Advances in the prediction of long-term effectiveness of immune checkpoint blockers for non-small-cell lung cancer

Sara Elena Rebuzzi^{1,2}, Alessandro Leonetti¹, Marcello Tiseo^{1,3} & Francesco Facchinetti^{*,4}

¹Medical Oncology Unit, University Hospital of Parma, 43122 Parma, Italy

²Medical Oncology Unit 1, Ospedale Policlinico San Martino of Genova, 16132 Genova, Italy

³Department of Medicine & Surgery, University of Parma, 43122 Parma, Italy

⁴INSERM U981, Gustave Roussy Cancer Campus, Université Paris Saclay, 94800 Villejuif, France

*Author for correspondence: francescofacchinetti2@gmail.com

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Immune checkpoint blockers (ICBs), namely programmed death-1 protein (PD-1) and programmed death ligand-1 (PD-L1) inhibitors, have dramatically changed the therapeutic landscape of advanced non-small-cell lung cancer (NSCLC). The anti-PD-1 agents nivolumab and pembrolizumab, together with the anti-PD-L1 atezolizumab are standard second-line treatment options based on the improvement in overall survival (OS) compared with docetaxel in previously treated NSCLC (CheckMate 017, CheckMate 057, Keynote-010, OAK trials) [1–4]. Moving to first-line setting, pembrolizumab is the standard front-line treatment of NSCLC patients with PD-L1 expression $\geq 50\%$ (Keynote 024) [5]. Nonetheless, combinations of anti-PD-1/PD-L1 agents with platinum-doublet chemotherapy (Keynote-189 [6] and Keynote-407 [7]) and bevacizumab (IMpower150 [8]), as well as the association of nivolumab with the anti-CTLA-4 ICB ipilimumab (CheckMate 227) [9], are the potential new options for the first-line treatment of NSCLC.

Despite the global improvement in survival outcomes, only a limited number of patients benefits from immunotherapy, experiencing durable response and long-term OS. Historically, long-term NSCLC survivors are defined as those patients who survive longer than 2 years from the date of diagnosis [10]. On the other hand, long responders are defined as having an objective complete or partial response lasting more than 6 months [11,12].

As follow-up of the first ICB trials in NSCLC patients increases, reaching up to the fifth year, more data on long-term OS are available. The Kaplan–Meier OS estimations of the pivotal ICB studies clearly reflect the trend in terms of long-term benefit of immunotherapy in NSCLC patients [13,14]. The tails of ICB curves reach indeed a plateau, indicating that a non-negligible quote of patients can experience an extended survival benefit, potentially turning out in cure. Moreover, recent data on NSCLC long-term survivors and responders can shed light on the identification of potential predictive biomarkers of long-term benefit from ICBs, which still represents an unmet need of ongoing clinical research.

Long-term effectiveness of ICBs in NSCLC: data from clinical trials

To date, increasing evidence on long-term survival estimations in NSCLC is available from many ICBs trials in the pretreated and in the first-line settings (Tables 1 & 2). In order to gather the most accurate long-term survival estimations, only studies whose updates have a median follow-up (mFU) of at least 2 years are included in this overview.

Pretreated NSCLC patients

Nivolumab

In the early CheckMate 003 trial, enrolling patients suffering from a wide range of malignancies, 129 pretreated NSCLC patients were treated [15]. In this study, nivolumab, across different doses, obtained 1-year (1-y), 2-year

Table 1. Trials of immune checkpoint blockers in pretreated advanced non-small-cell lung cancer patients with a minimum follow-up of 2 years.

Trial [Ref.]	Phase	Line of therapy	ICB	N	ORR	mDOR	mPFS	1y-PFS	2y-PFS	mOS	1y-OS	2y-OS	3y-OS	5y-OS
Nivolumab														
CheckMate 003 [15,16]	I	≥2nd	Nivolumab 1–10 mg/kg q2w	129	17%	17.0	2.3	22%	9%	9.9	42%	25%	18%	16%
			Nivolumab 1 mg/kg q2w	33	3%	14.7	-	19%	0%	9.2	33%	15%	15%	13%
			Nivolumab 3 mg/kg q2w	37	24%	17.0	-	30%	11%	14.9	56%	42%	27%	26%
			Nivolumab 10 mg/kg q2w	59	20%	19.1	-	19%	10%	9.2	38%	20%	14%	11%
CheckMate 063 [17,18]	II	≥3rd	Nivolumab 3 mg/kg q2w	117 (squamous)	15%	-	1.9	20%	-	8.1	39%	22%	-	-
CheckMate 017 [2,18–20]	III	2nd	Nivolumab 3 mg/kg q2w	135 (squamous)	20%	25.2	3.5	21%	16%	9.2	42%	23%	16%	-
CheckMate 057 [1,19,20]	III	2nd	Nivolumab 3 mg/kg q2w	292 (nonsquamous)	19%	18.3	2.3	19%	12%	12.2	51%	29%	18%	-
Pembrolizumab														
KEYNOTE-001 (pretreated cohort) [14,21–23]	Ib	≥2nd	Pembrolizumab 2, 10 mg/kg q3w 10 mg/kg q2w	449	23% [‡]	NR [†]	4.2	-	9% (3y-PFS)	10.5	-	30%	21%	16%
KEYNOTE-010 [3,24]	II/III	≥2nd	Pembrolizumab 2 mg/kg q3w	344	18%	NR	3.9	-	-	11.8 [†]	43%	-	23% [†]	-
			Pembrolizumab 10 mg/kg q3w	346	18%	NR	4.0	-	-	-	52%	-	-	-
Atezolizumab														
POPLAR [25,26]	II	≥2nd	Atezolizumab 1200 mg q3w	144	15%	22.3	2.7	-	-	12.6	-	32%	19%	-
OAK [4,27]	III	≥2nd	Atezolizumab 1200 mg q3w	424 (ITT 850)	15%	16.3	2.8	-	-	13.8	55%	31%	-	-
			Atezolizumab 1200 mg q3w	613 (ITT1225)	14%	23.9	2.7	-	-	13.3	53%	30%	-	-
FIR (pretreated cohorts) [28]	II	≥2nd	Atezolizumab 1200 mg q3w	93 without brain mts	21% [§]	17.0 [§]	3.7 [§]	29% [§]	10% [§] (30 months)	9.3	43%	25%	-	-
			Atezolizumab 1200 mg q3w	13 with brain mts	23% [§]	NE [§]	4.3 [§]	24% [§]	NE [§] (30 months)	6.8	38%	19%	-	-
Durvalumab														
Study 1108 [29]	I/II	≥2nd	Durvalumab 10 mg/kg q2w	240	-	-	-	-	-	-	-	25.2%	-	-

[†] Analysis conducted on pooled pembrolizumab doses cohorts after a median follow-up of 42.6 months.

[‡] Data reported are per immune-related response criteria per investigator assessment.

[§] Outcomes measured by mRECIST.

ICB: Immune checkpoint blocker; ITT 850: Intention-to-treat analysis on 850 patients; ITT 1225: Intention-to-treat analysis on 1225 patients; mFU: Median follow-up; mOS: Median overall survival; mPFS: Median progression-free survival; N: Number of patients treated with ICI; NE: Not evaluable; NR: Not reached; NSCLC: Non-small-cell lung cancer; ORR: Objective response rate; OS: Overall survival; PD-1: Programmed cell death-1; PD-L1: Programmed cell death-ligand 1; mts: Metastases.

Table 2. Trials of immune checkpoint blockers in treatment-naïve advanced non-small-cell lung cancer patients with a minimum follow-up of 2 years.													
Trial [Ref.]	Phase	ICB	N	ORR	mDOR	mPFS	1-year PFS	2-year PFS	mOS	1-year OS	2-year OS	3-year OS	5-year OS
Nivolumab													
CheckMate 012 [30–34]	I	Nivolumab 5–10 mg/kg q3w + platinum-based doublet CT	56	46%	10.4	6.0	-	-	19.2	-	-	25%	
		Nivolumab 3 mg/kg q2w + ipilimumab 1 mg/kg q12w/q6w	77	43%	NR	8.0	43%	29%	-	76%	49%	-	-
Pembrolizumab													
KEYNOTE-001 (treatment-naïve cohort) [14,21–23]	Ib	Pembrolizumab 2 or 10 mg/kg q3w 10 mg/kg q2w	101	42% [†]	NR [†]	6.0	-	-	22.3	-	49%	37%	23%
KEYNOTE-024 [35,36]	III	Pembrolizumab 200 mg q3w	154 (PD-L1 ≥50%)	45%	NR	10.3	-	-	30.0	70%	52%	-	-
KEYNOTE-021 (cohort G) [37,38]	II	Pembrolizumab 200 mg q3w + pemetrexed-carboplatin	60	57%	NR	24.0	57%	-	NR	77%	-	-	-
Atezolizumab													
BIRCH (treatment-naïve cohort) [39,40]	II	Atezolizumab 1200 mg q3w	138	26%	13.1	7.6	34%	20%	24.0	66%	50%	43% (30 months)	-
FIR (treatment-naïve cohort) [28]	II	Atezolizumab 1200 mg q3w	31 (PD-L1 TC2/3 or IC2/3)	32%	11.5 [‡]	5.5 [‡]	31% [‡]	12% [‡] (30 months)	14.4	74%	25% (30 months)	-	-
Durvalumab ± tremelimumab													
Study 1108 [29]	I/II	Durvalumab 10 mg/kg q2w	64	-	-	-	-	-	-	-	46%	-	-
MYSTIC [41]	III	Durvalumab 20 mg/kg q4w	374 (PD-L1 TC ≥25%)	-	-	-	-	-	16.3	-	38%	-	-
		Durvalumab 20 mg/kg q4w + tremelimumab 1 mg/kg	372 (PD-L1 TC ≥25%)	-	-	3.9	-	-	11.9	-	35%	-	-
† Data reported are per immune-related response criteria per investigator assessment. ‡ Outcomes measured by mRECIST. CT: Chemotherapy; ICB: Immune checkpoint inhibitor; ITT: Intention-to-treat; mOS: Median overall survival; mPFS: Median progression-free survival; mOS: Median overall survival; mPFS: Median progression-free survival;  													

[†] Data reported are per immune-related response criteria per investigator assessment.

[‡] Outcomes measured by mRECIST.

CT: Chemotherapy; ICB: Immune checkpoint inhibitor; ITT: Intention-to-treat; mOS: Median overall survival; mPFS: Median progression-free survival; N: Number of patients treated with ICI; NE: Not evaluable; NR: Not reached; NSCLC: Non-small-cell lung cancer; ORR: Objective response rate; OS: Overall survival; PD-1: Programmed cell death-1; PD-L1: Programmed cell death-ligand 1.

(2-y) and 3-year (3-y) OS rates of 42, 24 and 18%, respectively [16]. Better results were obtained among the 37 patients who received nivolumab 3 mg/kg, the dose chosen for further clinical development and now replaced by the flat dose of 240 mg every 2 weeks [42]. More recently, Gettinger and colleagues published the updated results of the above-mentioned trial, based on a minimum FU of almost 5 years [13]. The median OS (mOS) was confirmed as 9.9 months, with a 5-year (5-y) OS rate of 16%. Of note, a quarter of patients treated at the dose of 3 mg/kg were still alive after 5 years from nivolumab beginning [13]. Similarly, the updated CheckMate 063 Phase II trial (nivolumab in squamous NSCLC, in third or further treatment lines) reported a 1-y and 2-y OS of 39 and 22%, respectively [17,18].

In the two Phase III trials defining nivolumab as the new standard of care versus docetaxel for pretreated squamous (Checkmate 017) and nonsquamous (CheckMate 057) NSCLC patients, 1-y and 2-y OS ranged from 43 to 51% and to 23 to 29%, respectively [19]. In an updated pooled analysis of the two studies, the 3-y OS was 17%, confirming the survival benefit of nivolumab over docetaxel (3-y OS of 8%) [20]. Again, when pooling the results of the four studies of nivolumab monotherapy (CheckMate 017, CheckMate 057, CheckMate 063 and CheckMate 003) 4-y OS rate was almost threefold higher when patients were treated with nivolumab compared with docetaxel (14 vs 5%) [43].

Pembrolizumab

In the Phase I multicohort study KEYNOTE-001 pembrolizumab (2 or 10 mg/kg) was administered to pretreated and untreated NSCLC patients with PD-L1 expressed by at least 1% of tumor cells (PD-L1 $\geq 1\%$) [21]. Three updated analyses of the pretreated NSCLC cohorts were reported [14,22,23]. In the last one, after a mFU of 5 years, mOS was estimated at 10.5 months, with a 5-y OS rate of 15% [14].

Besides acknowledging the superiority of pembrolizumab compared with docetaxel in NSCLC patients with PD-L1 $\geq 1\%$ [3], the Phase II/III KEYNOTE-010 showed a 3-y OS rate for the anti-PD-1 agent of 23% (11% for docetaxel) [24].

Atezolizumab

Among the 144 patients who received atezolizumab in the randomized Phase II POPLAR trial, 32 and 19% were still alive at 2 and 3 years, respectively, with corresponding OS rates of 17 and 10% in the docetaxel arm [25,26].

Similar results were observed in the long-term survival data from Phase III OAK study, with 2-y OS rates of 30 and 21% for atezolizumab and docetaxel, respectively, after a mFU of 28 months [27].

In addition, the FIR trial investigated atezolizumab monotherapy in chemotherapy-naïve (cohort 1) and previously-treated patients (without and with brain metastases, cohort 2 and 3, respectively) who expressed PD-L1 (tumor cells 2/3 [TC2/3] or immune cells 2/3 [IC2/3] $\geq 5\%$ PD-L1 staining in TC or IC) [28]. After a mFU of 33 months, the 1-y OS and 30-month OS rate were 43 and 25% in cohort 1, 38 and 19% in cohort 2.

Durvalumab

Recently, survival updates concerning NSCLC patients included in the Phase I/II trial of durvalumab (anti-PD-L1 agent) have been published (mFU: 40 months) [29]. At 2 years since treatment beginning, 25% of the previously treated patients were still alive.

Untreated NSCLC patients

Nivolumab

The multicohort trial CheckMate 012 was the first Phase I trial designed to evaluate the potential benefit of nivolumab, given as monotherapy or in combination with other therapies, including ipilimumab and chemotherapy, for the first-line treatment of NSCLC [30,31]. The 3-y update of the three cohorts combining nivolumab with three platinum-based doublets reported an mOS of 19.2 months, with a quarter of patients still alive after 3 years [32]. The data concerning the nivolumab + ipilimumab cohort (77 patients) have also been recently updated. After 2 years, 49% of patients were alive and 29% of patients remained progression-free, with a median duration of response still not reached [33,34].

Pembrolizumab

The updated 5-y results of the treatment-naïve cohorts from the seminal KEYNOTE-001 trial are the last evidence of long-term efficacy of pembrolizumab as monotherapy for the frontline treatment of NSCLC, regardless of PD-L1

status (in a population still selected for PD-L1 $\geq 1\%$) [14,22,23]. When pembrolizumab was administered in first line, the mOS was 22.3 months and the 5-y OS rate reached 23% [14]. Looking at the data of pembrolizumab in PD-L1 $\geq 50\%$ (KEYNOTE 024) NSCLC patients [35], after a mFU of more than 2 years, the mOS, 1-y and 2-y OS rates reached 30 months, 70 and 51%, respectively (Table 2) [36]. These results were consistently longer than those reported with chemotherapy (mOS of 14 months; 1-y and 2-y OS rates of 55 and 35% respectively) [36].

When frontline pembrolizumab was combined to carboplatin and pemetrexed in nonsquamous histology (KEYNOTE 021), 57% of patients were progression-free at 1-y after, compared with 37% of the carboplatin-pemetrexed group, at a mFU of 18.7 months [37]. Moreover, in the same analysis, the 1-y OS rate of the combination strategy was 77% and remained still high at 18 months (70%) and confirmed to be superior to chemotherapy alone (1-y OS of 69% and 18-months OS of 56%) [37]. Notably, after five additional months of FU, the mOS was yet NR in the pembrolizumab plus chemotherapy arm, while in the chemotherapy arm was 21.1 months [38]. The findings from this Phase II study have subsequently been confirmed by the updated results from the Phase III KEYNOTE-189 study, on pembrolizumab plus pemetrexed and a platinum salt presented at ASCO 2019 [44]. After a mFU of 23 months, the mOS was doubled in the pembrolizumab combination compared with the chemotherapy arm (22 vs 11 months; HR: 0.65; 95% CI: 0.45–0.70) with a 2-y OS rate of 46 versus 30%.

Atezolizumab

In the first-line cohort of BIRCH trial (atezolizumab in NSCLC with PD-L1 $\geq 5\%$ of TC/IC), mOS was 24 months with 1-y, 2-y and 30-month OS rates of 66, 50 and 43%, respectively [40]. Similar results were observed with regard to FIR study [28].

Durvalumab, tremelimumab

A total of 64 NSCLC patients received durvalumab as first-line treatment in its early Phase study and 2-y OS in this group was 46% [29].

The Phase III MYSTIC trial compared durvalumab versus durvalumab plus tremelimumab (anti-CTLA-4) versus platinum-based doublet chemotherapy in untreated metastatic NSCLC. Despite the primary end point of superiority of durvalumab monotherapy and combination over chemotherapy in selected patients (PD-L1 expression $\geq 25\%$ in TC) was not met, a clinically meaningful improvement in OS was observed with durvalumab alone, compared with chemotherapy, while the combination of durvalumab plus tremelimumab did not add additional benefit to durvalumab alone (2-y OS of 38% with durvalumab vs 35% with durvalumab plus tremelimumab vs 23% with chemotherapy) [41].

Predictive biomarkers of long-term survival

Many potential prognostic and predictive biomarkers are currently under investigation in NSCLC patients treated with ICBs [45]. Among the several biological and clinical predictors of the benefit derivable from ICBs, PD-L1 expression is proven to be robustly correlated with long-term effectiveness thus far (Table 3). Several meta-analyses have been performed on PD-L1 prognostic/predictive role in NSCLC, showing that higher PD-L1 expression is associated with a higher likelihood of response to ICBs regardless of its threshold [46–49].

Besides PD-L1, the updated analyses on long-term survivors enrolled in the main ICB clinical trials may provide insights toward novel potential clinical and molecular biomarkers of long-term efficacy, which are discussed in the following section.

PD-L1

Nivolumab

In CheckMate 003 study, the survival benefit of nivolumab was seen across all PD-L1 levels, with similar 5-y OS rates for patients with $<1\%$ versus $\geq 1\%$ PD-L1 expression (20 vs 23%), reaching the 43% in patients with PD-L1 $\geq 50\%$ (out of 13 treated patients) [13]. In CheckMate 017 and CheckMate 057 [19], 2-y OS rates resulted again proportional to PD-L1 expression, ranging from 22 and 25% (squamous and nonsquamous histologies, respectively) in PD-L1 negative cohorts to 35 and 48% in PD-L1 $\geq 50\%$ groups. When the data of the four nivolumab trials CheckMate 003, 063, 017 and 057 were pooled, 4-y OS rates in PD-L1 $<1\%$ and $\geq 1\%$ groups were 11 and 19%, respectively [43].

Moving to nivolumab–ipilimumab combinations, the updated analysis of CheckMate 012 clearly demonstrated that increasing PD-L1 expression was associated with higher clinical activity and survival outcomes of the combi-

Table 3. PD-L1 expression subgroup results in trials of immune checkpoint blockers in pretreated/treatment-naïve advanced non-small-cell lung cancer with a minimum follow-up of 2 years.

Trial [Ref.]	ICB	PD-L1 [†]	N pts	2y-PFS	2y-OS	3y-OS	5y-OS
CheckMate 003 [13]	Nivolumab	<1%	30	-	-	-	20%
		≥1%	38	-	-	-	23%
		≥50%	13	-	-	-	43%
CheckMate 017 [19]	Nivolumab	<1%	54	-	22%	-	-
		≥1%	63	-	20%	-	-
		≥50%	17	-	35%	-	-
CheckMate 057 [19]	Nivolumab	<1%	108	-	25%	-	-
		≥1%	123	-	37%	-	-
		≥50%	66	-	48%	-	-
Pooled analysis of CheckMate 017, 057, 063 and 003 [43]	Nivolumab	<1%	192	-	-	-	-
		≥1%	224	-	-	-	-
KEYNOTE-001 (pretreated cohort) [14,22,23]	Pembrolizumab	<1%	80	-	23%	9%	4%
		1–49%	142	-	26%	17%	13%
		≥50%	100	-	39%	30%	25%
KEYNOTE-010 [24]	Pembrolizumab	≥1%	690	-	-	23%	-
		≥50%	290	-	-	35%	-
POPLAR [26]	Atezolizumab	TC3 or IC3	24	-	42%	38%	-
		TC2/3 or IC2/3	50	-	36%	21%	-
		TC1/2/3 or IC1/2/3	93	-	36%	18%	-
		TC0 and IC0	51	-	25%	21%	-
OAK (ITT1225 cohort) [27]	Atezolizumab	TC3 or IC3	89	-	43%	-	-
		TC2/3 or IC2/3	168	-	36%	-	-
		TC1/2/3 or IC1/2/3	347	-	32%	-	-
		TC0 and IC0	260	-	27%	-	-
FIR (pretreated cohorts) [28]	Atezolizumab	TC3 or IC3	38 (cohort 2)	20% [¶]	-	43% [¶]	-
			8 (cohort 3)	NE [¶]	-	19% [¶]	-
CheckMate 012 [32,34]	Nivolumab + CT	<1%	23	-	-	22%	-
		≥1%	23	-	-	22%	-
	Nivolumab + Ipilimumab	≥1%	47	38%	58%	-	-
		≥50%	13	54%	62%	-	-
KEYNOTE-001 (treatment-naïve cohort) [14,22,23]	Pembrolizumab	≥1%	79	-	-	22%	-
		1–49%	52	-	37%	28%	16%
		≥50%	27	-	67%	48%	30%
BIRCH (treatment-naïve cohort) [40]	Atezolizumab	TC2/3 or IC2/3	138	20%	50%	43% [¶]	-
		TC2 or IC2	73	13%	49%	39% [¶]	-
		TC3 or IC3	65	28%	52%	48% [¶]	-
FIR (treatment-naïve cohort) [28]	Atezolizumab	TC3 or IC3	7	14% [¶]	-	29% [¶]	-

[†]When not specified, PD-L1 expression refers to the percentage of tumor cells only.

[‡]Analysis conducted on pooled pembrolizumab doses cohorts after a median follow-up of 42.6 months.

[§]Data reported are per immune-related response criteria per investigator assessment.

[¶]30-month OS rate.

IC: Immune cell; ICB: Immune checkpoint blocker; mFU: Median follow-up; mOS: Median overall survival; mPFS: Median progression-free survival; N: Number of patients treated with ICI; NE: Not estimable; NR: Not reached; NSCLC: Non-small-cell lung cancer; ORR: Objective response rate; OS: Overall survival; PD-L1: Programmed cell death-ligand 1; TC: Tumor cell.

nation approach [34]. The response rate of 43% observed across PD-L1 expression levels increased to 57 and 92% in patients with PD-L1 ≥1% and ≥50%, respectively. Of note, OS rate at 1 year was 100% in ≥50% PD-L1 patients, compared with 87% in ≥1% PD-L1 patients. The 2-y OS rates were 62% in PD-L1 ≥50% patients compared with 58% in PD-L1 ≥1% patients, respectively.

On the contrary, in the CheckMate 012 cohort of patients who were treated with nivolumab plus platinum-based doublet chemotherapy, survival outcomes were similar across tumor PD-L1 expression levels (both 3-y OS rates of 22%). This data suggest that PD-L1 expression has no predictive value in patients treated with the combination of nivolumab and chemotherapy.

Pembrolizumab

In KEYNOTE 001 study, PD-L1 $\geq 50\%$ was associated with higher OS compared with PD-L1 1–49% and PD-L1 $< 1\%$, in both treatment-naïve and pretreated patients [14]. Looking at the pretreated cohort, PD-L1 $\geq 50\%$ was associated with an mOS of 15.4 months and a 5-y OS rate of 25%. Albeit PD-L1 $< 1\%$ and 1–49% groups shared similar mOS estimations (8.5 months), the 5-year OS was remarkably higher in the 1–49% subgroup (13 vs 3%). In the treatment-naïve group, PD-L1 $\geq 50\%$ patients experienced mOS of 35.4 months and 5-y OS rate of 30%, higher compared with PD-L1 1–49% patients (mOS of 19.5 months and 5-y OS rate of 15.7%), while survival analyses on PD-L1 $< 1\%$ patients were not performed due to the small cohort ($n = 12$).

At last, KEYNOTE-010 trial confirmed the higher survival outcomes of PD-L1 $\geq 50\%$ pretreated patients compared with the overall PD-L1 $\geq 1\%$ population (3-y OS of 35 vs 23%) [24]. KEYNOTE-189 was the only trial with updated results on pembrolizumab plus chemotherapy according to PD-L1 expression. Similarly to nivolumab–chemotherapy combinations, the survival benefit observed with the combination of pembrolizumab plus pemetrexed and platinum occurred regardless of PD-L1 expression [44].

Atezolizumab

In Phase II POPLAR and Phase III OAK trials, investigating atezolizumab activity in pretreated NSCLC patients, the survival benefit of atezolizumab was observed across all PD-L1 expression levels, including patients with TC0 and IC0 [26,27].

Moreover, in the updated analyses of OAK trial, the mutually exclusive analyses showed that the survival benefit in the TC2/3 or IC2/3 population was driven by TC3 or IC3 patients [27]. The TC2 or IC2 subgroup itself, therefore, did not experience survival benefit with atezolizumab. The greatest benefit was indeed observed in the TC3 or IC3 subgroup, where mOS was 20.5 months with 2-y OS of 43%, compared with 14.3 months with 2-y OS of 32% in the TC1/2/3 or IC1/2/3 subgroups. TC0 and IC0 subgroup had an mOS of 11.8 months with 2-y OS of 27%.

In the updated analysis of BIRCH trial, encouraging long-term OS rate at 30 months was observed regardless of PD-L1 status, with a range of 39–48% for 30-month OS rate in three PD-L1 subgroups (TC 0 or IC0, TC2 or IC2 and TC3 or IC3) [40].

Durvalumab, tremelimumab

Putting the threshold of PD-L1 ‘strong’ positivity at $\geq 25\%$ and gathering both pretreated and first-line patients, 2-y OS rates in Phase I/II durvalumab trial resulted 36% (PD-L1 $\geq 25\%$) and 22% (PD-L1 $< 25\%$) [29].

OS results of the MYSTIC trial across PD-L1 expression levels were reported at ASCO 2019 by Rizvi *et al.* [50], confirming the OS improvement for durvalumab alone compared with chemotherapy in patients with PD-L1 $> 25\%$. Moreover, no clear linear trend of OS improvement with increasing PD-L1 expression levels was observed with durvalumab plus tremelimumab versus chemotherapy.

Tumor mutational burden

Most recently, several clinical trials and retrospective analyses on NSCLC have highlighted tumor mutational burden (TMB) as a novel predictive and/or prognostic biomarker. TMB is the total number of nonsynonymous mutations per megabase (mut/Mb) in TC generally performed on tumor tissue or circulating tumor DNA through next-generation sequencing [51,52]. TMB has been associated, independently of the PD-L1 expression, with improved clinical benefit and survival outcomes of ICBs [51]. Albeit the role of TMB has been mostly envisaged in trials involving nivolumab (alone or in combination with ipilimumab) [9,53], its potential in predicting long-term outcomes has been recently presented for the association of durvalumab and tremelimumab [41].

In Phase III MYSTIC trial exploratory analyses on the predictive and prognostic role of tissue and blood TMB (tTMB and bTMB) were performed in the all population with survival updates at 2 years [41,50]. Long-term survival rates in patients receiving durvalumab plus tremelimumab increased with the threshold used for the definition of TMB-high cases, narrowing in parallel the proportion of patients definable ‘TMB high’. Patients with tTMB

≥ 10 mut/Mb experienced longer 2-y OS with the combination of durvalumab plus tremelimumab compared with durvalumab alone and to chemotherapy arms (42 vs 35 vs 23%). Conversely, patients with tTMB < 10 mut/Mb had similar 2-y OS across treatment groups, with slightly higher results obtained in the chemotherapy arm (19 vs 22 vs 25%) [41].

In the subgroup of patients with matched blood and tissue samples, bTMB showed good correlation with tTMB and subsequent analyses were conducted at different thresholds (cut-off of ≥ 16 mut/Mb and ≥ 20 mut/Mb), the OS estimations incrementally improving with increasing bTMB cut-offs for durvalumab plus tremelimumab. In the subgroup of patients with bTMB ≥ 20 mut/Mb, 2-y OS rates were 48, 34 and 19% in durvalumab plus tremelimumab, durvalumab and chemotherapy arms, respectively. On the other hand, patients with bTMB < 20 mut/Mb had similar survival outcomes, regardless of treatment regimen (2-y OS of 20 to 27%).

Of note, the first survival results with long-term FU based on the combined analysis of PD-L1 and bTMB were reported [50]. On one hand, durvalumab plus tremelimumab improved OS compared with chemotherapy across PD-L1 TC levels in patients with bTMB ≥ 16 or 20 mut/Mb, but not in lower thresholds (2y-OS ranging from 40 to 56% in bTMB ≥ 20 mut/Mb vs 20–23% in bTMB < 20 mut/Mb).

T-effector gene signature

In the updated analysis of the OAK study, von Pawel *et al.* assessed the expression of the T-effector gene signature (CD274, IFNG and CXCL9) – which is a marker of pre-existing immunity – in long-term survivors, defined as having an OS ≥ 2 years [54]. These patients were shown to harbor higher T effector gene signature expression compared with non-long-term survivors enrolled in the study. In long-term survivors, median T-effector gene signature expression was 1.7-fold higher than non-long-term survivors in the atezolizumab arm (1.61 vs 2.40). This finding was consistent with previous data reported on atezolizumab monotherapy [55] and the more recent data on the combination of atezolizumab plus chemotherapy [8].

Taken together, these results suggest that markers of pre-existing immunity, similarly to TMB, could be a novel biomarker of clinical benefit of ICBs.

Discussion

Due to the positive results of a series of recent clinical trials incorporating ICBs administration in the first-line NSCLC treatment, several therapeutic options are going to be available in this setting in the future [56]. The FU of these studies is still too short to figure out the long-term effectiveness of novel strategies, mainly represented by combinatorial ones. Nevertheless, the availability of predictors of long-lasting efficacy of anti-PD-1/PD-L1 agents, when administered as monotherapies in both untreated and pretreated populations, may add relevant information to better address NSCLC patients to ICBs.

The survival plateau observed in the updates of ICB trials globally proved that approximately 15 and 25% of previously treated and untreated patients, respectively, are alive at 5 years from treatment beginning (Tables 1 & 2). This proportion can meaningfully be enriched by selecting for PD-L1 expression (Table 3).

The recent potential recognition of cure in the metastatic setting of NSCLC patients is appealing, especially considering that the 5-y OS rates were stuck at 5% in the pre-immunotherapy era. In this sense, for each treatment strategy the *a priori* identification of patients who are meant to be alive at long-term time-points is crucial for two main reasons: in order to reduce exposure to ineffective strategies (i.e., increase efficacy) and to avoid overtreatment (i.e., decrease toxicity). With the exception of the recent evidence about TMB predictive role in the case of PD-1/PD-L1 and CTLA-4 concomitant inhibition, the most relevant biomarker is represented by PD-L1 expression. More recent evidence vouches moreover for the integration of diverse (and not correlated) biomarkers such as PD-L1 and TMB in addressing the long-lasting success of immunotherapy.

Due to the complex interface between the tumor and the immune system, the only PD-L1 evaluation could be diminishing, even if envisaging its potential in predicting long-term outcomes. More probably, the tumor and the host elements that concur to determine ICBs' activity participate in multifaceted interplays in determining ICBs' prolonged effects [57]. The dynamic nature of these forces may, in addition, be responsible of the differential duration of ICBs' benefit, still in the setting of long-lasting survival in NSCLC. Indeed, among the pretreated patients who drive prolonged OS benefit (≥ 2 years [12]) further successful fate is obtained by a quote only. From the second to the third year since beginning treatment, a modest drop in survival rates can still be recognized, whereas from the third to the fifth year a true plateau, still with some death events, can be recognized (Table 1).

Conclusion

The available updates of ICB studies are witness for their unprecedented long-term positive results in a critical disease setting such as advanced NSCLC. Five-year survival data are now robust enough to allow these prolonged positive outcomes to be envisaged, even more with the upcoming incorporation of immunotherapy in combinatorial, synergistic treatment strategies. In this sense, the appropriate patient selection and biomarker identification will be crucial, together with the integration of multimodal approaches to precise disease presentations, in order to potentially achieve cure in an increasing number of patients.

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