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Circulating Tumor DNA Dynamic Changes in Esophageal Squamous Cell Carcinoma Receiving Immunochemotherapy

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Keywords: circulating tumor DNA | esophageal squamous cell carcinoma | immunochemotherapy

ABSTRACT

Background: Immune checkpoint inhibitors have revolutionized the treatment strategy of esophageal squamous cell carcinoma (ESCC). The value of ctDNA dynamic changes in ESCC patients treated with immunochemotherapy was not clear.

Methods: A retrospective analysis was performed to analyze the association of ctDNA dynamic changes with the treatment efficacy of immunochemotherapy in patients with locally advanced, metastatic, or recurrent ESCC and who received immunochemotherapy at the Department of Medical Oncology, National Cancer Center from June 2023 to December 2024. Tumor mutation burden (TMB) and PD-L1 expression of tumor tissue were also explored.

Results: 57 patients with paired ctDNA at baseline and during treatment were analyzed. We found that patients with negative ctDNA during treatment demonstrated a higher tumor regression rate (96.8% vs. 73.1%; $p = 0.018$) and a higher cCR rate (45.2% vs. 15.4%; $p = 0.022$). Additionally, patients with continuously negative ctDNA ($p = 0.033$) or experienced ctDNA clearance during treatment ($p = 0.043$) had a higher cCR rate compared to those with persistently positive ctDNA. Moreover, among patients with TP53 mutations at baseline, those with TP53 mutations cleared during treatment showed a higher tumor regression rate (88.9% vs. 54.5%; $p = 0.031$) and cCR rate (33.3% vs. 0%; $p = 0.038$) compared to patients with persistent TP53 mutations. No correlation was observed between TMB and treatment efficacy, while a higher cCR rate was observed in patients with PD-L1 CPS ≥ 15 (63.6% vs. 24.4%; $p = 0.027$).

Conclusions: ctDNA dynamic changes demonstrated potential predictive value for the efficacy of immunochemotherapy in patients with ESCC. Further exploration through larger-scale studies is necessary.

1 | Introduction

Esophageal carcinoma is one of the most prevalent malignant tumors in China and globally, with esophageal squamous cell carcinoma (ESCC) being the predominant pathological subtype. The clinical symptoms of early ESCC are not distinctive; thus, the majority of patients are diagnosed at a locally advanced stage or with distant metastasis [1]. Immune checkpoint inhibitors have revolutionized the treatment strategy of ESCC. The combination of immunotherapy with chemotherapy has become the standard first-line treatment for patients with advanced ESCC [2–4]. Recently, two phase III randomized controlled clinical

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trials have demonstrated that neoadjuvant immunochemotherapy significantly improved the pathological complete response (pCR) rate in locally advanced ESCC compared to chemotherapy alone [5, 6]. Nevertheless, considerable heterogeneity was observed in treatment outcomes among patients, and there remains a lack of robust biomarkers to predict tumor response.

Most of the biomarkers investigated in previous studies were based on tumor tissue samples, such as PD-L1 expression, tumor mutation burden (TMB), and tumor-infiltrating lymphocytes. However, considering drug resistance and aggressive progression of ESCC, adjustment and optimization of the treatment regimen timely is an urgent issue. The difficulty in taking biopsy for tumor tissue repeatedly limits the feasibility of real-time dynamic monitoring. Circulating tumor DNA (ctDNA), a form of extracellular DNA released into the bloodstream by tumor cells, offers a promising alternative strategy. Detection of ctDNA from peripheral blood samples, commonly referred to as liquid biopsy, represents a non-invasive approach that enables dynamic monitoring of tumor load at multiple time points and clonal evolution of tumor mutations during therapy [7], and has great potential for disease monitoring, prognosis assessment, and treatment adjustment.

The development of next-generation sequencing (NGS) technology has significantly enhanced the sensitivity for detecting low-frequency mutations in ctDNA. Consequently, ctDNA has been widely recognized as a biomarker for identifying minimal residual disease (MRD) following curative treatment such as surgery or chemoradiotherapy (CRT) [8, 9]. The prognostic value of ctDNA dynamic changes has also been explored in several studies, especially in patients treated with CRT [10–12]. In a study enrolling 45 patients with esophageal cancer (including ESCC and esophageal adenocarcinoma), ctDNA detection was performed before and after CRT; the study revealed that detection of ctDNA following CRT was associated with tumor progression, formation of distant metastases, and shorter disease-specific survival [11]. In addition, the exploratory study of a phase II clinical trial (EC-CRT-001) enrolled locally advanced ESCC patients receiving toripalimab in combination with definitive CRT and evaluated ctDNA at three distinct time points: pre-treatment, during CRT, and post-CRT. The results demonstrated that ctDNA negativity during CRT or post-CRT was correlated with better tumor response and survival [12].

Progress in neoadjuvant immunochemotherapy enables the application of this combination more and more widely in ESCC. But the role of ctDNA dynamic changes in ESCC patients receiving immunochemotherapy remains unclear. Therefore, we conducted a retrospective study to explore ctDNA dynamic changes in ESCC patients treated with immunochemotherapy.

2 | Methods

2.1 | Study Design and Patients

A retrospective analysis was performed to analyze the association of ctDNA dynamic changes with the treatment efficacy of immunochemotherapy. Patients with locally advanced, metastatic, or recurrent ESCC who received neoadjuvant or first-line immunochemotherapy at the Department of Medical Oncology, National Cancer Center from June 2023 to December 2024 and met the following requirements were included: patients must have paired ctDNA detected both at baseline and during treatment; patients must have imaging-based efficacy evaluation after treatment. TMB and PD-L1 expression of tumor tissue were also explored.

Enhanced CT were performed every 6 weeks, and the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) were utilized to assess treatment efficacy in these patients. Considering that not all included patients have target lesions, to standardize the efficacy evaluation criteria for subsequent analyses, we defined tumor regression as enhanced CT demonstrating the relief of esophageal thickening, shrinkage of metastatic lymph nodes, or size reduction of other metastatic lesions. For patients who achieved CR, further endoscopic examination and biopsy were performed. Patients with no residual tumor confirmed by endoscopic examination and biopsy were evaluated as clinical complete response (cCR).

2.2 | Preparation of Plasma ctDNA and Tumor Tissue DNA

Ten milliliters of whole blood was centrifuged at 2000 g for 10 min at 4°C. Then the supernatant was centrifuged at 16000 g for another 10 min at 4°C for plasma collection. Approximately 4 mL of plasma was transferred to a new tube, and plasma ctDNA was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen, Valencia, CA, USA). Tumor tissue DNA was extracted using the QIAamp DNA tissue kit (Qiagen, Valencia, CA, USA). The concentrations of tissue DNA and ctDNA were measured by the Qubit 2.0 Fluorometer with the dsDNA HS assay kit (Life Technologies, Carlsbad, CA, USA).

2.3 | Library Preparation and Next-Generation Sequencing (NGS)

DNA fragmentation was performed using the M220 Focused-ultrasonicator (Covaris, Woburn, MA, USA), followed by end repair and adaptor ligation. Fragments of 200–400 bp in size were selected by AMPure beads (Agencourt AMPure XP kit; Beckman Coulter, Brea, CA, USA). Hybridization with bait probes was performed using a 168-gene panel and a 520-gene panel (Burning Rock Biotech, Guangzhou, China) for plasma samples and tumor tissue, respectively, followed by hybrid selection with magnetic beads and polymerase chain reaction amplification. Libraries were sequenced on an Illumina NextSeq 500 (Illumina Inc., San Diego, CA, USA) with paired-end reads.

2.4 | Sequencing Data Analysis

Sequencing data were mapped to the human genome (hg19) using Burrows-Wheeler aligner 0.7.10. Local alignment optimization, variant calling, and annotation were performed using the GATK 3.2 and MuTect (both from Broad Institute, Cambridge, MA, USA) and VarScan (Genome Institute, Washington University, USA) software. Variants were filtered using the VarScan filter pipeline. According to the ExAC, 1000 Genomes Project, dbSNP, and ESP6500SI-V2 databases, variants with a population frequency of over 0.1% were grouped as single-nucleotide polymorphisms

TABLE 1 | Patient characteristics.

Characteristics	*N* = 57
Median age (range)–year	62 (42–76)
Age group, No. (%)	
< 65	35 (61.4)
≥ 65	22 (38.6)
Sex, *n* (%)	
Male	47 (82.5)
Female	10 (17.5)
ECOG performance status score, No. (%)*	
0	25 (43.9)
1	31 (54.4)
2	1 (1.8)
Disease status, No. (%)	
Locally advanced	29 (50.9)
Distant metastatic	28 (49.1)
Sites of metastases, No. (%)	
Lymph node	46 (80.7)
Liver	3 (5.3)
Lung	2 (3.5)
Others	4 (7.0)
Previous treatment, No. (%)	
Treatment naive	52 (91.2)
Surgery	4 (7.0)
Chemoradiotherapy	1 (1.8)
PD-L1 expression, No. (%)	
NA	5 (8.8)
CPS < 1	11 (19.3)
CPS ≥ 1	41 (71.9)
CPS < 15	41 (71.9)
CPS ≥ 15	11 (19.3)
Smoking status, No. (%)	
Current or former	38 (66.7)
Never	19 (33.3)
Alcohol consumption, No. (%)	
Current or former	35 (61.4)
Never	22 (38.6)
PD-1/PD-L1 inhibitors, No. (%)	
Camrelizumab	26 (45.6)
Pembrolizumab	14 (24.6)
Adebrelimab	9 (15.8)
Cadonilimab	4 (7.0)
Sintilimab	2 (3.5)
Others	2 (3.5)
Chemotherapy regimens, No. (%)	
Nab-paclitaxel + cisplatin + fluoropyrimidines	39 (68.4)
Nab-paclitaxel + fluoropyrimidines	11 (19.3)
Nab-paclitaxel + cisplatin	5 (8.8)
Others	2 (3.5)

Abbreviations: CPS, combined positive score; ECOG, Eastern Cooperative Oncology Group; NA, not available; Nab-paclitaxel, albumin bound-paclitaxel; PD-L1, programmed death ligand 1; TMB, tumor mutation burden.
*Percentages may not add up to 100% owing to rounding.

(SNPs) and were excluded. Variants with variant allele frequency (VAF) ≥ 0.1% in plasma sample or VAF ≥ 1% in tumor tissue were defined as positive. Copy number should reach the minimum threshold as to gain and loss (as to copy number gain, CN > 2.25 for hotspot genes and CN > 2.5 for others; as to copy number loss, CN < 1.75 for hotspot genes and CN < 1.5 for others).

2.5 | Determination of Positive ctDNA and TMB Calculation

Positive ctDNA was defined as the detection of at least one somatic gene alteration. The maximal variant allele frequency (maxVAF), which was the highest VAF detected in ctDNA, was used for analysis.

TMB was calculated as the number of non-synonymous somatic single nucleotide variants (SNVs) and small insertions/deletions (InDels) in the coding regions of the targeted genes per million base pairs after excluding copy number variants (CNVs) and fusions.

2.6 | PD-L1 Expression

PD-L1 status was assessed by immunohistochemistry staining (22C3 PharmDx kit, Dako North America) and reported as a combined positive score (CPS). CPS was defined as the number of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) as a proportion of the total number of viable tumor cells multiplied by 100. CPS of 1, 5, 10, and 15 were determined as cutoff points for analysis.

2.7 | Statistical Analysis

Continuous variables were analyzed using the Mann–Whitney U test. Categorical variables were compared using the Chi-square test or Fisher's exact test. Spearman's correlation coefficient was

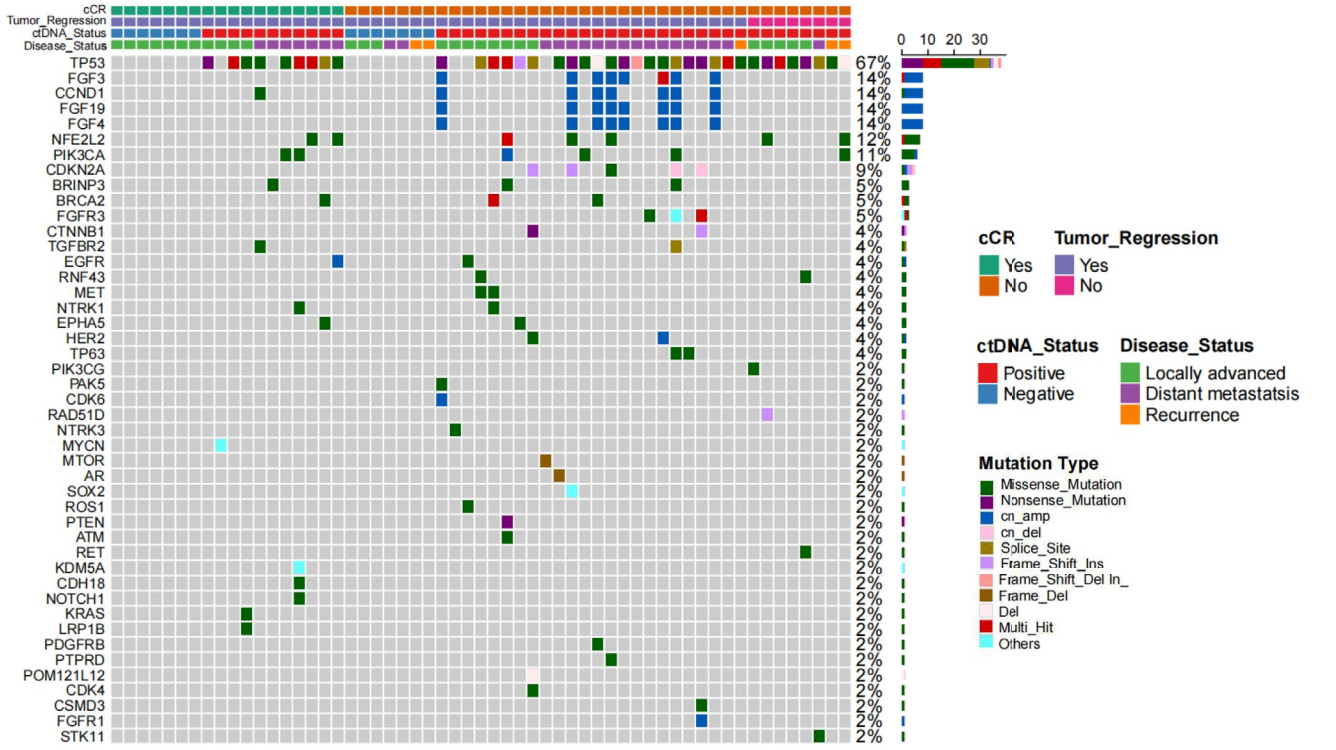

FIGURE 1 | Results of the ctDNA assay at baseline: Each column represents an individual patient. Clinical characteristics of cCR, tumor regression, ctDNA status, and disease status are shown at the top. Genes mutated in at least one (1.8%) of the patients in our cohort are depicted. The percentage of patients with mutations in each gene is shown on the left. ctDNA, circulating tumor DNA; cCR, clinical complete response.

utilized to assess the relationship between the VAF of mutations in tumor tissue and corresponding baseline plasma. All statistical tests were two-sided, and a p value <0.05 was considered statistically significant. Statistical analyses were performed by SPSS software (version 29.0). R software (version 4.3.3) was used to visualize the overall mutation landscape.

3 | Results

3.1 | Patient Characteristics and Efficacy

A total of 57 patients who received immunochemotherapy and had paired ctDNA detected both at baseline and during treatment were identified and were subsequently included in the analysis. The characteristics of these patients are summarized in Table 1. The median age of these patients was 62 years (range: 42–76 years), with the majority being male (47/57, 82.5%). Among them, 29 patients (50.9%) had locally advanced disease, and 28 patients (49.1%) had distant metastasis. The majority of patients (46/57, 80.7%) had lymph node metastasis. The most frequently administered PD-1/PD-L1 inhibitors included camrelizumab (26/57, 45.6%), pembrolizumab (14/57, 24.6%), and adebrelimab (9/57, 15.8%). The chemotherapy regimen most of the patients received was a triplet chemotherapy comprised of albumin-bound paclitaxel (nab-paclitaxel), cisplatin, and fluoropyrimidines (39/57, 68.4%), or a combination of two of these three drugs (16/57, 28.1%).

A total of 49 patients (86.0%) showed different degrees of tumor regression after treatment; 8 patients (14.0%) did not have tumor regression, of which 3 patients showed disease progression at the first efficacy evaluation after treatment. Eighteen patients (31.6%) achieved cCR confirmed by endoscopy and biopsy. At the data cutoff date (February 28, 2025), the median follow-up time was 7.59 months (range: 2.86–15.91 months); 13 patients had disease progression, and 2 patients died due to disease progression. Progression-free survival (PFS) and overall survival (OS) were not mature in these patients.

3.2 | Baseline ctDNA Analysis

Baseline ctDNA analysis revealed a total of 153 somatic alterations, including 96 SNVs, 16 InDels, 38 CNVs, and 3 translocations in 57 patients. The median number of alterations per patient was 2 (range: 0–11), the mean maxVAF was 8.1%, and the median maxVAF was 2.15%. Mutations were most frequently detected in TP53 (66.7%, $n=38$), followed by NFE2L2 (12.3%, $n=7$), PIK3CA (8.8%, $n=5$), and CDKN2A (5.3%, $n=3$) (Figure 1). CNVs were detected in 11 patients (19.3%). FGF3, FGF4, and FGF19 amplifications were the most common CNVs ($n=8$, 14.0%), followed by CCND1 amplification ($n=7$, 12.3%).

Paired tumor tissue and baseline plasma ctDNA were available in 53 patients. The 520 gene panel used in the tumor tissue test contains all the genes tested in the 168 gene panel used in the plasma test. In order to compare the consistency of baseline ctDNA with paired tumor tissue, mutations within the 168 gene panel were identified in the tumor tissue test results and then

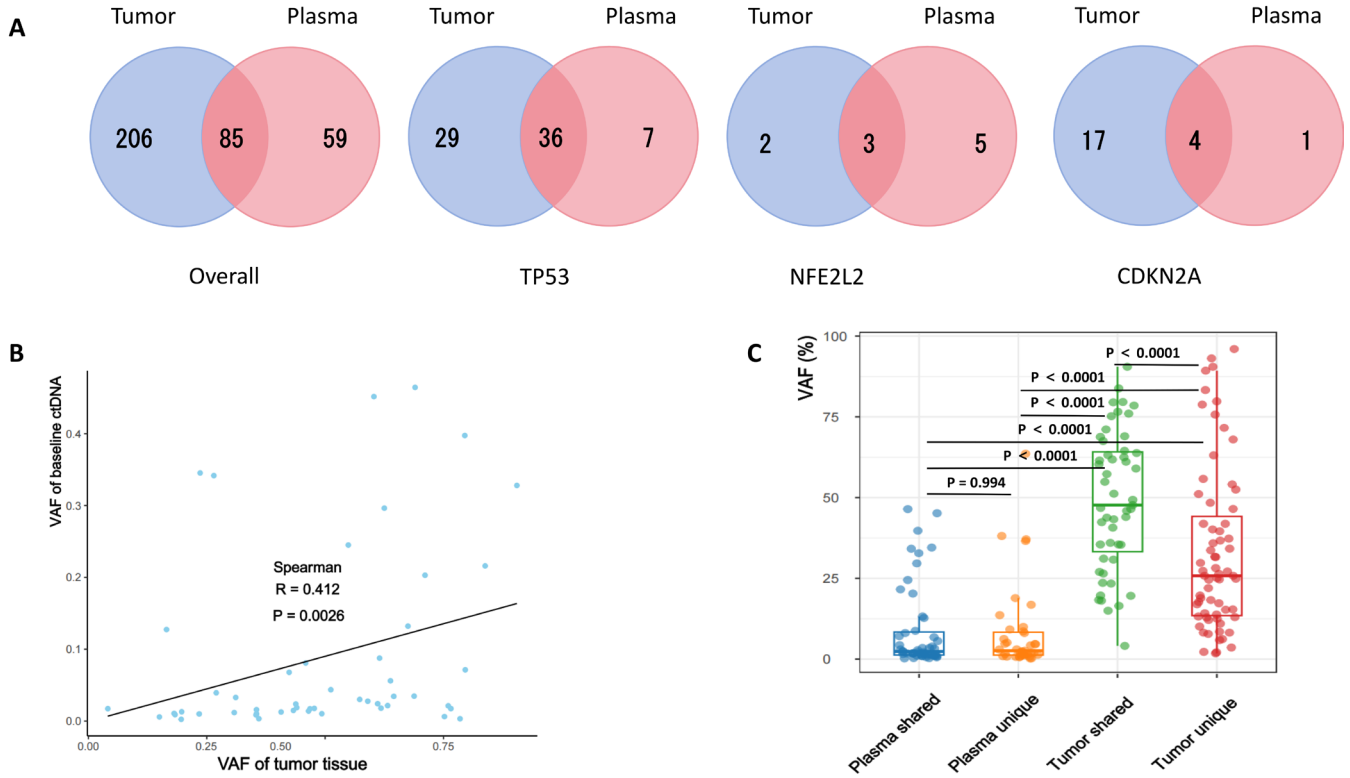

FIGURE 2 | (A) A Venn graph presented the number of shared variants of all, TP53, NFE2L2, and CDKN2A in baseline plasma and tumor tissue. (B) Correlation of VAF of the clone mutation in tumor tissue and the corresponding baseline plasma. (C) Comparison of the VAF of shared and unique variants in tumor tissue and the corresponding baseline plasma. VAF, variant allele frequency; ctDNA, circulating tumor DNA.

compared with the plasma ctDNA test results. A total of 144 somatic alterations were detected in the baseline ctDNA of the 53 patients, of which 85 alterations (59.0%) were shared by plasma and tumor tissue, and 59 (41.0%) of these alterations were unique to plasma ctDNA, suggesting that plasma can be a supplement to tumor tissue. The genetic alterations most frequently detected in plasma were also observed in tumor tissue, including TP53, NFE2L2, and CDKN2A (Figure 2A). Missense mutations were also the most commonly detected type of mutation in tumor tissue. The VAF of the dominant clone mutation in tumor tissue was positively correlated with its corresponding baseline plasma mutation, indicating that the abundance of ctDNA mutation was positively correlated with tumor DNA ($R=0.412$, $p=0.0026$, Figure 2B). The VAF of mutations shared by tumor tissue and plasma was significantly higher than mutations unique to tumor tissue (Figure 2C).

ctDNA was detected in 43 of 57 patients (75.4%) at baseline. The median number of alterations detected in these patients was 3 (range: 1–11). The ctDNA positive rate was higher in patients with distant metastatic diseases compared to patients with locally advanced diseases (92.9%, 26/28 vs. 58.6%, 17/29; $p=0.003$, Figure 3A). Similarly, patients with distant metastatic diseases also demonstrated higher maxVAF in baseline ctDNA compared to patients with locally advanced diseases ($p=0.0003$, Figure 3B).

The correlation between efficacy and most frequently detected genetic mutations in baseline ctDNA, including TP53, NFE2L2, PIK3CA, and CDKN2A, was analyzed; only TP53 mutations were related to a significantly lower tumor regression rate (78.9%, 30/38 vs. 100%, 19/19; $p=0.042$, Figure 3C), and none of these genetic mutations were related to cCR rate. In addition, none of the 8 patients with FGF3/4/19 amplifications achieved cCR, and the cCR rate was significantly lower compared to patients without FGF3/4/19 amplifications (0%, 0/8 vs. 36.7%, 18/49; $p=0.046$, Figure 3D).

3.3 | ctDNA Dynamic Changes and Efficacy

The median time between ctDNA detection at baseline and during treatment was 1.93 months, and the median treatment cycles patients completed before the second ctDNA detection was 3. The profile of plasma ctDNA detected during immunochemotherapy was displayed in Figure 4A. Totally, 47 alterations, 38 SNVs, 8 InDels, and 1 CNV were detected in these patients.

ctDNA positive rate decreased from 75.4% (43/57) at baseline to 45.6% (26/57) during treatment ($p=0.001$, Figure 4B). The mean max VAF of ctDNA also decreased from 8.1% to 3.4% ($p=0.0001$, Figure 4C), indicating a decrease in overall tumor load during treatment. There was no significant difference in tumor regression rate (negative: 100%, 14/14 vs. positive: 81.4%, 35/43; $p=0.179$, Figure 4D), or cCR rate (negative: 50%, 7/14 vs. positive: 25.6%, 11/43; $p=0.107$ Figure 4E) between patients with different ctDNA status at baseline. While patients with negative ctDNA during treatment demonstrated a higher proportion of patients with tumor regression (negative: 96.8%, 30/31 vs. positive: 73.1%, 19/26; $p=0.018$, Figure 4F) and a higher cCR

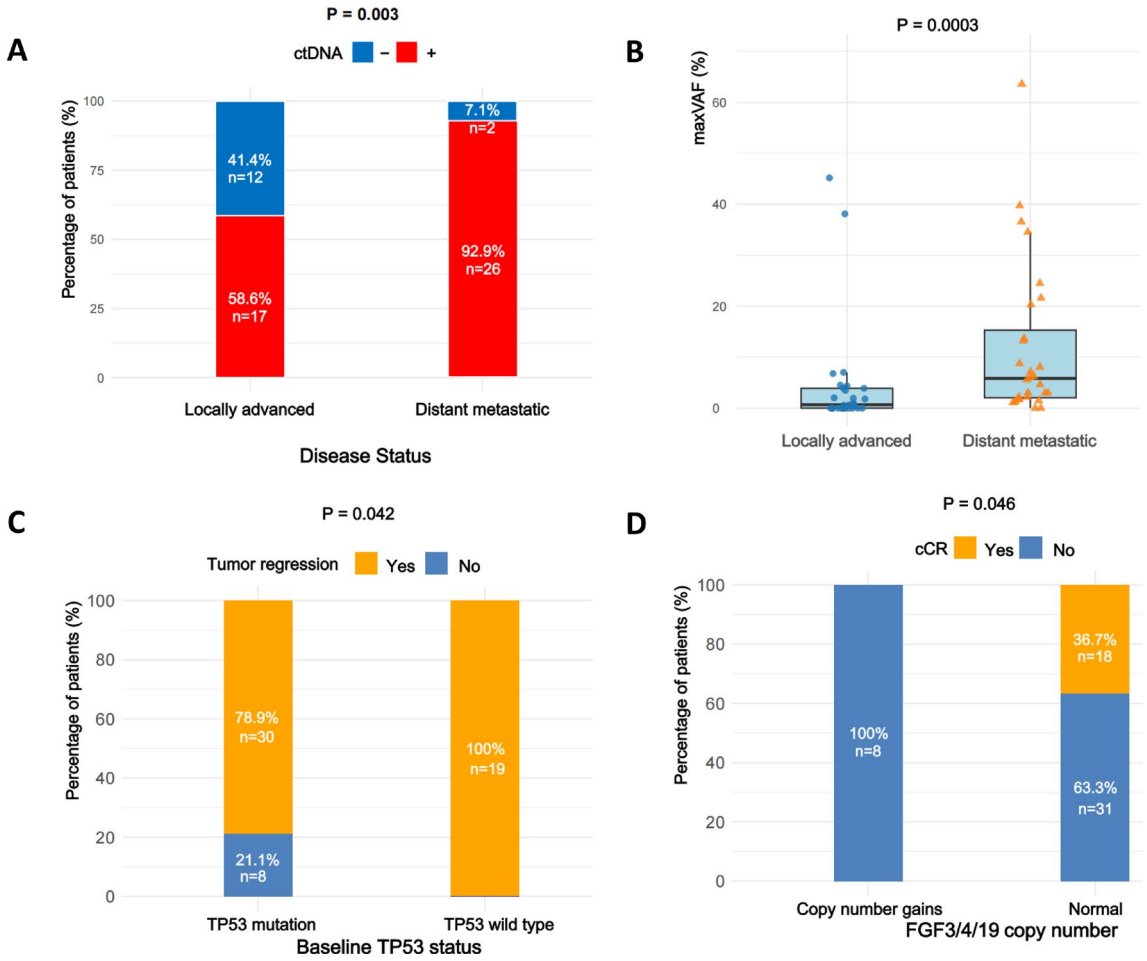

FIGURE 3 | (A) Disease status and ctDNA positive rate at baseline. (B) Disease status and maxVAF at baseline. (C) TP53 status at baseline and tumor regression rate. (D) FGF3/4/19 copy number variants at baseline and cCR rate. ctDNA, circulating tumor DNA; maxVAF, maximal variant allele frequency; cCR, clinical complete response.

rate (negative: 45.2%, 14/31 vs. positive: 15.4%, 4/26; $p = 0.022$, Figure 4G).

To analyze the correlation between ctDNA dynamic changes and efficacy, we divided the changes of ctDNA status at baseline and during treatment into four patterns: pattern 1 (ctDNA was negative both at baseline and during treatment, $n = 11$); pattern 2 (ctDNA was positive at baseline and cleared during treatment, $n = 20$); pattern 3 (ctDNA was negative at baseline but positive during treatment, $n = 3$); pattern 4 (consistently positive ctDNA, $n = 23$). The cCR rates of the four patterns were 54.5% (6/11), 40% (8/20), 33.3% (1/3), and 13.0% (3/23), respectively (Figure 5A–D). The tumor regression rates were 100% (11/11), 95% (19/20), 100% (3/3), and 69.6% (16/23), respectively. Compared with pattern 4, the cCR rate was higher in patients with persistently negative ctDNA (pattern 1; $p = 0.033$) and patients with ctDNA clearance during treatment (pattern 2; $p = 0.043$). However, there was no significant difference in the tumor regression rates between these four patterns.

We also analyzed the dynamic change of TP53 status in patients with TP53 mutation at baseline ($n = 38$). Patients were divided into two patterns: pattern 1 (TP53 mutation cleared during treatment, $n = 27$) and pattern 2 (TP53 mutation persistently exist at baseline and during treatment). Compared to pattern 2, patients with TP53 mutation clearance during treatment demonstrated a higher tumor regression rate (88.9%, 24/27 vs. 54.5%, 6/11; $p = 0.031$, Figure 5E) and a higher cCR rate (33.3%, 9/27 vs. 0%, 0/11; $p = 0.038$, Figure 5F).

3.4 | TMB and Efficacy

TMB was available in 54 patients, with a median TMB of 6 muts/Mb (range: 1–19 muts/Mb). The difference in TMB was not significant between patients with or without cCR ($p = 0.468$), or between patients with or without tumor regression ($p = 0.578$). In addition, there was no difference in the cCR rate ($p = 0.481$) and tumor regression rate ($p = 0.658$) between patients with TMB < 10 muts/Mb and TMB ≥ 10 muts/Mb.

3.5 | PD-L1 Expression and Efficacy

PD-L1 expression was available in 53 patients, and the majority of patients were with positive PD-L1 (CPS ≥ 1: 41/53, 77.4%). The difference in PD-L1 expression was not significant between patients with or without cCR ($p = 0.169$) or between patients with or without tumor regression ($p = 0.128$). The cCR rate

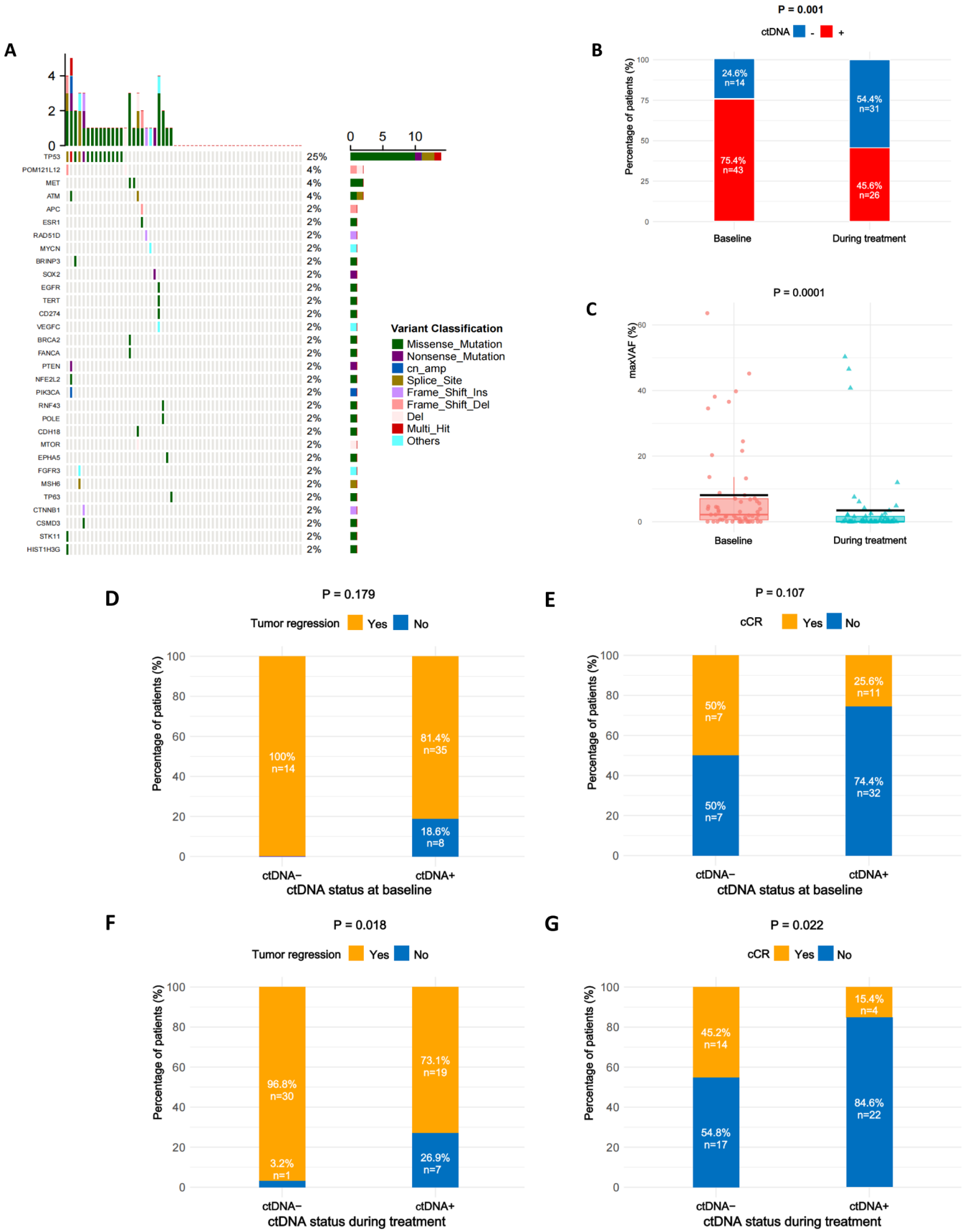

FIGURE 4 | (A) Results of the ctDNA assay during treatment. (B) ctDNA positive rate at baseline and during treatment. (C) maxVAF at baseline and during treatment. (D) ctDNA status at baseline and tumor regression rate. (E) ctDNA status at baseline and cCR rate. (F) ctDNA status during treatment and tumor regression rate. (G) ctDNA status during treatment and cCR rate. ctDNA, circulating tumor DNA; maxVAF, maximal variant allele frequency; cCR, clinical complete response.

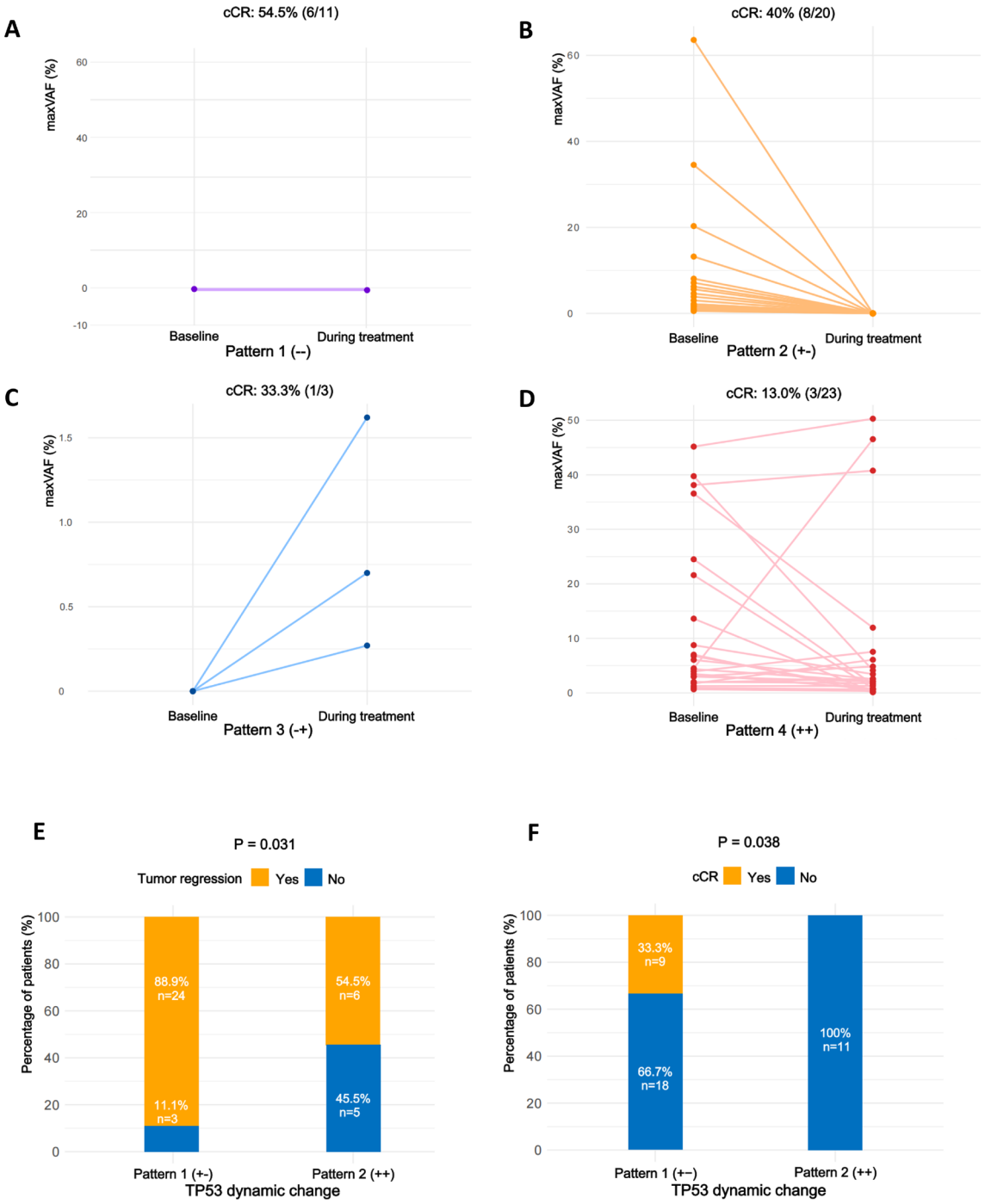

FIGURE 5 | cCR rates in four different ctDNA change patterns: (A) Pattern 1: Negative ctDNA at baseline and during treatment, $n = 11$. (B) Pattern 2: ctDNA was positive at baseline but cleared during treatment, $n = 20$. (C) Pattern 3: ctDNA was negative at baseline but positive during treatment, $n = 3$. (D) Pattern 4: Consistently positive ctDNA, $n = 23$. (E) Different TP53 mutation change patterns and cCR rate. (F) Different TP53 mutation change patterns and tumor regression rate.

was significantly higher in patients with CPS ≥ 15 compared to patients with CPS < 15 (63.6%, 7/11 vs. 24.4%, 10/41; $p = 0.027$, Figure 6), while the tumor regression rate did not differ significantly between these two groups ($p = 0.322$). Tumor regression rate and cCR rate between high and low PD-L1 expression groups based on the CPS cutoff points of 1, 5, and 10 were also compared, but no significant differences were observed.

4 | Discussion

The study analyzed the association of ctDNA dynamic changes, TMB, and PD-L1 expression with tumor response in patients with ESCC treated with immunochemotherapy and demonstrated the predictive potential of ctDNA dynamic changes for anti-tumor efficacy.

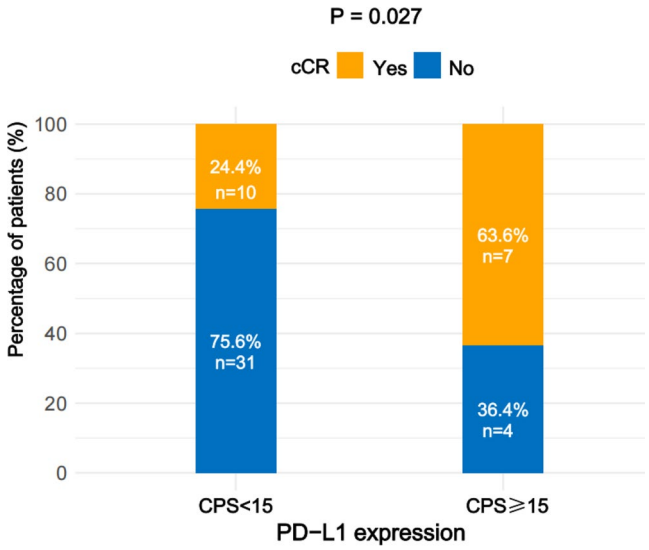

FIGURE 6 | cCR rate in patients with PD-L1 CPS < 15 and CPS ≥ 15.

The ctDNA variation profile of ESCC patients in our study was consistent with previous studies, with TP53, NFE2L2, PIK3CA, and CDKN2A being the most frequently mutated genes, and FGF3/4/19 amplifications were among the most commonly detected copy number variants. TP53 mutations have been associated with the development of various tumors, including esophageal cancer. Previous research has demonstrated that the predictive value of TP53 mutations varies with the types of cancer. For instance, TP53 mutations may serve as a positive predictor for immunotherapy efficacy in breast cancer and lung adenocarcinoma, but as a negative predictor in gastric adenocarcinoma, colorectal cancer, and head and neck squamous cell carcinoma (HNSCC) [13]. However, the predictive value of TP53 mutations in the efficacy of immunochemotherapy remains unclear in esophageal cancer. In our study, the baseline TP53 mutation status and its dynamic changes during treatment were demonstrated to predict the response to immunochemotherapy. Specifically, baseline TP53 mutations were related to a lower tumor regression rate, while the clearance of TP53 mutations during treatment was not only associated with a higher cCR rate but also a greater proportion of patients experiencing tumor regression.

The FGF3, FGF4, and FGF19 genes are located in the 11q13 region of chromosome 11, and the amplification of 11q13 represents one of the most frequently amplified chromosomal regions in tumors. Chromosome 11q13 amplification was found to correlate with carcinogenesis and the attenuation of effector immune cells in the tumor microenvironment [14]. In patients with HNSCC, 11q13 amplification was associated with decreased PFS and no clinical benefits after immunotherapy [15]. A previous phase II clinical trial also demonstrated the negative predictive value of 11q13 amplification in ESCC patients treated with immunotherapy. Among patients with advanced ESCC, those without 11q13 amplification exhibited higher objective response rates (ORR) and longer PFS compared to patients with 11q13 amplification [16]. The result was similar in our study; none of the eight patients with FGF3/4/19 amplifications achieved cCR, and the cCR rate was significantly lower than that of patients without FGF3/4/19 amplifications.

The half-life of ctDNA is only a few hours, which enables it to accurately reflect the tumor load in real time. In particular, the dynamic changes of ctDNA during treatment are closely related to the dynamic tumor burden, providing strong evidence for the use of ctDNA to monitor treatment response [17]. In recent years, several studies have investigated the potential of ctDNA for predicting therapeutic efficacy and monitoring tumor progression in ESCC, especially in patients who underwent chemoradiotherapy (CRT). A previous study supported the use of dynamic ctDNA changes, rather than baseline ctDNA, to predict short-term treatment response, monitor relapse, and survival in ESCC [12]. Consistent with their findings, our study also demonstrated that ctDNA status at baseline may not serve as a predictive factor for efficacy, while negative ctDNA during treatment and persistently negative ctDNA or ctDNA clearance during treatment were associated with a higher cCR rate after immunochemotherapy.

TMB and PD-L1 expression, which are promising predictors of immunotherapy efficacy, were also explored in this study. No correlation was observed between TMB and treatment efficacy. As for PD-L1 expression, a positive correlation between PD-L1 expression and the efficacy of PD-1 inhibitors combined with chemotherapy in patients with advanced esophageal cancer was observed in CheckMate 648, ORIENT-15, and ESCORT-1 [18–20]. Similarly, in our study, patients with PD-L1 CPS ≥ 15 were more likely to achieve cCR after immunochemotherapy. However, only 11 patients had PD-L1 CPS ≥ 15, which may limit the interpretation of the result.

Limitations of the study included the use of heterogeneous PD-1/PD-L1 inhibitors and chemotherapy regimens among patients included in this study. Secondly, the time point of ctDNA detection during treatment was not consistent, but most of the patients were detected after 3 or 4 cycles of treatment. Thirdly, the follow-up time was relatively short, and PFS and OS were not mature in these patients, which limited the exploration of ctDNA predictive value in survival outcomes.

In conclusion, we reported that ctDNA status during treatment, dynamic changes of ctDNA status and TP53 mutations, and PD-L1 expression were correlated with tumor response in ESCC patients receiving immunochemotherapy. Nevertheless, larger-scale studies are warranted in the future to further validate these findings and identify patients who are more likely to benefit from immunochemotherapy.

Author Contributions

Jing Huang and Bo Zhang designed the study. Wang Qu and Yun Liu treated patients involved in the study. Yijia Guo and Wang Qu collected and analyzed the data and drafted the manuscript. Tao Qu, Yan Song, and Jianping Xu revised the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

References

1. A. Pennathur, M. K. Gibson, B. A. Jobe, and J. D. Luketich, "Oesophageal Carcinoma," *Lancet* 381, no. 9864 (2013): 400–412, https://doi.org/10.1016/S0140-6736(12)60643-6.

2. J. M. Sun, L. Shen, M. A. Shah, et al., "Pembrolizumab Plus Chemotherapy Versus Chemotherapy Alone for First-Line Treatment of Advanced Oesophageal Cancer (KEYNOTE-590): A Randomised, Placebo-Controlled, Phase 3 Study," *Lancet* 398, no. 10302 (2021): 759–771.

3. Y. Song, B. Zhang, D. Xin, et al., "First-Line Serplulimab or Placebo Plus Chemotherapy in PD-L1-Positive Esophageal Squamous Cell Carcinoma: A Randomized, Double-Blind Phase 3 Trial," *Nature Medicine* 29, no. 2 (2023): 473–482.

4. J. Xu, K. Kato, E. Raymond, et al., "Tislelizumab Plus Chemotherapy Versus Placebo Plus Chemotherapy as First-Line Treatment for Advanced or Metastatic Oesophageal Squamous Cell Carcinoma (RATIONALE-306): A Global, Randomised, Placebo-Controlled, Phase 3 Study," *Lancet Oncology* 24, no. 5 (2023): 483–495.

5. J. Qin, L. Xue, A. Hao, et al., "Neoadjuvant Chemotherapy With or Without Camrelizumab in Resectable Esophageal Squamous Cell Carcinoma: The Randomized Phase 3 ESCORT-NEO/NCCES01 Trial," *Nature Medicine* 30, no. 9 (2024): 2549–2557.

6. Y. Zheng, G. Liang, D. Yuan, et al., "Perioperative Toripalimab Plus Neoadjuvant Chemotherapy Might Improve Outcomes in Resectable Esophageal Cancer: An Interim Analysis of a Phase III Randomized Clinical Trial," *Cancer Communications* 44, no. 10 (2024): 1214–1227.

7. J. C. M. Wan, C. Massie, J. Garcia-Corbacho, et al., "Liquid Biopsies Come of Age: Towards Implementation of Circulating Tumour DNA," *Nature Reviews. Cancer* 17, no. 4 (2017): 223–238.

8. N. Tarazona, F. Gimeno-Valiente, V. Gambardella, et al., "Targeted Next-Generation Sequencing of Circulating-Tumor DNA for Tracking Minimal Residual Disease in Localized Colon Cancer," *Annals of Oncology* 30, no. 11 (2019): 1804–1812.

9. T. Liu, Q. Yao, and H. Jin, "Plasma Circulating Tumor DNA Sequencing Predicts Minimal Residual Disease in Resectable Esophageal Squamous Cell Carcinoma," *Frontiers in Oncology* 11 (2021): 616209.

10. X. Wang, N. Yu, G. Cheng, et al., "Prognostic Value of Circulating Tumour DNA During Post-Radiotherapy Surveillance in Locally Advanced Esophageal Squamous Cell Carcinoma," *Clinical and Translational Medicine* 12, no. 11 (2022): e1116.

11. T. D. Azad, A. A. Chaudhuri, P. Fang, et al., "Circulating Tumor DNA Analysis for Detection of Minimal Residual Disease After Chemoradiotherapy for Localized Esophageal Cancer," *Gastroenterology* 158, no. 3 (2020): 494–505.e496.

12. B. Chen, S. Liu, Y. Zhu, et al., "Predictive Role of ctDNA in Esophageal Squamous Cell Carcinoma Receiving Definitive Chemoradiotherapy Combined With Toripalimab," *Nature Communications* 15, no. 1 (2024): 1919.

13. L. Li, M. Li, and X. Wang, "Cancer Type-Dependent Correlations Between TP53 Mutations and Antitumor Immunity," *DNA Repair* 88 (2020): 102785.

14. Y. Chen, Y. Huang, X. Gao, et al., "CCND1 Amplification Contributes to Immunosuppression and Is Associated With a Poor Prognosis to Immune Checkpoint Inhibitors in Solid Tumors," *Frontiers in Immunology* 11, no. 1620 (2020): 1620, https://doi.org/10.3389/fimmu.2020.01620.

15. S. Dou, L. Zhang, C. Wang, et al., "EGFR Mutation and 11q13 Amplification Are Potential Predictive Biomarkers for Immunotherapy in Head and Neck Squamous Cell Carcinoma," *Frontiers in Immunology* 13 (2022): 813732, https://doi.org/10.3389/fimmu.2022.813732.

16. F. Wang, C. Ren, Q. Zhao, et al., "Association of Frequent Amplification of Chromosome 11q13 in Esophageal Squamous Cell Cancer With Clinical Benefit to Immune Check Point Blockade," *Journal of Clinical Oncology* 37, no. 15_suppl (2019): 4036.

17. F. Diehl, K. Schmidt, M. A. Choti, et al., "Circulating Mutant DNA to Assess Tumor Dynamics," *Nature Medicine* 14, no. 9 (2008): 985–990.

18. K. Kato, Y. Doki, I. Chau, et al., "Nivolumab Plus Chemotherapy or Ipilimumab Versus Chemotherapy in Patients With Advanced Esophageal Squamous Cell Carcinoma (CheckMate 648): 29-Month Follow-Up From a Randomized, Open-Label, Phase III Trial," *Cancer Medicine* 13, no. 9 (2024): e7235.

19. Z. Lu, J. Wang, Y. Shu, et al., "Sintilimab Versus Placebo in Combination With Chemotherapy as First Line Treatment for Locally Advanced or Metastatic Oesophageal Squamous Cell Carcinoma (ORIENT-15): Multicentre, Randomised, Double Blind, Phase 3 Trial," *British Medical Journal* 377 (2022): e068714, https://doi.org/10.1136/bmj-2021-068714.

20. H. Luo, J. Lu, Y. Bai, et al., "Effect of Camrelizumab vs. Placebo Added to Chemotherapy on Survival and Progression-Free Survival in Patients With Advanced or Metastatic Esophageal Squamous Cell Carcinoma: The ESCORT-1st Randomized Clinical Trial," *JAMA* 326, no. 10 (2021): 916–925.