Exploring and modulating the tumor immune microenvironment: Towards improving patient outcomes of immunotherapy in lung cancer

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CHAPTER 6

Pembrolizumab with and without radiotherapy for metastatic non-small cell lung cancer: pooled analysis of two randomized trials

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RESEARCH IN CONTEXT

Evidence before this study

Immune checkpoint inhibition has a central role in the treatment of advanced NSCLC, but has only been beneficial in a minority of patients. We searched the scientific literature for a comparison of anti-PD-1 (e.g. pembrolizumab) with or without radiotherapy (RT) in the treatment of metastatic non-small cell lung cancer (mNSCLC). We used the search terms “pembrolizumab” AND “anti-PD-1” AND “non-small cell lung cancer” AND “response” AND “overall survival” to search for publications in PubMed from February 10, 2012 to June 17, 2020 and for abstracts presented at annual congresses of the American Association of Cancer Research (AACR), the American Society of Clinical Oncology (ASCO), American Society for Radiation Oncology (ASTRO), the European Society for Medical Oncology (ESMO), European Society for Radiotherapy and Oncology (ESTRO), and the World Conference on Lung Cancer (WCLC). We also searched the clinical trial registries of ClinicalTrials.gov and WHO International Clinical Trials Registry Platform. Only two randomized clinical trials evaluating the impact of combining pembrolizumab with RT on patient outcomes are currently published. The primary endpoint for both trials showed improvement in the combination therapy arm, but neither met the prespecified criteria for meaningful clinical benefit. This may indicate that a larger sample size is required to more accurately detect the effects of the addition of RT to immunotherapy on patient outcomes.

Added value of this study

Owing to their limited sample sizes, we hypothesized that the previous analyses of each individual trial lacked sufficient statistical power to detect practical, clinically attainable improvements in patient outcomes. This concern prompted re-evaluation with a pooled analysis to better evaluate this effect. We found that adding RT to pembrolizumab significantly increased response rates to unirradiated lesions, leading to a significant PFS and OS benefit.

Implications of all the available evidence

This pooled analysis shows that the combination of pembrolizumab with RT can be considered a treatment option for patients with mNSCLC, as it significantly increased treatment response and survival compared to pembrolizumab alone. These results warrant validation in a randomized phase III trial.

SUMMARY

Background

Radiation therapy (RT) may augment systemic antitumoral responses to immunotherapy. In metastatic non-small cell lung cancer (mNSCLC), several ongoing randomized studies are examining the addition of RT to various immunotherapy agents. However, the PEMBRO-RT and MDACC trials are the only known completed randomized comparisons of immunotherapy with or without radiation therapy (RT). When the trials were analyzed individually, a potential benefit was observed in the combination arms; however, the small sample size of each trial might have limited the detection of smaller than expected, but nevertheless clinically relevant, differences in response rates and outcomes. Hence, we performed a pooled analysis to infer whether RT improves responses to immunotherapy in mNSCLC patients.

Methods

Inclusion criteria for both completed trials were mNSCLC with at least one unirradiated lesion to monitor for out-of-field response. All patients were immunotherapy-naïve. The intention-to-treat population (ITTP) from both trials were included in this analysis. In the PEMBRO-RT trial (NCT02492568), patients were randomly assigned using a 1:1 ratio and stratified by smoking status (<10 vs ≥10 pack-years). In the MDACC trial (NCT02444741), patients were entered in one of two cohorts based on RT schema feasibility and subsequently randomized using a 1:1 ratio. Due to the nature of the intervention in the experimental arm (radiotherapy), blinding was not feasible in either trial. In both trials, pembrolizumab (200mg every 3 weeks) was administered with or without RT. In the PEMBRO-RT trial of previously chemotherapy-treated patients, the first dose of pembrolizumab was given sequentially <1 week after the last dose of RT (24Gy/3 fractions), while in the MDACC trial of both previously-treated and newly-diagnosed cases it was given concurrently with the first dose of RT (50Gy/4 fractions or 45Gy/15 fractions). Only unirradiated lesions were measured for response. The endpoints for this pooled analysis were out-of-field (abscopal) response rate (ARR), abscopal disease control rate (ACR), ARR at 12 weeks, ACR at 12 weeks, progression-free survival (PFS) and overall survival (OS).

Findings

Overall, 148 patients were analyzed (n=76 pembrolizumab; n=72 iRT). The median follow-up for all patients was 33 months. Most patients had non-squamous histology (84%) and received prior chemotherapy (75%). There were no differences between arms in terms of baseline variables, including PD-L1 status and metastatic disease volume. The most commonly irradiated sites were lung metastases (28/72, 39%), intrathoracic lymphatics (15/72, 21%), and lung primary disease (12/72, 17%). The ARR was 19.7% with pembrolizumab vs. 41.7% with iRT (odds ratio [OR] 2.96, 95% CI 1.42 to 6.20; p=0.004); ACR was 43.4% vs. 65.2% (OR 2.51, 1.28 to 4.91; p=0.009); median PFS was 4.4 months vs. 9.0 months (hazard ratio [HR] 0.67, 95% CI 0.45-0.99; p=0.026); and median OS was 8.7 months vs 19.2 months (HR 0.67, 0.54-0.84; p=0.006). No evidence for new safety concerns arose from this analysis.

Interpretation

Adding RT to immunotherapy significantly increased responses and outcomes in mNSCLC. These results warrant validation in a randomized phase III trial.

INTRODUCTION

The systemic treatment of metastatic non-small cell lung cancer (mNSCLC) continues to evolve rapidly, with immunotherapy (with or without chemotherapy) now being a cornerstone of first-line treatment [1-4]. However, the benefit of immunotherapy has been largely driven by a subset of patients with marked and durable responses to immunotherapeutic agents [5]. Just 17-48% of patients respond to immunotherapy-based approaches, leaving the need to explore further options for non-responders [1, 3, 4].

In order to improve outcomes for these patients, efforts have been aimed at increasing the response rate to immunotherapy, such as by combining immunotherapy with radiation therapy (RT). There is ample mechanistic evidence that RT can enhance the immune response in this setting [6-13]. Central to this notion is the concept of the abscopal effect, which refers to systemic (out of the RT field) anti-neoplastic effects caused by local RT. Biologically, RT enhances the systemic release of antigens from tumor tissue, which are then recognized by antigen-presenting cells and subsequently presented to T lymphocytes (especially CD8 cytotoxic T cells). Priming and activation of these cells causes a systemic immune response against tumor tissue both locally and systemically. Moreover, sublethal doses of RT have been mechanistically shown to more favorably modulate the tumor microenvironment so as to better attract T cells (e.g. potentially by means of reducing the inhibitory signal TGF-β), along with attenuating high-dose RT-induced immunosuppressive cell signaling (e.g. macrophage repolarization to the M1 subtype) [14-18].

Despite cumulative preclinical and clinical data, there remains relatively little randomized evidence of whether combining RT with immunotherapy (iRT) for mNSCLC improves response rates and/or outcomes over immunotherapy alone. The randomized PEMBRO-RT trial (n=78) conducted at the Netherlands Cancer Institute (NKI) suggested a trend towards improved response rates when pembrolizumab was combined with RT as compared to pembrolizumab alone, with a proportionally greater effect in PD-L1 negative patients [19]. A randomized study (n=80) from MD Anderson Cancer Center (MDACC) using either 50Gy/4 fractions or 45Gy/15 fractions did not discern outcome differences in the overall population, but did suggest proportionally greater effects on response rate and progression-free survival (PFS) when 50Gy/4 fractions was applied [20].

Analyzed individually, the relatively small sample size of both aforementioned clinical trials limited the detection of potentially significant differences in response rates and outcomes. While several ongoing randomized studies are examining the addition of RT to various immunotherapy agents, these are the only known completed randomized comparisons of immune checkpoint inhibition alone versus immune checkpoint inhibition combined with RT in mNSCLC. We therefore performed a pooled analysis of these two trials to better evaluate these clinical endpoints.

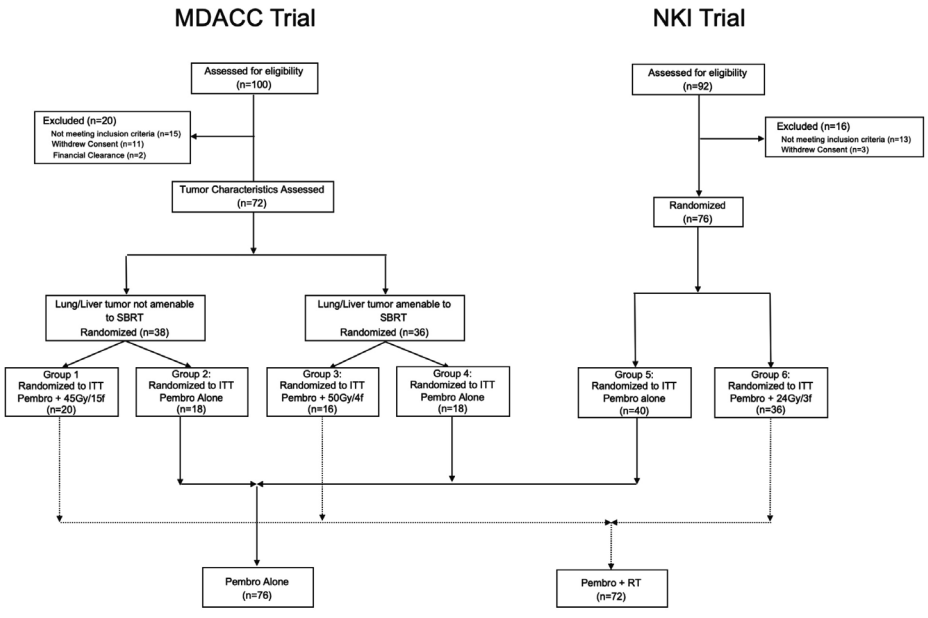
METHODS

Study design, participants and procedures

Both trials (NCT02492568 and NCT02444741) and this pooled post-hoc analysis were approved by the respective institutional review boards. Figure 1 shows a CONSORT diagram of the patient selection from both trials. This study analyzed outcomes for the overall intention-to-treat populations (ITTP), which were pooled based on receipt of pembrolizumab alone versus iRT, regardless of RT schema. Complete information regarding eligibility criteria, enrollment, randomization, and associated workup is included in the trial protocols (Supplemental file 1 and 2) and individual publications.

Of note, both studies required at least 1 unirradiated lesion to monitor the out-of-field response, and both trials administered 200 mg pembrolizumab every 3 weeks. In the PEMBRO-RT trial, the first dose of pembrolizumab was given sequentially <1 week after the last dose of RT, while in the MDACC trial it was given concurrently with the first dose of RT. All patients were immunotherapy-naïve. The Dutch PEMBRO-RT trial (2015-2018) examined only previously chemotherapy-treated patients, evaluated PD-L1 expression a(post-hoc) in nearly all patients, and utilized an RT dose of 24 Gy in 3 fractions (24Gy/3) for all patients in the RT arm [19]. The MDACC trial (2015-2018) encompassed both previously-treated and newly-diagnosed patients, did not mandate PD-L1 assessment, and utilized two fractionation schemas: 50 Gy in 4 fractions (50Gy/4) or (if 50 Gy was subjectively deemed unsafe owing to the size and/or location of the irradiated lesion) 45 Gy in 15 fractions (45Gy/15) with an optional simultaneous integrated boost (SIB) to gross disease of 60 Gy [20]. RT was delivered to 1 site in the PEMBRO-RT study and to a range of 1-4 sites concurrently and with the same dose/fractionation schema for each site in the MDACC study.

Figure 1. Consort diagram.



Randomization and masking

Both studies were open label; owing to the nature of the intervention in the experimental arms (radiotherapy), blinding was not feasible in either trial. In the NKI investigation, patients were randomly assigned using a 1:1 ratio carried out by Alea randomization software (FormsVision 2014) and stratified by smoking status (<10 vs ≥10 pack-years). In the MDACC study, patients were randomized using a 1:1 ratio by the MDACC Department of Biostatistics using the adaptive randomization method by Pocock and Simon with a minimization probability parameter of 0.90. The randomization process was controlled to ensure a balanced stratification by treatment arm.

Statistical analysis

For the PEMBRO-RT trial, the primary endpoint was overall response rate (ORR) at 12 weeks; for the MDACC trial, the primary endpoint was the best ORR. In this analysis, best out-of-field (abscopal) response rate (ARR), ARR at 12 weeks, best abscopal disease control rate (ACR), ACR at 12 weeks, progression free survival (PFS), and overall survival (OS) were evaluated as endpoints. ARR and ACR were defined in unirradiated lesions only based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 confirmed by independent radiologists with separate review at each center. PFS was calculated from the time of randomization to progression or death from any cause, or censored at the date of most recent imaging provided the lack of progressive disease. If the assigned treatment failed, and before patients switched treatment, PFS was censored on the date of the last on-study tumor assessment documenting absence of progressive disease for patients who were alive. OS was calculated from date of randomization to date of death from any cause.

Owing to the pooling of two separate trial populations, a fixed effect model was utilized in order to examine possible heterogeneity between trials; this was estimated by means of the I^2 statistic, which indicates heterogeneity caused by total variation across trials rather than chance. PFS and OS were estimated using the Kaplan-Meier method (KM). In exploratory subgroup analyses, the effect of RT on PFS and OS in predefined subgroups was evaluated using Cox proportional hazard models presented in a forest plot (the forest plot shows outcomes for all subgroup analyses, and findings reported in the Results are restricted to those with a 10% difference in effect size). Univariate and multivariable Cox analyses (covariates being the same variables as in the aforementioned forest plot) were performed to determine significant predictors for PFS and OS. Statistical analyses were performed with IBM SPSS v24 (Chicago, IL) and GraphPad Prism v8 (La Jolla, CA), and a p-value <0.05 was considered statistically significant. Power calculations are located in the original publications of each trial.

Role of the funding source

This analysis was designed by the principal and co-principal investigators of both trials. Both the MDACC and NKI trials were financially supported with an unrestricted grant by Merck Sharp & Dohme that included medication supply. The sponsors had no role in the analysis or interpretation of the data or in the writing of the report. The corresponding author had full access to all of the data and the final responsibility for the decision to submit for publication.

RESULTS

Altogether, 148 patients were analyzed, 72 from the MDACC trial and 76 from the PEMBRO-RT trial. The median follow-up for all patients was 33 months. Of these patients, 76 received pembrolizumab alone and 72 underwent iRT. Four of the twenty 45Gy/15 patients received SIB to 60Gy. Table 1 displays clinical characteristics of both cohorts and supplemental file 3 lists the details of unirradiated lesions.

Table 1. Characteristics between two treatment cohorts.

Parameter	Pembrolizumab	Pembrolizumab + RT	*P* Value
	76	72	
Age			
Median (range, y)	64 (33-82)	65 (33-91)	
≥65	36	35	0.88
<65	40	37	
Gender			
Male	43	42	0.83
Female	33	30	
Histology			
Squamous	13	11	0.76
Non-Squamous	63	61	
Lines of previous chemotherapy			
0	16	21	0.3
1	41	30	
≥2	19	21	
Smoking Status			
Current	13	18	
Former	49	41	0.48
Never smoker	14	13	
Sum of the baseline RECIST measurements			
≤median	42	40	0.97
>median	34	32	
Prior radiation therapy			
≤6 months	8	7	
>6 months	31	29	0.98
No	37	36	
PDL1 status			
Unknown	8	8	
<1%	36	31	0.79
1-49	16	20	
≥50%	16	13	
Radiated tumor site			
Lung, metastasis		28	
Lymph node, intra-thoracic		15	
Lung, primary tumor		12	
Lymph node, extra-thoracic		7	
Adrenal		7	
Bone		4	
Cutaneous		1	
Liver		2	
Retroperitoneal		2	
Pleural		1	

In MDA cohort, 2 patients received 2 lesions RT, 1 with 3 lesions RT and 1 with 4 lesions RT.

The response to treatment is shown in Table 2. For best overall response, the ARR (19.7% vs. 41.7% for pembrolizumab alone and iRT, respectively, p=0.004, odds ratio (OR) 2.96, 95% confidence interval (CI) 1.42-6.20; Supplemental Figure 1) and ACR (43.4% vs. 65.2%, p=0.007, OR 2.51, 95% CI 1.28-4.91; Supplemental Figure 2) were significantly higher in the iRT cohort. The ARR in the iRT arm was higher in each PD-L1 subgroup, but this was non- significant (p=0.08 for PD-L1<1%, OR 0.33, 95% CI 0.10-1.04; p=0.16 for PD-L1 1-49%, OR 0.30, 95% CI 0.06-1.44; p=0.70 for PD-L1 >50%, OR 0.58, 95% CI 0.13-2.69). For response at 12 weeks, ARR was 17.1% in the pembrolizumab alone group and 36.1% in the iRT group (p=0.09, OR 1.95, 95% CI 0.91-4.20; Supplemental Figure 3) and ACR was 38.1% in the pembrolizumab alone group and 62.5% in the iRT group (p=0.003, OR 2.71, 95% CI 1.39-5.28; Supplemental Figure 4).

Table 2. Response to Treatment

	Pembro alone	Pembro+RT	NTT	*P* Value	OR, 95% CI
Best overall response, No., %					
Best ARR	15/76 (19.7)	30/72 (41.7)	2	0.004	0.34 (0.16-0.72)
Best ACR	33/76 (43.4)	47/72 (65.2)	4.58	0.009	0.41 (0.21-0.79)
PD-L1 TPS, %					
<1%	6/36 (16.7)	11/29 (38)	4.69	0.08	0.33 (0.1-1.04)
1-49%	3/14 (21.4)	9/19 (47.4)	3.85	0.16	0.30 (0.06-1.44)
≥50%	5/15 (40)	6/13 (46.2)	16.13	0.70	0.58 (0.13-2.69)
Objective response at 12 wk, No., %					
Overall	14/76 (17.1)	25/72 (36.1)	5.26	0.03	0.42 (0.19-0.9)
Disease control	29/76 (38.1)	45/72 (62.5)	4.09	0.005	0.37(0.19-0.72)

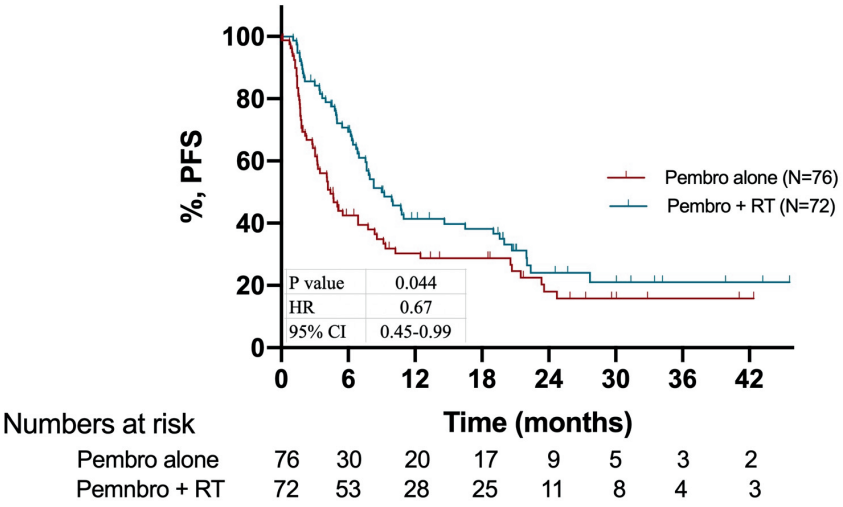
Pembro = pembrolizumab; RT = radiotherapy; NTT = number needed to treat; OR = odds ratio; CI = confidence interval; ARR = abscopal response rate; ACR = abscopal control rate; PD-L1 = programmed death–ligand 1; TPS, tumor proportion score; wk = week.

The median follow-up times were median follow-up times of 33.2 and 34.0 months in pembro alone and pembro+RT group.

Figures 2 and 3 shows comparative outcomes. The iRT cohort experienced a significantly higher median PFS compared to the pembrolizumab alone cohort (9.0 vs. 4.4 months, hazard ratio (HR) 0.67, 95% CI 0.45-0.99, p=0.044, Figure 2A; Supplemental Figure 5). Exploratory subgroup analyses (Figure 2B) suggested that the addition of RT was most beneficial in males (p=0.032), patients having received ≥2 lines of prior chemotherapy (p=0.016), or patients with low (1-49%) PD-L1 expression (p=0.012).

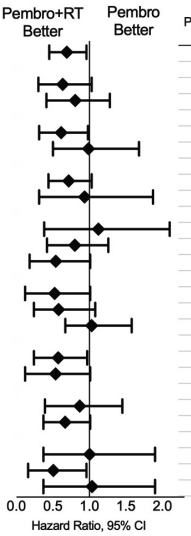
Figure 2. Kaplan-Meier curves for progression-free survival (A) between the pembrolizumab versus iRT cohorts, along with exploratory subgroup analysis (B) of associated factors.

A



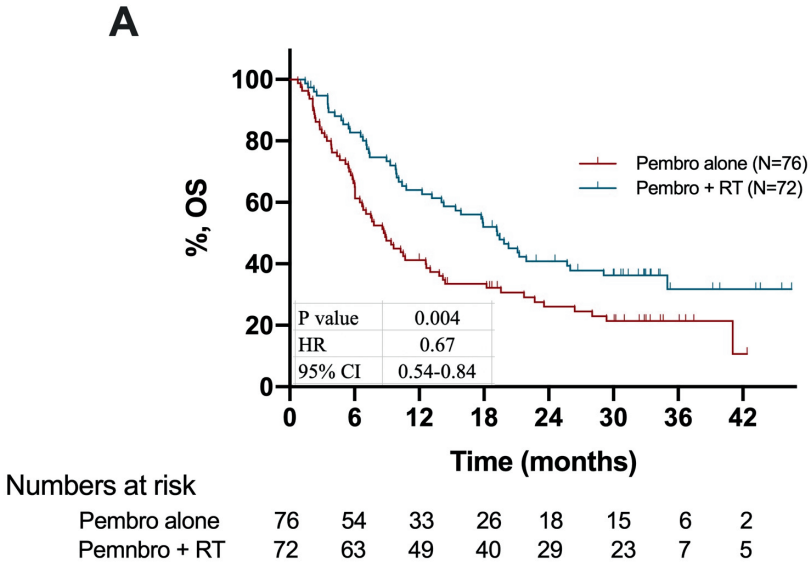
B

Subgroup	Pembro+RT Events, No. / Total No.	Pembro Events, No. / Total No.	HR, 95% CI	Pembro+RT Median PFS (months)	Pembro Median PFS (months)	*P*
Total	59/72	53/76	0.66 (0.45-0.96)	9.0	4.4	0.026
Age						
≥65	30/35	24/36	0.57 (0.30-1.03)	14.1	6.7	0.080
<65	29/37	29/40	0.73 (0.41-1.28)	4.6	6.7	0.330
Gender						
Male	31/42	32/43	0.55 (0.31-0.98)	10.2	6.1	0.032
Female	28/30	21/33	0.78 (0.50-1.68)	6.8	4.9	0.778
Histology						
Non-Squamous	7/11	5/13	0.68 (0.44-1.03)	7.9	4.5	0.054
Squamous	52/61	48/63	0.62 (0.31-1.87)	12.0	10.0	0.411
Previous chemotherapy						
0	15/21	11/16	0.89 (0.38-2.10)	12.3	6.5	0.700
1	28/30	30/41	0.72 (0.42-1.26)	7.4	5.0	0.231
≥2	16/21	12/19	0.43 (0.18-1.01)	8.3	1.6	0.016
Smoking Status						
Current	14/18	10/13	0.44 (0.12-1.01)	7.1	4.4	0.051
Former	37/41	36/49	0.41 (0.42-1.08)	8.4	4.4	0.060
Never	8/13	7/14	0.84 (0.67-1.58)	10.2	3.3	0.210
Sum of the baseline RECIST measurements						
≤median	31/40	26/42	0.51 (0.24-0.98)	11.7	6.5	0.048
>median	28/32	27/34	0.48 (0.12-1.01)	9.5	4.6	0.056
Prior RT						
Yes	23/36	21/39	0.76 (0.39-1.45)	7.4	4.4	0.390
No	36/40	32/37	0.62 (0.37-1.01)	9.6	4.7	0.053
PDL1 status						
<1%	27/31	22/36	0.74 (0.42-1.3)	6.8	4.7	0.290
1-49	14/20	12/16	0.4 (0.16-0.96)	10.4	3.3	0.012
≥50%	11/13	12/16	0.84 (0.37-1.9)	7.5	6.9	0.680

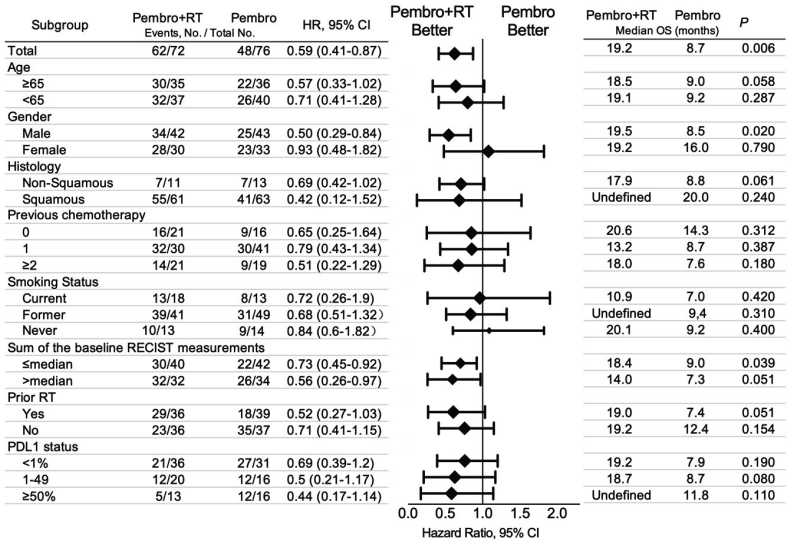


Patients who received iRT experienced significantly higher median OS compared to patients treated with pembrolizumab alone (19.2 vs. 8.7 months, HR 0.67, 95% CI 0.54-0.84, p=0.004, Figure 3A; Supplemental Figure 6). Exploratory subgroup analyses (Figure 3B) suggested a greater effect in males (p=0.02).

Figure 3. Kaplan-Meier curves for overall survival (A) between the pembrolizumab versus iRT cohorts, along with exploratory subgroup analysis (B) of associated factors.



B



A multivariable exploration of prognostic factors showed that never smokers and an RT schema of 50Gy/4 fractions were significantly associated with PFS (Table 3). There were no factors significantly associated with OS. Full details of adverse events are reported in the original publications of the two trials [19, 20]. Briefly, high-grade RT-related AEs were extremely uncommon and the pembrolizumab-related AEs were similar to those reported in other mNSCLC pembrolizumab monotherapy studies [3, 21, 22]; no new concerns regarding safety arose from this analysis.

Table 3. Univariate and multivariable Cox-analyses for intention-to-treat population.

	Univariate-	Multivariate-PFS			Univariate-OS			Multivariable-OS		
	P	HR	95% CI	*P*	HR	95%CI	*P*	HR	95% CI	*P*
Gender	0.39						0.26			
Male		0.85	0.57-1.25	0.44	0.78	0.50-1.21		0.77	0.50-1.21	0.26
Female		Ref			Ref			Ref		
Age	0.09						0.18			
≥65		1.33	0.89-1.98	0.17	0.97	0.64-1.48		0.97	0.64-1.48	0.90
<65		Ref			Ref			Ref		
Histology	0.16						0.07			
Non-Squamous		0.66	0.37-1.18	0.16	0.55	0.28-1.06		0.55	0.28-1.06	0.07
Squamous		Ref			Ref			Ref		
Smoking history	0.044						0.28			
Never		0.76	0.44-0.98	0.048	0.81	0.41-1.32		0.83	0.39-1.32	0.30
Former		0.91	0.37-1.33	0.097				1.03	0.80-1.76	0.40
Current		Ref			Ref			Ref		
Lines of previous chemotherapy	0.032						0.52			
0		0.81	0.46-1.22	0.28	1.03	0.61-1.81		1.03	0.61-1.81	0.52
1		0.87	0.54-1.12	0.071	Ref			0.81	0.56-1.53	0.62
≥2		Ref			0.81	0.56-1.53		Ref		
Previous radiotherapy	0.57						0.26			
Yes		1.36	0.90-2.04	0.14	1.28	0.83-1.96		1.28	0.83-1.96	0.26
No		Ref			Ref			Ref		
PD-L1, %	0.11						0			
0		Ref			Ref			Ref		
1-49		1.04	0.56-1.92	0.91	0.73	0.42-1.27		0.73	0.42-1.27	0.27
≥50		0.83	0.4-1.72	0.62	0.53	0.29-1.12		0.53	0.29-1.12	0.09
Treatment	0.03						0.038			
Pembrolizumab alone		Ref			1.57	1.03-2.40				
Pembrolizumab+45Gy/5f		0.98	0.43-1.53	0.57				1.169	0.73-2.40	0.34
Pembrolizumab+24Gy/3f		0.76	0.46-1.09	0.083				0.84	0.53-1.43	0.14
Pembrolizumab+50Gy/4f		0.67	0.36-0.98	0.047				0.82	0.34-1.87	0.23
Irradiated lesion	0.026						0.15			
Primary lung		Ref						Ref		
Metatistic lung		0.68	0.32-1.26	0.41				0.77	0.43-1.56	0.37
Lymph nodes		1.21	0.85-1.72	0.66				0.98	0.43-1.67	0.24
Others		0.83	0.43-1.39	0.34				1.07	0.72 -1.63	0.42

PFS = progression-free survival; OS = overall survival; HR=hazard ratio.
The four multi-RT patients were analyzed in the metastistic lung group as all of them had at least 1 lesion received RT in the lung. Progression-free survival was defined as the time from randomization to progression.

In the MDACC trial, RT scheme was chosen subjectively based on physicians' discretion and safety owing to the size and/or location of the irradiated lesion dose. Therefore, this pooled analysis is not suited to address the comparative efficacy of various RT schemas. However, it was notable that the ARR for the 45Gy/15 subgroup seemed similar to patients who received no RT, and that the ARR for both the 50Gy/4 and 24Gy/3 subgroups were similar as well, but over twice as high as the other 2 subgroups (Supplemental Figure 7). To further probe into this finding, we evaluated the difference in absolute lymphocyte count (ALC) before and after RT based on particular schema. As lymphocytes are important for an effective antitumor immune response, but are also very radiosensitive, specific RT schemas may also negatively influence the antitumor immune response induced by immunotherapy.

There was a significant drop in ALC for only the 45Gy/15 subgroup, whereas no effect on ALC was seen in both other schemas, suggesting a potentially detrimental effect with the 45Gy/15 schema (Supplementary Figure 8).

DISCUSSION

Despite the mounting pre-clinical and clinical data describing the augmenting effects of RT on immunotherapeutic treatment of mNSCLC, the only two existing randomized trials thus far were not able to show a significant improvement in patient outcomes, likely owing to limited sample size[19, 20]. This was the primary impetus to perform a pooled analysis of both studies, resulting in the largest prospectively collected cohort assembled to date. We found that adding RT to immunotherapy significantly increased the response rates of unirradiated lesions, which led to a significantly higher PFS and OS.

The abscopal effect is a relatively uncommon phenomenon, although it has been proposed that the addition of RT to immunotherapy could enhance the occurrence of abscopal responses and hence improve outcomes. To date, higher-volume randomized clinical data have been largely absent. This pooled analysis largely comprised irradiated intrathoracic disease; it shows that the abscopal effect was induced considerably more often with the addition of RT. The improved control of systemic disease likely drove the improved PFS and OS findings in the iRT arm.

Notably, both of the trials were powered to detect a 30% difference in response rate. While the primary endpoint for both trials showed improvement in the iRT arm, the results of neither study met the prespecified criteria for meaningful clinical benefit [19, 20]. A larger sample size would therefore likely be required to more accurately detect the effects of the addition of RT to immunotherapy on patient outcomes. Also, one of the major concerns in the PEMBRO-RT trial was the imbalance of PD-L1 distribution in favor of the iRT arm. Pooling the data of both trials eliminated this imbalance, strengthening the evidence that the observed improvement in patient outcomes was indeed due to the addition of RT.

Although this pooled analysis alleviates sample size concerns from each individual trial, it should be mentioned that the subgroup analyses are undoubtedly still limited by a low sample size and should thus be evaluated with caution. Notably, there was no correlation of PD-L1 expression with outcomes in our combined cohort. The improvements by iRT for the PD-L1 negative patients within the PEMBRO-RT study disappeared in this pooled analysis. This could be from bias due to the lack of PD-L1 scores for 19% (14/72) of the MDACC cases (as compared to only 3% (2/76) of PEMBRO-RT cases). Additionally, other data that may have a predictive role for response to immunotherapy, such as tumor mutational burden and baseline immune status, were not available. Taken together, it remains difficult to conclude whether a meaningful association between PD-L1 status and benefit from iRT exists, and larger-volume studies with mandatory PD-L1 assessment are required to address this unresolved question.

To date, many questions remain about the impact of different RT dose and fractionation schemas on the magnitude of the immune-boosting effect. RT schemas were variable in both trials largely because there is currently no consensus on optimal RT dosing in the mNSCLC setting. Because RT schemas were not applied randomly, but rather based on trial variability and/or physicians' discretion, statistical comparison between RT schemas was not feasible. Nevertheless, the large difference in ARR of the 50Gy/4 and 24Gy/3 subgroups compared to the 45Gy/15 and pembrolizumab alone subgroups remained striking. These results logically lead to inquiry regarding whether the findings were due to differences in ALC, unforeseen clinical factors associated with physician choice of RT schema, or both. With regard to ALC, the observation that 45Gy/15 fraction RT was associated with a more pronounced ALC decline, along with the similar ARR as pembrolizumab alone (20% for both) requires further investigation. With regard to unforeseen clinical factors, patients in the 24Gy/3 fraction cohort were more heavily pretreated, and were less likely to have received RT before study inclusion. There were also some important differences in trial design. The timing of RT and pembrolizumab was different between trials. Also, in the PEMBRO-RT study, only one lesion was treated with RT; in the MDACC study, up to 4 lesions were irradiated. Although only 4/16 (25%) patients in the 50Gy/4 cohort received concurrent multiple-site RT, these patients tended to perform better (data not shown) and could explain why the Cox multivariable analysis revealed that the 50Gy/4 fraction schema was significantly associated with PFS (but not OS). Additionally, this analysis did not show a differentially advantageous location or designated target lesion (e.g. primary vs metastasis) for application of RT owing to the similar PFS and OS in the corresponding subgroups. Taken together, owing to the multitude of aforementioned reasons, conclusions regarding the optimal dosing, timing or location of RT in order to induce an abscopal response cannot be drawn from this study.

In summary, this pooled analysis of two randomized trials examining pembrolizumab with or without RT in mNSCLC showed that the addition of RT to immunotherapy significantly increased the ARR, and was additionally associated with significant improvements in PFS and OS. These hypothesis-generating results should be corroborated in a dedicated, large-volume, randomized trial.

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SUPPLEMENTARY DATA

Figure S1. Results for the heterogeneity I2 test and the fixed effect model for best abscopal response rate (ARR).

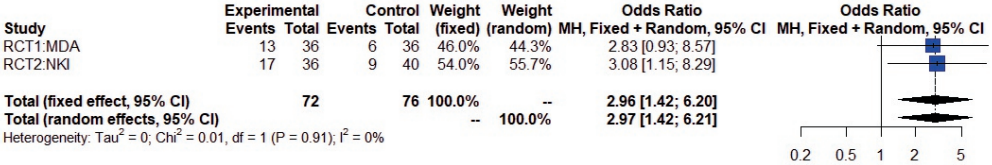

Figure S2. Results for the heterogeneity I2 test and the fixed effect model for best best abscopal control rate (ACR).

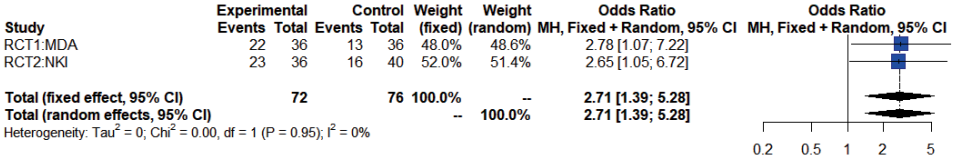

Figure S3. Results for the heterogeneity I2 test and the fixed effect mode for abscopal response rate (ARR) at 12 weeks.

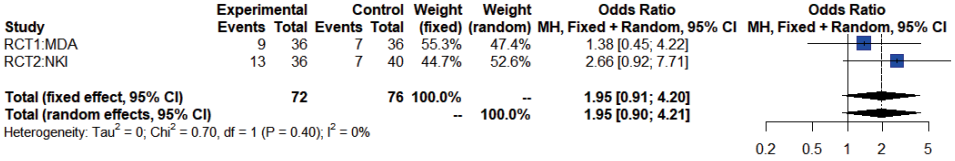

Figure S4. Results for the heterogeneity I2 test and the fixed effect modelfor abscopal control rate (ACR) at 12 weeks.

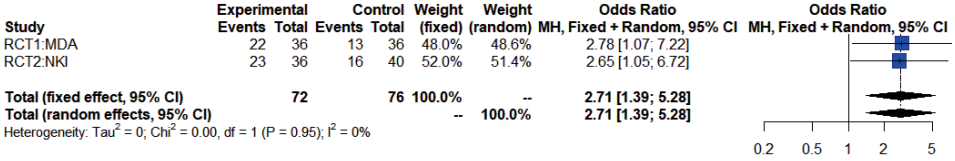

Figure S5. Results for the heterogeneity I2 test and the fixed effect model for abscopal control rate (ACR) at 12 weeks.

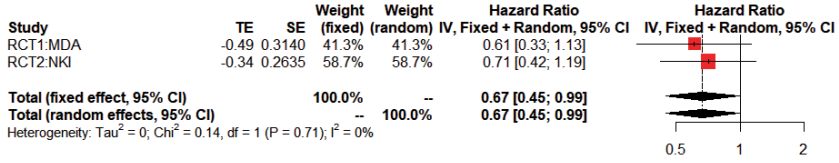

Figure S6. Results for the heterogeneity I2 test and the fixed effect model for median overall survival (OS).

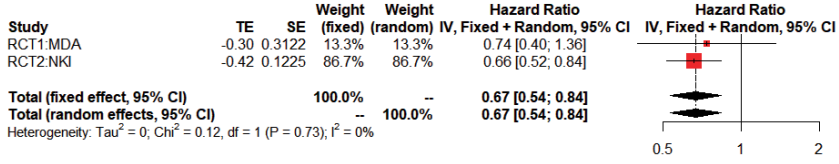

Figure S7. Best out-of-field (abscopal) response rates for various RT schemas and pembrolizumab alone.

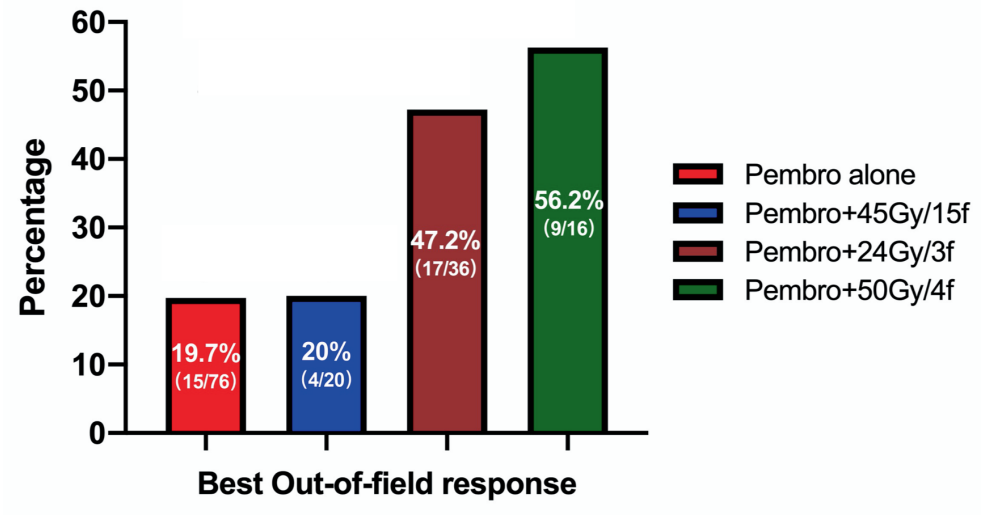

Figure S8. Absolute leucocyte count (ALC) change after RT for various RT schemes.