JAMA Oncology | Original Investigation

Effect of Pembrolizumab After Stereotactic Body Radiotherapy vs Pembrolizumab Alone on Tumor Response in Patients With Advanced Non–Small Cell Lung Cancer

Results of the PEMBRO-RT Phase 2 Randomized Clinical Trial

Willemijn S. M. E. Theelen, MD; Heike M. U. Peulen, MD, PhD; Ferry Lalezari, MD; Vincent van der Noort, PhD; Jeltje F. de Vries, PhD; Joachim G. J. V. Aerts, MD, PhD; Daphne W. Dumoulin, MD; Idris Bahce, MD, PhD; Anna-Larissa N. Niemeijer, MD; Adrianus J. de Langen, MD, PhD; Kim Monkhorst, MD, PhD; Paul Baas, MD, PhD

IMPORTANCE Many patients with advanced non–small cell lung cancer (NSCLC) receiving immunotherapy show primary resistance. High-dose radiotherapy can lead to increased tumor antigen release, improved antigen presentation, and T-cell infiltration. This radiotherapy may enhance the effects of checkpoint inhibition.

OBJECTIVE To assess whether stereotactic body radiotherapy on a single tumor site preceding pembrolizumab treatment enhances tumor response in patients with metastatic NSCLC.

DESIGN, SETTING, AND PARTICIPANTS Multicenter, randomized phase 2 study (PEMBRO-RT) of 92 patients with advanced NSCLC enrolled between July 1, 2015, and March 31, 2018, regardless of programmed death–ligand 1 (PD-L1) status. Data analysis was of the intention-to-treat population.

INTERVENTIONS Pembrolizumab (200 mg/kg every 3 weeks) either alone (control arm) or after radiotherapy (3 doses of 8 Gy) (experimental arm) to a single tumor site until confirmed radiographic progression, unacceptable toxic effects, investigator decision, patient withdrawal of consent, or a maximum of 24 months.

MAIN OUTCOMES AND MEASURES Improvement in overall response rate (ORR) at 12 weeks from 20% in the control arm to 50% in the experimental arm with $P < .10$.

RESULTS Of the 92 patients enrolled, 76 were randomized to the control arm (n = 40) or the experimental arm (n = 36). Of those, the median age was 62 years (range, 35-78 years), and 44 (58%) were men. The ORR at 12 weeks was 18% in the control arm vs 36% in the experimental arm ($P = .07$). Median progression-free survival was 1.9 months (95% CI, 1.7-6.9 months) vs 6.6 months (95% CI, 4.0-14.6 months) (hazard ratio, 0.71; 95% CI, 0.42-1.18; $P = .19$), and median overall survival was 7.6 months (95% CI, 6.0-13.9 months) vs 15.9 months (95% CI, 7.1 months to not reached) (hazard ratio, 0.66; 95% CI, 0.37-1.18; $P = .16$). Subgroup analyses showed the largest benefit from the addition of radiotherapy in patients with PD-L1–negative tumors. No increase in treatment-related toxic effects was observed in the experimental arm.

CONCLUSIONS AND RELEVANCE Stereotactic body radiotherapy prior to pembrolizumab was well tolerated. Although a doubling of ORR was observed, the results did not meet the study's prespecified end point criteria for meaningful clinical benefit. Positive results were largely influenced by the PD-L1–negative subgroup, which had significantly improved progression-free survival and overall survival. These results suggest that a larger trial is necessary to determine whether radiotherapy may activate noninflamed NSCLC toward a more inflamed tumor microenvironment.

TRIAL REGISTRATION ClinicalTrials.gov identifier: NCT02492568

JAMA Oncol. 2019;5(9):1276-1282. doi:10.1001/jamaoncol.2019.1478
Published online July 11, 2019.

Invited Commentary
page 1291

Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Willemijn S. M. E. Theelen, MD, Department of Thoracic Oncology, Netherlands Cancer Institute, NKI/AvL, Postbus 90203, 1006 BE Amsterdam, the Netherlands (w.theelen@nki.nl).

In recent years, treatment for non-small cell lung cancer (NSCLC) has changed significantly owing to the introduction of immunotherapy. The programmed death-ligand 1 (PD-L1)/programmed cell death 1 (PD-1) pathway is one of the most studied tumor immune escape mechanisms.[1] Targeting the PD-L1/PD-1 pathway with immune checkpoint inhibitors has produced long-lasting antitumor immune responses in a subset of NSCLC patients.[2-5] Unfortunately, most patients with NSCLC do not benefit from this treatment owing to primary resistance, possibly because certain tumor antigens are not recognized.

Stereotactic body radiotherapy (SBRT) is the delivery of a high radiation dose in generally 3 to 5 fractions with high accuracy to a single tumor site. Stereotactic body radiotherapy may synergize with immunotherapy. Several preclinical studies reported an increased tumor antigen release, improved antigen presentation, and T-cell infiltration in irradiated tumors. Combining radiotherapy with immune checkpoint inhibition showed more pronounced tumor regression in several solid tumor types, including in the nonirradiated tumors, than provided by either of these treatments alone.[6-12]

We present the results of the PEMBRO-RT study, the first randomized study, to our knowledge, of pembrolizumab, a highly selective humanized PD-1 monoclonal antibody, with or without prior SBRT to a single tumor site in patients with metastatic NSCLC. This study evaluates whether SBRT enhances the effect of immune checkpoint blockade by increasing tumor response in nonirradiated lung cancer lesions on PD-1 immune checkpoint blockade.

Key Points

Question Does stereotactic body radiotherapy enhance the effect of immune checkpoint inhibition by increasing tumor response in nonirradiated lung cancer lesions in metastatic non–small cell lung cancer?

Findings In this phase 2 clinical trial of 76 patients with recurrent metastatic non–small cell lung cancer randomized to either pembrolizumab alone or pembrolizumab after stereotactic body radiotherapy on a single tumor site, the overall response rate at 12 weeks was 18% in the control arm vs 36% in the experimental arm.

Meaning Stereotactic body radiotherapy prior to pembrolizumab was well tolerated; although a doubling of the overall response rate was observed, the results did not meet the study criteria for meaningful clinical benefit.

Methods

This multicenter, phase 2 randomized clinical trial was conducted at 3 medical sites in the Netherlands. Patients 18 years or older were eligible to participate if they had histological or cytological confirmed metastatic non-small cell lung cancer (NSCLC) that progressed after at least 1 regimen of chemotherapy but who were immunotherapy naive and had an Eastern Cooperative Oncology Group performance status of 1 or lower. At least 2 separate lesions were required, one of which was measurable according to the Response Evaluation Criteria in Solid Tumors and suitable for biopsy, and the other of which was amenable to irradiation. Patients were ineligible if they had (1) radiotherapy to any tumor site within 6 months before randomization; (2) known, active central nervous system metastases and/or carcinomatous meningitis; (3) untreated driver alterations of epidermal growth factor receptor or anaplastic lymphoma kinase; or (4) active autoimmune or interstitial lung disease. The trial protocol is provided in Supplement 1; the protocol and all amendments were approved by the institutional review board or independent ethics committee of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam. The trial was conducted in accordance with the provisions of the Declaration of Helsinki[13] and the Good Clinical Practice guidelines of the European Medicines Agency and the US Food and Drug Administration. All patients provided written informed consent before enrollment.

Patients were randomly assigned using a 1:1 ratio to receive treatment with pembrolizumab either after SBRT to a single tumor site (experimental arm) or without SBRT (control arm). Randomization was stratified to smoking status (<10 pack-years vs ≥10 pack-years). Pembrolizumab was administered intravenously at 200 mg every 3 weeks. In the experimental arm, the first course was given within 7 days after completion of SBRT, which consisted of 3 doses of 8 Gy delivered on alternate days to a single tumor site that did not overlap with the biopsy site and was deemed most safe and/or convenient for the patient. For details of the response evaluation, duration of treatment, and PD-L1 staining and scoring criteria, see the eMethods in Supplement 2.

The primary end point was overall response rate (ORR)—complete response and partial response—at 12 weeks from randomization. Secondary end points included safety, progression-free survival (PFS), overall survival (OS), and disease control rate at 12 weeks. End points were assessed in the intention-to-treat population, including all patients who underwent randomization with the exception of 2 patients in the experimental arm, who both withdrew consent (**Figure 1**). Adverse events were graded according to the Common Toxicity Criteria, version 4.0, and were registered from the date of informed consent until discontinuation of trial treatment. Exploratory end points included the effect of PD-L1 expression and prior radiotherapy on efficacy.

Efficacy was assessed in the intention-to-treat population, and safety was assessed in the as-treated population, which included all patients who had undergone randomization and received at least 1 dose of the assigned therapy. A statistical analysis indicated that with a sample of 74 patients, 37 in each arm, the trial would have a power of 82% with an odds ratio of 4 to detect the difference between a response rate of 20% in the control arm and 50% in the experimental arm at a 2-sided significance level of $P < .10$. For details regarding the statistical analyses of end points, see the eMethods in Supplement 2.

Results

Between July 1, 2015, and March 31, 2018, 92 patients were screened for enrollment, and 76 patients who met the eligi-

Figure 1. CONSORT Diagram

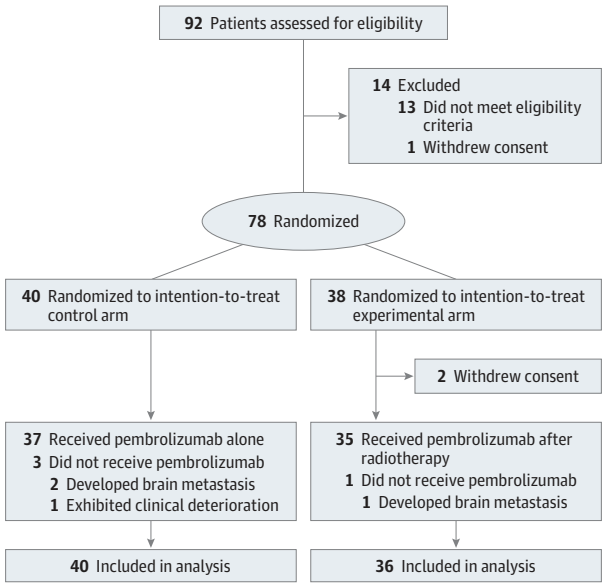


Table. Response to Treatment

Response	Experimental Arm, No./Total No. (%) (n = 36)[a]	Control Arm, No./Total No. (%) (n = 40)[b]
Best overall response, No.		
Complete response	3	1
Partial response	14	8
Stable disease	9	10
Progressive disease	10	21
Objective response rate at 12 wk		
Overall[c]	13/36 (36)	7/40 (18)
PD-L1 TPS, %		
0	4/18 (22)	1/25 (4)
1-49	3/8 (38)	3/8 (38)
≥50	6/10 (60)	3/5 (60)
Disease control rate at 12 wk[d]	23/36 (64)	16/40 (40)

Abbreviations: PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

[a] Patients who received pembrolizumab therapy after stereotactic body radiotherapy.

[b] Patients who received pembrolizumab therapy alone.

[c] $P = .07$.

[d] $P = .04$.

bility criteria were randomly assigned to either the control arm (n = 40) or the experimental arm (n = 36). Of those, the median age was 62 years (range, 35-78 years), and 44 (58%) were men. Patient demographics, including previous radiotherapy, were well balanced between the arms. The percentage of PD-L1-negative tumors was slightly higher in the control arm (25 of 38 [66%]) than in the experimental arm (18 of 36 [50%]), and the number of patients with a tumor proportion score of 50% or higher was lower in the control arm than in the experimental arm (5 of 38 [13%] vs 10 of 36 [28%]) ($P = .10$) (eTable 1 in Supplement 2). The tumor sites selected for SBRT were primarily lung lesions or lymph node metastases (eTable 2 in Supplement 2).

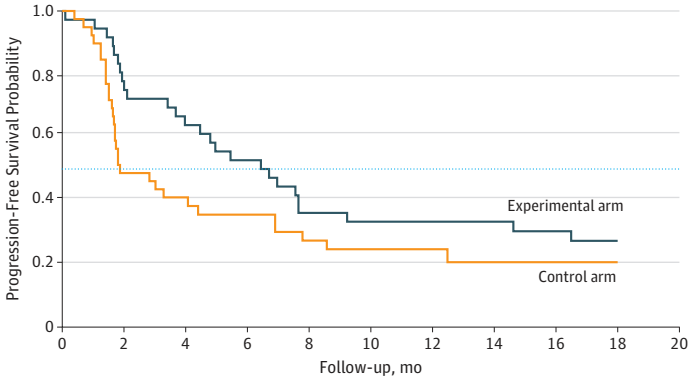
Thirty-seven patients (92%) in the control arm and 35 patients (97%) in the experimental arm received at least 1 course of pembrolizumab. All patients who did not receive pembrolizumab were categorized as having progressive disease for further analyses. One patient received palliative radiotherapy before the primary end point but remained part of the intention-to-treat population. At the cutoff date of July 1, 2018, the median follow-up time was 23.6 months (range, 0.1-34.4 months). Seven patients (18%) in the control arm and 4 patients (11%) in the experimental arm were still receiving treatment. The median duration of treatment for patients with at least 1 dose of pembrolizumab was 2.1 months (95% CI, 1.2-5.6 months) in the control arm and 4.2 months (95% CI, 2.7-11.0 months) in the experimental arm ($P = .30$).

In the intention-to-treat population, the ORR at 12 weeks was 18% (95% CI, 7%-33%) in the control arm and 36% (95% CI, 21%-54%) in the experimental arm ($P = .07$) (**Table**). The increased ORR in the experimental arm (22%) compared with the control arm (4%) was largely influenced by ORR in the PD-L1-negative subgroup, although this ORR in the PD-L1-negative subgroup was not significant ($P = .14$). Response rates in the 2 PD-L1-positive subgroups were similar in both arms. There was 1 complete response in the control arm and 3 in the experimental arm. In the control arm, the majority of patients (21 of 40 [53%]) showed progressive disease as best ORR compared with the experimental arm, in which partial response was most common (14 of 36 [39%]). Stable disease as best response was identical in both arms (10 of 40 [25%] and 9 of 35 [25%], respectively). In the overall population, significant improvement (64% vs 40%; $P = .04$) was observed in the disease control rate at 12 weeks in the experimental arm. The effect of SBRT on response rates in patients who were previously treated with radiotherapy (ie, >6 months before randomization) and patients who never received any radiotherapy was similar (odds ratios, 3.1 [95% CI, 0.5-23.5] vs 2.4 [95% CI, 0.5-13.1], both in favor of the experimental arm; $P = .81$), suggesting that previous radiotherapy did not strongly affect the study results (eTable 3 in Supplement 2). The distribution of baseline PD-L1 expression did not differ between patients who received radiotherapy more than 6 months before inclusion (PD-L1 expression of 0%, 27 patients; 1%-49%, 7 patients; and ≥50%, 8 patients) and patients who did not receive radiotherapy before inclusion (PD-L1 expression of 0%, 16 patients; 1%-49%, 9 patients; and ≥50%, 7 patients) ($P = .37$) (eTable 4 in Supplement 2). Two patients in the control arm had an initial increase in tumor burden of more than 20% at week 6 followed by partial response at week 12, which was considered pseudoprogression.

At the time of analysis, median PFS was 1.9 months (95% CI, 1.7-6.9 months) in the control arm and 6.6 months (95% CI, 4.0-14.6 months) in the experimental arm (**Figure 2**). The increased PFS in the experimental arm was not significant (hazard ratio [HR], 0.71; 95% CI, 0.42-1.18; $P = .19$). A significant benefit of SBRT with respect to PFS was seen in the PD-L1-negative subgroup (HR, 0.49; 95% CI, 0.26-0.94; $P = .03$); however, the limited number of responders must be taken into account. No benefit from the addition of SBRT

Figure 2. Progression-Free Survival in the Intent-to-Treat Population

A Progression-free survival

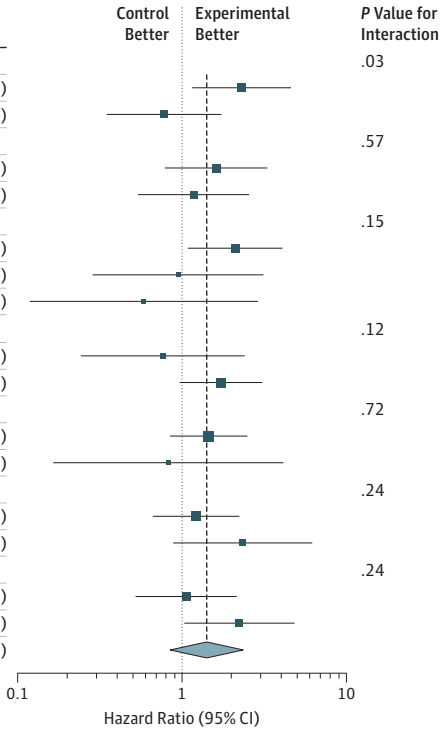


No. at risk

Experimental arm	36	28	23	19	13	12	12	11	10	9
Control arm	40	19	15	13	10	6	6	5	5	5

B Subgroup analysis

Subgroup	Control Events, No./ Total No.	Experimental Events, No./ Total No.	Hazard Ratio (95% CI)	*P* Value for Interaction
Sex				.03
Male	20/23	14/20	2.31 (1.15-4.62)	
Female	10/17	15/16	0.78 (0.35-1.74)	
ECOG performance score				.57
0	18/22	13/16	1.61 (0.78-3.32)	
1	11/17	15/19	1.18 (0.54-2.57)	
PD-L1, %				.15
0	22/25	17/18	2.11 (1.08-4.11)	
1-49	5/8	6/8	0.95 (0.28-3.14)	
≥50	2/5	6/10	0.58 (0.12-2.91)	
Smoking, pack-years				.12
<10	5/8	7/7	0.76 (0.24-2.41)	
≥10	25/32	22/29	1.73 (0.97-3.09)	
Histology				.72
Nonsquamous	27/36	26/31	1.45 (0.84-2.51)	
Squamous	3/4	3/5	0.82 (0.16-4.16)	
Lines of previous chemotherapy				.24
1	22/31	20/26	1.22 (0.66-2.24)	
≥2	8/9	9/10	2.35 (0.88-6.24)	
Age at randomization, y				.24
<65	14/22	17/21	1.06 (0.52-2.15)	
≥65	16/18	12/15	2.24 (1.03-4.86)	
Total	30/40	29/36	1.41 (0.85-2.36)	



A, Experimental arm comprised patients who received pembrolizumab after stereotactic body radiotherapy; control arm comprised patients who received pembrolizumab alone. Hazard ratio was 0.71 (95% CI, 0.42-1.18) with a 2-sided significance level of *P* = .19. B, ECOG indicates Eastern Cooperative Oncology Group; PD-L1, programmed death–ligand 1.

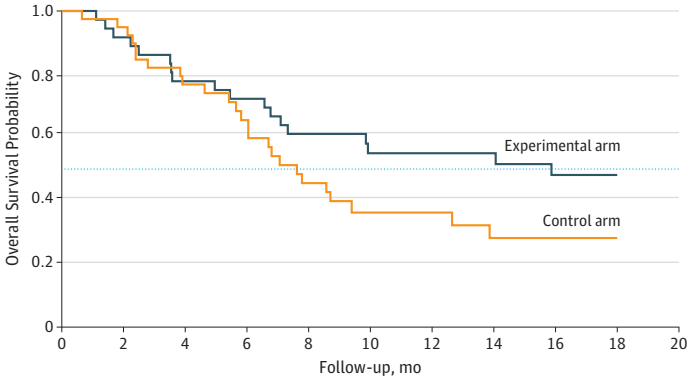
was seen in the PD-L1-positive subgroups (HR, 1.14; 95% CI, 0.45-2.89; *P* = .79) (Figure 2).

At the time of analysis, 51 patients had died. A median OS of 7.6 months (95% CI, 6.0-13.9 months) in the control arm and 15.9 months (95% CI, 7.1 months to not reached) in the experimental arm was observed (**Figure 3**). This increased OS was not significant (HR, 0.66; 95% CI, 0.37-1.18; *P* = .16). The benefit of SBRT with respect to OS was observed only in the PD-L1-negative subgroup (HR, 0.48; 95% CI, 0.24-0.99; *P* = .046), and no benefit was seen in the combined PD-L1-positive subgroups (HR, 1.4; 95% CI, 0.42-4.66; *P* = .58). Male patients (HR, 0.42; 95% CI, 0.19-0.96; *P* = .04) and smokers (HR, 0.48; 95% CI, 0.25-0.93; *P* = .03) performed significantly better in the experimental arm compared with the control arm (Figure 3). After correction for other variables, only PD-L1 status remained a predictive factor for OS in the experimental arm.

The most common adverse events were fatigue (28 of 72 patients [39%]), flulike symptoms (23 of 72 [32%]), and cough (20 of 72 [28%]). Fatigue (10 of 37 patients [27%] vs 18 of 35 [51%]; *P* = .05) and pneumonia (3 of 37 [8%] vs 9 of 35 [26%]; *P* = .06) occurred more often in the experimental arm than in the control arm. Pembrolizumab-related toxic effects were primarily fatigue (18%), flulike symptoms (15%), and pruritus (14%). Grade 3 to 5 pembrolizumab-related toxic effects were

Figure 3. Overall Survival in the Intent-to-Treat Population

A Overall survival

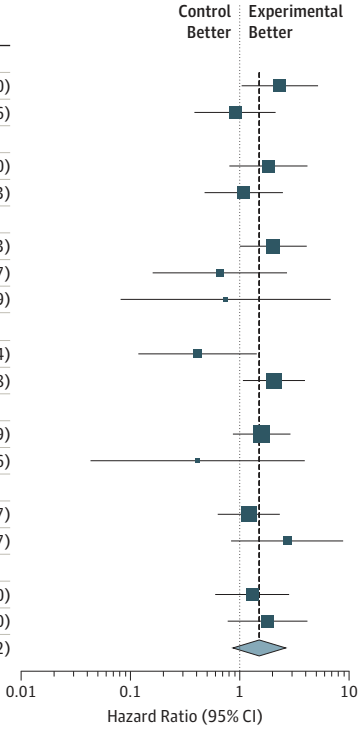


No. at risk

Experimental arm	36	33	28	26	20	18	18	16	14	14
Control arm	40	37	29	23	16	9	9	7	7	7

B Subgroup analysis

Subgroup	Control Events, No./ Total No.	Experimental Events, No./ Total No.	Hazard Ratio (95% CI)	*P* Value for Interaction
Sex				.08
Male	17/23	9/20	2.37 (1.04-5.40)	
Female	9/17	12/16	0.90 (0.38-2.16)	
ECOG performance score				.36
0	15/22	9/16	1.85 (0.80-4.30)	
1	10/17	12/19	1.09 (0.47-2.53)	
PD-L1, %				.13
0	21/25	13/18	2.06 (1.00-4.23)	
1-49	3/8	5/8	0.65 (0.15-2.77)	
≥50	1/5	3/10	0.74 (0.08-7.09)	
Smoking, pack-years				.02
<10	4/8	6/7	0.40 (0.11-1.44)	
≥10	22/32	15/29	2.09 (1.07-4.08)	
Histology				.47
Nonsquamous	24/36	18/31	1.61 (0.86-2.99)	
Squamous	2/4	3/5	0.40 (0.04-4.06)	
Lines of previous chemotherapy				.24
1	19/31	16/26	1.21 (0.62-2.37)	
≥2	7/9	5/10	2.77 (0.83-9.27)	
Age at randomization, y				.58
<65	13/22	12/21	1.31 (0.59-2.90)	
≥65	13/18	9/15	1.81 (0.77-4.30)	
Total	26/40	21/36	1.52 (0.85-2.72)	



A, Experimental arm comprised patients who received pembrolizumab after stereotactic body radiotherapy; control arm comprised patients who received pembrolizumab alone. Hazard ratio was 0.66 (95% CI, 0.37-1.18) with a 2-sided significance level of *P* = .16. B, ECOG indicates Eastern Cooperative Oncology Group; PD-L1, programmed death-ligand 1.

reported in 12 patients (17%), with no significant differences between arms. Adverse events that appeared in more than 10% of patients and relevant pembrolizumab-related toxic effects are presented in eTable 5 in Supplement 2.

Discussion

The PEMBRO-RT study is the first randomized trial, to our knowledge, to show an augmenting effect of SBRT on the response to PD-1 blockade in patients with metastatic NSCLC. The experimental arm showed an increase in ORR, disease control rate at 12 weeks, and median PFS and OS without an increase in toxic effects. The study did not meet its primary end point because the improvements did not meet the study's prespecified criteria—an increase of ORR from 20% in the control arm to 50% in the experimental arm at 12 weeks—for meaningful clinical benefit.

In recent trials, response rates of pembrolizumab-treated patients with advanced NSCLC were dependent on the PD-L1 expression levels of the tumor.[2,4,14,15] The response rate in the combined PD-L1-positive subgroups (PD-L1 ≥ 1%) in our study was much higher compared with other trials (52% [16 of 31] vs 18% to 27%).[2,14] Patient and

tumor characteristics in this study were comparable with previously reported studies. The reason for this study's high response rate remains unclear, but the excellent patient outcomes observed in both PD-L1-positive subgroups may have masked a potential augmenting effect of SBRT in this setting.

An imbalance of PD-L1 distribution in favor of the experimental arm must be taken into account for the overall cohort; however, when data from the PD-L1-negative subgroup were evaluated, a significant benefit was observed from the experimental approach. Blood and tumor samples collected during this trial may assist in gaining better insight regarding whether this improvement can be attributed to an augmenting effect from SBRT in these PD-L1-negative patients.

Limitations

Little is known about the effects of radiotherapy dose, fractionation, and treatment site on the antitumor immune response. Several immunogenic mice studies reported that the immune-modulating effect of hypofractionated radiotherapy was more pronounced compared with single-dose radiotherapy.[6,16-18] Thus, a dose of 3 × 8 Gy was chosen for SBRT preparation and delivery because of its high accuracy, which minimized the potential for toxic effects caused by the addition of radiotherapy. To further reduce the possibility of toxic effects, SBRT was administered to the experimental arm sequentially rather than concurrently, with no longer than 1 week between the last radiotherapy dose and the first pembrolizumab dose to minimize delay of systemic treatment. A study by Dovedi et al reported a decrease in PD-L1 expression and anergy of tumor-reactive T-cells 7 days after the last dose of fractionated radiotherapy in mice models.[8] Further research is needed to explore whether the radiotherapy dose and schedule used in this clinical trial were optimal with respect to the immune-modulating potential of radiation in combination with immune checkpoint inhibition in patients with cancer.

The safety profile observed in this clinical trial was consistent with previous studies of pembrolizumab treatment for advanced NSCLC.[2,4,14] Most immune-mediated events were grade 1 or 2. No significant differences in toxic effects between arms were observed. Only 1 patient experienced an immune-related adverse event that may have been augmented by SBRT. Nephritis developed in 1 patient after the administration of SBRT on a retroperitoneal lesion and the third course of pembrolizumab, resulting in discontinuation of treatment. Luke et al[19] reported safety data on 73 patients with solid tumors who were treated with pembrolizumab after SBRT to 2 to 4 tumor lesions. The timing of SBRT was similar to this study, but doses varied from 30 to 50 Gy in 3 to 5 fractions, depending on the tumor site. They concluded that the administration of SBRT before pembrolizumab treatment was well tolerated. In a KEYNOTE-001 phase 1 clinical trial, Shaverdian et al analyzed the effects of previous radiotherapy on the efficacy and safety of pembrolizumab treatment in patients with NSCLC.[20] They reported that the safety profile was acceptable, with a longer PFS and OS in the subgroup that received previous radiotherapy. The effects of previous radiotherapy on the efficacy and safety of pembrolizumab could not be established in this study, but this possible bias should be further investigated.

Conclusions

The results of this study are encouraging, and further evaluation in a larger phase 2/3 trial is recommended to confirm the findings and elucidate the processes by which SBRT may activate noninflamed NSCLC tumors toward an inflamed tumor microenvironment, rendering them receptive to immune checkpoint inhibition.

ARTICLE INFORMATION

Accepted for Publication: March 20, 2019.

Published Online: July 11, 2019. doi:10.1001/jamaoncol.2019.1478

Author Affiliations: Department of Thoracic Oncology, Netherlands Cancer Institute, Amsterdam (Theelen, de Langen, Baas); Department of Radiation Oncology, Netherlands Cancer Institute, Amsterdam (Peulen); Department of Radiation Oncology, Catharina Hospital, Eindhoven, the Netherlands (Peulen); Department of Radiology, Netherlands Cancer Institute, Amsterdam (Lalezari); Department of Biometrics, Netherlands Cancer Institute, Amsterdam (van der Noort, de Vries); Department of Pulmonology, Erasmus Medical Center, Rotterdam, Amsterdam, the Netherlands (Aerts, Dumoulin); Department of Pulmonology, VU Medical Center, Amsterdam, the Netherlands (Bahce, Niemeijer); Department of Pathology, Netherlands Cancer Institute, Amsterdam (Monkhorst).

Author Contributions: Dr Theelen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Theelen, Peulen, van der Noort, Aerts, Baas.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Theelen, Peulen, van der Noort, Aerts, Baas.
Critical revision of the manuscript for important intellectual content: Theelen, Peulen, Lalezari, van der Noort, de Vries, Aerts, Dumoulin, Bahce, Niemeijer, de Langen, Monkhorst.
Statistical analysis: Theelen, van der Noort.
Obtained funding: Baas.
Administrative, technical, or material support: Theelen, van der Noort, de Vries, Dumoulin, Niemeijer, de Langen.
Supervision: Bahce, de Langen, Baas.

Conflict of Interest Disclosures: Dr Theelen reported receiving grants from Merck Sharp & Dohme during the conduct of the study. Dr Peulen reported receiving grants from KWF during the conduct of the study. Dr Aerts reported receiving personal fees from Merck Sharp & Dohme, Lilly, Bristol-Myers Squibb, Boehringer Ingelheim, Amphera, Roche, Takeda, and AstraZeneca outside the submitted work; having a patent for allogenic tumor cell lysate licensed to Amphera; and having patents pending for combination treatment dendritic cell therapy, dendritic cell therapy with allogenic lysate in pancreatic tumors, and Janus kinase inhibition in solid tumors. Dr Bahce reported receiving grants from Merck Sharp & Dohme during the conduct of the study. Dr de Langen reported receiving grants from Merck Sharp & Dohme during the conduct of the study; grants from Merck Sharp & Dohme, Boehringer, Bristol-Myers Squibb, and AstraZeneca outside the submitted work; and nonfinancial support from Roche and Merck Serono outside the submitted work. Dr Monkhorst reported receiving personal and consultancy fees from Roche, Merck Sharp & Dohme, Bristol-Myers Squibb, AbbVie, AstraZeneca, and Takeda outside the submitted work. Dr Baas reported receiving grants and medication delivery from Merck Sharp & Dohme during the conduct of the study as well as grants and consultancy fees from Bristol-Myers Squibb outside the submitted work. No other disclosures were reported.

Funding/Support: This study was an investigator-initiated trial, designed by the authors and financially supported by an unrestricted grant from Merck Sharp & Dohme that included medication supply.

Role of the Funder/Sponsor: Merck Sharp & Dohme approved the manuscript, but they had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 3.

Additional Contributions: We wish to thank the study investigators, the site staff, and the participants and families who participated in this study. We would also like to acknowledge Netherlands Cancer Institute–Antoni van Leeuwenhoek Core Facility Molecular Pathology & Biobanking for supplying biobank material and laboratory support and the Netherlands Cancer Institute–Antoni van Leeuwenhoek Trial Bureau for clinical trial support.

REFERENCES

1. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26:677-704. doi:10.1146/annurev.immunol.26.021607.090331

2. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet*. 2016;387(10027):1540-1550. doi:10.1016/S0140-6736(15)01281-7

3. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. *N Engl J Med*. 2015;373(17):1627-1639. doi:10.1056/NEJMoa1507643

4. Reck M, Rodríguez-Abreu D, Robinson AG, et al; KEYNOTE-024 Investigators. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823-1833. doi:10.1056/NEJMoa1606774

5. Rittmeyer A, Barlesi F, Waterkamp D, et al; OAK Study Group. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet*. 2017;389(10066):255-265. doi:10.1016/S0140-6736(16)32517-X

6. Demaria S, Formenti SC. Radiation as an immunological adjuvant: current evidence on dose and fractionation. *Front Oncol*. 2012;2:153. doi:10.3389/fonc.2012.00153

7. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest*. 2014;124(2):687-695. doi:10.1172/JCI67313

8. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res*. 2014;74(19):5458-5468. doi:10.1158/0008-5472.CAN-14-1258

9. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature*. 2015;520(7547):373-377. doi:10.1038/nature14292

10. Gong X, Li X, Jiang T, et al. Combined radiotherapy and anti-PD-L1 antibody synergistically enhances antitumor effect in non–small cell lung cancer. *J Thorac Oncol*. 2017;12(7):1085-1097. doi:10.1016/j.jtho.2017.04.014

11. Zeng J, See AP, Phallen J, et al. Anti–PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol Biol Phys*. 2013;86(2):343-349. doi:10.1016/j.ijrobp.2012.12.025

12. Dovedi SJ, Cheadle EJ, Popple AL, et al. Fractionated radiation therapy stimulates antitumor immunity mediated by both resident and infiltrating polyclonal T-cell populations when combined with PD-1 blockade. *Clin Cancer Res*. 2017;23(18):5514-5526. doi:10.1158/1078-0432.CCR-16-1673

13. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

14. Garon EB, Rizvi NA, Hui R, et al; KEYNOTE-001 Investigators. Pembrolizumab for the treatment of non–small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018-2028. doi:10.1056/NEJMoa1501824

15. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al; KEYNOTE-189 Investigators. Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078-2092. doi:10.1056/NEJMoa1801005

16. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res*. 2009;15(17):5379-5388. doi:10.1158/1078-0432.CCR-09-0265

17. Schaue D, Ratikan JA, Iwamoto KS, McBride WH. Maximizing tumor immunity with fractionated radiation. *Int J Radiat Oncol Biol Phys*. 2012;83(4):1306-1310. doi:10.1016/j.ijrobp.2011.09.049

18. Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun*. 2017;8:15618. doi:10.1038/ncomms15618

19. Luke JJ, Lemons JM, Karrison TG, et al. Safety and clinical activity of pembrolizumab and multisite stereotactic body radiotherapy in patients with advanced solid tumors. *J Clin Oncol*. 2018;36(16):1611-1618. doi:10.1200/JCO.2017.76.2229

20. Shaverdian N, Lisberg AE, Bornazyan K, et al. Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: a secondary analysis of the KEYNOTE-001 phase 1 trial. *Lancet Oncol*. 2017;18(7):895-903. doi:10.1016/S1470-2045(17)30380-7