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Clinical Trial Results

Safety and Feasibility of Radiotherapy Plus Camrelizumab for Locally Advanced Esophageal Squamous Cell Carcinoma

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Key Words. Radiotherapy • Immunotherapy • PD-1 • Camrelizumab • Esophageal cancer

Trial Information

- **ClinicalTrials.gov Identifier:** NCT03222440
- **Sponsor:** Tianjin Medical University Cancer Institute & Hospital
- **Principal Investigators:** Ping Wang, Qingsong Pang
- **IRB Approved:** Yes

Lessons Learned

- Radiotherapy plus anti–PD-1 antibody as first-line therapy is safe and feasible in locally advanced esophageal squamous cell carcinoma (ESCC).
- Tumor-infiltrating and peripheral lymphocytes were associated with patient survival.
- Further studies combining chemoradiotherapy with immunotherapy in locally advanced ESCC and exploration of predictive biomarkers are warranted.

Abstract

Background. We conducted a phase Ib study of radiotherapy plus programmed cell death protein 1 (PD-1) monoclonal antibody camrelizumab as first-line treatment for locally advanced esophageal squamous cell carcinoma (ESCC).

Methods. We planned to enroll 20 patients with newly diagnosed locally advanced ESCC. Patients received 60 Gy radiation (2.0 Gy/fraction, 5 fractions/week), with camrelizumab (200 mg every 2 weeks) starting with radiotherapy and continuing for 32 weeks (i.e., for 16 cycles). The primary endpoints were safety and feasibility. Secondary endpoints were rates of radiologic and pathologic response, overall survival (OS), and progression-free survival (PFS). Study data were collected by the week during radiotherapy (RT), every month during the maintenance camrelizumab treatment, and every 3 months after treatment. Tumor microenvironment and peripheral blood were monitored at baseline and after 40 Gy radiation for association with efficacy.

Results. Twenty patients were enrolled and received treatment. One patient (patient 10) was excluded upon discovery of a second tumor in the bladder during treatment, leaving 19 patients for analysis. Toxicity was deemed tolerable. Fourteen (74%) patients had assessed objective response. At a median follow-up time of 31.0 months (95% confidence interval [CI], 27.0–35.1), median OS and PFS times were 16.7 months (95% CI, 5.9–27.9) and 11.7 months (95% CI, 0–30.3), respectively. OS and PFS rates at 24 months were 31.6% and 35.5%, respectively. Kaplan-Meier analysis revealed associations between the following factors and OS/PFS: tumor programmed cell death ligand 1 (PD-L1) expression, PD-1^+CD8^+, PD-1^+CD4^+ T cells, and PD-$L1^+CD4^+$ T cells; peripheral blood $CD4^+$, $CD8^+$, $CD4^+$ regulatory T cells, and their subsets.

Conclusion. Radiotherapy plus camrelizumab had manageable toxicity and antitumor efficacy for locally advanced ESCC. Several biomarkers were associated with clinical benefit and deserve further study. ***The Oncologist*** 2021;26:e1110–e1124

Discussion

Camrelizumab (SHR-1210) is a selective, humanized, IgG_4-kappa PD-1 monoclonal antibody. It was developed by Jiangsu Hengrui Medicine Co. Ltd. and received conditional approval in China for

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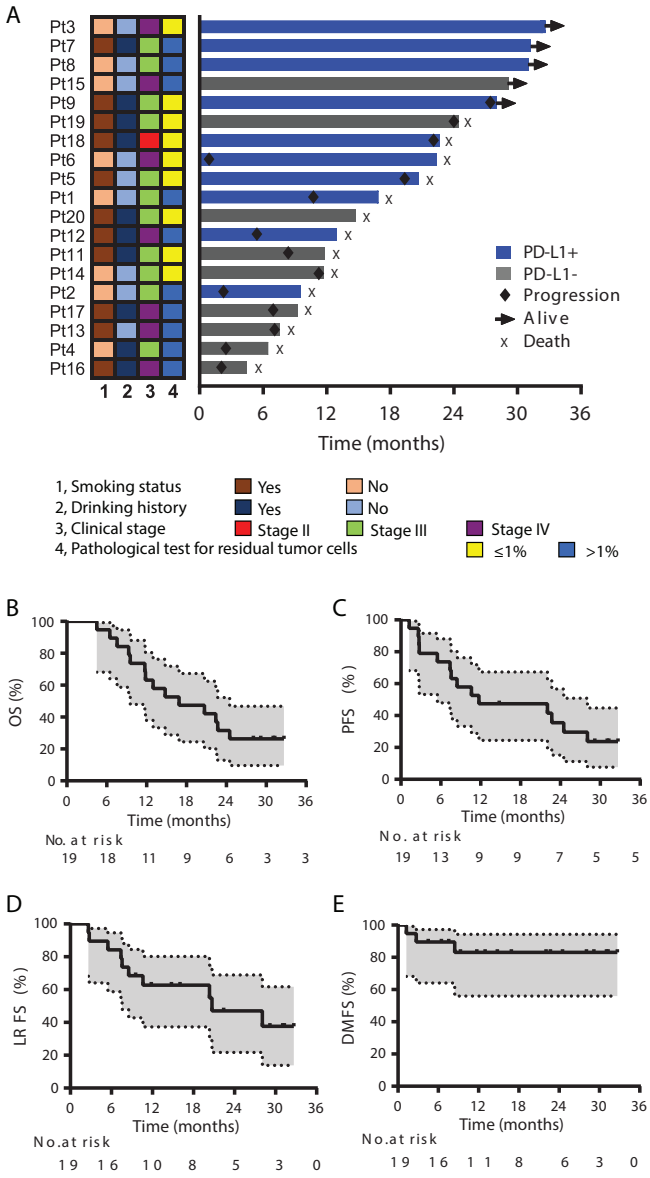

Figure 1. Antitumor efficacy of combining radiotherapy and SHR-1210. **(A):** Treatment exposure and response duration. The length of each bar represents the time to the last radiographic assessment according to RECIST version 1.1. Clinical and pathological features (smoking status, drinking history, clinical disease stage, and pathological test for residual tumor cells after 40 Gy) are shown for each patient (per RECIST version 1.1 by investigator review). **(B):** Overall survival. **(C):** Progression-free survival. **(D):** Local recurrence–free survival. **(E):** Distant metastasis–free survival.
Abbreviations: DMFS, distant metastasis–free survival; LRFS, local recurrence–free survival; OS, overall survival; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; Pt, patient.

the treatment of relapsed or refractory classical Hodgkin lymphoma in 2019. In 2020, another three indications were added for camrelizumab in China: (a) patients with advanced hepatocellular carcinoma who have previously received sorafenib and/or oxaliplatin-based systemic chemotherapy; (b) combined with pemetrexed and carboplatin as the first-line treatment of locally advanced or metastatic nonsquamous non-small cell lung cancer that has no *EGFR* or *ALK* mutation; and (c) patients with locally advanced or metastatic ESCC as second-line treatment.

In this phase Ib study, we investigated the safety and feasibility of definitive RT plus camrelizumab as first-line therapy for patients with locally advanced ESCC who were either ineligible for or had refused concurrent chemoradiotherapy (CCRT).

Of 19 patients enrolled from July 24, 2017, through January 25, 2018, 18 completed RT; 13 (68%) completed full cycles of camrelizumab. Objective responses were observed in 14 (74%) patients (2 [11%] complete responses and 12 [63%] partial responses). Among the 10 patients with PD-L1–positive tumors, 5 experienced a major pathologic response (indicated by ≤1% viable residual tumor cells in the tumor specimen) (Fig. 1A).

The target result expected was a median OS of 14 months. Our results showed median OS time at 16.7 months (95% CI, 5.9–27.9); 12-month and 24-month OS rates were 63.2% and 31.6% (Fig. 1B). Median PFS time was 11.7 months (95% CI, 0–30.3); 12-month and 24-month PFS rates were 47.4% and 35.5% (Fig. 1C). The relative shorter 24-month OS rate compared with 24-month PFS rate was because one patient died from cerebral infarction after 14.8 months of enrollment. Rates of locoregional recurrence–free survival were 62.7% at 12 months and 48.8% at 24 months (Fig. 1D), and corresponding distant metastasis–free survival rates were both 83.1% (Fig. 1E). At 24 months, locoregional recurrence occurred in nine (47%) patients, and distant metastasis occurred in three (16%) patients (two in liver and one in bone). Although CCRT is considered standard therapy for locally advanced ESCC, median OS times are only about 18.1–19 months. For patients who are intolerant to or refuse chemotherapy, RT is the main treatment. However, the median OS was 12 months. The median OS in our study was comparable to that after CCRT and improved compared with RT alone [1–3].

All patients experienced some form of treatment-related adverse events (AEs), but most were grade 1–2, and no grade 5 events were reported. The toxicity profile after RT plus camrelizumab was similar to previous reports of either modality given alone. Cutaneous capillary hemangioma was observed in 17 (89%) patients (15 [79%] with grade 1; 2 [10%] with grade 2), which was the immune-related AE. Episodes of grade 3 and grade 4 RT-related adverse events including lymphopenia, esophagitis, laryngitis, and leukopenia did not interrupt RT. These results indicate that the combination therapy did not increase RT-related toxicity compared with RT alone and that adverse events associated with the combination therapy were less severe than those associated with CCRT [1, 3].

We found that tumor microenvironment markers were associated with survival. High tumor PD-L1 (≥1%) expression at baseline was correlated with better OS. Interestingly, whole $CD8^+$ or $CD4^+$ T-cell population in tumor tissues was not associated with survival, but their subpopulations ($PD\text{-}1^+CD8^+$, $PD\text{-}L1^+CD4^+$, and $PD\text{-}1^+CD4^+$ T cells) were associated with OS/PFS. Our findings in peripheral blood supported the important role of $CD4^+$, $CD8^+$, and $CD4^+$ regulatory T (T_{reg}) cells in antitumor response. The association between OS/PFS and T-cell subsets of $Ki67^+CD8^+$, interferon-γ^+CD8^+, $Ki67^+T_{reg}$, and cytotoxic T-lymphocyte–associated protein 4–positive $CD4^+$ T cells in peripheral blood suggest that the T-cell status and function, such as proliferation, activation, or exhaustion, deserve more studies in future.

This first study of RT plus camrelizumab as first-line treatment showed promising efficacy and a manageable safety profile in patients with locally advanced ESCC.

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Trial Information	
Disease	Esophageal cancer
Stage of Disease/Treatment	Primary
Prior Therapy	None
Type of Study	Phase I/Ib
Primary Endpoints	Safety, toxicity
Secondary Endpoints	Efficacy, biomarker associations

Additional Details of Endpoints or Study Design

The main inclusion criteria were age ≥18 years; newly diagnosed, locally advanced ESCC (T3–4N0M0 or T1–4N^{+}M0, stage II–IVa according to the 8th [2017] edition of the American Joint Committee on Cancer staging system); no prior antitumor treatment; ineligible for or declined concurrent chemoradiotherapy; Eastern Cooperative Oncology Group score 0 or 1; adequate liver function; and normal blood cell counts. The main exclusion criteria were diagnosis of immunodeficiency, ongoing systemic immunosuppressive therapy, active autoimmune disease, human immunodeficiency virus, and clinically significant concurrent cancer.

Cervical, thoracic, and above-abdomen computed tomography scans and upper gastrointestinal radiography were obtained every 3 months after treatment until disease progression. All patients underwent endoscopic ultrasonography, the standard clinical practice for potential tumor biopsy, after receiving 40 Gy of radiation [20] (i.e., at the end of 4 weeks of RT) in order to confirm the pathological response rates and laboratory tests.

The primary endpoints were safety and feasibility. Treatment-related adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. We estimated that the risk of grade 5 adverse events would occur at a rate of about 5% according to the reported adverse events in concurrent chemoradiotherapy.

The secondary endpoints were radiologic and pathologic response, scored according to RECIST version 1.1 by individual clinicians. Outcome measures were objective response rate 4 weeks after the end of RT and PFS and OS. Locoregional recurrence–free survival (LRFS) and distant metastasis–free survival (DMFS) rates were also assessed in this study. We expected that median OS, being a benchmark, would exceed 14 months according to median OS of 12 months with RT alone.

The exploratory endpoints included biomarkers that associated with OS and PFS. Pairs of tumor tissue biopsy samples and EDTA-anticoagulant–treated peripheral blood specimens were to be collected before treatment (i.e., baseline) and during treatment (after the delivery of 40 Gy RT). Immunohistochemistry and six-color immunofluorescence were used to investigate PD-L1 expression and identify tumor-infiltrating lymphocytes in tumor tissues. Multiple-color flow cytometry was applied to investigate markers in peripheral T-cell populations.

Among enrolled and eligible patients, the truncated sequential probability test was used to evaluate objective response rate. Statistical tests included two-sided Fisher's exact tests and two-sided Mann-Whitney *U* tests. The Kaplan-Meier method was used to estimate PFS, OS, LRFS, and DMFS. The best cutoff of Kaplan-Meier survival analysis was calculated by the Youden index of the receiver operating characteristic curve. Differences in survival and recurrence rates were compared with log-rank tests for all markers. SPSS (version 21.0; STATA, College Station, TX) or R version 3.2.2 packages were used for all analyses. All reported *p* values were two-sided, and the significance level was set at .05.

Investigator's Analysis	Drug tolerable, hints of efficacy

Drug Information: Camrelizumab	
Generic Name	Camrelizumab
Drug Type	Antibody
Drug Class	Immune therapy
Dose	200 milligrams (mg) per flat dose
Route	i.v.
Schedule of Administration	SHR-1210 was infused intravenously at a fixed dose of 200 mg once every 2 weeks from the beginning of radiotherapy for up to 32 weeks (i.e., for 16 cycles). Radiotherapy was delivered as RapidArc (volumetric arc) intensity-modulated RT with a simultaneous integrated boost. The radiotherapy was given according to Chinese treatment guidelines for esophageal carcinoma and was prescribed to cover 95% of the planning gross tumor volume (PGTV), given at 2.0 Gy per fraction, five fractions per week, to a total of 60 Gy over 6 weeks. The dose prescribed to cover 95% of the planning target volume (PTV) was 1.8 Gy per fraction, five fractions per week, for a total of 54 Gy over 6 weeks. Target volumes were as described previously. Briefly, gross tumor volume (GTV) was determined based on the results of upper gastrointestinal radiography, esophageal endoscopy, and chest computed tomography. If lymphatic metastasis was present in the mediastinum, supraclavicular region, or abdominal cavity, GTV in involved lymph nodes was delineated. The clinical target volume (CTV) was

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delineated as GTV and plus 3-cm margins in the vertical direction, which covered the corresponding lymphatic drainage areas, and 0.6-cm margins in the anteroposterior and transverse directions, which did not exceed the anatomic boundary. PGTV and PTV was defined as GTV or CTV plus 5-mm margins, individually. No additional adjuvant or induction chemotherapy was performed.

Patient Characteristics	
Number of Patients, Male	12
Number of Patients, Female	7
Stage	Stage II: 1 Stage III: 11 Stage IV: 7
Age	Median (range): 64 (46–74) years
Number of Prior Systemic Therapies	0
Performance Status: ECOG	0 — 10 1 — 9 2 — 0 3 — 0 Unknown — 0
Other	Smoking status: Never: 8; Former or current: 11 Drinking status: Never: 9; Former or current: 10 Location: Cervical segment: 1; Upper thoracic segment: 5; Middle thoracic segment: 11; Inferior thoracic segment: 2
Cancer Types or Histologic Subtypes	Esophageal squamous cell carcinoma, 19

Primary Assessment Method	
Title	Safety and Feasibility
Number of Patients Screened	26
Number of Patients Enrolled	19
Number of Patients Evaluable for Toxicity	19
Number of patients Evaluated for Efficacy	19
Evaluation Method	NCI, CTCAE, version 4.0
Response Assessment CR	$n = 2$ (10.5%)
Response Assessment PR	$n = 12$ (63.2%)
Response Assessment SD	$n = 2$ (10.5%)
Response Assessment PD	$n = 3$ (15.8%)
Response Assessment OTHER	$n = 0$ (0%)
(Median) Duration Assessments PFS	11.7 months, CI: 0–30.3
(Median) Duration Assessments OS	16.7 months, CI: 5.9–27.9

Secondary Assessment Method	
Title	Objective Response Rate, OS, PFS
Number of Patients Screened	26
Number of Patients Enrolled	19
Number of Patients Evaluable for Toxicity	19
Number of Patients Evaluated for Efficacy	19
Evaluation Method	RECIST 1.1
Response Assessment CR	$n = 2$ (10.5%)
Response Assessment PR	$n = 12$ (63.2%)

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Response Assessment SD	$n = 2$ (10.5%)
Response Assessment PD	$n = 3$ (15.8%)
Response Assessment OTHER	$n = 0$ (0%)
(Median) Duration Assessments PFS	11.7 days, CI: 0–30.3
(Median) Duration Assessments OS	16.7 days, CI: 5.9–27.9

Outcome Notes

Evaluation of objective response rate (with RECIST 1.1) showed 2 (10%) complete responses, 12 (63%) partial responses, 2 (10%) stable disease, and 3 (16%) progressive disease. The median OS time was 16.7 months (95% CI, 5.9–27.9); OS rates were 63.2% at 12 months and 31.6% at 24 months (Figure 1B). The median PFS time was 11.7 months (95% CI, 0–30.3); PFS rates were 47.4% at 12 months and 35.5% at 24 months.

Adverse Events

All Cycles Name	NC/NA, %	Grade 1, %	Grade 2, %	Grade 3, %	Grade 4, %	Grade 5, %	All grades, %
Skin and subcutaneous tissue disorders	11	79	11	0	0	0	89
Lung infection	89	5	5	0	0	0	11
Pneumonitis	89	0	5	5	0	0	11
Esophagitis	53	32	16	0	0	0	47
Laryngitis	95	0	0	5	0	0	5
Constipation	74	16	11	0	0	0	26
Blurred vision	89	11	0	0	0	0	11
Proteinuria	63	37	0	0	0	0	37
Blood and lymphatic system disorders - Leukopenia	68	16	11	5	0	0	32
Blood and lymphatic system disorders - Neutrophilic granulopenia	68	0	32	0	0	0	32
Blood and lymphatic system disorders - Lymphopenia	53	0	11	32	5	0	47
Anemia	95	5	0	0	0	0	5
Alanine amino - transferase increased	89	11	0	0	0	0	11
Hyperglycemia	74	26	0	0	0	0	26
Fatigue	95	0	5	0	0	0	5
Cough	95	0	0	5	0	0	5
Respiratory, thoracic and mediastinal disorders	84	16	0	0	0	0	16
Hypothyroidism	84	16	0	0	0	0	16

Treatment-related adverse events occurring in all cycles.

All patients experienced some form of treatment-related AEs (Table 2), but most were grade 1–2, and no grade 5 events were reported. The most common type of toxicity was cutaneous capillary hemangioma (15 [79%] with grade 1 and 2 [10%] with grade 3), which was the immune-related AE; all cases were managed with local therapy or observation. Nine patients (47%) experienced grade 3 adverse events: six with lymphopenia (which was not treated), one with radiation pneumonitis, one with radiation laryngitis (treated with dexamethasone), and one with leukopenia (treated with granulocyte-macrophage colony-stimulating factor). The patient with radiation laryngitis had cervical esophageal cancer. One patient (5%) experienced grade 4 lymphopenia, which was not treated. Three patients experienced grade 1 hypothyroidism. Other organ-specific immune-related AEs, such as thyroiditis, stomatitis, and colitis, were not found.

Abbreviation: NC/NA, no change from baseline/no adverse event.

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Serious Adverse Events		
Name	**Grade**	**Attribution**
Radiation pneumonitis	3	Possible
Radiation laryngitis	3	Unrelated

Serious treatment-related adverse events occurred in all cycles.

Assessment, Analysis, and Discussion	
Completion	Study completed
Investigator's Assessment	Drug tolerable, hints of efficacy

In the current study, we report that radiotherapy (RT) plus camrelizumab, used as first-line therapy, has promising antitumor activity and a manageable toxicity profile for patients with locally advanced esophageal squamous cell carcinoma (ESCC). We further found associations between a variety of immune function–related biomarkers and overall survival (OS) and progression-free survival (PFS) (Fig. 2).

Although concurrent radiotherapy (CCRT) is considered standard therapy for locally advanced ESCC, local recurrence rates after such therapy are as high as 50%, and median OS times are only about 18.1–19 months [1–3]. For patients who refuse or were ineligible for CCRT, RT alone is the treatment of choice; however, 5-year OS rates after RT alone are <10% after two-dimensional RT and 20%–30% for three-dimensional conformal RT [1, 4, 5]. Recent clinical trials indicated that using anti–programmed cell death protein 1 (PD-1) antibodies (nivolumab, pembrolizumab, or camrelizumab) as second-line or consolidation therapy achieved objective clinical response rates of 22%–33% and a median PFS time of 1.8–3.6 months in patients with esophageal cancer [6–10]. In the current study of 19 enrolled patients, the combination of RT and camrelizumab resulted in median OS and PFS times of 16.7 months and 11.7 months in patients with newly diagnosed locally advanced ESCC who could not tolerate or declined CCRT (Table 1–3; Fig. 1). These durations are comparable to those after CCRT [1–3]. Indeed, clinical and preclinical studies of animal models have shown synergistic antitumor effects from adding RT to immunotherapy for advanced solid tumors [11–14]. Our findings support these studies.

We found that the toxicity profile after RT plus camrelizumab was similar to previous reports of either modality given alone (Table 4). The grade 3 and grade 4 RT-related adverse events in the current study consisted of lymphopenia, esophagitis, laryngitis, and leukopenia; these were managed without the need to interrupt RT. These results indicate the combination therapy did not increase RT-related toxicity compared with RT alone and that the adverse events of the combination therapy less severe than those associated with CCRT [1, 3]. Although we could not distinguish RT and immune-related pneumonitis on computed tomography scan, the incidence of pneumonitis in the present study (5% in grade 3) was not higher than that when camrelizumab was applied alone (6.7% in grade 3) [7]. The most common immune-related adverse event (AE) in our study was grade 1 and 2 cutaneous capillary hemangioma (17 [89%] patients). Cutaneous capillary hemangioma has been reported in other clinical trials of camrelizumab [7, 15, 16] but has not been reported after nivolumab or pembrolizumab; its mechanism, which may be specific to camrelizumab, is under investigation [17]. Three patients experienced grade 1 hypothyroidism. Other organ-specific immune-related AEs, such as thyroiditis, stomatitis, and colitis, were not found. These results indicated that the incidence of immune-related AEs did not increase when RT was combined with anti–PD-1 antibody.

We did observe some evidence of clinical benefit from RT plus camrelizumab in a few patients, suggesting that identifying those patients who are likely to benefit would be helpful. With tumor tissue samples from 17 patients at baseline and 16 patients during treatment assessed by multiplex immunofluorescence assay (Fig. 3), we found T-cell subsets in tumor tissues were disrupted by RT combined with camrelizumab treatment (Fig. 4). Programmed cell death ligand 1 (PD-L1) expression, density of PD-1^+CD8^+ cells, ratio of PD-$L1^+/CD4^+$ T cells in baseline tumors, and density of PD-1^+CD4^+ T cells in both baseline and on-treatment tumors were associated with patient survival (Fig. 5). We conclude that PD-$L1^+CD4^+$ tumor-infiltrating lymphocytes (TILs) (mostly consisting of regulatory T [T_{reg}] cells) and PD-1^+CD8^+ TILs contributed to the inhibitory immune microenvironment in ESCC. Infiltration of TILs has previously been linked with a positive survival benefit in esophageal cancer [18]. However, a recent study revealed patients with higher-grade renal cell carcinoma had high levels of exhausted PD-1 and T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) double-positive $CD8^+$ TILs [19]. The PD-1^+CD8^+ TILs were probably bystander TILs that recognized unrelated tumor antigens [20] and thus could not function as tumor-specific killers even though PD-1/PD-L1 signaling was inhibited. On the contrary, our result indicated that PD-1^+CD4^+ TILs probably generated antitumor activity, which was consistent with a recent study from triple negative breast cancer that showed PD-1^+CD4^+ TILs were associated with better survival [21]. It has been reported that $CD4^+$ T cells enhance the killer function of $CD8^+$ T cells mediated by interleukin-21 and adapting antigen presentation [22]. RT convert tumors into "in situ vaccines" [23], and RT plus PD-1 antibody promoted the release of tumor antigens [24]. These results suggested that the combination of RT and PD-1 antibody could result in a synergistic antitumor effect by both priming and reactivating immune response.

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With 18 pairs of peripheral blood T cells at baseline and during treatment evaluated by flow cytometric analysis, we found the ratios of $CD3^+$/lymphocytes, $CD8^+/CD3^+$, $CD4^+/CD3^+$, and $CD4^+Foxp3^+$ $T_{reg}/CD3^+$ cells were not significantly affected by RT plus camrelizumab, although the total lymphocytes decreased (Fig. 6). The decreased expression of PD-1 on T cells after combination treatment indicated regulatory effect of anti–PD-1 antibody (Fig. 6). Notably, we found the $CD4^+$ and $CD8^+$ T cells in the peripheral blood conversely associated with survival regardless of the time they were measured (baseline vs. during treatment) (Table 5; Fig. 7). Furthermore, we found the specific T-cell subsets, such as $Ki67^+CD8^+$, interferon-γ^+CD8^+, $Ki67^+$Treg, and cytotoxic T-lymphocyte–associated protein 4–positive $CD4^+$ T cells, also closely associated with patient survival (Table 5; Fig. 7).

These findings from tumor microenvironment and systemic immune status not only provide candidates for predictive biomarkers but also indicate that the specific status and function of T-cell subsets, such as proliferation, activation, or exhaustion, might play more important roles in antitumor immune response compared with "bulk" T cells and need to be emphasized in further studies.

Conclusively, this study of RT combined with camrelizumab showed promising efficacy and a manageable safety profile in patients with locally advanced ESCC. Our results further revealed several potential immune biomarkers in tumor tissues and peripheral blood. However, this study was limited by the small number of patients included. We have launched a single-arm phase I study (NCT03671265) to further explore the response to CCRT combined with camrelizumab as first-line therapy for locally advanced ESCC. Based on the preliminary result, a phase III, randomized, double-blind, placebo-controlled study of camrelizumab versus placebo in combination CCRT in these patients (NCT04426955) was carried out this year.

Disclosures

The authors indicated no financial relationships.

Acknowledgments

This work was supported by the National Nature Science Foundation of China (grants 81872462 and 81972772) and the Clinical Trial Supporting Foundation of Tianjin Medical University Cancer Institute & Hospital (grant C1707). We thank Jiangsu HengRui Medicine Co., China, for kindly providing the anti–PD-1 antibody camrelizumab. We thank Zhongxing Liao, M.D., Ph.D., and Christine Wogan, University of Texas MD Anderson Cancer Center, Houston, TX, for their careful and precise revision of the manuscript.

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Figures and Tables

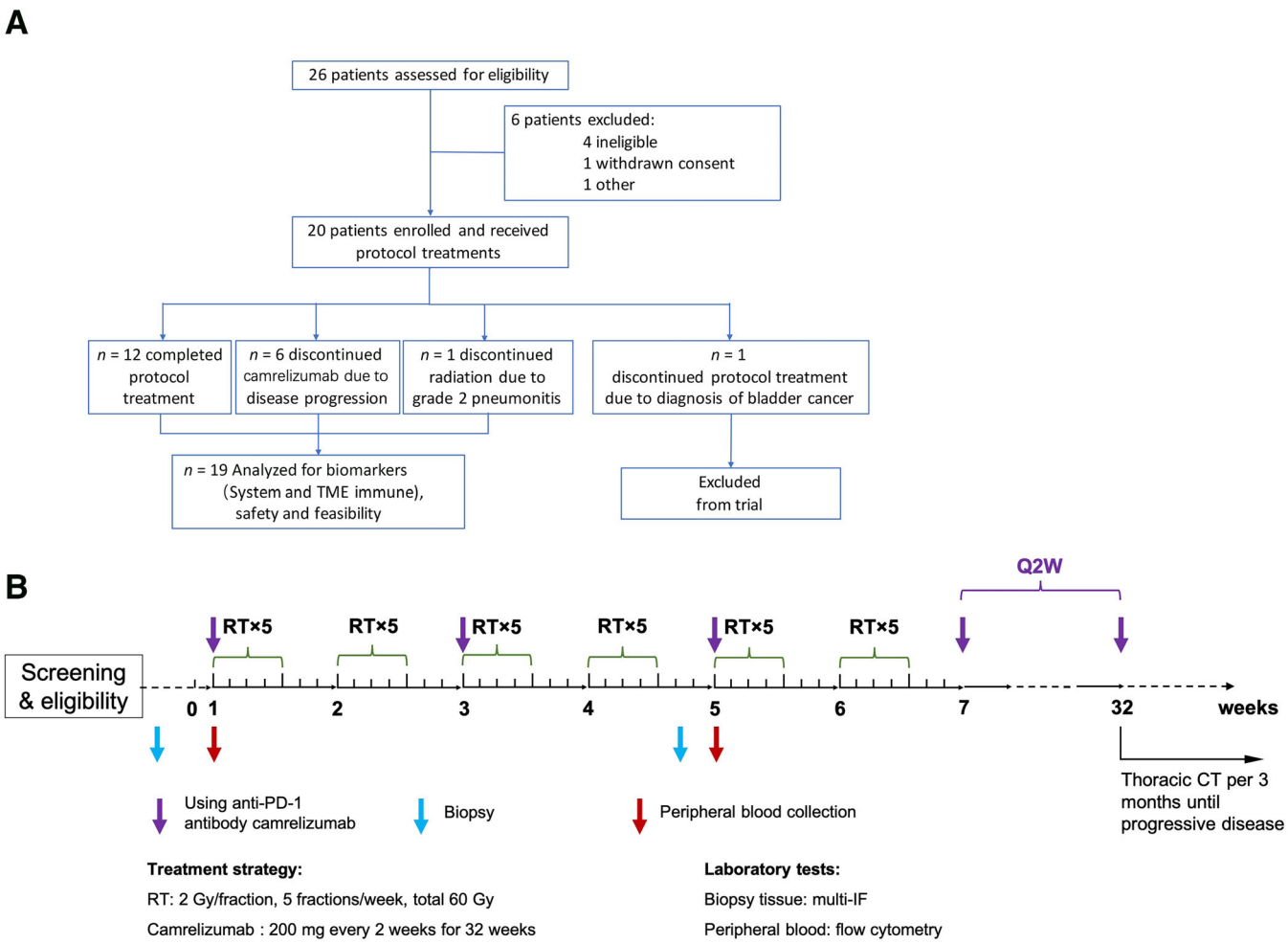

Figure 2. Consortium diagram and treatment schedule. **(A):** Consortium diagram. **(B):** Clinical treatment schedule.
Abbreviations: CT, chemotherapy; IF, immunofluorescence; PD-1, programmed cell death protein 1; Q2W, every 2 weeks; RT, radiotherapy; TME, tumor microenvironment.

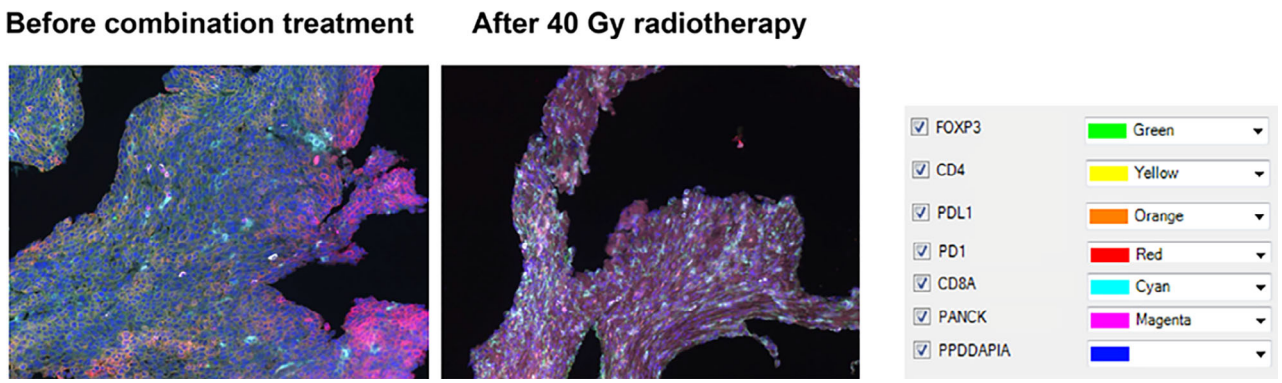

Figure 3. Multiplex staining for tumor-infiltrating T cells in esophageal squamous cell carcinoma tissue sections. Representative images of a patient (patient 3) who had a major pathological response. The PD-1 and PD-L1 expression during treatment decreased compared with that before treatment, whereas $CD4^+$ and $CD8^+$ T cells accumulated during treatment.
Abbreviations: FOXP3, forkhead box P3; PANCK, pan Cytokeratin; PD1, programmed cell death protein 1; PDL1, programmed cell death ligand 1; PPDDAPIA, DAPI.

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Table 1. Patient characteristics

Characteristic	*n* (%)
Age at enrollment, years	
Mean	62.6
Median (range)	64 (46–74)
Sex	
Female	7 (37)
Male	12 (63)
ECOG performance status score	
0	10 (56)
1	9 (47)
Smoking status	
Never	8 (42)
Former or current	11 (58)
Drinking status	
Never	9 (47)
Former or current	10 (56)
Location	
Cervical segment	1 (5)
Upper thoracic segment	5 (26)
Middle thoracic segment	11 (58)
Inferior thoracic segment	2 (11)
AJCC8 disease stage	
II	1 (5)
III	11 (58)
IV	7 (37)

Abbreviations: AJCC8, 8th edition of the American Joint Committee on Cancer Staging Manual (2017); ECOG, Eastern Cooperative Oncology Group.

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Table 2. Patient and treatment characteristics

ID	Age at Dx, yr	Sex	Hx of smoking	No. pack-years	Hx of drinking	ECOG score	Pre-treatment disease stage[a]	Pre-treatment disease stage[b]	Reason for no chemoRT	No. of SHR-1210 cycles	Pathology findings after 40 Gy	Response after 40 Gy	Respond at end of RT
1	71	F	No	0	No	1	III (T3N1M0)	III (T3N1M0)	Hypertension, age[c]	16	5% residual TCs	PR	PR
2	71	F	No	0	No	1	III (T3N1M0)	III (T3N1M0)	Hypertension, diabetes, age	11 (PD)	5% residual TCs	SD	PR
3	46	F	No	0	No	0	III (T3N1M0)	IVA (T3N2M0)	Achalasia cardia	16	0% residual TCs	PR	CR
4	49	M	No	0	Yes	0	III (T3N1M0)	III (T3N1M0)	Refused	9 (PD)	40% residual TCs	PR	PD
5	69	F	Yes	25	No	1	IIA (T3N0M0)	III (T3N0M0)	Vegetarian diet, Hx syphilis	16	1% residual TCs	PR	PR
6	62	M	No	0	Yes	0	III (T4N1M0)	IVA (T4N1M0)	Hepatitis B	16	0% residual TCs	SD	PD
7	56	M	Yes	38	Yes	0	III (T3N1M0)	III (T3N1M0)	Hypertension	16	10% residual TCs	PR	PR
8	61	F	No	0	No	1	IV (T3N1M1)	III (T3N1M0)	Pyramidal fracture	16	<10% residual TCs	PR	PR
9	66	M	Yes	40	Yes	0	IIB (T2N1M0)	III (T2N1M0)	Hypertension	16	0% residual TCs	PR	PR
10[d]													
11	72	M	Yes	40	Yes	1	III (T3N1M0)	III (T3N1M0)	Hepatitis B, age	16	1% residual TCs	PR	PR
12	71	M	Yes	75	Yes	0	IV (T3N1M1b)	IVA (T3N2M0)	Hypertension, age	14 (PD)	5% residual TCs	PR	PR
13	58	M	Yes	10	No	1	III (T3N1M0)	IVA (T3N2M0)	Paraplegia, hepatitis C	7 (anorexia)	>50% residual TCs	SD	SD
14	65	F	No	0	No	0	III (T3N1M0)	III (T3N1M0)	Refused	16	0% residual TCs	SD	SD
15	64	F	No	0	No	0	III (T3NIM0)	IVA (T3N2M0)	Hypertension, diabetes, hyperlipemia	16	5% residual TCs	SD	PR
16	59	M	Yes	30	Yes	1	III (T3NIM0)	IVA (T3N2M0)	Hypertension, coronary disease	10 (PD)	>10% residual TCs	PR	PD
17	50	M	Yes	30	Yes	1	IV (T4N1M1b)	IVA (T4N2M0)	Refused	16	5% residual TCs	PR	PR
18	74	M	Yes	50	Yes	1	IIB (T1N1M0)	IIA (T1N1M0)	Gastric ulcer, emphysema, age	14 (refused treatment)	0% residual TCs	SD	CR
19	72	M	Yes	14	Yes	0	IIA (T3N0M0)	III (T3N0M0)	Hypertension, age	16	1% residual TCs	SD	PR
20	54	M	Yes	1	Yes	1	III (T3NIM0)	III (T3NIM0)	Hypertension, cerebral infarction	16	0% residual TCs	PR	PR

Histopathologic diagnosis was squamous cell carcinoma in all cases. Radiation dose was 60 Gy in all cases except for patient 20, who received 52 Gy because of grade 2 radiation pneumonitis. SHR-1210 was given in 200-mg doses for the indicated number of cycles.

[a]Staged per American Joint Committee on Cancer (AJCC) 6th (2002) edition.

[b]Staged per AJCC 8th (2017) edition.

[c]More than 72 years.

[d]Excluded upon discovery of second primary cancer in bladder after 20 Gy RT and two cycles of SHR-1210.

Abbreviations: chemoRT, chemoradiotherapy; CR, complete response; Dx, diagnosis; ECOG, Eastern Cooperative Oncology Group; F, female; Hx, history; ID, identifier; M, male; PD, progressive disease; PR, partial response; RT, radiotherapy; SD, stable disease; TC, tumor cell.

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Table 3. Treatment failures

Patient ID	Type of failure	Treatment for failure	Vital status	Cause of death
1	Local recurrence	Docetaxel + cisplatin	Dead	Progressive disease
2	Distant metastasis in liver	Surgery and chemotherapy	Dead	Progressive disease
4	Local recurrence	Docetaxel + cisplatin	Dead	Progressive disease
5	Local and regional lymph node recurrence	Best supportive care	Dead	Progressive disease
6	Distant metastasis in liver	Radiofrequency ablation, docetaxel + cisplatin, maintenance pembrolizumab	Alive	—
9	Local recurrence	Best supportive care	Dead	Progressive disease
11	Distant metastasis in bone	Best supportive care	Dead	Progressive disease
12	Regional lymph node recurrence	Docetaxel + cisplatin	Dead	Progressive disease
13	Local recurrence and esophageal fistula	Stent + best supportive care	Dead	Progressive disease
14	Local recurrence	Best supportive care	Dead	Progressive disease
16	Local recurrence and esophageal fistula	Stent + best supportive care	Dead	Massive hemorrhage
17	Local recurrence	Best supportive care	Dead	Progressive disease
18	Regional lymph node recurrence	Best supportive care	Dead	Progressive disease

Table 4. Treatment-related adverse events

Adverse events	Grade 1,*n* (%)	Grade 2, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)	Total, *n* (%)
Reactive capillary hemangiomas	15 (79)	2 (10)	0 (0)	0 (0)	17 (90)
Pulmonary infection	1 (5)	1 (5)	0 (0)	0 (0)	2 (10)
Radiation pneumonitis	0 (0)	1 (5)	1 (5)	0 (0)	2 (10)
Radiation esophagitis	6 (32)	3 (16)	0 (0)	0 (0)	9 (47)
Radiation laryngitis	0 (0)	0 (0)	1 (5)	0 (0)	1 (5)
Constipation	3 (16)	2 (10)	0 (0)	0 (0)	5 (26)
Decreased appetite	1 (5)	1 (5)	0 (0)	0 (0)	2 (10)
Blurred vision	2 (10)	0 (0)	0 (0)	0 (0)	2 (10)
Skin albinism	1 (5)	1 (5)	0 (0)	0 (0)	2 (10)
Proteinuria	7 (37)	0 (0)	0 (0)	0 (0)	7 (37)
Leukopenia	3 (16)	2 (10)	1 (5)	0 (0)	6 (32)
Neutrophilic granulopenia	0 (0)	6 (32)	0 (0)	0 (0)	6 (32)
Lymphopenia	0 (0)	2 (10)	6 (32)	1 (5)	9 (47)
Anemia	1 (5)	0 (0)	0 (0)	0 (0)	1 (5)
Liver disorder	2 (10)	0 (0)	0 (0)	0 (0)	2 (10)
Albuminosis	1 (5)	1 (5)	0 (0)	0 (0)	2 (10)
Hyperglycemia	0 (0)	5 (26)	0 (0)	0 (0)	5 (26)
Fatigue	0 (0)	1 (5)	0 (0)	0 (0)	1 (5)
Cough	0 (0)	0 (0)	1 (5)	0 (0)	1 (5)
Hemoptysis	3 (16)	0 (0)	0 (0)	0 (0)	3 (16)
Hypothyroidism	3 (16)	0 (0)	0 (0)	0 (0)	3 (16)

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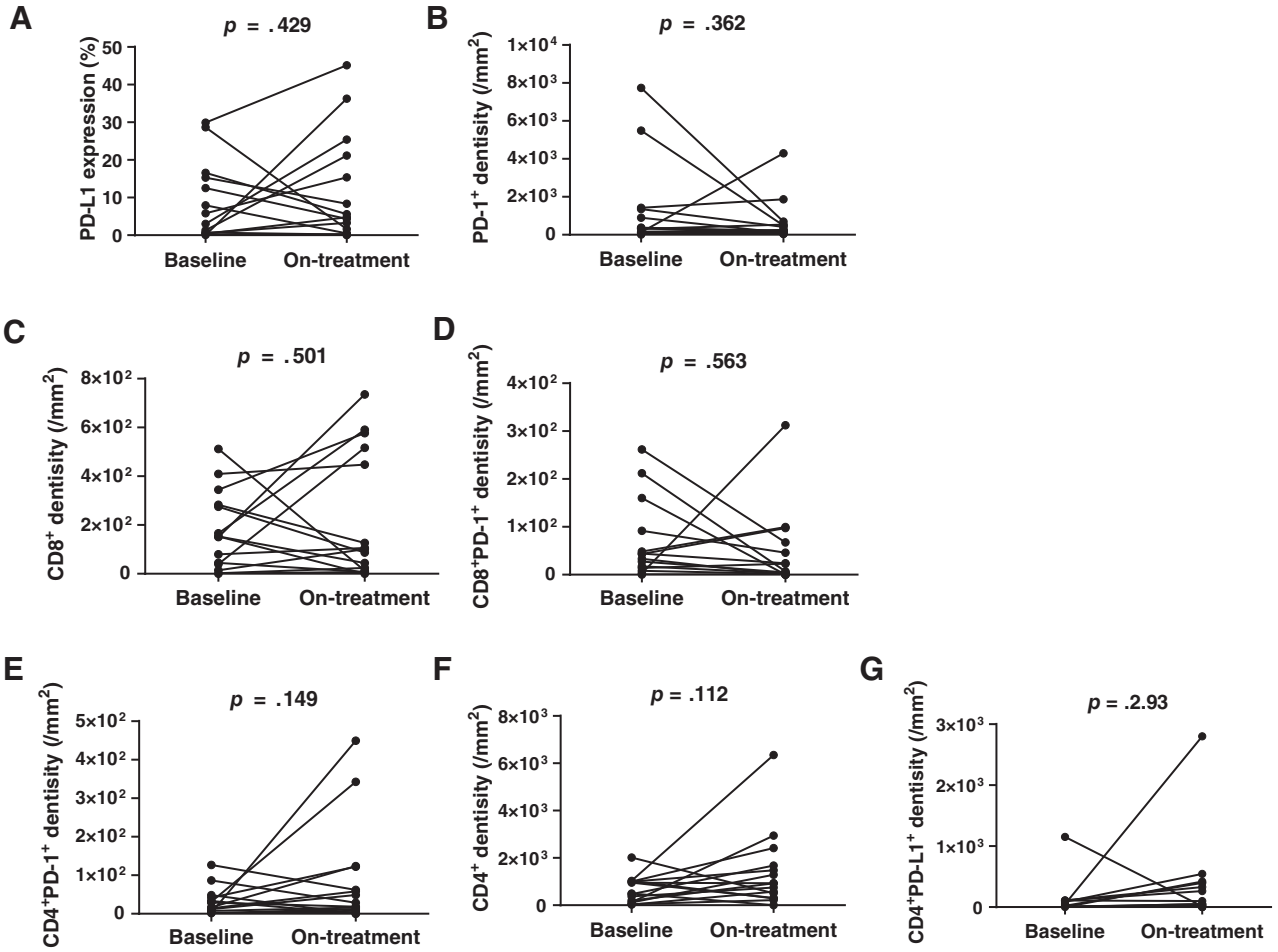

Figure 4. Dynamics of tumor-infiltrating T cells at baseline and during treatment. Pairs of biopsy tumor tissues that were collected before treatment (at baseline) and during treatment after 40 Gy radiotherapy were stained using a multiplex immunofluorescence method. Paired *t* test was used to evaluate the differences in multiple T-cell subsets between at baseline and on-treatment time-point. $p < .05$, significant difference.
Abbreviations: PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1.

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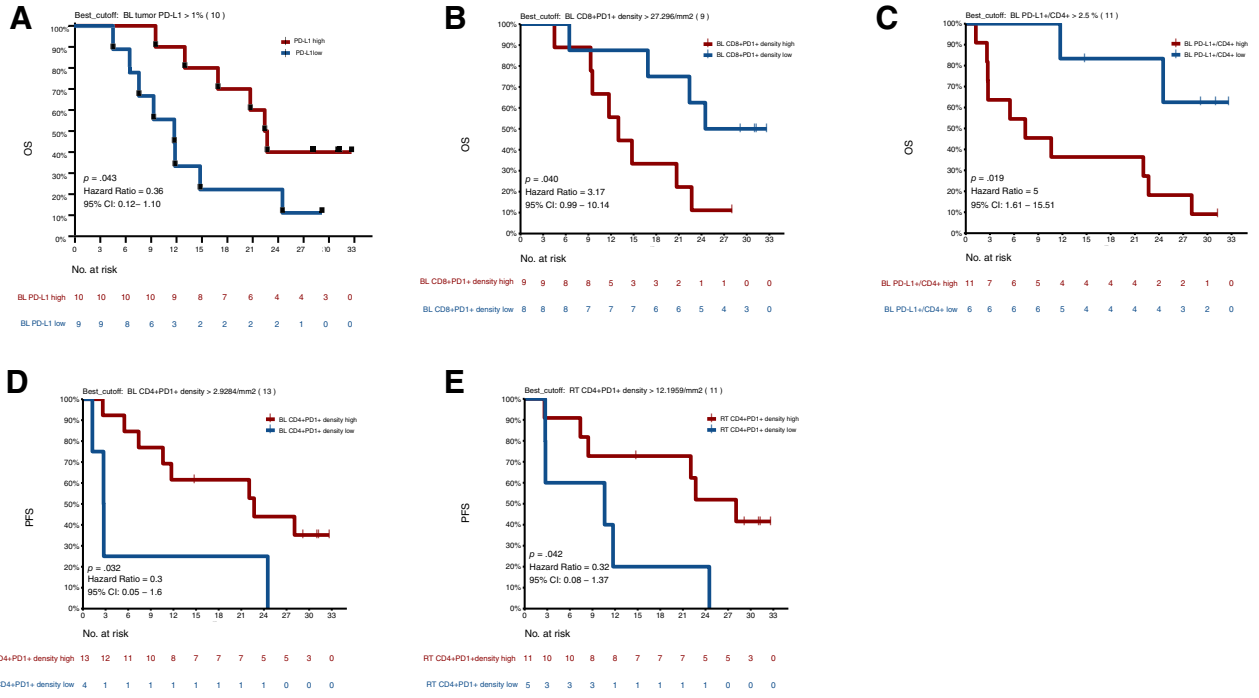

Figure 5. Intratumoral T cells were associated with clinical outcomes. Kaplan-Meier curves for overall survival and progression-free survival of patients in tumor tissue programmed cell death ligand 1 (PD-L1) expression **(A)**, programmed cell death protein 1 (PD-1)$^{+}$CD8^{+} T cells **(B)**, PD-L1^{+}CD4^{+} T cells **(C)**, and PD-1^{+}CD4^{+} T cells **(D)** at baseline and PD-1^{+}CD4^{+} T cells **(E)** during treatment. Log-rank *p* values are reported from an unadjusted analysis. The cutoff values were determined by calculated by the Youden index of the receiver operating characteristic curve.
Abbreviations: BL, baseline; CI, confidence interval; OS, overall survival; PFS, progression-free survival; RT, radiotherapy (during treatment).

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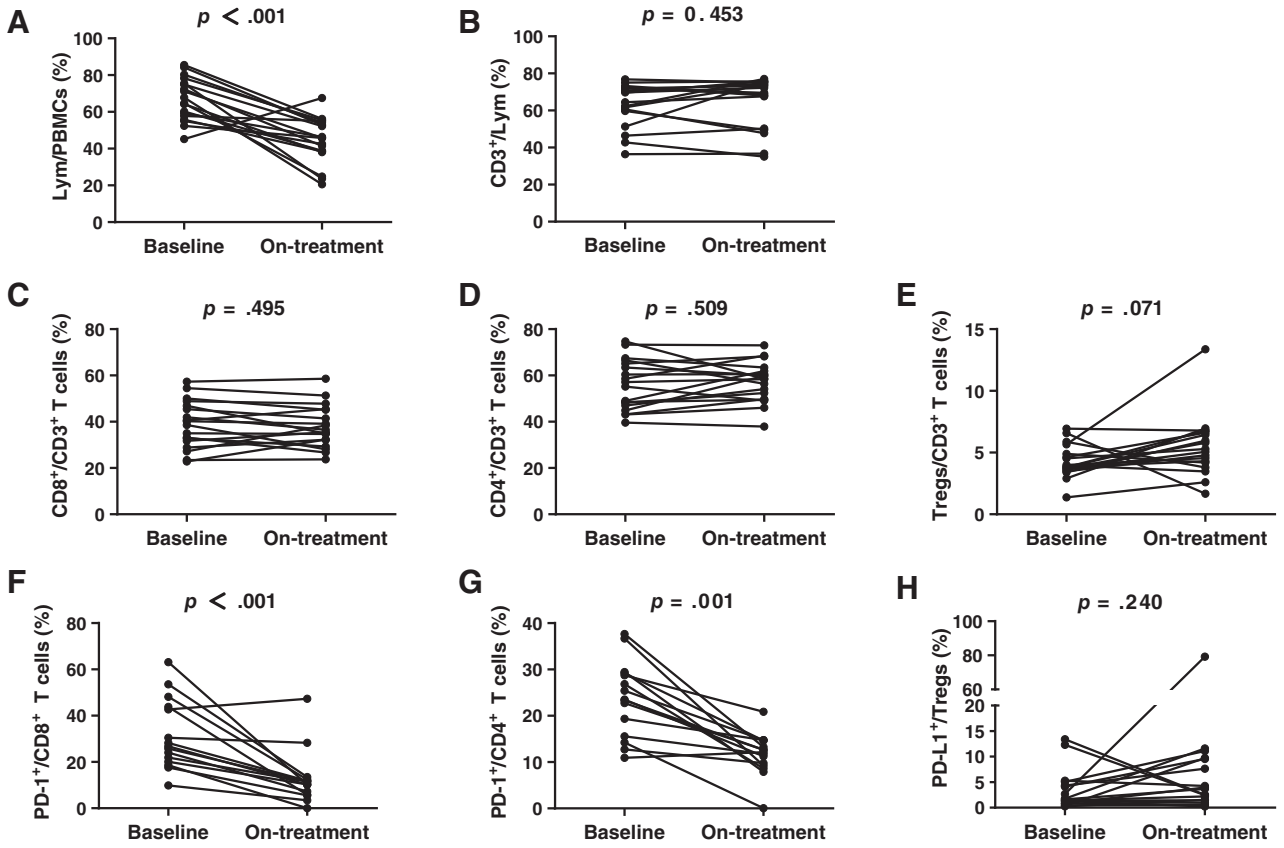

Figure 6. Dynamics of peripheral blood T cells at baseline and during treatment. Pairs of EDTA-anticoagulant peripheral blood specimens were collected at baseline and during treatment after 40 Gy radiotherapy. Flow cytometry was used to identify T-cell subsets and PD-1/PD-L1 expression. Paired *t* test was used to evaluate the differences in T-cell subpopulations between at baseline and during treatment timepoint. $p < .05$, significant difference.
Abbreviations: Lym, lymphocyte; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; Treg, regulatory T cell.

Table 5. Peripheral T cells were associated with clinical outcomes

	Baseline				During treatment			
	OS		PFS		OS		PFS	
T-cell subsets	*p* value[a]	HR[b] (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)
$CD4^+/CD3^+$	.007	0.17 (0.06–0.51)	.004	0.16 (0.05–0.47)	—[c]		.014	0.28 (0.80–1.00)
$CD8^+/CD3^+$	.004	9.95 (3.34–29.65)	.001	7.73 (2.56–23.28)	—		.002	4.49 (1.17–17.21)
$Ki67^+/CD8^+$	.011	0.25 (0.08–0.79)	.002	0.20 (0.06–0.64)	.038	0.24 (0.08–0.71)	.003	0.09 (0.03–0.28)
IFN-$\gamma^+/CD8^+$	—		.010	3.5 (0.90–14.22)	.034	0.31 (0.10–0.93)	—	
Treg[d]/lymphocytes	.002	0.20 (0.03–1.46)	.019	0.29 (0.05–1.59)	—		—	
$Ki67^+$/Treg	—		—		<.001	6.07 (0.94–39.30)	.019	3.31 (0.75–14.64)
$CTLA4^+/CD4^+$	.010	3.66 (1.03–13.01)	—		—		—	

[a]From log-rank test.
[b]Hazard in high group/hazard in low group.
[c]$p \geq .05$.
[d]Treg was defined as $CD4^+CD25^+Foxp3^+$ T cell.
Abbreviations: —, ≥0.05; CI, confidence interval; CTLA4, cytotoxic T-lymphocyte–associated protein 4; HR, hazard ratio; IFN-γ, interferon γ; OS, overall survival; PFS, progression-free survival; Treg, regulatory T cell.

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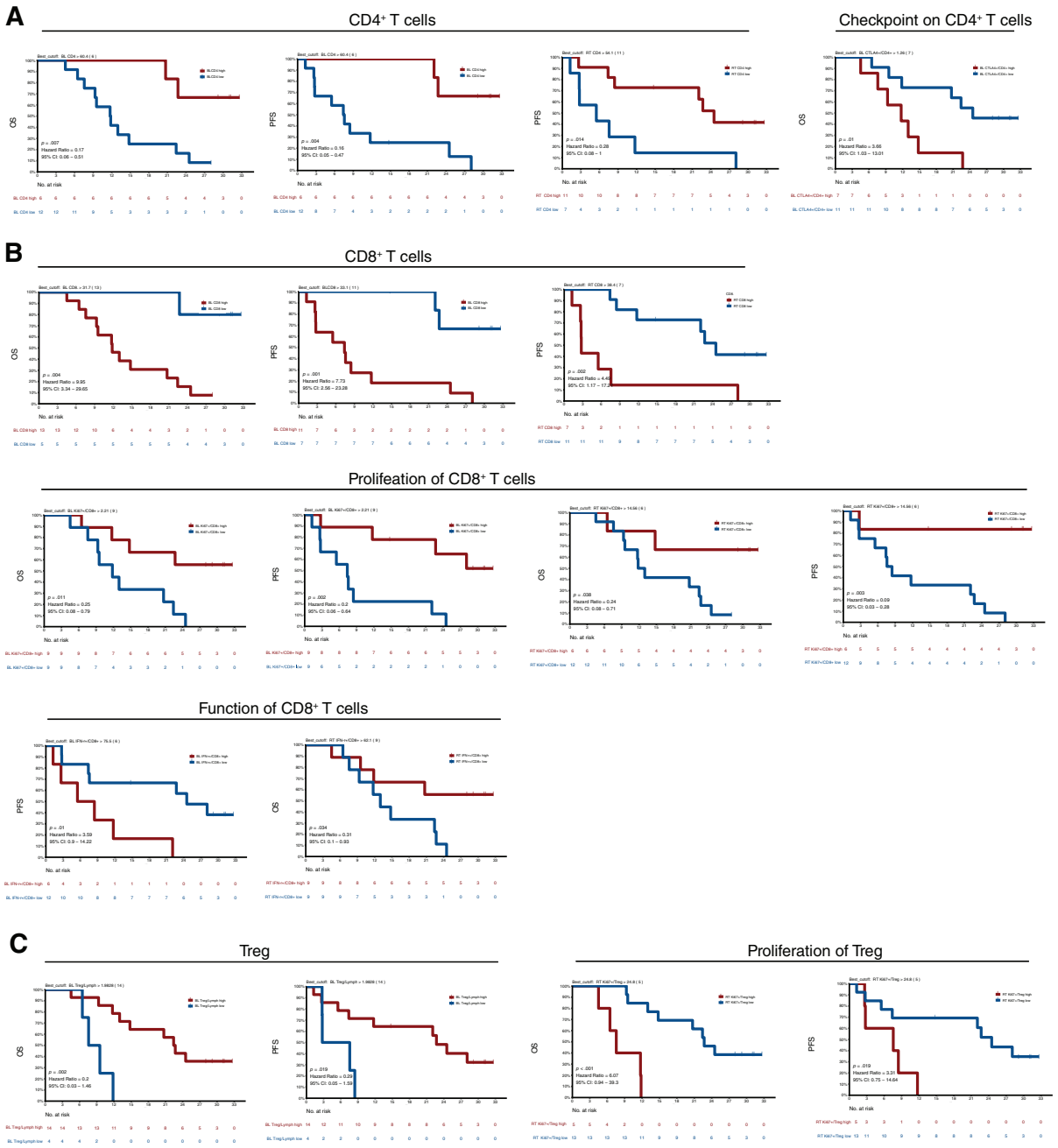

Figure 7. Kaplan-Meier estimates of overall survival and progression-free survival in patients in peripheral blood T-cell subsets predicting prognosis over time. **(A):** $CD4^+$ T cells and their subsets at baseline or during treatment were associated with OS and PFS. **(B):** $CD8^+$ T cells and their subsets at baseline or during treatment were associated with OS and PFS. **(C):** $CD4^+CD25^+Foxp3^+$ Treg cells and their subsets during treatment were associated with OS and PFS. The cutoff values were determined by calculated by the Youden index of the receiver operating characteristic curve. Log-rank *p* values are reported from an unadjusted analysis.
Abbreviations: BL, baseline; CI, confidence interval; CTLA4, cytotoxic T-lymphocyte–associated protein 4; IFN-r, Interferon-gamma; Lymph, lymphocyte; OS, overall survival; PFS, progression-free survival; RT, radiotherapy (during treatment); Treg, regulatory T cell.

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