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ORIGINAL ARTICLE

Short-term CTLA-4 blockade directly followed by PD-1 blockade in advanced melanoma patients: a single-center experience

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Background: Combination of T cell checkpoint blockade by CTLA-4- and PD-1-blockade is one of the most promising therapies in patients with advanced melanoma. It induces superior response rates when compared with single-agent therapy, but at the cost of a high percentage of grade 3 and 4 adverse events (AEs). This combination therapy was until July 2016 not available in the Netherlands, which prompted several physicians to treat patients with less than standard numbers of courses of ipilimumab followed directly by nivolumab or pembrolizumab.

Patients and methods: In this retrospective analysis, patients were included who were treated with two courses (day 0 and 21) anti-CTLA-4 (ipilimumab 3 mg/kg q3wk), directly followed by anti-PD-1 (starting at day 22 with nivolumab 3mg/kg q2wk or pembrolizumab 2 mg/kg q3wk). Data on treatment-related AEs were collected from electronic patient records and scored according to CTCAE 4.03 criteria. Overall response was evaluated using RECIST 1.1 for CT-scans and EORTC criteria for PET-scans.

Results: Forty advanced melanoma patients could be included (29/40 pembrolizumab, 11/40 nivolumab). Median follow-up (FU) was 51 weeks (range: 4–63 weeks) with a minimum FU of 26 weeks. Treatment-related AEs of grade 3 and 4 occurred in 38% of the patients. The best overall response rate (BORR) was 55% (95% CI 39–70) and disease control rate was 75% (95% CI 59–87). Ongoing responses were observed in 82% of responding patients.

Conclusion: Treatment with short-term CTLA-4 blockade directly followed by PD-1 blockade may have similar efficacy but potentially lower toxicity when compared with concurrent therapy with anti-CTLA-4 and anti-PD-1. These results warrant further investigation in a prospective randomized controlled clinical trial.

Key words: melanoma, CTLA-4 blockade, PD-1 blockade, checkpoint inhibition, combination therapy

Introduction

Checkpoint inhibition, by monoclonal antibodies targeting CTLA-4 or PD-1/PD-L1, has become one of the most promising therapies to achieve long-term benefit in patients with advanced melanoma. While the objective response rate upon CTLA-4 blockade by ipilimumab has been low with 6%–19% [1, 2], ~20% of patients achieve nevertheless long-term survival [3]. Targeting PD-1, by pembrolizumab or nivolumab, has been better-tolerated and resulted in higher response rates of 21%–44% [2, 4–7], a higher 3-year overall survival (OS) rate of 38%–45% [8, 9] and a 5-year OS rate of 34% for nivolumab, fueling the hope that the plateau of long-term survivors can be elevated over that of ipilimumab [9]. The concurrent combination of CTLA-4 and PD-1 blockade increased the response rate further to 53%–61%, but at the cost of significantly higher frequencies of treatment-related grade 3 and 4 adverse events (AEs) of 53%–55% [2, 10, 11]. Updated data from the phase 2 CheckMate 069 trial indicate a 2-year overall survival rate of 64%, which seems to be higher than that for anti-PD-1 monotherapies [12].

Preclinical data point towards an important immune modulating role for CTLA-4 signaling early in T cell activation, while PD-1 signals can be overcome at this stage by IL-2 and CD28 signaling (reviewed in [13, 14]). Within the tumor microenvironment,

however, PD-1/PD-L1 interaction appears to be dominant due to the regularly found PD-L1 expression on tumor cells shaping the T cell effector functions, whereas CTLA-4 seems to play a lesser role, due to the absence of B7 on tumor cells [15–17]. These preclinical data argue for a possible treatment regimen starting with CTLA-4 blockade followed by PD-1 blockade. However, whether this is relevant for the clinic (concurrent versus sequential application of CTLA-4 and PD-1 blockade) has not been evaluated so far in detail.

The phase I trial testing different doses of ipilimumab in combination with nivolumab, recommended concurrent ipilimumab 3 mg/kg plus nivolumab 1 mg/kg as dosing to proceed to phase 2 testing [11]. This recommendation was based on a very small number of patients. The certainty of having obtained the optimal doses for the combination of CTLA-4 and PD-1 blockade is further challenged by the fact that in metastatic renal cell cancer no difference in best overall response rate (BORR) and progression-free survival was observed between ipilimumab 3 mg/kg + nivolumab 1 mg/kg versus ipilimumab 1 mg/kg + nivolumab 3 mg/kg, whereas the latter appears to have a better safety profile [2, 18]. This has now led to two trials testing a lower dose of ipilimumab (1 mg/kg) in combination with PD-1 blockade in advanced melanoma (KEYNOTE-029, NCT02089685 and CheckMate 511, NCT02714218, www.clinicaltrials.gov).

Sequential application of standard doses of ipilimumab (3 mg/kg, four courses), followed by nivolumab (3 mg/kg) versus vice versa has recently been tested (CheckMate 064 trial [19]). The best overall responses were 31% in the ipilimumab →nivolumab arm and 56% in the nivolumab → ipilimumab arm.

In our work, here, we describe the retrospective analysis of another sequential combination possibility, namely standard dosing of two courses of ipilimumab directly followed by standard dosing of PD-1 blockade (pembrolizumab or nivolumab), inducing transient combination of ipilimumab and PD-1 blockade. Such early switch was offered to patients at our institute when the CheckMate 067 data became available, but synchronous combination was not yet approved in the Netherlands.

When analyzing patients retrospectively that received two courses of ipilimumab and were switched directly to anti-PD-1 we found a similar BORR, but less grade 3 and 4 AEs, when compared with the data published for the phase 2 and 3 trial testing concurrent CTLA-4 and PD-1 blockade [2, 10].

Being aware of the retrospective character and small sample size of our analysis, our observation so far may well argue for further prospective and randomized evaluation of this possibly equipotent, but less toxic treatment scheme.

Patients and methods

In the Netherlands, anti-PD-1 was only available in second line after CTLA-4 blockade, and the concurrent combination was not until July 2016. This has led physicians at our institute to offer patients to switch earlier after one or two courses of ipilimumab 3 mg/kg directly to standard dosing of PD-1 blockade (pembrolizumab 2 mg/kg q3wk or nivolumab 3 mg/kg q2wk).

Considering the fact, that after two course of ipilimumab the steady state level is achieved (investigator brochure ipilimumab), we included in this retrospective analysis only late stage melanoma patients treated at the Netherlands Cancer institute (NKI) between May and December 2015 with two courses of ipilimumab (day 0 and 21) directly followed by anti-PD-1 (day 22). Subsequent infusions of nivolumab or pembrolizumab were 2, respectively, 3 weekly.

Patients were identified by using pharmacy records and electronic patient records (EPR) of the Netherlands Cancer Institute. Eligible for analysis were patients who received at least one infusion of anti-PD-1.

Patients were evaluated by CT-scan or PET-scan according to our institute's standard. Tumor response was assessed in retrospect by a radiologist according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (CT-scans), and according to EORTC criteria (PET-scans) to determine BORR (CR rate plus PR rate) and disease control rate (DCR) (CR rate plus PR rate plus rate of SD).

Data on toxicity were collected from EPR, checking for each patient their whole EPR. According to the institute's general standard for immunotherapies, a physician or nurse practitioner saw the patients before each infusion, documenting toxicity and approving the next infusion. Toxicity was scored retrospectively according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. The diagnosis of colitis was based on colonoscopy with histological confirmation. Laboratory parameters that were evaluated before every infusion, according to the institute's standards, included hematology, kidney and liver functions, electrolytes, CRP, ESR, albumin, total protein, glucose, lipase, amylase, TSH, fT4, ACTH, cortisol, LH, FSH. Additionally S100 was tested before every infusion as it might be an additional marker of early response [20].

Data-lock was set at 16 March 2016. The Human Research Ethics Committee (HREC) of the NKI-AVL judged that approval of a HREC was not necessary for this retrospective analysis.

Results

Patient characteristics

Forty adult patients with irresectable stage III and stage IV melanoma were treated in the period of May until December 2015 at the NKI with short-term ipilimumab, directly followed by PD-1 blockade (29/40 pembrolizumab, 11/40 nivolumab). One patient was planned to receive this schedule but detoriated rapidly after the first cycle of ipilimumab and was therefore excluded from this analysis. The median follow-up (FU) at data-lock was 51 weeks (range 4–63). Minimum FU of patients who were alive at time of data-lock was 26 weeks. Baseline characteristics are listed in Table 1. Median patient age was 54 years, 55% were male, and 75% had an ECOG performance score of 0. Eighty percent had stage M1c disease and 20% had brain metastasis at baseline (75% treated, but 50% presenting with cerebral progression at start of treatment). Primary location was skin in 29/40 patients (72%), mucosal in 4/40 (10%) and unknown primary in 7/40 patients (18%).

Eight patients (20%) had undergone prior treatment by MAPK pathway inhibition (selective BRAF inhibitor +/– MEK inhibitor). Three patients progressed before switch and in four patients a switch was made at the time point of maximum response. One patient with a c-KIT mutated mucosal melanoma had been treated with imatinib.

Baseline LDH was elevated above upper limited of normal (ULN) in 25% of patients and above 2× ULN in 5%. Relative lymphocyte count was below 17.5% in 23% of patients, and relative eosinophil count was below 1.5% in 23% of patients. S100 was elevated in 63% of patients, with a mean of 0.64 μg/l (normal < 0.10 μg/l; range 0.03–7.90) (Table 1).

Table 1. Baseline characteristics of the patients

Characteristic	
Age—years	
Mean (range)	53.8 (27–76)
Sex	
Male	22 (55)
Female	18 (45)
ECOG performance status	
0	30 (75)
1	8 (20)
2	2 (5)
Type of melanoma	
Cutaneous	29 (73)
Mucosal	4 (10)
Unknown primary	7 (17)
Metastasis stage[a]	
M1c	32 (80)
M1a, M1b	8 (20)
Brain metastases	
Yes	8 (20)
No	31 (78)
Not determined	1 (2)
BRAFV600 status	
Mutation	23 (58)
No mutation	17 (42)
Prior systemic treatment of metastatic disease	
None	29 (73)
BRAF-i ± MEK-i	8 (20)
TIL	2 (5)
Imatinib	1 (2)
Lactate dehydrogenase	
≤ULN	29 (73)
>ULN	10 (25)
>2× ULN	2 (5)
Unknown	1 (2)
S100	
≤ULN	15 (37)
>ULN	25 (63)
ESR	
≤ULN	16 (40)
>ULN	19 (48)
Unknown	5 (12)
Relative lymphocyte count	
<17.5%	9 (23)
≥17.5%	24 (60)
Unknown	7 (17)
Relative eosinophil count	
<1.5%	9 (23)
≥1.5%	24 (60)
Unknown	7 (17)

Data are *n* (%) unless stated otherwise.
[a]Disease stage was defined according to the tumor–node–metastasis system of the American Joint Committee on Cancer 7th Edition.
BRAF-i, BRAF-inhibitor; MEK-i, MEK-inhibitor; TIL, tumor infiltrating lymphocytes; ULN, upper limit of normal; ESR, erythrocyte sedimentation rate.

Safety

Treatment-related toxicity of any grade was observed in 35 of the 40 patients (88%). The most frequent treatment-related toxicities of any grade were fatigue (43%), diarrhea (35%), pruritus (33%), and skin rash (25%). Onset of the immune-related AEs occurred most often after the combination of the second dose of anti-CTLA-4 and the first infusion of anti-PD-1.

Treatment-related grade 3 or 4 AEs occurred in 15/40 patients (38%) (Table 2). The most common grade 3 and 4 AEs were colitis (18%), elevated lipase and amylase (8%), elevation of liver enzymes (5%), and maculopapular rash (5%).

Systemic immunosuppressive medication for the management of AEs was required for 11 patients (28%).

In six patients (15%), treatment was permanently discontinued due to toxicity. Of these patients, three had ongoing disease control after treatment discontinuation.

Efficacy

In 37 of the 40 patients, response could be evaluated by imaging. Three patients could not be evaluated due to death before first evaluation.

Due to the non-pre-specified character of this treatment regimen, radiological evaluation was not consistent. Thirty-one

Table 2. Treatment-related adverse events

Event	Any grade[a]	Grade 3–4
	No. of patients with event (%)	
Any	35 (88)	15 (38)
Fatique	17 (43)	0
Diarrhea	14 (35)	6 (15)
Pruritus	13 (33)	1 (3)
Rash	10 (25)	2 (5)
Colitis	8 (20)	7 (18)
Hypothyroidism	8 (20)	0
Vitiligo	8 (20)	0
flu-like symptoms	8 (20)	1 (3)
ALT increased	7 (18)	2 (5)
AST increased	7 (18)	0
Adrenal insufficiency	5 (13)	0
Serum amylase increased	5 (13)	3 (8)
Lipase increased	5 (13)	3 (8)
Arthralgia	4 (10)	0
Dry mouth	4 (10)	0
Hyperthyroidism	4 (10)	0
Nausea	4 (10)	0
Chills	3 (8)	0
Dry skin	3 (8)	0
Fever	3 (8)	0
Creatinine increased	2 (5)	1 (3)
Headache	2 (5)	0

[a]Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.
AST, aspartate aminotransferase level; ALT, alanine aminotransferase level.

Table 3. Response to treatment

Response	
Best overall response—*n* (%)[a]	
Complete response	6 (15)
Partial response	16 (40)
Stable disease	8 (20)
Progressive disease	7 (18)
Died before first evaluation	3 (8)
Time to objective response—weeks	
Median	14
Range	8–40

[a]Best overall response rate was assessed by an independent radiologist according to the Response Evaluation Criteria in Solid Tumors, version 1.1 for CT-scans or, in case of PET-scans, by best metabolic response according to EORTC criteria for PET-scans.

patients were evaluated by CT-scan and six patients by PET-scan. The first radiological evaluation generally took place at week 11 or week 12, when most patients had received two cycles of ipilimumab and two to four cycles of anti-PD-1 (some patients had already had a treatment break due to toxicity at that time point).

BORR so far is 55% (95% CI 39–70) with 15% complete responders and 40% partial responders, and DCR was 75% (95% CI 59–87) (Table 3). Median time to first response was 14 weeks (range 8–40). Response data per imaging modality are displayed in supplementary Table S1, available at *Annals of Oncology* online. We observed a higher percentage of responses in patients evaluated by CT-scan ($n = 34$) than in patients ($n = 6$) evaluated by PET-scan (supplementary Table S1, available at *Annals of Oncology* online). Ongoing responses were observed in 82% of responding patients. Ongoing disease control was observed in 73% (22/30 patients) at a median FU of 51 weeks (range 26–63). Details of the individual responses are given in Figure 1A and B.

Discussion

The concurrent combination of CTLA-4 and PD-1 blockade (ipilimumab + nivolumab) has been shown to be superior to single ipilimumab treatment of advanced melanoma in the phase 2 and 3 trial [2, 10]. Updated data from the phase 2 trial indicate a 2-year overall survival rate of 64% for patients treated with the combination [12], a number not seen before in the systemic treatment of advanced melanoma. These promising results, however, come at the cost of significantly higher treatment-related grade 3 and 4 toxicity, when compared with single CTLA-4 or PD-1 blockade (55% versus 27% versus 16%, respectively) [2].

This raises the question whether these highly effective checkpoint inhibitors can be combined in a better way, with the aim of keeping the high response rates, while lowering the toxicity. As discussed in the introduction, based on pre-clinical data our current understanding of the timing of checkpoint molecule interactions argues for a sequential blockade of CTLA-4 first, followed by PD-1-blockade. Direct switch-sequencing of CTLA-4 and PD-1 blockade (with the aim of also maintaining some synergistic effect of CTLA-4 and PD-1 blockade) has not been tested yet. In the two sequential cohorts of the phase 1 combination study, patients started nivolumab 4–12 weeks after the last cycle of ipilimumab [11].

The only randomized switch trial, so far, showed that six courses nivolumab followed by four courses ipilimumab was superior to the reverse ipilimumab → nivolumab sequence [19]. However, the time periods to switch were different (3 weeks for ipilimumab → nivolumab and 2 weeks for nivolumab → ipilimumab). In addition, the half-lives of the antibodies are different (ipilimumab 15.4 days versus nivolumab 26.7 days). These two facts likely result in a stronger overlap of the antibodies in the nivolumab → ipilimumab than in the ipilimumab → nivolumab arm. Pharmacodynamics of anti-PD-1 showed PD-1 receptor occupancy of >70% on circulating T-cells, more than 2 months after infusion (receptor occupancy for ipilimumab is unknown, personal communications, BMS) [21]. This possible higher combination levels of the antibodies present in the serum in the nivolumab → ipilimumab arm might be also the explanation for the higher treatment-related grade 3 and 4 toxicity rate (63% versus 50%) and the higher BORR (56% versus 31%). In that way this study unfortunately compares different grades of combination therapy, but cannot address convincingly the question of therapy sequences.

The sequential scheme presented here purposely accepted an overlap of the antibodies. In that way this scheme is more an alternative combination scheme and indeed with a synergistic efficacy (BORR) above what is expected from monotherapies [2, 7].

It also seems to be more feasible than the CheckMate 064 schemes, as 40 of in total 41 patients (97%) starting treatment received subsequent PD-1 blockade and 37/41 (90%) were evaluable after the combination, compared with only 53/70 (76%) in the ipilimumab → nivolumab arm of the CheckMate 064 trial [19].

All other current attempts focus on dose adjustments. For example, the KEYNOTE-029 study (NTC02089685, www.clinicaltrials.gov) combined a lower dose of ipilimumab (1 mg/kg) concurrent with the currently used standard dose of pembrolizumab (2 mg/kg). Preliminary data indicate a lower grade 3 and 4 toxicity rate (42%) while preserving the BORR (57%) and DCR (79%) [22], when compared with the 53%–55% grade 3 and 4 toxicity rate within the phase 2 and 3 trial testing ipilimumab plus nivolumab [2, 10]. Another example is the CheckMate 511 trial (NCT02714218, www.clinicaltrials.gov) comparing concurrent ipilimumab at a lower dose of 1 mg/kg plus nivolumab 3 mg/kg (a dosing scheme used in renal cell carcinoma trials), to the standard combination scheme.

Our retrospective analysis suggests that our approach might induce a similar BORR when compared with the concurrent application schemes of ipilimumab plus anti-PD-1 [2, 22], but at potentially lower toxicity rates than the standard scheme of ipilimumab plus nivolumab [2, 10] or concurrent combinations using lower doses of ipilimumab [22]. The retrospective character of this study implies that toxicities can be underreported and grading retrospectively can lead to bias. However, the occurrence of grade 3 or 4 AEs with need for hospitalization, postponement of immunotherapy or start of systemic corticosteroids can be very accurately retrieved from EPR.

We are aware of the fact that there might be some classification bias due to the retrospective scoring, although patients were

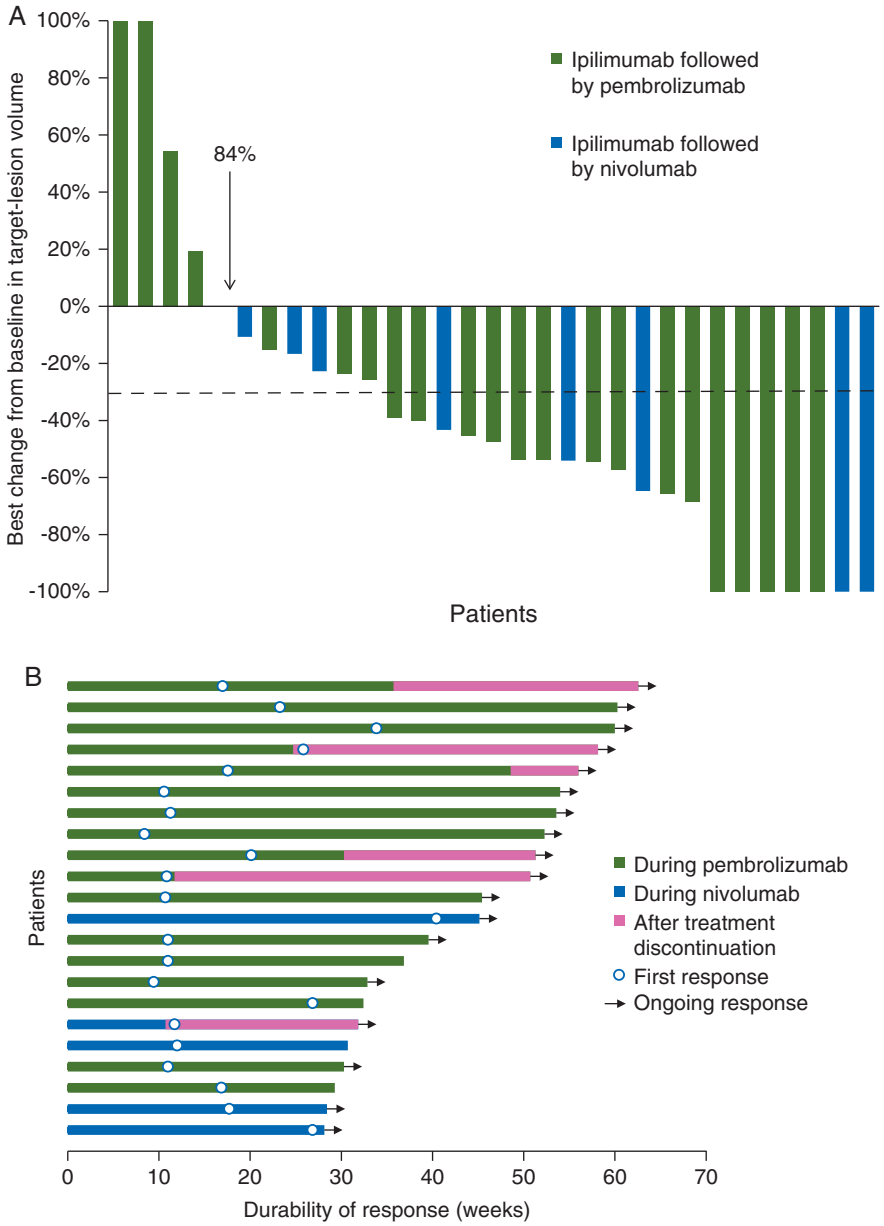

Figure 1. Change in tumor burden and durability of tumor regressions. (A) Best change from baseline in the sum of the reference diameters of the target lesion(s) in the 31 patients evaluated by CT-scan, receiving pembrolizumab after ipilimumab (green) and those receiving nivolumab after ipilimumab (blue). The dashed line indicates the 30% reduction in tumor burden that is consistent with a response to treatment according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. (B) Durability of tumor regressions in patients who had an objective response to the regimen with nivolumab (blue) or pembrolizumab (green). Open circles indicate the first evidence of objective response and arrows indicate an ongoing response at the time of the analysis. The pink colors represent the period of time of response after discontinuation of treatment.

assessed for toxicity before every cycle as patients in prospective studies, and grade 3 and 4 toxicities always require medical interventions that normally could not have been missed in retrospect.

Our data are striking in the light, that in contrast to patients treated in the phase 2 and 3 trial, many of our patients had received prior systemic treatment of advanced disease and/or had (symptomatic) cerebral metastases, both of which are considered to be negative prognostic markers for response, respectively, survival upon immunotherapy [3, 22]. We acknowledge the limitation that patients included in this analysis are evaluated by two different imaging modalities and response criteria. However, the response rate of patients evaluated by CT-scan according to RECIST 1.1 (as used in other immunotherapy studies) is even higher than that of the total cohort. Therefore, and considering the retrospective nature of these data, this scheme strongly deserves further investigation, which should take place in a randomized controlled clinical trial comparing the standard combination of ipilimumab and nivolumab to our sequential scheme.

Aside better quality of life for our patients, such a less toxic combination scheme of CTLA-4 and PD-1 blockade could create space for triple combinations with additional checkpoint inhibitors (e.g. LAG-3, TIM-3), e.g. in patients not achieving complete responses upon the doublet. One might envision that our

sequential scheme using in addition the adjusted anti-CTLA-4 plus anti-PD-1 dosing (like tested in the KEYNOTE-029 and CheckMate 511 trial), could be even less toxic and equally effective. Such scheme would be then the favorable backbone doublet for addition of new checkpoint inhibitors.

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Disclosure

AM-E, EAR, FL—no conflicts to disclose; JVVT—advisory role: MSD, BMS; JBAGH—advisory role: BMS, MSD, Pfizer, Roche, Novartis, Neon Therapeutics, research grants: BMS, MSD, GSK; CUB—advisory role: MSD, BMS, GSK, Roche, Novartis, Lilly, Pfizer, research grant: Novartis.

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