Original Study

ANNALS OF SURGERY OPEN

OPEN

Neoadjuvant Chemoradiotherapy Upregulates the Immunogenicity of Cold to Hot Tumors in Esophageal Cancer Patients

Yushi Nagaki, MD, PhD,*† Satoru Motoyama, MD, PhD,‡ Yusuke Sato, MD, PhD,*† Akiyuki Wakita, MD, PhD,*† Hiromu Fujita, MD, PhD,*† Kohei Kemuriyama, MD,*† Ryohei Sasamori, MD,*† Shu Nozaki, MD,*† Kyoko Nomura, MD, PhD,§ and Yoshihiro Minamiya, MD, PhD*†

Objective: To test the hypothesis that neoadjuvant chemoradiotherapy (NACRT) is more effective against hot esophageal squamous cell carcinoma (ESCC) and that it may upregulate tumor immunogenicity.

Background: There have been several recent reports showing the efficacy of immune check-point inhibitors (ICIs) against esophageal cancer, especially immunologically hot tumors. In addition, several studies have suggested that chemotherapy and radiotherapy may convert cold tumors to hot tumors.

Methods: Of 105 ESCC patients who underwent surgery after NACRT between 2010 and 2018 at our hospital, 99 whose biopsy tissue samples were obtained were enrolled. Based on immunohistochemical analysis, tumors that were FOXA1 (+) and/or EYA2 (+) were defined as hot tumors, others were cold tumors. We then investigated the association between tumor immunogenicity and clinicopathological features.

Results: The 29 patients with hot tumors before NACRT had a significantly better 5-year disease-specific survival (DSS) rate than the remaining 70 patients with cold tumors (85% vs 64%; $P = 0.036$). In a multivariate analysis, tumor immunogenicity was a significant independent predictor of DSS. Of 68 patients without a pathological complete response (non-pCR) in their primary tumor, 51 (75%) had hot tumors after NACRT. Moreover, 75% (36/48) of tumors that were cold before NACRT were converted to hot tumors after NACRT.

Conclusions: Patients with hot ESCC tumors treated with NACRT plus esophagectomy had a better prognosis than those with cold tumors. NACRT upregulated cold tumor immunogenicity to hot tumors, suggesting NACRT may increase the sensitivity of ESCC to adjuvant ICIs.

Keywords: cold tumor, esophageal squamous cell carcinoma (ESCC), EYA2, FOXA1, hot tumor, immune check-point inhibitors (ICIs), immunogenicity, neoadjuvant chemoradiotherapy (NACRT)

INTRODUCTION

Deaths from esophageal cancer (EC) reached more than half a million worldwide in 2020.[1] Esophageal squamous cell carcinoma (ESCC) is the most common EC subtype in many parts of the world, especially Asia, Africa, and South America.[2] The development of trimodal therapy entailing combined surgery, conventional chemotherapy, and radiotherapy has improved outcomes, but the overall prognosis for ESCC patients remains poor.[3–5] This trimodal therapy has proved most effective for patients with immunogenic tumors.[6]

Immune check-point inhibitors (ICIs), including ipilimumab, nivolumab, and pembrolizumab, have revolutionized the treatment of multiple cancer types with a subset of patients experiencing long-lasting favorable tumor responses.[7] ICIs are currently being used alone or in combination with conventional chemotherapy as first or second-line therapy for patients with unresectable EC or as postoperative adjuvant therapy after EC resection.[8–12] ICIs reportedly provide a survival benefit in these patients, especially those with squamous cell carcinomas.[12]

Responders to ICIs display the "hot tumor" immunoreactive phenotype, whereas nonresponders display the "cold tumor" immunosuppressive phenotype.[13] Hot tumors are characterized by a high tumor mutation burden, neo-antigen expression, T lymphocyte infiltration, and increased PD-L1 expression as a result of the IFN-γ production, all of which are absent from cold tumors.[14–18] For ESCC patients, however, these biomarkers are still insufficiently predictive for patient selection for ICI therapy. Moreover, treatments that would turn nonimmunogenic cold tumors into immunogenic hot tumors, making them more susceptible to ICIs, would be highly desirable. Wang et al[19] stratified 2 molecular subtypes of ESCC using gene expression profiling data in Asian populations. Subtype I was enriched in pathways associated with immune responses (ie, potentially hot tumors), while subtype II was enriched in pathways associated

*From the Division of Esophageal Surgery, Akita University Hospital, Akita, Japan; †Department of Thoracic Surgery, Akita University Graduate School of Medicine, Akita, Japan; ‡Department of Gastrointestinal Surgery, Japanese Red Cross Akita Hospital, Akita, Japan; and §Department of Health Environmental Science and Public Health, Akita University Graduate School of Medicine, Akita, Japan.

Disclosure: The authors declare that they have nothing to disclose.

Guarantors of integrity of entire study and study concepts: Y.N., S.M., and Y.M. Study design or data acquisition or data analysis: Y.N., S.M., Y.S., A.W., H.F., K.K., R.S., S.N., K.N., and Y.M. Statistical analysis: Y.N. and K.N. Manuscript editing: Y.N. and S.M. All authors read and approved the final manuscript.

Reprints: Yushi Nagaki, MD, PhD, Department of Thoracic Surgery, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita, 010-8543, Japan. E-mail: nagaki@med.akita-u.ac.jp

Annals of Surgery Open (2024) 1:e385

Received: 7 September 2023; Accepted 3 January 2024

Published online 2 February 2024

DOI: 10.1097/AS9.0000000000000385

with different biological pathways. In addition, FOXA1 and EYA2 were subtype-specific diagnostic immunohistochemical (IHC) markers of subtype I ESCCs.[19] Several studies also suggested that chemotherapy and radiotherapy administered with ICIs or before ICI initiation may convert cold to hot tumors.[20] In the present study, we tested the hypothesis that neoadjuvant chemoradiotherapy (NACRT) was more effective in patients with hot than cold tumors, but it also had the potential to upregulate tumor immunogenicity, thereby improving survival in patients with ESCC.

METHODS

Patients

This was an observational study. Of 105 patients who received esophagectomy with curative intent (R0 esophagectomy) after NACRT for thoracic ESCC between 2010 and 2018 in our hospital, 99 whose biopsy tissue samples before NACRT were obtained were enrolled. No enrolled patients received ICI therapy before surgery. NACRT was recommended for patients staged as clinical T3–4 based on depth of invasion of the primary tumor or regional lymph node metastasis (cT3–4 or cN+) and with an Eastern Cooperative Oncology Group performance status of 0–1. This included patients with supraclavicular lymph node metastasis (cM1 lymph node).[21] Clinical staging was determined according to the TNM classification of the UICC (8th edition),[22] based on esophagogastroduodenoscopy, contrast-enhanced computed tomography, and fluorodeoxyglucose-positron emission tomography. Cervical and abdominal ultrasonography and endoscopic ultrasound were performed for staging as necessary. This study was approved by the Ethics Committee of Akita University Graduate School of Medicine (No. 2406). All participants provided informed consent and signed human subject institutional review board consent forms.

Neoadjuvant Chemoradiotherapy

The NACRT protocol entailed radiotherapy (40 Gy in 20 fractions, 41.4 Gy in 23 fractions, or 42 Gy in 21 fractions) with 2 courses of combined chemotherapy with 5-fluorouracil 800 mg/m^2/day on days 1–5 and cisplatin or nedaplatin 80 mg/m^2/day on day 1. High-energy X-rays (10 MV) were used for the radiotherapy. All patients underwent 2- or 3-dimensional radiotherapy planning, and the radiotherapy target was set around the gross tumor volume and metastatic lymph nodes. As a result, in nearly all patients, the upper-to-lower mediastinum was included in the radiated fields. The radiotherapy consisted of 1.8–2.0 Gy/day for 5 days each week.

Surgery

Esophagectomy was scheduled to be performed more than 3 weeks after completing NACRT, by which time patients had no treatment-related adverse events worse than grade 2 according to the Common Terminology Criteria for Adverse Events Version 4.0.[23] When deciding the interval before surgery, we did not take into consideration the residual carcinoma after NACRT. Esophagectomy under right thoracotomy or thoracoscopic (including robot-assisted thoracoscopic) esophagectomy with extended 3-field lymph node dissection (bilateral cervical (including supraclavicular), mediastinal, and abdominal lymph nodes) was performed. In other words, McKeown esophagectomy with extended 3-field lymph node dissection was performed as the standard operative method in this study. Our standard reconstruction was with a gastric tube in open surgery via the posterior mediastinal or retrosternal route.

Pathological Analysis Including Tumor Regression Grade

Surgically resected ESCC specimens were subjected to routine pathological examination. Pathological stage after neoadjuvant therapy (ypStage) was determined according to the TNM classification of the UICC (8th edition).[22] The level of tumor regression in response to NACRT was evaluated based on the Japanese Classification of Esophageal Cancer.[24] Tumor regression grade (TRG) was classified into 4 categories: TRG0, no recognized cytological or histological therapeutic effect; TRG1, slightly effective with apparently viable cancer cells accounting for 1/3 or more of the tumor tissue; TRG2, moderately effective with viable cancer cells accounting for less than 1/3 of the tumor tissue; and TRG3, highly effective with no evidence of viable cancer cells.

Follow-Up

Almost none of the patients received postoperative adjuvant chemotherapy, though a small portion of the patients received adjuvant nivolumab therapy or placebo in a clinical trial. All patients visited our hospital every 2 months. At each visit, they received a follow-up examination that included physical examinations, blood tests, and chest X-rays. Chest and abdominal contrast-enhanced computed tomography were performed every 4 months during the first 5 years, then every 6 months thereafter. We performed esophagogastroduodenoscopy annually except in cases where there were signs of anastomotic stenosis or obstruction. For patients with recurrence, chemotherapy was performed and, if possible, surgical resection and reradiation were also performed.

Determination of Tumor Immunoreactivity: Hot or Cold Tumors

We determined tumor immunoreactivity based on the intensity of IHC staining for FOXA1 and EYA2. Wang et al[19] showed FOXA1 and EYA2 to be potential markers of the immunoreactive ESCC subtype. We defined tumors that were FOXA1 (+) and/or EYA2 (+) as hot tumors and those that were FOXA1 (–) and EYA2 (–) as cold tumors. These definitions were applied to both biopsy tissue samples before NACRT (n = 99) and residual tumor samples from patients without a complete pathological response (non-pCR) resected after NACRT (n = 68). Biopsy tissue samples before NACRT were obtained from areas without necrotic tissue at the margin of the tumors under pretreatment endoscopy, as these areas contained numerous viable cells. The samples were fixed in 10% formalin neutral buffer solution overnight at room temperature and then embedded in paraffin. Resected postoperative tumor samples after NACRT were prepared similarly. After IHC staining, areas without necrotic tissue at the margin of the tumors were evaluated.

The IHC Staining

IHC staining was performed as previously described.[19] In brief, 4-μm-thick sections from the tissue samples were deparaffinized in xylene and rehydrated through a graded ethanol series. After antigen retrieval in citrate buffer (pH 6.0) and blocking with 3% H_2O_2 for 30 minutes, the sections were stained by incubation first with anti-FOXA1 (1:300 dilution, ab55178; Abcam plc, Cambridge, UK) or anti-EYA2 (1:400 dilution, ab95875; Abcam plc) antibody and then with HRP-conjugated secondary antibody (K4001, DAKO, Santa Clara, CA). After developing with diaminobenzidine, the sections were counterstained with hematoxylin, dehydrated and mounted. The staining was scored by 2 investigators blinded to the clinical data as follows: 1+, weak/faint staining; 2+, moderate staining; or 3+, strong

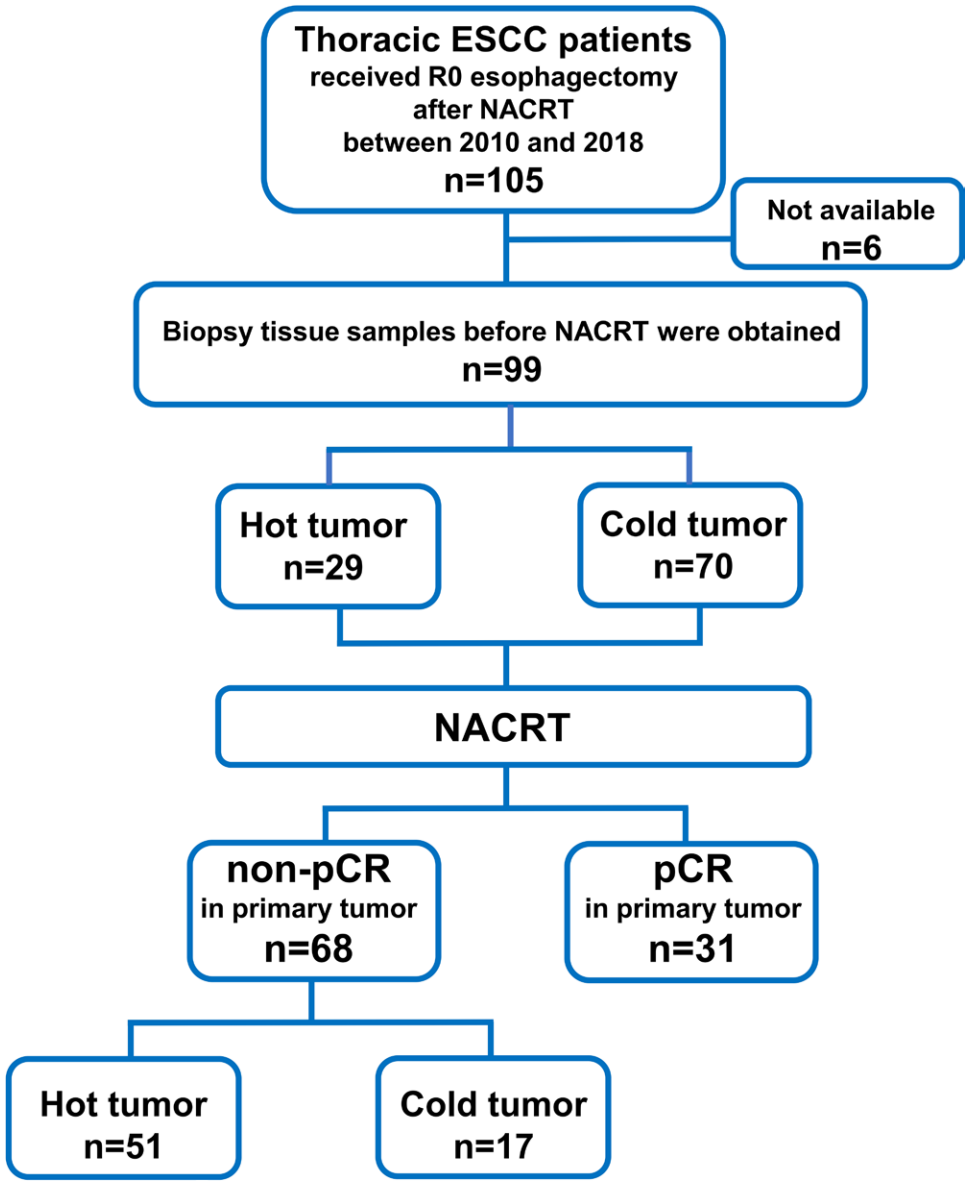

FIGURE 1. Study flow chart is shown as a CONSORT diagram.

staining. Samples were classified as high expression (3+) or low expression (2+ or 1+) and defined as positive (+) or negative (–) accordingly.

Statistical Analysis

A χ^2 test or Fischer's exact test for categorical variables and a Wilcoxon test for continuous variables were used for statistical testing to detect any differences between hot and cold tumor groups. Kaplan–Meier method was applied to depict overall survival (OS) and disease-specific survival (DSS) between hot and cold tumor groups by using the log-rank test. OS was calculated as the date from the surgery to death regardless of any cause, and DSS was calculated as the time from the surgery to death from ESCC. Patients known to be alive or lost to follow-up on the date of last contact were treated as censored. To investigate the impact of hot tumor compared to the cold tumor group, we applied a Cox proportional hazard model to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs). To select an optimal Cox model, we adjusted for all variables selected at $P < 0.05$ in the univariate models.

All the P values are reported as 2-sided with a significance level of 0.05. All statistical analyses were performed using JMP14 software (SAS Institute, Cary, NC).

RESULTS

Tumor Immunogenicity and Clinicopathological Features

The study flow chart is shown as a CONSORT diagram in Figure 1. Based on the intensity of IHC staining for FOXA1 and/or EYA2 before NACRT, 29 ESCC patients (29%) were classified as having hot tumors and 70 (71%) were classified as having cold tumors (Fig. 2). The clinicopathological features of all 99 enrolled participants are summarized in Table 1. The median age of the patients was 64 (41–75) years, and the female-to-male ratio was 1:6. Interestingly, half of the female patients were classified as having hot tumors (7/15 patients), while only one-third of the males had hot tumors (22/84 patients), though the difference was not statistically significant. There was also no significant difference in age, tumor location, depth of invasion, lymph node metastasis, cM1 lymph node, clinical stage, or tumor differentiation between the 2 groups. Following NACRT

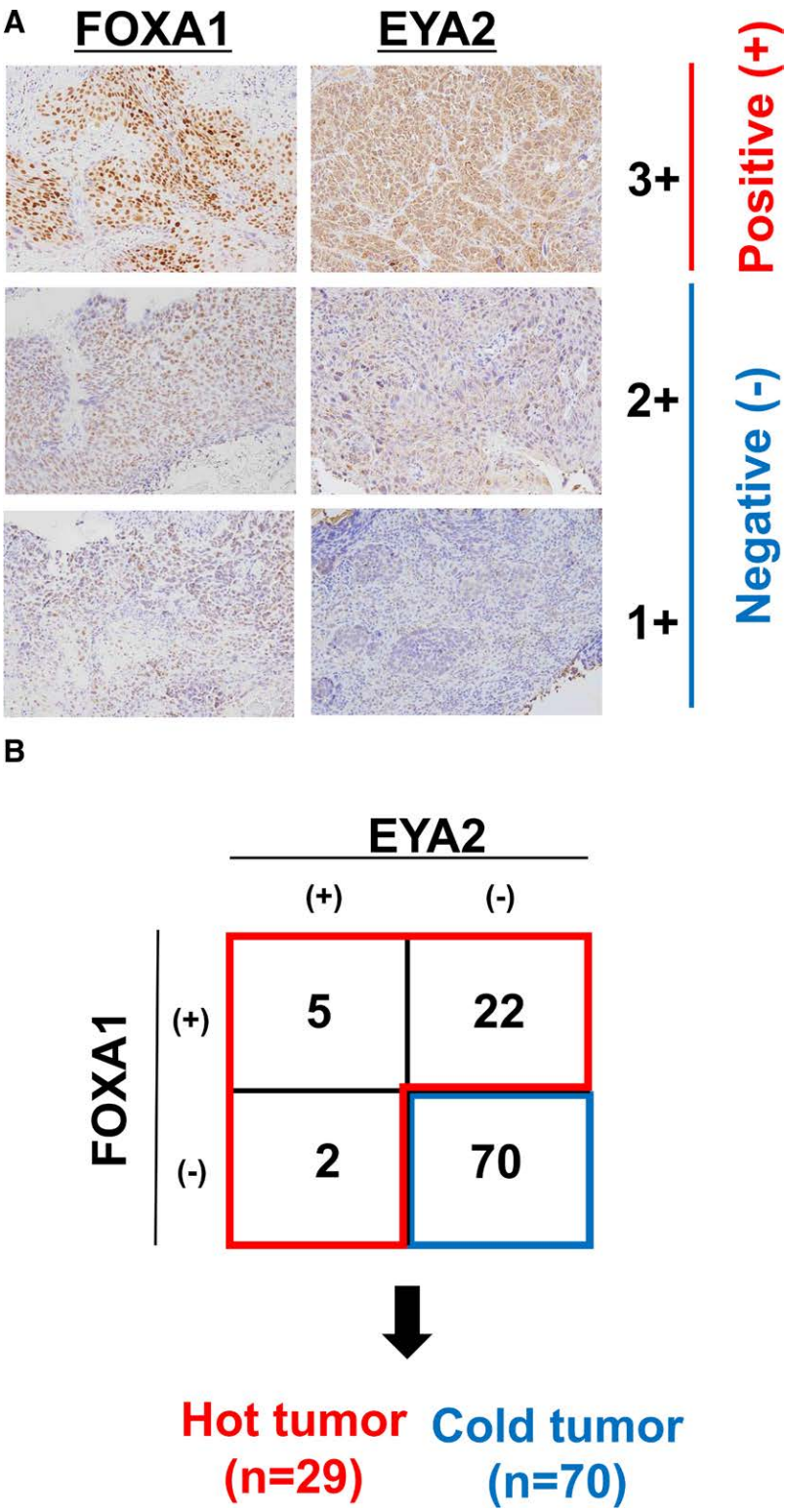

FIGURE 2. A, Tumor immunogenicity was assessed based on the intensity of immunohistochemical staining for FOXA1 and EYA2. Samples classified as high expression (3+) and low expression (2+ or 1+) were defined as positive (+) and negative (–), respectively. Tumors showing FOXA1 (+) and/or EYA2 (+) immunogenicity were defined as hot tumors; those showing FOXA1 (–) and EYA2 (–) immunogenicity were defined as cold tumors. B, Numbers of hot and cold tumors before neoadjuvant chemoradiotherapy (NACRT). Among 99 patients, 29 (29%) had hot tumors while 70 (71%) had cold tumors.

and then surgery entailing complete esophageal resection (R0), a pathological complete response (pCR, pT0N0) was achieved in 23% (23/99 cases) of the patients. There was no statistically significant difference in TRG between hot and cold tumors, but there was a trend toward more effective in hot. Forty-two patients (42%) subsequently experienced recurrence and it was, not significantly, a trend toward a lower recurrence rate after surgery in a hot tumor. After recurrence, they were treated with chemotherapy, radiotherapy and ICI therapy, alone or in combination.

TABLE 1.

Clinicopathological Characteristics (n = 99)

Characteristics	Total n = 99	Hot Tumor n = 29	Cold Tumor n = 70	*P*
Age at surgery (year)				0.24
Median	64	66	63	
Range	41–75	43–75	41–75	
Gender				0.12
Female	15 (15%)	7 (25%)	8 (11%)	
Male	84 (85%)	22 (75%)	62 (89%)	
Tumor location				0.97
Upper	19 (19%)	6 (21%)	13 (19%)	
Middle	52 (53%)	15 (52%)	37 (53%)	
Lower	28 (28%)	8 (28%)	20 (29%)	
Depth of invasion (cT)				0.29
T1	4 (4%)	0 (0%)	4 (6%)	
T2	4 (4%)	2 (7%)	2 (3%)	
T3	91 (92%)	27 (93%)	64 (91%)	
Lymph node metastasis (cN)				0.24
N0	11 (11%)	5 (17%)	6 (9%)	
N1	58 (59%)	13 (45%)	45 (64%)	
N2	29 (29%)	11 (38%)	18 (26%)	
N3	1 (1%)	0 (0%)	1 (1%)	
Distant metastasis (cM)				0.54
M0	85 (86%)	24 (83%)	61 (87%)	
M1 (Lymph node)	14 (14%)	5 (17%)	9 (13%)	
Clinical stage (cStage)				0.27
I	3 (3%)	0 (0%)	3 (4%)	
II	15 (15%)	7 (24%)	8 (11%)	
III	64 (65%)	17 (59%)	47 (67%)	
IVA	3 (3%)	0 (0%)	3 (4%)	
IVB	14 (14%)	5 (17%)	9 (13%)	
Tumor differentiation				0.47
Not poorly	89 (90%)	25 (86%)	64 (91%)	
Poorly	10 (10%)	4 (14%)	6 (9%)	
TRG				0.48
Grade 1	30 (30%)	7 (24%)	23 (33%)	
Grade 2–3	69 (70%)	22 (76%)	47 (67%)	
ypStage				0.59
I	42 (42%)	13 (45%)	29 (41%)	
II	16 (16%)	4 (14%)	12 (17%)	
IIIA	11 (11%)	5 (17%)	6 (9%)	
IIIB	19 (19%)	4 (14%)	15 (21%)	
IVA	3 (3%)	0 (0%)	3 (4%)	
IVB	8 (8%)	3 (10%)	5 (7%)	
Recurrence				0.37
Present	42 (42%)	10 (34%)	32 (46%)	
Absent	57 (56%)	19 (66%)	38 (54%)	
Prognosis				0.12
Alive	59 (60%)	21 (72%)	38 (54%)	
Dead with ESCC	28 (28%)	4 (14%)	24 (34%)	
Dead with other diseases	12 (12%)	4 (14%)	8 (11%)	

TRG indicates tumor regression grade; ypStage, pathological stage after NACRT.

Tumor Immunogenicity and Patient Prognosis

The median length of follow-up after surgery for censored cases was 60 (46–148) months. There were 40 deaths among the 99 patients (40%), including 28 ESCC-specific deaths (28%), during the follow-up period (Table 1). Kaplan–Meier analysis comparing the survival rates after esophagectomy revealed the 5-year DSS rate among patients with a hot tumor to be 85%, which was significantly better that the DSS rate of 64% among those with a cold tumor (P = 0.036, Fig. 3). However, there was no statistically significant difference in the 5-year OS rate between patients with a hot tumor (72%) and cold tumor (54%), but there was a trend toward better in hot tumor (P = 0.079, not shown). There was no significant difference in TRG or ypStage between the 2 immunogenic groups (Table 1). Univariate analysis of DSS taking into consideration age, gender, tumor location, tumor differentiation, ypStage, TRG, and tumor immunogenicity revealed ypStage, TRG, and tumor immunogenicity before NACRT to be significant factors affecting 5-year DSS in ESCC patients after NACRT followed by surgery (Table 2). Variables with a P value < 0.05 in the univariate analysis were entered into the subsequent multivariate Cox model and all 3 variables, including tumor immunogenicity before NACRT, were significant independent predictors of DSS (ypStage: HR = 2.613; 95% CI = 1.218–5.606; TRG: HR = 2.553; 95% CI = 1.209–5.392; tumor immunogenicity before NACRT: HR = 2.925; 95% CI = 1.012–8.460) (Table 2).

Tumor Immunogenicity After NACRT

Among the 99 patients, pCR was achieved for the primary tumors in 31 cases (31%). We next analyzed the immunogenicity after NACRT in the remaining patients, who did not

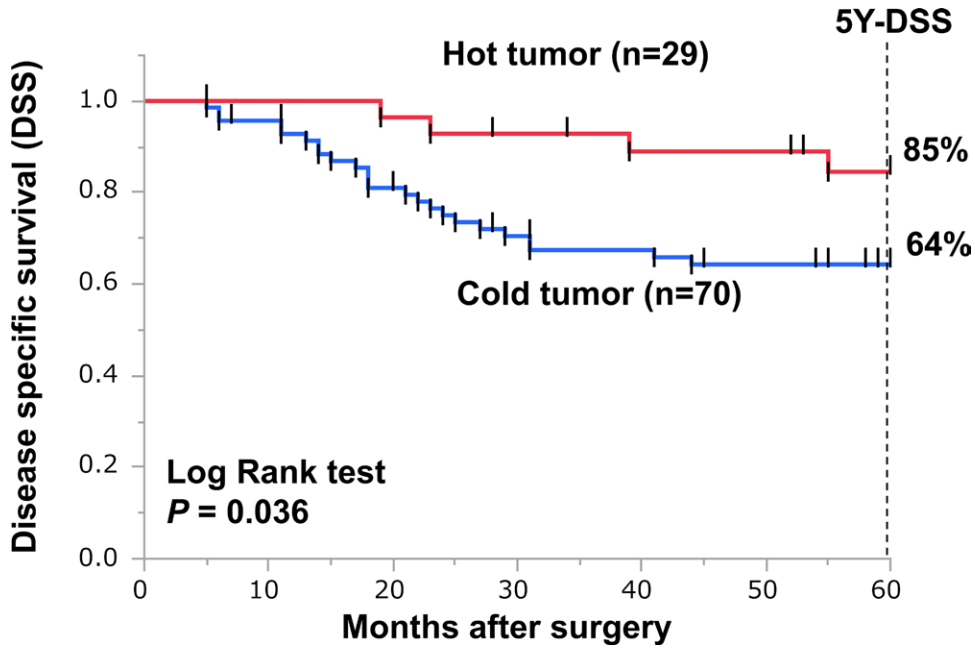

FIGURE 3. Kaplan–Meier curves comparing disease-specific survival (DSS) among esophageal squamous cell carcinoma (ESCC) patients with hot or cold tumors before NACRT. The log-rank test was used to compare the curves.

have a pCR (non-pCR) (Fig. 1). Of the 68 patients with residual cancer, 20 (29%) had hot tumors before NACRT, whereas 51 (75%) had hot tumors after NACRT (Figs. 4A, B). Of the 48 patients with a cold tumor before NACRT, 36 (75%) were converted to a hot tumor after NACRT (Fig. 4A), which suggests NACRT converted tumors from cold to immunoreactive hot tumors. Kaplan–Meier analysis showed the 5-year DSS rate after esophagectomy to be 58% among those whose cold tumor was converted to hot and 50% among those whose cold tumor was not converted (Fig. 5). This suggests a possible trend toward better DSS in patients whose tumors converted from cold to hot after NACRT, though the effect was not significant in this small patient sample ($P = 0.446$).

DISCUSSION

The present study analyzed the impact of tumor immunogenicity assessed based on the expression of 2 diagnostic markers, FOXA1 and EYA2, in ESCC patients who received esophagectomy with curative intent after NACRT. We found that patients with hot tumors before NACRT had significantly better 5-year DSS than those with cold tumors. In addition to ypStage, TRG, and tumor immunogenicity before NACRT were significant independent predictors of DSS. Notably, NACRT converted cold tumors to hot tumors in 75% of patients. And although the effect was not statistically significant, there was a trend toward a better DSS in the patients whose cold tumors were converted to hot after NACRT as compared to patients whose cold tumors were unchanged.

Although trimodal therapy entailing combined surgery and conventional chemotherapy and radiotherapy is standard for EC patients, the efficacy of this approach differs depending on the immunogenicity of a patient's tumor. By analyzing the gene expression profile of Asian populations, including a Japanese cohort, Wang et al[19] previously identified 2 ESCC molecular subtypes. Moreover, they found that FOXA1 and EYA2 were diagnostic markers of subtype I ESCCs, enriched in pathways that include immune responses, while LAMC2 and KRT14 were diagnostic markers of subtype II ESCCs, overexpressing genes involved in ectoderm development, glycolytic processes, and cell proliferation.[19] This suggests that subtype I ESCCs may be more susceptible to trimodal therapy. Consistent with that idea, Tanaka et al[6] reported that a subgroup of ESCCs overexpressing various immune activation-related genes exhibited greater susceptibility to chemoradiotherapy. Our finding that patients with hot tumors had a significantly better prognosis after NACRT plus surgical treatment agrees with those of earlier studies.

TABLE 2.

Univariate and Multivariate Survival Analyses of 5-Year Disease-Specific Survival (n = 99)

	Univariate Survival Analyses				Multivariate Survival Analyses		
		95% (CI)				95% (CI)	
Variables	HR	L	U	P	HR	L	U
Age: >65 (n = 47) vs <65 (n = 52)	1.461	0.695	3.073	0.318	–	–	–
Gender: male (n = 84) vs female (n = 15)	NA	NA	NA	0.999	–	–	–
Tumor location: upper (n = 19) vs other (n = 80)	1.054	0.427	2.601	0.909	–	–	–
Tumor differentiation: not poorly (n = 89) vs poorly (n = 10)	NA	NA	NA	0.999	–	–	–
ypStage: III–IV (n = 41) vs I–II (n = 58)	2.693	1.261	5.753	0.011	2.613	1.218	5.606
TRG: grade 1 (n = 30) vs grades 2–3 (n = 69)	2.869	1.366	6.027	0.005	2.553	1.209	5.392
Tumor immunogenicity: cold tumor (n = 71) vs hot tumor (n = 28)	2.932	1.017	8.456	0.047	2.925	1.012	8.460

CI indicates confidence interval; HR, hazard ratio; TRG, tumor regression grade; ypStage, pathological stage after NACRT.

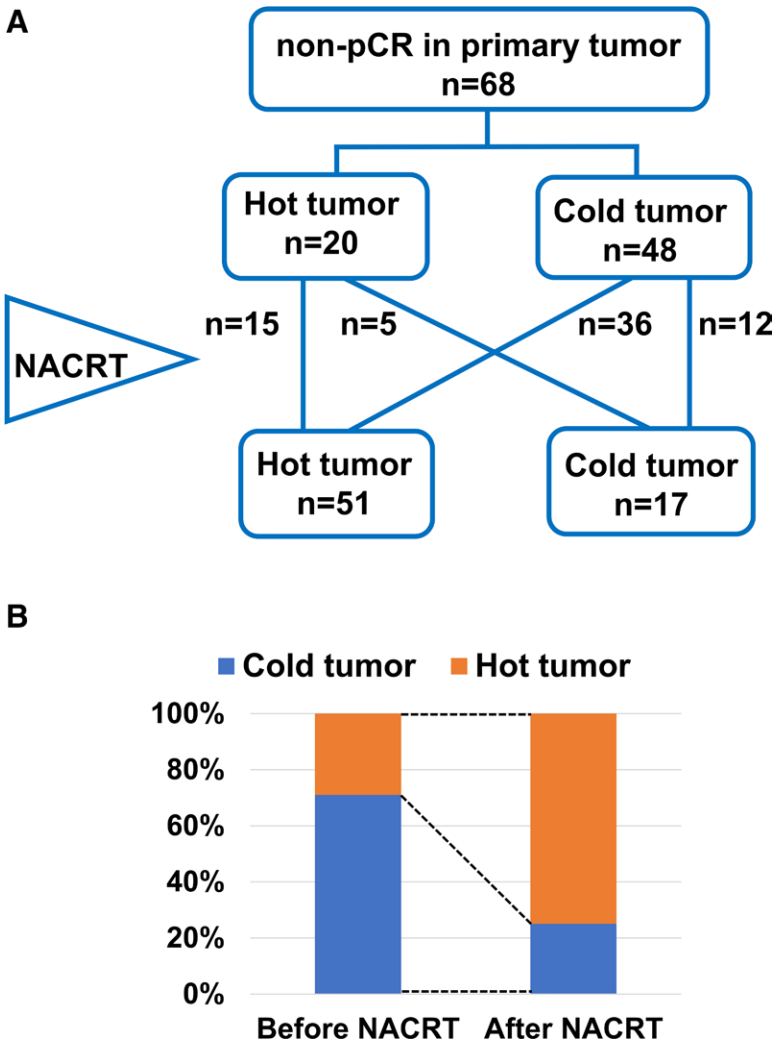

FIGURE 4. A and B, Flow chart (A) and bar chart (B) showing the change in tumor immunogenicity before and after NACRT in patients without a pathological complete response (non-pCR) in their primary tumors (n = 68). Twenty patients (29%) had hot tumors before NACRT, whereas 51 patients (75%) had hot tumors after NACRT; that is, NACRT upregulated tumor immunogenicity from cold to hot.

Multimodal therapy for EC is now facing a major turning point. Immunotherapy has become a key approach for EC patients and now ranks with traditional therapies for EC.[8–12] Biomarkers indicative of tumor immunogenicity and predictive of treatment response are more important with immunotherapy than with conventional therapies. At present, PD-L1 expression assessed based on an IHC Combined Positive Score[11,25,26] or Tumor Proportion Score,[8] tumor mutation burden,[27] deficient DNA mismatch repair, and microsatellite instability[28] are all reported to be predictive biomarkers associated with the efficacy of ICIs. Our findings suggest that FOXA1 and EYA2 are predictive biomarkers for conventional therapy and that combining these biomarkers for subtype I ESCCs may also be predictive of immunotherapeutic efficacy. However, no patients enrolled in the present study received ICI therapy before surgery and patients were not necessarily treated with ICIs after surgery. This is a major limitation of this study.

A crucial finding of this study is that radiotherapy and/or chemotherapy may convert a nonimmunogenic tumor to an immunogenic tumor. For ESCC patients with cold tumors, intervention leading to conversion to a hot tumor with high immunogenicity is necessary to optimize the response to ICIs. Then combining ICIs with conventional radiotherapy and/or chemotherapy reportedly improves antitumor responses and has a subsequent survival benefit as compared to ICIs alone.[18] Release of neoantigens induced by tumor cell death caused by localized radiotherapy or cytotoxic chemotherapy promotes activation of T cells and migration of T cells into tumors, which can induce antitumor-specific immune responses. In that regard, Demaria et al[29] showed that, in mice, local radiation of a tumor also impairs the growth of nonirradiated portions of the tumor at a distance and that this abscopal effect is immunologically mediated. Moreover, this effect was shown to be tumor-specific, as the growth of a nonirradiated lymphoma coimplanted in the same mice was unaffected.[29] The clinical benefit of combined ICI therapy with radiotherapy as compared to radiotherapy alone has also been observed in multiple retrospective analyses of patients with brain metastasis, mainly from melanoma, nonsmall cell lung cancer (NSCLC) or renal cell carcinoma.[30] In addition, a retrospective analysis by Koller et al[31] showed that radiotherapy plus ipilimumab improves survival and the response rate in patients with metastatic melanoma as compared to ipilimumab alone. A pooled analysis of 2 prospective randomized phase 3 trials also demonstrated that adding radiotherapy to pembrolizumab improves clinical benefit without increasing toxicity in patients with NSCLC as compared to pembrolizumab alone.[32] These studies suggest combining radiotherapy and ICIs could improve the prognosis of patients with cancer. However, another randomized phase 2 trial showed that radiotherapy combined with durvalumab and tremelimumab, a PD-L1 and CTLA-4 inhibitor,

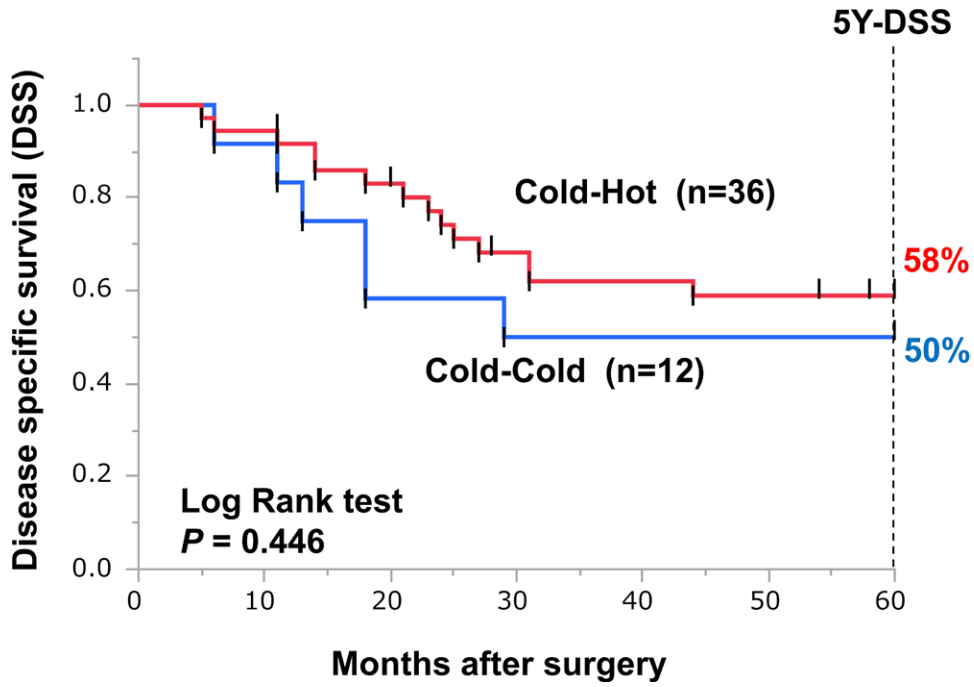

FIGURE 5. Kaplan–Meier curves comparing DSS among ESCC patients with cold tumors before NACRT (n = 48) and then cold or hot tumors after NACRT. The log-rank test was used to compare the curves.

respectively, did not improve the response rate as compared to ICIs alone in patients with NSCLC.[33] What's more, the rate of treatment-related serious adverse events (including 1 death from respiratory failure) was increased in patients in the ICIs plus radiotherapy group (15–19% vs 4%, respectively).[33] The difference in the efficacy and toxicity of adding radiotherapy to immunotherapy may reflect a difference in the type or timing of the radiotherapy. The ELEKTRA trial, an exploratory phase 2 trial for patients with melanoma brain metastasis, focused on the timing of irradiation with respect to the administration of ipilimumab and nivolumab. It was observed that radiotherapy followed by ICIs had clinical benefits associated with increasing numbers of activated T cells compared to ICI treatment before radiotherapy.[34] This is consistent with our present finding that neoadjuvant therapy, which included radiotherapy, could enhance tumor immunogenicity. The development of combination therapies employing ICIs is needed to drive an antitumor immune response not only in patients presenting with a hot tumor but also in those presenting with a cold tumor.

Evidence attesting to the effectiveness of ICIs for patients with ESCC has accumulated from multiple randomized clinical trials carried out around the world.[8–12] As first-line therapy, patients with metastatic or recurrent unresectable ESCC are being treated with pembrolizumab or nivolumab combined with conventional chemotherapy, or with doublet ICIs, ipilimumab plus nivolumab. The results of the CheckMate 577 trial are particularly important for patients with resected ESCC.[12] Patients treated with NACRT followed by R0 esophagectomy, but showing an incomplete pathological response were enrolled in this clinical trial. The results established adjuvant treatment with nivolumab to be effective for patients who remain at high risk for recurrence after NACRT. The background of the patients in the present study resembled those of the patients in the CheckMate 577 trial; indeed, some patients in the present study were enrolled in the CheckMate 577 trial. Consequently, our finding that FOXA1 and EYA2 are useful immunogenicity biomarkers may support the results of the CheckMate 577 trial. Future studies examining how tumor immunogenicity changes after neoadjuvant chemotherapy (standard treatment for resectable advanced ESCC in Japan from JCOG1109[35]) will be required to verify our findings.

In this study, we investigated whether NACRT used for patients with ESCC affects tumor immunogenicity assessed based on expression of 2 previously reported biomarkers. As noted above, 1 major limitation of this study is that only a small percentage of patients enrolled in this study were treated with ICIs after surgery. Twelve patients enrolled in the CheckMate 577 trial received nivolumab or a placebo as adjuvant therapy after surgery. In addition, after recurrence, a few other patients were treated with nivolumab plus ipilimumab, with nivolumab monotherapy, or with chemotherapy in the CheckMate 648 trial. A second limitation is the retrospective nature of this study, which could potentially introduce selection bias. In addition, because this study included only a small number of cases (only 99 thoracic ESCC patients) at a single center, its impact is limited. Moreover, immunogenicity results before NACRT might have been inadequate to decide on the immune profile of the whole tumor since the biopsy tissue volume obtained by esophagogastroduodenoscopy was small. Lastly, we chose DSS defined as the time from the surgery date to death from EC as the primary endpoint. We also analyzed the OS in this cohort, but there were no significant outcomes. In general, patients with ESCC frequently have multiple primary cancers, so death from other cancers inevitably increases. In addition, because death from other diseases after NACRT can't be neglected such as lung and heart disorders, DSS as the primary endpoint for the pure evaluation of the impact of chemoradiotherapy on survival rate and tumor immunogenicity.

CONCLUSIONS

Our findings indicate that NACRT is effective in hot tumors and that it converted cold tumors to immunoreactive hot tumors. This suggests NACRT could improve the efficacy of ICIs administered as adjuvant therapy in ESCC. We anticipate that assessing tumor immunoreactivity will help us establish a perioperative treatment strategy that improves the survival of thoracic ESCC patients.

Acknowledgments

We thank all members of our laboratories for their technical assistance and helpful discussions. This work was supported by Grants-in-Aid for Scientific Research (Kaken) from the Japanese Ministry of Science to Y.N. (23K15512).

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209–249.
2. Arnold M, Ferlay J, van Berge Henegouwen MI, et al. Global burden of oesophageal and gastric cancer by histology and subsite in 2018. *Gut*. 2020;69:1564–1571.
3. Pasquali S, Yim G, Vohra RS, et al. Survival after neoadjuvant and adjuvant treatments compared to surgery alone for resectable esophageal carcinoma: a network meta-analysis. *Ann Surg*. 2017;265:481–491.
4. Moehler M, Maderer A, Thuss-Patience PC, et al. Cisplatin and 5-fluorouracil with or without epidermal growth factor receptor inhibition panitumumab for patients with non-resectable, advanced or metastatic oesophageal squamous cell cancer: a prospective, open-label, randomised phase III AIO/EORTC trial (POWER). *Ann Oncol*. 2020;31:228–235.
5. Leng X-F, Daiko H, Han Y-T, et al. Optimal preoperative neoadjuvant therapy for resectable locally advanced esophageal squamous cell carcinoma. *Ann N Y Acad Sci*. 2020;1482:213–224.
6. Tanaka Y, Aoyagi K, Minashi K, et al. Discovery of a good responder subtype of esophageal squamous cell carcinoma with cytotoxic T-Lymphocyte signatures activated by chemoradiotherapy. *PLoS One*. 2015;10:e0143804.
7. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359:1350–1355.
8. Doki Y, Ajani JA, Kato K, et al; CheckMate 648 Trial Investigators. CheckMate 648 trial investigators nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *N Engl J Med*. 2022;386:449–462.
9. Sun J-M, Shen L, Shah MA, et al; KEYNOTE-590 Investigators. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. *Lancet*. 2021;398:759–771.
10. Kato K, Cho BC, Takahashi M, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol*. 2019;20:1506–1517.
11. Kojima T, Shah MA, Muro K, et al; KEYNOTE-181 Investigators. Randomized Phase III KEYNOTE-181 study of pembrolizumab versus chemotherapy in advanced esophageal cancer. *J Clin Oncol*. 2020;38:4138–4148.
12. Kelly RJ, Ajani JA, Kuzdzal J, et al; CheckMate 577 Investigators. Adjuvant nivolumab in resected esophageal or gastroesophageal junction cancer. *N Engl J Med*. 2021;384:1191–1203.
13. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature*. 2017;541:321–330.
14. Chan TA, Yarchoan M, Jaffee E, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol*. 2019;30:44–56.
15. Jardim DL, Goodman A, de Melo Gagliato D, et al. The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell*. 2021;39:154–173.
16. Teng MWL, Ngiow SF, Ribas A, et al. Classifying cancers based on T-cell Infiltration and PD-L1. *Cancer Res*. 2015;75:2139–2145.
17. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
18. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;348:56–61.
19. Wang F, Yan Z, Lv J, et al. Gene expression profiling reveals distinct molecular subtypes of esophageal squamous cell carcinoma in Asian populations. *Neoplasia*. 2019;21:571–581.
20. Zhang J, Huang D, Saw PE, Song E. Turning cold tumors hot: from molecular mechanisms to clinical applications. *Trends Immunol*. 2022;43:523–545.
21. Sato Y, Motoyama S, Wada Y, et al. Neoadjuvant chemoradiotherapy followed by esophagectomy with three-field lymph node dissection for thoracic esophageal squamous cell carcinoma patients with clinical stage III and with supraclavicular lymph node metastasis. *Cancers*. 2021;13:983.
22. Rice TW, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: application to clinical practice. *Ann Cardiothorac Surg*. 2017;6:119–130.
23. US Department of Health and Human Services. *Common Terminology Criteria for Adverse Events (CTCAE). Version 4.0*. National Institutes of Health; 2009.
24. Japan Esophageal Society. *Japanese Classification of Esophageal Cancer*. 12th ed; 2022.
25. Ott PA, Bang Y-J, Piha-Paul SA, et al. T-Cell–Inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J Clin Oncol*. 2019;37:318–327.
26. Shah MA, Kojima T, Hochhauser D, et al. Efficacy and safety of pembrolizumab for heavily pretreated patients with advanced, metastatic adenocarcinoma or squamous cell carcinoma of the esophagus: the phase 2 KEYNOTE-180 study. *JAMA Oncol*. 2019;5:546–550.
27. McGranahan N, Furness AJS, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science*. 2016;351:1463–1469.
28. Zhao P, Li Li, Jiang X, et al. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J Hematol Oncol*. 2019;12:54.
29. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys*. 2004;58:862–870.
30. Voronova V, Lebedeva S, Sekacheva M, et al. Quantification of scheduling impact on safety and efficacy outcomes of brain metastasis radio- and immuno-therapies: a systematic review and meta-analysis. *Front Oncol*. 2020;10:1609.
31. Koller KM, Mackley HB, Liu J, et al. Improved survival and complete response rates in patients with advanced melanoma treated with concurrent ipilimumab and radiotherapy versus ipilimumab alone. *Cancer Biol Ther*. 2017;18:36–42.
32. Theelen WSME, Chen D, Verma V, et al. Pembrolizumab with or without radiotherapy for metastatic non-small-cell lung cancer: a pooled analysis of two randomised trials. *Lancet Respir Med*. 2021;9:467–475.
33. Schoenfeld JD, Giobbie-Hurder A, Ranasinghe S, et al. Durvalumab, tremelimumab alone or in combination with low-dose or hypofractionated targeted radiotherapy in metastatic non-small cell lung cancer refractory to prior PD(L)-1 therapy: a multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol*. 2022;23:279–291.
34. Hassel JC, Schank TE, Smetak H, et al. Evaluation of radio-immunotherapy sequence on immunological responses and clinical outcomes in patients with melanoma brain metastases (ELEKTRA). *Oncoimmunology*. 2022;11:2066609.
35. Kato K, Ito Y, Daiko H, et al. A randomized controlled phase III trial comparing two chemotherapy regimen and chemoradiotherapy regimen as neoadjuvant treatment for locally advanced esophageal cancer, JCOG1109 NExT study. *J Clin Oncol*. 2022;40(4_suppl):238–238.