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ORIGINAL ARTICLE

NEPTUNE: Phase 3 Study of First-Line Durvalumab Plus Tremelimumab in Patients With Metastatic NSCLC

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ABSTRACT

Introduction: NEPTUNE, a phase 3, open-label study, evaluated first-line durvalumab plus tremelimumab versus chemotherapy in metastatic NSCLC (mNSCLC).

Methods: Eligible patients with *EGFR* and *ALK* wild-type mNSCLC were randomized (1:1) to first-line durvalumab (20 mg/kg every 4 weeks until progression) plus tremelimumab (1 mg/kg every 4 weeks for up to four doses) or standard chemotherapy. Randomization was stratified by tumor programmed death-ligand 1 expression (≥25% versus <25%), tumor histologic type, and smoking history. The amended primary end point was overall survival (OS) in patients with blood tumor mutational burden (bTMB) greater than or equal to 20 mutations per megabase (mut/MB). Secondary end points included progression-free survival (PFS) in patients with bTMB greater than or equal to 20 mut/MB and safety and tolerability in all treated patients.

Results: As of June 24, 2019, 823 patients were randomized (intention-to-treat [ITT]); 512 (62%) were bTMB-evaluable, with 129 of 512 (25%) having bTMB greater than or equal to 20 mut/MB (durvalumab plus tremelimumab [n = 69]; chemotherapy [n = 60]). Baseline characteristics were balanced in the intention-to-treat. Among patients with bTMB greater than or equal to 20 mut/MB, OS improvement with durvalumab plus tremelimumab versus chemotherapy did not reach statistical significance (hazard ratio 0.71 [95% confidence interval: 0.49–1.05; *p* = 0.081]; median OS, 11.7 versus 9.1 months); the hazard ratio for PFS was 0.77 (95% confidence interval, 0.51–1.15; median PFS, 4.2 versus 5.1 months). In the overall safety population, incidence of grade 3 or 4 treatment-related adverse events was 20.7% (durvalumab plus tremelimumab) and 33.6% (chemotherapy).

Conclusions: NEPTUNE did not meet its primary end point of improved OS with durvalumab plus tremelimumab versus chemotherapy in patients with mNSCLC and bTMB greater than or equal to 20 mut/MB. Despite the amended study design, with a resultant small primary analysis population, therapeutic activity was aligned with expectations based on mechanistic biology and previous studies.

Introduction

Immunotherapies targeting programmed cell death protein-1 (PD-1) and its ligand (programmed cell death-ligand 1 [PD-L1]), either as monotherapy or in combination with chemotherapy, have transformed the first-line treatment of metastatic NSCLC (mNSCLC).[1–4] Investigation of novel immunotherapy combinations and identification of additional biomarkers may broaden the range of treatment options and enhance precision of first-line treatment selection. Tumor cell (TC) PD-L1 expression has been used to guide treatment decisions, but it is not always predictive of response to immunotherapy, especially for combinations of anti–PD-(L)1 and anti–CTLA-4 agents.[5,6] More recently, tumor mutational burden (TMB), derived from the total somatic mutation count (a surrogate for tumor neoantigen load) and measured in the blood or tissue, has emerged as a potential predictive biomarker of response to immunotherapy that is independent of PD-L1.[5–9] Previous studies have found high tissue TMB (tTMB ≥10 mutations per megabase [mut/Mb]) to be predictive of progression-free survival (PFS) benefit with anti–PD-1 plus anti–CTLA-4 in patients with mNSCLC; however,

overall survival (OS) benefit was similar in patients with high and low tTMB.[6,8,10] Evaluation of blood TMB (bTMB) allows for rapid, less invasive testing,[11,12] with feasibility demonstrated in multiple clinical studies.[5,9,12–15]

Durvalumab, a selective, high-affinity human immunoglobulin G1 monoclonal antibody that blocks PD-L1 binding to PD-1 and CD80,[16] was found to have clinical activity in patients with mNSCLC, with or without the anti–CTLA-4 therapy, tremelimumab, in the advanced treatment-line setting.[17–19] In exploratory analyses from the phase 3 MYSTIC study in patients with mNSCLC, incremental improvement in OS and PFS at increasing bTMB thresholds was observed with first-line durvalumab plus tremelimumab versus chemotherapy.[5] The bTMB cutoff of greater than or equal to 20 mut/Mb provided optimal OS benefit (alongside improvements in PFS and response rate), with a good balance between survival benefit and prevalence in this population.[5]

Here, we report the final results of the phase 3 NEPTUNE study (NCT02542293) evaluating the efficacy and safety of first-line durvalumab plus tremelimumab versus platinum-based chemotherapy in patients with mNSCLC. On the basis of the above-mentioned findings in MYSTIC, the primary end point of NEPTUNE was amended to evaluate OS in patients with bTMB greater than or equal to 20 mut/Mb instead of the intention-to-treat (ITT) population. We also report the results of prespecified secondary and exploratory analyses to evaluate the effects of PD-L1 expression, including bTMB and tTMB, on outcomes.

Materials and Methods
Patients
Adults with stage IV NSCLC were eligible if they had no previous systemic therapy for advanced or metastatic NSCLC; Eastern Cooperative Oncology Group performance status of 0 to 1; measurable disease by investigator assessment (central review not required) per Response Evaluation Criteria in Solid Tumors version 1.1; and confirmed tumor PD-L1 expression status, assessed using the VENTANA PD-L1 (SP263) immunohistochemistry assay, before randomization. Patients with sensitizing *EGFR* mutations or *ALK* rearrangements and those with unstable brain metastases or spinal cord compression were excluded (baseline brain computed tomography or magnetic resonance imaging was not mandated [consistent with current clinical practice guidelines][20]). Full eligibility criteria are provided in Supplementary Table 1. All patients provided written informed consent for participation.

Study Design and Treatment
NEPTUNE, a global, phase 3, open-label study, was conducted at 182 sites in 29 countries. Patients were randomized (1:1) between November 19, 2015, and May 27, 2017, to durvalumab 20 mg/kg every 4 weeks until disease progression plus tremelimumab 1 mg/kg every 4 weeks for up to four cycles, or investigator's choice of platinum-based chemotherapy every 3 weeks for 4 to 6 cycles (Supplementary Fig. 1). Randomization was stratified by PD-L1 TC expression (≥25% versus <25%), tumor histologic type (squamous versus nonsquamous), and smoking history (never versus ever smoker). Chemotherapy options comprised carboplatin plus paclitaxel for patients with either tumor histologic type, and cisplatin or carboplatin plus gemcitabine or pemetrexed, respectively, for patients with squamous and nonsquamous histologic type. Patients with nonsquamous histologic type in the chemotherapy arm who had not progressed after four cycles of platinum plus pemetrexed were eligible to receive pemetrexed maintenance therapy. Patients continued treatment until disease progression per investigator assessment, unacceptable toxicity, or withdrawal of consent.

The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The protocol and all modifications were approved by the institutional review boards or ethics committees of all participating centers and the relevant regulatory authorities.

End Points and Assessments
The primary end point per the original NEPTUNE protocol was OS (time from randomization to death from any cause), assessed in the ITT population (later amended to include both the ITT and PD-L1 TC ≥25% populations; amendment date: February 3, 2017). More recently, exploratory analyses from MYSTIC suggested that bTMB may represent a predictive biomarker for durvalumab plus tremelimumab.[5] In response, the primary end point in NEPTUNE was amended to evaluate OS with durvalumab plus tremelimumab versus chemotherapy in patients with bTMB greater than or equal to 20 mut/Mb (the cutoff at which optimal OS benefit was observed in MYSTIC; amendment date: April 3, 2019).

Key secondary end points were OS in patients with bTMB greater than or equal to 16 mut/Mb, bTMB greater than or equal to 12 mut/Mb, and PD-L1 TC less than 1%. Furthermore, OS was assessed in the ITT and bTMB less than 20 mut/Mb populations as secondary end points and in the bTMB-evaluable population. The secondary end points of PFS (time from randomization to investigator-assessed objective disease progression or death), investigator-assessed objective response rate (ORR), and duration of response (DoR) were assessed in patients with bTMB greater than or equal to 20 mut/

Mb and in the ITT, bTMB less than 20 mut/Mb, and bTMB-evaluable populations. Exploratory analyses included assessment of OS and PFS at different bTMB cutoffs (8–28 mut/Mb) and tTMB cutoffs (4–18 mut/Mb).

Blood and tissue TMB were evaluated as described previously[5] (additional details in the Supplementary Materials). Briefly, bTMB was evaluated using the GuardantOMNI plasma next-generation sequencing platform (Guardant Health, Redwood City, CA) and tTMB using the FoundationOne CDx platform (Foundation Medicine, Cambridge, MA).

Statistical Analysis

On the basis that the original primary OS end point would be assessed in the ITT and PD-L1 TC greater than or equal to 25% populations, enrollment of approximately 1330 patients was planned in order to randomize 800 eligible patients, including approximately 336 and 520 patients with PD-L1 TC greater than or equal to 25% and TC greater than or equal to 1%, respectively. in light of emerging data from MYSTIC, however,[5] the primary analysis population was changed to patients with bTMB greater than or equal to 20 mut/Mb. According to the amended statistical analysis plan, the primary analysis was to be performed when approximately 87% maturity in the bTMB greater than or equal to 20 mut/Mb population was achieved. As the protocol amendment occurred after the completion of enrollment, the sample size for this population could not be changed. Assuming a true OS average hazard ratio (HR) of 0.49 and median OS in the chemotherapy arm of 10 months after an exponential distribution for both durvalumab plus tremelimumab and chemotherapy arms, with approximately 140 patients, it was calculated that approximately 122 OS events (87% maturity) would provide greater than 90% power to reveal statistical significance at the two-sided overall alpha level of 5%. Two planned interim analyses were removed in separate protocol amendments (September 2017 and February 2018) to ensure sufficient follow-up for both efficacy and safety before conducting the primary analysis. To control the type I error at 5% (two-sided), a hierarchical multiple testing procedure with gatekeeping strategy was used across the primary end point and key secondary OS end points (Supplementary Materials and Supplementary Fig. 2).

The primary OS analysis was performed using an unstratified log-rank test, with HRs and 95% confidence intervals (CIs) estimated using a Cox proportional hazards model. The Kaplan-Meier method was used to generate survival curves. Additional details for the secondary analyses are summarized in the Supplementary Materials. Efficacy was analyzed on an ITT basis, including all randomized patients, or subsets of this population based on PD-L1 expression or TMB levels. All patients who received at least one dose of study treatment (safety population) were included in the safety analyses; the data were summarized descriptively.

Results

Patients and Treatment

Of 1350 patients enrolled, 823 were randomized to durvalumab plus tremelimumab (n = 410) or chemotherapy (n = 413) (Fig. 1). Reasons for exclusion included screen failure (n = 482), death (n = 16), and patient decision (n = 27). The most common reasons for screen failure were an *EGFR* mutation or *ALK* rearrangement and unknown tumor PD-L1 status (Supplementary Table 2); known tumor PD-L1 status was required before randomization (see Methods and Supplementary Table 1). There were 640 patients (77.8% of ITT) with plasma samples available, of whom 512 (62.2% of ITT) were bTMB-evaluable (80% assay success rate) (Fig. 1). The prevalence of bTMB and tTMB scores at or above various prespecified and exploratory cutoffs is summarized in Supplementary Table 3. The primary analysis population (bTMB ≥20 mut/Mb) comprised 129 patients (durvalumab plus tremelimumab [n = 69]; chemotherapy [n = 60]). Most randomized patients received study treatment (durvalumab plus tremelimumab, 410 [100%]; chemotherapy, 399 [96.6%]) (Fig. 1). The median (range) total duration of treatment was 4.6 (0.1–41.1) and 4.3 (0.3–40.9) months for the durvalumab plus tremelimumab and chemotherapy arms, respectively.

At data cutoff (June 24, 2019), most patients had discontinued study treatment (ITT: 379 [92.4%] of 410 and 396 [99.2%] of 399 treated patients in the durvalumab plus tremelimumab and chemotherapy arms, respectively; bTMB ≥20 mut/Mb: 63 [91.3%] of 69 and 59 [100%] of 59 treated patients, respectively), mainly due to disease progression (Fig. 1). The number of patients remaining on study treatment in the durvalumab plus tremelimumab and chemotherapy arms was 31 (7.6%) and three (0.8%), respectively, in the ITT population and six (8.7%) and zero, respectively, in the bTMB greater than or equal to 20 mut/Mb population.

In the ITT population, 173 (42.2%) patients in the durvalumab plus tremelimumab arm and 185 (44.8%) patients in the chemotherapy arm received subsequent anticancer therapies (30 [43.5%] and 30 [50.0%] patients, respectively, in the bTMB ≥20 mut/Mb population) (Supplementary Table 4). The number of patients who received subsequent immunotherapy in the durvalumab plus tremelimumab and chemotherapy arms,
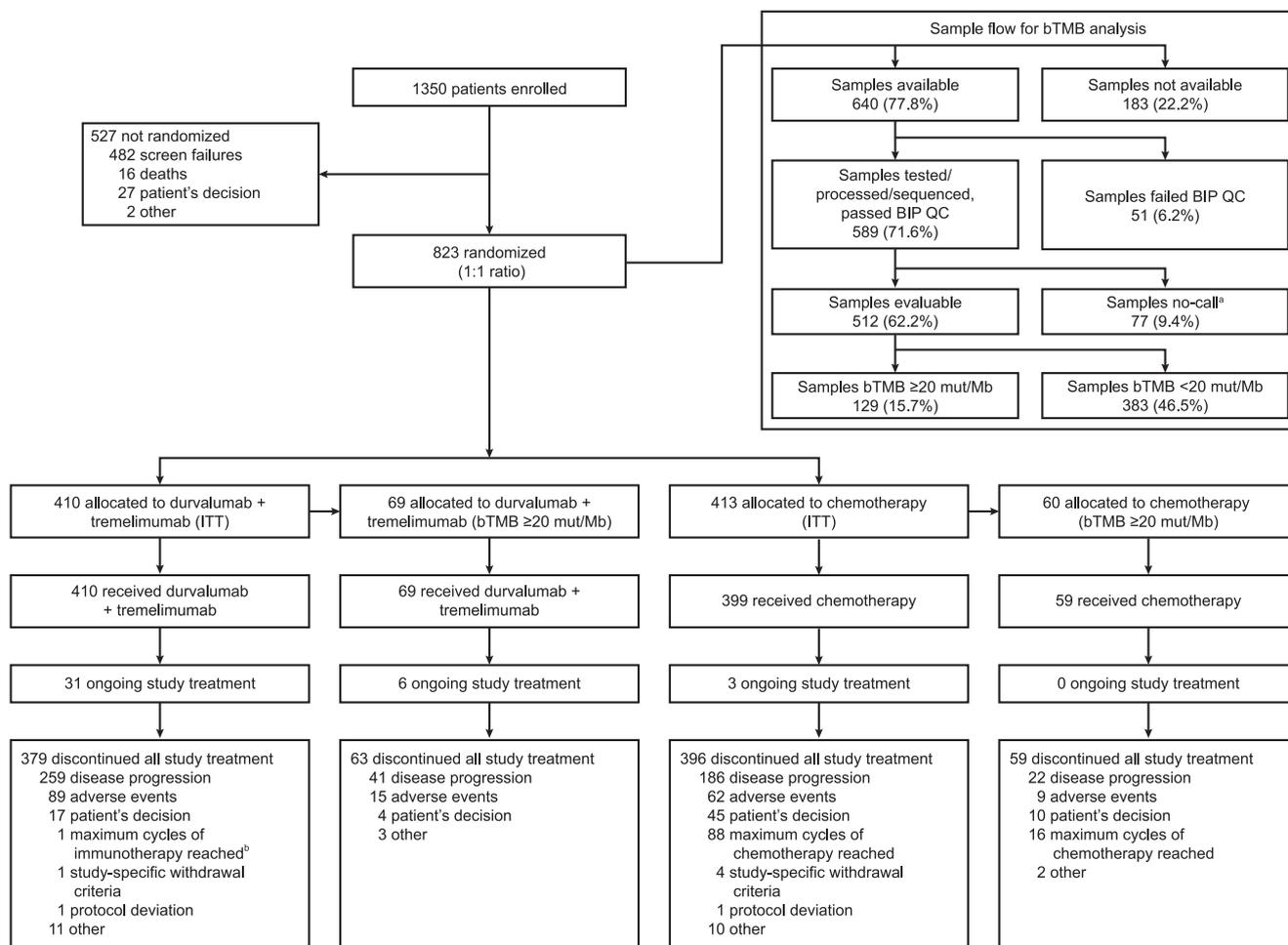
Figure 1. CONSORT diagram. Data cutoff was June 24, 2019. [a]Samples were assigned "no-call" if they had low diversity (due to low circulating cell-free DNA input) or low tumor shedding leading to the inability to assign a TMB high or TMB low call. [b]Only applicable for patients completing study treatment before implementation of clinical study protocol amendment, which allowed patients to continue receiving immunotherapy until disease progression when previously a maximum of 12 months was allowed. BIP, bioinformatics pipeline (automated software process that calculates and reports bTMB status); bTMB, blood tumor mutational burden; ITT, intention-to-treat; Mb, megabase; mut, mutations; QC, quality control.

respectively, was 18 (4.4%) and 88 (21.3%) in the ITT population (seven [10.1%] and 15 [25.0%] in the bTMB greater than or equal to 20 mut/Mb population). In the durvalumab plus tremelimumab arm, 30 patients (7.3%) in the ITT population and seven (10.1%) in the bTMB greater than or equal to 20 mut/Mb population received retreatment with durvalumab plus tremelimumab.

Baseline demographics and disease characteristics in the ITT population were generally balanced between the treatment arms (Table 1). Within the bTMB greater than or equal to 20 mut/Mb population, however (which was not a preplanned subgroup at the time of randomization), there were imbalances between the durvalumab plus tremelimumab and chemotherapy arms, particularly for Asian race, Eastern Cooperative Oncology Group performance status, tumor histologic type PD-L1 expression status, never smokers, and number of target lesions.

Overall Survival

At data cutoff, the median (range) follow-up for OS in censored patients was 32.9 (0–42.5) months (ITT) and 35.0 (4.2–42.5) months (bTMB ≥20 mut/Mb population).

In the primary analysis population (bTMB ≥20 mut/Mb), treatment with durvalumab plus tremelimumab did not statistically significantly improve OS versus chemotherapy; however, there was a numerically reduced risk of death with an HR of 0.71 (95% CI: 0.49–1.05; $p = 0.081$). The median OS was 11.7 (95% CI: 8.6–15.2) and 9.1 (95% CI: 7.8–12.6) months in the durvalumab plus tremelimumab and chemotherapy arms, respectively, with 24-month OS rates of 26.1% and 13.6% (Fig. 2A). A sensitivity analysis using a multivariate Cox proportional hazards model adjusting for multiple baseline covariates (including age at randomization, smoking status, tumor histologic type race, and number of target lesions at baseline) resulted in an HR for OS of 0.58 (95% CI: 0.38–

Table 1. Baseline Patient Demographics and Disease Characteristics in the ITT Population and in Patients With bTMB Greater Than or Equal to 20 mut/Mb

Characteristic	ITT			bTMB ≥20 mut/Mb		
	Durvalumab + Tremelimumab (n = 410)	Chemotherapy (n = 413)	Total (N = 823)	Durvalumab + Tremelimumab (n = 69)	Chemotherapy (n = 60)	Total (N = 129)
Median age (range), y	63.0 (27-83)	65.0 (30-90)	64.0 (27-90)	64.0 (35-79)	65.5 (49-82)	64.0 (35-82)
Sex, n (%)						
Male	297 (72.4)	305 (73.8)	602 (73.1)	56 (81.2)	49 (81.7)	105 (81.4)
Female	113 (27.6)	108 (26.2)	221 (26.9)	13 (18.8)	11 (18.3)	24 (18.6)
Race, n (%)						
White	307 (74.9)	289 (70.0)	596 (72.4)	56 (81.2)	53 (88.3)	109 (84.5)
Asian	86 (21.0)	99 (24.0)	185 (22.5)	11 (15.9)	5 (8.3)	16 (12.4)
Black or African American	3 (0.7)	7 (1.7)	10 (1.2)	1 (1.4)	0	1 (0.8)
American Indian or Alaska Native	10 (2.4)	12 (2.9)	22 (2.7)	0	0	0
Other or missing	4 (1.0)	6 (1.5)	10 (1.2)	1 (1.4)	2 (3.3)	3 (2.3)
ECOG performance status, n (%)						
0	159 (38.8)	155 (37.5)	314 (38.2)	26 (37.7)	17 (28.3)	43 (33.3)
1	251 (61.2)	256 (62.0)	507 (61.6)	43 (62.3)	43 (71.7)	86 (66.7)
Missing	0	2 (0.5)	2 (0.2)	0	0	0
Tumor histologic type n (%)						
Squamous	166 (40.5)	170 (41.2)	336 (40.8)	31 (44.9)	32 (53.3)	63 (48.8)
Nonsquamous	244 (59.5)	243 (58.8)	487 (59.2)	38 (55.1)	28 (46.7)	66 (51.2)
Disease stage classification, n (%)						
Metastatic	96 (23.4)	100 (24.2)	196 (23.8)	9 (13.0)	9 (15.0)	18 (14.0)
Locally advanced	0	1 (0.2)[a]	1 (0.1)[a]	0	0	0
Locally advanced and metastatic	314 (76.6)	312 (75.5)	626 (76.1)	60 (87.0)	51 (85.0)	111 (86.0)
Smoking history, n (%)						
Never smoker	72 (17.6)	74 (17.9)	146 (17.7)	3 (4.3)	0	3 (2.3)
Former smoker	200 (48.8)	222 (53.8)	422 (51.3)	36 (52.2)	31 (51.7)	67 (51.9)
Current smoker	138 (33.7)	117 (28.3)	255 (31.0)	30 (43.5)	29 (48.3)	59 (45.7)
Brain metastases, n (%)	41 (10.0)	37 (9.0)	78 (9.5)	7 (10.1)	7 (11.7)	14 (10.9)
Liver metastases, n (%)	82 (20.0)	77 (18.6)	159 (19.3)	17 (24.6)	15 (25.0)	32 (24.8)
Number of target lesions, n (%)						
1	96 (23.4)	107 (25.9)	203 (24.7)	10 (14.5)	11 (18.3)	21 (16.3)
2	115 (28.0)	121 (29.3)	236 (28.7)	16 (23.2)	15 (25.0)	31 (24.0)
≥3	199 (48.5)	184 (44.6)	383 (46.5)	43 (62.3)	34 (56.7)	77 (59.7)
Missing	0	1 (0.2)	1 (0.1)	0	0	0
Maximum number of previous chemotherapy regimens,[b] n (%)						
0	391 (95.4)	385 (93.2)	775 (94.2)	69 (100.0)	58 (96.7)	127 (98.4)
1	15 (3.7)	23 (5.6)	39 (4.7)	0	2 (3.3)	2 (1.6)
≥2	4 (1.0)	5 (1.2)	9 (1.1)	0	0	0
PD-L1 expression status, n (%)						
TC <25%	245 (59.8)	249 (60.3)	494 (60.0)	35 (50.7)	34 (56.7)	69 (53.5)
TC ≥25%	165 (40.2)	164 (39.7)	329 (40.0)	34 (49.3)	26 (43.3)	60 (46.5)
TC <1%	91 (22.2)	104 (25.2)	195 (23.7)	12 (17.4)	13 (21.7)	25 (19.4)
TC ≥1%	319 (77.8)	309 (74.8)	628 (76.3)	57 (82.6)	47 (78.3)	104 (80.6)

[a]One patient was incorrectly randomized with locally advanced (non-metastatic) disease stage; a protocol deviation was reported.
[b]Patients were not permitted to have received prior chemotherapy or other systemic therapy for advanced or metastatic NSCLC; however, prior platinum-containing adjuvant, neoadjuvant, or definitive chemoradiation was permitted, provided that progression had occurred more than 6 months from the last therapy.
bTMB, blood tumor mutational burden; ECOG, Eastern Cooperative Oncology Group; ITT, intention-to-treat; PD-L1, programmed cell death-ligand 1; TC, tumor cell.

0.88) for durvalumab plus tremelimumab versus chemotherapy (Supplementary Table 5). A further post hoc sensitivity analysis adjusting only for the number of target lesions at baseline produced an HR for OS of 0.65 (95% CI: 0.44–0.96). There was no OS improvement with durvalumab plus tremelimumab versus chemotherapy in the
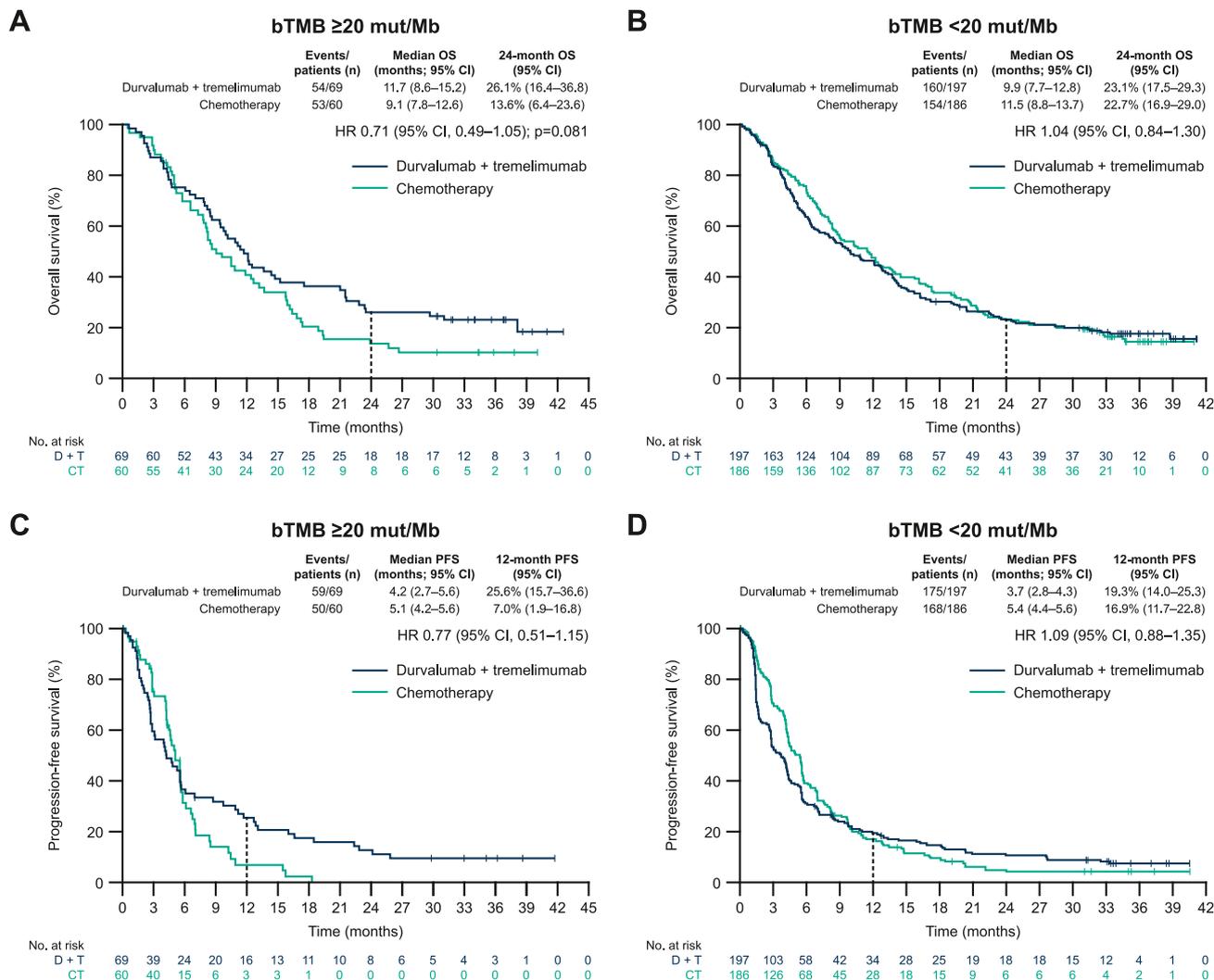
Figure 2. Overall survival and progression-free survival in patients with bTMB greater than or equal to 20 and less than 20 mut/Mb. Overall survival in patients with (*A*) bTMB greater than or equal to 20 mut/Mb (primary end point) and (*B*) bTMB less than 20 mut/Mb (secondary end point). Progression-free survival in patients with (*C*) bTMB greater than or equal to 20 mut/Mb and (*D*) bTMB less than 20 mut/Mb (secondary end points). HRs and 95% CIs were calculated using an unstratified Cox proportional hazards model, with ties handled by the Efron approach. bTMB, blood tumor mutational burden; CI, confidence interval; CT, chemotherapy; D, durvalumab; HR, hazard ratio; Mb, megabase; mut, mutations; OS, overall survival; PFS, progression-free survival; T, tremelimumab.

bTMB less than 20 mut/Mb population (HR 1.04 [95% CI: 0.84–1.30]; median OS, 9.9 [95% CI: 7.7–12.8] versus 11.5 [95% CI: 8.8–13.7] months; 24-month OS rate, 23.1% versus 22.7%) (Fig. 2*B*).

The HRs for OS with durvalumab plus tremelimumab versus chemotherapy were 0.94 (95% CI: 0.73–1.21) and 0.87 (95% CI: 0.63–1.20) in the bTMB greater than or equal to 12 and 16 mut/Mb populations, respectively, indicating (together with the HR for the bTMB ≥20 mut/Mb population; see previous text) a trend of improved HRs for OS with higher bTMB cutoffs. No improvement in OS was observed for durvalumab plus tremelimumab versus chemotherapy in patients with PD-L1 TC less than 1% (HR 1.07 [95% CI: 0.79–1.46]) (Supplementary Fig. 3). No OS improvements were observed for

durvalumab plus tremelimumab versus chemotherapy in the ITT population; results in the bTMB-evaluable population were similar to those observed in the ITT population (HR [95% CI] 0.95 [0.78–1.15] and 1.02 [0.87–1.19], respectively) (Supplementary Fig. 4*A* and *B*).

In the bTMB greater than or equal to 20 mut/Mb and ITT populations, the HRs for OS with durvalumab plus tremelimumab versus chemotherapy across prespecified patient subgroups defined by baseline demographics, disease characteristics, and PD-L1 TC expression were generally consistent with the respective overall populations (Supplementary Fig. 5*A* and *B*). The sample sizes were small for some of the subgroups in the bTMB greater than or equal to 20 mut/Mb population (e.g., female sex, PD-L1 TC <1%), as reflected by the wide 95% CIs.

Progression-Free Survival

In the bTMB greater than or equal to 20 mut/Mb population, the HR for PFS with durvalumab plus tremelimumab versus chemotherapy was 0.77 (95% CI: 0.51–1.15); median PFS was 4.2 (95% CI: 2.7–5.6) versus 5.1 (95% CI: 4.2–5.6) months and 12-month PFS rate was 25.6% versus 7.0% (Fig. 2C). In a sensitivity analysis using a multivariate Cox proportional hazards model adjusting for baseline covariates (including age at randomization, smoking status, tumor histologic type, race, and number of target lesions at baseline), an HR for PFS of 0.69 (95% CI: 0.45–1.05) was observed with durvalumab plus tremelimumab versus chemotherapy (Supplementary Table 5). In the bTMB less than 20 mut/Mb population, there was no difference in PFS for durvalumab plus tremelimumab versus chemotherapy (HR 1.09 [95% CI: 0.88–1.35]; median PFS, 3.7 [95% CI: 2.8–4.3] versus 5.4 [95% CI: 4.4–5.6] months; 12-month PFS rate, 19.3% versus 16.9%) (Fig. 2D).

No differences in PFS for durvalumab plus tremelimumab versus chemotherapy were observed in the ITT population (HR 1.08 [95% CI: 0.92–1.25]) or bTMB-evaluable population (HR 1.01 [95% CI: 0.84–1.22]) (Supplementary Fig. 4C and D).

Tumor Response

The ORR in the bTMB greater than or equal to 20 mut/Mb population was lower with durvalumab plus tremelimumab versus chemotherapy (27.5% [95% CI: 17.46–39.62] versus 43.3% [95% CI: 30.59–56.76]; odds ratio 0.50 [95% CI: 0.24–1.03]). DoR was longer for patients treated with durvalumab plus tremelimumab versus chemotherapy (median DoR [95% CI], 11.6 [6.7–21.5] versus 4.2 [3.0–6.9] months; patients remaining in response at 12 months, 44.4% versus 9.5%) (Fig. 3).

Exploratory Analysis of bTMB and tTMB

Among the ITT population, 512 (62.2%) and 369 (44.8%), respectively, patients had plasma and tissue samples that were evaluable for TMB. In the OS analysis based on bTMB cutoffs (8–28 mut/Mb), higher bTMB was associated with improved HRs for durvalumab plus tremelimumab versus chemotherapy (Fig. 4A and B); similar trends were observed for PFS (Fig. 4C and D). In the analysis of OS and PFS based on tTMB cutoffs (4–18 mut/Mb), higher tTMB was associated with improved treatment benefit with durvalumab plus tremelimumab versus chemotherapy, although the data sets were smaller at the higher cutoffs (Supplementary Fig. 6). Among 236 patients with matched samples (28.7% of ITT), bTMB and tTMB were moderately correlated (Spearman's rho = 0.64; Pearson's r = 0.80) (Supplementary Fig. 7).
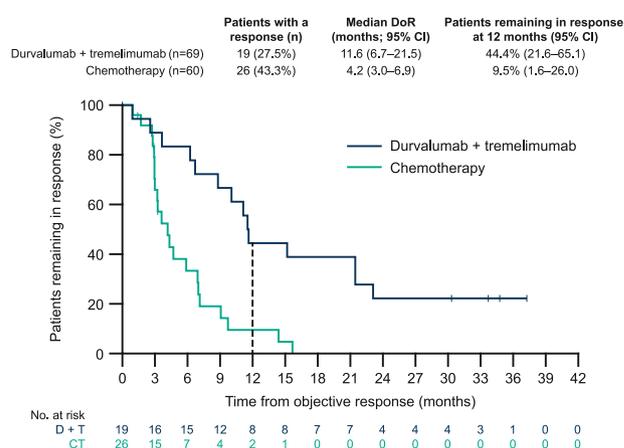
Figure 3. Duration of response in patients with bTMB greater than or equal to 20 mut/Mb. Measured from time of first documented response. Responses were investigator-assessed per RECIST version 1.1 and include unconfirmed responses. bTMB, blood tumor mutational burden; CI, confidence interval; CT, chemotherapy; D, durvalumab; DoR, duration of response; Mb, megabase; mut, mutations; RECIST, Response Evaluation Criteria in Solid Tumors; T, tremelimumab.

Correlation Between bTMB and PD-L1

In an exploratory analysis in 512 patients with bTMB and PD-L1 TC results, no correlation was observed between the two parameters (Spearman's rho = 0.018; Pearson's r = 0.040) (Supplementary Fig. 8).

Safety

A safety summary for patients in the overall safety population and patients with bTMB greater than or equal to 20 mut/Mb is provided in Supplementary Table 6. All-grade adverse events (AEs) considered by the investigator to be treatment-related AEs (TRAEs) occurred in 68.3% and 81.5% of patients treated with durvalumab plus tremelimumab and chemotherapy, respectively, in the overall safety population. Fewer patients had grade 3 or 4 TRAEs with durvalumab plus tremelimumab than with chemotherapy (20.7% versus 33.6%). Treatment-related deaths occurred in 2.4% and 1.5% of patients treated with durvalumab plus tremelimumab and chemotherapy, respectively. For durvalumab plus tremelimumab and chemotherapy, respectively, treatment-related serious AEs occurred in 19.8% and 15.5% of patients and TRAEs leading to treatment discontinuation occurred in 14.6% and 11.3% of patients. Immune-mediated AEs (imAEs) occurred in 32.9% of patients treated with durvalumab plus tremelimumab versus 2.3% treated with chemotherapy (grade 3 or 4 imAEs, 10.7% versus 0.3%; grade 5 imAEs, 1.0% versus 0%). Safety results in the bTMB greater than or equal to 20 mut/Mb population were generally consistent with the overall safety population.

The most frequent TRAEs and imAEs are summarized in Table 2. Notably, pneumonitis imAEs of any grade

A bTMB high: OS

bTMB (mut/Mb)	n (%)		OS HR (95% CI)
≥8	387 (76)		0.94 (0.75–1.17)
≥10	326 (64)		0.87 (0.69–1.11)
≥12	281 (55)		0.94 (0.73–1.21)
≥14	223 (44)		0.88 (0.66–1.18)
≥16	182 (36)		0.87 (0.63–1.20)
≥18	157 (31)		0.79 (0.56–1.12)
≥20	129 (25)		0.71 (0.49–1.05)
≥22	98 (19)		0.70 (0.45–1.09)
≥24	82 (16)		0.62 (0.38–1.02)
≥26	71 (14)		0.61 (0.35–1.03)
≥28	60 (12)		0.49 (0.27–0.89)

0.25   0.5   1.0   1.5   2.5

← Favors durvalumab + tremelimumab   Favors chemotherapy →

B bTMB low: OS

bTMB (mut/Mb)	n (%)		OS HR (95% CI)
<8	125 (24)		0.98 (0.66–1.45)
<10	186 (36)		1.10 (0.79–1.51)
<12	231 (45)		0.96 (0.72–1.28)
<14	289 (56)		1.00 (0.77–1.29)
<16	330 (64)		0.99 (0.78–1.26)
<18	355 (69)		1.02 (0.81–1.29)
<20	383 (75)		1.04 (0.84–1.30)
<22	414 (81)		1.02 (0.83–1.26)
<24	430 (84)		1.04 (0.84–1.27)
<26	441 (86)		1.03 (0.84–1.26)
<28	452 (88)		1.04 (0.85–1.27)

0.25   0.5   1.0   1.5   2.5

← Favors durvalumab + tremelimumab   Favors chemotherapy →

C bTMB high: PFS

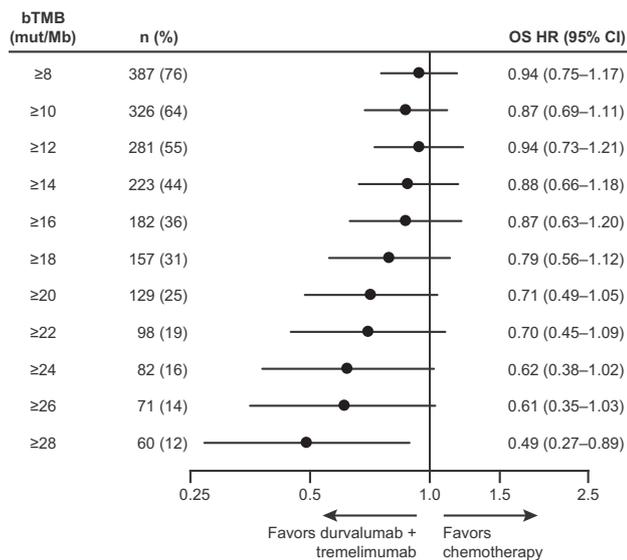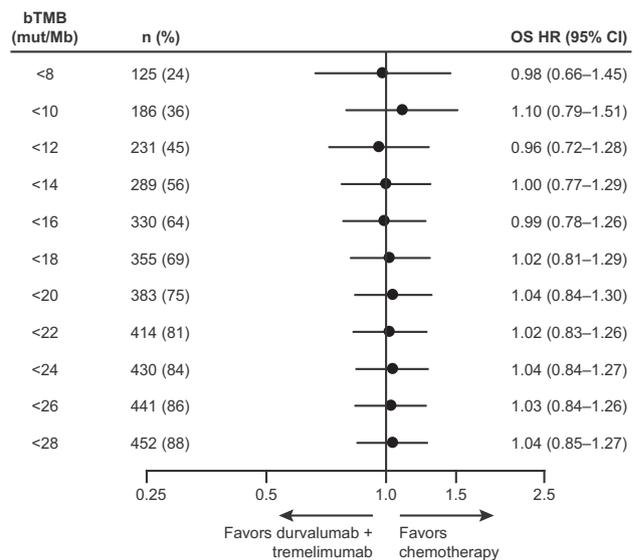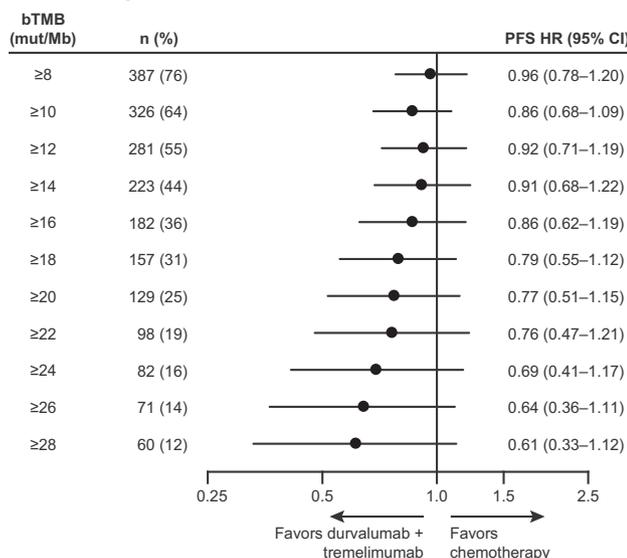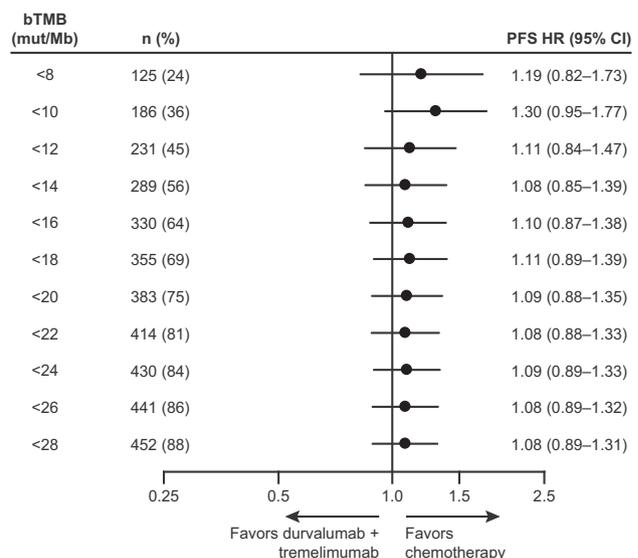| bTMB (mut/Mb)	n (%)		PFS HR (95% CI)
≥8	387 (76)		0.96 (0.78–1.20)
≥10	326 (64)		0.86 (0.68–1.09)
≥12	281 (55)		0.92 (0.71–1.19)
≥14	223 (44)		0.91 (0.68–1.22)
≥16	182 (36)		0.86 (0.62–1.19)
≥18	157 (31)		0.79 (0.55–1.12)
≥20	129 (25)		0.77 (0.51–1.15)
≥22	98 (19)		0.76 (0.47–1.21)
≥24	82 (16)		0.69 (0.41–1.17)
≥26	71 (14)		0.64 (0.36–1.11)
≥28	60 (12)		0.61 (0.33–1.12)

0.25   0.5   1.0   1.5   2.5

← Favors durvalumab + tremelimumab   Favors chemotherapy →

D bTMB low: PFS

bTMB (mut/Mb)	n (%)		PFS HR (95% CI)
<8	125 (24)		1.19 (0.82–1.73)
<10	186 (36)		1.30 (0.95–1.77)
<12	231 (45)		1.11 (0.84–1.47)
<14	289 (56)		1.08 (0.85–1.39)
<16	330 (64)		1.10 (0.87–1.38)
<18	355 (69)		1.11 (0.89–1.39)
<20	383 (75)		1.09 (0.88–1.35)
<22	414 (81)		1.08 (0.88–1.33)
<24	430 (84)		1.09 (0.89–1.33)
<26	441 (86)		1.08 (0.89–1.32)
<28	452 (88)		1.08 (0.89–1.31)

0.25   0.5   1.0   1.5   2.5

← Favors durvalumab + tremelimumab   Favors chemotherapy →

Figure 4. Overall survival and progression-free survival across bTMB cutoffs. HRs and 95% CIs were calculated using an unstratified Cox proportional hazards model, with treatment as the only covariate and with ties handled by the Efron approach. The percentage of patients within each bTMB cutoff group was calculated based on the total bTMB-evaluable population. bTMB, blood tumor mutational burden; CI, confidence interval; HR, hazard ratio; Mb, megabase; mut, mutations; OS, overall survival; PFS, progression-free survival.

were reported in 5.9% and 0.3% (grade 3 or 4, 1.7% and 0%) of patients treated with durvalumab plus tremelimumab versus chemotherapy, respectively, and colitis imAEs of any grade were reported in 2.2% and 0% (grade 3 or 4, 1.2% and 0%). Most frequent all-cause AEs and treatment-related serious AEs are summarized in Supplementary Tables 7 and 8.

Discussion

In NEPTUNE, first-line treatment with durvalumab plus tremelimumab did not statistically significantly improve OS versus chemotherapy in patients with mNSCLC and bTMB greater than or equal to 20 mut/Mb (HR 0.71 [95% CI: 0.49–1.05]; median OS, 11.7 versus 9.1 months). A numerically higher proportion of patients treated with durvalumab plus tremelimumab versus chemotherapy were estimated to be alive at 24 months (26.1% versus 13.6%), suggesting a longer-term treatment effect. PFS improvement for durvalumab plus tremelimumab versus chemotherapy was consistent with the OS result (HR 0.77 [95% CI: 0.51–1.15]).

NEPTUNE was initially designed to evaluate the primary OS end point in the ITT population. In response to the evolving understanding of immune checkpoint

Table 2. Treatment-Related AEs and Immune-Mediated AEs in the Safety Population

n (%)	Durvalumab + Tremelimumab (n = 410)		Chemotherapy (n = 399)	
	Any Grade[a]	Grade 3 or 4	Any Grade[a]	Grade 3 or 4
Treatment-related AEs[b]	280 (68.3)	85 (20.7)	325 (81.5)	134 (33.6)
Events occurring in ≥10% of patients in either arm[c]				
Nausea	34 (8.3)	1 (0.2)	115 (28.8)	1 (0.3)
Anemia	21 (5.1)	0	127 (31.8)	39 (9.8)
Fatigue	29 (7.1)	1 (0.2)	56 (14.0)	4 (1.0)
Diarrhea	51 (12.4)	3 (0.7)	29 (7.3)	3 (0.8)
Decreased appetite	27 (6.6)	4 (1.0)	52 (13.0)	2 (0.5)
Neutropenia	3 (0.7)	1 (0.2)	70 (17.5)	30 (7.5)
Rash	51 (12.4)	2 (0.5)	21 (5.3)	2 (0.5)
Vomiting	14 (3.4)	1 (0.2)	48 (12.0)	4 (1.0)
Pruritus	42 (10.2)	2 (0.5)	7 (1.8)	1 (0.3)
Alopecia	2 (0.5)	0	46 (11.5)	2 (0.5)
Thrombocytopenia	3 (0.7)	1 (0.2)	45 (11.3)	14 (3.5)
Hypothyroidism	46 (11.2)	1 (0.2)	0	0
	Any Grade[d]	Grade 3 or 4	Any Grade[d]	Grade 3 or 4
Immune-mediated AEs (grouped terms)[e]	135 (32.9)	44 (10.7)	9 (2.3)	1 (0.3)
Events occurring in ≥2 patients in either arm[c]				
Hypothyroidism	39 (9.5)	1 (0.2)	2 (0.5)	0
Pneumonitis	24 (5.9)	7 (1.7)	1 (0.3)	0
Rash	18 (4.4)	4 (1.0)	4 (1.0)	0
Hyperthyroidism	17 (4.1)	0	0	0
Diarrhea	12 (2.9)	3 (0.7)	0	0
Colitis	9 (2.2)	5 (1.2)	0	0
Dermatitis	7 (1.7)	1 (0.2)	1 (0.3)	1 (0.3)
Hepatic laboratory parameters reported as AEs	8 (2.0)	6 (1.5)	0	0
Hepatitis	8 (2.0)	7 (1.7)	0	0
Thyroiditis	8 (2.0)	0	0	0
Hypophysitis	4 (1.0)	2 (0.5)	0	0
Pancreatic laboratory investigations reported as AEs	4 (1.0)	4 (1.0)	0	0
Myositis	3 (0.7)	3 (0.7)	0	0
Thyroid laboratory parameters reported as AEs (decreased thyroid activity)	3 (0.7)	1 (0.2)	0	0
Type 1 diabetes mellitus	3 (0.7)	3 (0.7)	0	0

(continued)

Table 2. Continued				
	Any Grade[d]	Grade 3 or 4	Any Grade[d]	Grade 3 or 4
Pancreatitis	2 (0.5)	1 (0.2)	0	0
Other rare or miscellaneous	3 (0.7)	1 (0.2)	1 (0.3)	0

Note: Included are AEs that occurred during the treatment period and up to 90 days after the last dose of immunotherapy (30 days after the last dose of chemotherapy) or up to the start of any subsequent therapy (whichever occurred first).

[a]Treatment-related AEs leading to death in the durvalumab plus tremelimumab arm were as follows: pneumonitis (four patients); acute kidney injury, acute respiratory failure, cardiac arrest, chronic obstructive pulmonary disease, diarrhea, hepatitis, multiple organ dysfunction, and septic shock (one patient each). Of these, two patients had more than one AE that led to death (one patient had acute kidney injury and pneumonitis and one patient had multiple organ dysfunction and septic shock). Treatment-related AEs leading to death in the chemotherapy arm were as follows: neutropenic sepsis and septic shock (two patients each); cardiac arrest, cerebrovascular accident, febrile neutropenia, pancytopenia, and pneumonia (one patient each). Of these, three patients had more than one AE that led to death (one patient had febrile neutropenia and septic shock, one patient had neutropenic sepsis and cardiac arrest, and one patient had pancytopenia and septic shock).

[b]AEs assessed by the investigator as possibly related to any study treatment (AEs were counted as related if there was a missing causality assessment for any treatment).

[c]The events are listed in descending order of frequency across both treatment arms.

[d]Immune-mediated AEs leading to death in the durvalumab plus tremelimumab arm were as follows: pneumonitis (three patients) and hepatitis (one patient). There were no immune-mediated AEs leading to death in the chemotherapy arm.

[e]An AE consistent with an immune-mediated mechanism of action, where there is no clear alternate cause, and requiring the use of systemic steroids or other immunosuppressants or, for specific endocrine events, endocrine therapy.

AE, adverse event.

inhibitor therapy and exploratory analyses of TMB in MYSTIC,[5] the primary analysis population in NEPTUNE was changed from the ITT to patients with bTMB greater than or equal to 20 mut/Mb. The survival results in the bTMB greater than or equal to 20 mut/Mb population in NEPTUNE were generally consistent with those from MYSTIC; however, the magnitude of the OS benefit in MYSTIC for durvalumab plus tremelimumab versus chemotherapy (HR 0.49 [95% CI: 0.32–0.74])[5] was greater than that observed in NEPTUNE. Because the primary end point of NEPTUNE was amended after the completion of recruitment and randomization, the sample size of the primary analysis population was relatively small (n = 69 and n = 60 in the durvalumab plus tremelimumab and chemotherapy arms, respectively), and some imbalances were introduced in patient characteristics between the two treatment arms. Thus, the lack of statistical significance may be explained by the amendment to use a smaller subset of the ITT as the primary analysis population. Given the small size of the primary analysis population, there was increased risk of even numerically small imbalances having a meaningful impact on the observed OS benefit, making interpretation difficult. the effect of the between-arm imbalances was evident in the multivariate analyses, in which adjusting for baseline characteristics resulted in improved OS and PFS for durvalumab plus tremelimumab versus chemotherapy in the bTMB greater than or equal to 20 mut/Mb population; notably, a further post hoc analysis adjusting for number of target lesions alone resulted in an improved OS HR of 0.65 (95% CI: 0.44–0.96). It is nonetheless important to note that the performance of both the treatment and control arms in NEPTUNE was generally lower than that in some previous studies of immunotherapy in this setting, including MYSTIC, with shorter median OS outcomes.[1,5,6,9] This may have been due to the relatively high proportions of patients with squamous histologic type and with liver metastases at baseline in this study population.

The ORR was lower with durvalumab plus tremelimumab versus chemotherapy (27.5% [95% CI: 17.46–39.62] versus 43.3% [95% CI: 30.59–56.76]) in patients with bTMB greater than or equal to 20 mut/Mb in NEPTUNE, which differs from previous results with this combination.[5,19] In contrast, median DoR was longer with durvalumab plus tremelimumab versus chemotherapy (11.6 versus 4.2 months) and a greater proportion of patients had an ongoing response at 12 months (44.4% versus 9.5%). Similarly, although median PFS was shorter with durvalumab plus tremelimumab versus chemotherapy (4.2 versus 5.1 months), a greater proportion of patients were progression free at 12 months (25.6% versus 7.0%). The tails of both the OS and PFS Kaplan-Meier curves suggest that within the bTMB greater than or equal to 20 mut/Mb population there may be a subset who experience long-term clinical benefit with durvalumab plus tremelimumab. Early crossing of the OS and PFS Kaplan-Meier curves for durvalumab plus tremelimumab and chemotherapy was observed. This is similar to observations in multiple previous trials of anti–PD-(L)1 antibodies, with or without anti–CTLA-4 agents, suggesting an excess number of deaths occur with immunotherapy versus chemotherapy within the first 12 weeks after initiation of therapy.[1,5,8,21] The addition of chemotherapy to combination immunotherapy may help overcome the lower response rates and early drop in PFS.[22,23] Results from

the phase 3 POSEIDON study indicated a statistically significant and clinically meaningful PFS and OS benefit with first-line durvalumab plus chemotherapy and a limited course of tremelimumab versus chemotherapy alone in patients with mNSCLC.[23] No new safety signals were identified for this combination regimen, and the addition of tremelimumab to durvalumab plus chemotherapy did not lead to an increased rate of treatment discontinuation.

There is an unmet need to identify patients who require a combination of anti–PD-(L)1 and anti–CTLA-4 to achieve optimal therapeutic benefit. Although minimal benefit has been observed in patients with PD-L1 TC less than 1% treated with anti–PD-(L)1 as monotherapy and in combination with chemotherapy,[2,4,5] they seemed to derive greater OS benefit than the ITT population with anti–PD-(L)1 plus anti–CTLA-4 versus chemotherapy in the ARCTIC, MYSTIC, and CheckMate 227 studies.[5,6,19] Of note, in the CheckMate 9LA study, OS benefit was observed with nivolumab plus ipilimumab plus chemotherapy versus chemotherapy alone consistently across all PD-L1 levels, including patients with PD-L1 TC less than 1%.[22] In the present study, OS in the PD-L1 TC less than 1% population was similar to that in the ITT population, with no observed difference between the durvalumab plus tremelimumab arm and the chemotherapy arm. In the subgroup analysis within the primary analysis population however, the HR for OS favored durvalumab plus tremelimumab versus chemotherapy in patients with PD-L1 TC less than 1% and bTMB greater than or equal to 20 mut/Mb, although the small sample size limits interpretation. Further investigation of combination immunotherapy in this population may be warranted. Consistent with previous studies,[5,12] no correlation was observed between bTMB and PD-L1 TC expression levels, suggesting they are independent biomarkers.

In line with earlier studies, a moderate correlation was observed between bTMB and tTMB in NEPTUNE.[5,12] TMB is a quantitative biomarker, with inherent variability according to the specific assay used[24,25]; as such, a correlation between bTMB and tTMB scores does not necessarily imply qualitative concordance in the mutations identified by blood and tissue assays.[12] This may in part be driven by the limits of detection in assays used for bTMB quantification; specifically, variability in levels of tumor DNA shedding can alter the proportion of genomic alterations meeting the limit of detection for a given assay, in turn affecting the bTMB score.[11] Despite these potential limitations, in NEPTUNE OS improvements for durvalumab plus tremelimumab versus chemotherapy were generally greater at higher TMB cutoffs, whether measured in blood or tissue. This is in agreement with the hypothesis that higher TMB levels may represent higher tumor neoantigen load (and, therefore, stronger tumor antigenicity), which in turn has been associated with increased efficacy of immunotherapy, in particular with anti–PD-(L)1 plus anti–CTLA-4.[26,27] It is important, however, to acknowledge that only indirect measurement of tumor neoantigen load can be achieved through quantification of TMB. TMB is considered a surrogate biomarker for variables more directly related to the efficacy of immunotherapies, which is an important caveat that may partially account for inter- and intra-study differences in outcomes for patient populations defined by the same TMB cutoffs. The bTMB threshold of greater than or equal to 20 mut/Mb was found to be optimal for clinical benefit with durvalumab plus tremelimumab in both MYSTIC and NEPTUNE, although neither study was initially designed to evaluate TMB. The recently presented BFAST study prospectively selected patients with bTMB greater than or equal to 16 mut/Mb and randomized them to atezolizumab or chemotherapy in cohort C; however, no statistically significant difference in investigator-assessed PFS (HR 0.77 [95% CI: 0.59–1.00; $p = 0.053$]) or OS (HR 0.87 [95% CI: 0.64–1.17; $p = 0.35$]) was observed.[15] Additional investigation is required to refine the evaluation of bTMB, along with further harmonization of TMB testing methodologies.[24,25]

In the present study, durvalumab plus tremelimumab was found to have a well-tolerated and manageable safety profile that was consistent with previous findings.[5,17–19,28,29] Safety findings in the bTMB greater than or equal to 20 mut/Mb subset of the safety population were representative of the overall safety population.

Limitations of NEPTUNE included the relatively small sample size of the primary analysis population resulting from the amendment to the study design, which also led to imbalances in baseline characteristics between the treatment arms. Subsequent immunotherapy was received in a higher proportion of patients in the chemotherapy arm compared with the durvalumab plus tremelimumab arm in both the ITT (21.3% versus 4.4%) and bTMB greater than or equal to 20 mut/Mb (25.0% versus 10.1%) populations, which may be considered as a potential bias for the OS analysis.[30]

In conclusion, NEPTUNE did not meet its primary end point of a statistically significant improvement in OS with first-line durvalumab plus tremelimumab versus chemotherapy in patients with mNSCLC and bTMB greater than or equal to 20 mut/Mb. Therapeutic activity by bTMB level based on OS, PFS, and DoR was in line with the expectations based on mechanistic biology and previous results from MYSTIC[5]; however, it was not possible to confirm statistical significance owing to limitations resulting from the amended study design,

including the small size of the primary analysis population and imbalances between the treatment arms.

CRediT Authorship Contribution Statement

Gilberto de Castro, Jr.: Investigation, Resources, Writing—review and editing, Supervision.

Naiyer A. Rizvi: Conceptualization, Writing—review and editing.

Peter Schmid: Conceptualization, Investigation, Writing—review and editing, Supervision.

Konstantinos Syrigos: Investigation, Data curation, Writing—review and editing.

Claudio Martin: Investigation, Writing—review and editing.

Nobuyuki Yamamoto: Investigation, Writing—review and editing.

Ying Cheng: Investigation, Writing—review and editing.

Vladimir Moiseyenko: Investigation, Writing—review and editing.

Yvonne Summers: Investigation, Writing—review and editing.

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Maciej Bryl: Investigation, Writing—review and editing.

Alona Zer: Investigation, Resources, Data curation, Writing—review and editing.

Mustafa Erman: Investigation, Writing—review and editing.

Constanta Timcheva: Investigation, Writing—review and editing.

Rajiv Raja: Methodology, Investigation, Writing—review and editing, Funding acquisition.

Kirsha Naicker: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing—review and editing, Supervision.

Urban Scheuring: Conceptualization, Formal analysis, Data curation, Writing—review and editing, Supervision.

Jill Walker: Conceptualization, Methodology, Formal analysis, Writing—review and editing.

Helen Mann: Formal analysis, Writing—review and editing.

Vikram Chand: Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing—review and editing, Supervision.

Tony Mok: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing—original draft, Writing—review and editing.

Data Sharing

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data-sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2022.09.223.

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