major ligands, PD-L1 and PD-L2, are commonly overexpressed on the surface of tumour cells. This interaction results in an effective inhibition of the effector T-cell response. In contrast, binding of CTLA4 with its two major ligands CD80 and CD86

(mainly expressed in antigen-presenting cells) preferentially modulates T-cell activation and expansion in lymph nodes [123]. Importantly, blocking these co-inhibitory signals, particularly the PD-1/PD-L1 interaction, has changed treatment paradigms of lung cancer.

Agonistic monoclonal antibodies targeting the co-inhibitory PD-1/PD-L1 interaction have recently impacted the treatment landscape of advanced NSCLC. Five drugs accumulate the vast majority of the clinical evidence to date: two anti PD-1 monoclonal antibodies (nivolumab and pembrolizumab) and three anti-PD-L1 monoclonal antibodies (atezolizumab, durvalumab and avelumab). Both safety and early activity seem similar with either PD-1 or PD-L1 blockade. Efficacy data from the initial phase I trial of nivolumab [124] have been confirmed in five large randomised trials for nivolumab, pembrolizumab and atezolizumab in pre-treated patients so far [125–129]. Consistent conclusions can be drawn regarding the efficacy of these drugs in unselected patients [125, 126, 128, 129] (table 4). First, the overall response rate is ∼20% and responses, which are usually detected early (2 months), are profound and durable (median 15 months *versus* ∼6 months with docetaxel). In addition, a substantial proportion of patients also achieve long-lasting disease stabilisation with clinical benefits. Second, median PFS (∼4 months) probably does not capture the true benefit of these drugs either. Finally, PD-1/PD-L1 blockers robustly demonstrate a median ∼3-month improvement in OS compared to second-line chemotherapy. Remarkably, about 15–20% of the patients treated with these drugs appear to survive for >24 months [125–129] (table 4), although longer follow-up is required to more definitively conclude on the potential for long-term survival. The toxicity profile also favours anti-PD-1/PD-L1 therapy over chemotherapy, despite longer treatment exposures. The most frequently reported adverse event under PD-1 pathway blockade is asthenia (15–20%), commonly a tumour-related symptom. Immune-related adverse events can be relatively common (∼20%), but are mostly mild (>90%) and rarely motivate drug withdrawal (<5%). These toxicities typically involve the skin (erythema and rash), gastrointestinal tract (colitis and diarrhoea), endocrine glands (hypophisitis, thyroiditis and adrenalitis), liver (hepatitis) and lungs (pneumonitis). Although rare (∼1–4%), special attention is merited by interstitial pneumonitis in lung cancer patients. To date, nivolumab has obtained regulatory approval (FDA and EMA) for NSCLCs progressing on first-line chemotherapy. Pembrolizumab is FDA approved in second or further lines of treatment for PD-L1 positive (⩾1% of cells) NSCLCs. Atezolizumab has been also recently approved by the FDA for NSCLC patients progressing on platinum-containing chemotherapy.

Very recently, data from two large randomised phase III trials in the front-line setting have been reported [130, 131] (table 5). In one of these trials, pembrolizumab showed superior clinical activity to platinum-based doublet chemotherapy in terms of response rates (44.8% *versus* 27.8%), PFS (10.3 *versus* 6 months; HR 0.50, p<0.001) and OS (HR 0.60, p=0.005) among *EGFR* and *ALK* wild-type NSCLCs with PD-L1 expression on at least 50% of tumour cells [130]. These are practice-changing results, and might lead to a change in the standard of care of advanced-stage NSCLC. Other randomised trials against first-line platinum-based chemotherapy (*e.g.* NCT02477826) or studies assessing their role in earlier stages of the disease (*e.g.* adjuvant setting; NCT02595944, NCT02504372, NCT02486718 and NCT02273375) are already ongoing.

Several potential predictive biomarkers for optimal patient selection for anti-PD-1/PD-L1 therapy are actively being investigated. Among them, PD-L1 expression measured by immunohistochemistry, albeit not without some limitations that are out of the scope of this review, is the most feasible and applicable to the clinic at present. Its potential predictive role has been assessed in most of the randomised controlled trials (table 4). At least two studies in pre-treated patients that have enrolled PD-L1^{-} NSCLCs demonstrate a positive predictive role for PD-L1 expression, showing marked survival improvements for nivolumab compared to docetaxel among PD-L1^{+} NSCLCs (14.9–15.5 months with nivolumab compared to 8.2–9.2 months with docetaxel; HR ∼0.60) but overlapping survival curves in PD-L1^{-} subsets (∼9 months; HR ∼1) [126, 128]. Conversely, in the OAK study, the OS improvement of atezolizumab over docetaxel was also observed in PD-L1^{-} tumours, with a similar degree of benefit as in PD-L1^{+} NSCLC (HR 0.75, 95% CI 0.59–0.96; p=0.02) [129] (table 4). However, issues related to a potentially lower analytical performance of the SP142 clone used in this trial with respect to labelling percentages of PD-L1^{+} tumour-cells might limit the interpretation of these data [133]. Furthermore, among PD-L1^{-} NSCLCs, response rates and PFS seem somewhat consistently higher for docetaxel (10–15% and 3.6–4.1 months, respectively) compared to anti-PD-1/PD-L1 drugs (8–9% and 1.7–2.6 months, repectively) [126, 128, 129] (table 4). Notably, this predictability might be different for nonsquamous and squamous cell lung cancers, as at least in the relatively small cohort of squamous cell lung cancer patients enrolled in the CheckMate 017 trial, treatment benefits in terms of response rates, PFS and overall were superior for nivolumab compared to docetaxel, irrespective of PD-L1 expression [125] (table 4). In any case, what randomised trials reported to date almost invariably show is that treatment benefits with these drugs are proportional to the grade of expression, that is, response rates and survival tend to increase with increasing levels of tumour cell PD-L1 positivity (less studied for immune cell PD-L1) [126–131, 134]. For instance, those patients with ⩾50% of tumour cell PD-L1 positivity, objective responses reached 45% [134], and median OS in pre-treated

TABLE 4 Practice-changing randomised trials with anti-PD-1/PD-L1 drugs in advanced, pre-treated (second-line) nonsmall cell lung cancer (NSCLC) patients

Trial	Subjects	Histology	PD-L1 expression			Therapy	RR			mPFS months			mOS months		
			Selection	Positivity	Test/clone		All	PD-L1$^+$	PD-L1$^-$	All	PD-L1$^+$	PD-L1$^-$	All	PD-L1$^+$	PD-L1$^-$
CheckMate 017 (phase III) [125]	272	SCC	No	⩾1%	Dako/28-8	Docetaxel	9%	11%	10%	2.8	*2.8*	*3*	*6*	*7.2*	5.9
						Nivolumab	20%	17%	17%	3.5 (HR 0.62)	*3.3 (HR 0.67)*	*3.1 (HR 0.66)*	9.2 (HR 0.59)	*9.3 (HR 0.69)*	8.7 (HR 0.58)
CheckMate 057 (phase III) [126]	582	Non-SCC	No	⩾1%	Dako/28-8	Docetaxel	12%	12%	15%	*4.2*	4.5	*3.6*	9.4	*9*	*10.1*
						Nivolumab	19%	31%	9%	*2.3 (HR 0.92)*	4.2 (HR 0.7)	*2.1 (HR 1.19)*	12.2 (HR 0.73)	17.7 (HR 0.58)	*10.5 (HR 0.87)*
KEYNOTE-010 (phase III) [127]	1034	NSCLC	Yes (⩾1%)	⩾1%	Dako/22C3	Docetaxel	9%	8%		4	4.1		8.5	8.2	
						Pembrolizumab 2 mg·kg^{-1}	18%	30%		3.9 (HR 0.88)	5 (HR 0.59)		10.4 (HR 0.71)	14.9 (HR 0.54)	
						Pembrolizumab 10 mg·kg^{-1}	19%	29%		*4 (HR 0.79)*	5.2 (HR 0.59)		12.7 (HR 0.61)	17.3 (HR 0.50)	
POPLAR (phase II) [128]	287	NSCLC	No	TC1-3 or IC1-3	VENTANA/ SP142	Docetaxel	15%	16%	10%	*3*	*3*	*4.1*	9.7	9.2	*9.7*
						Atezolizumab	15%	18%	8%	*2.7 (HR 0.94)*	*2.8 (HR 0.85)*	*1.7 (HR 1.12)*	12.6 (HR 0.73)	15.5 (HR 0.59)	*9.7 (HR 1.04)*
OAK (phase III) [129]	850	NSCLC	No	TC1-3 or IC1-3	VENTANA/ SP142	Docetaxel	13%	16%	11%	*4*	*4.1*	*4*	9.6	10.3	8.9
						Atezolizumab	13%	18%	8%	*2.8 (HR 0.95)*	*2.8 (HR 0.91)*	*2.6 (HR 1)*	13.8 (HR 0.73)	15.7 (HR 0.74)	12.6 (HR 0.75)

PD-L1$^+$ data from KEYNOTE-010 refer to patients with at least 50% tumour-cell positivity; hazard ratio (HRs) take nivolumab as reference. RR: response rate; mPFS: median progression-free survival; mOS: median overall survival; SCC: squamous cell carcinoma. Italics indicate statistically nonsignificant results.

TABLE 5 Randomised trials with anti-PD-1/PD-L1 drugs in advanced, previously untreated nonsmall cell lung cancer (NSCLC) patients

Trial	Subjects	Histology	PD-L1 expression			Therapy	RR			mPFS months			mOS months		
			Selection	Positivity	Test/clone		All	PD-L1$^+$	PD-L1$^-$	All	PD-L1$^+$	PD-L1$^-$	All	PD-L1$^+$	PD-L1$^-$
KEYNOTE-024 (phase III) [130]	305	NSCLC	Yes (⩾50%)	⩾50%	Dako/22C3	Platinum doublet	27.8%			6			NR		
						Pembrolizumab	44.8%			10.3 (HR 0.50)			NR (HR 0.60)		
CheckMate 026 (phase III) [131]	541	NSCLC	Yes (⩾1%)	⩾1–5%	Dako/28-8	Platinum doublet	*33.5%*			*5.9*			*13.2*		
						Nivolumab	*26.1%*			*4.2 (HR 1.15)*	*(HR 1.07)*		*14.4 (HR 1.02)*	*(HR 0.90)*	
KEYNOTE-021 (phase II) [132]	123	Non-SCC	No	⩾1–50%	Dako/22C3	Carboplatin +pemetrexed	29%	35%	13%	8.9			NR		
						Carboplatin +pemetrexed +pembroliozumab	55%	80%	57%	13 (HR 0.53)			NR		

PD-L1$^+$ data from CheckMate 026 and KEYNOTE-021 trials refer to patients with at least 50% of tumour-cell positivity; hazard ratios (HRs) data take nivolumab as reference. RR: response rate; mPFS: median progression-free survival; mOS: median overall survival; NR: not reached; SCC: squamous cell carcinoma. Italics indicate statistically nonsignificant results.

patients was ⩾17.2 months in the biomarker selected KEYNOTE-001 and KEYNOTE-010 trials [127, 134] (~9 months with docetaxel) (table 4). Furthermore, anti-PD-1 inhibitors showed superiority against first-line platinum-based chemotherapy in NSCLCs with ⩾50% of tumour PD-L1 positivity [130] but not among those with ⩾5% PD-L1 positivity (HR for PFS 1.15, p=0.25) [131] (table 5). Somewhat surprisingly, in the small cohort of ⩾50% PD-L1$^+$ NSCLC in this trial, nivolumab did not demonstrate superiority either [131]. These results are inconsistent with other nivolumab trial data [135] and should be cautiously interpreted.

Although it is a challenging issue to address in the clinic, not only the quantity but also the context in which PD-L1 is expressed should be taken into consideration. Thus, PD-L1 might be intrinsically overexpressed as a result of the activation of aberrant oncogenic pathways or induced upon interferon (IFN)-γ release as a mechanism of anti-tumour T-cell immune evasion (adaptive immune resistance). Accumulating evidence does suggest that the latter is more relevant and probably needed in order to obtain a response with anti-PD-1/PD-L1 drugs [136, 137]. In fact, other surrogates of pre-existing immunity such as high tumour mutational load [138], presence of clonal tumour infiltrating lymphocytes [139] and Th1-type or IFN-based transcriptomic signatures [140] have shown to be positive predictive markers for the benefit from these drugs [137].

Treatment outcomes seem largely independent from histology [125–128]. With regard to other clinical or molecular markers analysed, smokers (HR 0.70 *versus* HR 1.02 in never-smokers), *EGFR* wild-type (HR 0.66 *versus* HR 1.18 in *EGFR*-mutant tumours) and *KRAS*-mutant lung adenocarcinomas (HR 0.52 *versus* HR 0.98 in *KRAS* wild-type tumours), that is, tumour subtypes that are normally associated with higher mutational burden tend to derive greater treatment benefits from anti-PD-1/PD-L1 drugs [126].

Combinatorial therapies with PD-1 pathway blockade

For PD-1/PD-L1 blockade, combination strategies incorporate another treatment modality aiming to generate or enhance a stronger anti-tumour T-cell immune response and/or reverse the immune-suppressive tumour microenvironment. Multiple clinical studies are ongoing in this field, testing combinatorial treatment strategies that include: immunogenic cancer cell death inducers (chemotherapy, radiotherapy, targeted therapy and oncolytic viruses); strategies to increase anti-tumour T-cell activation (anti-CTLA4 monoclonal antibodies and cancer vaccines); strategies to increase T-cell trafficking into tumours (*e.g.* epigenetic reprogramming drugs, cytokines and antiangiogenics); strategies to stimulate T-cell cytotoxic effects (*e.g.* monoclonal antibodies targeting co-stimulatory checkpoint proteins (*e.g.* CD137, CD40, OX40 and GITR); adoptive T-cell therapy; and drugs targeting other immunosuppressive tumour pathways (*e.g.* monocloncal antibodies targeting other co-inhibitory checkpoints (*e.g.* CTLA4, TIM3, LAG3, BTLA and TIGIT) or idoleamine-2,3-dioxigenase inhibitors) [137].

The combination of anti-PD-1/PD-L1 plus anti-CTLA4 blockade has already shown to be synergistic and highly active in metastasic melanoma [141]. Preliminary results from three phase I studies in NSCLC have also been reported. These trials show that toxicities are dose and schedule dependent, and might be severe in a significant proportion of patients (17–50%) [135, 142, 143]. The most updated efficacy outcomes come from the CheckMate 012 trial, where modified combination treatment schedules are being investigated in treatment-naïve, advanced NSCLC patients (n=130). Again, treatment benefits were higher for patients with PD-L1$^+$ tumours (pooled response rates 57%; median PFS of 10.6 and 8.1 months, respectively) compared to PD-L1$^-$ NSCLCs (pooled response rates 18%; median PFS of 2.4 months and 4.7 months, respectively). Of note, efficacy was also higher, with increasing levels of PD-L1 expression (⩾50% PD-L1$^+$ tumours: pooled response rates 92%; median PFS >12 months; 1-year OS rate 90–100%). All these data compared favourably with the results obtained in the independent cohort of patients treated with nivolumab monotherapy and, at least for PD-L1$^+$ NSCLCs, largely exceed the results expected with standard first-line platinum-based chemotherapy [135].

Immature efficacy data are also available for the combination of PD-1/PD-L1 inhibitors plus conventional anticancer therapies. In a recently published small phase II trial (n=123), the combination of pembrolizumab plus carboplatin and pemetrexed significantly increased response rates (55% *versus* 29%) and median PFS (13 *versus* 8.9 months; HR 0.53, p=0.01) compared to chemotherapy alone, with an acceptable toxicity profile [132] (table 5). A phase III trial testing this combination is currently ongoing (NCT02578680). Remarkable response rates (30–75%) have been reported with other anti-PD-1/PD-L1 drugs plus platinum-based first-line chemotherapy in several phase I trials [144–146]. Multiple studies are also in progress testing the combination of checkpoint inhibitors plus oncogene-targeted therapies in molecularly selected patients (*e.g.* NCT02013219 and NCT02584634).

Conclusions and future perspectives

Biological therapies, particularly genotype-tailored treatments and immune checkpoint inhibitors, have improved treatment outcomes of a substantial proportion of NSCLC patients. At present, the selection of

these treatments is based on several predictive biomarkers that are tested in tumour specimens, underscoring the need for a multidisciplinary management of these patients for prompt diagnosis, tissue collection and sample priorisitation. Importantly, the development of novel technologies for detecting circulating tumour biomarkers (*e.g.* free serum tumour DNA genotyping methods) is rapidly evolving [147]. These technologies will be particularly useful when there is limited access to tumour biopsies, and will surely constitute important complementary sources of tumour material for genomic, molecular and immune-profiling analysis in the near future [3].

As the complexity and number of targetable genomic events increases, optimisation of molecular profiling technologies for the clinic and conducting innovative biomarker-driven clinical trials are needed for the success of precision oncology and novel genotype-tailored drug development. In addition, in order to achieve long-term survival benefits in oncogene-selected patients, understanding the sources of tumour heterogeneity and acquired resistance with continuous tumour monitoring and post-progression tumour genotyping is needed. This approach has important clinical limitations, as serial re-biopsies are not always feasible in the clinic and not free from risk in some cases. Clinical validation of plasma genotyping methods will be very important in this context in the upcoming years. Combinatorial therapies instead of sequential treatments might be another way to delay aggressive forms of resistance and positively impact treatment outcomes in this regard.

The recent success of immune therapies in lung cancer underscores the importance of profiling and targeting the tumour microenvironment as well. Development of novel immune-based treatments is exponentially expanding, and treatment combinations on the pillar of therapies reversing tumour immune-suppression (*e.g.* PD-1 blockade plus conventional cancer therapies or other immune approaches) are promising future strategies for NSCLC patients. The integration of immunobiologists into the multidisciplinary teams will be necessary in the near future in order to select for the potentially most effective and less toxic combinations, and a close multidisciplinary collaboration with other medical disciplines will be paramount to prevent and early treatment of immune-related adverse events.

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