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Opportunities and challenges of head and neck cancer treatment in the era of immune checkpoint inhibitors

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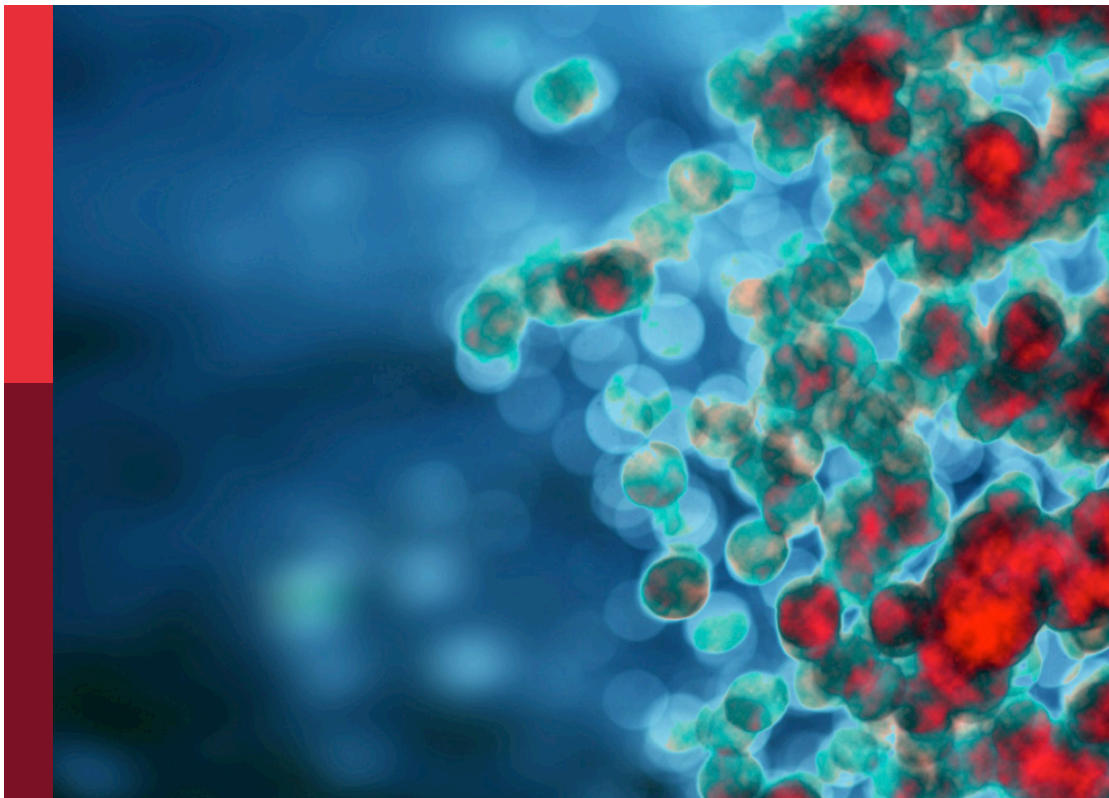

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Opportunities and challenges of head and neck cancer treatment in the era of immune checkpoint inhibitors

Topic editors

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Editorial: Opportunities and challenges of head and neck cancer treatment in the era of immune checkpoint inhibitors

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head and neck cancer, immune checkpoint inhibitors, tumor micro environment, immunotherapy, cancer

Editorial on the Research Topic

Opportunities and challenges of head and neck cancer treatment in the era of immune checkpoint inhibitors

Head and neck squamous cell carcinoma (HNSCC) remains one of the most challenging solid tumors to manage, with persistently suboptimal survival outcomes despite advancements in surgery, radiotherapy, and chemotherapy (1). Over the past decade, immune checkpoint inhibitors (ICIs)—particularly those targeting programmed cell death protein 1 (PD-1) and its ligand PD-L1—have revolutionized the treatment landscape of multiple malignancies, including HNSCC (2, 3). By reinvigorating cytotoxic T-cell responses, ICIs have demonstrated durable clinical benefits in subsets of patients with recurrent and metastatic disease, and in some contexts, have even redefined standards of care. Nevertheless, the response rates to ICIs in HNSCC remain limited to a fraction of patients, and the complexity of immune resistance, tumor heterogeneity, and adverse immune-related events pose significant barriers to universal efficacy (4, 5).

In this context, our Research Topic "*Opportunities and Challenges of Head and Neck Cancer Treatment in the Era of Immune Checkpoint Inhibitors*" was launched to provide a platform for cutting-edge research and clinical observations aimed at addressing these unresolved issues. The goal of this Research Topic is to deepen our understanding of immunotherapy mechanisms in HNSCC, highlight emerging biomarkers for patient stratification, explore innovative therapeutic combinations, and provide real-world insights into the optimization of treatment-related toxicity and efficacy. We believe this Topic provides a timely synthesis of current advancements and critical gaps in immuno-oncology as it relates to head and neck cancer.

A prominent theme in this Research Topic is the advancement of ICI-based neoadjuvant and multimodal therapies. Several studies examined the feasibility and efficacy of integrating ICIs with chemotherapy or radiotherapy in locally advanced HNSCC. Li et al. and Ding et al. demonstrated that neoadjuvant immunochemotherapy led to high rates of major or complete pathological responses in oral squamous cell

carcinoma (OSCC), with improved locoregional control and survival. Yao et al. retrospectively compared PD-1 versus EGFR inhibitors in hypopharyngeal cancer, showing superior response and organ preservation in the PD-1 cohort. Sun et al. reported a case of paranasal sinus carcinoma achieving primary pathological response with neoadjuvant nivolumab and chemotherapy, providing clinical insight into rare subtypes. Yu et al. demonstrated that adding a PD-1 inhibitor to induction chemotherapy in nasopharyngeal carcinoma significantly improved complete response rates and survival outcomes. Collectively, these studies support the inclusion of ICIs in early-stage treatment, while highlighting the need for predictive biomarkers to guide patient selection.

Another key focus of this Research Topic lies in the discovery of predictive biomarkers and the application of computational approaches to inform immunotherapy strategies. He et al. applied multi-cohort transcriptomic analysis and machine learning to identify ten exosome-related genes relevant to immune evasion and prognosis, with ANGPTL1 showing promise as a novel biomarker. Tran et al. used electronic health record data and machine learning to detect ICI-induced inflammatory arthritis and associated immune-related adverse events, emphasizing the utility of data-driven approaches in toxicity prediction and management.

This Topic also features timely reviews addressing current challenges and therapeutic innovations. Aboaid et al. provided a comprehensive overview of immunotherapy trials in locally advanced and recurrent/metastatic HNSCC, including dual ICI combinations and novel agents such as virotherapy and CAR-T. Chen et al. synthesized meta-analysis data from randomized trials to compare ICI regimens in the first- and second-line settings for recurrent/metastatic HNSCC, with subgroup analyses based on PD-L1 expression. Their findings suggest that pembrolizumab combined with chemotherapy offers the most substantial PFS benefit among patients with high PD-L1 expression. Wu et al. reviewed small-molecule immunomodulators as potential adjuvants to overcome resistance, and Zhang et al. discussed the emerging application of NK cell therapies in OSCC, particularly CAR-NK technologies. Zheng et al. reviewed the immune microenvironment in papillary thyroid carcinoma, providing mechanistic insights relevant to immune modulation beyond classical HNSCC.

Real-world and rare case reports included in this Topic further illustrate the complexity of ICI-based treatment. Qing et al. described long-term immunotherapy with EBV-DNA monitoring in nasopharyngeal carcinoma, while Song et al. reported successful ICI rechallenge after anaphylaxis. Li et al. presented a case of sarcomatoid transformation following sequential ICI-targeted therapy, underscoring the importance of dynamic tumor profiling during treatment. Chen et al. analyzed thyroid dysfunction risks in patients receiving anti-PD-1 with or without radiotherapy, identifying key clinical factors associated with immune-related endocrinopathy.

Together, these contributions underscore both the promise and the complexity of immunotherapy in head and neck cancer. While ICIs offer durable responses and potential curative effects, their integration into multimodal regimens requires careful consideration of toxicity, resistance, and patient selection (6). Moving forward, the development of robust biomarkers, integration of multi-omics data, and application of AI-based tools will be essential to guide individualized therapy (7). This Research Topic highlights a dynamic and rapidly evolving field, and we hope it will inspire further translational efforts and collaborative innovation to optimize immunotherapy strategies for patients with HNSCC.

Author contributions

ZL: Writing – original draft, Formal Analysis, Project administration, Conceptualization, Investigation, Data curation, Writing – review & editing. LC: Supervision, Writing – original draft, Writing – review & editing. XP: Supervision, Writing – review & editing, Validation, Investigation, Writing – original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Rapid identification of inflammatory arthritis and associated adverse events following immune checkpoint therapy: a machine learning approach

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Introduction: Immune checkpoint inhibitor-induced inflammatory arthritis (ICI-IA) poses a major clinical challenge to ICI therapy for cancer, with 13% of cases halting ICI therapy and ICI-IA being difficult to identify for timely referral to a rheumatologist. The objective of this study was to rapidly identify ICI-IA patients in clinical data and assess associated immune-related adverse events (irAEs) and risk factors.

Methods: We conducted a retrospective study of the electronic health records (EHRs) of 89 patients who developed ICI-IA out of 2451 cancer patients who received ICI therapy at Northwestern University between March 2011 to January 2021. Logistic regression and random forest machine learning models were trained on all EHR diagnoses, labs, medications, and procedures to identify ICI-IA patients and EHR codes indicating ICI-IA. Multivariate logistic regression was then used to test associations between ICI-IA and cancer type, ICI regimen, and comorbid irAEs.

Results: Logistic regression and random forest models identified ICI-IA patients with accuracies of 0.79 and 0.80, respectively. Key EHR features from the random forest model included ICI-IA relevant features (joint pain, steroid prescription, rheumatoid factor tests) and features suggesting comorbid irAEs (thyroid function tests, pruritus, triamcinolone prescription). Compared to 871 adjudicated ICI patients who did not develop arthritis, ICI-IA patients had higher odds of developing cutaneous (odds ratio [OR]=2.66; 95% Confidence Interval [CI] 1.63-4.35), endocrine (OR=2.09; 95% CI 1.15-3.80), or gastrointestinal (OR=2.88; 95% CI 1.76-4.72) irAEs adjusting for demographics, cancer type, and ICI regimen. Melanoma (OR=1.99; 95% CI 1.08-3.65) and renal cell carcinoma (OR=2.03; 95% CI 1.06-3.84) patients were more likely to develop ICI-IA compared to lung cancer patients. Patients on nivolumab+ipilimumab were more likely to develop ICI-IA compared to patients on pembrolizumab (OR=1.86; 95% CI 1.01-3.43).

Discussion: Our machine learning models rapidly identified patients with ICI-IA in EHR data and elucidated clinical features indicative of comorbid irAEs. Patients with ICI-IA were significantly more likely to also develop cutaneous, endocrine, and gastrointestinal irAEs during their clinical course compared to ICI therapy patients without ICI-IA.

KEYWORDS

immune checkpoint inhibitors, immune-related adverse events, immune checkpoint inhibitor-induced inflammatory arthritis, machine learning, electronic health records, big data

1 Introduction

Immune checkpoint inhibitors (ICIs) have become a pillar of cancer therapy, with demonstrated efficacy in many malignancies (1–9). ICIs are antibodies that antagonize checkpoints of T-cell development, enabling tumor-reactive T-cells to attack cancer cells (1, 2, 8, 10, 11). There are currently two major classes of ICIs. The first, approved in 2011, targets cytotoxic T-lymphocyte antigen 4 (CTLA-4). The second, first approved in 2014, targets programmed cell death protein 1 (PD-1) or its counterpart, programmed cell death ligand 1 (PD-L1). These ICIs may be used alone or combined with each other and other cancer therapies and are approved for a wide panel of cancers. An estimated 44% of US cancer patients are eligible for ICI therapy (4–7, 9, 12, 13).

However, while ICIs are effective anti-cancer agents, checkpoint blockade is associated with development of immune-related adverse events (irAEs) that affect a wide spectrum of organ systems (14–17). IrAEs can pose a significant barrier to ICI usage. They can prevent patients from continuing ICIs, diminish patient quality of life, and in severe cases, lead to death (3, 12, 13, 18). There are two major deficits in our current understanding of irAEs and our ability to care for patients with irAEs. First, irAEs are difficult to identify both in the patient care setting and for research studies. Outside of large clinical trials, many published studies are single-site with small cohorts identified and characterized by labor-intensive chart review (19–24). Second, we currently have a very limited ability to predict which patients will develop irAEs. Rheumatologic irAEs, including ICI-induced inflammatory arthritis (ICI-IA), epitomizes both of these limitations (24–35). ICI-IA has been recognized as an irAE for less than a decade with case studies and small cohort descriptions first appearing in the literature in 2017 (24–26). With a reported

Abbreviations: ANA, Anti-nuclear antibody; AUROC, Area under the receiver operating characteristic curve; CCP, Cyclic citrullinated peptide; CI, Confidence interval; CPT, Current Procedural Terminology; CRP, C-reactive protein; CTLA-4, Cytotoxic T-lymphocyte antigen 4; DMARD, Disease-modifying antirheumatic drug; HER, Electronic health record; ESR, Erythrocyte sedimentation rate; ICD, International Classification of Disease; ICI, Immune checkpoint inhibitor; ICI-IA, Immune checkpoint inhibitor-induced inflammatory arthritis; IL, Interleukin; irAE, Immune-related adverse event; LOINC, Logical Observation Identifiers Names and Codes; ML, Machine learning; NM, Northwestern Medicine; NMEDW, Northwestern Medicine Enterprise Data Warehouse; NPV, Negative predictive value; OMOP, Observational Medical Outcomes Partnership; OR, Odds ratio; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death ligand 1; PPV, Positive predictive value; TNF, Tumor necrosis factor; TSH, Thyroid stimulating hormone; UMLS, Unified Medical Language System.

prevalence of 1-7%, it is relatively rare (36). However, it can have significant impact for patients with 12-13% of cases resulting in termination of ICI therapy (34, 35). Previous studies have described ICI-IA affecting the knees or small joints, tending to be seronegative for anti-cyclic citrullinated peptide (CCP) antibodies and rheumatoid factor, and most commonly treated with corticosteroids with elevation to disease-modifying antirheumatic drugs (DMARDs), tumor necrosis factor (TNF) inhibitors, and interleukin (IL)-6 receptor inhibitors (24–27, 29, 32, 37). Though development of ICI-IA cannot be well predicted, in a previous study, melanoma and genitourinary cancer as well as receiving combination ICI therapy were found to be associated with ICI-IA development compared to lung cancer and PD-1 monotherapy, respectively (38).

Prompt identification of patients with ICI-IA is essential for providing rapid referrals for effective clinical care and for performing research into the etiology of ICI-IA. However, ICI-IA is difficult to identify in clinical data, owing to its rarity, lack of dedicated diagnosis code, and heterogeneous presentation (24–32, 35). Furthermore, ICI-IA's typically lower severity compared to other irAE such as myocarditis or pneumonitis means that patients may be less likely to be seen by a rheumatologist for their symptoms. While there have been numerous studies published on ICI-IA, ICI-IA cohort definition has primarily relied on manual identification of these patients by rheumatologists and/or filtering for patients who have been seen by a rheumatologist or been prescribed specific immunomodulatory drugs (26, 27, 29, 32, 34, 38). As a result, cohorts have remained relatively small and single site and run the risk of missing many ICI-IA patients who may have had a less severe presentation or other barriers to seeing a rheumatologist. Additionally, while many studies on ICI-IA include description of comorbid irAEs (26–28, 32), no previous study has tested the association of ICI-IA with these comorbid irAEs compared to a control cohort of ICI patients who did not develop arthritis, making it difficult to understand if the prevalence of comorbid irAEs experienced by ICI-IA patients is statistically different from the rest of the ICI population.

To address these fundamental gaps in knowledge and facilitate identification of ICI-IA and associations with the development of ICI-IA, we developed an electronic health record (EHR)-based machine learning strategy to rapidly identify patients who have possible ICI-IA and discover hidden features associated with ICI-IA. With the wide adoption of EHRs in inpatient and ambulatory settings (39), EHR data and data modeling strategies present an opportunity to develop tools to identify ICI-IA, as well as uncover clinical features of ICI-IA or associated conditions that may not be immediately obvious. Many previous studies have demonstrated success in identifying conditions including hypertension, stroke, systemic lupus erythematosus, asthma, and leukemia (40–44). To investigate the relationship between ICI-IA and other irAEs elucidated by our machine learning model, we also fully adjudicated a control cohort of 871 patients receiving ICI therapy for all irAEs, and used this control cohort to examine the association between the development of ICI-IA and other comorbid irAEs as well as cancer type and ICI regimen.

2 Materials and methods

2.1 Patient population and data source

The study population included patients seen in the Robert H. Lurie Comprehensive Cancer Center at Northwestern Medicine (NM), a large healthcare system providing inpatient, outpatient, and specialty care throughout Chicago and Northern Illinois. Data was acquired from the Northwestern Medicine Enterprise Data Warehouse (NMEDW), NM's clinical research database containing data on over 10 million patients as of October 2023. This study was governed by the Northwestern University institutional review board, protocol #STU00210502 and STU00206779.

We retrospectively identified an ICI cohort of all patients aged 18 to 99 with a diagnosis of cancer (melanoma, renal cell carcinoma, non-small cell and small cell lung carcinoma, urothelial cancer, head and neck cancer, gastric cancer, colon cancer, liver cancer, cervical cancer, uterine cancer, breast cancer, Hodgkin's lymphoma, Merkel cell carcinoma, rectal cancer, prostate cancer, esophageal cancer, leukemia, or lymphoma) who received at least one dose of ICI therapy (pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab, ipilimumab, or tremilumumab) between March 1, 2011 and January 1, 2021 (Figure 1A). Cancer was identified in the NMEDW by International Classification of Disease-9th revision-Clinical Modification (ICD-9-CM) and ICD-10-CM diagnosis codes. ICIs were identified in the NMEDW by a regular expression search for the generic and brand name in the medication data table (Supplementary Table 1). Sex, race, and ethnicity were gathered from patient demographic data present in the NMEDW. Prior autoimmune diseases were collected from NMEDW diagnosis codes (Supplementary Table 2).

2.2 Adjudication of ICI-induced inflammatory arthritis and statistical test control cohorts

Patient charts in NM's Epic EHR were manually reviewed (SDT) with clinician guidance (CG, JL, AK, JS) to identify a case cohort of patients with ICI-IA (Figure 1A). We reviewed the charts of all patients who had the keywords: "arthritis", "arthralgia", or "joint" in the assessment and plan section of any clinical notes written after the patient received their first ICI dose. Cases were classified with ICI-IA if the patient had *de novo* joint pain/arthralgia/arthritis (no history of arthritis or presentation different from what the patient has experienced in the past), and an oncologist or rheumatologist noted suspicion of the presentation being secondary to ICI therapy. Case status, date of ICI-IA onset, cancer, ICI regimen, joint involvement, rheumatoid factor, anti-CCP, anti-nuclear antibodies (ANA), and treatment for ICI-IA were recorded in a REDCap database (45). Cases were additionally reviewed for other irAEs the patients experienced – cutaneous AEs, thyroid dysfunction, hypophysitis or adrenal insufficiency, diabetes, hepatic AEs, diarrhea, colitis, pneumonitis, cardiovascular AEs, and encephalitis. They were classified with an irAE if an oncologist or

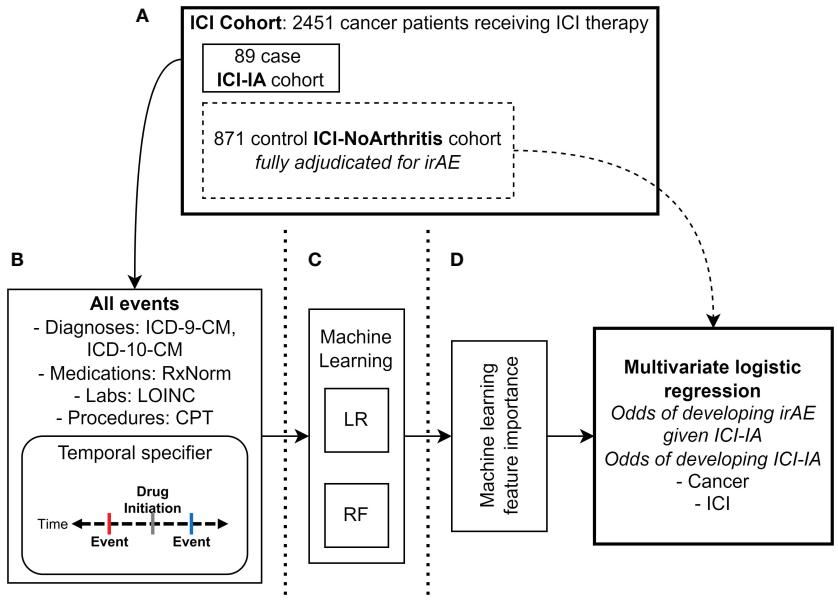

FIGURE 1
Methods diagram. **(A)** Manual adjudication of ICI cohort (N=2451 patients) for immune checkpoint inhibitor-induced inflammatory arthritis (ICI-IA) cases (N=89 patients). From the ICI cohort, 871 random patients without ICI-IA (ICI-NoArthritis) were adjudicated for all irAE. **(B)** Electronic health record data extraction of all diagnosis (ICD-9-CM, ICD-10-CM), medication (RxNorm), laboratory test (LOINC), and procedure (CPT) codes. Individual code occurrences were modified to specify whether they occurred before or after ICI initiation, and dichotomized to presence/absence of the code. Data was extracted for the full ICI cohort of 2451 patients. **(C)** Logistic regression (LR) and random forest (RF) machine learning models were trained on the EHR codes to identify ICI-IA. **(D)** Feature importance was analyzed to characterize ICI-IA patients in the EHR. Multivariate logistic regression was used to calculate odds ratios for development of ICI-IA given cancer and ICI regimen, as well as development of non-arthritis irAEs given ICI-IA versus ICI-noIA.

the relevant specialist noted suspicion of the presentation being secondary to ICI therapy without other likely etiologies. See Supplementary Methods for details.

The remaining patients without ICI-IA in the full ICI cohort were used as controls for our machine learning models. To compare irAE associations with ICI-IA, a random sample of 871 patients without ICI-IA (ICI-NoArthritis) from the overall ICI cohort of 2451 were chart reviewed for all irAEs (same irAEs and classification threshold as above) to serve as the control cohort for the statistical tests (SDT, GMP, KJR, CDM, JDJ, JT, KV, PD, SM, UR) (Figure 1A). Following initial adjudication, SDT reviewed a random sample of 10% of the charts and found greater than 90% agreement.

2.3 EHR code selection for machine learning

We collected all EHR clinical codes for every patient in our ICI cohort: ICD-9-CM and ICD-10-CM diagnosis codes, Logical Observation Identifiers Names and Codes (LOINC) laboratory codes, Unified Medical Language System (UMLS) RxNorm medication codes, and Current Procedural Terminology (CPT) procedure codes (Figure 1B). To determine if a machine learning model could select sensible ICI-IA-relevant codes and discover new predictors of ICI-IA, we input all EHR codes into our models. ICD-9-CM diagnosis codes were translated to ICD-10-CM to prevent duplication of diagnosis codes, using the concept relationships in the Observational Medical Outcomes Partnership (OMOP) common data model vocabulary tables (46). For each code, we added a temporal modifier specifying whether the code occurred before or after ICI initiation. We then dichotomized each to presence or absence of the code.

2.4 Machine learning to identify ICI-induced inflammatory arthritis in EHR data

The EHR codes were fed into Logistic Regression with Ridge (L2) penalty and Random Forest machine learning models to classify patients who experienced ICI-IA from the ICI cohort (Figure 1C). These models were selected for their capacity to provide clinically interpretable models. We bootstrapped model development 100 times to calculate model performance metrics with 95% confidence intervals and consensus code contribution. For each round of model development (each 1 of 100 bootstrap rounds), we used a random 50/50 training-testing split, stratified for consistent case/control proportions in the training and test sets. Cases were up-sampled during cross-validation and model training to balance the low ratio of ICI-IA cases to ICI controls (47). Five-fold cross-validation with 25 iterations was used to optimize model parameters in the training set. The test set was left untouched for performance evaluation. Models were evaluated using area under the receiver operating characteristic curve (AUROC). Accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were measured for all models, optimizing

Youden's J to balance sensitivity and specificity (48). EHR code contribution was calculated from the logistic regression beta coefficients and the random forest feature importance.

2.5 Key EHR codes in the ICI-induced inflammatory arthritis machine learning model

Feature importance from the random forest model was analyzed for the key EHR codes used to identify ICI-IA, averaging feature importance across the 100 bootstrapped models – codes with higher feature importance contribute more to the model for identifying ICI-IA (Figure 1D). While not a direct equivalence, these codes could indicate clinical features potentially describing ICI-IA and allow us to determine if the models were using ICI-IA-relevant information to capture patients with ICI-IA. The Fisher Exact test was used to determine the association between ICI-IA and the codes. Models were trained on decreasing percentages of the top codes (50% - 0.003%) and performance compared to the full models.

2.6 Cancer type and immune checkpoint inhibitor association with ICI-induced inflammatory arthritis

Multivariate logistic regression was used to calculate unadjusted and adjusted odds ratios (ORs) of developing ICI-IA given cancer type and first ICI regimen (specific ICI drug) (Figure 1D). Lung cancer and pembrolizumab were used as reference groups for cancer type and ICI regimen, respectively, as they represented our largest cancer type and treatment regimen. Covariates included in the calculation of adjusted ORs were sex, age, race, and ethnicity, cancer type for ICI ORs and ICI regimen for cancer ORs. Cancer type was determined by ICD-9/10 codes and ICI was determined by regex search as described in the patient population section (Supplementary Table 1). Cancer determination by ICD code compared to chart review showed <10% discrepancy. An ICI regimen was determined combination ICI therapy if two different ICIs were infused on the same date.

2.7 Determining irAE associations with ICI-induced inflammatory arthritis

Multivariate logistic regression was used to calculate unadjusted and adjusted ORs of developing non-arthritis irAEs given ICI-IA, compared to ICI-NoArthritis control patients (Figure 1D). IrAE associations were tested at three timeframes relative to ICI-IA development: irAE development any time after ICI initiation, only irAEs occurring prior to ICI-IA development, and only irAEs occurring post ICI-IA development. In the ICI-NoArthritis controls, irAE were included at any time after ICI initiation. Covariates included in the calculation of adjusted ORs were sex, age, race, ethnicity, cancer type, first ICI regimen, and presence of autoimmune disease prior to ICI initiation.

2.8 Analytical software

Statistical analysis and machine learning was performed using R 4.2.2 and Python 3.9.7 with *scikit-learn*, *imbalanced-learn*, *scipy*, and *statsmodels* packages. Statistical test results were considered statistically significant where $p < 0.05$. Models are available on GitHub (https://github.com/stevetran99/ICI-IA_ML_Classification).

3 Results

3.1 ICI-induced inflammatory arthritis cohort clinical characteristics

From the NMEDW, 2451 patients with a diagnosis of cancer who received at least one ICI dose between March 1, 2011 and January 1, 2021 were identified. Expert-guided chart review identified 89 cases of ICI-IA based on clinical suspicion without other likely etiologies in the oncology and rheumatology clinical notes. There were 2362 patients without ICI-IA remaining from the ICI cohort to serve as machine learning controls, with 871 of those patients adjudicated for other irAE serving as controls for our association tests.

Table 1 presents the demographic and cancer characteristics of the ICI-IA cohort and machine learning control cohort. We saw no significant differences across primary demographic characteristics (sex, age, race, ethnicity). Lung cancer (N=34, 38%), melanoma (N=29, 33%), and kidney cancer (N=20, 23%) were the most common cancers in our ICI-IA cohort. Pembrolizumab (N=28, 32%), nivolumab (N=23, 26%), and combination nivolumab-ipilimumab (N=27, 30%) were the predominant first ICI regimens and the majority of patients had no prior autoimmune disease (N=87, 98%). ICI-IA involved the knees (N=42, 47%), hand (N=31, 35%) and shoulder (N=28, 31%) (Table 2). The knees (N=30, 34%) and hands (N=22, 25%) were the most common first joints affected. Twenty-seven patients had diffuse joint pain. Of patients with specific joint involvement mentioned (N=71, 80%), the median number of distinct joint locations affected was 2, range 1 to 7. Of those tested for markers of rheumatologic disease, 5 of 20 patients were positive for rheumatoid factor, 0 of 16 patients were positive for anti-CCP antibodies, 9 of 15 patients were positive for ANA (Table 2). Eighteen (20%) patients were seen by a rheumatologist. ICI-IA onset was a median of 20 weeks (IQR 8-45 weeks) after initiating checkpoint therapy. Primary treatment modalities for the ICI-IA symptoms were steroids (N=43, 48%), and NSAIDs (N=33, 37%). In 18 (20%) patients, ICI therapy was held or stopped entirely due to arthritis.

3.2 EHR-based machine learning reliably identified ICI-induced inflammatory arthritis

Machine learning models were trained to identify ICI-IA in EHR data on the 89 ICI-IA cases and 2362 machine learning controls. We identified 32,682 EHR codes representing all

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TABLE 1 Demographic and clinical variables for cancer patients receiving Immune Checkpoint Inhibitor Therapy between March 1, 2011 and January 1, 2021.

	Overall	ICI-IA	ML Control
	N=2451	N=89	N=2362
Sex, n (%)			
Male	1355 (55.3)	48 (53.9)	1307 (55.3)
Female	1096 (44.7)	41 (46.1)	1055 (44.7)
Age, n (%)			
20 – 29	10 (0.4)		10 (0.4)
30 – 39	43 (1.8)	1 (1.1)	42 (1.8)
40 – 49	101 >(4.1)	5 (5.6)	96 (4.1)
50 – 59	284 (11.6)	15 (16.9)	269 (11.4)
60 – 69	716 (29.2)	29 (32.6)	687 (29.1)
70 – 79	776 (31.7)	25 (28.1)	751 (31.8)
80 – 89	441 (18.0)	13 (14.6)	428 (18.1)
>= 90	80 (3.3)	1 (1.1)	79 (3.3)
Race, n (%)			
White	1935 (78.9)	77 (86.5)	1858 (78.7)
Black or African American	198 (8.1)	4 (4.5)	194 (8.2)
Asian	96 (3.9)	1 (1.1)	95 (4.0)
Other	111 (4.5)	5 (5.6)	106 (4.5)
Unknown	111 (4.5)	2 (2.2)	109 (4.6)
Ethnicity, n (%)			
Hispanic or Latino	109 (4.4)	2 (2.2)	107 (4.5)
Not Hispanic or Latino	2220 (90.6)	84 (94.4)	2136 (90.4)
Unknown	122 (5.0)	3 (3.4)	119 (5.0)
Cancer, n (%)			
Lung Cancer	1278 (52.1)	34 (38.2)	1244 (52.7)
Melanoma	495 (20.2)	29 (32.6)	466 (19.7)
Renal Cell Carcinoma	323 (13.2)	20 (22.5)	303 (12.8)
Breast Cancer	23 (0.9)	1 (1.1)	22 (0.9)
Urothelial Cancer	143 (5.8)	2 (2.2)	141 (6.0)
Endometrial Cancer	13 (0.5)	1 (1.1)	12 (0.5)
Other Malignancy	57 (2.3)	2 (2.2)	55 (2.3)
Cervical Cancer	1 (0.0)		1 (0.0)
Colon Cancer	17 (0.7)		17 (0.7)
Esophageal Cancer	4 (0.2)		4 (0.2)
Gastric Cancer	4 (0.2)		4 (0.2)
Head and Neck Cancer	52 (2.1)		52 (2.2)
Hodgkins Lymphoma	1 (0.0)		1 (0.0)
Leukemia	6 (0.2)		6 (0.3)

(Continued)

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TABLE 1 Continued

	Overall	ICI-IA	ML Control
	N=2451	N=89	N=2362
Cancer, n (%)			
Liver Cancer	6 (0.2)		6 (0.3)
Merkel Cell Carcinoma	6 (0.2)		6 (0.3)
Other Lymphoma	6 (0.2)		6 (0.3)
Prostate Cancer	15 (0.6)		15 (0.6)
Rectal Cancer	1 (0.0)		1 (0.0)
First ICI, n (%)			
Pembrolizumab	941 (38.4)	28 (31.5)	913 (38.7)
Nivolumab	635 (25.9)	23 (25.8)	612 (25.9)
Ipilimumab	119 (4.9)	3 (3.4)	116 (4.9)
Combination*	332 (13.5)	27 (30.3)	305 (12.9)
Durvalumab	147 (6.0)	5 (5.6)	142 (6.0)
Atezolizumab	271 (11.1)	3 (3.4)	268 (11.3)
Avelumab	5 (0.2)		5 (0.2)
Cemiplimab	1 (0.0)		1 (0.0)
Prior Autoimmune Disease, n (%)			
No Prior	2339 (95.4)	87 (97.8)	2252 (95.3)
Prior	112 (4.6)	2 (2.2)	110 (4.7)

ICI-IA, Immune checkpoint inhibitor-induced inflammatory arthritis; ML, Machine learning.
*Combination: Nivolumab-Ipilimumab.

diagnoses, procedures, labs, and medications, with the temporal modifiers for codes occurring before and after ICI initiation as variables for the models. Trained on the full code set, logistic regression modeling achieved an AUROC of 0.77 (95% Confidence Interval [CI] 0.73-0.82) while random forest modeling achieved an AUROC of 0.81 (95% CI 0.76-0.86) (Table 3). Training on decreasing percentages of the top codes derived from the random forest model showed consistently high performance for logistic regression and random forest models before dropping in performance below 31 codes (Figure 2). Our highest performing model, random forest with the top 31 codes, achieved an AUROC of 0.81 (95% CI 0.75-86), accuracy of 0.79 (95% CI 0.63-0.94), sensitivity of 0.71 (95% CI 0.54-0.88), specificity of 0.79 (95% CI 0.62-0.95), PPV of 0.13 (95% CI 0.03-0.22), and NPV of 0.99 (95% CI 0.98-0.99) (Table 3).

3.3 Key EHR codes in the ICI-induced inflammatory arthritis model were potentially related to other irAEs

From the random forest model, we evaluated the 55 most important codes to elucidate clinical features relevant to identifying ICI-IA. Codes were categorized as ICI-IA relevant (codes matching elements of ICI-IA presentation, diagnosis, or management in the literature), potentially relevant to other irAEs, and those that were related to other medical history elements. Twenty-two were relevant to ICI-IA, including diagnosis codes for unspecified joint pain, knee pain, and unspecified osteoarthritis (osteoarthritis was likely a placeholder early in the diagnostic workflow); laboratory test and procedure codes for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor, and anti-CCP antibody tests; medication codes for prednisone and methylprednisolone. ('ICI-IA' codes in Figure 3). Sixteen were potentially relevant to other irAEs, including codes for endocrine disorder screening, thyroid function tests, cortisol labs, medication codes for triamcinolone, and diagnosis codes for pruritus and myositis ('irAE' codes in Figure 3). Seventeen were related to other medical history elements such as COVID-19 or chemotherapy administration ('Other' codes in Figure 3).

3.4 Melanoma, renal cell carcinoma, and combination nivolumab-ipilimumab therapy were associated with development of ICI-induced inflammatory arthritis

Melanoma, OR = 1.99 (95% CI 1.08-3.65) and renal cell carcinoma patients, OR = 2.03 (95% CI 1.06-3.84), had higher odds of developing ICI-IA compared to lung cancer patients,

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TABLE 2 Immune checkpoint inhibitor-induced inflammatory arthritis (ICI-IA) laboratory tests, joint involvement, and comorbid irAEs for our cohort of patients with ICI-IA.

	ICI-IA N=89
Rheumatoid factor, n (%)	
Positive	5 (6)
Negative	15 (17)
Anti-CCP Antibodies, n (%)	
Positive	0 (0)
Negative	16 (18)
Anti-Nuclear Antibodies, n (%)	
Positive	9 (10)
Negative	6 (7)
Arthritis Treatment, n (%)	
Steroids	43 (48)
NSAID	33 (37)
Joint Injection	9 (10)
DMARD	6 (7)
Apremilast	1 (1)
None Documented	31 (35)
Joint Involvement, n (%)	
Hand	31 (35)
Wrist	12 (13)
Elbow	5 (6)
Shoulder	28 (31)
Neck	5 (6)
Back	9 (10)
Hip	18 (20)
Knee	42 (47)
Ankle	13 (15)
Foot	7 (8)
Diffuse	27 (30)
Comorbid irAE, n (%)	
Cutaneous	38 (43)
Endocrine	
Thyroid	16 (18)
Hypophysitis/Adrenal Insufficiency	10 (11)
Diabetes	1 (1)
Gastrointestinal	
Diarrhea/Constipation	26 (29)
Colitis	9 (10)
Hepatic	8 (9)
Pneumonitis	4 (4)
Cardiac	1 (1)
Encephalitis	1 (1)

(Continued)

TABLE 2 Continued

adjusting for sex, age, race, ethnicity, and first ICI regimen. Combination nivolumab-ipilimumab treatment was associated with higher odds of developing ICI-IA, OR = 1.86 (95% CI 1.01-3.43), compared to patients who received pembrolizumab, adjusting for sex, age, race, ethnicity, and cancer type (Table 4).

3.5 ICI-induced inflammatory arthritis was associated with development of cutaneous, endocrine, and gastrointestinal irAEs

We further explored the key irAE related EHR codes from our ICI-IA machine learning model. The irAEs most commonly documented as co-occurring with ICI-IA were cutaneous irAE: pruritus, rash, and vitiligo (N=38); gastrointestinal irAE: diarrhea and constipation (N=26) and colitis (N=9); endocrine irAE: thyroid dysfunction (N=16), hypophysitis and adrenal insufficiency (N=10), and diabetes (N=1); and hepatic irAE: transaminitis and hepatitis (N=8). Four ICI-IA patients experienced pneumonitis. Pericarditis and encephalitis were documented in one separate patient each. Twenty-one ICI-IA patients had no other documented irAE.

ICI-IA patients had higher odds of having any additional documented irAE, odds ratio (OR) = 2.53 (95% CI 1.49-4.31), compared to ICI-NoArthritis patients, adjusting for sex, age, race, ethnicity, cancer type, first ICI regimen, and presence of autoimmune disease prior to ICI therapy (Table 5). They specifically had higher odds of experiencing cutaneous irAE, OR = 2.66 (95% CI 1.63-4.35), endocrine irAE, OR = 2.09 (95% CI 1.15-3.80), and gastrointestinal irAE, OR = 2.88 (95% CI 1.76-4.72) (Table 5).

In unadjusted models, ICI-IA patients had higher odds of developing cutaneous irAE, OR = 1.65 (95% CI 1.00-2.72), and gastrointestinal irAE, OR = 1.76 (95% CI 1.07-2.90), prior to ICI-IA development compared to the ICI-NoArthritis control developing these irAEs at any time. ICI-IA patients had higher odds of developing thyroid dysfunction, OR = 2.01 (95% CI 1.01-4.00), post ICI-IA development compared to the ICI-NoArthritis control developing thyroid dysfunction at any time (Table 5). However, these temporal associations were no longer statistically significant when adjusting for sex, age, race, ethnicity, cancer type, first ICI regimen, and presence of prior autoimmune disease.

TABLE 3 Logistic Regression and Random Forest machine learning models performance metrics for identifying ICI-induced inflammatory arthritis (ICI-IA).

Full models (N features = 32,682)						
Model	AUROC	Accuracy	Sensitivity	Specificity	PPV	NPV
Logistic Regression	0.77 (0.73-0.82)	0.72 (0.51-0.92)	0.73 (0.49-0.96)	0.71 (0.50-0.93)	0.09 (0.05-0.14)	0.99 (0.98-1.00)
Random Forest	0.81 (0.76-0.86)	0.77 (0.60-0.93)	0.74 (0.56-0.91)	0.77 (0.59-0.94)	0.11 (0.05-0.18)	0.99 (0.98-0.99)
Models trained on top 31 EHR codes						
Model	AUROC	Accuracy	Sensitivity	Specificity	PPV	NPV
Logistic Regression	0.80 (0.75-0.86)	0.80 (0.65-0.95)	0.70 (0.53-0.87)	0.81 (0.64-0.97)	0.13 (0.06-0.20)	0.99 (0.98-0.99)
Random Forest	0.81 (0.75-0.86)	0.79 (0.63-0.94)	0.71 (0.54-0.88)	0.79 (0.62-0.95)	0.13 (0.03-0.22)	0.99 (0.98-0.99)

Accuracy, sensitivity, specificity, PPV, and NPV were calculated optimizing Youden's J.
AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.
Confidence intervals in parentheses are 95% confidence intervals calculated by bootstrapping model development 100 times.

4 Discussion

Using an ICI cohort of 2451 patients, 89 of which had ICI-IA, we developed an EHR-based machine learning model that could rapidly identify patients who had possible ICI-IA with AUROCs of 0.80-0.81 and accuracy of 0.79-0.80. Our machine learning model captured key EHR codes relevant to ICI-IA as well as codes indicative of cutaneous and endocrine irAEs. On further investigation of these irAE related codes, we found that ICI-IA was associated with development of additional cutaneous, endocrine, and gastrointestinal irAEs independent of cancer type and ICI regimen.

To develop our EHR-based machine learning model of ICI-IA, we created a cohort of 2451 patients who had cancer and received an ICI, including 89 ICI-IA patients, and 871 patients without ICI-IA, who were fully adjudicated for irAEs by manual chart review.

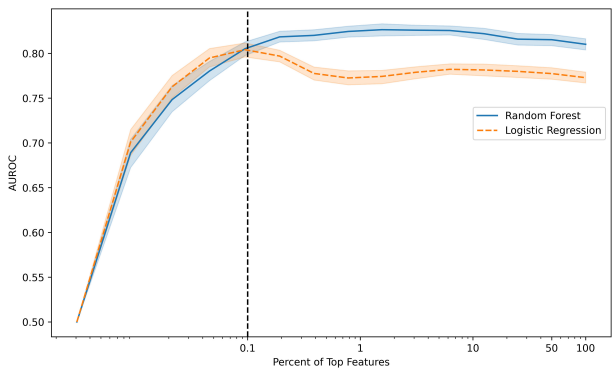

FIGURE 2
Model performance (area under the receiver operating characteristic curve, AUROC) versus percentage of the top features used to develop random forest and logistic regression models. Models maintain high performance with decreasing percentage of top features included before dropping performance with fewer than 0.1% of features or 31 features (vertical dotted line).

We expanded on an approach described by Thangaraj et al. and used all diagnosis, laboratory test, procedure, and medication codes in the EHR to develop an ICI-IA identification algorithm (43). Both logistic regression and random forest machine learning models performed well on the full code set (32,682 codes) and maintained high performance while reducing the number of EHR codes used to develop the models – at 31 codes, logistic regression had an AUROC of 0.80 (95% CI 0.75-0.86) while random forest had an AUROC of 0.81 (95% CI 0.75-0.86) However, both models had low PPVs of 0.13, despite good AUROC and accuracy. This is an inherent limitation of PPV in rare conditions such as ICI-IA, with a prevalence only 3.6% in our ICI cohort. This also speaks to the purpose of our ICI-IA model as a filter for potential ICI-IA cases for clinician verification rather than as a replacement of clinical expertise.

A major advantage of this approach compared to traditional manual selection of model features is that our EHR model provided information on codes useful for identifying ICI-IA patients. While these codes are not a direct equivalence, they were indicators of clinical features that could describe ICI-IA. Both models maintained high performance down to 0.1% of the total code set, suggesting the majority of information important for identifying ICI-IA patients was held by a small fraction of EHR codes. We evaluated the top codes from the random forest model to understand the key predictive elements. Twenty-two of the 55 top codes were determined to be relevant to ICI-IA including lab and procedure codes for ESR, CRP, and rheumatoid factor, diagnosis codes for joint pain, and medication codes for steroids. These factors are consistent with the ICI-IA literature regarding ICI-IA presentation, diagnostic tests, and therapy (27, 29, 37, 49). The density of ICI-IA relevant codes in the group of features with the highest feature importance – the top 8 codes were ICI-IA relevant codes – indicates that our strategy constructed a clinically sensible model to capture ICI-IA relevant information without manual guidance on which codes should be used, increasing the credibility of the other unexpected key EHR codes we found.

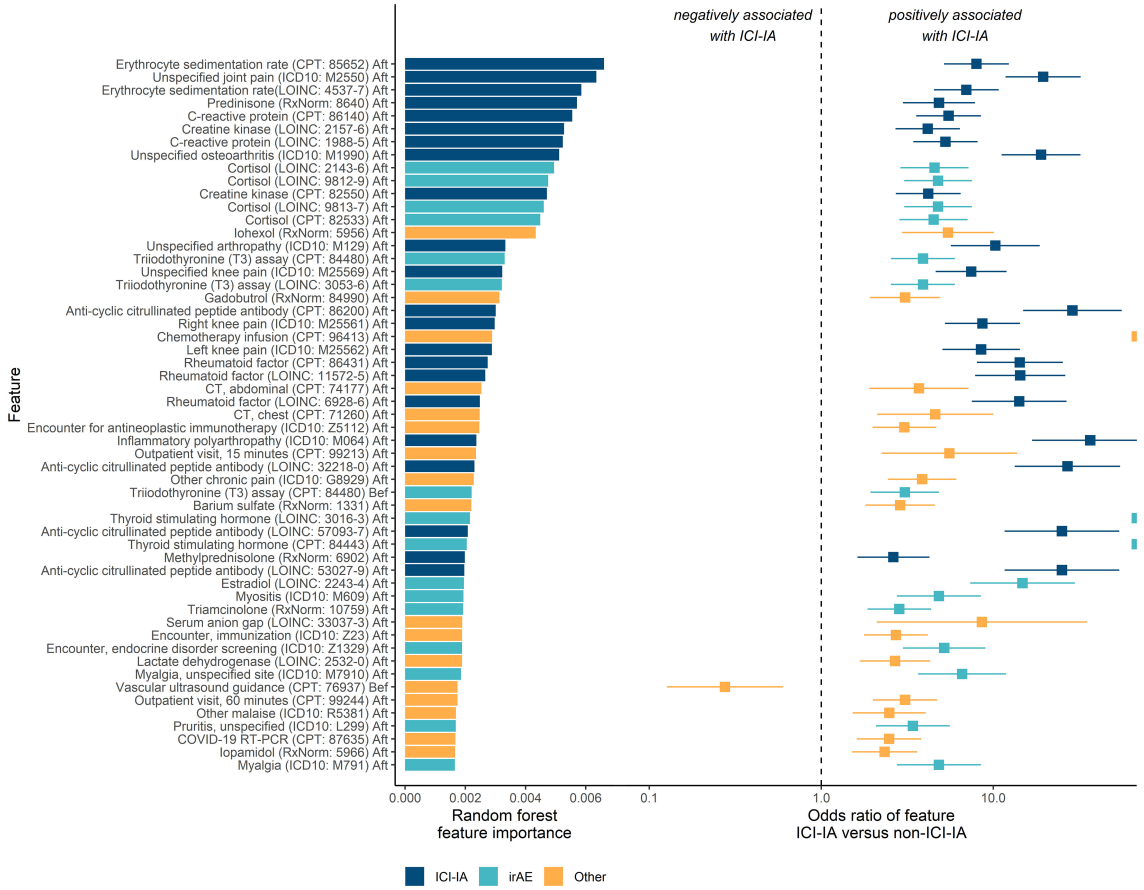

FIGURE 3
Key EHR codes in the machine learning models and association with ICI-induced inflammatory arthritis (ICI-IA). Left: the random forest model's feature importance for identifying ICI-IA patients. Right: odds of the patient having an EHR code if they developed ICI-IA versus if they did not, by Fisher Exact test. Error bars are 95% confidence intervals. 'ICI-IA' codes are those directly relevant to ICI-IA. 'irAE' codes are those potentially describing other irAEs. 'Other' codes are those describing other parts of the patient medical history. The top codes are predominantly ICI-IA relevant codes, with a high concentration of relevant codes occupying the topmost importance. The top irAE related codes are endocrine (cortisol, thyroid function tests, estradiol), myositis, and cutaneous (medication order for triamcinolone and pruritus). The majority of the top codes are positively associated with ICI-IA. Codes are labeled by name, code vocabulary, code, and temporal modifier (before or after ICI therapy initiation).

TABLE 4 Odds of developing ICI-induced inflammatory arthritis (ICI-IA) given cancer and first ICI.

Cancer	Unadjusted OR (95% CI)	P-value†	Adjusted OR (95% CI)	P-value†
Lung Cancer	1.00 (reference)		1.00 (reference)	
Melanoma	2.28 (1.36-3.78)	**0.001**	1.99 (1.08-3.65)	**0.026**
Renal Cell Carcinoma	2.42 (1.35-4.22)	**0.002**	2.03 (1.06-3.84)	**0.031**
Breast Cancer	1.66 (0.09-8.29)	0.624	1.67 (0.09-8.91)	0.627
Endometrial Cancer	3.05 (0.17-16.16)	0.291	2.21 (0.12-12.33)	0.460
Urothelial Cancer	0.52 (0.08-1.73)	0.371	0.70 (0.11-2.43)	0.638
Other Malignancy	1.33 (0.21-4.53)	0.700	1.25 (0.20-4.40)	0.763
First ICI	**Unadjusted OR (95% CI)**	**P-value†**	**Adjusted OR (95% CI)**	**P-value†**
Pembrolizumab	1.00 (reference)		1.00 (reference)	
Nivolumab	1.23 (0.69-2.14)	0.478	0.95 (0.52-1.71)	0.855
Ipilimumab	0.84 (0.20-2.43)	0.782	0.49 (0.11-1.57)	0.282

(Continued)

TABLE 4 Continued

First ICI	Unadjusted OR (95% CI)	P-value†	Adjusted OR (95% CI)	P-value†
Combination*	2.89 (1.67-4.99)	**<0.001**	1.86 (1.01-3.43)	**0.046**
Durvalumab	1.15 (0.39-2.78)	0.780	1.22 (0.40-3.07)	0.703
Atezolizumab	0.37 (0.09-1.04)	0.099	0.40 (0.09-1.16)	0.137

Covariates included for adjusted odds ratios (ORs) were sex, age, race, ethnicity, as well as cancer type for ICI ORs and first ICI regimen for cancer type ORs.
*Combination: Nivolumab-Ipilimumab.
†P-values < 0.05 significant (bold).

TABLE 5 Odds ratio (OR) of developing irAEs given ICI-induced inflammatory arthritis (ICI-IA).

IrAE anytime	Unadjusted Odds of irAE (95% CI) ICI-IA vs controls	Unadjusted p-value†	Adjusted Odds of irAE (95% CI) ICI-IA vs controls	Adjusted p-value†
Any IrAE	3.10 (1.87-5.15)	**<0.001**	2.91 (1.74-4.87)	**<0.001**
Cutaneous	3.34 (2.12-5.25)	**<0.001**	3.18 (2.00-5.06)	**<0.001**
Endocrine	3.18 (1.85-5.48)	**<0.001**	2.70 (1.54-4.72)	**<0.001**
Thyroid	3.13 (1.71-5.73)	**<0.001**	2.67 (1.43-4.99)	**0.002**
Hypophysitis/Adrenal Insuff.	4.28 (1.99-9.24)	**<0.001**	3.35 (1.52-7.37)	**0.003**
Diabetes	1.97 (0.23-17.04)	0.539	2.53 (0.27-23.64)	0.415
Gastrointestinal	3.09 (1.95-4.90)	**<0.001**	2.90 (1.81-4.66)	**<0.001**
Diarrhea/Constipation	2.71 (1.65-4.46)	**<0.001**	2.59 (1.55-4.31)	**<0.001**
Colitis	2.47 (1.15-5.28)	**0.020**	2.18 (0.99-4.80)	0.054
Hepatic	2.17 (0.98-4.80)	0.057	1.97 (0.86-4.49)	0.107
Pneumonitis	0.61 (0.22-1.73)	0.356	0.64 (0.22-1.81)	0.397
Cardiac	9.89 (0.61-159.42)	0.106	10.74 (0.62-186.55)	0.103
Encephalitis	1.40 (0.17-11.53)	0.753	1.28 (0.15-10.95)	0.820
IrAE Prior to ICI-IA	**Unadjusted Odds of irAE (95% CI) ICI-IA vs controls**	**Unadjusted p-value†**	**Adjusted Odds of irAE (95% CI) ICI-IA vs controls**	**Adjusted p-value†**
Cutaneous	1.65 (1.00-2.72)	**0.048**	1.58 (0.95-2.63)	0.077
Endocrine	1.02 (0.47-2.18)	0.963	0.81 (0.37-1.77)	0.595
Thyroid	0.85 (0.33-2.18)	0.735	0.67 (0.26-1.77)	0.421
Hypophysitis/Adrenal Insuff.	2.45 (0.98-6.13)	0.056	1.81 (0.70-4.65)	0.218
Diabetes	1.97 (0.23-17.04)	0.539	2.53 (0.27-23.64)	0.415
Gastrointestinal	1.76 (1.07-2.90)	**0.027**	1.62 (0.97-2.71)	0.065
Diarrhea/Constipation	1.55 (0.88-2.73)	0.126	1.44 (0.81-2.56)	0.215
Colitis	1.87 (0.81-4.32)	0.142	1.66 (0.70-3.93)	0.251
Hepatic	1.03 (0.36-2.96)	0.954	0.91 (0.31-2.69)	0.863
Pneumonitis*				
Cardiac*				
Encephalitis*				

(Continued)

TABLE 5 Continued

IrAE Post ICI-IA	Unadjusted Odds of irAE (95% CI) ICI-IA vs controls	Unadjusted p-value†	Adjusted Odds of irAE (95% CI) ICI-IA vs controls	Adjusted p-value†
Cutaneous	0.91 (0.51-1.62)	0.744	0.82 (0.45-1.48)	0.510
Endocrine	1.76 (0.94-3.32)	0.079	1.45 (0.76-2.79)	0.262
Thyroid	2.01 (1.01-4.00)	**0.045**	1.67 (0.82-3.39)	0.155
Hypophysitis/Adrenal Insuff.	1.59 (0.54-4.68)	0.398	1.24 (0.41-3.69)	0.703
Diabetes*				
Gastrointestinal	0.74 (0.39-1.40)	0.358	0.68 (0.36-1.30)	0.243
Diarrhea/Constipation	0.83 (0.42-1.65)	0.600	0.77 (0.38-1.55)	0.463
Colitis	0.50 (0.12-2.12)	0.351	0.44 (0.10-1.90)	0.271
Hepatic	1.03 (0.36-2.96)	0.954	0.88 (0.30-2.59)	0.815
Pneumonitis	0.61 (0.22-1.73)	0.356	0.64 (0.22-1.81)	0.397
Cardiac	9.89 (0.61-159.44)	0.106	10.74 (0.62-186.55)	0.103
Encephalitis	1.40 (0.17-11.53)	0.753	1.28 (0.15-10.95)	0.820

Covariates included for adjusted odds ratios (ORs) were sex, age, race, ethnicity, cancer type, first ICI regimen, and prior autoimmune disease. IrAE anytime: in cases, irAEs included were those that developed at any time in the patient medical history. IrAE Prior to ICI-IA: in cases, irAEs included were those that developed prior to ICI-IA. IrAE post ICI-IA: irAEs included where those that developed after ICI-IA. In controls, irAEs that developed at any time in the medical history were included for all comparisons. Endocrine irAEs included thyroid, hypophysitis/adrenal insufficiency (Insuff.), and diabetes. Gastrointestinal irAEs included diarrhea/constipation and colitis.
*These irAE had zero cases in our ICI-IA cohort at these timepoints.
†P-values < 0.05 significant (bold).
Cases were 89 patients with ICI-IA. Controls were 871 patients without ICI-IA.

While many of the top codes identified by our models were similar to those identified by clinical experts in ICI-IA, 16 of the 55 top codes appeared related to other irAEs, suggesting a possible relationship between ICI-IA and other irAE. We found codes for thyroid stimulating hormone (TSH) and T3 thyroid function tests, cortisol tests, pruritus, and triamcinolone, all after ICI initiation. These codes indicated potential association of ICI-IA with endocrine and cutaneous irAE. Indeed the literature suggests comorbid cutaneous, endocrine, gastrointestinal, and other irAEs with development of ICI-IA (26, 27). However, to our knowledge we are the first to statistically associate that ICI-IA patients were significantly more likely than ICI patients without ICI-IA to also develop cutaneous, OR = 2.66 (95% CI 1.63-4.35), endocrine, OR = 2.09 (95% CI 1.15-3.80), and gastrointestinal irAEs, OR = 2.88 (95% CI 1.76-4.72), adjusting for sex, age, race, ethnicity, cancer type, first ICI regimen, and presence of autoimmune disease prior to ICI initiation. ICI-IA patients were also more likely to experience any other irAE, OR = 2.53 (95% CI 1.49-4.31). To our knowledge, this is the first study that has conducted tests of association of irAEs comparing ICI-IA to a control cohort of ICI patients without ICI-IA, with both cohorts fully adjudicated for irAEs. This confirms the results of previous studies (26, 30, 32, 38) and shows that patients with ICI-IA are actually experiencing higher rates of cutaneous, endocrine, and gastrointestinal irAE compared to the general ICI population. Additionally, our findings suggest that the association between ICI-IA and cutaneous, endocrine, and gastrointestinal irAEs is independent of cancer type, ICI regimen, and prior autoimmune disease.

Conducting temporal association tests for irAEs that occurred before and after ICI-IA, we found that ICI-IA patients were more likely to have developed cutaneous irAEs and gastrointestinal irAEs prior to ICI-IA, in an unadjusted model. Conversely, ICI-IA patients were more likely to develop thyroid dysfunction after ICI-IA. Our temporal association findings indicate a possible common phenotype of patients who receive ICI therapy, develop skin and/or gastrointestinal irAEs, followed by ICI-IA, and finally thyroid dysfunction. However, when adjusting for demographics, cancer type, ICI regimen, and prior autoimmune disease, these temporal associations were no longer statistically significant, suggesting that the temporal phenotypes may be dependent on cancer type and ICI regimen or specific to our cohort and will require larger cohort sizes and future study.

As part of our analysis, we recapitulated findings in the literature of associations between cancer and ICI regimen and development of ICI-IA. We found similar associations in our ICI-IA cohort to that observed previously by Cunningham-Bussel et al. (38) even with our differing inclusion protocol for ICI-IA, increasing our confidence in the representativeness of our ICI-IA and ICI cohorts. Melanoma patients, OR = 1.99 (95% CI 1.08-3.65), and renal cell carcinoma patients, OR = 2.03 (95% CI 1.06-3.84), had higher odds of developing ICI-IA compared to lung cancer patients, adjusting for sex, age, race, ethnicity, and first ICI regimen. We also found that patients whose first ICI regimen was combination nivolumab-ipilimumab had higher odds of developing ICI-IA, OR = 1.86 (95% CI 1.01-3.43), compared to patients who received pembrolizumab, adjusting for sex, age, race, ethnicity, and cancer type. We additionally found similar time elapsed from ICI initiation to ICI-IA development compared to the ICI-IA literature – median of 20 weeks (5 months) compared to 2.7 – 5 months (30, 34, 50).

Our study is not without limitations. Despite being larger than most published studies, our ICI-IA cohort is still relatively small (N=89) and constrained to a single site. This small cohort relative to the number of EHR codes could make training our machine learning models difficult. We mitigated this issue by performing feature reduction based on the feature importance provided by the initial random forest model and saw high-performance using only 0.1% of the total code set. Another limitation is that our ICI-IA adjudication was done by retrospective chart review, therefore relying on clinician documentation of ICI-IA, and included patients that were not seen by a rheumatologist. Thus, some of these patients may have arthralgia or polymyalgia rheumatica rather than arthritis. However, as we expand this study to further sites and patients, we will be able to better make this distinction. We chose to accept these limitations to capture a broader cohort of patients with potentially less severe ICI-IA or who had other barriers to seeing a rheumatologist. Our finding that only 18 of the 89 ICI-IA patients were seen by a rheumatologist further highlights the shortcomings of the current standard for defining ICI-IA cohorts and the importance of our model for identifying ICI-IA patients.

In summary, we developed a novel EHR-based machine learning algorithm that was able to identify ICI-IA patients in the EHR with high performance. This EHR algorithm could be adapted to other EHR systems to facilitate cohort definition for ICI-IA for multicenter research studies and to assist in clinical practice with recommending patients suspected to have ICI-IA to follow-up care with a rheumatologist. Our machine learning strategy revealed hidden relationships between ICI-IA and other irAE that were corroborated in association tests. It provided insight into the clinical features that were descriptive of ICI-IA, including features potentially pertaining to comorbid irAEs. By further examining these irAEs, we found that cutaneous, endocrine, and gastrointestinal irAEs were significantly more likely to be found in patients with ICI-IA compared to cancer patients receiving ICI therapy who did not experience ICI-IA, independent of cancer type and ICI regimen. We also found potential temporal relationships between ICI-IA and other irAEs. This indicates that other such temporal phenotypes may exist in patients who develop irAEs that could be captured in EHR data. Overall, further exploration of irAEs in EHR data and investigation of irAE temporal phenotypes may reveal leads for mechanistic studies of irAE development and improve care for these patients through more rapid identification of irAEs and timelier recommendation for follow-up care.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data used and analyzed during the current study are available from the corresponding author on reasonable request. Note that row level data is access controlled and cannot be provided publicly due to data use restrictions. Models are available on GitHub (https://github.com/stevetran99/ICI-IA_ML_Classification). Requests to access these datasets should be directed to TLW, t-walunas@northwestern.edu.

Ethics statement

The studies involving humans were approved by Northwestern University Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because impracticality of consent and low risk of the study.

Author contributions

SDT: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing, Conceptualization. JL: Data curation, Writing – review & editing, Methodology. CG: Data curation, Methodology, Writing – review & editing. LVR: Data curation, Methodology, Writing – review & editing. JP: Data curation, Methodology, Writing – review & editing. GMP: Data curation, Writing – review & editing. KJR: Data curation, Writing – review & editing. CDM: Data curation, Writing – review & editing. JDJ: Data curation, Writing – review & editing. JT: Data curation, Writing – review & editing. KV: Data curation, Writing – review & editing. PVD: Data curation, Writing – review & editing. SM: Data curation, Writing – review & editing. UR: Data curation, Writing – review & editing. KT: Data curation, Writing – review & editing. NM: Investigation, Writing – review & editing. JLJ: Investigation, Writing – review & editing. YL: Investigation, Methodology, Writing – review & editing. AK: Investigation, Writing – review & editing. JS: Investigation, Writing – review & editing. TLW: Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision.

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Conflict of interest

TLW receives unrelated research funding from Gilead Sciences. AK is a strategic advisor for Datavant, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1331959/full#supplementary-material

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Additional PD-1 inhibitor improves complete response to induction chemotherapy in locally advanced nasopharyngeal carcinoma

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Purpose: To investigate the treatment response and toxicity of the combination of induction chemotherapy (IC) and PD-1 inhibitor in locally advanced nasopharyngeal carcinoma (LANPC).

Methods: Patients with stage III–IVA NPC who received IC or IC + PD-1 inhibitor were included. The chi-square test and multivariate logistic regression analysis were used for statistical analysis.

Results: A total of 225 patients were identified, including 193 (85.8%) and 32 (14.2%) who received IC alone and IC + PD-1 inhibitor, respectively. The addition of PD-1 inhibitor to IC significantly improved the tumor response than those treated with IC alone. The complete response (CR), partial response, stable disease, and progressive disease rates of 4.7% vs. 31.3%, 69.4% vs. 62.5%, 24.9% vs. 6.3%, and 1.0% vs. 0% in patients receiving IC alone and IC + PD-1 inhibitor, respectively (P<0.001). The results of the multivariate logistic regression showed that receiving PD-1 inhibitor was an independent predictor influencing the CR rate of patients (odds ratio 9.814, P<0.001). The most common toxicity by using IC and PD-1 inhibitor was hematological toxicity. In terms of non-hematological toxicity, 7 (21.9%) patients experienced thyroid dysfunction and all of them were hyperthyroidism. No grade 5 toxicities were found. In those who received IC and PD-1 inhibitor, the one-year locoregional recurrence-free survival, distant metastasis-free survival, disease-free survival, and overall survival were 100%, 96.9%, 96.9%, and 100%, respectively.

Conclusion: The addition of PD-1 inhibitor to IC has promise as an effective treatment approach for LANPC. More studies are expected to provide further insights into the optimal use of this treatment strategy, paving the way for more personalized and effective treatment options for patients with LANPC.

KEYWORDS

nasopharyngeal carcinoma, chemotherapy, induction therapy, immunotherapy, response

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor originating from the epithelial cells of the nasopharynx. It is relatively rare worldwide but has a higher incidence in certain regions, particularly Southeast Asia, including China (1). There were approximately 70% of patients diagnosed with locally advanced nasopharyngeal carcinoma (LANPC) (2). The optimal treatment approach for LANPC according to the current guidelines is induction chemotherapy (IC) following concurrent chemoradiotherapy (CCRT), with a 5-year overall survival (OS) rate of approximately 85% (3). However, there were approximately 20% of patients may develop disease recurrence after comprehensive treatment, especially for those with inferior response to IC (4–6). According to the second analysis from the prospective trial, those with complete response (CR) to IC had significantly lower recurrence rates and higher survival rates (5). However, the CR rate was only 2.8–11.3% after IC in several prospective studies (5, 7, 8).

Immunotherapy has emerged as a transformative treatment approach for various malignancies, including NPC (9). The rationale behind immunotherapy in NPC lies in the unique immunogenicity and immune evasion mechanisms associated with the disease. NPC is characterized by a high frequency of Epstein-Barr virus (EBV) association, which induces the upregulation of immune checkpoint proteins, such as programmed cell death ligand-1 (PD-L1), leading to immune evasion. Immunotherapeutic agents targeting these immune checkpoints, particularly anti-PD-1/PD-L1 antibodies, have demonstrated remarkable efficacy in NPC (10–12). Several phase III studies have shown that chemotherapy plus PD-1 inhibitor had a significantly higher clinical response, progression-free survival (PFS), and OS compared to those treated with chemotherapy alone (10–12). The unique epidemiology, pathogenesis, and treatment challenges associated with NPC have prompted the exploration of novel therapeutic approaches, including the integration of IC and PD-1 inhibitor in LANPC. Therefore, this study aimed to investigate the treatment response, toxicity, and short-term survival of the combination of IC and PD-1 inhibitor in LANPC.

Materials and methods

Patients

We retrospectively collected data from LANPC patients who were treated at our institution from January 2019 to September 2023. Patients who met the following criteria were included: 1) histologically confirmed NPC; 2) diagnosed with LANPC (stage III-IVA disease) according to the 8th edition of the American Joint Committee on Cancer staging system); 3) received induction therapy including two-three cycles of IC and PD-1 inhibitor. We excluded patients with the following criteria: 1) underwent surgical treatment to the primary nasopharyngeal tumors and/or the metastatic cervical lymph nodes; 2) had second primary cancers simultaneously diagnosed with NPC or had other malignancy before NPC diagnosis. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Xiamen University and informed consent was obtained from all the patients before treatment (approval number: 2023050).

Variables

We include the following variables in the analysis: age, gender, smoking history, alcohol history, histology, clinical stage, tumor (T) stage, nodal (N) stage, IC regimen, PD-1 inhibitor as well as the plasma EBV-DNA levels before and after the induction treatment.

Treatment

In our institution, the IC regimens included the TPF (docetaxel 75 mg/m^2 or nab-paclitaxel 260mg/m^2 on day 1, cisplatin 25 mg/m^2 on days 1–3, and 5-FU 600–750 mg/m^2 per day as a continuous 120 hours infusion or S1 capsules 40 mg/m^2 bid on day 1–14), TP (docetaxel 75 mg/m^2 or nab-paclitaxel 260mg/m^2 on day 1, cisplatin 25 mg/m^2 on days 1–3), or GP regimens (gemcitabine 1000 mg/m^2 on days 1 and 8, cisplatin 25 mg/m^2 on days 1–3). We added the PD-1 inhibitor including Camrelizumab (200 mg on day 1) or Tislelizumab (200 mg on day 1) into IC during the phase of induction therapy. Pegylated recombinant human granulocyte colony-stimulating factor could be used for the prevention of bone marrow suppression.

All patients began definitive CCRT within three weeks after the completion of induction therapy. Radiotherapy was delivered using volumetric modulated arc therapy or helical tomotherapy. Target volumes were delineated following guidelines from the Chinese Society of Clinical Oncology (CSCO) and our institution (13, 14). We primarily delineated the grosstumor volume in the nasopharynx (GTVp), gross tumor volume in the neck (GTVn), high-risk clinical target volume in the neck (CTVn1) (GTVn + 3 mm, with corresponding modifications made when adjacent to important organs at risk [OARs]), high-risk clinical target volume (CTVp1) (GTVp + 5–10mm, with corresponding modifications made when adjacent to important OARs), and low-risk clinical target volume (CTV2) (CTVp1 + 5–10 mm and cervical lymph nodes, with corresponding modifications made based on the extent of tumor invasion or proximity to important OARs). The total radiation dose for GTVp, GTVn, CTVn1, CTVp1, and CTVp2 was 70.29 Gray (Gy), 70.29 Gy, 62.04 Gy, 62.04 Gy, and 56.10 Gy, respectively, delivered in 33 fractions given five times per week. GTVp and GTVn were delineated according to the tumor area after IC, while the extent of bone and paranasal sinus infiltration was delineated based on the tumor area before IC. Concurrent chemotherapy was recommended and cisplatin (80 mg/m2 given on days 1–3, every 3 weeks) or lobaplatin (30 mg/m2 on day 1, every 3 weeks) were used with a total of two cycles.

Assessment of treatment response

The effectiveness evaluation after induction therapy was performed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Two experienced radiation oncologists (SGW) and radiologists (YKS) evaluated the changes

in the imaging of the primary nasopharyngeal tumors and the metastatic cervical lymph nodes before and after induction therapy. The evaluation criteria include CR, partial response (PR), stable disease (SD), and progressive disease (PD). The overall response rate (ORR) was calculated as the sum of CR and PR.

Assessment of treatment toxicity

Acute toxicities during induction therapy were graded according to the Common Terminology Criteria for Adverse Events version 4.0. The toxicities related to PD-1 inhibitor were also evaluated at each treatment cycle according to the guidelines (15).

Follow-up

In this study, survival data were collected retrospectively by reviewing medical records. All patients underwent regular follow-up assessments, including monitoring EBV-DNA levels, MRI, chest CT scans, and abdominal sonography every three months for a minimum of three years. Immediate imaging examinations, such as PET/CT, were conducted for patients showing signs of disease progression. The endpoints analyzed included locoregional recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), disease-free survival (DFS), and OS. LRFS was defined as the time from diagnosis to the occurrence of locoregional recurrence, while DMFS was the time from diagnosis to the occurrence of distant metastasis. DFS was defined as the time from diagnosis to the occurrence of locoregional relapse, distant metastasis, or death from any cause, whichever occurred first. OS duration was determined as the time from diagnosis to the date of death from any cause or the last recorded date when the patient was known to be alive.

Statistical analysis

The differences in patient characteristics between the two treatment arms were compared using the chi-square test or Fisher's exact test. Multivariate logistic regression analysis was used to determine the independent factors influencing the CR rate after induction therapy. The survival curves were plotted using the Kaplan-Meier method. Statistical analysis in this study was conducted using the IBM SPSS 26.0 software package (IBM Corp., Armonk, NY), with a significance level of $P < 0.05$ indicating statistical significance.

Results

Patient characteristic

A total of 225 patients were included in this study (Table 1). Among these patients, 166 (72.5%) were male, 204 (90.7%) were WHO type III subtype, 163 (72.4%) were at T3–4 stage, 175 (77.8%) were at N2–3 stage, and 201 (89.3%) had detectable EBV-DNA before treatment. Regarding induction treatment, 43 (19.1%), 119 (52.9%), and 63 (28.0%) received GP, TP, and TPF regimens, respectively. There were 193 (85.8%) patients treated with IC alone and 32 (14.2%) patients received IC + PD-1 inhibitor. Of

TABLE 1 Patient characteristics between those treated with induction chemotherapy alone and induction chemotherapy plus PD-1 inhibitor.

Variables	n	IC	IC + PD-1 inhibitor	P
Age (years)				
<50	127	108 (56.0)	19 (59.4)	0.718
≥50	98	85 (44.0)	13 (40.6)	
Gender				
Male	59	53 (27.5)	6 (18.8)	0.299
Female	166	140 (72.5)	26 (81.3)	
Smoking history				
No	115	102 (52.8)	13 (40.6)	0.200
Yes	110	91 (47.2)	19 (59.4)	
Alcohol history				
No	148	121 (62.7)	27 (84.4)	0.017
Yes	77	72 (37.3)	5 (15.6)	
Histology				
WHO II	21	20 (10.4)	1 (3.1)	0.324
WHO III	204	173 (89.6)	31 (96.9)	
T stage				
T1	24	22 (11.4)	2 (6.3)	0.674
T2	38	34 (17.6)	4 (12.5)	
T3	117	98 (50.8)	19 (59.4)	
T4	46	39 (20.2)	7 (21.9)	
N stage				
N0	1	0 (0.0)	1 (3.1)	0.100
N1	49	43 (22.3)	6 (18.8)	
N2	87	74 (38.3)	13 (40.6)	
N3	88	76 (39.4)	12 (37.5)	
AJCC stage				
III	104	88 (45.6)	16 (50.0)	0.644
IVA	121	105 (54.4)	16 (50.0)	
Pretreatment EBV-DNA level				
Undetected	24	21 (10.9)	3 (9.4)	1.000
Detective	201	172 (89.1)	29 (90.6)	

IC, induction chemotherapy; WHO, World Health Organization; T, tumor; N, nodal; AJCC, American Joint Committee on Cancer; EBV-DNA, Epstein Barr virus-deoxyribonucleic acid.

those receiving PD-1 inhibitor, 17 (53.1%) received Camrelizumab, and 15 (46.9%) received Tislelizumab. There were no significant differences in age, gender, smoking history, alcohol history, T stage, N stage, histological subtype, and EBV-DNA levels between patients receiving IC and IC + PD-1 inhibitor (Table 1).

Treatment response

All patients underwent efficacy evaluation after induction therapy (Figure 1A). The addition of PD-1 inhibitor to IC significantly improved the tumor response than those receiving IC alone. The CR, PR, SD, and PD rates of 4.7%, 69.4%, 24.9%, and 1.0%, respectively, for those treated with IC alone. In those with IC + PD-1 inhibitor, the CR, PR, SD, and PD rates were 31.3%, 62.5%, 6.3%, and 0%, respectively (P<0.001). There was a significant difference in ORR between those treated with IC alone and IC + PD-1 inhibitor (74.1% vs. 93.8%, P=0.012). Figure 2 shows a patient who achieved CR after IC + PD-1 inhibitor.

We also evaluated the primary nasopharyngeal tumors and metastatic cervical lymph nodes separately. The addition of PD-1 inhibitor to IC also significantly improved the response to the primary nasopharyngeal tumors (P<0.001) (Figure 1B) and metastatic cervical lymph nodes (P=0.002) (Figure 1C), respectively. Regarding ORR, IC + PD-1 inhibitor could improve the ORR of metastatic cervical lymph nodes than those treated with

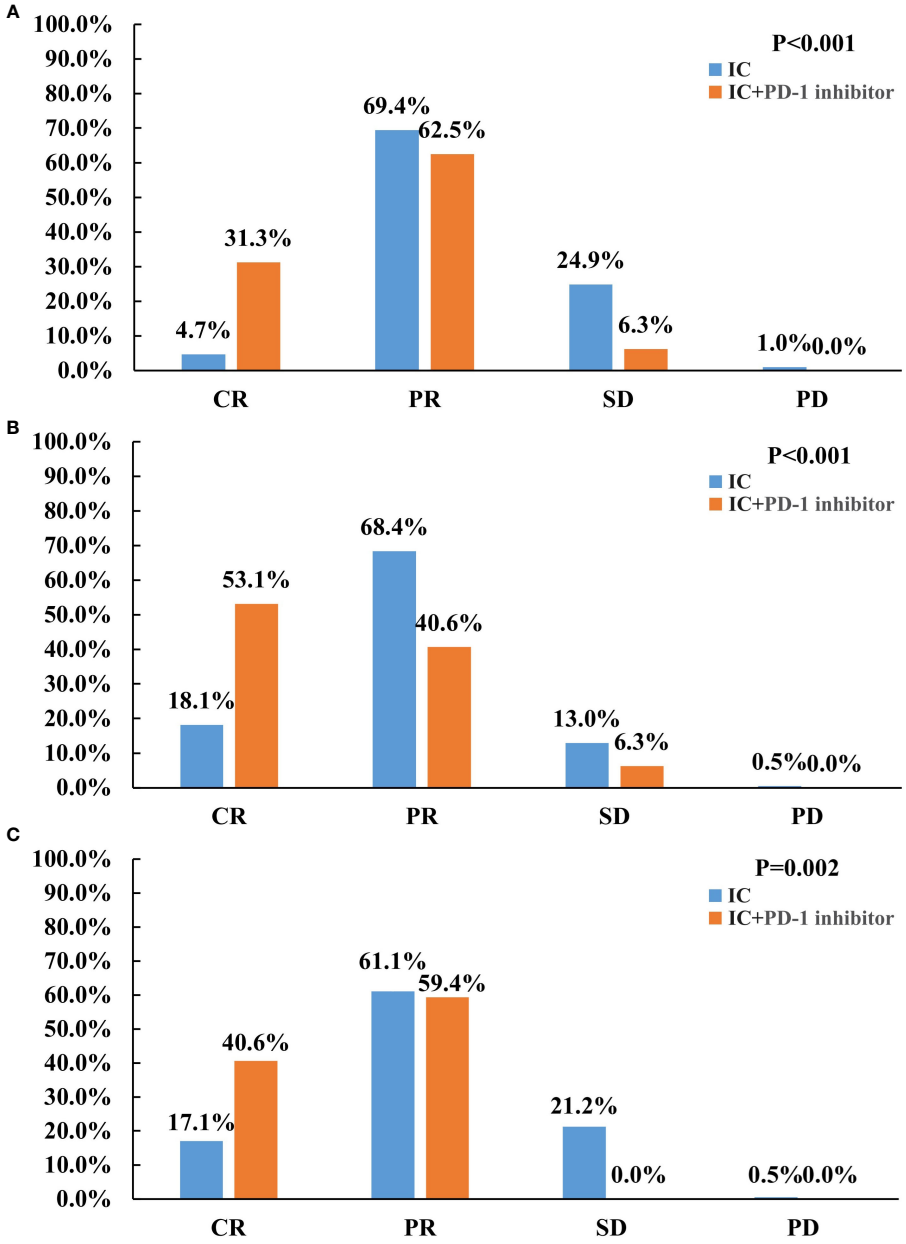

FIGURE 1
Treatment response between induction chemotherapy alone and induction chemotherapy plus PD-1 inhibitor in the entire cohort **(A)**, primary nasopharyngeal tumors **(B)**, and metastatic cervical lymph nodes **(C)**.

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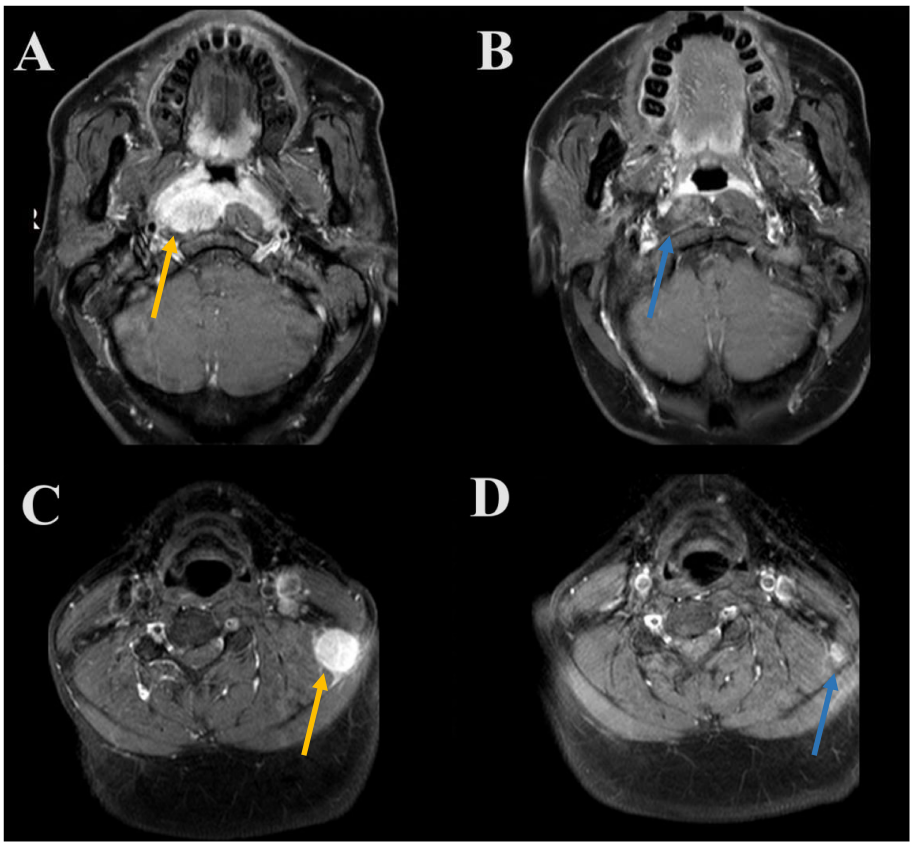

FIGURE 2
A patient who achieved complete response after induction chemotherapy plus PD-1 inhibitor (**A**, primary nasopharyngeal tumors before induction therapy [orange arrow]; **B**, complete response to primary nasopharyngeal tumors after induction therapy [orange arrow]; **C**, metastatic cervical lymph node before induction therapy [blue arrow]; **D**, complete response to metastatic cervical lymph node after induction therapy [blue arrow]).

IC alone (P=0.001), but there was a similar ORR for primary nasopharyngeal tumors between the treatment arms (P=0.386).

Predictive factors associated with complete response after induction therapy

We conducted a multivariate logistic regression analysis to assess the independent predictors influencing the CR rate of patients after induction therapy (Table 2). The results revealed that receiving PD-1 inhibitor was an independent predictor influencing the CR rate of patients (odds ratio [OR] 9.814, 95% confidence interval [CI] 3.464–27.804, P<0.001). We also found that the CR rate of stage III patients was significantly higher than that of stage IVA patients (OR 3.886, 95% CI 1.269–11.906, P=0.017). The sensitivity analyses also showed that the additional PD-1 inhibitor to IC was the independent predictor influencing the CR of the primary nasopharyngeal tumors (OR 5.378, 95% CI 2.413–11.989, P<0.001) (Table 3) and metastatic cervical lymph nodes (OR 3.317, 95% CI 1.492–7.374, P=0.003) (Table 4).

Toxicity

We assessed the treatment toxicities in those receiving PD-1 inhibitor (n=32) (Figure 3). Regarding hematological toxicity, 11 (34.4%), 6 (18.8%), 3 (9.4%), and 3 (9.4%) patients experienced anemia, thrombocytopenia, leukopenia, and neutropenia, respectively. Five patients (15.7%) experienced grade 3–4 hematological toxicity, but all returned to normal after symptomatic treatment.

In terms of non-hematological toxicity, 7 (21.9%), 7 (21.9%), 5 (15.6%), and 2 (6.3%) patients experienced alanine aminotransferase elevation, thyroid dysfunction, aspartate aminotransferase elevation, and creatinine elevation, respectively, all of which were grade 1 or 2 toxicities without any grade 3 or higher toxicities were found.

Among the 17 patients who received treatment with Camrelizumab, three patients (17.6%) experienced reactive cutaneous capillary endothelial proliferation, all of which were grade 1 or 2. One patient (3.1%) developed scattered rashes during treatment but improved after symptomatic treatment.

TABLE 2 Multivariate logistic regression analysis for independent predictors influencing the complete response rate of the entire cohort.

Variables	OR	95%CI	P
Age (year)			
<50	1		
≥50	1.014	0.327–3.147	0.981
Gender			
Male	1		
Female	1.446	0.323–6.484	0.630
Smoking history			
No	1		
Yes	1.352	0.370–4.943	0.649
Alcohol history			
No	1		
Yes	0.245	0.048–1.249	0.091
Histology			
WHO II	1		
WHO III	2.631	0.615–11.301	0.998
AJCC stage			
III	1		
IVA	3.886	1.269–11.906	0.017
Pretreatment EBV DNA level			
Undetected	1		
Detective	1.112	0.125–9.909	0.924
PD-1 inhibitor			
No	1		
Yes	9.814	3.464–27.804	<0.001

IC, induction chemotherapy; WHO, World Health Organization; AJCC, American Joint Committee on Cancer; EBV-DNA, Epstein Barr virus-deoxyribonucleic acid; OR, odds ratio; CI, confidence interval.

TABLE 3 Multivariate logistic regression analysis for independent predictors influencing the complete response rate to primary nasopharyngeal tumors.

Variables	OR	95%CI	P
Age (years)			
<50	1		
≥50	1.850	0.947–3.617	0.072
Gender			
Male	1		
Female	1.100	0.409–2.956	0.851
Smoking history			
No	1		
Yes	1.220	0.520–2.859	0.648
Alcohol history			
No	1		
Yes	0.874	0.397–1.922	0.737
Histology			
WHO II	1		
WHO III	2.899	0.621–13.532	0.176
AJCC stage			
III	1		
IVA	1.817	0.932–3.544	0.080
Pretreatment EBV DNA level			
Undetected	1		
Detective	1.006	0.333–3.038	0.991
PD-1 inhibitor			
No	1		
Yes	5.378	2.413–11.989	<0.001

IC, induction chemotherapy; WHO, World Health Organization; AJCC, American Joint Committee on Cancer; EBV-DNA, Epstein Barr virus-deoxyribonucleic acid; OR, odds ratio; CI, confidence interval.

Short-term survival

In those treated with IC and PD-1 inhibitor, all patients received and completed the recommended radiotherapy and concurrent chemotherapy. The median follow-up period was 17.0 months (range, 9–37 months). One patient experienced an elevation of plasma EBV-DNA levels 7.3 months after NPC diagnosis, and PET/CT confirmed thoracic vertebral metastasis. The patient received a combination of Tislelizumab and the GP regimen. After two cycles of treatment, EBV-DNA became undetectable, and PET/CT showed no metabolic activity in the thoracic vertebral metastases. At the time of data publication, the patient had completed the fifth cycle of treatment. Another patient was diagnosed with primary hepatocellular carcinoma 17.9 months after NPC diagnosis and underwent surgical treatment. None of the patients experienced locoregional recurrence or death during the follow-up period. The one-year LRFS, DMFS, DFS, and OS were 100%, 96.9%, 96.9%, and 100%, respectively (Figure 4).

Discussion

Currently, the recommendation for PD-1 inhibitor in NPC mainly focuses on recurrent or metastatic patients and shows improved tumor response rates and survival rates compared to those treated with chemotherapy alone (9–12). Theoretically, integrating PD-1 inhibitor into the induction therapy may achieve a better response for LANPC compared to those treated with IC alone. To test this hypothesis, we explored the clinical response and toxicity of IC + PD-1 inhibitor in patients with LANPC. We found that adding PD-1 inhibitor to IC significantly improved the CR of patients while maintaining acceptable treatment toxicities.

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TABLE 4 Multivariate logistic regression analysis for independent predictors influencing the complete response rate to metastatic cervical lymph nodes.

Variables	OR	95%CI	P
Age (years)			
<50	1		
≥50	0.913	0.449–1.856	0.801
Gender			
Male	1		
Female	2.028	0.732–5.615	0.174
Smoking history			
No	1		
Yes	0.928	0.403–2.138	0.862
Alcohol history			
No	1		
Yes	0.75	0.339–1.661	0.479
Histology			
WHO II	1		
WHO III	0.545	0.187–1.593	0.268
AJCC stage			
III	1		
IVA	1.629	0.823–3.225	0.161
Pretreatment EBV DNA level			
Undetected	1		
Detective	0.711	0.248–2.034	0.525
PD-1 inhibitor			
No	1		
Yes	3.317	1.492–7.374	0.003

IC, induction chemotherapy; WHO, World Health Organization; AJCC, American Joint Committee on Cancer; EBV-DNA, Epstein Barr virus-deoxyribonucleic acid; OR, odds ratio; CI, confidence interval.

The response to IC is closely associated with the survival of patients. Results from the secondary analysis of the prospective study have shown that patients achieving a CR after IC with a GP regimen have significantly better OS than those with PR or SD/PD, with 5-year OS of 100%, 88.4%, and 61.5% respectively (P=0.005) (5). Achieving a CR is crucial in cancer treatment as it indicates the eradication of visible tumor cells and is associated with improved long-term outcomes. However, it should be noted that in prospective studies, the CR rates after GP, TPF, and TP regimen were only 10% (5), 11.3% (8), and 2.8% (7), respectively. Several retrospective studies, including ours, have also found that the CR rate after IC did not exceed 5% (6, 16). In this study, the CR rate was only 4.8% in patients receiving IC, which was consistent with the findings of the above studies. However, when we added PD-1 inhibitor to IC, the CR rate reached 34.4%, and the ORR after induction therapy reached 93.8%. In two prospective studies combining the GP regimen with Tislelizumab, the overall CR rate was 41.3–50.0%, and the ORR was 88.9% to 95.8% (17, 18). Based on our results and the findings of the above prospective studies, integrating PD-1 inhibitor into induction therapy may improve the CR rate and potentially have an impact on survival outcomes.

Our study further showed that the CR rate was 56.3% and 43.8% in the primary nasopharyngeal tumors and metastatic cervical lymph nodes after IC + PD-1 inhibitor, respectively. Furthermore, adding PD-1 inhibitor was identified as an independent predictive factor affecting the CR rate of patients. Currently, there is still a lack of separate evaluations of responses to IC + PD-1 inhibitor in prospective studies. A retrospective study by Xiang et al. found that adding PD-1 inhibitor to IC significantly improved the CR rate of the primary nasopharyngeal tumors (0.8% vs. 14%, P<0.001) and metastatic cervical lymph nodes (22.3% vs. 36.8%, P=0.021) compared to IC alone. However, for patients receiving IC with the GP regimen, adding PD-1 inhibitor only improved the CR rate of the primary nasopharyngeal tumors (17.0% vs. 1.5%, P=0.002), while it did not affect the CR rate of metastatic cervical lymph nodes (31.9% vs. 27.3%, P=0.400) (19). In our previous study, we found that patients achieving CR in the primary nasopharyngeal tumors or metastatic cervical lymph nodes after IC had better progression-free survival (6). Therefore, in the era of immunotherapy, more studies are required to assess the effect of treatment response to IC + PD-1 inhibitor on survival outcomes in LANPC.

Several prospective studies on head and neck cancer have found that adding PD-1/PD-L1 inhibitor (Pembrolizumab or Avelumab) to CCRT and subsequent maintenance PD-1/PD-L1 inhibitor for one year did not significantly improve survival outcomes compared to those treated with CCRT alone (20, 21). However, patients enrolled in both studies did not receive induction therapy, therefore the response to IC and PD-1 inhibitor could not be evaluated. Since cervical lymph nodes are a standard target volume for radiotherapy of the NPC, radiation to the lymph node drainage area may impair the immune response caused by PD-1 antibodies. Therefore, initiating PD-1 inhibitor treatment before radiotherapy may better activate the immune system and enhance the efficacy of immunotherapy. For LANPC patients, initial findings from the CONTINUUM study suggested that the use of Sintilimab (PD-1 inhibitor) in combination with IC, CCRT, and adjuvant therapy significantly improves the 3-year event-free survival rate compared to those treated with IC plus CCRT (86.1% vs. 76.0%) (22). In our study, we found short-term survival was excellent by using IC and PD-1 inhibitor with a median follow-up time of 17.0 months. Several studies have shown that chemotherapy could induce antigen presentation and induce expression of immune checkpoints (23). In addition, chemotherapeutic agents-induced immunogenic cell death and their immune stimulation activity are considered the main mechanisms of combination therapy (24). Therefore, early initiation of immunotherapy may be beneficial in LANPC.

In this study, 53.1% of patients received treatment with Camrelizumab, and 46.9% received treatment with Tislelizumab. Multivariate analysis showed no significant difference in the CR rate between the two PD-1 inhibitors. Li et al. found that IC with GP regimen had an immune modulation effect in LANPC and did not weaken the cytotoxic activity and proliferative capacity of T cells

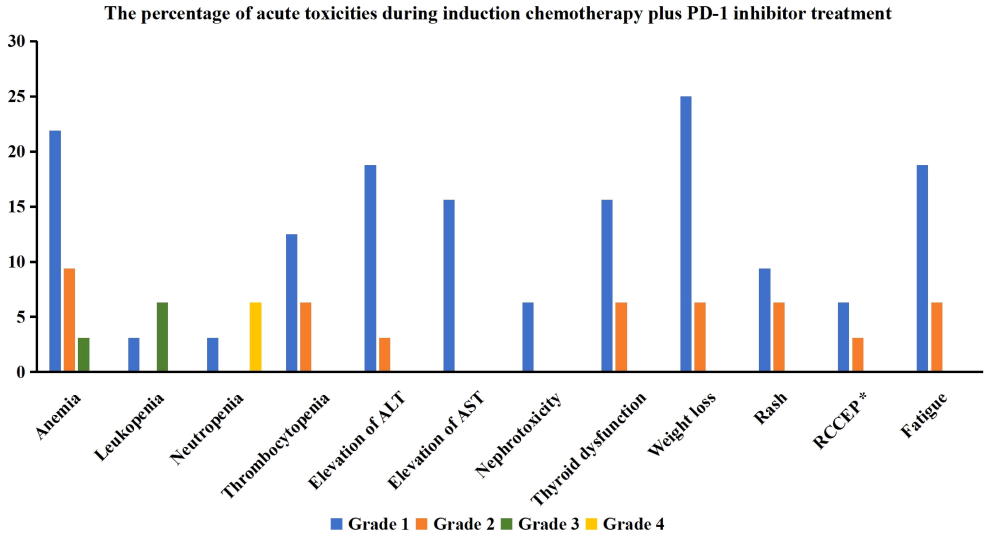

FIGURE 3
The percentage of acute toxicities during induction chemotherapy plus PD-1 inhibitor treatment (ALT, alanine aminotransferase; AST, aspartate aminotransferase; RCCEP, reactive cutaneous capillary endothelial proliferation) (*17 patients received treatment with Camrelizumab).

(25). In addition, the effects of nab-paclitaxel on modulation of the cancer-immunity cycle provide potential avenues for a combined therapeutic rationale to improve the efficacy of PD-1 inhibitor (26). Several studies on lung and breast cancer have shown that the combination of nab-paclitaxel and PD-1 inhibitor results in significantly better survival than nab-paclitaxel alone (27, 28). The use of corticosteroids in immunotherapy may affect the efficacy of PD-1 inhibitors, while paclitaxel and docetaxel often require corticosteroid pretreatment. Therefore, the combination of nab-paclitaxel and PD-1 inhibitor may have advantages. Currently, we are conducting a prospective Phase II study to explore the impact of the TPF regimen based on nab-paclitaxel combined with Camrelizumab on tumor response rate and survival in patients with LANPC.

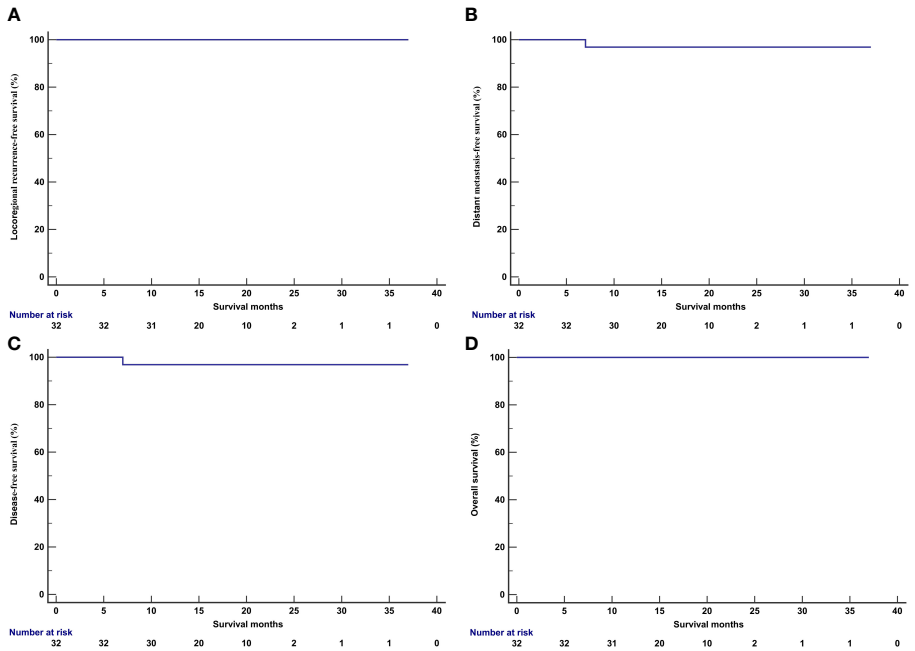

FIGURE 4
Kaplan-Meier curve of locoregional recurrence-free survival **(A)**, distant metastasis-free survival **(B)**, disease-free survival **(C)**, and overall survival **(D)** in patients receiving induction chemotherapy plus PD-1 inhibitor.

In this study, the common toxicities were hematological toxicity and liver function damage, which were similar to the common toxicities of chemotherapy. Thyroid dysfunction is a common toxicity to PD-1 inhibitor, especially hypothyroidism (29, 30). In this study, we observed that 21.9% of patients experienced hyperthyroidism during IC and PD-1 inhibitor, and no cases of hypothyroidism were observed. The study by Zhang et al. also found that out of 25 patients, 9 (36%) had thyroid dysfunction, with 8 patients having hyperthyroidism and 1 patient having hypothyroidism (18). Studies on NPC and lung cancer have found that patients who experience thyroid dysfunction during PD-1 inhibitor treatment have better disease control rates (29, 30). However, due to the small sample size, we did not observe the correlation between thyroid dysfunction and the CR rate of patients. In the future, more samples need to be accumulated to explore the correlation between thyroid dysfunction and the efficacy of treatment in patients.

We needed to acknowledge several limitations of our study. First, the combination of IC and PD-1 inhibitor has not yet been approved for LANPC, and our study only included a small sample size. Second, the recording of toxicities during treatment may be insufficient due to the retrospective analysis. Therefore, we did not compare the differences in adverse reactions between IC and IC combined with PD-1 inhibitor. However, based on the results from the prospective randomized controlled studies (10–12), the incidence of all adverse events, grade 3 or greater adverse events, and fatal adverse events were similar between those treated with GP and GP + PD-1 inhibitor in recurrent or metastatic NPC. Third, the immune infiltration characteristics such as PD-L1 expression were not routinely assessed in patients with LANPC in our institution, thus we were unable to evaluate the relationship between the CR rate and PD-L1 expression status. However, several prospective randomized controlled studies in recurrent or metastatic NPC have found no significant correlation between baseline PD-L1 expression and objective response rate or progression-free survival (10–12). Finally, our study has a relatively short follow-up time, and a longer follow-up is needed to clarify the impact of a combination of IC and PD-1 inhibitor on the survival of patients.

Conclusions

In conclusion, the addition of PD-1 inhibitor to IC has promise as an effective treatment approach for LANPC. More studies are expected to provide further insights into the optimal use of this treatment strategy, paving the way for more personalized and effective treatment options for patients with LANPC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The First Affiliated Hospital of Xiamen University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

Y-FY: Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. G-ZL: Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. R-JW: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Y-KS: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. S-GW: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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Small molecule inhibitors as adjuvants in cancer immunotherapy: enhancing efficacy and overcoming resistance

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Adjuvant therapy is essential in cancer treatment to enhance primary treatment effectiveness, reduce adverse effects, and prevent recurrence. Small molecule inhibitors as adjuvants in cancer immunotherapy aim to harness their immunomodulatory properties to optimize treatment outcomes. By modulating the tumor microenvironment, enhancing immune cell function, and increasing tumor sensitivity to immunotherapy, small molecule inhibitors have the potential to improve patient responses. This review discusses the evolving use of small molecule inhibitors as adjuvants in cancer treatment, highlighting their role in enhancing the efficacy of immunotherapy and the opportunities for advancing cancer therapies in the future.

KEYWORDS

adjuvant, adjuvant therapy, neoadjuvant therapy, anti-tumor drug, cancer immunotherapy, small molecule inhibitor

1 Introduction

Adjuvant therapy in cancer treatment refers to the use of additional treatments such as chemotherapy, radiation, or targeted therapies following primary treatments like surgery. The main objectives of adjuvant therapy are to enhance the effectiveness of the primary treatment, reduce adverse effects, and prevent disease recurrence (1–3). This approach targets residual cancer cells post-surgery, helping to reduce the risk of cancer returning and spreading, and thereby improving the overall success rates of cancer eradication (4, 5). In the context of cancer immunotherapy, small molecule inhibitors serve as immune adjuvants. These inhibitors aim to modulate the tumor microenvironment, enhance

immune cell function, and increase tumor sensitivity to immunotherapy (6, 7). By leveraging their immunomodulatory properties, small molecule inhibitors can optimize treatment outcomes, improve patient responses, and provide new opportunities for advancing cancer therapies (8).

In cancer immunotherapy, the concept of using small molecule inhibitors as adjuvants involves leveraging the immunomodulatory effects of these drugs to enhance the effectiveness of immunotherapy. For example, small molecule inhibitors can modulate the tumor microenvironment, boost immune cell function, increase tumor sensitivity to immunotherapy, and achieve better treatment outcomes (9–11). Using small molecule inhibitors as adjuvants in cancer treatment is a rapidly evolving and expanding field. By researching how small molecule inhibitors interact with immunotherapy, optimizing treatment regimens, predicting patient responses to treatment, it can provide more opportunities and improvements for future cancer treatments. In this comprehensive review, we delve into the evolving role of small molecule inhibitors as adjuvants in cancer immunotherapy, exploring their mechanisms of action, clinical applications, and potential for improving treatment outcomes.

2 Mechanisms of action of small molecule inhibitors in cancer immunotherapy

Small molecule inhibitors play a significant role in cancer immunotherapy by targeting specific pathways and molecules involved in regulating the immune response to tumors. These inhibitors act through various mechanisms to modulate the tumor microenvironment and enhance the anti-tumor immune response. Some common mechanisms of action of small molecule inhibitors in cancer immunotherapy have been summarized.

2.1 Immune checkpoint blockade

Immune checkpoint blockade is a cutting-edge cancer immunotherapy that targets molecules like CTLA-4 and PD-1 to activate T cells and boost anti-tumor immunity. By blocking inhibitory signals, checkpoint inhibitors unleash the immune system to recognize and eliminate cancer cells (12, 13). Monoclonal antibodies targeting CTLA-4, such as ipilimumab, disrupt this inhibitory signal, enhancing T cell activation and anti-tumor immune responses (14). Small molecule inhibitors, on the other hand, are designed to interfere with intracellular signaling pathways, thus modulating immune responses indirectly. PD-1, expressed on T cells upon activation, interacts with PD-L1 to inhibit T cell function (15). Small molecule inhibitors targeting the PD-1/PD-L1 pathway can modulate intracellular signaling pathways, leading to T cell activation and immune-mediated tumor cell killing (16). By targeting these key immune checkpoint molecules with small molecule inhibitors, we can modulate immune responses to overcome tumor-induced immune suppression and expand the therapeutic landscape in cancer immunotherapy.

2.2 Signal transduction pathways

Signal transduction pathways play a critical role in regulating immune responses in cancer, including immune cell activation, proliferation, and effector functions. Small molecule inhibitors targeting key signaling molecules within these pathways have emerged as promising adjuvants in cancer immunotherapy (17) (18). For instance, inhibitors of the PI3K-Akt-mTOR pathway can modulate T cell activation and differentiation, enhancing anti-tumor immunity (19). Inhibitors of the MAPK pathway, such as MEK inhibitors, can regulate T cell function and cytokine production to optimize anti-tumor immune responses (20). Additionally, inhibitors of the NF-κB pathway can modulate inflammatory responses and immune cell activation. By selectively targeting specific nodes within these signaling pathways, small molecule inhibitors can fine-tune immune responses to promote effective anti-tumor immunity (21). Understanding the intricate interplay of signal transduction pathways and harnessing the therapeutic potential of small molecule inhibitors offer exciting avenues to expand the therapeutic landscape of cancer immunotherapy and improve patient outcomes.

2.3 Enhancing immune cell infiltration

Enhancing immune cell infiltration into tumors is a critical mechanism by which small molecule inhibitors act as adjuvants in cancer immunotherapy. Effective infiltration of immune cells into the tumor microenvironment is essential for mounting a robust anti-tumor immune response. Small molecule inhibitors target key pathways and mechanisms, such as angiogenesis, extracellular matrix remodeling, and immune cell infiltration, to enhance the immune response within the tumor microenvironment.

2.3.1 Targeting angiogenic pathways

Tumor growth and progression are heavily dependent on the formation of new blood vessels, a process known as angiogenesis. Tumors secrete vascular endothelial growth factor (VEGF) to promote angiogenesis, which also creates an abnormal and disorganized vascular network that impedes immune cell infiltration. Small molecule inhibitors, such as those targeting VEGF receptors (VEGFR), can normalize the tumor vasculature. By inhibiting VEGF signaling, these inhibitors can reduce the formation of new blood vessels, disrupt existing abnormal vessels, and improve the overall vascular structure within the tumor. This normalization of the tumor vasculature facilitates better penetration and infiltration of immune cells, such as cytotoxic T lymphocytes

(CTLs) and natural killer (NK) cells, into the tumor microenvironment (22).

2.3.2 Modulating the extracellular matrix

The extracellular matrix (ECM) within tumors often presents a physical barrier to immune cell infiltration. Small molecule inhibitors can modulate components of the ECM to enhance immune cell penetration. For instance, inhibitors targeting enzymes such as matrix metalloproteinases (MMPs) can degrade ECM components, thereby reducing the physical barriers that prevent immune cells from reaching the tumor core. By altering the ECM composition, these inhibitors create pathways for immune cells to infiltrate more effectively (23).

2.3.3 Reducing immunosuppressive cells

The tumor microenvironment often contains a high number of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which inhibit the activity and infiltration of effector immune cells. Small molecule inhibitors can selectively target and reduce the population of these immunosuppressive cells. For example, inhibitors of the colony-stimulating factor-1 receptor (CSF-1R) can decrease the number of MDSCs, thereby reducing their suppressive effects on immune cell infiltration and function. This reduction in immunosuppressive cells enhances the ability of effector immune cells to infiltrate the tumor and exert their anti-tumor effects (24).

2.3.4 Enhancing chemokine signaling

Chemokines are signaling molecules that guide the migration of immune cells to sites of inflammation, including tumors. Small molecule inhibitors can enhance chemokine signaling pathways to promote the recruitment and infiltration of immune cells into tumors. For instance, inhibitors that upregulate the expression of chemokines such as CXCL9 and CXCL10 can attract more CTLs to the tumor site. By increasing the concentration of these chemokines in the tumor microenvironment, small molecule inhibitors enhance the directional migration of immune cells into the tumor, improving their infiltration and subsequent anti-tumor activity (25).

2.4 Immunomodulation

Small molecule inhibitors, by targeting immunomodulatory pathways, can shape the immune landscape within the tumor microenvironment to bolster immune-mediated tumor eradication (26). These inhibitors can modulate the activity of various immune cell populations, such as T cells, regulatory T cells, and myeloid-derived suppressor cells, to tip the balance in favor of anti-tumor immune responses. Furthermore, small molecule inhibitors can impact cytokine signaling networks, influencing the immune cell functions and interactions critical for mounting effective anti-tumor immune responses (27). By fine-tuning immune responses through targeted immunomodulation, small molecule inhibitors can overcome immune evasion mechanisms employed by tumors and enhance the efficacy of cancer immunotherapy. Leveraging the power of immunomodulation in conjunction with other therapeutic strategies, such as immune checkpoint blockade or targeted therapies, offers a multifaceted approach to expand the therapeutic landscape of cancer immunotherapy and improve patient outcomes.

By targeting these key pathways and mechanisms, small molecule inhibitors can synergize with immunotherapy approaches to improve treatment outcomes in cancer patients. Further research into the precise mechanisms of action of small molecule inhibitors in cancer immunotherapy holds promise for developing more effective and targeted cancer treatments.

3 Clinical applications of small molecule inhibitors as adjuvants in cancer immunotherapy

Small molecule inhibitors have demonstrated promising clinical applications in cancer immunotherapy across various types of cancer (9). These inhibitors play a crucial role as adjuvants by enhancing immune responses, overcoming resistance mechanisms, and improving overall treatment outcomes. Their ability to modulate the tumor microenvironment and improve immune cell infiltration makes them valuable assets in combination with existing immunotherapy approaches.

For instance, studies have shown that small molecule inhibitors targeting VEGFR can normalize tumor vasculature, facilitating better immune cell infiltration and enhancing the effectiveness of immune checkpoint inhibitors in clinical settings. In a phase II clinical trial, the combination of the VEGFR inhibitor axitinib with the PD-1 inhibitor pembrolizumab showed significant improvement in response rates and overall survival in patients with metastatic renal cell carcinoma (28). Similarly, the PI3K inhibitor idelalisib has been used successfully in combination with rituximab for the treatment of relapsed chronic lymphocytic leukemia, demonstrating the potential of small molecule inhibitors in enhancing the efficacy of immunotherapy (29).

3.1 Combination therapy

Combining small molecule inhibitors with other immunotherapeutic approaches, such as immune checkpoint inhibitors or adoptive T cell therapy, offers a synergistic approach to amplify immune responses and overcome resistance mechanisms (30). By targeting distinct signaling pathways or immune checkpoints simultaneously, combination therapy has the potential to broaden the spectrum of anti-tumor immune responses and improve treatment outcomes. Furthermore, combining small molecule inhibitors with traditional cancer treatments like chemotherapy or radiation therapy can create a multifaceted attack on tumor cells, leading to more comprehensive and durable responses (31). The rational design of combination regimens that leverage the strengths

of different therapeutic modalities holds promise in expanding the therapeutic landscape of cancer immunotherapy and addressing the challenges of immune evasion and tumor heterogeneity.

3.2 Overcoming resistance

Resistance mechanisms, such as immune evasion and tumor heterogeneity, can limit the success of immunotherapeutic approaches. Small molecule inhibitors can help overcome resistance by targeting pathways involved in immune evasion and tumor immune escape (32). By disrupting these critical signaling pathways in the tumor microenvironment, small molecule inhibitors can enhance immune cell infiltration, reprogram immune responses, and restore immune recognition of tumor cells (33). Additionally, combination therapies that incorporate small molecule inhibitors alongside immunotherapies or other treatments present a comprehensive strategy to combat resistance and enhance treatment outcomes. Overall, by targeting resistance mechanisms, small molecule inhibitors play a vital role in expanding the therapeutic landscape of cancer immunotherapy and improving patient responses to treatment.

3.3 Personalized medicine and adjuvant therapy

By leveraging small molecule inhibitors in cancer immunotherapy, personalized medicine aims to identify specific molecular targets or pathways unique to each patient's tumor (34). This precision medicine approach allows for the selection of the most effective small molecule inhibitors based on the molecular characteristics of the tumor, genetic profile of the patient, and immune response (35). By customizing treatment regimens to match the individual tumor biology and immune landscape, personalized medicine maximizes therapeutic efficacy while minimizing side effects.

Additionally, overcoming drug resistance is a critical aspect of personalized medicine. Certain genetic changes caused by drug resistance can be precisely regulated through targeted small molecule inhibitors. By identifying and targeting these specific genetic alterations, small molecule inhibitors can help to overcome resistance and restore sensitivity to treatment. The use of small molecule inhibitors as adjuvants in cancer immunotherapy represents a promising strategy to expand the therapeutic landscape, overcome treatment resistance, and improve patient responses to immunotherapy.

3.4 New targets and indications

Small molecule inhibitors offer the potential to target novel pathways and molecular targets that have not been previously exploited in immunotherapeutic approaches. By identifying and leveraging these new targets, researchers can broaden the scope of immunotherapy strategies, address the challenges of treatment resistance, and enhance therapeutic efficacy (36). Additionally, the discovery of new indications for small molecule inhibitors in cancer immunotherapy opens up opportunities to treat a wider range of cancer types and patient populations (37). The pursuit of novel targets and indications for small molecule inhibitors in cancer immunotherapy holds great promise for expanding the therapeutic landscape and improving outcomes for individuals with cancer.

The clinical applications of small molecule inhibitors in cancer immunotherapy represent a rapidly evolving field, with ongoing research focusing on optimizing treatment regimens, identifying biomarkers, and expanding the therapeutic potential of these inhibitors in various cancer types (38). As our understanding of the tumor microenvironment and immune response continues to advance, small molecule inhibitors are poised to play a pivotal role in shaping the future of cancer immunotherapy.

4 Examples of small molecule inhibitors with immunomodulatory effects in cancer immunotherapy

Small molecule inhibitors are increasingly recognized for their role in cancer immunotherapy due to their ability to modulate immune responses and enhance anti-tumor activity. These inhibitors target specific proteins and pathways involved in cancer cell proliferation and survival, as well as the tumor microenvironment, which can suppress the immune system (39). By inhibiting these targets, small molecule inhibitors can restore or enhance the immune system's ability to recognize and destroy cancer cells.

Some well-known examples include Vemurafenib, which targets BRAF and modulates the tumor microenvironment to promote T cell infiltration in melanoma (40); Dasatinib, which targets multiple tyrosine kinases and enhances immune cell function in leukemia and solid tumors (41); and Ibrutinib, which inhibits BTK and modulates B-cell receptor signaling in B-cell malignancies (42). Other notable inhibitors, such as Sunitinib and Pazopanib, target multiple receptor tyrosine kinases and have shown efficacy in reducing regulatory T cells and myeloid-derived suppressor cells, thereby enhancing the overall immune response in various cancers (43, 44). These small molecule inhibitors, by targeting critical pathways involved in immune regulation and tumor growth, offer significant potential to improve the effectiveness of cancer immunotherapies and patient outcomes. In Table 1, selected examples of these small molecule inhibitors, which have been proven to exert excellent immunomodulatory effects, have been summarized with discussion of their action mechanisms.

These examples highlight the diverse mechanisms of action and clinical applications of small molecule inhibitors with immunomodulatory effects in cancer immunotherapy. By targeting

TABLE 1 Selected examples of small molecule inhibitors with immunomodulatory effects and their action mechanisms.

Small Molecule Inhibitor	Target	Mechanism of Action	Cancer Types	Refs
Vemurafenib (Zelboraf)	BRAF	Inhibits mutated BRAF protein; modulates tumor microenvironment and promotes T cell infiltration	Melanoma	(40)
Dasatinib (Sprycel)	BCR-ABL, SRC family kinases	Inhibits multiple tyrosine kinases; modulates immune cell function and enhances anti-tumor immune response	Leukemia, solid tumors	(41)
Imatinib (Gleevec)	BCR-ABL	Inhibits BCR-ABL tyrosine kinase; alters tumor microenvironment and influences immune response	Chronic myeloid leukemia (CML), GIST	(42)
Sunitinib (Sutent)	VEGFR, PDGFR, KIT	Multi-targeted receptor tyrosine kinase inhibitor; reduces Tregs and MDSCs, enhancing immune response	Renal cell carcinoma, gastrointestinal stromal tumors	(43)
Idelalisib (Zydelig)	PI3Kδ	Inhibits PI3Kδ; affects immune cell subsets and tumor microenvironment	B-cell malignancies (e.g., CLL, FL)	(44)
Ibrutinib (Imbruvica)	BTK	Inhibits BTK; modulates B-cell receptor signaling and immune cell function	B-cell malignancies (e.g., CLL, MCL)	(45)
Acalabrutinib (Calquence)	BTK	Inhibits BTK; similar to ibrutinib but with potentially fewer off-target effects	B-cell malignancies (e.g., CLL, MCL)	(46)
Cabozantinib (Cometriq)	VEGFR, MET, AXL	Inhibits multiple tyrosine kinases; reduces immunosuppressive cells and enhances anti-tumor immunity	Renal cell carcinoma, medullary thyroid cancer	(47)
Pazopanib (Votrient)	VEGFR, PDGFR, KIT	Inhibits multiple receptor tyrosine kinases; modulates immune cell infiltration and function	Renal cell carcinoma, soft tissue sarcoma	(48)
Sorafenib (Nexavar)	RAF, VEGFR, PDGFR	Multi-kinase inhibitor; affects tumor angiogenesis and immune cell function	Hepatocellular carcinoma, renal cell carcinoma	(49)
Crizotinib (Xalkori)	ALK, ROS1	Inhibits ALK and ROS1; modulates tumor microenvironment and immune response	Non-small cell lung cancer (NSCLC)	(50)
Ceritinib (Zykadia)	ALK	Inhibits ALK; similar to crizotinib but more potent	Non-small cell lung cancer (NSCLC)	(51)
Alectinib (Alecensa)	ALK	Inhibits ALK; effective in crizotinib-resistant cases	Non-small cell lung cancer (NSCLC)	(52)
Brigatinib (Alunbrig)	ALK, EGFR	Inhibits ALK and EGFR; modulates immune cell function and tumor microenvironment	Non-small cell lung cancer (NSCLC)	(53)
Nilotinib (Tasigna)	BCR-ABL	Inhibits BCR-ABL tyrosine kinase; affects immune responses and tumor microenvironment	Chronic myeloid leukemia (CML)	(54)
Erlotinib (Tarceva)	EGFR	Inhibits EGFR; modulates tumor cell growth and immune cell infiltration	Non-small cell lung cancer (NSCLC), pancreatic cancer	(55)
Gefitinib (Iressa)	EGFR	Inhibits EGFR; affects tumor cell proliferation and immune responses	Non-small cell lung cancer (NSCLC)	(56)
Afatinib (Gilotrif)	EGFR, HER2	Inhibits EGFR and HER2; modulates immune cell function and tumor microenvironment	Non-small cell lung cancer (NSCLC)	(57)
Osimertinib (Tagrisso)	EGFR	Inhibits EGFR T790M mutation; modulates immune responses	Non-small cell lung cancer (NSCLC)	(58)
Venetoclax (Venclexta)	BCL-2	Inhibits BCL-2; promotes apoptosis of cancer cells and influences immune responses	Chronic lymphocytic leukemia (CLL), AML	(59)
Selinexor (Xpovio)	XPO1	Inhibits nuclear export protein XPO1; modulates immune responses and tumor cell growth	Multiple myeloma, diffuse large B-cell lymphoma	(60)
Alpelisib (Piqray)	PI3Kα	Inhibits PI3Kα; affects tumor cell proliferation and immune cell function	Breast cancer	(61)
Trametinib (Mekinist)	MEK	Inhibits MEK; affects tumor cell signaling and immune cell infiltration	Melanoma	(62)
Cobimetinib (Cotellic)	MEK	Inhibits MEK; similar to trametinib, enhances immune cell function	Melanoma	(63)

(Continued)

TABLE 1 Continued

Small Molecule Inhibitor	Target	Mechanism of Action	Cancer Types	Refs
Dabrafenib (Tafinlar)	BRAF	Inhibits BRAF; similar to vemurafenib, affects immune cell function and tumor microenvironment	Melanoma	(64)
Everolimus (Afinitor)	mTOR	Inhibits mTOR; modulates immune responses and tumor cell proliferation	Renal cell carcinoma, breast cancer	(65)
Temsirolimus (Torisel)	mTOR	Inhibits mTOR; similar to everolimus, affects immune cell function	Renal cell carcinoma	(66)
Ruxolitinib (Jakafi)	JAK1, JAK2	Inhibits JAK1/2; modulates immune cell function and cytokine signaling	Myelofibrosis, polycythemia vera	(67)
Tofacitinib (Xeljanz)	JAK1, JAK3	Inhibits JAK1/3; affects immune cell signaling and function	Rheumatoid arthritis, being investigated for cancer	(68)
Abemaciclib (Verzenio)	CDK4, CDK6	Inhibits CDK4/6; affects cell cycle progression and modulates immune responses	Breast cancer	(69)

specific pathways involved in immune regulation and tumor growth, these inhibitors have the potential to enhance the efficacy of immunotherapy and improve outcomes for cancer patients.

5 Challenges and future directions in small molecule inhibitors as adjuvants in cancer immunotherapy

5.1 Resistance mechanisms

One of the challenges in using small molecule inhibitors as adjuvants in cancer immunotherapy is the development of resistance mechanisms by tumors (32). Tumors can acquire mutations or activate alternative signaling pathways to bypass the effects of these inhibitors. For example, resistance to BTK inhibitors like ibrutinib in B-cell malignancies often involves mutations in the BTK binding site or activation of PLCγ2 signaling (70). Future research should focus on understanding these resistance mechanisms and developing strategies to overcome them, such as combination therapies with other targeted agents or immunotherapies.

5.2 Specificity and off-target effects

Small molecule inhibitors may have off-target effects on normal cells, leading to toxicities and adverse effects (71). For instance, the multi-kinase inhibitor sunitinib has been associated with cardiotoxicity and hypertension due to its off-target effects on other kinases (72). Improving the specificity of these inhibitors to target tumor cells while sparing healthy tissues is crucial for minimizing toxicity and improving the safety profile of combination therapies.

5.3 Biomarker identification

Biomarkers that predict response to small molecule inhibitors as adjuvants in cancer immunotherapy are still evolving (73). Identifying reliable biomarkers to predict response to small molecule inhibitors as adjuvants in cancer immunotherapy is another unique challenge. For example, PD-L1 expression is a known biomarker for response to checkpoint inhibitors, but similar biomarkers for small molecule inhibitors are still being explored (74). Research efforts should prioritize the discovery and validation of biomarkers that can guide treatment selection and monitor response to therapy.

5.4 Optimal dosing and scheduling

Determining the optimal dosing and scheduling of small molecule inhibitors in combination with immunotherapy is essential for maximizing therapeutic efficacy while minimizing toxicity. This is particularly important for inhibitors that may have cumulative toxicities when used in combination regimens. Studies have shown that staggered dosing schedules can reduce toxicity and improve outcomes in combination therapies involving kinase inhibitors and immunotherapies (75).

5.5 Combination therapy strategies

Developing rational combination therapy strategies with small molecule inhibitors and immunotherapy agents is a complex and evolving field (30). For instance, combining VEGFR inhibitors with checkpoint inhibitors has shown promise in preclinical models, but optimal combinations and sequences need to be established through clinical trials (76). Future directions should explore novel combinations, target multiple pathways simultaneously, and

leverage advances in tumor immunology to enhance the anti-tumor immune response and overcome treatment resistance.

5.6 Translational research and clinical trials

Translating preclinical findings into clinical practice and conducting well-designed clinical trials are essential for evaluating the safety and efficacy of small molecule inhibitors as adjuvants in cancer immunotherapy (77). Future research directions should prioritize rigorous clinical testing and validation of promising combination therapies.

Overall, addressing these challenges and advancing research efforts in biomarker identification, treatment optimization, combination therapy strategies, and clinical trial design will be critical for harnessing the full potential of small molecule inhibitors as adjuvants in cancer immunotherapy and improving outcomes for cancer patients. Collaborative efforts between researchers, clinicians, and industry stakeholders will be essential for driving progress in this rapidly evolving field.

6 Conclusion

In conclusion, small molecule inhibitors have emerged as promising adjuvants in cancer immunotherapy, offering the potential to enhance the anti-tumor immune response and improve treatment outcomes for cancer patients. By targeting specific signaling pathways involved in tumor growth and immune evasion, these inhibitors can modulate the tumor microenvironment, sensitize tumors to immune-mediated destruction, and potentiate the effects of immunotherapy agents.

Despite the significant progress in the development and clinical use of small molecule inhibitors in cancer treatment, several challenges remain to be addressed. Resistance mechanisms, off-target effects, biomarker identification, optimal dosing and scheduling, as well as rational combination therapy strategies are important considerations that need to be carefully addressed in future research and clinical practice. Moving forward, future directions in small molecule inhibitors as adjuvants in cancer immunotherapy should focus on overcoming resistance mechanisms, improving specificity and safety profiles, identifying predictive biomarkers, optimizing treatment regimens, developing innovative combination therapies, and conducting robust translational research and clinical trials.

It is essential that collaborative efforts and multidisciplinary approaches be employed to advance the field of small molecule inhibitors in cancer immunotherapy. By addressing these challenges and pursuing innovative research strategies, we can harness the full potential of small molecule inhibitors to improve patient outcomes, enhance treatment response rates, and ultimately pave the way for more effective and personalized cancer therapies in the future.

Author contributions

HJ: Writing – original draft, Writing – review & editing. XW: Writing – original draft, Writing – review & editing. NF: Data curation, Writing – review & editing. CW: Data curation, Writing – review & editing. ZG: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NK cell based immunotherapy against oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC), a major subtype of head and neck cancers, presents significant challenges due to its aggressive feature and limited therapeutic efficacy of conventional treatments. In response to these challenges, Natural Killer (NK) cells, a vital component of the innate immune system, are being explored for their therapeutic potential in OSCC due to their inherent ability to target and eliminate cancer cells without prior sensitization. This review uniquely focuses on the evolving role of NK cells specifically in OSCC, incorporating recent advancements in CAR-NK cell engineering and personalized therapy approaches that have not been comprehensively covered in previous reviews. The mechanisms through which NK cells exert cytotoxic effects on tumor cells include direct killing through the engagement of natural cytotoxic receptors and antibody-dependent cellular cytotoxicity (ADCC), making them promising agents in cancer immunotherapy. Additionally, the article explores recent advancements in engineering NK cells to enhance their antitumor activity, such as the modification with chimeric antigen receptors (CARs) to target specific tumor antigens. Clinical implications of NK cell-based therapies, including the challenges of integrating these treatments with existing protocols and the potential for personalized therapy, are examined. The review highlights the promise of NK cell therapies in improving outcomes for OSCC patients and outlines future directions for research in this dynamic field of oncological immunotherapy.

KEYWORDS

oral squamous cell carcinoma, immunotherapy, natural killer cells, tumor microenvironment, CAR-NK

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Introduction

OSCC represents a common and formidable cancer in the head and neck area, marked by malignant growths arising from the squamous epithelium of the oral cavity (1–3). Occupying the sixteenth position worldwide in incidence and mortality rates, OSCC presents substantial public health challenges across various demographics (4). The oral cavity comprises multiple potential locales for these carcinomas' emergence, encompassing the jaw's mucosa, anterior tongue, posterior molars, mouth's floor, hard palate, and the inner surfaces of the lips (3, 5).

Research indicates that over 90% of oral cancers manifest as squamous cell carcinomas, which underscores the predominant cellular origin of these tumors (2, 6). The genesis of OSCC involves multiple factors, with principal risk elements being the consumption of tobacco products, both smoked and smokeless, and the presence of high-risk strains of human papillomavirus (HPV) (7, 8). Notably, tobacco usage is strongly correlated with the development of OSCC, exhibiting a dose-response relationship where increased tobacco exposure elevates the risk of this malignancy (9). Furthermore, the prevalence of OSCC is notably affected by age, with individuals over the age of 40 facing a heightened risk, thereby underlining age as a significant demographic risk factor (10, 11). The global incidence of OSCC varies, with higher rates observed in areas where tobacco usage is widespread and in regions where socio-economic conditions hinder timely diagnosis and treatment (12). Despite advancements in diagnostic and therapeutic techniques, the prognosis for OSCC remains relatively dismal, especially for cases identified in advanced stages (12). Prompt detection and an integrated approach to treatment, combining surgery, radiation therapy, and chemotherapy, are imperative for enhancing survival outcomes.

Traditional treatment modalities for oral squamous cell carcinoma carry inherent limitations (Table 1) (3). Surgical interventions can cause significant trauma, impacting both the functionality and aesthetic appearance of the oral and maxillofacial regions (13, 14). Moreover, the concealed nature of primary tumor development often results in the imprecise identification of positive margins during surgical resection, potentially leaving residual cancerous cells (13). Chemotherapy may lead to adverse effects such as hair loss, nausea, vomiting, and increased susceptibility to infections (3). Similarly, radiotherapy can inflict temporary or permanent damage to the healthy tissues surrounding the cancer cells, markedly diminishing the patient's quality of life (15, 16). Additionally, about one-third of patients continue to face the risks of recurrence and resistance to radiation and chemotherapy following these conventional treatments.

TABLE 1 Comparison of traditional treatment of OSCC.

Treatment	Advantages	Disadvantages
Surgery	Often completely removes the tumor; Immediate control the progress of OSCC; Can prevent further spread of cancer	Risk of significant side effects including disfigurement and loss of function; Requires hospitalization and recovery time; Not suitable for all stages or locations of tumors
Radiation Therapy	Non-invasive Can be targeted to minimize damage to surrounding tissues Effective for local control	Can cause long-term damage to surrounding healthy tissues; Side effects include dry mouth, sore throat, and potential for secondary cancers; Requires multiple sessions
Chemotherapy	Can target cancer cells throughout the body Can be combined with other treatments to improve outcomes	Systemic side effects including nausea, fatigue, hair loss; May not be effective against all cancer cells; Risk of developing resistance to drugs

Recent advancements have focused on enhancing the five-year survival rate of OSCC, which has improved from 59% to 70% over the period from 1990 to 2011 (5, 17). Nevertheless, survival outcomes are still significantly influenced by factors such as the stage of the tumor at diagnosis, its anatomical location, and the presence of regional or distant metastases (18–20). The management of OSCC is further complicated by its high rate of recurrence and the potential for developing secondary primary tumors, necessitating a comprehensive, multidisciplinary approach and continuous monitoring (6, 15, 21). A profound understanding of OSCC's biological behavior and the molecular and cellular mechanisms underlying its development is crucial for the creation of targeted therapies that enhance clinical results (22, 23).

The aim of this review is to comprehensively explore and highlight the therapeutic potential of NK cells in the treatment of OSCC. The review will focus on the unique abilities of NK cells to target and eliminate cancer cells, particularly in the context of OSCC, and will cover recent advancements in NK cell engineering, such as the development of Chimeric Antigen Receptor NK (CAR-NK) cells. Thus, we conducted a comprehensive search in PubMed using the keywords "natural killer cells and Oral Squamous Cell Carcinoma," covering all relevant studies published until July 2024. This search aimed to gather the latest research findings and developments in the role of natural killer (NK) cells in the context of Oral Squamous Cell Carcinoma (OSCC). It will also examine the integration of NK cell-based therapies with other treatment modalities, the challenges posed by the tumor microenvironment, and the future directions for research to enhance the clinical outcomes of NK cell therapies in OSCC.

The OSCC tumor cells interactive with NK cells

The crosstalk between OSCC tumor cells and NK cells is pivotal for patient outcomes and disease progression (24). Since immune cells form the cellular foundation of immunotherapy, a profound understanding of immune infiltration within TME is essential to unravel the underlying molecular mechanisms and develop novel immunotherapeutic strategies to enhance clinical outcomes (25). Shao P et al. identified NK cell-associated genes, including SSNA1, TRIR, PAXX, DPP7, WDR34, EZR, PHLDA1, and ELOVL1, by quantifying NK cells and exploring their single-cell expression patterns in the HNSCC microenvironment. Their findings

indicated that patients with high EZR expression might have a poor prognosis and worse clinical features. John S et al. conducted an immunohistochemical study to evaluate the distribution of cytotoxic T lymphocytes and natural killer cells in OSCC and oral epithelial dysplasia (OED) (26). The study aimed to assess the expression of CD8 and CD57 immune cells in OSCC, OED, and normal oral mucosa. OED with moderate or severe dysplasia and OSCC samples had higher levels of infiltrating immune cells, including T cells, B cells, NK cells, and macrophages, compared to normal mucosa. The results indicated that CD8 and CD57 expression increased from normal mucosa to OED, with the highest expression in OSCC. CD8 and CD57 could serve as surrogate markers to assess the malignant potential of lesions and determine the prognosis of patients with oral cancer (26).

Zhu et al. delves into the effects of oral cancer cell-derived exosomes (OcEXs) on the activity of NK cells, particularly their influence on NK cell receptors' expression and functionality. Exosomes were extracted from oral cancer cell lines WSU-HN4 and SCC-9 via ultrafiltration, and their protein contents were analyzed using mass spectrometry, which identified a high concentration of transforming growth factor (TGF)-β1 (27, 28). Initial interactions with OcEXs resulted in the upregulation of activating receptors (NKG2D and NKp30) and downregulation of the inhibitory receptor (NKG2A) in NK cells, suggesting an enhancement in NK cell cytotoxicity. However, this expression waned over seven days, hinting at a potential induction of NK cell dysfunction over time. The cytotoxic capabilities of NK cells against oral cancer cells initially increased but declined following prolonged exposure to OcEXs. These findings underscore that while oral cancer-derived exosomes can temporarily boost NK cell activity, extended exposure leads to diminished cytotoxicity and functional impairment of NK cells. This investigation provides critical insights into the intricate interactions between cancer-derived exosomes and NK cells, presenting promising directions for advancing immunotherapy approaches in oral cancer (27).

Similarly, another study examines the influence of OCEXs on NK cell functions via the IRF-3 signaling pathway (29). This study provides profound insights into how exosomes from oral cancer cells can augment the cytotoxic capabilities of NK cells, an essential component of immune surveillance against tumors. Upon internalization by NK cells, the OCEXs facilitated increased NK cell proliferation and enhanced the release of cytotoxic molecules such as perforin and granzyme M, indicating a stimulatory effect on NK cells. The study identified NAP1, a protein highly concentrated in OCEXs, as pivotal in activating the IRF-3 pathway in NK cells. This activation bolstered the expression of IFN genes and chemokines, thereby enhancing NK cell functions. This mechanism not only deepens our understanding of cellular interactions within the tumor microenvironment but also indicates potential therapeutic targets for boosting NK cell activity against oral cancer (10). Yu X et al. investigated NUP62CL as an immunological and prognostic biomarker for OSCC (30). Tumor tissue samples from 319 OSCC patients, along with their clinical information, were retrospectively collected. The study identified high NUP62CL expression in OSCC tissues, which was associated with larger tumor size, advanced clinical stage, and poor prognosis. Additionally, NUP62CL protein expression was positively correlated with the abundance of $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $CD56^+$ NK cells, $CD68^+CD86^+$ macrophages, and $CD68^+CD163^+$ macrophages, as well as immune checkpoints, including PD-1, PD-L1, and CTLA-4 protein expression. NUP62CL could serve as an effective prognostic and immunological biomarker for OSCC patients (30).

Immunotherapy for OSCC

The expanding comprehension of TME in the context of immunotherapy for OSCC lays a vital groundwork for refining treatment modalities (1, 22). Particularly, the strategic modulation of NK cells within the TME uncovers promising avenues to boost the efficacy of immunotherapeutic interventions (31–33). Building upon these insights, the scope of immunotherapy for OSCC is broadening to encompass not only strategies centered on NK cells but also a diverse array of other immunotherapeutic agents (34). These advances strive to exploit the intricate interactions within the TME to enhance the precision and effectiveness of targeting and eradicating cancer cells (35). This evolving landscape offers renewed optimism for the development of more potent and tailored treatment options for patients with OSCC (36–38).

In the field of immunotherapy for OSCC, the TME is crucial, particularly affecting the efficacy of therapies that utilize NK cells (32, 34). Composed of a diverse assembly of cells, extracellular matrix elements, and signaling molecules, the TME frequently manifests an immunosuppressive influence that can impair the functionality of NK cells (24, 39). Nonetheless, the inherent capacity of NK cells to identify and annihilate malignant cells without the need for prior sensitization positions them as potent agents in the fight against OSCC (40). Recent breakthroughs in comprehending the dynamics between NK cells and the TME have catalyzed the formulation of approaches to amplify NK cell activity (35, 40). These include obstructing inhibitory signals within the TME and engineering NK cells to bear chimeric antigen receptors (CARs) (22, 24, 41). Such enhancements are designed to augment the natural cytotoxic abilities of NK cells, thus bolstering their capacity to effectively target and eliminate tumor cells in the formidable milieu of OSCC.

The immune system plays a pivotal role in combating cancer, involving both innate and adaptive immune responses. B and T cells are integral to adaptive immunity, while macrophages, eosinophils, NK cells, and dendritic cells (DCs) constitute the components of innate immunity (42–44). Cancer cells manipulate their surface antigen expression and suppress immune factor secretion, thereby evading and inhibiting immune-mediated destruction, fostering tumor progression (45). Advances in the understanding of NK cells, coupled with developments in immunology and genetic engineering technologies, have positioned NK cells as primary agents in cancer therapy (41). NK cells are increasingly recognized for their unique immunological responses, becoming pivotal figures in tumor immunotherapy (24). The development of OSCC is intricately linked to the immune microenvironment, making immunotherapy an increasingly utilized approach in this

context (46). To elucidate the significance and potential of NK cell immunotherapy in the treatment of OSCC can provide a reference for its clinical application (47, 48).

Caruntu A et al. studied the persistent changes in peripheral blood lymphocyte subsets in patients with OSCC (49). They assessed the proportions of $CD3^+$ total T lymphocytes, $CD3^+CD4^+$ helper T lymphocytes, $CD3^+CD8^+$ suppressor/cytotoxic T lymphocytes, $CD3^-CD19^+$ total B lymphocytes, and $CD3^-CD16^+CD56^+$ NK cells in the peripheral blood of OSCC patients. The data, collected both pre- and post-therapy, indicated that the level of total $CD3^+$ T lymphocytes in OSCC patients remained similar to that of control subjects, highlighting the stability of this immune parameter. However, pre-therapeutic data revealed a lower proportion of $CD4^+$T, a significantly higher level of cytotoxic/suppressive $CD8^+$T, and a much lower $CD4^+$/CD8 T lymphocyte ratio compared to controls. In contrast, circulating NK $CD16^+$ cells were markedly higher pre-therapy compared to the control group. These findings provide new insights into the immune alterations in the peripheral blood of OSCC patients, contributing to the understanding of the complex interplay between immuno-inflammatory processes and carcinogenesis (32, 49). While, Santos EM et al. evaluated the defense mechanisms of $CD8^+$ and NK cells in oral and oropharyngeal squamous cell carcinoma (OSCC and OPSCC) (50). Fifty-four cases of squamous cell carcinoma (42 OSCC and 12 OPSCC) were treated immunohistochemically with CD8 and CD57 monoclonal antibodies. The study examined the relationship of CD8+ and NK cells with tumor size, lymph node metastasis (LNM), clinical staging (CS), overall survival (OS), and disease-free survival (DFS). Results showed that CD8 expression was higher in T1 and T2 tumors compared to T3 and T4 tumors, and in tumors without LNM and with CS II or III. However, there was no association between the biomarkers and OS or DFS. These findings suggest that the differential infiltration of CD8+ cells in OSCC and OPSCC may reflect a distinct tumor microenvironment with a favorable local cytotoxic immune response against neoplastic cells (50–52).

NK cell activity is dependent on the balance between activating and inhibitory receptors on their surface

NK cells, a fundamental subset of innate lymphoid cells, originate in the bone marrow and reach maturity in secondary lymphoid tissues like the spleen, tonsils, and lymph nodes (53, 54). Distinguished from T cells by their lack of CD3 expression, NK cells are characterized by the presence of CD56 and CD16, which serve as definitive markers of their lineage (55). As pivotal agents in the immune system's frontline defense, NK cells activate without prior sensitization, playing an essential role in protecting the body against pathogens and malignancies, notably against virally infected cells and tumors.

The primary activating receptors on NK cells include CD16, NKG2D, DNAM-1, and the natural cytotoxicity receptors (NCRs) (56, 57). These are counterbalanced by inhibitory receptors such as CD94, NKG2A, and a variety of killer immunoglobulin-like receptors (KIRs) (58). Additionally, NK cell functionality is modulated by checkpoint inhibitors like PD1, TIGIT, LAG3, and TIM3, which can dampen NK cell activity (41, 59, 60). These checkpoint inhibitors represent both a challenge and a critical target for therapeutic interventions. The efficacy of NK cells hinges on the intricate dynamics between these inhibitory and activating signals (61, 62). Harnessing these interactions is crucial for the advancement of NK cell-based immunotherapeutic strategies, particularly in the context of OSCC (46, 63). Enhancing NK cell activity in OSCC could significantly improve cancer management and patient outcomes, leveraging the potent capabilities of these immune cells to combat malignancy effectively (64) (Figure 1).

The tumoricidal mechanism of NK cells against OSCC tumor cells

NK cells utilize a diverse array of mechanisms to eradicate tumor cells, playing a crucial role in the immune system's defense against malignancies such as OSCC (65). One fundamental strategy is the "Missing-self" recognition, where NK cells target and destroy tumor cells that lack MHC-I molecules (66). This is accomplished through the release of cytotoxic molecules like granzymes and perforin (67). Upon forming an immune synapse with a target cell, NK cells orchestrate the reorganization of the actin cytoskeleton, facilitating the expulsion of perforin and granzymes (68). Perforin creates pores in the target cell membrane, enabling granzymes to penetrate and initiate apoptosis by cleaving cellular substrates (69). Additionally, NK cells can induce cell death via surface molecules such as Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), which bind to their respective receptors on tumor cells, fostering apoptosis (55, 70). This not only aids in eliminating cancer cells but also promotes an inflammatory response that can attract further immune cells to the tumor site (71). Moreover, NK cells can trigger pyroptosis, a highly inflammatory form of cell death, through the activation of caspase 3 and gasdermin E, thus enhancing the immune clearance of tumor cells.

Another critical cytotoxic mechanism employed by NK cells is ADCC (72, 73). This process is facilitated by the high-affinity Fc receptor CD16 on NK cells, which interacts with antibodies bound to tumor cell antigens. The engagement of CD16 triggers the release of cytotoxic granules and pro-inflammatory cytokines, directly leading to the destruction of the target cells. ADCC is particularly significant in the context of cancer therapy, where monoclonal antibodies are designed to target specific tumor antigens (74). For instance, drugs like trastuzumab and rituximab target cancer cells for destruction via ADCC, with NK cells playing an essential role. Enhancing ADCC, whether by increasing CD16 expression or by

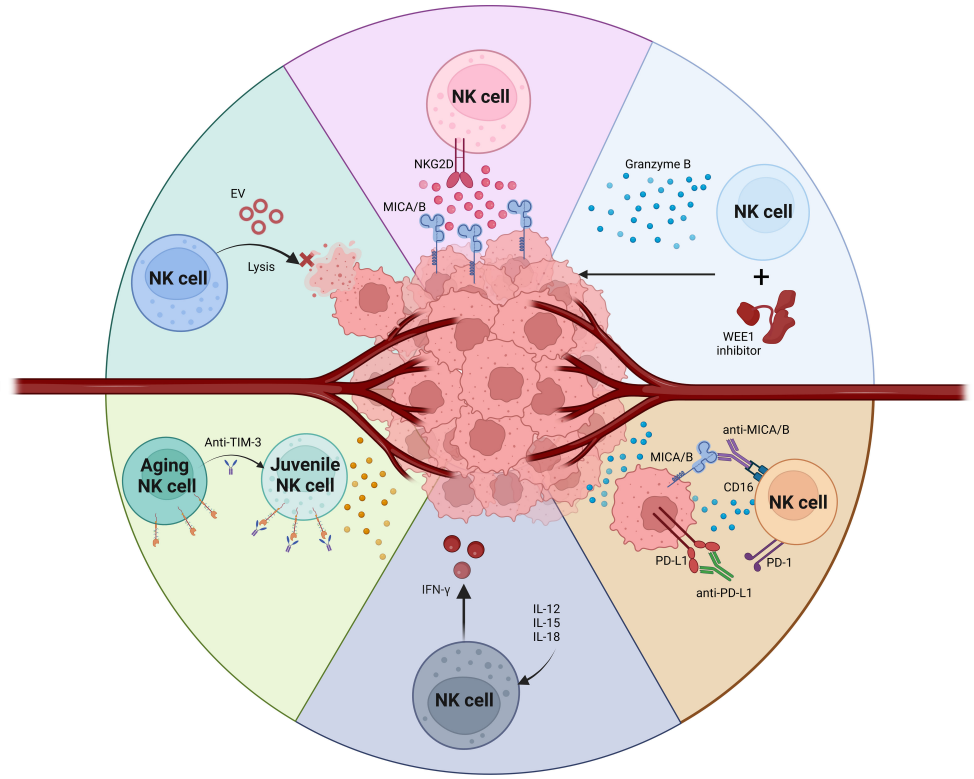

FIGURE 1
NK cells based therapy for OSCC tumor.

improving NK cell affinity for antibodies, presents a promising avenue in cancer immunotherapy (75). By optimizing the natural capabilities of NK cells through ADCC, novel therapeutic strategies can be developed to improve the precision and effectiveness of tumor cell elimination, potentially enhancing outcomes in cancer treatment.

NK cells related immunotherapy against oral squamous cell carcinoma

The research conducted by Gupta et al. explores the role of NK cells in monitoring and controlling OSCC, with a specific focus on the prognostic significance of histomorphological features (64). This study highlights the correlation between the presence of CD57 immunopositive NK cells and various markers of OSCC progression, including tumor budding, the size of tumor cell nests, and the lymphocytic response from the host. It demonstrates that increased NK cell activity is associated with favorable prognostic characteristics in OSCC, suggesting that the profiling of NK cells could inform therapeutic approaches and potentially act as indicators of OSCC progression. The findings advocate for the potential benefits of modulating NK cell activity to boost anti-tumor immune responses in OSCC, reinforcing the therapeutic promise of targeting these immune cells to enhance cancer treatment outcomes.

In oral cancer, circulating tumor cells (CTCs) deploy mechanisms that enable them to escape destruction by NK cells (76). A critical strategy involves the upregulation of the protein N-cadherin in self-seeded CTCs, which enhances their ability to evade immune detection. This upregulation induces functional exhaustion in NK cells through interactions with the KLRG1 receptor on NK cells. Self-seeded tumor cells, a subset of CTCs capable of returning to and proliferating within the primary tumors, exhibit elevated levels of N-cadherin. The soluble N-cadherin released from these cells engages the KLRG1 receptor on NK cells, leading to a state of exhaustion marked by diminished cytotoxicity and cytokine production. This interaction between N-cadherin and KLRG1 impairs NK cell functions, facilitating the circulation and seeding of new tumors by the tumor cells. Overexpression of N-cadherin in tumor cells not only increases their evasion from NK cell-mediated destruction but also enhances their seeding efficiency and potential for metastasis. Given these dynamics, targeting the N-cadherin/KLRG1 interaction emerges as a promising strategy to augment NK cell activity against oral cancer CTCs. By blocking N-cadherin or disrupting its interaction with KLRG1, it may be possible to restore NK cell functionality, potentially curtailing tumor metastasis and recurrence. This approach underscores the importance of understanding and manipulating key molecular interactions in the immune evasion strategies of tumor cells to develop more effective cancer therapies.

Researchers have discovered that these dysplastic cells frequently demonstrate aberrant activation of the Wnt/β-catenin pathway, primarily due to the overexpression of Wnt ligands that are dependent on Porcupine (PORCN) (77).The inhibition of

PORCN, a key enzyme in the secretion of Wnt ligands, plays a critical role in the treatment strategy for OSCC, which often originates from potentially malignant lesions such as oral dysplasia. By pharmacologically targeting PORCN, the secretion of Wnt ligands is effectively inhibited, thereby reducing the activity of the Wnt/β-catenin pathway. The findings from this research suggest that targeting PORCN not only disrupts this critical signaling pathway but also significantly reduces the progression from oral dysplasia to OSCC. This points to a promising therapeutic strategy that aims to prevent the development of OSCC by intervening early in the cellular alterations within the oral cavity.

Despite advancements with immune checkpoint inhibitors (ICI) across various cancers, there is a pressing need for new strategies to broaden treatment efficacy, particularly for patients who do not develop effective antitumor T-cell responses. Radiation and pharmacological treatments can significantly alter the tumor immune microenvironment, prompting investigations into synergistic immunotherapeutic approaches. Patin EC et al. explored the enhancement of antitumor responses through the combined application of ataxia telangiectasia and Rad3-related kinase inhibition (ATRi) and radiotherapy (RT) (78). Utilizing the HPV-negative murine oral squamous cell carcinoma model, MOC2, we assessed the nature of the antitumor response post-ATRi/RT treatment through RNA sequencing and detailed flow cytometry analyses. The potential benefits of immunotherapies, particularly those targeting the T cell immunoreceptor with Ig and ITIM domains (TIGIT) and Programmed cell death protein 1 (PD-1) following ATRi/RT, were evaluated in the MOC2 model and corroborated in another model, SCC7. The results highlight that ATRi amplifies the inflammation induced by radiotherapy within the tumor microenvironment, with NK cells playing a pivotal role in enhancing treatment outcomes. It was demonstrated that the antitumor efficacy of NK cells could be significantly increased with ICI targeting TIGIT and PD-1. Analyses of clinical samples from patients receiving ATRi (ceralasertib) validate the translational potential of these preclinical findings (79). This study uncovers a previously unrecognized role of NK cells in the antitumor immune response to radiotherapy, which can be further augmented by leveraging small-molecule DNA damage-response inhibitors alongside immune checkpoint blockade, offering a novel avenue to enhance cancer therapy efficacy.

CAR-NK cells for OSCC

Chimeric Antigen Receptor (CAR) NK cells refer to NK cells that have been genetically modified to express chimeric antigen receptors (CARs) on their surfaces (80, 81). CARs are synthetic proteins that consist of an extracellular antigen-binding domain, usually derived from a single-chain variable fragment (scFv) of an antibody, linked to an intracellular signaling domain of a T-cell receptor complex protein, such as CD3ζ. By introducing CARs into NK cells, researchers can redirect the specificity and activity of these cells to target specific antigens on OSCC tumor cells or other diseased cells. This approach has the potential to enhance the therapeutic efficacy of NK cells by making them more selective and potent against cancer or other diseases (81, 82). CAR-NK cells are currently being investigated as a potential treatment for various types of cancer and other diseases. They offer the advantage of being able to recognize and kill tumor cells directly, without the need for prior activation or antigen presentation by other immune cells (83). However, further research is needed to optimize the design and function of CAR-NK cells and to understand their safety and efficacy in clinical trials.

Jacobs MT et al. explored how memory-like differentiation, tumor-targeting monoclonal antibodies (mAbs), and chimeric antigen receptors (CARs) can enhance natural killer (NK) cell responses to head and neck squamous cell carcinoma (HNSCC) (84). To address this, the study hypothesized that memory-like (ML) NK cell differentiation, tumor targeting with cetuximab, and engineering with an anti-EphA2 CAR could improve NK cell responses against HNSCC. In this study, ML NK and conventional (cNK) cells from healthy donors were used. Cytokine production IFNγ, TNF, degranulate, and kill HNSCC cell lines and primary HNSCC cells were compated, both alone and in combination with cetuximab, *in vitro* and *in vivo* using xenograft models. Additionally, they engineered ML and cNK cells to express anti-EphA2 CAR-CD8A-41BB-CD3z and assessed their functional responses against HNSCC cell lines and primary tumor cells. Human ML NK cells exhibited enhanced production of IFNγ and TNF, as well as improved short- and long-term killing of HNSCC cell lines and primary targets compared to cNK cells. These responses were further enhanced by cetuximab. ML NK cells expressing anti-EphA2 CAR showed increased IFNγ production and cytotoxicity against EphA2+ cell lines and primary HNSCC targets compared to controls. These preclinical findings indicate that ML differentiation alone or combined with cetuximab-directed targeting or EphA2 CAR engineering can be effective against HNSCC. The results provide a strong rationale for investigating these combination approaches in early-phase clinical trials for patients with HNSCC (84, 85).

CAR-NK cells offer significant advantages in the treatment of OSCC, capitalizing on their derivation from a variety of sources such as peripheral blood, umbilical cord blood, and induced pluripotent stem cells (44, 53). This versatility and the potential for mass production address some of the challenges faced with patient-derived therapies. Notably, CAR-NK cells exhibit a safer risk profile, largely free from the severe toxicities often associated with CAR-T cell therapies, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) (86). Additionally, they do not provoke graft-versus-host disease (GVHD), rendering them suitable for allogeneic use in clinical applications.

The capability of CAR-NK cells to effectively target and eliminate tumor cells, while navigating through and often overcoming the inhibitory mechanisms of the tumor microenvironment, positions them as a promising avenue for advancing treatment strategies in OSCC (41, 87, 88). Their lower incidence of severe toxicities and the absence of GVHD further underscore their potential as a transformative approach in oncology, offering a potent, scalable, and safer alternative to traditional CAR-T cell therapies (Table 2).

Utilizing CAR natural killer (CAR-NK) cells derived from induced pluripotent stem cells (iPSCs) and directed against MUC1, a protein

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TABLE 2 Comparison of CAR-NK cells and CAR-T cells against OSCC.

Feature	CAR-NK Cells	CAR-T Cells	References
Source	Derived from peripheral blood, umbilical cord blood, Stem cell differentiation or cell lines	Derived from patient's own T cells (autologous) or donor T cells (allogeneic)	(53, 89, 90)
Activation and Expansion	Can be activated and expanded ex vivo with cytokines (e.g., IL-2, IL-15)	Activated and expanded ex vivo using anti-CD3/CD28 beads and cytokines (e.g., IL-2)	(89, 91, 92)
Killing Mechanism	Direct cytotoxic effects through release of perforin and granzyme, and antibody-dependent cellular cytotoxicity (ADCC)	Direct cytotoxic effects through release of perforin and granzyme, and induction of apoptosis in target cells	(56, 89)
Advantages	Lower risk of cytokine release syndrome (CRS) and graft-versus-host disease (GVHD); Can target a broad range of tumors; Multiple NK cells sources.	Highly specific to target antigens; Proven efficacy in hematologic malignancies; Long-lasting persistence and memory formation.	(55, 89, 93, 94)
Disadvantages	Shorter lifespan and persistence *in vivo*; Potential for limited efficacy in solid tumors due to tumor microenvironment	Higher risk of CRS and GVHD; Complex and costly manufacturing process Potential for severe toxicities	(81, 89, 95, 96)
Current Research Status	Still in experimental stages, with ongoing research to optimize and validate their use in clinical settings.	Several FDA-approved therapies for hematologic cancers, with ongoing trials and research for efficacy in solid tumors, including OSCC	(83, 89, 97)

commonly overexpressed in OTSCC (98). The efficacy of iPSC-derived MUC1-targeted CAR-NK cells was rigorously tested both *in vitro* and *in vivo*. MUC1 expression in OTSCC tissues and cell lines was verified through immunohistochemical and immunofluorescence analyses. Subsequent assessments demonstrated that these engineered NK cells could effectively target and annihilate MUC1-expressing cancer cells, exhibiting significantly enhanced cytotoxicity compared to iPSC-derived NK cells without the CAR modification. In a BNDG mouse xenograft model, the MUC1-targeted CAR-NK cells markedly curtailed tumor growth without causing substantial weight loss or hematological toxicity, indicating a favorable safety profile. The study suggests that MUC1-targeted CAR-NK cell therapy holds considerable promise as a new treatment modality for OTSCC, potentially offering higher efficacy and reduced toxicity compared to existing options. These findings advocate for the advancement to clinical trials to more comprehensively evaluate the effectiveness and safety of this innovative therapy in human subjects. This research not only underscores the potential of CAR-NK cell technology in treating solid tumors but also represents a significant stride forward in developing more effective therapies for patients with ad.

Potential immuno-targets for NK cells related immunotherapy over OSCC

In the evolving landscape of immunotherapy for OSCC, identifying potential immune targets for NK cells presents a promising avenue for enhancing treatment efficacy (7). Among the key targets are the tumor associated antigens, like EGFR, which has been broadly used in clinical (99). Additional, stress-induced ligands MICA and MICB, which are recognized by the activating receptor NKG2D on NK cells. Overexpression of these ligands on OSCC cells can markedly enhance NK cell-mediated cytotoxicity. Furthermore, the blockade or modulation of inhibitory receptors such as PD1, TIGIT, TIM3, KIRs (Killer-cell Immunoglobulin-like Receptors) on NK cells, which interact with MHC class I molecules on tumor cells (100, 101). These molecules can restain NK cells via delivery immunosuppressive signals (53, 91). Exploring these targets within the tumor microenvironment of OSCC can lead to the development of targeted therapies that activate or enhance the innate cytotoxic responses of NK cells, offering a robust strategy to combat this challenging malignancy.

Utilizing a cytobrush providing a less traumatic alternative to traditional biopsies and can be executed without the need for specialized medical facilities. The samples are then analyzed using an advanced ELISA method, noted for its high sensitivity and specificity, facilitating the detection of specific biomarkers critical for early diagnosis of OSCC (102). The study targeted six biomarkers, including well-established ones like EGFR, p53, and Ki67 used in clinical diagnostics, alongside newer markers such as PD-L1, HLA-E, and B7-H6, which are pertinent to the tumor microenvironment and mechanisms of immune evasion. Implementing this novel diagnostic approach could become a pivotal tool for screening and early diagnosis, potentially decreasing the morbidity and mortality associated with OSCC. Its non-invasive nature, coupled with rapid processing times, positions it as an excellent option for routine monitoring and early intervention. This innovation marks a significant stride towards transforming current practices in the detection and management of oral cancer, making early diagnostics more accessible and effective.

The plasma levels of CASC15 are elevated in patients with stage I and II OSCC compared to those with oral ulcers and healthy controls, with no significant differences observed between the latter two groups (103). This upregulation of CASC15 effectively distinguishes OSCC patients from those with oral ulcers and healthy individuals. Further investigation revealed an inverse correlation between CASC15 and another LncRNA, MEG3, within OSCC tissues. Specifically, overexpression of CASC15 in

OSCC cells led to a suppression of MEG3 expression, while overexpression of MEG3 did not affect CASC15 levels. The study suggests that CASC15 promotes the proliferation of OSCC cells by negatively regulating MEG3. These insights underscore the importance of CASC15 and MEG3 in the pathology of OSCC and highlight their potential as targets for therapeutic intervention (103).

Epidermal growth factor receptor

The study investigates the impact of cold atmospheric pressure plasma (CAP)-induced radicals on the epidermal growth factor receptor (EGFR), which is notably overexpressed in OSCC, aiming to understand the mechanism behind the selective cytotoxicity observed (104, 105). CAP treatment generates highly reactive radicals within both the plasma plume and the cell culture media. This results in a distinct selective killing effect on OSCC cells compared to normal human gingival fibroblasts. The selective cytotoxicity is specifically observed in OSCC cells that overexpress EGFR, where degradation and dysfunction of EGFR occur (105). This effect is absent in normal cells, highlighting the targeted action of CAP. Furthermore, the introduction of a nitric oxide scavenger prior to CAP treatment in the cell culture effectively mitigates the degradation and dysfunction of EGFR, as well as the associated cytotoxicity in OSCC cells (106). This evidence suggests that CAP could serve as a promising cancer treatment strategy by specifically inducing dysfunction in EGFR through nitric oxide radicals in OSCC cells that overexpress this receptor. This targeted approach offers potential for developing treatments that spare normal cells while effectively combating cancer cells, thereby improving therapeutic outcomes in oral squamous cell carcinoma (105).

The therapeutic efficacy of cetuximab in treating OSCC is significantly attributed to its ability to activate NK cells, thereby inducing ADCC and promoting cytokine secretion (107). Specifically, cetuximab-activated NK cells enhance dendritic cell (DC) maturation through the secretion of interferon-gamma (IFN-γ) (108). This process increases the cross-presentation of tumor antigens to CD8+ T cells, leading to the expansion of EGFR-specific T cells and bolstering the immune response against tumor cells (99). Moreover, this interaction between NK cells and DCs is characterized by bidirectional crosstalk, wherein increased DC expression further stimulates NK cell activation. This synergistic relationship enhances the overall immune response within the tumor microenvironment (109, 110). Research is ongoing to explore combinations of cetuximab with various drugs, such as IL-12, lenalidomide, monalizumab (an anti-NKG2A antibody), and the CD137/4-1BB agonist urea, to further augment the efficacy of cetuximab by boosting NK cell activation and the ADCC effect (108, 111, 112). These combinations aim to optimize cetuximab's therapeutic potential in OSCC. Additionally, cetuximab treatment has been linked to increased expression of CTLA-4, TIM-3, and TGF-β on intratumoral regulatory T cells (Tregs) (113, 114). These changes may also play a role in NK-mediated DC maturation, contributing to a more robust immunological attack on tumor cells. This multifaceted impact underscores the complex interplay between various components of the immune system in cetuximab's mechanism of action against OSCC, highlighting potential targets for enhancing treatment efficacy (115).

Boosting NKG2D activation signal for NK cells

HNSCC tumors tend to shed NKG2D ligands, leading to suppression of NK cells. Counteracting the effects of NKG2D ligand shedding to enhance NK cell activity includes apheresis of peripheral blood ligands (116), and antibody-mediated inhibition of MIC cleavage (117, 118). These studies highlight the significant role of the natural killer group 2D (NKG2D) receptors on NK cells and certain T cell subsets in the immunosurveillance of head and neck squamous cell carcinoma (HNSCC). These receptors are targeted by cancer evasion strategies, notably through the shedding of NKG2D ligands (NKG2DLs). Analysis of plasma and tumor samples from 44 HNSCC patients revealed that high levels of NKG2DLs in the plasma correlate with NK cell inhibition and disease progression. This finding was further substantiated by observations that NK cells are unable to infiltrate HNSCC tumors with high NKG2DL levels, suggesting a novel NKG2DL-dependent mechanism for tumor immune escape (119, 120). Moreover, the study also explores the potential of monitoring plasma NKG2DL levels for diagnostic and prognostic purposes, identifying patients who might benefit from therapies aimed at restoring NKG2D-dependent tumor immunosurveillance (121). Furthermore, experimental interventions in the study demonstrated that removing shed NKG2DLs (sNKG2DLs) from the plasma could restore NK cell function *in vitro* and enhance patient outcomes post-surgery. A proof-of-concept study involving adsorption apheresis to remove sNKG2DLs from plasma in rhesus monkeys was successful, suggesting this method could be a promising preconditioning strategy to boost the effectiveness of autologous and adoptive cellular cancer immunotherapies.

In another study, the role of CHMP2A in regulating tumor resistance to NK cell-mediated cytotoxicity was explored using a sophisticated "two cell type" whole-genome CRISPR-Cas9 screening system focused on human glioblastoma stem cells (GSC) and OSCC. The research identified CHMP2A as a pivotal regulator of GSC resistance to NK cell attacks, and these findings were further validated in a OSCC model (122). The investigation revealed that CHMP2A deletion in tumor cells activates the NF-κB pathway, which in turn enhances the secretion of chemokines. This increased chemokine production significantly boosts NK cell migration towards the tumor cells, thereby enhancing the immune response against the tumor. In the OSCC context, specifically within the CAL27 tumor model, it was demonstrated that CHMP2A mediates tumor resistance through a different mechanism-by the secretion of extracellular vesicles (EVs). These vesicles carry MICA/B and TRAIL, ligands known to induce apoptosis in NK cells, thus effectively reducing NK cell viability and inhibiting their anti-tumor functions. To substantiate these *in vitro* results, the study also included *in vivo* experiments where CHMP2A was deleted in CAL27 OSCC cells. This modification led to significantly increased NK cell-mediated killing in a xenograft model using immunodeficient mice, confirming the crucial role of CHMP2A in modulating tumor sensitivity to NK cell cytotoxicity.

These findings highlight a complex mechanism of tumor immune escape facilitated by CHMP2A through the secretion of EVs that impair NK cell function. This study not only sheds light on the intricacies of tumor-immune system interactions but also identifies CHMP2A as a promising target for enhancing the efficacy of NK cell-based immunotherapies (122).

Using an immunocompetent mouse model and the syngeneic 4MOSC head and neck squamous cell carcinoma model, CHMP2A was knocked out (KO) via CRISPR/Cas9 in 4MOSC1 cells. These modified cells were then transplanted into immunocompetent hosts. The CHMP2A KO in 4MOSC1 cells enhanced NK cell-mediated tumor cell killing *in vitro*. Following transplantation, $CHMP2A^{KO}$ in 4MOSC1 cells improved both T cell and NK cell antitumor activity compared to wild type tumors. There was no difference in tumor development between WT and CHMP2A KO tumors in immunodeficient mice. Mechanistically, CHMP2A KO tumors in immunocompetent mice showed increased $CD4^+$ and $CD8^+$ T cells, NK cells, and fewer myeloid-derived suppressor cells (MDSCs) (123). Thus, inhibiting CHMP2A and related pathways, it may be possible to counteract tumor resistance mechanisms and improve the therapeutic outcomes for patients suffering from various forms of cancer, including glioblastoma and head and neck squamous cell carcinoma (124).

Immune checkpoint blockade therapy

Immune checkpoint blockade therapy is a form of cancer immunotherapy that enhances the immune system's ability to fight cancer by inhibiting the checkpoints that regulate immune responses (125). These checkpoints are often exploited by cancer cells to avoid being attacked by the immune system. Common targets of checkpoint inhibitors include the PD-1/PD-L1 and CTLA-4 pathways, which are crucial in maintaining immune homeostasis and preventing autoimmunity (125). By blocking these pathways, checkpoint inhibitors unleash the potential of T cells or NK cells to effectively recognize and destroy cancer cells (126). This approach has revolutionized the treatment of various cancers, including melanoma, lung cancer, and renal cell carcinoma, offering significant improvements in patient outcomes (127, 128). Despite its success, the therapy can also lead to immune-related adverse effects due to increased immune activity, necessitating careful management and monitoring.

CD38, a member of the ribosyl cyclase family, is expressed on various hematological cells and is known to contribute to immunosuppression and tumor promotion (129). While targeting CD38 with antibodies has been approved for treating multiple myeloma, its role in solid tumors like OSCC has not been extensively studied (130, 131).Ding Z et al. investigated the multifunctionality of CD38 in OSCC, focusing on its prognostic implications, immune balance, and interaction with immune checkpoints (132). This retrospective study analyzed 92 OSCC samples using immunohistochemistry (IHC) to determine the spatial distribution of CD38 and assess its diagnostic and prognostic value. Additionally, preoperative peripheral blood samples from 53 OSCC patients were analyzed via flow cytometry. The study also utilized the Tumor Immune Estimation Resource (TIMER) and cBioPortal databases to examine CD38 levels in various tumors and their correlation with the tumor immune microenvironment in HNSCC. CD38 was found ubiquitously in tumor cells (TCs), fibroblast-like cells (FLCs), and tumor-infiltrating lymphocytes (TILs). Patients with high CD38 expression in TCs (CD38(TCs)) had higher TNM stages and an increased risk of lymph node metastasis. Elevated CD38 in FLCs (CD38(FLCs)) was significantly associated with poor WPOI. Increased CD38 in TILs (CD38(TILs)) correlated with higher Ki-67 levels in tumor cells. Moreover, patients with high CD38(TCs) were more susceptible to postoperative metastasis, and those with high CD38(TILs) independently predicted shorter overall and disease-free survival. Interestingly, patients with high CD38(TILs), but not CD38 (TCs) or CD38(FLCs), had significantly lower levels of $CD3^+CD4^+$T cells and a higher ratio of $CD3^-CD16^+CD56^+$NK cells. This immune imbalance was linked to dysregulated immune checkpoint molecules (VISTA, PD-1, LAG-3, CTLA-4, TIGIT, GITR) and specific immune cell subsets, which were positively correlated with CD38 expression in HNSCC. CD38 is a poor prognostic biomarker for OSCC patients and plays a crucial role in modulating the immune microenvironment and maintaining circulating lymphocyte homeostasis (132). The co-expression of CD38 and immune checkpoint molecules offers new insights into immune checkpoint therapy (131).

Anti-PD-(L)1

Although most previous research has not demonstrated programmed cell death protein 1 (PD-1) induction on human NK cells, emerging studies have reported PD-1 expression under specific clinical conditions, including OSCC (133). This suggests a nuanced role for PD-1 in the context of NK cell function within certain tumors (134, 135). The activation of NK cells has been shown to significantly bolster the anti-tumor efficacy of PD-1/PD-L1 blocking antibodies in animal models. This enhancement indicates that effective NK cell activity could be crucial for optimizing PD-1-based immunotherapy in OSCC (136, 137). Enhancing NK cell function might involve disrupting their immunosuppressive interactions within the tumor microenvironment (TME), particularly with PD-1-expressing myeloid-derived suppressor cells (MDSCs), although direct interactions between these cells have not yet been documented (138). Additionally, the tumor response to NK cell-produced interferon-gamma (IFN-γ) includes upregulated expression of PD-L1, which can contribute to an immunosuppressive environment. Therefore, understanding and manipulating the dynamics of NK cell interactions and PD-1/PD-L1 pathways in the TME could provide significant therapeutic advantages in treating OSCC. This approach highlights the potential of integrating NK cell modulation into existing and developing immunotherapeutic strategies, aiming to enhance overall treatment outcomes (139).

Anti-TIGIT

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an inhibitory receptor expressed on NK cells, T cells, and T regulatory (Treg) cell subsets (79, 140). The signaling through TIGIT inhibits NK cell-mediated cytotoxicity and is linked to decreased cytokine production and degranulation capacity. Targeting TIGIT through antibody blockade has shown

promising results *in vitro* and in animal models (141, 142). Blocking TIGIT reduces NK cell exhaustion, inhibits tumor growth, and enhances the production of proinflammatory cytokines by NK cells, indicating its potential as a therapeutic target to boost the immune response against tumors (141, 143).

Anti-TIM3

TIM3 (T cell immunoglobulin mucin-3) checkpoint inhibition is being explored as a treatment for advanced solid malignancies, including OSCC (144, 145). Previous studies, particularly in melanoma, have demonstrated that inhibiting TIM3 can alleviate NK cell exhaustion, enhancing their cytotoxic function (146). Several early-stage clinical trials (e.g., NCT02608268, NCT03744468) are currently investigating the efficacy of TIM3 inhibition in the treatment of advanced solid tumors, including OSCC. While the clinical effectiveness of these inhibitors has yet to be fully established, emerging evidence suggests that TIM3 plays a significant role in the progression of OSCC and may represent a valuable target for enhancing antitumor immunity (7).

Anti-LAG3

Inhibition of LAG3 using monoclonal antibodies has shown promising results in preclinical models. For instance, in a mouse model of OSCC, blocking LAG3 was able to limit tumor growth, suggesting that LAG3 could be a viable target for immunotherapy (147, 148). However, the precise contribution of NK cells in this context remains somewhat ambiguous. While NK cells are affected by LAG3 inhibition, LAG3 is also expressed on adaptive tumor-infiltrating lymphocytes, such as T cells, which are known to significantly influence tumor dynamics (144). The dual expression of LAG3 on both innate and adaptive immune cells complicates the interpretation of how LAG3 blockade benefits are mediated. Thus, while LAG3 blockade holds potential as a therapeutic strategy in treating advanced solid tumors, further research is necessary to disentangle the effects attributable to NK cells from those due to other immune cell types. This distinction is crucial for optimizing the therapeutic strategies targeting LAG3 and enhancing the overall effectiveness of cancer immunotherapy (125).

Anti-NKG2A

NKG2A is a receptor that carries an immunoreceptor tyrosine-based inhibitory motif (ITIM) and pairs with CD94 (111, 149). When NKG2A binds to its ligand HLA-E, it recruits the tyrosine phosphatase SHP-1, which subsequently suppresses NK cell-mediated cytotoxicity, including ADCC (150). *In vitro* studies have demonstrated that monalizumab can enhance the effector functions of both NK cells and $CD8^+$ T cells. Its effect is found to be synergistic when used in combination with imrvalumab and cetuximab, enhancing overall immune response against tumors (111, 151). However, monalizumab alone does not effectively promote ADCC, but its combination with cetuximab significantly amplifies ADCC, pointing towards a synergistic approach to boost anti-tumor activity (152).

Anti-KIR inhibitory receptors

Lirilumab is a monoclonal antibody targeting KIR2D (killer-cell immunoglobulin-like receptors 2D), which are inhibitory receptors found on NK cells (153–155). These receptors normally interact with HLA-C on target cells to inhibit NK cell activity, thus restraining their cytotoxic response. Lirilumab binds to and blocks the activity of the inhibitory receptors KIR2DL1, KIR2DL2, and KIR2DL3 on peripheral NK cells, thereby reducing their inhibitory effects and enhancing NK cell anti-tumor response (153). This blockade also includes some interaction with KIR2DS1 and KIR2DL2, reducing their off-target effects. In clinical settings, particularly in patients with certain hematological malignancies, allogeneic transfer of NK cells lacking these inhibitory KIRs, facilitated by lirilumab, has shown potential in preventing relapse by amplifying the NK cells' tumor-fighting capabilities (155, 156). These approaches illustrate the strategic targeting of NK cell inhibitory pathways as a means to potentiate their natural cytotoxic capabilities against cancer cells, offering promising enhancements to existing cancer immunotherapies (154).

Adenosine 2B receptor

Wang B et al. investigated the impact of co-inhibiting the adenosine 2b receptor (A2BR) and PD-L1 on the recruitment and cytotoxicity of NK cells in OSCC (157). Adenosine is known to modulate anti-tumor immune responses by affecting T-cells and NK cells within the tumor microenvironment (158, 159). However, the role of adenosine receptors in OSCC progression and their influence on immune checkpoint therapy is not well understood. In this study, tumor tissues from 80 OSCC patients admitted to Shandong University Qilu Hospital between February 2014 and December 2016 were analyzed. The expression of A2BR and PD-L1 in different regions of the tumor tissues, such as the tumor nest, border, and paracancer stroma, was detected using immunohistochemical staining. Treatment with BAY60-6583 increased PD-L1 expression in CAL-27 cells, an effect partially inhibited by PDTC, indicating that A2BR induces PD-L1 expression via the NF-κB signaling pathway. Furthermore, high A2BR expression in OSCC was linked to lower NK cell infiltration. Treatment with MRS-1706 (an A2BR inverse agonist) and/or a PD-L1-neutralizing antibody (CD274) enhanced NK cell recruitment and cytotoxicity against OSCC cells. Overall, the findings highlight the synergistic effect of co-inhibiting A2BR and PD-L1 in treating OSCC by modulating NK cell recruitment and cytotoxicity (157, 158).

Unlike T cells, NK cells are not restricted by major histocompatibility complex (MHC) molecules. Their activation is controlled through a balance of surface activating and inhibitory receptors. By blocking these immune checkpoints, such as TIGIT and TIM3, it is possible to prevent the immune escape of tumors, thereby enabling NK cells to more effectively exert their antitumor effects. This strategy aims to bolster the immune system's natural ability to fight cancer, enhancing the efficacy of cancer immunotherapy (125, 160).

NK cells kill the OSCC cancer stem cells

The induced chemotherapy resistance and differentiation in oral cancer stem cells are associated with increased expression of CD54, B7H1, and MHC class I molecules (161). This process is mediated by a combination of membrane-bound or secreted IFN-γ and TNF-α from the NK cells. Interestingly, blocking these cytokines with specific antibodies to both IFN-γ and TNF-α, and not to each one alone, was necessary to inhibit the differentiation or resistance to NK cells. Furthermore, the use of these antibodies was required to prevent NK-mediated inhibition of stem cell growth, restoring their numbers to levels observed when the stem cells were cultured without anergized NK cells. The study also highlights that the effect of blocking IFN-γ, in the absence of TNF-α blocking, was particularly influential in preventing the increase in surface receptor expression, as adding an anti-IFN-γ antibody alone significantly reduced the upregulation of CD54, B7H1, and MHC class I. While antibodies to CD54 or LFA-1 did not inhibit differentiation, antibodies targeting MHC class I, but not B7H1, enhanced the cytotoxicity of NK cells against well-differentiated oral squamous carcinoma cells and OSCC that had differentiated following treatment with IL-2 and anti-CD16 monoclonal antibodies. Conversely, this approach inhibited the cytotoxicity of NK cells against undifferentiated OSCC. These findings suggest that NK cells, through their ability to kill or induce differentiation, may play a crucial role in preventing the progression of cancer by targeting cancer stem cells (161). This action could significantly impede cancer growth, invasion, and metastasis, offering potential therapeutic avenues for targeting cancer stem cells to control disease progression.

NK cell combine with icon immunotherapy against OSCC

Icon immunotherapy—a novel dual-targeting agent that focuses on both neovascular and cancer cell targets—in treating OSCC. The study used the human tongue cancer line TCA8113, both *in vitro* and *in vivo* within severe combined immunodeficiency (SCID) mice models (162). Icon, a chimeric immunoconjugate combining factor VII and human IgG1 Fc, was investigated for its potential to induce murine natural killer (NK) cell activity and activate the complement system to eradicate cancer cells. The results underscored the pivotal role of NK cells in mediating the cytotoxic effects of Icon. Further *in vivo* studies reinforced these findings. When tested on human tongue tumor xenografts in CB-17 strain of SCID mice—which possess normally functioning NK cells—Icon successfully eradicated the established tumors. Conversely, in SCID/Beige mice, which lack functional NK cells, Icon's effectiveness was markedly reduced. This contrast highlights the essential role of NK cells in the therapeutic efficacy of Icon immunotherapy. The study concludes that NK cells are indispensable for the success of Icon immunotherapy in cancer treatment. The results also suggest that insufficient NK cell levels or activity could be a contributing factor to resistance against therapeutic antibodies, a finding that has implications for ongoing preclinical and clinical research into antibody-based therapies. This insight into the mechanism of Icon underscores the importance of NK cells and suggests that enhancing NK cell function could improve the outcomes of immunotherapeutic strategies targeting cancers like OSCC.

Jung EK et al. investigated the efficacy of natural killer (NK) cell therapy combined with chemoradiotherapy (CRT) in murine models of head and neck squamous cell carcinoma (HNSCC) (163). CRT successfully recruited mouse NK cells to the tumor site. Additionally, expanded and activated human NK cells (eNKs) were recruited to the tumor site in response to CRT, with CRT enhancing the anti-tumor activity of eNKs in an NOD/SCID IL-2Rγnull mouse model. Various HNSCC cell lines displayed different NK cell ligand activation patterns in response to CRT, which correlated with NK cell-mediated cytotoxicity. Identifying these activation patterns during CRT may improve patient selection for adjuvant NK cell immunotherapy combined with CRT. This study is the first to explore the antitumor function and recruitment of NK cells with CRT in an HNSCC mouse model (163), which provide evidence of major anti-tumor capacity of NK cells in solid tumor.

Inhibition myeloid-derived suppressor cells to augment the anti-tumor effects of NK cells

A particular focus is placed on the role of myeloid-derived suppressor cells (MDSCs) in inhibiting NK cell function within the tumor microenvironment of OSCC. Greene S, et al. explored the potential of NK-cell-based immunotherapy in overcoming limitations faced by T-cell-based therapies in treating OSCC (164). The research involves both murine models and human clinical samples to assess the suppressive actions of MDSCs derived from peripheral blood and tumor sites. In murine models, the study demonstrated that neutrophilic-MDSCs (PMN-MDSCs) expressing CXCR2 are pathologically accumulated in peripheral areas and within tumors, where they inhibit NK cell function through mechanisms such as TGFβ secretion and hydrogen peroxide production. A small-molecule inhibitor of CXCR1 and CXCR2, SX-682, was found to significantly reduce the accumulation of these suppressive cells in tumors. This facilitated enhanced infiltration, activation, and therapeutic efficacy of adoptively transferred murine NK cells. In the clinical setting, significant levels of circulating and tumor-infiltrating CXCR1/2+ PMN-MDSC and monocytic-MDSC were observed in patients with OSCC. These tumor-associated MDSCs displayed stronger immunosuppressive effects than their circulating counterparts, mediated through multiple independent mechanisms including TGFβ and nitric oxide. The findings suggest a promising therapeutic approach combining CXCR1/2 inhibitors with adoptively transferred NK cells, highlighting the need for clinical trials to evaluate this strategy's efficacy in enhancing NK cell-mediated immunotherapy in OSCC (165).

While immune checkpoint inhibitors have revolutionized cancer treatment, their clinical benefits have been limited to a subset of patients (160, 166). Therefore, developing more effective methods to target tumor cells expressing immune checkpoint molecules is crucial (125). Fabian KP et al. studied the antitumor effects of PD-L1 targeting high-affinity natural killer (t-haNK) cells, which also target suppressive MDSC cells (167). For the first time, this study reports a novel NK cell line, PD-L1 targeting high-affinity

natural killer (t-haNK) cells, derived from NK-92 cells. These cells were engineered to express high-affinity CD16, endoplasmic reticulum-retained interleukin (IL)-2, and a PD-L1-specific CAR. PD-L1 t-haNK cells retained the expression of native NK receptors and contained high levels of granzyme and perforin granules. Their results showed that PD-L1 t-haNK cells expressed PD-L1-targeting CAR and CD16, retained native NK receptors, and carried high levels of granzyme and perforin granules. Irradiated PD-L1 t-haNK cells were able to lyse all 20 human cancer cell lines tested, including triple-negative breast cancer (TNBC) and lung, urogenital, and gastric cancer cells. The cytotoxicity of PD-L1 t-haNK cells correlated with the PD-L1 expression on tumor targets and could be enhanced by pretreating the targets with interferon (IFN)-γ. *In vivo*, irradiated PD-L1 t-haNK cells inhibited the growth of engrafted TNBC and lung and bladder tumors in NSG mice. The combination of PD-L1 t-haNK cells with N-803 and anti-PD-1 antibody showed superior tumor growth control in engrafted oral cavity squamous carcinoma tumors in C57BL/6 mice. Additionally, when cocultured with human PBMCs, PD-L1 t-haNK cells preferentially lysed the MDSC population without affecting other immune cell types (167).

Cytokine and chemokine signal pathway enhance NK cell based therapy against OSCC

The chemokine and cytokine signal have received significant attention due to their role in cancer development (168). Liu H et al. explored the effects of adenovirus-mediated overexpression of interleukin-21 (IL-21) on the development of OSCC *in vitro* (169). In tumor cells, IL-21 enhances the immune response by increasing the cytotoxic activity of natural killer cells, B cells, and $CD8^+$ T cells, leading to tumor cell apoptosis. The therapeutic effects of IL-21 have been studied in various diseases, with numerous clinical trials underway (168). This study was to determine the role of IL-21 in OSCC *in vitro*. IL-21 expression in OSCC tissues was detected using RT-qPCR, western blotting, and immunohistochemistry analyses, which revealed decreased IL-21 protein expression in OSCC tissues. IL-21 was overexpressed in CAL-27 cells using adenovirus. IL-21 overexpression inhibited OSCC cancer cell proliferation. Additionally, wound healing assays indicated that IL-21 overexpression suppressed cell migration, while TUNEL staining and flow cytometry analysis demonstrated that IL-21 overexpression promoted cell apoptosis via activation of the JNK signaling pathway. These findings suggest that IL-21 may serve as a potent antitumor agent in OSCC (169).

Upregulation of CCL2/CCR2 is linked to cancer progression, metastasis, and relapse (168, 170). By integrating scRNA-seq data with TCGA data, we discovered that the IL6/IL6R and CCL2/CCR2 signaling pathways have a more significant impact on immune evasion by NK cells in the HPV-negative HNSCC cohort compared to the HPV-positive cohort. In orthotopic mouse models, blocking IL6 with a neutralizing antibody suppressed HPV-negative tumors but not HPV-positive ones, and this suppression was accompanied by increased infiltration and proliferation of $CD161^+$ NK cells. Notably, combining the CCR2 chemokine receptor antagonist RS504393 with IL6 blockade resulted in a more pronounced antitumor effect, characterized by more activated intratumoral NK cells in HPV-negative HNSCC compared to either agent alone. These findings demonstrate that dual blockade of the IL6 and CCR2 pathways effectively enhances NK cell-mediated antitumor activity in HPV-negative HNSCC, offering a novel strategy for treating this type of cancer (171).

Another study from Crist M et al. investigated how metformin enhances natural killer (NK) cell functions in HNSCC by inhibiting CXCL1 (172). This study included results from two phase I open-label trials involving HNSCC patients treated with metformin (NCT02325401, NCT02083692). Peripheral blood samples were collected from patients before and after metformin treatment or from newly diagnosed HNSCC patients. NK cells were treated with either a vehicle or metformin and then analyzed by RNA sequencing (RNA-seq). Significant pathways identified by RNA-seq were inhibited, and NK cells were further analyzed using NKCA, ELISA, and western blot analyses. The study found increased activated peripheral NK cell populations in patients treated with metformin and enhanced NK cell tumor infiltration in preoperatively treated HNSCC patients. Metformin increased the production of antitumorigenic cytokines ex vivo, particularly perforin. It also enhanced NK cell cytotoxicity against HNSCC cells, inhibited the CXCL1 pathway, and stimulated the STAT1 pathway. Exogenous CXCL1 was found to prevent metformin-enhanced NK cell-mediated cytotoxicity. Metformin-mediated NK cell cytotoxicity was independent of AMP-activated protein kinase but dependent on both the mechanistic target of rapamycin and pSTAT1. These findings reveal a new role for metformin in promoting immune antitumorigenic function through NK cell-mediated cytotoxicity and CXCL1 downregulation in HNSCC, informing future immunomodulating therapies in this context.

Reduce the epithelial-mesenchymal transition

Epithelial–mesenchymal transition (EMT) is a critical process in embryonic development, fibrosis, and cancer invasion (173). Despite recent advances in treatment, the 5-year overall survival rate of oral squamous cell carcinoma (OSCC) has not improved. EMT plays a significant role in the local recurrence and lymph node metastasis of oral cancer. Wang C et al. studied how heparanase (HPSE) promotes malignant characteristics in human oral squamous carcinoma cells by regulating EMT-related molecules and levels of infiltrating NK cells (174). Knocking down HPSE expression reduced the proliferation rate of SCC-25 cells, leading to a significant increase in the percentage of cells in the G0/G1 phase, and suppressed cell migration and invasion. E-cadherin mRNA and protein expression increased, while Snail and Vimentin expression decreased. RNA sequencing between the small interfering RNA and negative control groups identified 42 differentially expressed genes, including syndecan binding protein, RAB11A (a member of the RAS oncogene family), and DDB1 and CUL4-associated factor 15. These findings indicate that HPSE knockdown suppresses SCC-25 cell proliferation, invasion, migration, and EMT, potentially through syndecan binding protein and RAB11A. Additionally, HPSE may regulate the activation levels of infiltrating NK cells, possibly via DDB1 and CUL4-associated factor 15.

Modulate the RNA-network combine with NK cell therapy

The networks involving circle RNAs (circRNAs) and small RNAs can impact numerous molecular targets, driving specific cellular responses and determining cell fates. In cancer, ncRNAs have been identified as oncogenic drivers and tumor suppressors across all major cancer types (175).. Liu L et al. studied the role of SP2-induced circPUM1 in modulating chemoresistance and natural killer (NK) cell toxicity in OSCC (176). Previous research has established that NAP1L1 plays critical roles in various cancers and is involved in chemoresistance in hepatocellular carcinoma and glioma. The study identified NAP1L1 as a downstream target of miR-770-5p and crucial for circPUM1-mediated chemoresistance and NK cell toxicity in OSCC cells (175, 177). circPUM1 in OSCC cells and generated dysregulated circPUM1 cell models, demonstrating that circPUM1 promotes chemoresistance and NK cell toxicity. Furthermore, the transcription factor SP2 regulates circPUM1 expression in OSCC cells, with circPUM1 acting as a molecular sponge for miR-770-5p. NAP1L1, a downstream target of miR-770-5p, is essential for circPUM1-mediated cisplatin resistance and NK cell cytotoxicity in OSCC cells. The network comprising SP2, circPUM1, miR-770-5p, and NAP1L1 presents a promising avenue for developing novel diagnostic or therapeutic targets for OSCC. NAP1L1 overexpression promoted cell viability, which was reduced by downregulated circPUM1, and NAP1L1 downregulation alleviated the increased cell viability promoted by upregulated circPUM1 in cisplatin-treated OSCC cells. These findings suggest that NAP1L1 plays a crucial role in circPUM1-mediated chemoresistance and NK cell toxicity in OSCC cells (178).

Tertiary lymphoid structures

Tertiary lymphoid structures (TLS) are ectopic lymphoid structures in cancers typically associated with favorable prognosis, but their prognostic significance in OSCC is not well understood, and the relationship between TILs and TLSs in OSCC has been seldom explored (179, 180). Li Q et al. investigated the prognostic value of TLS andTILs in OSCC (181). In this study, markers associated with TLS, including peripheral node addressin (PNAd) in high endothelial venules, CD20 in B cells, and CD3 in T cells, were examined in 168 OSCC patients. Survival analysis was conducted to compare TLS-positive and TLS-negative cohorts. Additionally, TILs were identified by staining $CD8^+$ cytotoxic T cells and $CD57^+$ NK cells. TLSs were found to be highly organized structures in 45 (26.8%) cases. Patients with TLS-positive tumors had significantly better 5-year overall survival (OS) rates (88.9% vs. 56.1%, $P < 0.001$) and relapse-free survival (RFS) rates (88.9% vs. 63.4%, $P = 0.002$). The presence of TLS was identified as an independent prognostic factor for both 5-year OS (hazard ratio [HR] = 3.784; 95% confidence interval [CI], 1.498-9.562) and RFS (HR = 3.296; 95% CI, 1.279-8.490) in multivariate analysis. Additionally, a higher density of CD8+ T cells and $CD57^+$ NK cells was observed in TLS-positive sections compared to TLS-negative ones ($P < 0.001$), and their combination provided higher predictive accuracy (AUC = 0.730; 95% CI, 0.654-0.805). In conclusion, the results suggest that TLS is an independent positive prognostic factor for OSCC patients, providing a theoretical basis for the future diagnostic and therapeutic value of TLS in OSCC treatment.

Limitations of NK cells against OSCC

While NK cell-based immunotherapies offer promising potential in treating OSCC, several limitations and challenges must be addressed to improve their efficacy (86, 182).

Tumor microenvironment challenges

The TME in OSCC is rich in immunosuppressive factors such as TGF-β, IL-10, and myeloid-derived suppressor cells (MDSCs). These factors inhibit NK cell activation and function, reducing their ability to attack tumor cells effectively. Additionally, the dense extracellular matrix (ECM) and stromal components in the TME physically impede NK cell infiltration and migration to the tumor site, limiting their cytotoxic effects on cancer cells.

Intrinsic limitations of NK cells

Heterogeneity and Variability. NK cells are not a homogeneous population, and their activity can vary significantly between individuals. This variability affects the consistency and predictability of NK cell-based therapies.

Short lifespan and persistence

NK cells have a relatively short lifespan in the bloodstream, and maintaining their persistence and activity within the TME is challenging. This necessitates repeated infusions or genetic modifications to enhance their longevity and efficacy.

Therapeutic delivery challenges

Targeting and Specificity: Ensuring that engineered NK cells, such as CAR-NK cells, specifically target OSCC cells without affecting normal tissues remains a significant challenge. Off-target effects can lead to unintended damage to healthy cells and tissues.

Resistance mechanisms

Tumor cells can develop mechanisms to evade NK cell detection, such as downregulating stress ligands or upregulating inhibitory signals that prevent NK cell activation. This adaptive resistance can reduce the overall effectiveness of NK cell therapies.

Clinical and practical limitations

Cost and Complexity: The production and administration of NK cell-based therapies are complex and costly. This includes the isolation, expansion, and genetic modification of NK cells, which require specialized facilities and expertise.

Regulatory hurdles

NK cell therapies are relatively new, and regulatory approval processes can be lengthy and stringent. Ensuring safety, efficacy, and quality control for these therapies adds to the development timeline and cost.

Nevertheless, overcoming the limitations of NK cells against OSCC has become a significant focus in cancer research. Integrating NK cell-based therapies with other treatments, such as immune checkpoint inhibitors, can significantly enhance their efficacy. Modulating the tumor microenvironment to be more favorable to NK cell activity is another promising approach (183, 184). Furthermore, advancements in genetic engineering, such as the development of CAR-NK cells, can improve targeting and persistence. Additionally, modifying NK cells to express cytokines or receptors that boost their activity within the tumor microenvironment can address some inherent limitations. Ongoing research and innovative strategies are crucial for overcoming these barriers and optimizing NK cell therapies for superior clinical outcomes.

Future outlook

The field of immunotherapy for OSCC has evolved dramatically, with Natural Killer (NK) cell-based therapies emerging as a promising frontier. NK cells, a critical component of the innate immune system, possess inherent cytotoxic abilities that can be harnessed and enhanced to target cancer cells. As research delves deeper into the unique properties of NK cells, their role in combating OSCC is being redefined, offering new therapeutic avenues and hope for improved patient outcomes. NK cells exhibit a natural ability to detect and kill cells undergoing stress, such as cancerous cells, without the need for prior sensitization. This capability makes them an attractive option for immunotherapy, particularly in OSCC, where the early detection and treatment of cancer cells are crucial for improving survival rates.

Furthermore, NK cells' mechanisms of action do not rely on antigen presentation by Major Histocompatibility Complex (MHC) molecules, which are often downregulated in OSCC cells to evade immune detection. This allows NK cells to overcome one of the primary mechanisms of immune escape utilized by tumor cells. Recent advances in biotechnology have enabled the engineering of NK cells to enhance their anticancer activity. CAR can be expressed on NK cells, creating CAR-NK cells that combine the specificity of antibody-based recognition with the potent cytotoxic activity of NK cells. These engineered NK cells can be directed to target specific antigens expressed on the surface of OSCC cells, increasing their efficacy and specificity. Clinical trials involving CAR-NK cells have shown promising results, indicating significant potential for their use as a treatment modality for OSCC.

However, challenges remain in the clinical application of NK cell therapies. One of the main hurdles is the immunosuppressive tumor microenvironment (TME) of OSCC, which can inhibit NK cell function. Strategies to overcome this include the use of adjuvant therapies that modify the TME to be more conducive to NK cell activity. For instance, combining NK cell therapy with checkpoint inhibitors or modulators of the TME can enhance the effectiveness of NK cells. Looking forward, the integration of NK cell-based therapies into the standard care for OSCC appears promising. Ongoing research aims to optimize the delivery, specificity, and persistence of NK cells within the TME. Furthermore, as our understanding of the molecular and cellular interactions within the OSCC TME improves, so too will strategies for enhancing NK cell function.

The ultimate goal is to develop a tailored immunotherapy approach that can be integrated with existing surgical and chemotherapeutic treatments to provide a comprehensive treatment strategy for OSCC patients. In conclusion, NK cell-based immunotherapy holds a bright future in the management of OSCC. Continued research and clinical trials are essential to harness the full potential of NK cells, refine their application, and solidify their place in the oncological arsenal against oral cancer.

Author contributions

YZ: Methodology, Supervision, Writing – original draft. JX: Formal analysis, Software, Writing – original draft. HW: Data curation, Resources, Writing – original draft. JH: Software, Writing – original draft. DZ: Resources, Software, Writing – original draft. SW: Resources, Writing – original draft. XJ: Data curation, Methodology, Writing – original draft. ZH: Formal analysis, Investigation, Resources, Writing – original draft. YG: Funding acquisition, Supervision, Validation, Writing – review & editing. LJ: Supervision, Validation, Writing – review & editing. QS: Conceptualization, Data curation, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Immune microenvironment in papillary thyroid carcinoma: roles of immune cells and checkpoints in disease progression and therapeutic implications

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Papillary thyroid cancer (PTC) is the most common type of primary thyroid cancer. Despite the low malignancy and relatively good prognosis, some PTC cases are highly aggressive and even develop refractory cancer in the thyroid. Growing evidence suggested that microenvironment in tumor affected PTC biological behavior due to different immune states. Different interconnected components in the immune system influence and participate in tumor invasion, and are closely related to PTC metastasis. Immune cells and molecules are widely distributed in PTC tissues. Their quantity and proportion vary with the host's immune status, which suggests that immunotherapy may be a very promising therapeutic modality for PTC. In this paper, we review the role of immune cells and immune checkpoints in PTC immune microenvironment based on the characteristics of the PTC tumor microenvironment.

KEYWORDS

papillary thyroid cancer, immune microenvironment, immunization therapy, immune checkpoints, immune cells

1 Introduction

Thyroid cancer (TC) represents a prevalent malignancy within the endocrine system, demonstrating a higher incidence in women compared to men and predominantly affecting individuals aged 40 to 50 (1, 2). The biological properties of various thyroid cancer subtypes span a broad spectrum. Based on their histological characteristics and cellular origins, thyroid cancers are classified into papillary, medullary, and follicular carcinomas (3). Papillary thyroid carcinoma (PTC) is a differentiated cancer subtype in the thyroid,

constituting most form of primary thyroid malignancy (4). Over recent decades, the incidence of PTC has exhibited an increasing trend and a shift towards younger age groups (5, 6). For PTC, the current traditional therapies include surgical resection, radiotherapy, chemotherapy, endocrine inhibition and other therapeutic means, but the efficacy of various treatment methods has different degrees of limitations (7, 8). Despite their slow tumor growth, low malignancy, and overall favorable prognosis, over 10% of patients had tumor recurrence or metastasized to other sites after surgery (9). Some cases appear highly aggressive and may even progress to refractory thyroid cancer (10). Immune cell infiltration is frequently observed in the vicinity or within primary PTC tissue. The prognosis of PTC might be associated with the surrounding inflammatory response (11). Increasing evidence suggests that the immune microenvironment influences tumor biological behavior.

2 Immune cells In PTC tumor microenvironment

In 2002, Dunn proposed the immune editing hypothesis, which categorized the reciprocity between the tumors and immune system into "elimination", "equilibrium", and "escape". The "elimination" phase, also called "surveillance", involves the immune system clearing tumor cells before diagnosis. During the "equilibrium" phase, Tumor cells vary in the direction of low immunogenicity, which makes themselves not easily detected by the body's immune surveillance mechanism (12). Studies (13, 14) have shown that tumor cells can "camouflage" themselves by reducing MHC I expression, thus evading immune system surveillance. Another study (15) analyzed the influence of the immune environment on the clinical manifestations of patients and found that immune cells in PTC patients' thyroids differed from healthy ones. Specifically, the proportions of B cells, T cells (mainly CD8+ T cells) and M1 macrophages showed obvious reduction. The larger the difference between these immune cells and healthy thyroid tissue, the greater the likelihood of PTC progression and recurrence, and the lower the patients' overall survival rate.

The tumor microenvironment (TME) contains tumor cells and their living environment (including immune cells, stromal cells and blood vessels), which cooperate with each other (16). Each component in TME plays a crucial role in tumor initiation and progression. Their quantity as well as proportion vary with the host's immune status (17). In most cancers, a high proportion of M2/M1 macrophages is strongly associated with poor clinical prognosis (18). In thyroid cancer, tumor-related macrophages (Tumor-associated macrophages, TAMs) are dominated by M2 polarized macrophages, providing a good tumor microenvironment for tumor growth, survival and angiogenesis. Experimental results of various tumors, including thyroid cancer, show that high-density TAMs are associated with poor prognosis of tumors (19, 20). At present, many cytokines, chemokines and their signaling pathways also have been found in PTC. For example, activation of IL-6/JAK2/STAT3 pathway could promote PTC cell proliferation and migration, and IL-34 promotes PTC cell proliferation (21), epithelial-stromal transition and extracellular regulatory kinase signaling pathway and inhibits apoptosis (22). In PTC tumor microenvironment, overexpression of IL-6 promotes the growth of PTC (23). The infiltration of plasma cells in the DTC microenvironment was positively correlated with a favorable prognosis (24). Immature Dendritic cells in the PTC microenvironment can secrete immunosuppressive cytokines, such as IL-10 and TGF- β, so as to inhibit the immune response and result in the development of PTC, while $CD8^+$ T cells recognize tumor cells to express antigen and thus participate in the killing of tumor cells, exhibiting protective effects on PTC (25, 26).

Xie Z et al. (27) investigated immune-related cells in TME, focusing on the relationship between PTC and chronic inflammation. The study included 799 PTC patients and 194 healthy ones. It was found that compared with normal thyroids, the overall immune level of PTC tissues was stronger, and many cells in TME such as Tregs and M0 macrophages were elevated. Furthermore, the more advanced the tumor, the greater the proportion and abundance above normal levels. Higher immune group had a later stage than the lower one, with a larger tumor size, increased metastasis of lymph node, and a higher frequency of BRAF mutations. This suggests that changes in immune status within the TME are closely related to tumor progression, and that various immune cells can either promote or inhibit PTC metastasis and recurrence to different extents.

2.1 Natural killer cells

NK cells are essential components of inherent immunity that express various regulatory receptors associated with activation or inhibition. These receptors facilitate the distinction between "self" and "non-self," enabling them to selectively "eliminate" (28). NK cell infiltration in tumors is often linked to the initiation or progression of cancer of early and metastatic stages of tumor development, and is generally predictive of a favorable prognosis (29).

In PTC, NK cells in TME are elevated in comparison to normal thyroid tissue, but not in peripheral blood (30). The abundance of NK cells in TME is significantly negatively associated with tumor progression. NK cells are able to kill cancer cells directly, and also responsible for the immune surveillance (31, 32). They may provide new ideas for PTC diagnosis and therapy. However, their efficacy is somewhat limited during the anti-tumor process due to the secretion of immunosuppressive factors by tumor cells, which reduce the activation receptors on NK cells while upregulating inhibitory receptors, making NK cell activation difficult. Tumor cells can also evade immune surveillance by reducing MHC I molecule expression, which blocks tumor antigen presentation (33). Additionally, the number and functionality of NK cells in the TME typically decline with tumor progression (34), and NK cells may be rendered dysfunctional due to metabolic disorders (35). These limitations of NK cells within the TME should be considered when utilizing them for PTC diagnosis, staging, and treatment.

2.2 T lymphocytes

T lymphocytes can be classified into helper T cells (Th), cytotoxic T cells (CTL), and regulatory T cells (Treg) according to their various functions. They mature from lymphoid progenitor cells in the thymus and are central to cellular immunity. CD4 is expressed in all Th cells. Naive CD4+T cells, known as Th0 cells, can differentiate into Th1, Th2, and Th17 lineages that have distinct immune roles through antigen stimulation and cytokine regulation. Th1 cells enhance and amplify cellular responses by secreting regulatory molecule, including interleukin (IL)-2 and IFN-γ, and induce other immune cells to exhibit antitumor activity (36). In contrast, Th2 cells inhibit the antitumor effects of cellular immunity by secreting IL-4 and suppressing NK cell activation (37). The Th1/Th2 ratio serves as a useful indicator of dynamic changes in the antitumor immune process. Moreover, Th17 levels in PTC tissue samples are higher than in healthy thyroid tissue, with this difference also observed in patients' peripheral blood. More Th17 in peripheral blood tend to predict larger tumor volume (38).

The primary function of CTLs is to specifically recognize endogenous antigen peptide-MHC I molecular complexes and subsequently kill tumor cells. This has become an essential marker for evaluating tumor prognosis (39–41). PTC patients with a higher expression of CD8+ CTLs show lower tumor stages and higher survival rates, while the reduction of CD8+ T cells weakens the immune system's ability to eliminate tumor cells, making tumors more aggressive (42). In the study by Modi J et al (43). PTC patients with CD8+ T cell infiltration experienced slower tumor progression, reduced tumor growth, and fewer recurrences.

Tregs, commonly referred to as CD4+CD25+Foxp3+ T cells, primarily weaken immune level through direct contact to target cells and cytokine secretion. High Tregs expression in cancer tissue is typically related to poor prognosis. Tregs are highly aggregated in the tumor site and peripheral blood of cancer patients (44, 45), and their inhibitory effect on the immune function of cancer patients is stronger than in healthy individuals (46). Tregs in PTC patients' peripheral blood are significantly increased compared to normal thyroid tissue and thyroid adenoma patients (47, 48). In the TME, Tregs can weaken the body's immune response to tumors through various mechanisms, including affecting cytokine secretion (49, 50), increasing cAMP-mediated immunosuppression via adenosine and prostaglandin (51, 52), regulating signal transduction through receptor-ligand binding (53, 54), and mediating immunosuppression through the exosome pathway (55). French JD et al. (42) using immunohistochemical analysis, quantitatively counted lymphocytes in the TME of PTC tissues, and found that T cells in the PTC tissues of patients were mainly composed of CD4 + T cells. The quantity of Foxp3+ regulatory T cells was related to lymph node metastasis ($r = 0.858$; $P = 0.002$), and the ratio of CD8 to Treg was strongly negatively associated with tumor size.

In the future, the frequency of Treg cells in TME is likely to become an important factor in predicting, diagnosing, and evaluating the prognosis of PTC. Furthermore, the suppressive effect of Treg cells should be taken into account when designing immunotherapy for PTC. Overall, a better understanding of the complex interactions between various immune cell types in the TME is significant for the exploration of more effective diagnostic and therapeutic strategies for PTC and other cancers.

2.3 Mast cells

Mast cells are tissue-resident component ubiquitously distributed across nearly all tissues. Their regulatory role in the tumor microenvironment (TME) is often multifaceted, exhibiting both pro-tumorigenic and anti-tumorigenic effects (56). The tumor-promoting effects primarily involve the secretion of vascular endothelial growth factors (VEGF) to promote neovascularization, the secretion of matrix metalloproteinases (MMPs) to enhance cancer progression, and the release of regulatory molecules to facilitate immune tolerance. Conversely, their anticancer effects include direct inhibition of tumor growth, immune stimulation, and reduction of cell motility (57). Mast cells situated within or surrounding tumors may exhibit different roles. While mast cells generally play a pro-carcinogenic role in most tumors (58, 59), their contributions to cancer progression can vary depending on which stage the tumors are at and where they are in tumor tissue (60).

Limited studies (61) have assessed the correlation between mast cells and PTC. One study reported that mast cell accumulation was observed in 95% of PTC samples, with the density positively correlated with cancer aggressiveness. Other studies demonstrated that mast cell derivatives, such as histamine and chemokines, accelerated the progression of PTC as well as distant metastasis *in vitro*. But this phenomenon will be exactly the opposite when inhibitors of mast cells are applied (62), potentially providing novel therapeutic strategies for PTC treatment.

2.4 Tumor-associated macrophages

Tumor-associated macrophages (TAMs) are the most abundant in the tumor microenvironment (TME). They can differentiate into two subpopulations that exert opposing effects on the host's immune response to tumors. M1 macrophages predominantly suppress tumor growth and angiogenesis by producing cytokines like IL-1. In contrast, M2 macrophages generate IL-13, IL-10, and other factors that foster tumor development and enhance the invasive capabilities of tumor cells (63). Within the TME, cancer cells secrete signaling factors, mediated by exosomes, that induce mononuclear macrophages to differentiate into the M2 subtype (64), resulting in an imbalance between M1 and M2 populations and ultimately promoting cancer progression (65).

Elevated TAM in PTC is closely related with biological behavior of the tumors (66). Studies (67, 68) have revealed the macrophage infiltration rate in PTC is significantly higher than that in benign tumors, with the extent of infiltration positively correlating with lymph node metastasis. The underlying mechanism remains incompletely understood; however, it may involve TAMs promoting tumor cells of PTC metastasis through the cytokine CXCL8 and its paracrine interaction with CXCR1/2 (69).

Consequently, a comprehensive understanding of the functional differences between distinct TAM subtypes in the thyroid gland may potentially establish TAMs as new idea for thyroid tumor therapy.

2.5 Dendritic cells

Dendritic cells (DCs) are the most functionally specialized APCs in immune system. They serve as initiators of the adaptive immune response and act as a "bridge" connecting innate and adaptive immunity.

Normally, DCs are scarcely present in thyroid tissue. However, their prevalence increases in human papillary thyroid carcinoma (PTC) tissue (70). Immature DCs possess robust antigen-processing capabilities but are less effective in promoting immune responses. Interestingly, they may even weaken immune responses by secreting inhibitory cytokines including IL-10 and TGF-β (71).

Moreover, Tregs and DCs can interact and collaboratively involve in immune regulation in TME. In PTC tissues, Tregs can inhibit DC function, co-stimulatory ligands expression, CD8+ T cells activation (72). DCs are able to restore their function by blocking PD-1 pathways, IL-10 secretion, and production of lactic acid (73). Therefore, disrupting the interaction between Tregs and DCs in PTC may shed new light on immune therapy.

2.6 Neutrophils

Neutrophils have long been recognized for their pivotal role in acute phase of inflammatory. Recently, they've emerged as a new subject of investigation in the field of oncology. Accumulating experimental evidence suggests that neutrophils may exert both antitumor and protumor effects by releasing various regulatory molecules within the tumor microenvironment (74). Neutrophils exhibit a dual role in PTC development and progression. On one hand, they promote genetic instability, proliferation, invasion (75), and vascular remodeling of cancer cells by releasing neutrophil elastase (76). Conversely, neutrophils have demonstrated antitumor properties, possessing the capacity to "eliminate" through antibody-dependent cellular cytotoxicity (ADCC) (77). Maria et al. found that PTC tissue extended the survival of human neutrophils and enhances its activity and reactive oxygen species (ROS) generation, suggesting that neutrophils can acquire a cytotoxic antitumor phenotype under the influence of thyroid tumor microenvironment. Notably, during tumor progression, the neutrophil population increases, and their phenotype undergoes alterations. Several subsets of circulating neutrophils with distinct maturity and immunological properties can be identified in advanced cancer, each playing a unique role in tumor immunity (78).

In PTC tissues, tumor cells recruit neutrophils by releasing CXCL8/IL-8 and reduce apoptosis rate of neutrophils through secretion of granulocyte colony-stimulating factor (GM-CSF) (79). The ratio of neutrophil count to lymphocyte count (neutrophil to lymphocyte ratio; NLR) in peripheral blood is associated with tumor development and progression (80), and higher NLR is associated with larger tumor volume and higher risk of recurrence in thyroid cancer (81).

3 Immune checkpoints of PTC

Lymphocyte activation primarily relies on the specific recognition of antigens by antigen receptors, with the strength, duration, and nature of the activation signal often regulated by cell surface receptor molecules. Immune checkpoints act as regulatory components, controlling timing and intensity of immune responses, maintaining self-tolerance, and preventing immune hyperactivity. In TME, these regulators inhibits immune responses, rendering the body incapable of mounting an efficient immune response against cancer, thus facilitating immune evasion (82). Common immune checkpoints in PTC include programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), cytotoxic T lymphocyte antigen 4 (CTLA-4), and indoleamine 2,3-dioxygenase (IDO) (83).

A recent study (15) revealed that several key immune checkpoints, including LAG3, PD-1, and IDO1, are inhibited in early PTC compared to normal thyroid tissue, potentially associated to the prevention of immune cell-mediated damage to healthy thyroid tissue. Interestingly, during the pathological stage, most of the immune checkpoints were upregulated, particularly the N stage, advanced. Likewise, the BRAFV600E mutation has been associated with the elevation of most checkpoints (84, 85).

3.1 Programmed cell death protein 1/ Programmed cell death ligand 1

The PD-1/PD-L1 pathway has emerged as a vital suppressive regulator in cancer. The overexpression of PD-L1 suggests that PD-L1 undermines immune surveillance of tumor in TME (86). Due to the cell and tissue-specific distribution of PD-L1, PD-1 play its part in at distinct stages of T cell activation, altering T cell function under antigen-specific stimulation, inhibiting CTLs, and enhancing tumor proliferation and invasion (87, 88). When T cells are recognized with PD-L1-positive tumor cells, tumor cells can cause programmed T cell death. In addition, tumor cells can produce cytokines including IL-10, allowing tumor cells to escape the clearance of CTL (47).. These mechanisms facilitate immune evasion by thyroid cancer cells and play a critical role in the transformation of normal cells into tumor cells (89).

PD-1 is widely expressed on lymphocytes capable of receiving antigen stimulation, acting as a "rheostat" for immune responses and regulating lymphocyte reactions to antigens. During antigen recognition, PD-1 binds to its ligands, recruiting tyrosine phosphatase (SHP-2), which can dephosphorylate and inactivate proximal effector molecules of antigen receptors on lymphocyte surfaces (87), such as inactivating Zap70 in T lymphocytes to inhibit TCR signaling (90) or inactivating Syk in B lymphocytes to inhibit BCR signaling (91).

PD-1's effects on biochemical signaling pathways also promote T cell conversion of naive into inducible Treg (iTreg) cell through various mechanisms. Firstly, PD-1 enhances Foxp3 expression by inhibiting Akt activation (92). Secondly, by inhibiting cyclin-

dependent kinase 2 (Cdk2), PD-1 amplifies Smad3-mediated transactivation by transforming growth factor β (TGF-β) (93, 94), promoting Foxp3 transcription (95). Thirdly, through metabolic reprogramming of activated T cells, PD-1 inhibits glucose metabolism (96) and promotes fatty acid β-oxidation (97), specifically activating metabolic programs that support Treg cell generation while inhibiting Th0 cell differentiation into Th1 or Th17 cells (98, 99). Therefore, targeting PD-1 and its downstream signaling pathways is an effective means of improving immunity in cancers. The PD-1 pathway represents one of the primary factor in immune escape. Given their specificity and significance, PD-1-blocking agents have shown considerable promise in cancer immunotherapy. Currently, these agents are widely employed in diagnosing and treating clinical diseases, exhibiting high clinical value for advanced cancers. They hold the potential to control other immune diseases through PD-1 signaling as well (100) (Figure 1).

3.2 Cytotoxic T lymphocyte antigen-4

CTLA-4 is a transmembrane protein implicated in immune regulation, typically occur on activated T cells. It attenuates T cell activation primarily by inhibiting the CD28 costimulatory signal (Figure 2). This is partially due to its competition with CD28 for recognition to CD80 and CD86 on APCs, which obstructs costimulatory signals essential for T cell activation and prevents downstream signal transduction promoting T cell activation and proliferation (101, 102). Consequently, CTLA-4 makes it difficult for T cells to activate. Upon CTLA-4 activation, T cell activation and IL-2 secretion are diminished, exerting a negative regulatory effect on tumor immunity (Figure 3). Recent studies have also demonstrated that PD-1+Tim-3+CD8+ T lymphocytes exhibit varying degrees of functional impairment in patients with regional metastatic PTC (103).

In comparison, PD-1 indirectly hinders TCR or BCR responses to antigens via intracellular signaling, while CTLA-4 entirely obstructs CD28 costimulation through competitive inhibition, acting more comprehensively and rapidly (87).

3.3 Indoleamine 2, 3-dioxygenase 1

Indoleamine 2,3-dioxygenase 1 (IDO1) is a oxidoreductase responsible for catalyzing. In papillary thyroid microcarcinoma (PTMC), 31% of the cells were positive for IDO, which may be associated with tumor metastasis (104). In cancer, IDO1 can exert an immunosuppressive function, and its expression is significantly correlated with FoxP3. This relationship promotes tumor immune

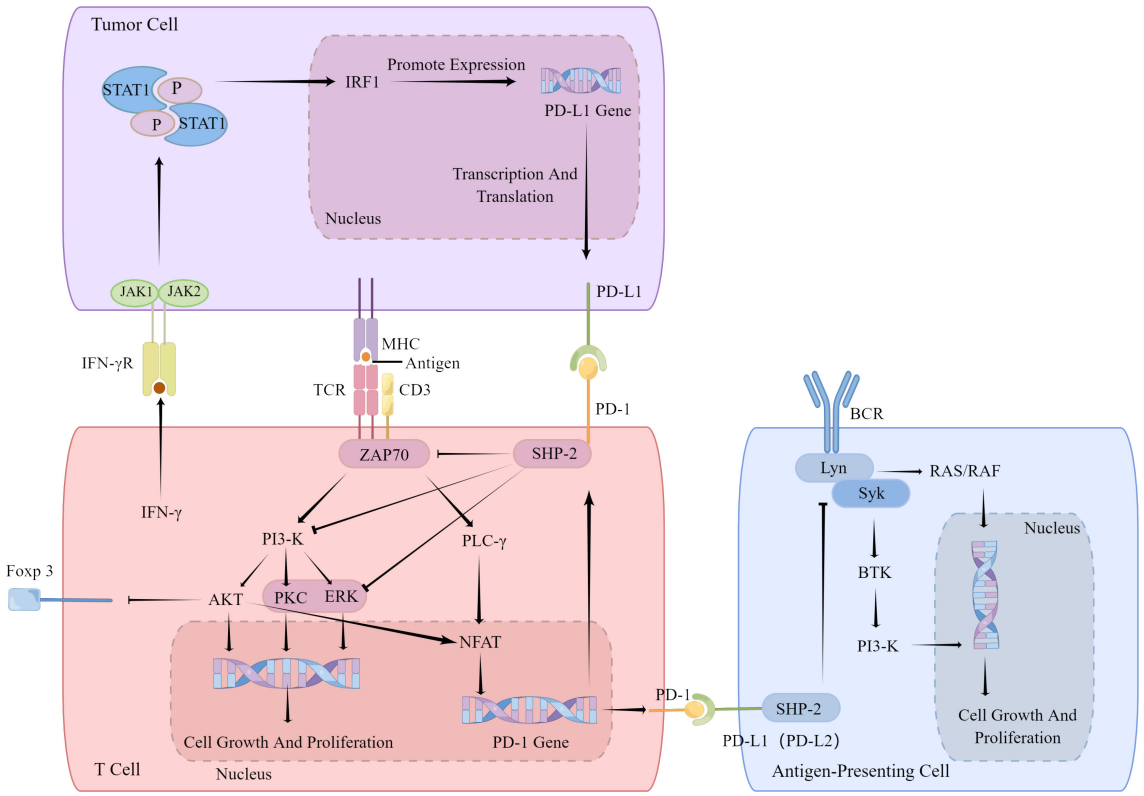

FIGURE 1
PD-1 inhibit TCR and BCR signaling. PD-1 inhibits the co-stimulatory signal of T cell activation by raising SHP-2,so that T cells cannot be activated normally and lead to increases Foxp3 expression. IFN-γ secreted by T cells will induce tumor cells to express PD-1 receptor PD-L1. PD-1 inhibits B cell activation by inhibiting downstream signal of BCR. IFN-γ, interferon-γ; IRF1, Interferon regulatory factor 1; CD3, coreceptor; PI3-K, SHP-2, ZAP70, JAK1 and JAK2, kinases; PLC-γ, phospholipase C-γ; AKT, kinase; PKC, Protein kinase C; ERK, extracellular regulated protein kinases; NFAT, activating T nuclear factor; NF-κB, transcription factor; Lyn, Syk, BTK, kinases.

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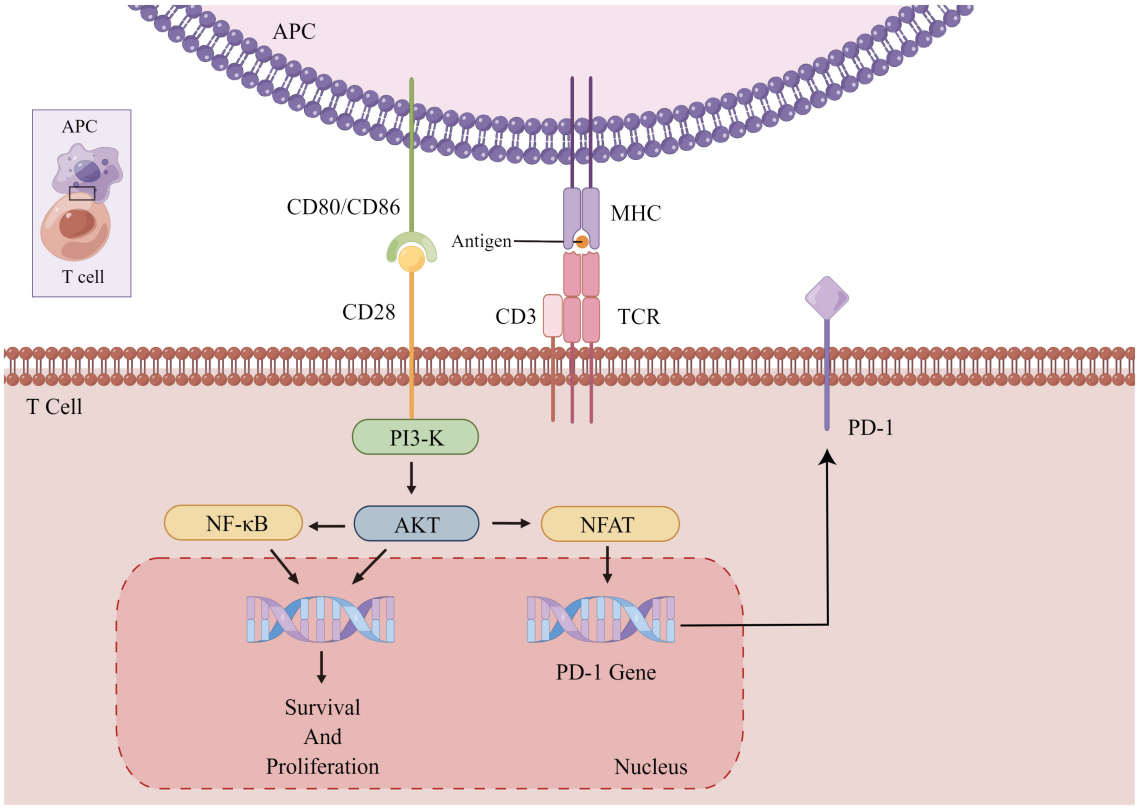

FIGURE 2
Activation and proliferation of normal T cells. The binding of CD28 and CD80/86 provides a co-stimulatory signal for T cell activation, causing T cell activation and proliferation. Chronically activated T cells increase the expression of PD-1 to prevent immune overshoot. APC, antigen-presenting cells; PI3-K, AKT, kinase; NF-κB, transcription factor; CD3, coreceptor; NFAT, activating T nuclear factor.

evasion by inducing FoxP3 phenotype regulation, consequently suppressing the immune microenvironment (105).

4 Regulatory effect of BRAF V600E mutation

BRAF is an activator of the RAS-regulated serine-threonine kinase and the MAPK signaling cascade. This pathway mediates the regulation of cell proliferation, differentiation, and survival in response to extracellular signals. The BRAFV600E mutation simulates phosphorylation in the activating fragment of BRAF, resulting in the dysregulation of cell proliferation (106).

The BRAFV600E gene mutation is closely related to elevated quantity of immunosuppressive regulators in PTC cells. Studies have reported (24) that CTLA-4 and PD-L1 expression levels are inversely associated with thyroid differentiation score (TDS) in PTC, a relationship more pronounced in tumors harboring the BRAFV600E mutation. BRAFV600E tumors expressed higher levels of PD-1 compared to BRAF wild-type tumors (53% vs. 12.5%). BRAFV600E promotes thyroid cancer development by increasing myeloid-derived suppressor cells (MDSCs) (107). As a heterogeneous population of immature myeloid cells, MDSCs are the primary coordinator of the immunosuppressive environment in cancer. MDSCs, primarily through CXCR2, show ligand recruitment to the TME (108). MDSCs are amplified during cancer progression and has the remarkable ability to inhibit T cell function in the tumor microenvironment (109), which is able to produce mediators necessary for neoangiogenesis and tissue invasion (110). In the peripheral circulation, MDSCs promote PTC progression. By inhibiting miR-486-3p, MDSCs promoted the activity of the NF-κB2 signaling pathway, leading to the accelerated invasion (111).

In addition, BRAFV600E upregulated T-box transcription factor 3 (TBX3) induced MAPK pathway activation. Therefore, TBX3 could be associated with BRAFV600E-related tumor genesis (112). TBX3 belongs to the T-box transcription factors family, associated with tumor progression and metastasis (113). Analysis of PTC patient specimens revealed that TBX3 is highly expressed in cancerous thyroid cells, indicating down regulation of TBX3 could delay the G1/S phase transition, decreased cell growth *in vitro* and inhibited tumor formation *in vivo* (114).

Considering the strong correlation between BRAF and the pathological characteristic of PTC, BRAF mutation status has the potential to serve as a risk assessment indicator and prognostic marker for PTC. However, similar prediction models are challenging to adapt to multivariate factors, such as patient age and gender, which may increase the cost and complexity of evaluation. These limitations necessitate further exploration (115). Beyond risk assessment and prognosis, the BRAF mutation may

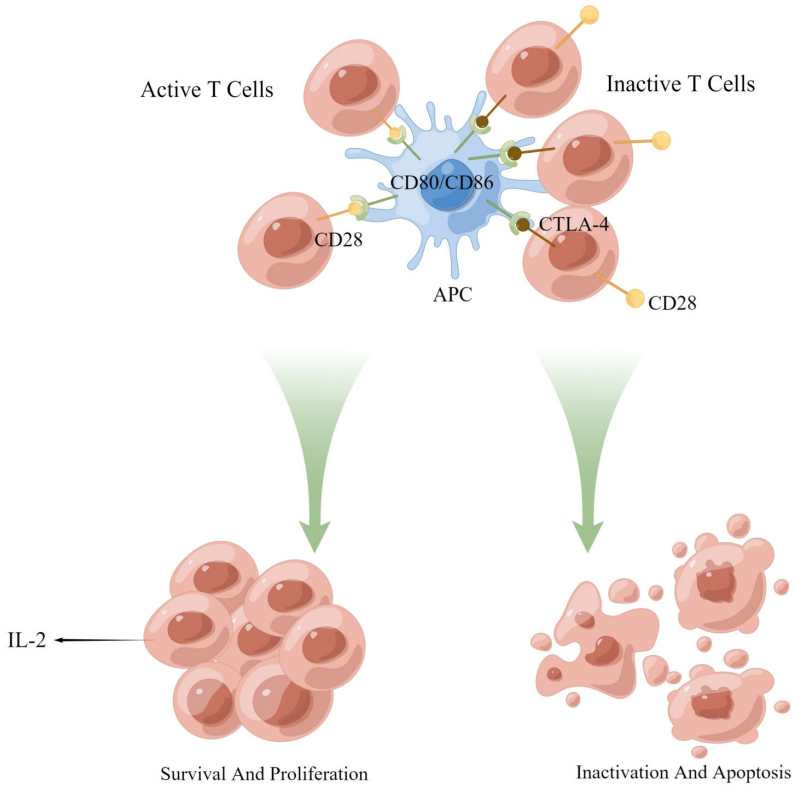

FIGURE 3
Activation and proliferation of normal T cells. The binding of CD28 and CD80/86 provides a co-stimulatory signal for T cell activation, causing T cell activation and proliferation. Chronically activated T cells increase the expression of PD-1 to prevent immune overshoot. APC, antigen-presenting cells; PI3-K, AKT, kinase; NF-κB, transcription factor; CD3, coreceptor; NFAT, activating T nuclear factor.

play a crucial role as a therapeutic target for PTC. Currently, BRAF kinase inhibitors have been utilized in non-small cell lung cancer and melanoma, while research on PTC treatment remains in its early stages (116).

5 Immunotherapy strategies for PTC

For patients with advanced PTC or distant metastases, conventional therapies, including chemotherapy and radiotherapy, are prone to developing tolerance (117), thereby limiting their effectiveness. Consequently, treatment options for patients with advanced disease or distant metastases are restricted. Harnessing the immune system appears to be a highly promising strategy for addressing these challenges.

5.1 Adoptive cell therapy

Adoptive cell therapy (ACT) involves the extraction of precursor cells from autologous or allogeneic anti-tumor effector cells, followed by their *in vitro* induction, activation, and expansion using activators such as IL-2 and specific peptides. Proliferating cells are then transfused back into cancer patients and enhance their anti-tumor immunity, aiming to achieve therapeutic effects and prevent recurrence (118, 119).

Phase I clinical trial results have demonstrated that dendritic cells stimulated with autologous PTC tumor lysates can effectively control tumor progression without significant adverse effects (120). In this study, patients with refractory PTC and distant metastases were selected, and some experienced stabilization after treatment, confirming the feasibility of ACT for advanced PTC management.

Apart from DCs, chimeric antigen receptor T (CAR-T) cell immunotherapy has also undergone modifications and been applied in clinical practice in recent years. Genetic engineering techniques enable the addition of chimeric antibodies to T cells, allowing T cells to recognize and simultaneously activate tumor cell killing. There has been preclinical validation on the therapy for intercellular adhesion molecule (ICAM)-1 in thyroid cancer. Based on previous study findings (121), some investigators (122) have verified the feasibility of ICAM-1 as a CAR-targeting antigen by examining its relationship with tumor malignancy in patients with recurrent advanced PTC lacking other treatment options. Other studies (123, 124) have also reported a favorable safety profile for this therapy, suggesting the potential of ICAM-1 as a target for treatment of advanced recurrent thyroid tumors.

Since T cells upregulate ICAM-1 expression upon activation, ICAM-1 CAR-T cells may engage in mutual attacks, potentially reducing T cell infiltration into PTC tissues and causing collateral

tissue damage (125). Therefore, further refinement is necessary before this therapy can be widely adopted in clinical practice.

5.2 Immune checkpoint inhibitors

Immune checkpoint inhibitors (ICIs) are monoclonal antibody (mAb) drugs developed to target specific immune checkpoints. Tumor cells cannot interact with immune cells through immune checkpoints above when ICIs are applied, which can block immune checkpoint-mediated immune escape. There has been monoclonal antibodies against PD-1/PD-L1 and CTLA-4, such as pembrolizumab and ipilimumab (126).

Existing trials have demonstrated that ICIs exhibit good efficacy and safety in PTC treatment (126, 127). The potential of combining ICIs with currently available drugs for advanced thyroid cancer has garnered interest. Animal studies have confirmed that combinations of BRAF inhibitors and checkpoint inhibitor immunotherapies synergistically reduce tumor volume in mouse models of carcinoma (128). However, mAbs can sometimes cause immune-related adverse events resembling autoimmune reactions (129), prompting consideration of small molecule inhibitors as alternative therapeutic strategies. Unlike mAbs, small molecule inhibitors can interact with both receptor on the surface and intracellular molecular targets (26), making them a promising therapeutic approach.

The efficacy of ICIs is influenced by the host's immune status, as they target immune checkpoints and the function of immune cells and molecules in TME changes accordingly. Intrinsic microorganisms contribute to the body's overall and local immunological regulation and can significantly impact the efficacy of ICIs (130). In PTC, VEGF can inhibit DC antigen presentation, enhance Treg amplification, and mediate the upregulation of PD-1 on T cells in TME. Combining VEGF inhibitors with ICIs can synergistically promote immune checkpoint blockade effect (131–133). Given the unique influence of the immune microenvironment on tumor progression, the combination of anti-inflammatory drugs and ICIs is also common. For instance, aspirin is widely used in cancer treatment and can reduce the mortality rate of various adenocarcinomas (134). Metformin and phenformin affect angiogenesis (135), regulate immune responses (136), and can be used in combination with ICIs. Consequently, to widely apply ICIs in the clinical treatment of PTC, a comprehensive assessment of the patient's immune status is necessary.

6 Conclusion

In summary, immune cells and molecules in TME are of vital importance in papillary thyroid carcinoma (PTC) progression by modulating immune response against cancer. Immune checkpoints are regulatory molecules in the immune system, with the PD-1/PD-L1 and CTLA-4 pathways emerging as significant contributors to tumor immunosuppression. Furthermore, the BRAFV600E mutation is intimately linked to PTC development and progression, potentially leading to aberrant cell proliferation and subsequent PTC onset. BRAFV600E also exerts a regulatory effect on immune checkpoints. CTLA-4 and PD-L1 levels are inversely associated with TDS, particularly in tumors harboring the BRAFV600E mutation. Consequently, BRAFV600E may serve as a critical target and prognostic marker for PTC treatment.

Patients with advanced disease or distant metastases face limited treatment options, making the utilization of the immune system a particularly promising approach. Adoptive cell therapy, utilizing dendritic cells (DC) and chimeric antigen receptor T (CAR-T) cells, has proven effective for patients with advanced PTC. Employing immune checkpoint inhibitors (ICIs) to modulate PD-1 targets and their downstream signaling pathways effectively enhances the host's immunity to cancer; however, ICIs can sometimes result in immune-related adverse events, warranting consideration of small molecule inhibitors as an alternative. Moreover, ICI efficacy is easily influenced by gut microorganisms and the body's immune levels, necessitating the assessment of the host's immune status during treatment. Combination of ICIs with vascular endothelial growth factor (VEGF) inhibitors or anti-inflammatory drugs has demonstrated improved efficacy and is expected to offer potential therapeutic value for PTC management.

Author contributions

XZ: Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. RS: Formal Analysis, Investigation, Methodology, Writing – original draft. TW: Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Sarcomatoid carcinoma transformation in oral undifferentiated carcinoma following sequential immune combined targeted therapy: a case report

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The diagnosis and treatment of head and neck undifferentiated carcinoma (HNUC) present significant challenges. Herein, we present the case of a patient with advanced HNUC who underwent conversion surgery following treatment with a combination of pembrolizumab and nimotuzumab. During therapy, histological transformation from undifferentiated to sarcomatoid carcinoma was detected at the primary site. This case not only highlights the potential of immune combination-targeted therapy to reduce tumour burden and increase the surgical options for patients, but also reveals the complex alterations in tumour biology that may occur during treatment. It emphasizes the necessity for routine pathological assessments throughout the therapeutic regimen to guide personalised therapeutic strategies and optimise patient prognoses.

KEYWORDS

undifferentiated carcinoma, sarcomatoid carcinoma, pathological transformation, immunotherapy, targeted therapy, conversion therapy

Introduction

Head and neck undifferentiated carcinoma (HNUC) is a rare subtype of head and neck cancer (HNC) characterised by low incidence and poor prognosis (1, 2). While HNUC is more common in the nasopharynx, its occurrence in the oral cavity is extremely rare (2–4). Despite exploration of multimodal therapies for HNUC, no consensus has been reached regarding the optimal treatment regimen or sequence. The 2022 first edition of the National Comprehensive Cancer Network (NCCN) guidelines recommends immune combination targeted therapy for special cases of head and neck squamous cell carcinoma (HNSCC). In some cases, Pembrolizumab (PD-1 inhibitor), is reported to have potential efficiency in

treatment of HNUC (5). Although Sakamoto et al. reported a successful case of combined targeted therapy and chemotherapy for undifferentiated carcinoma (UC) of the tongue (6), the efficacy of combined immune and targeted therapies for UC of the oral cavity remains unclear.

Pathological transformation has been reported in various solid tumours and may be correlated with a poor prognosis (7, 8). Recently, immune and/or targeted therapy-induced pathological transformations have been associated with potential acquired resistance in patients (9). For example, the transformation of non-small cell lung cancer (NSCLC) to small-cell lung cancer (SCLC) represents a significant mechanism of resistance to chemotherapy, immunotherapy, and targeted therapy (10). Combined immune target-related pathological transformations are rare in HNC. Here, we report a rare case of a patient with advanced PD-L1+ and EGFR+ HNUC who underwent pathological transformation from HNUC to sarcomatoid carcinoma (SC) following combined immune and targeted treatment. This case evaluates the potential efficacy of combined immune and targeted therapy for UC of the oral cavity and subsequent treatment strategies following pathological transformation.

Case report

A 60-year-old male patient, with a long history of smoking and alcohol consumption presented with a painful lesion on the left lower gingiva lasting over one year, and a left submandibular mass for more than one month. On May 31, 2023, he consulted the Department of Oral and Maxillofacial Surgery at the Affiliated Hospital of Qingdao University, where a pathological biopsy revealed a malignant tumour of the left lower gingiva (Figures 1A, a–d). An enhanced computed tomography (CT) scan of the neck showed osteolytic destruction in the body and ramus of the left mandible, measuring approximately 34mm x 42mm x 77mm, and multiple enlarged lymph nodes in the neck (Figure 2B, a-b). The patient initially declined the recommended surgical intervention and opted for chemotherapy instead. On June 10, 2023, he started chemotherapy with the TPF regimen (Docetaxel, 350mg, d1; Cisplatin, 60mg, d1-d2; Capecitabine, 1.5g, d1-d14) in the Oncology Department. However, on the sixth day of taking Capecitabine, the patient experienced seizures and developed severe anemia, requiring blood transfusions due to unstable vital signs. And Capecitabine was discontinued on June 16, 2023. Subsequently, chemotherapy was discontinued, and the patient chose to receive palliative care at home. The tumour size increased drastically, with the left submandibular mass growing rapidly. It eventually breached the skin, leading to ulceration and bleeding (Figures 2A, a).

On July 18, 2023, the patient presented to the oral emergency department with uncontrolled bleeding from the lesion. Hemostasis was achieved through packing and application of pressure. Microscopic examination of the biopsy tissue showed tumour cells with an epithelial-like morphology, diffusely distributed in sheets, with no evidence of squamous differentiation or glandular structures. Additional immunohistochemistry (IHC) on the biopsy sample of the

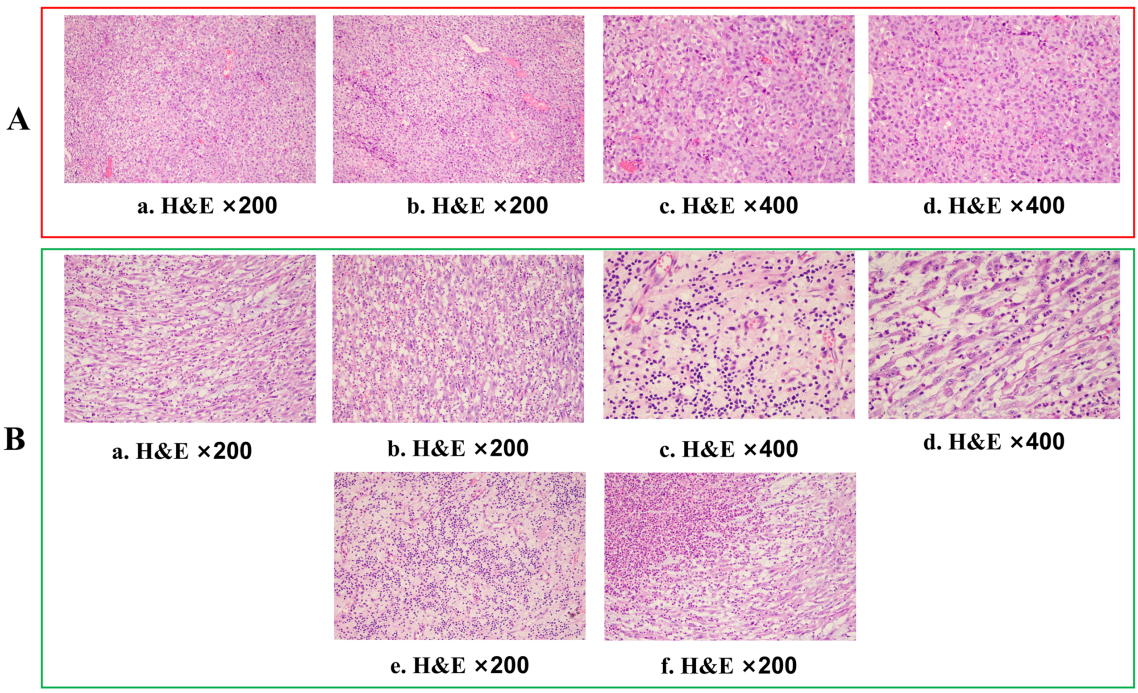

FIGURE 1
Histopathological assessment of tumour tissue. **(A)** Haematoxylin and eosin (H&E) staining (×200 and ×400) of a gingiva biopsy showed tumour cells with an epithelial-like morphology, diffusely distributed in sheets, with no evidence of squamous differentiation or glandular structures. (**B**, a–d). Haematoxylin and eosin (H&E) staining (×200 and ×400) of the gingival biopsy revealed that the tumour cells predominantly exhibited a spindle-shaped morphology. (e, f) In some areas, the tumour exhibited significant degeneration and necrosis, accompanied by histiocytic response and marked lymphocytic infiltration.

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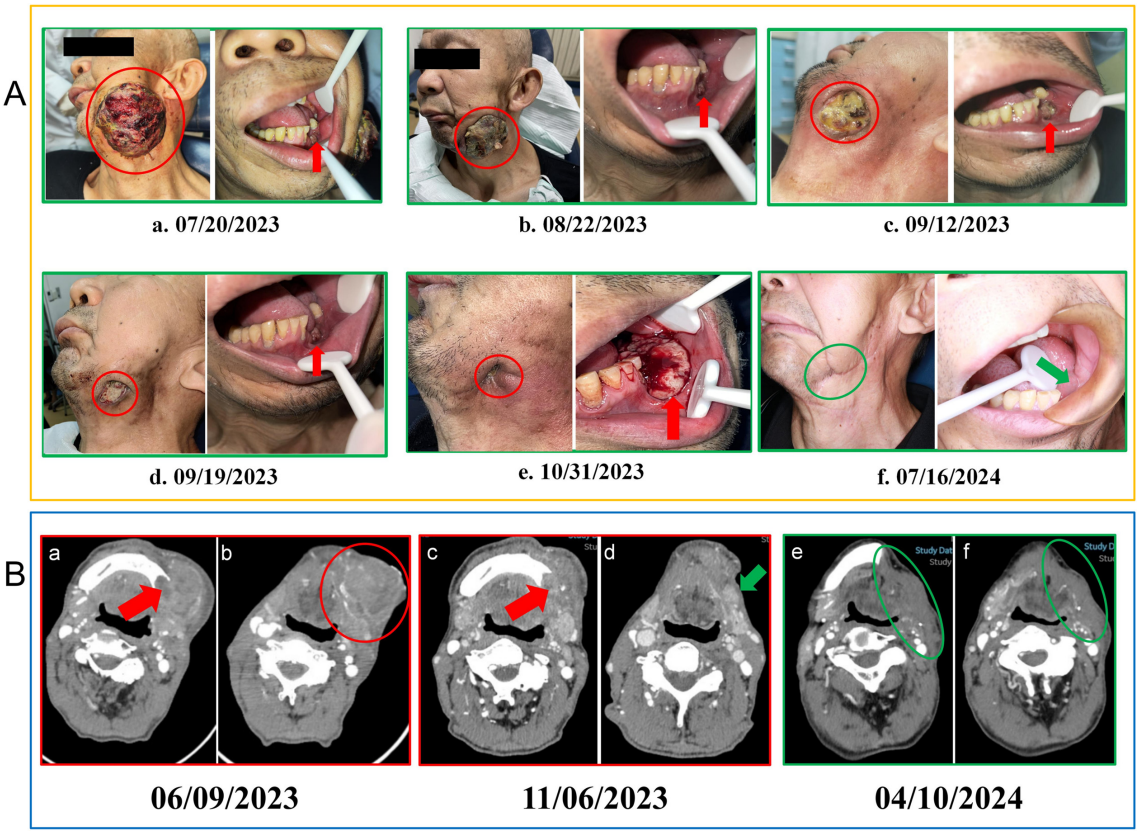

FIGURE 2
(A) Changes in the lesions during treatment. (a) Prior to treatment, the intraoral lesion displayed tooth loss in the left lower molar region with destruction of the alveolar ridge, while the left submandibular metastatic lesion showed surface ulceration and bleeding. (b) After two cycles of treatment, the intraoral lesion showed minor reduction, and the left submandibular metastatic lesion decreased significantly. (c) Nine days after three cycles, the intraoral lesion remained relatively unchanged, while the submandibular lesion demonstrated showed further reduction. (d) Sixteen days after three cycles, the intraoral lesion remained stable, and the submandibular lesion maintained a PR status. (e) Fourteen days after five cycles of treatment, the tumour in the left lower gingiva showed rapid growth, while the submandibular lesion had nearly disappeared. (f) Five months post-surgery, no recurrence of the lesion was observed. Red circles mark the extraoral lesion, red arrows point to the intraoral lesion, while green arrows and circles indicate the intraoral and extraoral wound status 5 months post-surgery. PR, partial response; CT, computed tomography; **(B)** Patient imaging examinations during the course of the disease. (a, b) At the time of initial diagnosis, a CT scan of the neck revealed osteolytic destruction of the left mandibular body and ramus, along with left-sided lymphadenopathy. (c, d) Following five cycles of combined immune-targeted therapy, no significant change was noted in the bony destruction, but the surrounding soft tissue mass had reduced. (e, f) A follow-up CT scan on April 10, 2023, five months post-surgery, showed no signs of recurrence. Red arrows indicate the mandibular destruction area, red circles mark the submandibular metastatic lesion, green arrows show the submandibular lesion after 5 cycles of combined targeted therapy, and green circles represent the original lesion site 5 months post-surgery.

patient's intraoral lesion showed Ki-67 (+, 30%), CK (cytoplasmic +), CK5/6 (-), P40 (-), EBER (-), P16 (-), HMB45 (-), LCA (-), EMA (-), ERG (-), U145 PU.1 (-), PD-L1 (22C3) (CPS:80), and EGFR (+) (Figures 3A, a–h). Based on the histological morphology and IHC results, malignancies such as melanoma, lymphoma, and angiosarcoma were ruled out, and the patient was diagnosed with advanced-stage HNUC. Given the patient's current overall condition, treatment tolerance, tumour imaging, and personal preference, a multidisciplinary team (MDT) recommended immune combined targeted therapy (pembrolizumab 200 mg, q3w, IV drip; nimotuzumab 400 mg, q3w, IV drip). The patient first received Pembrolizumab + Nivolumab on July 21, 2023, followed by subsequent cycles every 21 days: Second cycle on August 11, 2023, Third cycle on September 3, 2023, Fourth cycle on September 24, 2023, Fifth cycle on October 16, 2023.

After two cycles of immune combined targeted therapy, both the left submandibular and primary lesions exhibited significant reduction (Figures 2A, b). After the third cycle, the lesions further reduced (Figures 2A, c, d). By the end of four cycles, the intraoral and submandibular lesions had regressed almost completely. According to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, the patient achieved a partial response (PR). Nonetheless, after the fifth cycle, tumour proliferation recurred at the primary site of the left lower gingiva (Figures 2A, e). After immune combined targeted therapy, the patient's general condition significantly improved, and the willingness for treatment markedly increased owing to the perceived hope of the treatment. Additionally, the patient's Eastern Cooperative Oncology Group Performance Status (ECOG PS) score improved from 3 to 1. Based on the patient's physical condition and disease status, MDT recommended

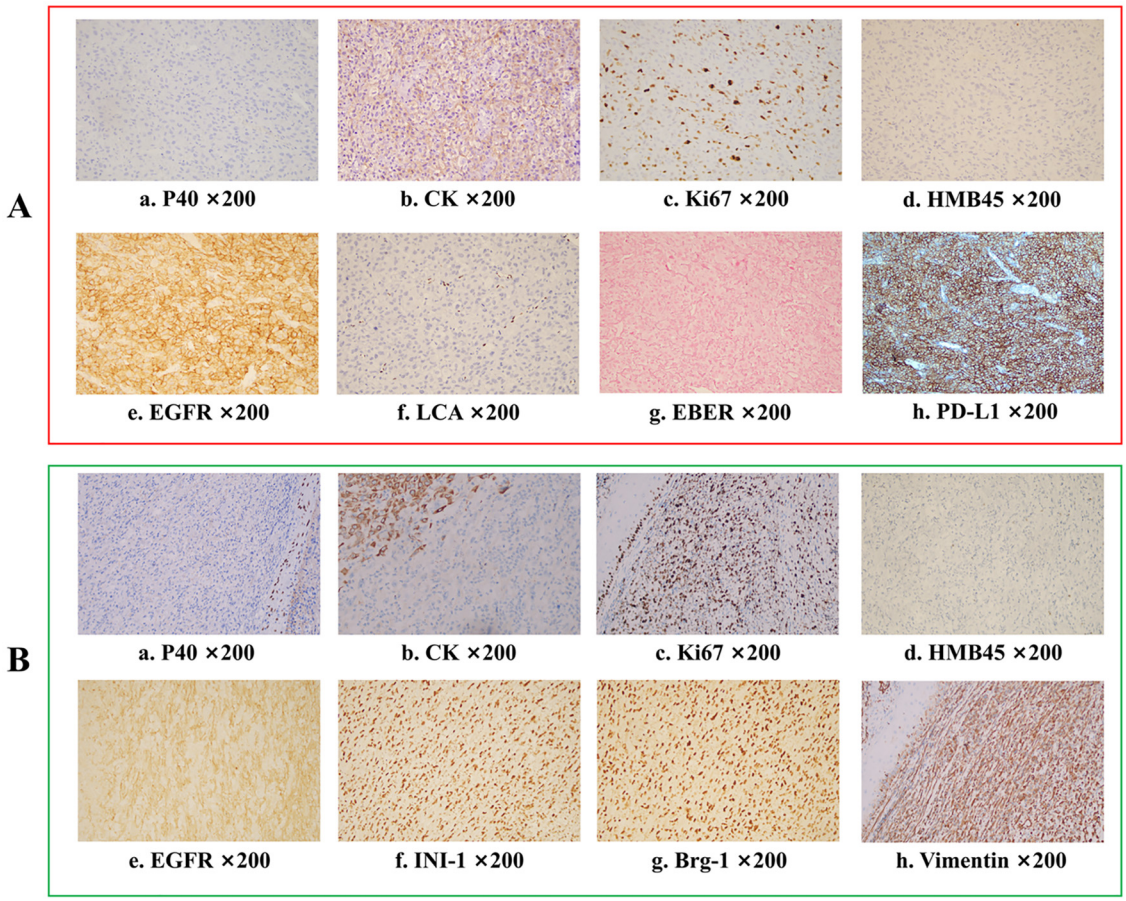

FIGURE 3
Immunohistochemistry analysis for tumours before and after pembrolizumab combined with nimotuzumab treatment. **(A)** Pathological results before treatment indicate undifferentiated carcinoma Ki-67 (+, 30%), CK (cytoplasmic +), CK5/6 (-), P40 (-), EBER (-), P16(-), HMB45(-), LCA(-), EMA(-), ERG (-), U145 PU.1(-), PD-L1(22C3)(CPS:80), EGFR (+). **(B)** Final pathological results after surgery showed a sarcomatoid carcinoma Ki-67 (+, 70%), CK (focal +), CK5/6 (-), p40 (-), p16 (-), HMB45 (-), p63 (-), S100(-), INI-1 (+), MyoD1 (-), Vimentin (+), Brg-1(+), p53 (++, 60%), EGFR (+).

a local surgical intervention. Subsequently, extended resection of the primary tumour site and neck lymphadenectomy were performed. Preoperative CT scans revealed a significant reduction in the lesion size (Figure 2B, c-d). On November 8, 2023, pathological examination following conversion surgery revealed that the tumour cells were predominantly spindle-shaped (Figures 1B, a–d), with significant tumour degeneration and necrosis in certain areas (Figures 1B, e), accompanied by histiocytic reaction and abundant lymphocytic infiltration (Figures 1B, f), indicative of post-treatment changes. No tumour metastasis was observed in the dissected cervical lymph nodes. IHC results showed: CK (focal +), Ki-67 (+, 70%), CK5/6 (-), p40 (-), p16 (-), HMB45 (-), p63 (-), S100 (-), INI-1 (+), MyoD1 (-), Vimentin (+), Brg-1 (+), p53 (++, 60%), and EGFR (+) (Figures 3B, a–h). Based on the histological morphology and IHC results, the pathological diagnosis was confirmed as SC. Twenty days postoperatively (November 28, 2023), the patient continued adjuvant monotherapy with pembrolizumab. A CT scan five months post-surgery showed good recovery at the original lesion site (Figure 2B, e-f), and the patient has been followed up for one year without recurrence. No adverse reactions were observed during the treatment. The comprehensive timeline of the patient's diagnosis, treatment, and lesion changes is depicted in Figure 4.

Discussion

HNUC is a poorly differentiated and aggressive disease, with limited consensus on optimal management owing to its rarity (2, 11). Sakamoto et al. found that cetuximab-targeted therapy could produce favourable outcomes in oral UC (6). Studies indicate that over 50% of patients with UC aberrantly express the PD-L1 protein, suggesting potential sensitivity to immune checkpoint inhibitors (12). Consequently, immune checkpoint inhibitors and targeted therapies have emerged as promising therapeutic strategies for UC management. According to our clinical experience with 150 patients treated with immune-based treatment regimens, we found that some patients achieved positive outcomes after 2-4 cycles of treatment (13). However, immune resistance and disease progression may be observed in cycles 4-6, highlighting the need for incorporating local therapy. Consistent with this trend, our

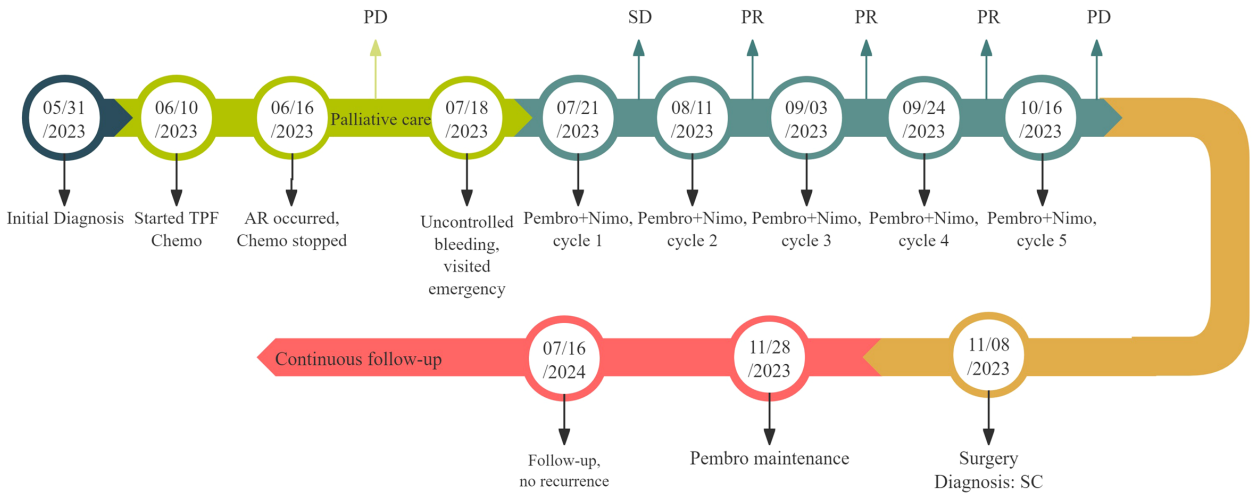

FIGURE 4
Timeline of clinical management and disease evolution. The patient began TPF Chemo on June 10, 2023. On June 16, 2023, severe AR (severe anemia) occurred, leading to the cessation of Chemo and the decision to opt for palliative care. During palliative treatment, the lesion gradually enlarged, and on July 18, 2023, the patient presented to the emergency department due to uncontrolled bleeding, which was managed with pressure bandaging. On July 21, 2023, the first cycle of Pembro combined with Nimo therapy was initiated, followed by the second cycle on August 11, the third cycle on September 3, and the fourth cycle on September 24. Over these four cycles, the lesions showed significant shrinkage. However, after the fifth cycle on October 16, 2023, the intraoral lesion suddenly enlarged, prompting a MDT recommendation for additional local surgery, which was performed on November 8, 2023. Twenty days post-surgery, on November 28, 2023, Pembro maintenance therapy commenced. Follow-up continued with no recurrence or metastasis observed. AR, adverse reaction; Chemo, chemotherapy; Pembro, pembrolizumab; Nimo, nimotuzumab; PD, progressive disease; PR, partial response; MDT, multidisciplinary team.

patient experienced significant tumour shrinkage and partial response after 4 cycles of combined immune-targeted therapy. Nonetheless, the emergence of a new tumour at the primary site following cycle 5 was indicative of disease progression.

Currently, determining the precise timing and optimal approach for incorporating local therapies after immunotherapy or targeted therapy remains a challenge. In our case, immunotherapy and targeted therapies provide new hope by converting initially inoperable tumours into surgically manageable lesions through a process known as 'conversion therapy' (14). Additionally, we considered the risks associated with radiation therapy, such as osteonecrosis and skin infections, and the potential increase in surgical complexity and complications if radiotherapy were to fails (15, 16). Finally, the MDT recommended surgery as a local treatment strategy. The findings of this unique case provide empirical evidence supporting the use of conversion therapy for HNUC.

Histological transformation can be considered a form of acquired resistance, and may be correlated with phenotypic alterations in the tumour induced by immunotherapy or targeted therapy (7, 17–19). In the context of lung cancer treatment, lung adenocarcinoma has been observed to transform into SCLC or more aggressive SC (7, 20–23). Liang et al. suggested a potential association between the transformation of lung adenocarcinoma to SC and the use of immune checkpoint inhibitors (7). Additionally, existing literature indicates that the transformation of lung adenocarcinomas into SC may also be linked to EGFR-targeted therapy (8). These histological changes may contribute to therapeutic resistance and a poor prognosis (10). A clear transition from UC to SC is crucial for guiding treatment strategies. A notable aspect of this case is the significant difference in the tumour's histological morphology before and after surgery. Preoperatively, the tumour exhibited an epithelial-like cell morphology, whereas postoperatively, it showed spindle cell morphology, with no evidence of squamous or glandular differentiation (24, 25). Comprehensive IHC testing was performed both preoperatively and postoperatively, ruling out other rare malignancies such as melanoma (HMB45 and S100 negative), lymphoma (LCA negative), angiosarcoma (ERG negative), and rhabdomyosarcoma (MyoD1 negative) (24–26). The preoperative diagnosis of UC was confirmed, and the postoperative loss of INI-1 or BRG-1 further supported the diagnosis of SC. In our case, the patient successfully underwent conversion therapy before surgery, and postoperative pathology revealed a transition from UC to SC.

Although SC is predominantly found in the lungs and kidneys, it is uncommon in the head and neck region and accounts for approximately 1% of all HNC cases (2, 27, 28). SC may require a distinct therapeutic approach compared to UC. The histological transformation observed in this case suggests that repeated pathological examinations may be necessary for disease progression to potentially guide adjustments in therapeutic strategies for improved outcomes. Additionally, we observed that SC transformation induced by immune-targeted therapies may differ from that induced by radiotherapy. Based on relevant reports and our case, we found that the latency of radiotherapy-induced SC transformation may be over a period of years, whereas immune-targeted therapies can induce more rapid transitions (7, 29). This finding has potential implications for clinical work. Further investigation is needed to understand the mechanisms and clinical implications of this phenomenon, including its predictive

value for prognosis and its impact on treatment strategies. Interestingly, preoperative imaging revealed enlarged cervical lymph nodes with enhancements suggestive of metastasis. However, postoperative pathology revealed thickened lymph node capsules without evidence of carcinoma. This discrepancy between imaging and pathology may indicate fibrotic repair following the regression of metastatic cancer cells in the lymph nodes owing to immunotherapy. In such cases, a fine-needle aspiration biopsy of the lymph nodes is recommended to minimise patient trauma.

This study highlights the importance of molecular matched therapies for rare malignancies without a standard of care. The combination of a PD-1 inhibitor with an EGFR inhibitor was effective and well tolerated by the patient, making conversion surgery feasible. Additionally, monitoring pathological transformations related to combined immune and targeted therapies is essential. Once disease progression occurs, rebiopsy can aid in diagnosing and managing the disease and providing timely guidance for adjusting the treatment plan.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of Affiliated Hospital of Qingdao University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JL: Conceptualization, Data curation, Formal analysis, Software, Writing – original draft. XZ: Conceptualization, Data curation, Methodology, Writing – original draft. WS: Conceptualization, Methodology, Resources, Writing – review & editing. KS: Formal analysis, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Neoadjuvant immunotherapy plus chemotherapy for squamous cell carcinoma of the paranasal sinus: a case report

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Background: Immune checkpoint inhibitors (ICIs) such as pembrolizumab and nivolumab are recommended as first-line therapies for recurrent and metastatic head and neck squamous cell carcinoma (HNSCC). However, their efficacy in neoadjuvant therapy remains uncertain.

Case presentation: We report the case of a 68-year-old male diagnosed with HNSCC who received neoadjuvant nivolumab (anti-PD-1 inhibitor) plus nab-paclitaxel and carboplatin. Biomarkerswere assessed by immunohistochemistry, and apoptosis-related molecules were analyzed via Western blotting. The patient achieved significant tumor regression and major pathological response (MPR) without severe adverse events. Post-treatment analyses revealed PD-L1 expression increased from 30% to 50% in tumor cells, CD8+ lymphocyte infiltration significantly improved, and Ki-67 expression was markedly reduced.

Conclusions: This case highlights the potential of combining ICIs with chemotherapy in neoadjuvant settings for HNSCC, providing mechanistic insights and clinical evidence for this emerging approach. Further studies are needed to establish the optimal neoadjuvant treatment regimen and identify patient populations most likely to benefit.

KEYWORDS

neoadjuvant, immunotherapy, chemotherapy, head and neck squamous cell carcinoma, major pathological response

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a significant global health burden, accounting for more than 800,000 new cases in 2020 and causing 400,000 deaths annually (1). Despite advances in surgery and chemoradiotherapy, approximately 60% of patients present with locally advanced disease at diagnosis, and nearly half experience

relapse within two years (2). This highlights an urgent need for more effective treatment strategies.

The standard neoadjuvant regimen, TPF (cisplatin + paclitaxel + 5-fluorouracil), has shown limited success in improving prognosis for all head and neck tumors (3, 4). Recently, growing evidence has demonstrated the promise of neoadjuvant immune checkpoint inhibitors (ICIs) across various malignancies. ICIs have achieved favorable pathological response rates (PCR) by harnessing the immune system's ability to target and eliminate cancer cells (5–7). For instance, the Checkmate 816 study highlighted the benefits of combining immunotherapy with chemotherapy in the neoadjuvant treatment of lung cancer, achieving significant clinical benefits, including stage reduction and improved R0 resection rates, with a 13.6% increase in PCR compared to chemotherapy alone (6).

In the context of recurrent and metastatic HNSCC, ICIs have demonstrated survival benefits and are now integral to first-line treatment strategies (8–10). Key studies such as Checkmate-141, Keynote-040, and Keynote-048 have driven a paradigm shift in the treatment of relapsed/metastatic HNSCC, paving the way for immunotherapy to play a transformative role in earlier disease stages (8, 11–13). These findings suggest that neoadjuvant immunotherapy, particularly in combination with chemotherapy, could offer novel therapeutic avenues for patients with unresectable HNSCC.

In this case report, we describe an HNSCC patient who received neoadjuvant immunochemotherapy (nivolumab plus Nab-paclitaxel/carboplatin), resulting in a major pathological response (MPR). This case highlights the potential of combined immunotherapy and chemotherapy to achieve significant clinical benefits in HNSCC and underscores the need for further exploration of this treatment approach.

Method

Clinical data were collected from a patient diagnosed with ethmoid sinus carcinoma, treated at the Department of Medical Oncology, Qilu Hospital (Qingdao, China). The inclusion criteria for this study included patients diagnosed with locally advanced, non-metastatic squamous cell carcinoma of the head and neck (HNSCC) (cT4N0M0), confirmed by imaging and pathological examination. Patients were required to have adequate organ function and Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. Exclusion criteria included prior exposure to immunotherapy, presence of distant metastases, severe comorbidities contraindicating immunotherapy or chemotherapy, or inability to provide informed consent. Tumor biomarkers, including PD-L1, Ki67, CD8, and IFN-γ, were evaluated through immunohistochemistry (IHC) on baseline and postoperative samples. For PD-L1 detection, SP263 staining was utilized following standard protocols.

Protein expression analysis was performed using Western blotting, as described in previous studies. Antibodies targeting Caspase-7, Caspase-3, Bcl-2, and Bax were obtained from Proteintech and diluted at ratios ranging from 1:800 to 1:2000. GAPDH was used as a loading control to ensure consistent protein quantification.

During follow-up, craniocerebral magnetic resonance imaging (MRI) was conducted every three months for the first two years to monitor disease progression. Treatment-related toxicities were assessed in accordance with the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Ethical approval for this study was obtained from the Ethics Committee of Qilu Hospital (Qingdao, China) under the registration number KYLL-2023083. Written informed consent was obtained from the patient before participation.

Results

In January 2022, a 68-year-old Asian male worker presented to our hospital with a two-month history of headache, diplopia, and restricted eye movement. His Eastern Cooperative Oncology Group (ECOG) Performance Status was 1. The patient had a 10-year history of hypertension, controlled with oral ACE inhibitors, and a 20-year history of diabetes, managed with insulin. He also reported a 30-year smoking history (approximately five cigarettes per day) and a 30-year history of alcohol consumption (about 100 g per day).

Pre-treatment magnetic resonance imaging (MRI) revealed an occupying lesion in the ethmoid sinus, involving the frontal bone, sphenoid bone, left orbit, and anterior cranial fossa meninges. The lesion encased the left superior rectus muscle, superior oblique muscle, and optic nerve. A nasopharyngeal biopsy confirmed moderately differentiated papillary squamous cell carcinoma (SCC, cT4N0M0). Immunohistochemistry showed CK5/6(+), p63(+), p40(+), EGFR(+), Ki-67 (+50%), and p16(-). *In situ* hybridization demonstrated EBER(-), while PD-L1 expression was 30% in tumor cells (TC) and 10% in immune cells (IC).

Given the extensive tumor burden and the patient's severe symptoms (e.g., headache, diplopia, and restricted eye movement), organ preservation was a high priority for the patient and his family. Considering the patient's advanced age and inability to tolerate an aggressive TPF regimen, we opted for a combination of nivolumab and chemotherapy based on evidence suggesting improved objective response rates (ORR) with this approach in other cancer types. On February 16, 2022, the patient began treatment with nivolumab (360 mg on day 1), albumin-bound paclitaxel (125 mg/m² on days 1 and 8), and carboplatin (AUC = 5 on day 1), administered every three weeks for three cycles.

Following three cycles of immunochemotherapy, MRI demonstrated significant tumor shrinkage (Figure 1). In April 2022, the patient underwent left whole-group sinus opening, bilateral middle turbinectomy, and left sinus tumor composite resection via nasal endoscopy. Postoperative pathological analysis revealed only well-differentiated SCC scattered in the left orbital wall, with no residual tumor cells in the left ethmoid sinus, frontal sinus, or nasal cavity.

Subsequently, the patient received concurrent chemoradiotherapy because only major pathological response (MPR) rather than

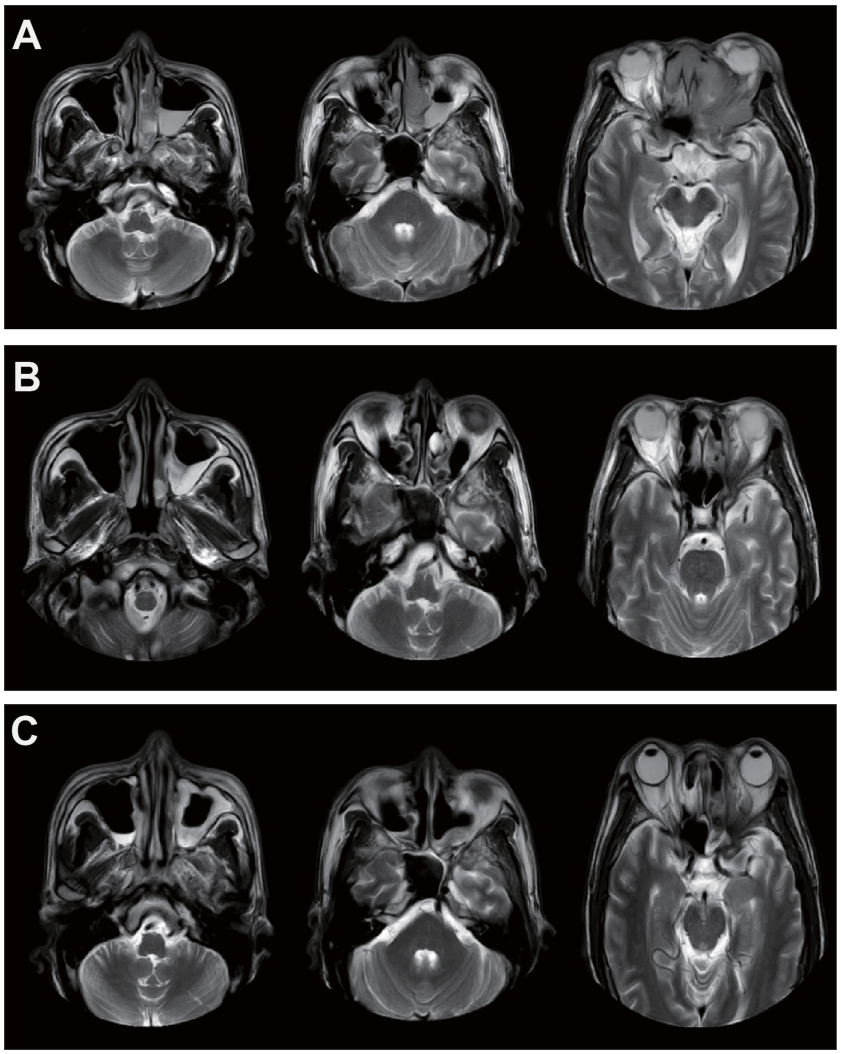

FIGURE 1
Imaging examinations of the patient during treatment. **(A)** MRI of the primary tumor before treatment. **(B)** MRI after three cycles of neoadjuvant therapy with nivolumab. **(C)** Imaging results following neoadjuvant therapy, surgery, and concurrent chemoradiotherapy.

pathological complete response (PCR) was achieved. Intensity-modulated radiotherapy (IMRT) was delivered with the following parameters: 60.06 Gy for 95% PTV in 33 fractions (1.82 Gy per fraction) and 66 Gy for gross tumor volume (GTV) in 33 fractions (2 Gy per fraction). Radiotherapy was administered five days a week using a Varian linear accelerator (6 MV X-ray). Concurrent chemotherapy consisted of cisplatin (40 mg weekly). At the last follow-up, the patient remained progression-free for over 20 months.

Throughout treatment, adverse events (AEs) were well-tolerated. No grade 3–4 AEs were observed, while grade 1–2 AEs included myelosuppression, nausea, vomiting, and radiation-induced skin injury.

To investigate the therapeutic mechanism, we assessed molecular markers of proliferation and apoptosis in baseline and post-treatment tumor specimens. Immunohistochemical analysis revealed a significant increase in PD-L1 and IFN-γ expression in tumor-infiltrating immune cells, along with elevated CD8+ cell infiltration and a reduction in Ki-67 expression (Figure 2). Western blot analysis demonstrated increased expression of pro-apoptotic proteins Caspase-7, Caspase-3, and Bax, accompanied by decreased expression of the anti-apoptotic protein Bcl-2 (Figure 3).

Discussion

Neoadjuvant immunotherapy has demonstrated promising safety and tolerability profiles in HNSCC patients, alongside significant tumor shrinkage effects (14–17). In recent years, clinical studies investigating combinations of neoadjuvant immunotherapy with chemotherapy, radiotherapy, and targeted therapies in HNSCC have shown broad application potential for earlier-stage disease. Approximately 75% of HNSCC patients treated with neoadjuvant

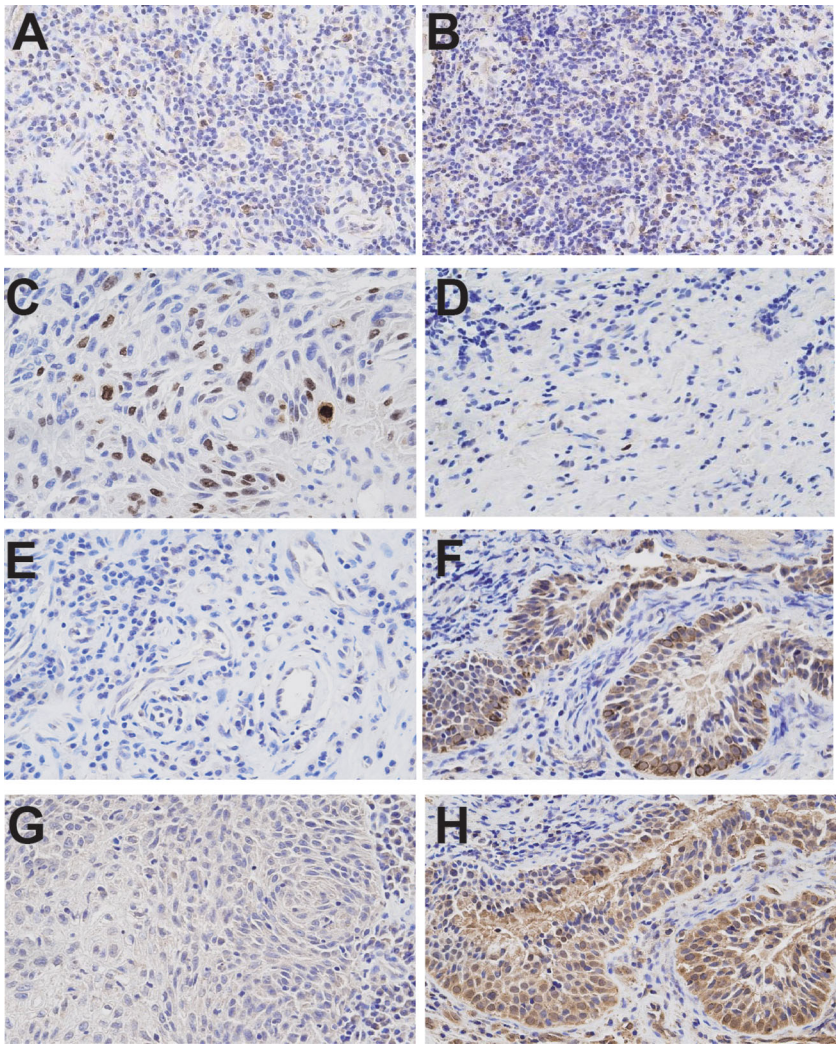

FIGURE 2
Immunohistochemical analyses of tumor samples before and after treatment. **(A)** PD-L1 immunohistochemistry (Ventana SP263 assay) from the initial diagnostic specimen. **(B)** PD-L1 immunohistochemistry from the final resection specimen. **(C)** Ki-67 immunohistochemistry from the initial diagnostic specimen. **(D)** Ki-67 immunohistochemistry from the final resection specimen. **(E)** IFN-γ expression in the initial diagnostic specimen. **(F)** IFN-γ expression in the final resection specimen. **(G)** CD8+ infiltrates in the initial diagnostic specimen. **(H)** CD8+ infiltrates in the final resection specimen. Images were taken at 400× magnification. Immunohistochemical analyses demonstrated a significant increase in PD-L1 and IFN-γ expression on tumor-infiltrating immune cells and CD8+ infiltrates, alongside a reduction in Ki-67 expression.

immunotherapy have achieved partial pathological responses, and preliminary data suggest that partial or major pathological responses may correlate with improved clinical outcomes (18).

HNSCC can be broadly classified into conventional types, which are predominantly associated with environmental factors such as tobacco and alcohol, and human papillomavirus (HPV)-related subtypes (19). Prognosis varies based on HPV status; patients with p16-positive HNSCC generally have better outcomes, while those with p16-negative HNSCC face poorer prognoses (20). For patients with p16-negative HNSCC and tumor invasion into adjacent organs, achieving R0 resection through surgery can be particularly challenging. Chemoradiotherapy, although effective, is associated with significant toxicities, such as myelosuppression, mucositis, and radiation-induced injuries, which can impair patient quality of life. These challenges emphasize the importance of exploring alternative strategies, such as neoadjuvant immunotherapy, to improve clinical outcomes while minimizing treatment-related morbidities.

Currently, five primary neoadjuvant strategies for HNSCC have been described: definitive immunotherapy plus concurrent chemoradiotherapy, definitive immunotherapy plus radiotherapy, neoadjuvant immunotherapy combined with radiotherapy, neoadjuvant immunotherapy with chemotherapy, and immunotherapy alone (21). In this case, a neoadjuvant regimen combining nivolumab with albumin-bound paclitaxel and carboplatin achieved a major pathological response (MPR), highlighting the potential efficacy of this approach.

Moreover, the patient and his family expressed a strong desire for organ preservation due to the tumor's location and associated

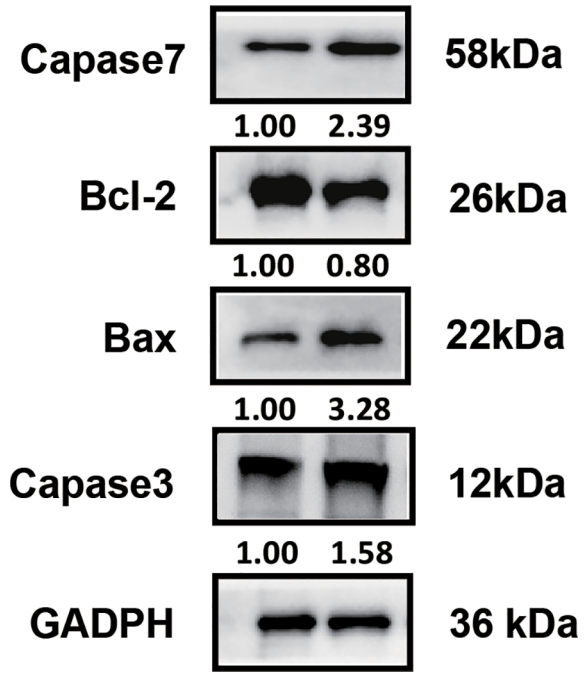

FIGURE 3
Western blot analysis of apoptosis-associated molecules. Elevated expression levels of pro-apoptotic molecules (Caspase-7, Caspase-3, Bax) and reduced expression of the anti-apoptotic molecule Bcl-2 were observed in post-treatment tumor samples.

symptoms, including headache, diplopia, and restricted eye movement. This perspective significantly influenced the choice of treatment, favoring a less invasive yet effective neoadjuvant immunochemotherapy regimen. Post-treatment, the patient reported significant symptom relief and expressed satisfaction with the treatment outcome.

The identification of predictive biomarkers for immunotherapy in HNSCC remains a critical area of research. While PD-L1 positivity, high tumor mutational burden (TMB), and the presence of CD8+ lymphocytes are currently recognized as promising markers, further exploration is needed (22–25). Achieving pCR appears more likely with neoadjuvant stereotactic body radiotherapy (SBRT), where pCR rates have been reported (26). For patients without SBRT, MPR rates range from 2.9% (20) to 31% (14). In this case, the patient treated with nivolumab in combination with chemotherapy came close to achieving pCR. These findings suggest that combining neoadjuvant immunotherapy with other modalities could provide additional benefits to HNSCC patients (27).

Our case revealed several key biological changes following treatment. Postoperative analyses showed increased PD-L1 expression, elevated IFN-γ levels on tumor-infiltrating immune cells, and a significant increase in CD8+ lymphocyte infiltration, coupled with decreased Ki-67 expression. Western blot analyses further demonstrated upregulation of pro-apoptotic molecules, including Caspase-7, Caspase-3, and Bax, along with downregulation of anti-apoptotic Bcl-2, suggesting enhanced tumor cell apoptosis as a key mechanism driving therapeutic efficacy.

Despite these promising results, this case study has inherent limitations. As a single case, the findings may not be generalizable to the broader HNSCC population. Moreover, the challenges associated with surgery and chemoradiotherapy, such as high morbidity and incomplete pathological responses, further highlight the need for novel therapeutic strategies. Mechanistic analyses in this study suggested enhanced tumor cell apoptosis as a potential mode of action, providing a foundation for future investigations. Larger cohort studies are warranted to validate the clinical benefits of neoadjuvant immunotherapy combined with chemotherapy and to explore its broader applicability in HNSCC.

Conclusion

In summary, this case highlights the successful application of neoadjuvant nivolumab combined with chemotherapy in an HNSCC patient, achieving significant tumor regression and organ preservation. Post-treatment analyses revealed increased PD-L1 expression, enhanced CD8+ lymphocyte infiltration, and decreased Ki-67 expression, indicating that the combination of immunotherapy and chemotherapy promotes tumor cell apoptosis and reshapes the immune microenvironment. These findings support the potential of neoadjuvant immunotherapy to improve clinical outcomes for HNSCC patients. However, this study has inherent limitations, as it is based on a single case, which limits the generalizability of the findings. Future larger cohort studies are needed to validate these results, optimize treatment regimens, and identify biomarkers for predicting therapeutic response. Establishing robust evidence will be critical for integrating neoadjuvant immunotherapy into standard clinical practice for HNSCC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Qilu Hospital (Qingdao, China), registration number: KYLL-2023083. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YS: Data curation, Formal analysis, Investigation, Writing – original draft. GY: Data curation, Formal analysis, Investigation,

Writing – original draft. RS: Conceptualization, Writing – review & editing. FC: Conceptualization, Funding acquisition, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Evaluating the influence of anti-PD-1 immunotherapy combined with IMRT on thyroid dysfunction in nasopharyngeal carcinoma

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Background: Immunotherapy represents a major breakthrough in malignant tumor treatment in recent years. Anti-PD-1 immunotherapy has significantly prolonged Event-free Survival (EFS) in Nasopharyngeal Carcinoma (NPC). However, its potent anti-tumor effects can also attack normal tissues and organs, leading to immune-related adverse effects (irAE), with the thyroid being one of the most commonly affected organs. This study aims to analyze the incidence and related factors of thyroid dysfunction in NPC patients receiving anti-PD-1 immunotherapy with/without Intensity-modulated radiotherapy (IMRT), and further explore whether radiotherapy interacts with thyroid immune-related adverse reactions.

Methods: 108 NPC patients receiving immunotherapy combined with chemotherapy or chemoradiotherapy were retrospectively included. Data collected included smoking status, BMI, presence of thyroid nodules, staging, treatment modality, thyroid mean dose (Dmean), percentage of thyroid volume receiving more than x Gy, pituitary mean dose (Dmean), and TSH and FT4 levels per cycle. T-tests, rank-sum tests, multivariate logistic regression analysis, ROC curves, and Cox proportional hazards models were used to evaluate the effects of anti-PD-1 immunotherapy combined with chemoradiotherapy on thyroid function.

Results: Patients with pre-treatment smoking history, thyroid nodules, and cervical lymph node metastasis were more likely to develop thyroid dysfunction ($P<0.05$). During treatment, 81 patients developed varying degrees of thyroid dysfunction. Subclinical hyperthyroidism (33.9%) was most common in the immunotherapy plus chemoradiotherapy group, while subclinical hypothyroidism (23.9%) was most common in the immunotherapy plus chemotherapy group. Compared to the immunotherapy plus chemotherapy group, the immunotherapy plus chemoradiotherapy group showed higher incidence and severity of hyperthyroidism (median peak FT4 concentration: 19.11 pmol/L vs 16.21 pmol/L) ($P=0.001$). The immunotherapy plus chemoradiotherapy group showed lower incidence but increased severity of hypothyroidism compared to the immunotherapy plus chemotherapy group, though these differences were not statistically significant.

Conclusion: NPC patients with smoking history, thyroid nodules, and cervical lymph node metastasis have significantly increased risk of thyroid dysfunction when receiving anti-PD-1 immunotherapy combined with IMRT. The combination of anti-PD-1 immunotherapy and IMRT increases both the incidence and severity of thyroid dysfunction.

KEYWORDS

PD-1, radiotherapy, thyroid dysfunction, nasopharyngeal carcinoma, IMRT (intensity modulated radiation therapy)

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial cancer originating in the nasopharyngeal mucosa. According to the International Agency for Research on Cancer, there were approximately 133,000 new cases and 80,000 deaths from NPC in 2020, with China accounting for 47% of global new cases (1). Radiotherapy is the primary treatment for NPC, with concurrent chemoradiotherapy or combined neoadjuvant chemotherapy considered the standard treatment for locally advanced NPC. Despite these intensive treatment approaches, 20%-30% of patients still experience disease recurrence (2–4), resulting in suboptimal survival outcomes.

Immunotherapy has emerged as a major breakthrough in cancer treatment in recent years. Immune checkpoint inhibitors (ICIs) have significantly extended progression-free survival (PFS) and overall survival (OS) in recurrent/metastatic NPC. According to the 2022 NCCN guidelines (4), PD-1 inhibitors are now recommended as first-line and second-line treatment options for recurrent/metastatic NPC. Additionally, clinical trials investigating first-line treatment (pre-, during, or post-radiotherapy) for non-recurrent/metastatic NPC are widely ongoing, showing promising preliminary results (5).

As one of the most widely used immunotherapy drugs, PD-1 inhibitors may trigger autoimmune responses in multiple systems, including the endocrine system, due to excessive immune cell activation. These reactions are known as immune-related adverse events (irAEs). Endocrine dysfunction is among the most common adverse events reported in ICI clinical trials, including hypothyroidism, hyperthyroidism, pituitary inflammation, primary adrenal insufficiency, and insulin resistance, with thyroid-related immune adverse events being the most frequent (6).

Due to NPC high radiosensitivity, radiotherapy has long been the preferred treatment method. During radiotherapy for NPC patients, the thyroid gland is often partially or completely included in the radiation field due to its unique anatomical position. This results in high-dose radiation exposure, leading to various early and late reactions and causing multiple acute and chronic functional abnormalities, primarily manifesting as hypothyroidism, including both subclinical and clinical cases. These thyroid dysfunctions can cause various discomforts, affecting physical health and, in severe cases, significantly reducing quality of life. While maintaining tumor coverage, the thyroid typically receives doses exceeding 50Gy, easily causing damage (7). The median time to hypothyroidism after intensity-modulated radiotherapy is 1.4-1.8 years, with an incidence rate of 23%-53% (8). Under current radiation techniques, most tumor and normal tissue cell death occurs through mitotic death, where cells may undergo several divisions before dying, explaining why thyroid dysfunction may manifest long after completing radiotherapy.

Current research generally attributes radiation-induced hypothyroidism to four main mechanisms: 1. Direct radiation damage to thyroid cells, causing DNA strand damage and subsequent cell death (9). 2. Vascular damage to the thyroid, where ionizing radiation increases apoptosis of vascular endothelial and smooth muscle cells, inhibits proliferation, and triggers inflammatory responses through various cytokines, mediators, and inflammatory cells (10, 11). 3. Thyroid capsule fibrosis, preventing compensation for thyroid cell damage and leading to atrophy and chronic inflammation (8). 4. Pituitary damage affecting thyroid hormone regulation, as the radiation field may include part of the pituitary gland in addition to the thyroid. While post-radiotherapy thyroid dysfunction primarily presents as hypothyroidism, some cases manifest as hyperthyroidism. The mechanisms for hyperthyroidism may include:1. Direct thyroid tissue damage causing inflammation or other pathological changes, leading to excessive thyroid hormone secretion. 2. Impact on the immune system causing autoimmune responses, such as the production of thyroid autoantibodies. 3. Structural changes in thyroid tissue affecting thyroid hormone regulation.

With increasing use of anti-PD-1 immunotherapy in NPC treatment, experts are focused on whether radiotherapy worsens immunotherapy-induced thyroid dysfunction and if there's synergy between them. No consensus exists yet. This study analyzes thyroid function changes in 108 NPC patients receiving chemo-immunotherapy ± radiotherapy to provide clinical guidance.

Methods and materials

A retrospective study of 108 NPC patients treated at Shenzhen People's Hospital between January 2019 and August 2024. Metastatic patients received chemotherapy plus immunotherapy (immunotherapy group), while non-metastatic/recurrent patients received concurrent chemoradiotherapy plus immunotherapy (combined therapy group). The median follow-up was 152 days, with immunotherapy given in 21-day cycles for 7 total cycles, and a median of 33 radiation fractions.

Inclusion criteria: Patients aged 25-70 (median 42), including 76 males and 32 females, with pathologically confirmed NPC (WHO type: undifferentiated non-keratinizing squamous cell carcinoma), willing to receive radiotherapy and PD-1 immunotherapy, and PS score 0-2. Exclusion criteria: pre-existing thyroid dysfunction or related diseases/surgeries, previous head/neck radiation or immunotherapy, hypothalamic-pituitary axis disorders, and incomplete clinical data. Clinical staging followed AJCC 8th edition TNM system.

The 108 patients were divided into two groups based on treatment modality: immunotherapy group (immunotherapy plus chemotherapy) and combined therapy group (immunotherapy plus chemoradiotherapy). Based on thyroid function changes, patients were further categorized into hyperthyroid, hypothyroid, and no-change groups.

Observation parameters: Demographic data (age, sex, smoking status, BMI), thyroid nodule status, staging, and treatment modality. Treatment types included chemotherapy, radiotherapy, and immunotherapy. Radiotherapy parameters measured included thyroid mean dose (Dmean), percentage of thyroid volume receiving >x Gy [Vx(%) for x=35-60 in 5Gy increments], and pituitary mean dose. Thyroid function was assessed through TSH (most sensitive early indicator of dysfunction) and FT4 (most sensitive for hypothyroidism diagnosis) blood levels.

Study endpoint was defined as thyroid function assessment after the 7th immunotherapy cycle. Time of onset was measured from first ICI dose to initial thyroid dysfunction. Thyroid dysfunction was classified as clinical hyperthyroidism (low TSH with elevated FT4), subclinical hyperthyroidism (low TSH with normal FT4), clinical hypothyroidism (high TSH with low FT4), or subclinical hypothyroidism (high TSH with normal FT4). Patients experiencing transient hyperthyroidism before developing hypothyroidism were classified in the corresponding hypothyroid group. Laboratory reference ranges were set at TSH: 0.56-5.91 mIU/L and FT4: 97.98-16.02 pmol/L.

Radiotherapy setup: Patients were positioned supine with thermoplastic head-neck-shoulder mask fixation from skull vertex to shoulders, using 4-5 point fixation. Enhanced CT scanning (3mm slices) ranged from skull vertex to 2cm below clavicular head. Target volumes were contoured using MRI reference: GTVnx (primary tumor and retropharyngeal nodes), GTVnd (positive neck nodes), CTV1 (high-risk areas), CTV2 (low-risk areas). Post-neoadjuvant therapy GTVnx was based on pre-treatment MRI, GTVnd on post-treatment MRI. CTVs had 5mm margins, PTVs 3-5mm (reduced to 1-2mm near critical structures). Prescribed doses: PGTVnx/nd 68-70Gy, PCTV1 60-64Gy, PCTV2 54-58Gy/30-33F. Organ constraints included thyroid Dmean ≤ 45Gy. Treatment delivered via IMRT (Eclipse) using 6MV X-ray linear accelerator (VARIAN), median 33 fractions. Thyroid contouring and treatment planning followed the 2017 international guidelines for nasopharyngeal cancer target delineation (12). Thyroid dose parameters including volume, maximum dose, minimum dose, and mean dose were precisely calculated.

All 108 nasopharyngeal cancer patients received platinum monotherapy chemotherapy combined with anti-PD-1 immunotherapy concurrent with radiotherapy.

All patients received anti-PD-1 immunotherapy (as shown in Table 1) administered every three weeks until disease progression or intolerable toxicity, to control for potential variations in outcomes among different types of immune checkpoint inhibitors (ICIs) such as PD-1, PD-L1, and CTLA-4.

Statistical analysis utilized SPSS 26.0 software. Count data were expressed as numbers and percentages. Normally distributed continuous variables were described as mean ± standard deviation, while non-normally distributed variables used interquartile ranges. Mann-Whitney U and Kruskal-Wallis tests were used for group comparisons. Multivariate logistic regression analysis and ROC curves were used to analyze clinical and biochemical characteristics of thyroid dysfunction. Statistical significance was set at $P<0.05$.

Results

Study included 108 NPC patients with median age 42 years at first ICI treatment, 70.4% male. Thyroid dysfunction distribution: 54 developed hyperthyroidism, 27 hypothyroidism, and 27 remained unchanged. In the combined therapy group (n=62): 9 (14.5%) developed hypothyroidism, 38 (61.3%) hyperthyroidism, and 15 (24.2%) no change. In immunotherapy-only group (n=46): 18 (39.1%) developed hypothyroidism, 16 (34.8%) hyperthyroidism, and 12 (26.1%) no change. Thyroid dysfunction was significantly associated with treatment modality ($P<0.05$). Baseline thyroid nodules correlated with hyperthyroidism development ($P<0.05$, Table 2), while N staging influenced hypothyroidism occurrence ($P<0.05$, Table 3).

Multivariate analysis of post-treatment hyperthyroidism in NPC patients identified smoking, pre-treatment thyroid nodules, and N staging as significant risk factors ($P<0.05$), as shown in Table 4.

TABLE 1 PD-1 drugs of all types.

Immunodrug	N	Proportion
Sintilimab	55	50.9%
Toripalimab	28	25.9%
Tislelizumab	14	13.0%
Pembrolizumab	7	6.5%
Nivolumab	4	3.7%

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TABLE 2 Baseline characteristics of patients with hyperthyroidism.

	hyperthyroidism group (n=54)	Non- hyperthy-roidism group (n=54)	P value
Gender			1.000
Male	38	38	
Female	16	16	
Age			.060
<40 years	27	15	
~ 59 years	22	32	
ge; 60 years	5	7	
Smoke			.071
No	15	24	
Yes	39	30	
BMI			.479
<18.5 kg/m2	9	13	
18.5 ~ 23.9 kg/m2	36	30	
≥ 24 kg/m2	9	11	
Thyroid nodule			.001
No	12	29	
Yes	42	25	
T staging			.318
1	4	2	
2	10	8	
3	25	20	
4	15	24	
N staging			.960
0	0	0	
1	9	8	
2	22	23	
3	23	23	
M staging			.069
0	40	30	
1	14	21	
Treatment mode			.011
R+C+I [a]	38	24	
C+I [b]	16	30	

[a]Radiotherapy +Chemotherapy + Immunotherapy. [b]Chemotherapy +Immunotherapy.

TABLE 3 Baseline characteristics of patients with hypothyroidism.

	hypothyroidism group (n=27)	Non- hypothy-roidism group (n=81)	P value
Gender			.808
Male	20	56	
Female	7	25	
Age			.125
<40 years	8	34	
40 ~ 59 years	13	41	
≥ 60 years	6	6	
Smoke			.107
No	6	33	
Yes	21	48	
BMI			.734
<18.5 kg/m2	4	17	
.5 ~ 23.9 kg/m2	17	50	
≥ 24 kg/m2	6	14	
Thyroid nodule			.651
No	9	32	
Yes	18	49	
T staging			.910
1	2	4	
	4	14	
3	11	35	
4	10	28	
N staging			.015
	0	0	
1	0	16	
2	16	31	
3	11	34	
M staging			.061
0	13	57	
1	14	24	
Treatment mode			.006
R+C+I [a]	9	53	
C+I [b]	18	28	

[a]Radiotherapy + Chemotherapy + Immunotherapy. [b]Chemotherapy + Immunotherapy.

Independent risk factors for post-treatment hyperthyroidism in NPC patients were identified through multivariate analysis: smoking, pre-treatment thyroid nodules, and N staging. ROC curve analysis showed AUC values of 0.694, 0.759, and 0.780 respectively, indicating good predictive capability for these risk factors (P<0.05), as shown in Figure 1; Table 5.

Multivariate analysis of post-treatment hypothyroidism in NPC patients identified smoking, pre-treatment thyroid nodules, and N staging as significant risk factors (P<0.05), as shown in Table 6.

Independent risk factors for post-treatment hypothyroidism in NPC patients were identified through multivariate analysis: smoking, pre-treatment thyroid nodules, and N staging. ROC curve analysis showed AUC values of 0.722, 0.704, and 0.669 respectively, indicating good predictive capability for these risk factors (P<0.05), as shown in Figure 2; Table 7.

Median time to thyroid dysfunction onset was 49 days overall - 50 days in combined therapy group and 46 days in immunotherapy-only group. Hyperthyroidism developed earlier in combined therapy group compared to immunotherapy group (85d vs 105d), though difference was not statistically significant (P>0.05), as shown in Figures 3, 4. Various patterns of thyroid dysfunction were observed: hyperthyroidism was most common (54 patients, 50%), while hypothyroidism and no change each affected 27 patients (25%). In combined therapy group (n=62), 38 cases (61.3%) developed hyperthyroidism (21 subclinical, 17 clinical) and 9 cases (14.5%) developed primary hypothyroidism, mostly clinical cases. In immunotherapy-only group (n=46), 16 cases (34.8%) developed hyperthyroidism (9 subclinical, 7 clinical) and 18 cases (39.1%) developed hypothyroidism (11 subclinical, 7 clinical), as illustrated in Figure 5.

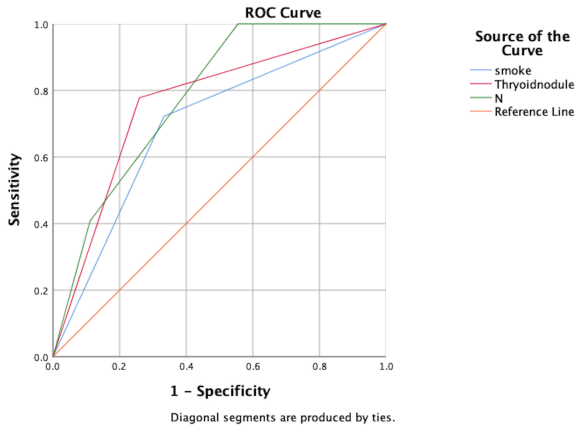

FIGURE 1
ROC analysis of the predictive value of smoking, thyroid nodules, and N-staging for the development of hyperthyroidism.

TABLE 4 Multifactorial logistic regression analysis of hyperthyroidism.

	B	S.E.	Wald	P	Exp(B)
Gender	-.310	1.263	.060	.806	.733
Age	.534	.764	.488	.485	1.705
Smoke	-2.519	.942	7.149	.008	.081
Weight	.006	.067	.009	.923	1.006
BMI	-1.100	1.224	.808	.369	.333
Thyroid nodule	-2.483	1.015	5.980	.014	.084
T	.197	.650	.092	.762	1.217
N	-3.641	1.065	11.700	.001	.026
M	1.483	1.107	1.793	.181	4.405
PD-1	.500	.474	1.114	.291	1.650
Constant	8.440	5.283	2.552	.110	4627.930

TABLE 5 AUC values for each factor of hyperthyroidism.

	AUC	S. E.	P	95% CI	
				Lower Bound	Upper Bound
smoke	.694	.064	.005	.570	.819
Thyroid nodule	.759	.059	.000	.644	.875
N	.780	.058	.000	.666	.894

TABLE 6 Multifactorial logistic regression analysis of hypothyroidism.

	B	S.E.	Wald	P	Exp(B)
Gender	-.080	1.103	.005	.942	.923
Age	-.017	.043	.156	.692	.983
Smoke	1.883	.832	5.128	.024	6.575
Weight	.137	.109	1.571	.210	1.147
BMI	-.395	.347	1.296	.255	.674
Thyroid nodule	2.064	.861	5.744	.017	7.876
T	.093	.586	.025	.874	1.098
N	1.450	.695	4.357	.037	4.265
M	.306	.905	.114	.735	1.358
PD-1	-.109	.298	.134	.715	.897
constant	-4.064	5.542	.538	.463	.017

TABLE 7 AUC values for each factor of hypothyroidism.

	AUC	S. E.	P	95% CI	
				Lower Bound	Upper Bound
smoke	.722	.071	.005	.583	.861
Thyroid nodule	.704	.072	.010	.562	.846
N	.669	.074	.033	.525	.814

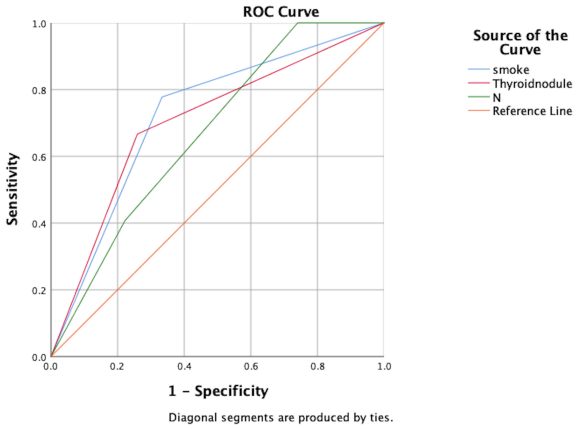

FIGURE 2
ROC analysis of the predictive value of smoking, thyroid nodules, and N-staging for the development of hypothyroidism.

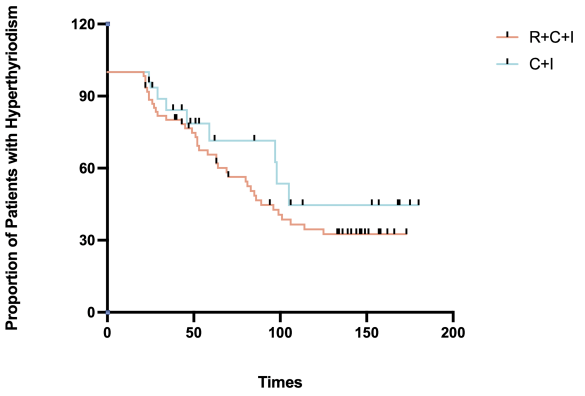

FIGURE 3
Time to hyperthyroidism in the immunotherapy combined with radiotherapy group versus the immunotherapy alone group.

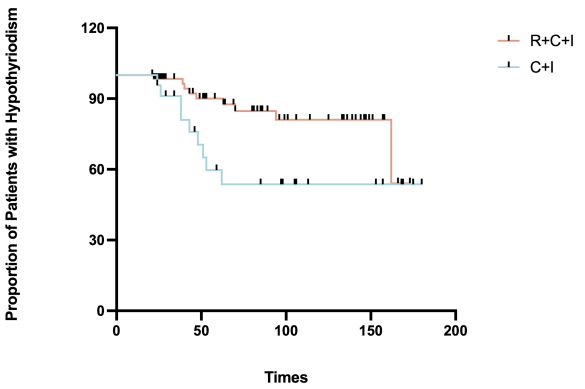

FIGURE 4
Time to onset of hypothyroidism in the immunotherapy combined with radiotherapy group vs. immunotherapy alone group.

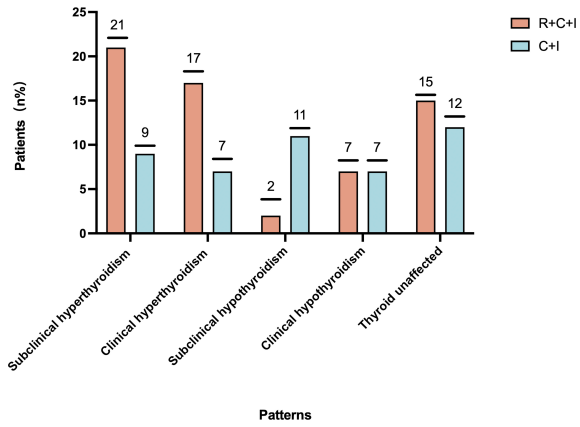

FIGURE 5
Patterns and incidence of thyroid dysfunction.

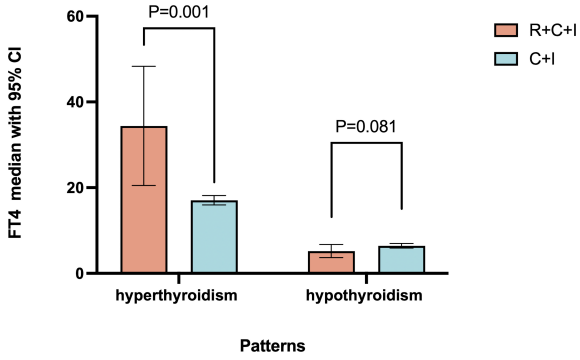

FIGURE 6
FT4 comparison of abnormal thyroid function.

Among hyperthyroid patients, the immunotherapy group showed median peak FT4 of 16.21 pmol/L (1.2% increase, mean rank 9.5), while the combined therapy group showed median peak FT4 of 19.11 pmol/L (19.3% increase, mean rank 21.4, 55.6% higher than immunotherapy group), showing significant difference (P=0.001) as shown in Figure 3. For hypothyroid patients, the immunotherapy group had median lowest FT4 of 6.47 pmol/L (18.9% decrease, mean rank 24.37), while the combined therapy group showed median lowest FT4 of 5.835 pmol/L (26.9% decrease, mean rank 18.5, 24.1% lower than immunotherapy group), though difference was not significant (P=0.081) as shown in Figure 6. Four patients progressed from subclinical hyperthyroidism to clinical hypothyroidism during treatment.

Discussion

Recent years have shown improved NPC patient prognosis through combined radiotherapy and immunotherapy. Growing preclinical and clinical data support the combination of radiotherapy and immune checkpoint inhibitors (13, 14). Studies suggest ICI-related thyroid dysfunction correlates with longer progression-free survival (PFS) and overall survival (OS) (15, 16).

In this short-term follow-up study, thyroid dysfunction incidence was 31.2% in the immunotherapy group, slightly lower than current largest cohort studies of ICI-related thyroid irAE, with potential for increased incidence during longer follow-up. The combined therapy group showed significantly higher thyroid dysfunction incidence at 43.5%. This study aimed to compare clinical and biochemical characteristics of thyroid dysfunction after combined radiotherapy and immunotherapy, suggesting potential heterogeneity between clinical and subclinical thyroid dysfunction occurrence.

In this study, smoking, thyroid nodules, and N staging were identified as risk factors for thyroid dysfunction. Tobacco, containing nicotine and thiocyanate, interferes with TSH levels, thyroid hormone metabolism, and immune system function, promoting thyroid dysfunction during NPC treatment. Non-functioning thyroid nodules may develop secretory function after radiation exposure or PD-1 inhibitor treatment, affecting thyroid hormone synthesis and release, leading to thyroid dysfunction. Lymph node positivity is a factor influencing radiation-induced hypothyroidism in head and neck cancer radiotherapy patients (17). NPC patients with N2-3 vs N0-1 patients (37.38% vs 13.11%) (18). In a large cohort study, gender and age were strong predictors of thyroid dysfunction (19). However, our study did not find such significance, possibly due to small sample size and lack of statistical power.

Several studies have demonstrated that mean thyroid radiation dose is an independent risk factor for radiation-induced hypothyroidism (13, 20). The threshold dose for radiation-induced thyroid secretory dysfunction is 30Gy, with doses above 30Gy generally considered sufficient to cause thyroid secretory damage (21). In this study, the mean thyroid dose was 49.63 ± 2.69Gy (Table 8). Research has shown that patients with the entire thyroid gland included in the target area have significantly higher rates of radiation-induced hypothyroidism compared to those with partial thyroid irradiation (22). Many scholars believe there is a linear relationship between thyroid dose-volume and radiation-induced hypothyroidism. Research has shown that V25 ≤ 60%, V35 ≤ 55%, and V45 ≤ 45% are independent risk factors for predicting radiation-induced hypothyroidism (23). Studies have reported that pituitary radiation doses exceeding 45Gy can cause pituitary damage (24). In this cohort study, the mean pituitary radiation dose was 32.41 ± 6.39Gy (Table 8).

TABLE 8 Irradiation dose in the combined treatment group.

	Dose(Gy)
Thyroid Dmean	49.6257 ± 2.69013
Thyroid V35	52.1248 ± 2.11599
Thyroid V40	51.0544 ± 2.29639
Thyroid V45	50.0852 ± 2.60207
Thyroid V50	49.2881 ± 2.77693
Thyroid V55	46.7633 ± 4.27295
Thyroid V60	41.3580 ± 6.49687
Pituitary Dmean	32.4095 ± 6.39475

Thyroid dysfunction following ICI treatment can appear within weeks to months after the first dose, currently understood as immune-mediated destructive thyroiditis, typically presenting as transient hyperthyroidism followed by hypothyroidism. Clinical manifestations of thyroid irAE are diverse, including not only thyroiditis-like presentations but also isolated thyrotoxicosis, hypothyroidism, and subclinical thyroid dysfunction (6). ICI-related thyroid dysfunction manifests as hypothyroidism and hyperthyroidism, with thyroiditis and thyroid storm being relatively rare, and no large-scale studies have reported on these yet. ICI-related hyperthyroidism mainly presents with hypermetabolic symptoms, while ICI-related hypothyroidism shows similar manifestations to conventional hypothyroidism, characterized by decreased metabolic rate and reduced sympathetic nervous system activity.

Radiation-induced thyroid dysfunction presents similarly to conventional hypothyroidism, classified as subclinical or clinical based on severity. Clinical hypothyroidism shows decreased metabolic rate and reduced sympathetic nervous activity, with typical symptoms including fatigue, weakness, cold intolerance, constipation, depression, slow reactions, and dull expressions. Radiation-induced hypothyroidism can occur as early as 1.9 months post-radiotherapy, with incidence reaching 40-50% within 2 years. Subclinical hypothyroidism predominates (approximately 70%) (25, 26) and may progress to clinical hypothyroidism, completely resolve, or remain stable (27). Radiation-induced hyperthyroidism is relatively rare, with an incidence of only 0.6%, and is typically self-limiting. Its clinical symptoms are identical to conventional hyperthyroidism, presenting with hypermetabolic symptoms due to excessive thyroid hormone in circulation.

The earlier onset and increased severity of hyperthyroidism in radiochemotherapy combined with immunotherapy compared to immunotherapy with chemotherapy alone may involve multiple factors. Immune checkpoint inhibitors activate the immune system against cancer cells but can trigger autoimmune side effects, while radiotherapy induces thyroid-specific autoantibodies and enhances systemic immune response, increasing the probability of immune attacks on thyroid tissue. Through high-energy radiation's direct cell-killing effects, structural changes occur in irradiated thyroid regions, disrupting endocrine function and leading to excessive hormone secretion, which amplifies immunotherapy-induced hyperthyroidism. Additionally, radiotherapy triggers inflammatory cytokine storm in thyroid tissue, releasing pro-inflammatory cytokines like IL-6 and TNF-α, which enhance the systemic immune inflammatory response from immunotherapy. These mechanisms work synergistically, resulting in increased systemic immune activation, enhanced inflammatory responses, and individual variations in thyroid radiation dose, ultimately leading to more severe hyperthyroidism.

The increased severity of hypothyroidism in radiochemotherapy combined with immunotherapy compared to immunotherapy with chemotherapy alone can be attributed to several factors. Radiotherapy for nasopharyngeal cancer can directly damage thyroid tissue through high-energy radiation, affecting not only tumor cells but also surrounding normal tissue, leading to thyroid cell damage or apoptosis and reduced hormone secretion. The immune system's synergistic destructive effect occurs when immunotherapy activates the immune system, potentially triggering immune-mediated

thyroiditis, while radiation-induced tissue damage increases thyroid susceptibility to immune attacks, resulting in more severe dysfunction. This is a primary reason for the objective results seen in phase 3 clinical trials of combined radiotherapy and immunotherapy. Radiation-induced fibrosis can replace normal thyroid tissue with scar tissue, further compromising thyroid function and hormone secretion. Chronic inflammation is enhanced by radiotherapy, particularly in the context of immunotherapy, affecting both thyroid function and recovery capacity, leading to more pronounced and persistent hypothyroid symptoms. The complexity of combination therapy increases the difficulty of individualizing treatment plans, and in some cases in this study, larger radiation doses and fields increased the risk of unnecessary thyroid damage, exacerbating hypothyroidism severity. Thus, the synergistic effect of radiotherapy's direct physical damage, fibrosis, and immune-mediated injury from immunotherapy results in more severe hypothyroidism with higher incidence and severity rates.

In radiochemotherapy with immunotherapy, four patients progressed from subclinical hyperthyroidism to clinical hypothyroidism. This progression could result from either anti-PD-1 immunotherapy alone or combined effects with radiation therapy. "Normal" thyroid hormone levels during treatment may not represent true normal function, as clinical symptoms can still occur. Preventive levothyroxine is typically avoided to prevent potential hyperthyroidism. Management focuses on precise thyroid protection during radiation, optimized treatment timing, antioxidant supplementation, appropriate nutrition, and collaborative care between specialties for continuous monitoring.

Current limitations in this study of thyroid dysfunction following immunotherapy combined with radiochemotherapy for nasopharyngeal cancer include:1. Small sample size and short follow-up period, potentially underestimating late-onset thyroid dysfunction. 2. Irregular thyroid function monitoring in some patients. 3. Some patients received brief ICI treatment without subsequent thyroid monitoring, leading to inaccurate onset timing and missed thyroid irAE diagnoses. 4. Lack of thyroid ultrasound and antibody results, preventing comparison of thyroid volume and echo changes across different functional states. 5. Future studies could benefit from including FDG-PET/CT examination, which provides valuable systemic metabolic information for monitoring immune therapy response, distinguishing active tumors from pseudoprogression, and identifying checkpoint inhibitor-related adverse effects (28).

This report finds that nasopharyngeal cancer patients receiving immunotherapy combined with radiochemotherapy showed higher incidence and severity of thyroid dysfunction compared to those receiving immunotherapy with chemotherapy alone. The study's limitations include its single-center nature with small sample size and potential selection bias. The lack of information about other subgroups, particularly those with different demographic or clinical characteristics, may affect the strength of associations. Additionally, the short follow-up period and center-specific patient population and institutional practices may impact results, reducing external validity. Future research should employ multi-center approaches with longer study periods to ensure more representative sampling and improve reliability of conclusions.

Conclusions

NPC patients receiving anti-PD-1 immunotherapy combined with radiochemotherapy show increased incidence and severity of thyroid dysfunction. The risk of thyroid dysfunction is significantly higher in nasopharyngeal cancer patients who smoke, have thyroid nodules, and cervical lymph node metastases when receiving anti-PD-1 immunotherapy combined with IMRT.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Shenzhen People's hospital institutional and research ethics committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LC: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. LG: Conceptualization, Writing – original draft. GX: Supervision, Writing – review & editing. YZ: Data curation, Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft. SC: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. ZL: Supervision, Writing – review & editing. XL: Supervision, Writing – review & editing. SW: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Evaluating the efficacy and safety of immune checkpoint inhibitors in first and second-line treatments for recurrent and metastatic head and neck squamous cell carcinoma: a systematic review and network meta-analysis of RCTs with a focus on PD-L1 expression

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Introduction: This study systematically reviewed and conducted a network meta-analysis to assess the efficacy and safety of first-line and second-line immunotherapy treatments for recurrent and metastatic head and neck squamous cell carcinoma (R/M HNSCC). The findings aim to provide robust evidence to guide clinical decision-making.

Methods: We conducted an comprehensive literature search in PubMed, Embase, Cochrane Library, and Web of Science. The outcome measures included overall survival (OS), progression-free survival (PFS), overall response rate (ORR), and grade 3 or higher adverse events (AEs ≥3). To compare the efficacy and safety of various first-line and second-line immunotherapy regimens for R/M HNSCC with different PD-L1 expression levels, we conducted a Bayesian network meta-analysis. This study is registered in the Prospective Register of Systematic Reviews (CRD42024551711).

Results: This analysis included 9 randomized controlled trials (RCTs) involving 5,946 patients and seven immunotherapy regimens. Among patients with R/M HNSCC, pembrolizumab combined with chemotherapy as a first-line treatment was the only immunotherapy regimen to show a PFS benefit compared to SOC (HR = 0.92, 95% CI: 0.77–1.10); however, the difference was not statistically

significant. Meanwhile, nivolumab provided the most pronounced OS benefit (HR=0.71,95%CI:0.52-0.98). Additionally, pembrolizumab exhibited the most favorable safety profile relative to SOC (OR=0.12, 95% CI: 0.05-0.29). In second-line therapy, nivolumab outperformed SOC in multiple aspects, including OS (HR=0.68, 95% CI: 0.54-0.86), ORR (OR=0.40, 95% CI: 0.17-0.95), and grade ≥3 adverse events (OR=0.32, 95% CI: 0.19-0.54). Subgroup analysis by PD-L1 expression revealed that nivolumab, compared to SOC, conferred the greatest OS benefit (HR=0.59, 95% CI: 0.34-1.00) as a first-line therapy in patients with PD-L1 expression ≥1%, while pembrolizumab combined with chemotherapy(pem-chemo) showed the most substantial PFS benefit (HR=0.82, 95% CI: 0.67-1.00). For patients with PD-L1 expression ≥20%, pem-chemo delivered the optimal OS (HR=0.60, 95% CI: 0.44-0.81) and PFS (HR=0.73, 95% CI: 0.55-0.97) outcomes compared to SOC. Furthermore, in patients with PD-L1 expression ≥1%, nivolumab as a second-line treatment demonstrated superior OS (HR=0.55, 95% CI: 0.39-0.78) and PFS (HR=0.59, 95% CI: 0.41-0.84) compared to SOC.

Conclusions: These results suggest that immunotherapy may improve survival outcomes compared to SOC for patients with R/M HNSCC, while maintaining a comparable safety profile. For patients, pembrolizumab combined with chemotherapy and nivolumab as first-line treatments may represent the most optimal options, with nivolumab also showing promise as a second-line therapy. In patients with PD-L1 expression ≥1% or ≥20%, pembrolizumab combined with chemotherapy may be the preferred first-line therapy, while nivolumab remains the most favorable second-line treatment.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42024551711.

KEYWORDS

R/M HNSCC, ICIs, efficacy, safety, network meta-analysis, PD-L1 expression

1 Introduction

Head and neck cancer ranks as the sixth most common malignancy globally, with over 891,000 new cases and more than 458,000 deaths annually, predominantly due to head and neck squamous cell carcinoma (HNSCC) (1). HNSCC arises from the mucosal epithelial cells of the oral cavity, pharynx, larynx, and sinonasal tract (2). The recurrence and metastasis of these malignant tumors significantly contribute to the high mortality rate associated with HNSCC, often leading to a poor prognosis, with a median survival of less than one year (3). Currently, the EXTREME regimen (cisplatin or carboplatin combined with 5-fluorouracil and cetuximab) is the standard first-line treatment for R/M HNSCC. Although it improves the overall response rate (ORR) and median survival, its tolerability is poor, with increased incidences of adverse reactions such as skin reactions (9%), sepsis (19%), and thrombocytopenia (11%), significantly reducing patients' quality of life (4). After disease progression following first-line therapy, standard second-line treatments include monotherapies with methotrexate, docetaxel, or cetuximab, but the median overall survival (OS) is generally less than six months (5). Consequently, researchers have been striving to develop new therapeutic strategies to prolong patient survival.

Over the past decade, studies have demonstrated the benefits and safety of immune checkpoint inhibitors (ICIs) in various tumors (6, 7). ICIs restore T-cell activity and enhance anti-tumor immune responses by binding to protein receptors on T cells (8). The FDA's approval of nivolumab and pembrolizumab in 2016 marked the beginning of an era of immunotherapy for R/M HNSCC patients (9, 10). As more large-scale RCTs are conducted, the landscape of first-line and second-line immunotherapy for R/M HNSCC is becoming increasingly diverse. However, the optimal immunotherapy regimen balancing efficacy and safety remains unclear. PD-L1 expression, as a biomarker, can predict which patients are more likely to respond to immunotherapy, thus optimizing the potential benefit for targeted populations (11). For R/M HNSCC patients with varying levels of PD-L1 expression, there are multiple immunotherapy options available. However, it remains unclear which first-line and second-line immunotherapy regimens provide the greatest benefit for these patients.

Most RCTs directly compare immunotherapy with standard treatments, lacking direct comparative studies among different immunotherapies (5, 12). The Bayesian approach is particularly well-suited for comparing multiple treatments in the absence of direct head-to-head trials, as it seamlessly integrates direct and indirect evidence while providing probabilistic rankings (13). Unlike frequentist methods, Bayesian credible intervals offer a more intuitive quantification of uncertainty. Additionally, the Bayesian framework supports consistency checks and sensitivity analyses, ensuring that results are both robust and clinically meaningful (14). Therefore, this study employs a Bayesian framework to indirectly compare the efficacy and safety of various immunotherapy regimens and conducts a network meta-analysis to identify the optimal first-line and second-line treatments for different patient populations based on PD-L1 expression, providing evidence-based support for clinical decision-making.

2 Materials and methods

This network meta-analysis (NMA) follows the guidelines set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for network meta-analyses (Supplementary Table 1) (15). Given the scarcity of direct comparative RCTs for different immunotherapy regimens, the Bayesian method is employed to predict the ranking of efficacy and safety through indirect comparisons (16). To ensure transparency, reliability, and novelty, the study protocol is registered with the Prospective Register of Systematic Reviews (CRD42024551711).

2.1 Data sources and search strategy

A systematic search was conducted in the PubMed, EMBASE, Cochrane Library, and Web of Science databases. The search terms included "head and neck squamous cell carcinoma", "Squamous Cell Carcinoma of the Larynx", "randomized clinical trial", "immune checkpoint inhibitors", "PD-L1 inhibitor","PD-1 inhibitor", "CTLA-4 inhibitor", "pembrolizumab", "camrelizumab", "nivolumab", "ipilimumab", "durvalumab", "tremelimumab", "toripalimab" and "tislelizumab". (Supplementary Table 2). The search covered publications from database inception until September 1, 2024, utilizing a combination of free-text and MeSH terms, without language restrictions.

2.2 Selection criteria

Inclusion Criteria:

1. RCTs involving patients with R/M HNSCC confirmed by histology or cytology.
2. RCTs employing immunotherapy alone or in combination as first-line or second-line treatment regimens.
3. RCTs comparing immunotherapy alone or in combination with other treatment regimens for R/M HNSCC.
4. RCTs that report at least one of the following outcome measures:

OS, defined as the time from randomization to death from any cause; progression-free survival (PFS), defined as the time from randomization to disease progression or death from any cause; ORR, defined as the proportion of patients achieving an objective response; and grade 3 or higher AEs (AEs ≥3) as defined by the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute.

Exclusion Criteria:

1. RCTs based on different phases of the same patient cohort.
2. RCTs with unclear outcome measures.
3. Reviews or case reports.

RCTs were screened based on titles and abstracts before inclusion. All included RCTs were double-checked by two reviewers to ensure that the data were the most recently published.

2.3 Data extraction and quality assessment

Three researchers independently extracted data from the RCTs in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Any discrepancies were resolved through discussion with a fourth author. The data extracted from each article included the trial name, NCT number, publication journal, randomization ratio, year of publication, trial phase, tumor stage, histological type, sample size, patient age and gender distribution, racial composition, PD-L1 expression status, Eastern Cooperative Oncology Group (ECOG) performance status, and treatment regimens for both the experimental and control groups. The outcomes extracted included hazard ratios (HRs) with 95% confidence intervals (CIs) for OS and PFS, and odds ratios (ORs) with 95% CIs for ORR and AEs ≥ 3.

The quality of the included RCTs was assessed using the Cochrane Risk of Bias Tool (2.0). This tool evaluates five domains: risk of bias arising from the randomization process, risk of bias due to deviations from the intended interventions, risk of bias from missing outcome data, risk of bias in the measurement of the outcome, and risk of bias in the selection of the reported result. Each RCT was categorized into one of three risk levels: low risk, high risk, and having "some concerns."

2.4 Statistical analysis

The primary outcomes are OS and PFS, while the secondary outcomes include ORR and AEs≥3. The effect size for OS and PFS is expressed as HRs with 95% CIs, and the effect size for ORR and grade 3 or higher AEs is expressed as ORs with 95% CIs.

A NMA was conducted using a Bayesian model in R software, employing the "rjags" and "gemtc" packages to evaluate the efficacy and safety of immunotherapy in first and second-line treatments for R/M HNSCC (17). A random-effect model was used, establishing three independent Markov chains, each with 20,000 burn-ins and 100,000 sample iterations, with a thinning interval for each chain. The overall ranking probability of different treatment regimens' efficacy and safety was derived from the Markov chain iterations, and results were visualized through graphical representations. Funnel plots were created using STATA 18.0 software to assess publication bias. To verify the accuracy of indirect comparisons in the NMA, a pairwise meta-analysis based on frequentist methods was conducted comparing head-to-head studies and NMA indirect comparisons (Supplementary Table 3).

Additionally, Revman 5.4 software was used to perform a pairwise meta-analysis based on frequentist methods, aiming to re-evaluate the efficacy and safety of first-line or second-line immunotherapy versus chemotherapy alone in R/M HNSCC patients with and without PD-L1 expression. Heterogeneity was assessed using the Q-test and I^2 statistic, with $I^2 \leq 50\%$ or $P \geq 0.1$ indicating low heterogeneity, and $I^2 > 50\%$ or $P < 0.1$ indicating high heterogeneity. For studies with high heterogeneity, a random-effects model was applied; otherwise, a fixed-effect model was used. Sensitivity analyses were performed on high heterogeneity studies by sequentially excluding studies from the model and comparing heterogeneity changes before and after exclusion to ensure result reliability. If significant heterogeneity changes were observed, the sources of heterogeneity were analyzed. Subgroup analyses were conducted based on OS and PFS outcomes for different PD-L1 positive patients receiving first-line/second-line treatment, comparing the efficacy of various immunotherapy regimens versus chemotherapy. The significance level was set at $\alpha = 0.05$.

3 Results

3.1 Systematic review and characteristics of the included studies

In the initial literature search, a total of 769 records were identified from the databases. After screening the abstracts to remove duplicates and irrelevant articles, 554 studies remained eligible for full-text review. Among these, 497 were excluded due to irrelevance (n = 226), review/meta-analyses (n = 259), or non-English publications (n = 12). Of the remaining 57 reports, 48 were excluded during eligibility assessment for reasons such as non-RCT designs (n = 21), study protocols (n = 12), duplicate clinical trials (n = 7), or inappropriate control groups (n = 8). Ultimately, 9 studies met our eligibility criteria (Figure 1), enrolling a total of 5,946 patients who received any of the following 8 treatments: nivolumab plus ipilimumab (nivo-ipi), durvalumab plus tremelimumab (durva-treme), durvalumab (durva), pembrolizumab (pem), pembrolizumab plus chemotherapy (pem-chemo), nivolumab (nivo), tremelimumab (treme), and standard of care (soc). Detailed information on all included studies is provided in Tables 1, 2, and Supplementary Table 4.

Quality assessment using the ROB 2.0 tool showed that 5 of the 9 included studies were evaluated as low risk, while 4 were classified as having some concerns. CheckMate 141 has certain variations in baseline patient data, leading to 'some concerns' regarding deviations from intended interventions. CheckMate 141, CheckMate 651, EAGLE, Keynote-048, and Keynote-040 are all open-label studies that were not double-blinded. Additionally, more than 10 patients in each study either withdrew or were lost to follow-up, thereby raising concerns regarding potential deviations from the intended interventions. Overall, all the RCTs were meticulously designed, demonstrating a high level of research quality. The specific assessment results are detailed in Supplementary Figures 1, 2.

3.2 Pairwise meta-analysis

3.2.1 Comparisons of OS, PFS, ORR

Four studies investigating first-line treatment strategies reported OS, revealing moderate statistical heterogeneity (P=0.05, I^2=55%). A random-effects model was utilized for the meta-analysis (Supplementary Figure 3). The findings indicated a trend toward improved OS in patients with R/M HNSCC who received immunotherapy, compared to the SOC (HR=0.89, 95% CI: 0.79-1.01), although the difference did not reach statistical significance. Subgroup analyses demonstrated no significant OS advantage for either ICI monotherapy (HR=0.85, 95% CI: 0.66-1.09) or dual ICI therapy (HR=0.99, 95% CI: 0.88-1.12) relative to SOC. However, significant OS benefits were observed in patients with PD-L1 expression levels ≥1% (HR=0.75, 95% CI: 0.66-0.86) and ≥20% (HR=0.78, 95% CI: 0.62-0.99) treated with immunotherapy compared to SOC (Figure 2; Supplementary Figure 7).

PFS was also assessed in the same four studies, which demonstrated significant heterogeneity (P=0.001, I^2=75%). A random-effects model was applied to this analysis as well (Supplementary Figure 4). The results showed no significant PFS improvement in R/M HNSCC patients without PD-L1 selection who were treated with immunotherapy compared to SOC (HR=1.13, 95% CI: 0.98-1.31). Subgroup analyses further indicated no PFS benefit for ICI monotherapy (HR=1.18, 95% CI: 0.98-1.42) or dual ICI therapy (HR=1.21, 95% CI: 0.90-1.63) over SOC. Similarly, no significant PFS improvement was observed in patients with PD-L1 expression ≥1% (HR=1.07, 95% CI: 0.86-1.33) or ≥20% (HR=1.00, 95% CI: 0.89-1.12) receiving immunotherapy compared to SOC (Figure 2; Supplementary Figure 8).

Four studies on first-line treatment strategies reported ORR, with significant statistical heterogeneity among the studies (P<0.1, I^2=94%). A random-effects model was applied for the meta-analysis (Figure 3; Supplementary Figure 5). The results indicated that, in patients with R/M HNSCC, treatment with immunotherapy was associated with an increased ORR compared to SOC (OR=3.02, 95% CI: 1.47–6.18). Subgroup analysis revealed that both ICI monotherapy (OR=3.54, 95% CI: 1.41–8.87) and combination therapy with two ICIs (OR=4.53, 95% CI: 2.64–7.78) significantly increased the ORR compared to SOC.

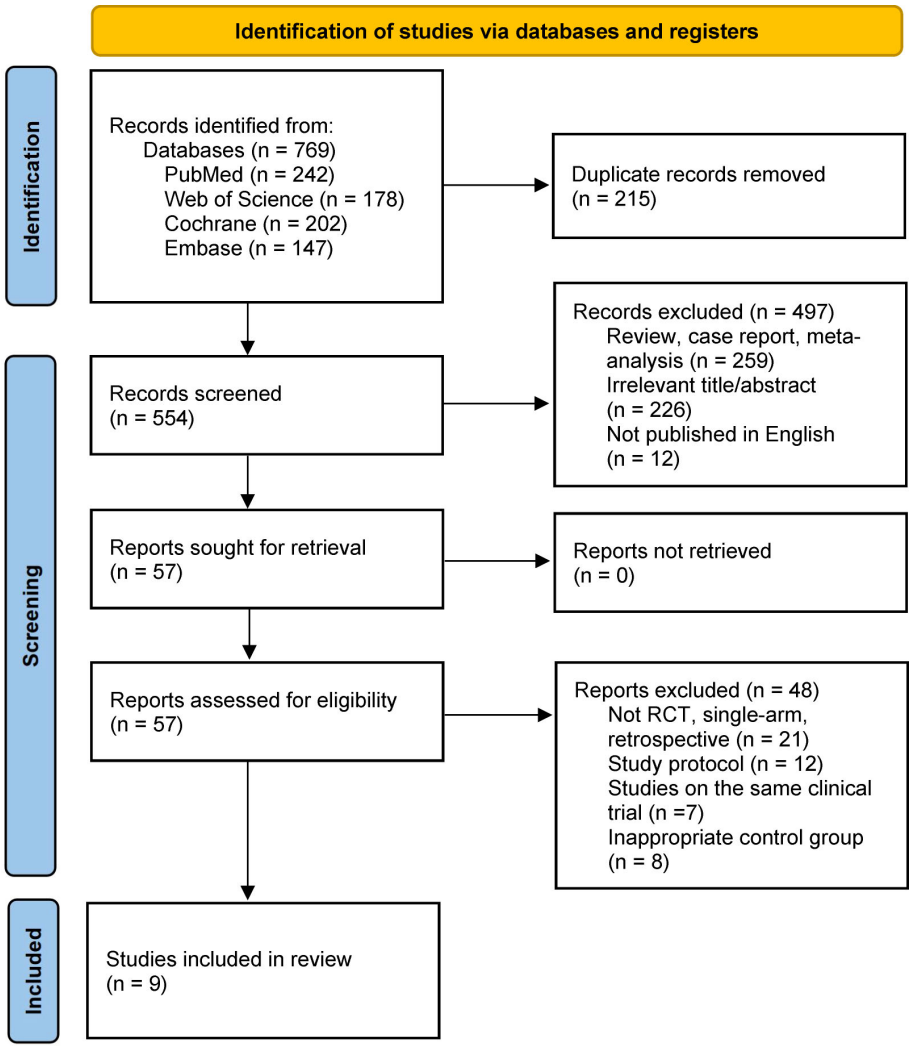

FIGURE 1
Literature search and screening flow diagram.

Four studies on second-line treatment reported OS, with low heterogeneity (P=0.34, I^2=10). A fixed-effect model was used for the meta-analysis (Supplementary Figure 9). The results indicated that R/M HNSCC patients (HR=0.84, 95% CI: 0.78-0.95) treated with immunotherapy had an OS benefit compared to the control group. Subgroup analysis based on PD-L1 expression showed that patients with PD-L1 expression ≥1% (HR=0.67, 95% CI: 0.55-0.82) had a significant OS benefit with immunotherapy compared to SOC. R/M HNSCC patients receiving single ICI (HR=0.81, 95% CI: 0.72-0.90) had an OS benefit over SOC, whereas dual immunotherapy and SOC had comparable efficacy (HR=1.04, 95% CI: 0.85-1.26) (Figure 2; Supplementary Figure 13).

Four studies on second-line treatment reported PFS, with low statistical heterogeneity (P=0.35, I^2=11). A fixed-effect model was used for the meta-analysis (Supplementary Figure 10). The results indicated that R/M HNSCC patients without PD-L1 expression selection (HR=1.02, 95% CI: 0.92-1.12) had comparable PFS with immunotherapy compared to SOC. For R/M HNSCC patients with PD-L1 expression ≥1% (HR = 0.76, 95% CI: 0.50–1.17), treatment with ICIs showed a PFS benefit compared to the control group; however, the difference was not statistically significant. R/M HNSCC patients receiving single ICI (HR=1.00, 95% CI: 0.89-1.11) and dual immunotherapy (HR=1.09, 95% CI: 0.90-1.33) did not show significant PFS benefit compared to SOC (Figure 2; Supplementary Figure 14).

Four studies on second-line treatment reported ORR, with low statistical heterogeneity (P=0.27, I^2=23). A fixed-effect model was used for the meta-analysis (Figure 3; Supplementary Figure 11). The results indicated no significant ORR benefit for R/M HNSCC patients (OR=0.83, 95% CI: 0.65-1.06) treated with immunotherapy compared to SOC. Subgroup analysis showed that neither single ICI (OR=0.79, 95% CI: 0.59-1.05) nor dual immunotherapy (OR=0.96, 95% CI: 0.61-1.53) provided an ORR benefit compared to SOC.

3.2.2 Safety and toxicity

The incidence of AEs≥3 was used to assess the safety and toxicity of first-line and second-line ICI treatments.

TABLE 1 Baseline Characteristics of Studies Included in the Network Meta-Analysis.

Study (Phase, Design)	Source (y)	Registered ID (Randomization)	Sample Size (Median Age/y)	Stage (Male/ Female)	Histology	Ethnicity (%)	Intervention Arm(s)	Control Arm
CheckMate 651 (18)	JCO	NCT02741570	468/441	IV	Squamous	American (100.0)	Nivolumab 3 mg/kg Q2W	cisplatin 100 mg/m^2 or carboplatin area under the curve 5 mg/ml/min on day 1
(open-label, III)	2022	(1:1)	(61/62)	777/170			+ipilimumab 1 mg/kg Q6W	+5-fluorouracil 1000 mg/m^2/day on days 1-4 Q3W + cetuximab 400 mg/m^2 on day 1, 250 mg/m^2 Q1W
KESTREL (19)	ANN	NCT02551159	204/413/206	IV	Squamous	White (73.3) Asian (24.9)	Durvalumab 1500 mg Q4W	cisplatin 100 mg/m2 or carboplatin at an area under the curve of 5 mg/ml/min on day 1
(open-label, III)	2023	(1:2:1)	(62/61/61)	689/134		Black or African American (1.3) Other (0.5)	or +tremelimumab 75 mg Q4W (for a maximum of four doses)	+5-fluorouracil 1000 mg/m^2/day on days 1-4 Q3W + cetuximab 400 mg/m^2 on day 1, then 250 mg/m^2 Q1W
KEYNOTE048 (12)	Lancet	NCT02358031	301/281/300	IV	Squamous	Europe (31.7)	Pembrolizumab 200 mg Q3W	Cetuximab 400 mg/m^2, then 250 mg/m^2 Q1W
(open-label, III)	2019	(1:1:1)	(62/61/61)	735/147		North America (22.3) Other (46.0)	or +carboplatin area under the curve 5 mg/m^2 or cisplatin 100 mg/m^2 +5-fluorouracil 1000 mg/m^2/day 4 consecutive days Q3W	+carboplatin area under the curve 5 mg/m^2 or cisplatin 100 mg/m^2 +5-fluorouracil 1000 mg/m^2/day 4 consecutive days Q3W
KEYNOTE-040 (5)	Lancet	NCT02252042	247/248	IV	Squamous	Europe (61.6)	Pembrolizumab 200 mg Q3W	Methotrexate 40 mg/m^2 Q1W or docetaxel 75 mg/m^2 Q3W
(open-label, III)	2018	(1:1)	(60/60)	412/83		North America (26.9) Other (11.5)		or cetuximab 250 mg/m^2 Q1W, then loading dose 400 mg/m^2
CheckMate 714 (20)	JAMA Oncol	NCT02823574	282/143	IV	Squamous	United Kingdom (100.0)	Nivolumab 3 mg/kg Q2W	Nivolumab 3 mg/kg Q2W
(double-blind, II)	2023	(2:1)	(60/60)	346/79			+ipilimumab 1 mg/kg Q6W	+placebo Q2W
EAGLE (21)	ANN Oncol	NCT02369874	240/247/249	IV	Squamous	White (80.4)	Durvalumab 10 mg/kg Q2W	Standard of care
(open-label, III)	2020	(1:1:1)	(59/61/61)	618/118		Asian (15.4) Other (4.2)	or durvalumab 20 mg/kg Q4W +tremelimumab 1 mg/kg Q4W up to four doses, then durvalumab 10 mg/kg Q2W	(include: cetuximab, docetaxel, paclitaxel, methotrexate, 5-fluorouracil, TS-1 or capecitabine)
CONDOR (22)	JAMA Oncol	NCT02319044	133/67/67	IV	Squamous	Canada (100.0)	Durvalumab 20 mg/kg Q4W +tremelimumab 1 mg/kg Q4W, followed by durvalumab 10 mg/kg Q2W	Tremelimumab 10 mg/kg Q4W for 7 doses then every 12 weeks for 2 additional doses
(open-label, II)	2018	(2:1:1)	(62/62/61)	220/47			or durvalumab 10 mg/kg Q2W	

(Continued)

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TABLE 1 Continued

Study (Phase, Design)	Source (y)	Registered ID (Randomization)	Sample Size (Median Age/y)	Stage (Male/ Female)	Histology	Ethnicity (%)	Intervention Arm(s)	Control Arm
KEYNOTE-122 (23)	ANN Oncol	NCT02611960	117/116	III- IV	Squamous	North America (14.2)	Pembrolizumab 200 mg Q3W	Capecitabine 1000 mg/m^2 on days 1-14 Q3W
(open-label, III)	2022	(1:1)	(51/53)	(193/40)		Asia (85.8)		or gemcitabine 1250 mg/m^2 on days 1-8 Q3W or docetaxel 75 mg/m^2 on days 1 Q3W
CheckMate 141 (24)	Oral Oncol	NCT02105636	240/121	IV	Squamous	North America (40.2)	Nivolumab 3 mg/kg Q2W	Methotrexate 40–60 mg/m^2 Q1W
(open-label, III)	2018	(2:1)	(60/61)	(61/300)		Europe (47.4) Other (12.4)		or docetaxel 30–40 mg/m^2 Q1W or cetuximab 400 mg/m^2 once then 250 mg/m^2 Q1W

Four studies evaluating first-line treatments reported the incidence of grade ≥3 AEs, with significant statistical heterogeneity observed across studies (P<0.1, I^2=96%). A random-effects model was employed for the meta-analysis (Figure 3; Supplementary Figure 6). The results showed that in patients with R/M HNSCC, ICI therapy was associated with a significantly lower incidence of grade ≥3 AEs compared to the SOC (OR=0.17, 95% CI: 0.08-0.40). Subgroup analysis demonstrated that combination therapy with two ICIs (OR=0.25, 95% CI: 0.11-0.60) significantly reduced the incidence of grade ≥3 AEs compared to SOC. In contrast, ICI monotherapy (OR=0.14, 95% CI: 0.01-1.37) did not show a statistically significant safety advantage over SOC.

Four studies on second-line treatment reported the incidence of grade 3 or higher AEs, with low statistical heterogeneity (P=0.14, I^2=42%). A fixed-effect model was used for the meta-analysis (Figure 3; Supplementary Figure 12). The results showed that for R/M HNSCC patients, the use of immunotherapy (OR=0.38, 95% CI: 0.28-0.50) was associated with a lower incidence of grade 3 or higher AEs compared to the control group. Subgroup analysis of intervention regimens indicated that single ICI (OR=0.33, 95% CI: 0.25-0.42) and dual immunotherapy (OR=0.61, 95% CI: 0.39-0.96) both demonstrated better safety profiles compared to SOC.

3.3 Network meta-analyses

3.3.1 Comparisons of OS, PFS and ORR

The primary efficacy endpoints of this study were OS and PFS, with ORR as a secondary outcome. The NMA included seven first-line immunotherapy regimens (Figure 4A) and six second-line immunotherapy regimens (Figure 4C) for patients with R/M HNSCC.

For OS in first-line therapy (Figure 5A), nivolumab (HR=0.71, 95% CI: 0.52-0.98), pem-chemo (HR=0.77, 95% CI: 0.63-0.94), and pembrolizumab monotherapy (HR=0.83, 95% CI: 0.70-0.99) demonstrated significant OS benefits compared to the standard of care (SOC). In second-line therapy (Figure 6A), only nivolumab (HR=0.68, 95% CI: 0.54-0.86) and pembrolizumab (HR=0.83, 95% CI: 0.70-0.99) exhibited significant OS improvements over SOC.

For PFS in first-line therapy (Figure 5A), nivo-ipi showed the poorest PFS outcomes across all treatment regimens. None of the immunotherapy regimens conferred a significant PFS benefit compared to SOC. Similarly, in second-line therapy (Figure 6A), no significant PFS improvements were observed for any immunotherapy regimen compared to SOC. However, nivolumab (HR=0.58, 95% CI: 0.37-0.90) provided the most notable PFS benefit when compared to tremelimumab.

With respect to ORR in first-line therapy (Figure 5B), none of the immunotherapy regimens offered an ORR advantage over SOC. The SOC showed a notably superior ORR compared to nivo-ipi (OR=0.19, 95% CI: 0.13-0.27). For second-line therapy (Figure 6B), pembrolizumab (OR=0.32, 95% CI: 0.22-0.45), nivolumab (OR=0.32, 95% CI: 0.19-0.54), and durvalumab (OR=0.37, 95% CI: 0.23-0.60) demonstrated significant ORR advantages over SOC.

3.3.2 Safety and toxicity

Safety and toxicity were evaluated based on the incidence of grade 3 or higher AEs. The NMA included nine first-line ICI

TABLE 2 Characteristics of included randomized controlled trials.

Study	PD-L1 Detection	PD-L1≥1% Patients (%)			PD-L1 ≥20% Patients (%)			Reported Outcomes
		Intervention (s),n(%)		Control, n (%)	Intervention (s),n(%)		Control, n (%)	
CheckMate 651	CPS	355(75.2)		372(78.3)	185(39.2)		178(37.5)	OS, PFS, ORR, grade≥3 AEs
KESTREL	TPS	/		/	63(30.9)	128(31.0)	65(31.6)	OS, PFS, ORR, grade≥3 AEs
KEYNOTE-048	CPS, TPS	257(85)	242(86)	255(85)	133(44)	126(45)	232(40.1)	OS, PFS, ORR, grade≥3 AEs
KEYNOTE-040	TPS, CPS	196(79)		191(77)	/		/	OS, PFS, ORR, grade≥3 AEs
CheckMate 714	TPS	157(55.7)		79(55.2)	/		/	OS, PFS, ORR, grade≥3 AEs
EAGLE	TPS	/		/	68(28.3)	72(29.1)	72(28.9)	OS, PFS, ORR, grade≥3 AEs
CONDOR	TPS	61(45.9)	30(44.8)	30(44.8)	/		/	OS, PFS, ORR, grade≥3 AEs
KEYNOTE-122	CPS	87(74.4)		73(62.9)	55(47.0)		46(39.7)	OS, PFS, ORR, grade≥3 AEs
CheckMate 141	TPS	88(36.7)		61(48.8)	/		/	OS, PFS, ORR, grade≥3 AEs

regimens for grade ≥3 AEs (Figure 4B) and five second-line ICI regimens (Figure 4D).

In first-line therapy (Figure 5B), all ICI monotherapies significantly reduced the incidence of grade ≥3 AEs compared to the SOC. The most notable safety benefits were observed with pembrolizumab (OR=0.12, 95% CI: 0.05-0.29), nivo-ipi (OR=0.19, 95% CI: 0.08-0.42), and nivolumab monotherapy (OR=0.19, 95% CI: 0.08-0.42). For second-line therapy (Figure 6B), pembrolizumab (OR=0.32, 95% CI: 0.22-0.54), nivolumab (OR=0.32, 95% CI: 0.19-0.54), and durvalumab (OR=0.37, 95% CI: 0.23-0.60) demonstrated significant safety advantages over SOC.

No new safety signals emerged during the study. The most commonly reported grade ≥3 AEs associated with immunotherapy were anemia, nausea, vomiting, decreased neutrophil count, neutropenia, fatigue, and asthenia (Figure 7; Supplementary Table 5). Frequently reported grade ≥3 immune-mediated AEs included rash, hypothyroidism, hyperthyroidism, and immune-mediated lung disease (Supplementary Table 5). Among all grade

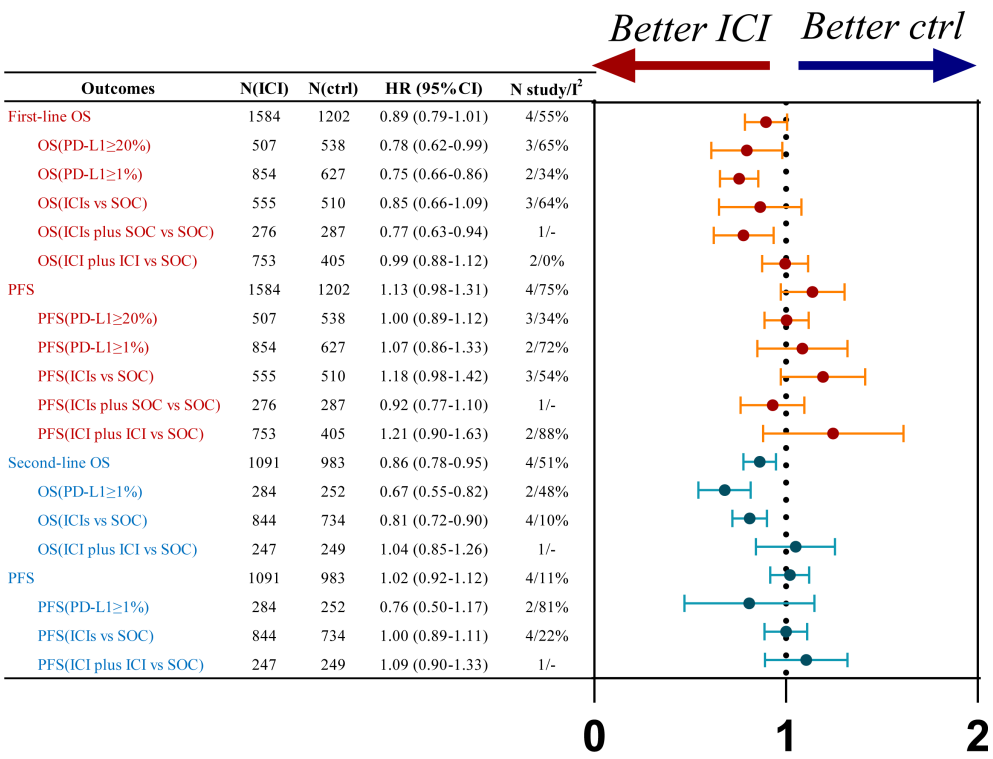

FIGURE 2
Summary forest plot of OS and PFS for first-line (Red) and second-line (Blue) treatments, ctrl, control.

Outcomes	n/N(ICI)	n/N(ctrl)	OR (95%CI)	N study/I^2
First-line ORR	1393/1721	833/1491	3.02 (1.47-6.18)	4/94%
ORR(ICIs vs SOC)	459/555	236/510	3.54 (1.41-8.87)	3/86%
ORR(ICIs plus SOC vs SOC)	181/281	192/300	1.02 (0.73-0.43)	1/-
ORR(ICI plus ICI vs SOC)	753/885	405/681	4.53 (2.64-7.78)	2/77%
AE≥3	566/1704	1069/1433	0.17 (0.08-0.40)	4/96%
AE≥3(ICIs vs SOC)	113/552	383/509	0.14 (0.01-1.37)	3/98%
AE≥3(ICIs plus SOC vs SOC)	131/276	239/287	0.18 (0.12-0.27)	1/-
AE≥3(ICI plus ICI vs SOC)	322/876	447/637	0.25 (0.11-0.60)	2/93%
Second-line ORR	911/1091	838/983	0.83 (0.65-1.06)	4/23%
ORR(ICIs vs SOC)	708/844	632/734	0.79 (0.59-1.05)	4/37%
ORR(ICI plus ICI vs SOC)	203/247	206/249	0.96 (0.61-1.53)	1/-
AE≥3	182/1081	314/937	0.38 (0.28-0.50)	4/42%
AE≥3(ICIs vs SOC)	142/835	256/697	0.33 (0.25-0.42)	4/0%
AE≥3(ICI plus ICI vs SOC)	40/246	58/240	0.61 (0.39-0.96)	1/-

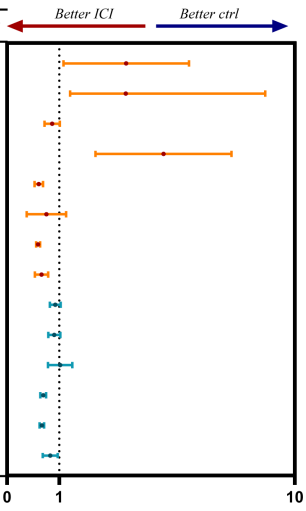

FIGURE 3
Summary forest plot of ORR and grade 3 or higher AEs for first-line (red) and second-line (blue) treatments, ctrl, control.

≥3 AEs, pem-chemo was most likely to induce anemia, neutropenia, and decreased neutrophil count. For immune-mediated grade ≥3 AEs, durva-treme was most likely to result in rash and hypothyroidism. The incidence of treatment-related AEs, such as anemia, neutropenia, and decreased neutrophil count, varied significantly across regimens, while the spectrum of immune-mediated AEs, including hyperthyroidism and immune-mediated lung disease, was more consistent across treatments.

3.3.3 Subgroup analysis

The NMA included six first-line immunotherapy regimens (Figures 8A, B) and three second-line immunotherapy regimens

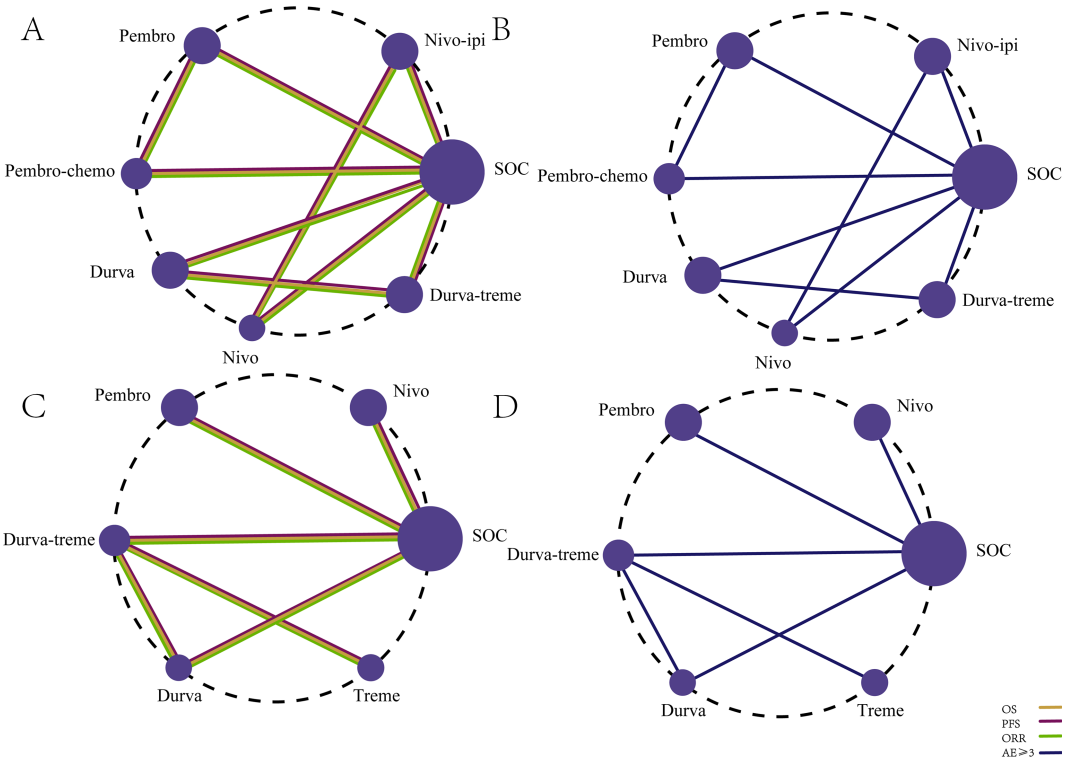

FIGURE 4
Network diagram comparing the efficacy and safety of first-line and second-line immunotherapy regimens in R/M HNSCC without PD-L1 expression selection. **(A)** OS, PFS, and ORR for first-line immunotherapy regimens. **(B)** Incidence of AEs ≥3 for first-line immunotherapy regimens. **(C)** OS, PFS, and ORR for second-line immunotherapy regimens. **(D)** Incidence of AEs ≥3 for second-line immunotherapy regimens.

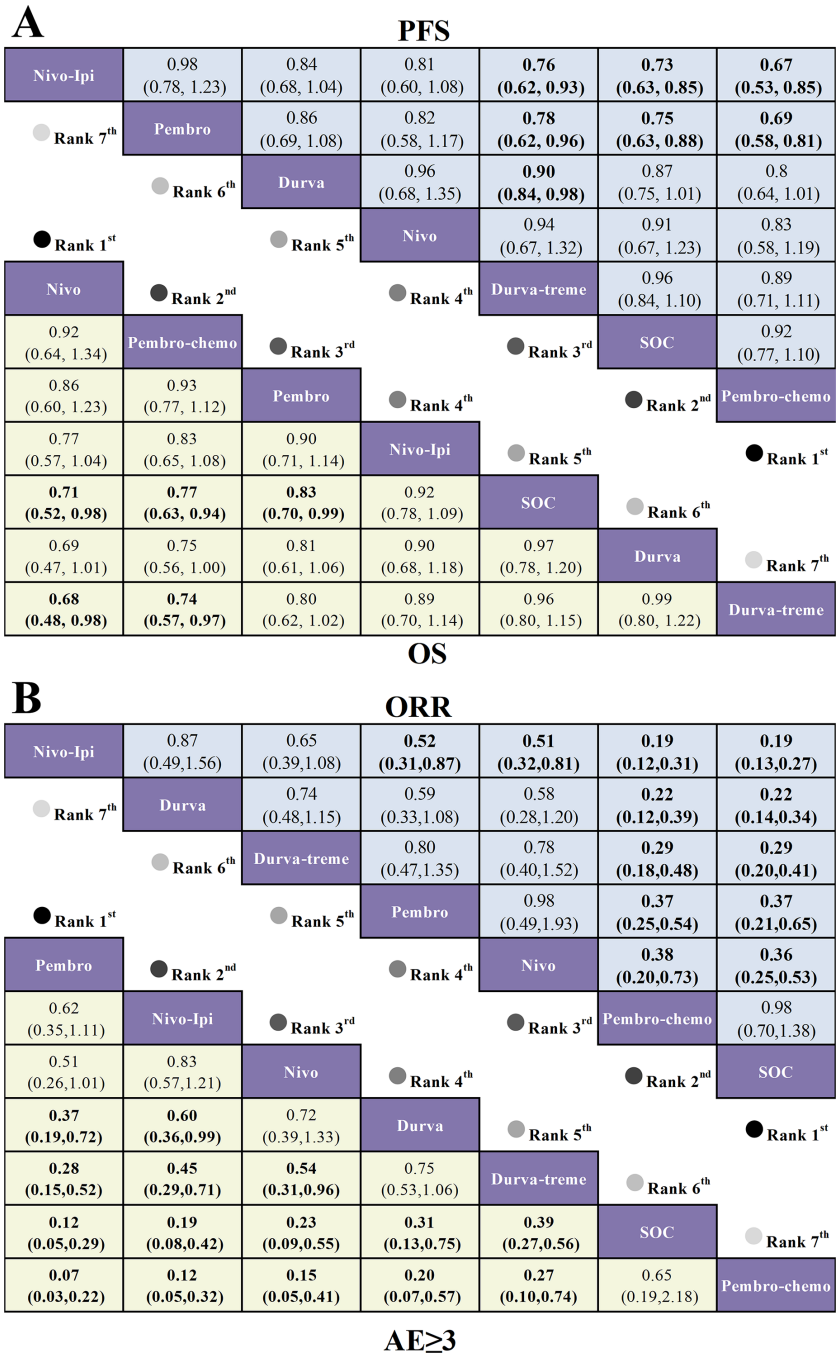

FIGURE 5
League table based on Bayesian network meta-analysis comparing the efficacy and safety of first-line immunotherapy in R/M HNSCC patients. **(A)** HR and 95% CI for OS (yellow lower triangle) and PFS (blue upper triangle), with HR < 1.00 indicating a better survival benefit. **(B)** OR and 95% CI for AEs ≥3 and ORR, with OR < 1.00 indicating a better benefit.

(Figure 8C) for R/M HNSCC patients with different levels of PD-L1 expression.

For first-line treatment regarding OS (Figures 9A, C), R/M HNSCC patients with PD-L1 expression ≥1% showed significant OS benefits with pembrolizumab plus chemotherapy (HR=0.65, 95% CI: 0.53-0.80) and pembrolizumab (HR=0.78, 95% CI: 0.64-0.96) compared to SOC. In patients with PD-L1 expression ≥20%, both pembrolizumab plus chemotherapy (HR=0.60, 95% CI: 0.44-0.81) and pembrolizumab (HR=0.61, 95% CI: 0.45-0.83) demonstrated significant OS benefits over SOC. For second-line treatment (Figure 9B), patients with PD-L1 expression ≥1% had significantly prolonged OS with nivolumab (HR=0.55, 95% CI: 0.39-0.78) and pembrolizumab (HR=0.74, 95% CI: 0.58-0.94) compared to SOC.

Regarding PFS for first-line treatment (Figures 9A, C), in R/M HNSCC patients with PD-L1 expression ≥1%, only pembrolizumab

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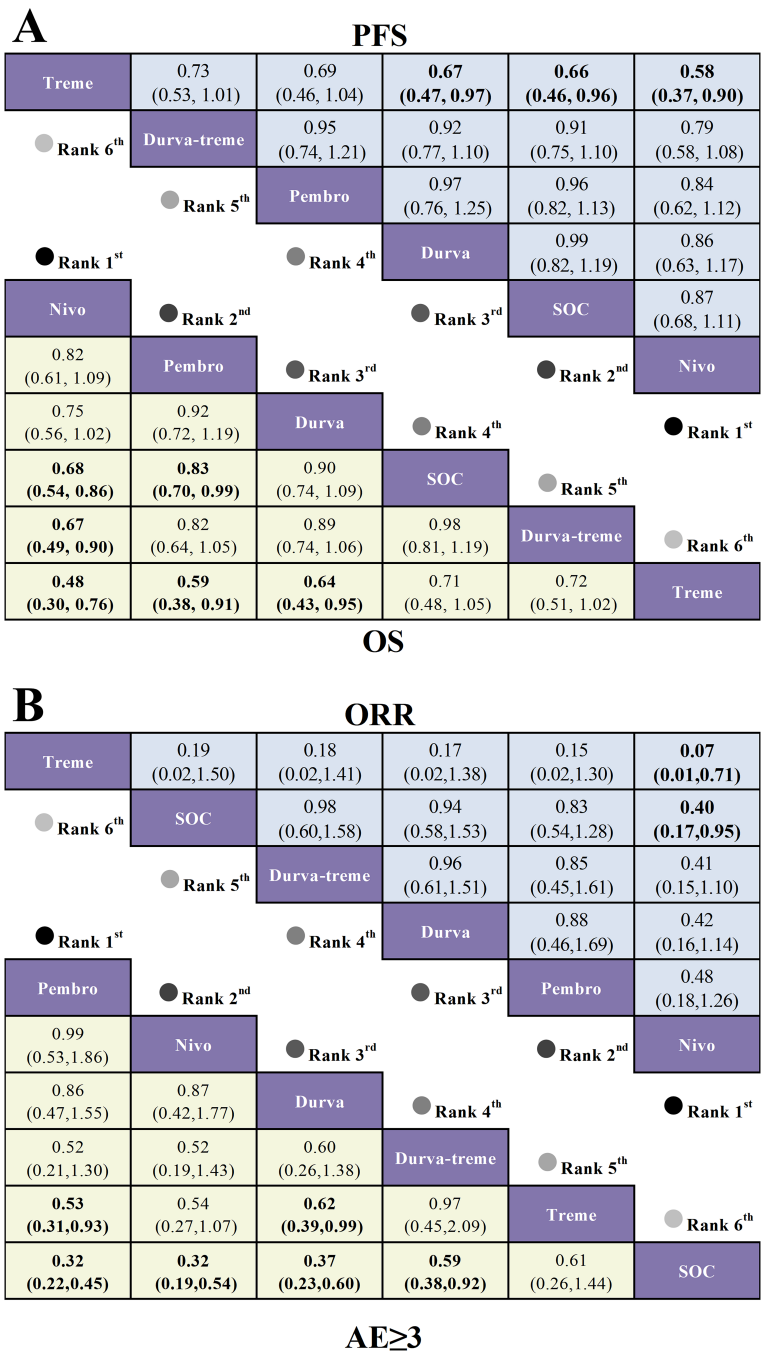

FIGURE 6
League table based on Bayesian network meta-analysis comparing the efficacy and safety of second-line immunotherapy in R/M HNSCC patients. **(A)** HR and 95% CI for OS and PFS, with HR < 1.00 indicating better survival benefit. **(B)** OR and 95% CI for AEs ≥3 and ORR, with OR < 1.00 indicating better benefit.

plus chemotherapy (HR=0.82, 95% CI: 0.67-1.00) showed a statistically significant extension of PFS compared to SOC. For patients with PD-L1 expression ≥20%, only pembrolizumab plus chemotherapy (HR=0.73, 95% CI: 0.55-0.97) significantly extended PFS compared to SOC. For second-line treatment (Figure 9B), nivolumab (HR=0.59, 95% CI: 0.41-0.84) showed significant PFS benefits over SOC in patients with PD-L1 expression ≥1%.

3.4 Rank

Ranking analysis based on Bayesian ranking profiles was conducted (Figures 10–12; Supplementary Tables 6–9). Among patients with R/M HNSCC without PD-L1 selection, nivolumab was most likely to rank first for OS with a cumulative probability of 64.17%.Pem-chemo ranked first for PFS (69.42%) and ORR

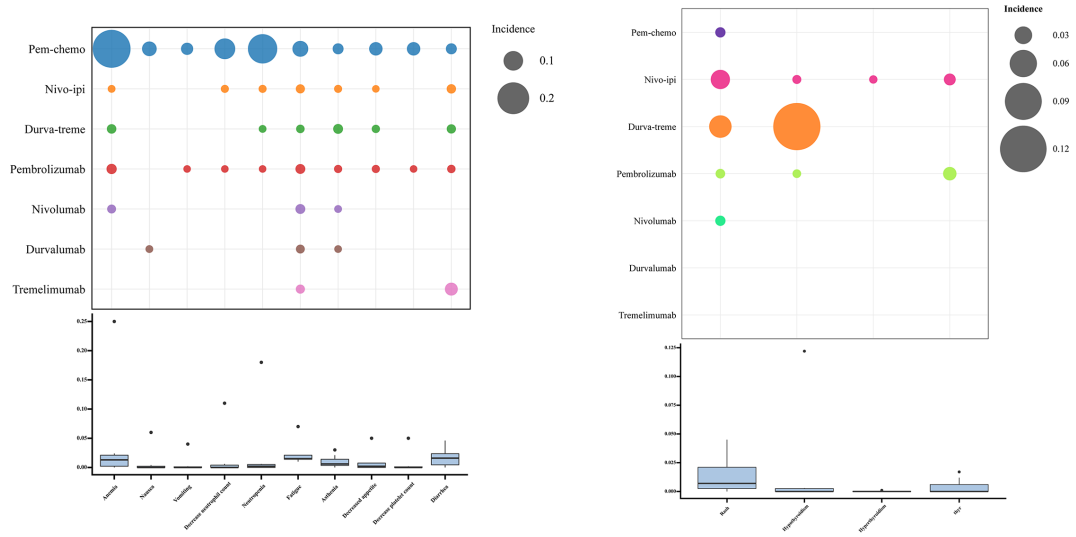

FIGURE 7
Safety Profile of Various immunotherapy Regimens. **(A)** Incidence of treatment-related Grade ≥3 adverse events. **(B)** Incidence of immune-mediated Grade ≥3 adverse events.

(35.06%), while pembrolizumab monotherapy was most likely to rank first for grade ≥3 AEs (56.42%). Notably, pem-chemo demonstrated the best efficacy in first-line treatment, ranking first for both PFS and ORR, and second for OS.

For second-line treatment in R/M HNSCC patients, nivolumab was most likely to rank first for OS (89.91%), PFS (75.10%), and ORR (76.94%), while also ranking first for grade ≥3 AEs (37.14%). In second-line treatment, nivolumab exhibited excellent performance in both efficacy and safety, ranking first across all key outcomes: OS, PFS, ORR, and grade ≥3 AEs.

Among R/M HNSCC patients with PD-L1 expression ≥1%, nivolumab as a first-line treatment was most likely to provide the best OS benefit (63.37%), while pem-chemo was most likely to offer the greatest PFS benefit (91.76%). In second-line treatment, nivolumab demonstrated the most favorable outcomes for both OS (91.76%) and PFS (98.96%).For patients with PD-L1 expression ≥20%, pem-chemo was the most likely first-line therapy to provide the best OS (49.65%) and PFS benefit (91.42%).

3.5 Heterogeneity and Inconsistency

The results of the pairwise meta-analysis based on the frequentist approach are consistent with the corresponding aggregated results within the Bayesian framework (Supplementary Table 3). Heterogeneity was assessed using the Q test and I^2 statistics, indicating high heterogeneity with $I^2 > 50\%$ (Figures 2, 3). After sequential exclusion of individual studies, the heterogeneity did not significantly decrease, suggesting the reliability of the conclusions. A funnel plot was used to analyze publication bias with OS as the outcome indicator, showing a symmetrical distribution of study points without scattered distribution, indicating a low likelihood of publication bias in this study (Supplementary Figures 15, 16).

4 Discussion

To the best of our knowledge, this study represents the first comprehensive systematic review and network meta-analysis assessing the safety and efficacy of both first-line and second-line immunotherapies in patients with R/M HNSCC, including detailed evaluations of efficacy in subgroups with PD-L1 expression levels of ≥1% and ≥20%. Our extensive analysis yields evidence-based insights for clinical practice, summarized as follows:

1. First-line immunotherapy demonstrated a clear safety advantage compared to the SOC, but no significant efficacy benefit. In contrast, second-line immunotherapy showed significant advantages in both OS and grade ≥3 adverse events compared to SOC.
2. Combination therapy with chemotherapy and ICIs led to a marked improvement in efficacy compared to ICI monotherapy; however, this came at the cost of increased toxicity.
3. In first-line treatment for R/M HNSCC patients, pembrolizumab combined with chemotherapy provided the greatest efficacy benefit, with no statistically significant difference in safety compared to SOC, while pembrolizumab monotherapy showed the best safety profile. For patients with PD-L1 expression ≥20%, pem-chemo demonstrated the most significant efficacy benefit. Among patients with PD-L1 expression ≥1%, nivolumab offered the best OS benefit, while pem-chemo provided the greatest PFS advantage.
4. In second-line treatment, for patients, nivolumab exhibited the most outstanding performance in both efficacy and safety among all treatment options. Similarly, for patients with PD-L1 expression ≥1%, nivolumab again demonstrated the best efficacy outcomes.

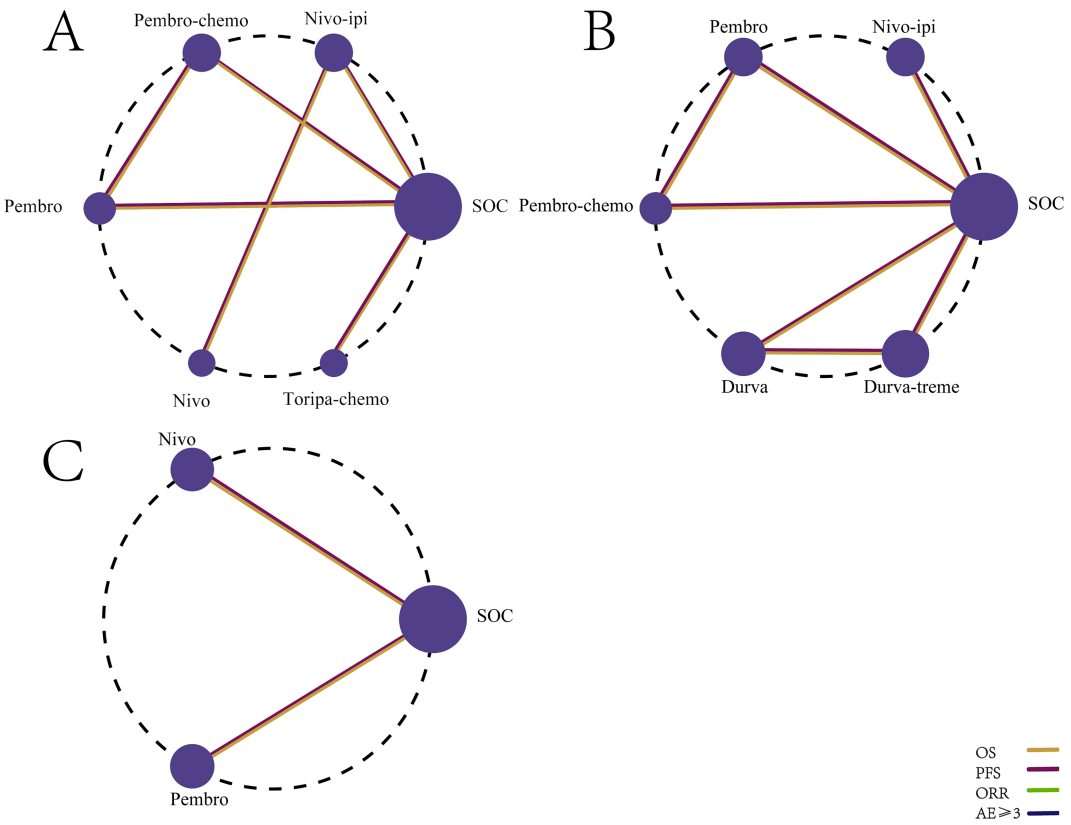

FIGURE 8
Network diagram comparing the efficacy of first-line and second-line immunotherapy regimens in R/M HNSCC with different PD-L1 expression levels. **(A)** First-line treatment regimens in patients with PD-L1 expression ≥1%. **(B)** First-line treatment regimens in patients with PD-L1 expression ≥20%. **(C)** Second-line treatment regimens in patients with PD-L1 expression ≥1%.

Overall, immunotherapy demonstrated significant advantages in OS and Grade ≥3 adverse events compared to SOC, consistent with previous meta-analysis results (25). Given the distinct differences between first-line and second-line treatments, we analyzed them separately and performed subgroup analyses for monotherapy and combination therapy, enriching and objectifying our conclusions. Immunotherapy specifically activates the anti-tumor activity of T lymphocytes by blocking the interactions between PD-1, PD-L1, and CTLA-4, thereby enabling T cells to specifically recognize and eliminate tumor cells while sparing normal tissues (26, 27). This selective mechanism contrasts with chemotherapy, which directly destroys cancer cells but, due to its non-specific nature, may lead to increased resistance and toxicity (28). This explains the observed efficacy and safety advantages of immunotherapy over SOC. The differing mechanisms of immunotherapy and chemotherapy suggest a synergistic effect when combined, enhancing anti-tumor efficacy and explaining the significant improvement in outcomes with combined therapy (29).

This study also performed a statistical analysis of Grade ≥3 and immune-mediated adverse events, with no new safety issues identified. The incidence of severe adverse events was higher with ICI combined with chemotherapy compared to ICI monotherapy and dual immunotherapy, consistent with Dang's meta-analysis (30). Unlike Dang's study, our research included immune-mediated adverse events, noting a significant increase in such events with dual immunotherapy. This may be attributed to the additive effects of CTLA-4 and PD-L1/PD-1 pathways (31).

Interestingly, dual immunotherapy regimens, such as durvalumab plus tremelimumab, did not show clinical benefit over SOC in both first-line and second-line settings, and nivolumab plus ipilimumab also did not show benefit in the first-line setting compared to SOC. In contrast, ICI monotherapy demonstrated significant clinical benefits over SOC. The lack of clinical efficacy with dual immunotherapy compared to monotherapy indicates the need for further research to understand the effects of combining PD-(L)1 and CTLA-4 inhibitors in R/M HNSCC.

PD-L1 expression levels serve as biomarkers for predicting the clinical efficacy of immunotherapy in various malignancies (32). Our study found that for patients with PD-L1 expression levels of ≥20% or ≥1%, pembrolizumab combined with chemotherapy as a first-line treatment provided excellent efficacy benefits. Compared to the meta-analysis by Rodrigo et al., which only compared high

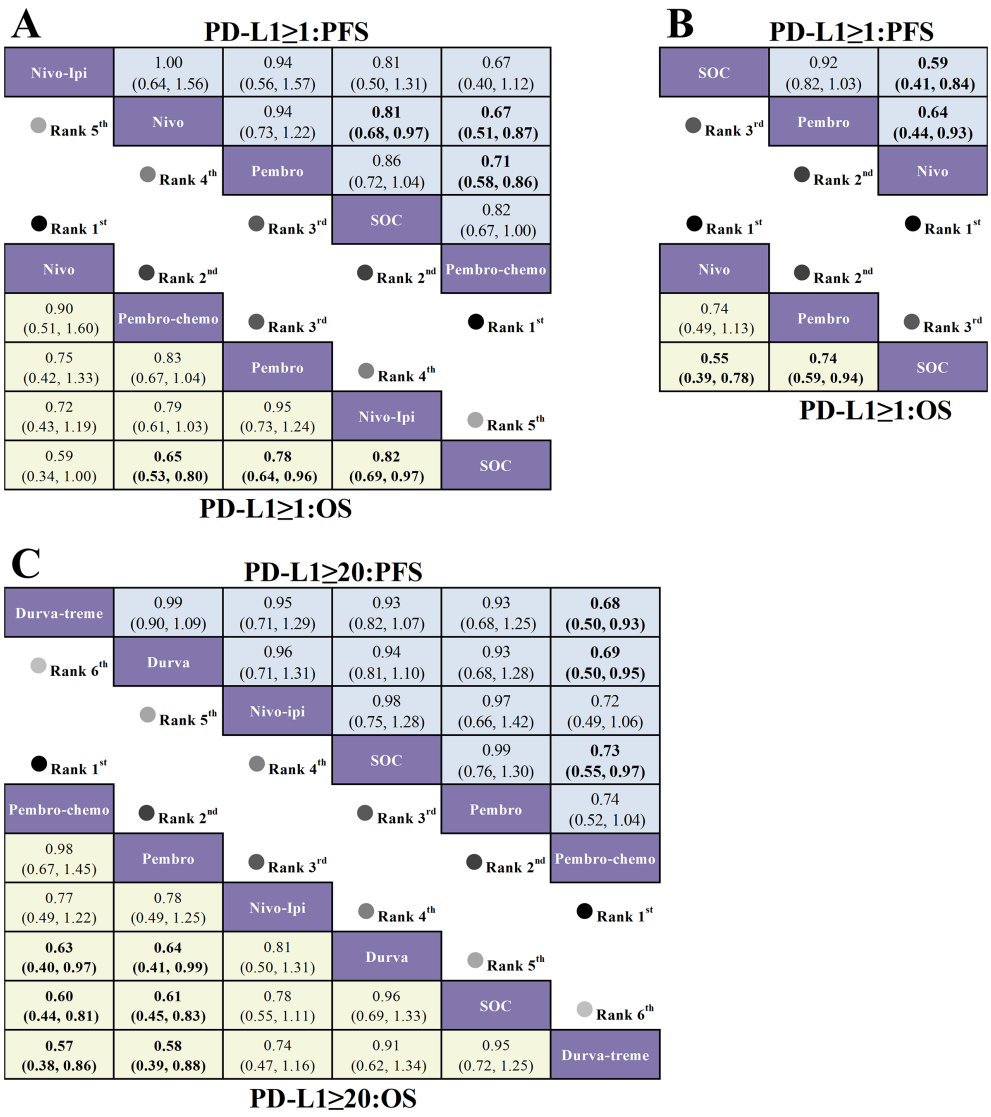

FIGURE 9
League table based on Bayesian network meta-analysis comparing the efficacy of first-line and second-line immunotherapy in R/M HNSCC patients with different PD-L1 expression levels. **(A)** HR and 95% CI for OS (yellow lower triangle) and PFS (blue upper triangle) in first-line treatment for patients with PD-L1 expression ≥1%, with HR < 1.00 indicating better survival benefit. **(B)** HR and 95% CI for OS and PFS in second-line treatment for patients with PD-L1 expression ≥1%. **(C)** HR and 95% CI for OS and PFS in first-line treatment for patients with PD-L1 expression ≥20%, with HR < 1.00 indicating better survival benefit.

PD-L1 expression(PD-L1 ≥10%) in R/M HNSCC patients using immunotherapy versus SOC, our study stratified PD-L1 expression levels and identified the optimal immunotherapy regimens (33).

This study provides a significant contribution to the existing knowledge on immunotherapy for R/M HNSCC by leveraging the Bayesian framework to yield novel insights into the comparative efficacy and safety of various regimens. Unlike prior analyses that primarily emphasized the relationship between higher PD-L1 expression and improved outcomes, this study goes beyond by stratifying PD-L1 expression levels (≥1%, ≥20%) and identifying optimal first-line and second-line treatments tailored to these subgroups. The Bayesian approach offers distinct advantages over traditional frequentist methods, including the integration of direct and indirect evidence, probabilistic treatment rankings, and robust consistency checks (34). This enables a more nuanced understanding of treatment efficacy and safety, facilitating evidence-based, personalized treatment strategies for diverse patient populations. When weighing clinical efficacy and safety, pembrolizumab combined with chemotherapy emerges as a strong first-line treatment option for R/M HNSCC patients without PD-L1 selection, while nivolumab stands out as the optimal second-line therapy. Additionally, our results demonstrate that selecting the appropriate first- or second-line immunotherapy regimen for patients with PD-L1 expression levels of ≥20% or ≥1% can lead

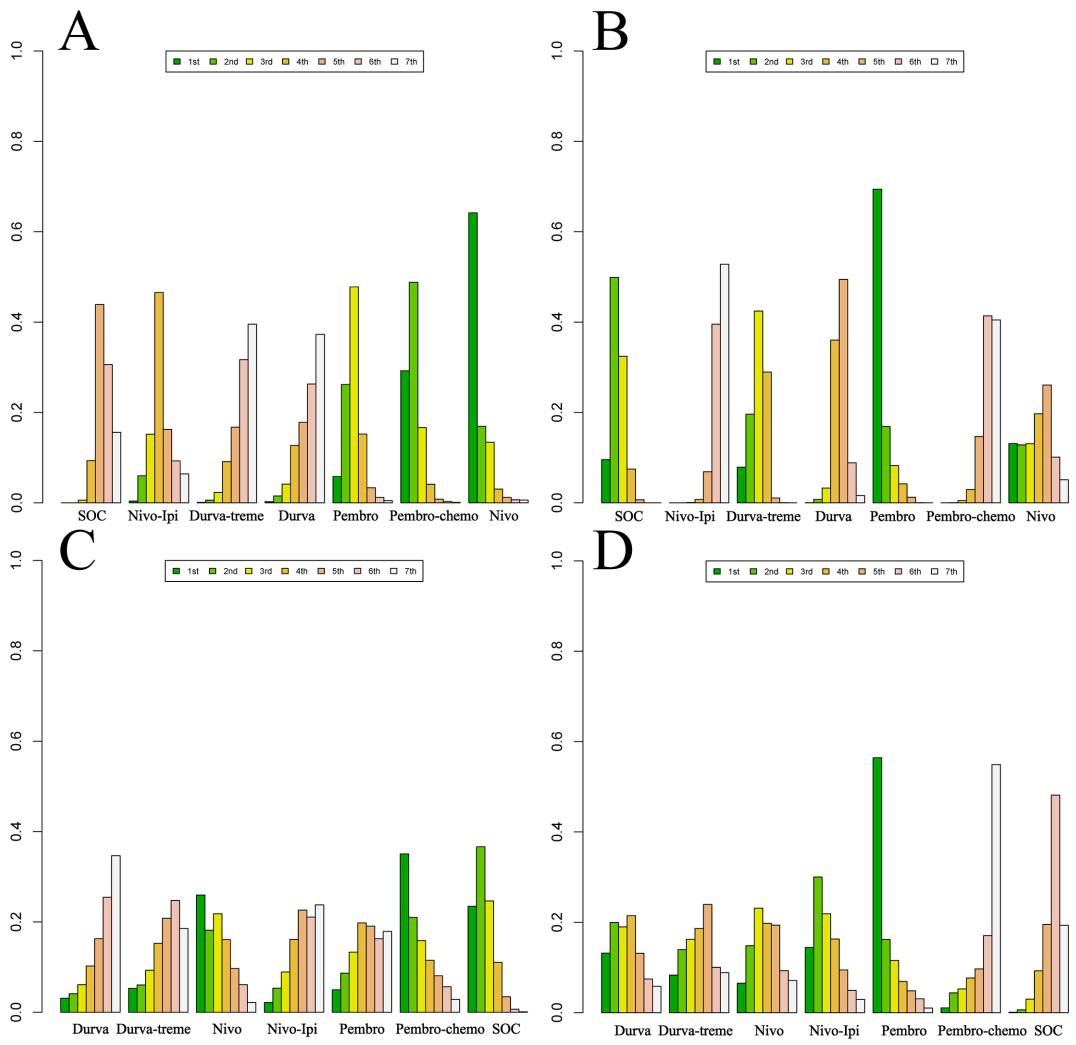

FIGURE 10
Bayesian ranking profiles for efficacy and safety of various first-line immunotherapy regimens in R/M HNSCC patients. **(A)** OS Ranking. **(B)** PFS Ranking. **(C)** ORR Ranking. **(D)** Grade ≥3 Adverse Events Ranking.

to improved survival outcomes. These findings can complement NCCN guidelines by providing additional evidence on the most effective treatment approaches for R/M HNSCC patients based on PD-L1 expression. Future research should focus on more second-line studies combining immunotherapy with chemotherapy, such as pembrolizumab plus chemotherapy and nivolumab plus chemotherapy, to potentially expand the options for second-line treatments. Although the included studies were multicenter RCTs with patients from diverse ethnic backgrounds, we found that the majority of participants were from Europe, North America, and Asia. Less than 1% of the included patients were Black, and fewer than 3% were Hispanic. Future RCTs that include a greater number of participants from African populations or other underrepresented minority groups would enhance the comprehensiveness and generalizability of the findings. Current RCTs mainly stratify PD-L1 expression levels at 1% and 20%. Stratified analysis for patients with PD-L1 expression ≥50% in future studies could greatly aid in personalized treatment for this subgroup.

4.1 Limitations

Although this study draws several important conclusions, it is important to acknowledge a few limitations. First, there were variations in the SOC regimens used across the different control groups. For instance, the CheckMate 651 and KESTREL trials utilized cisplatin or carboplatin plus 5-fluorouracil in combination with cetuximab, while CheckMate 141 employed methotrexate, docetaxel, or cetuximab. While both regimens are recognized as standard first-line treatments, these differences may have introduced some degree of bias into the results. Secondly, as mentioned earlier, less than 1% of the included patients were Black,

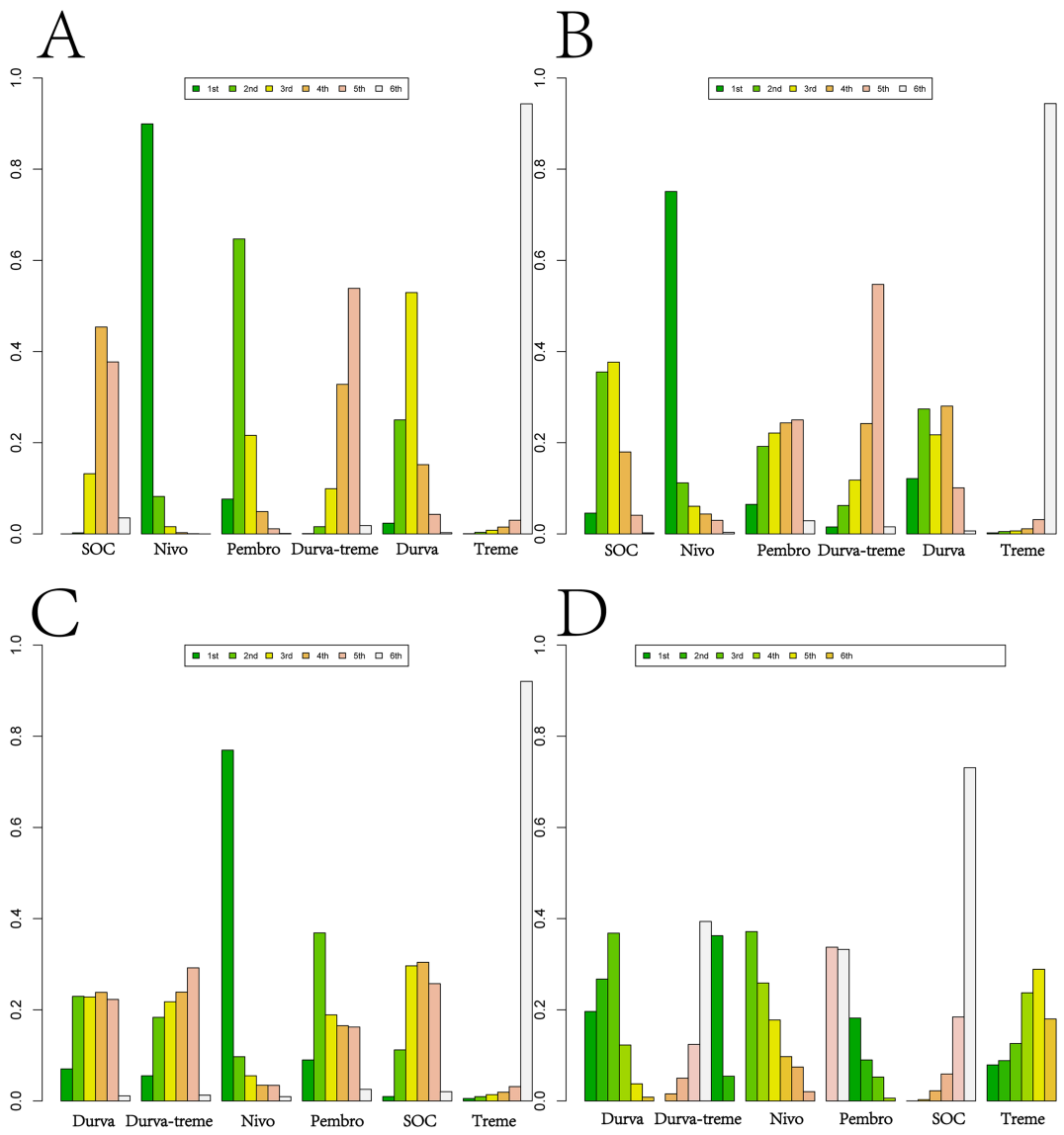

FIGURE 11
Bayesian ranking profiles for efficacy and safety of various second-line immunotherapy regimens in R/M HNSCC patients. **(A)** OS Ranking. **(B)** PFS Ranking. **(C)** ORR Ranking. **(D)** Grade ≥3 Adverse Events Ranking.

and fewer than 3% were Hispanic. The applicability of the study's conclusions to Black individuals or other minority populations requires further consideration. Third, despite our efforts to include all relevant RCTs investigating immunotherapy for R/M HNSCC, the limited number of available trials means that some interventions were represented by only a single RCT, which may restrict the robustness of our conclusions. Fourth, as only two studies reported the safety outcomes of combination therapy compared to SOC, the finding that dual ICIs significantly reduced the incidence of grade ≥3 AEs compared to SOC should be interpreted with caution. Fifth, the included RCTs employed different methods for assessing PD-L1 expression, with some using the tumor proportion score (TPS) and others using the combined positive score (CPS). Given that CPS provides a more comprehensive reflection of the tumor microenvironment and PD-L1 expression status, it is generally preferred. However, in trials such as KESTREL or CheckMate 714, which only reported TPS, we were constrained to using TPS for PD-L1 evaluation. Finally, head and neck cancers comprise a diverse array of subtypes, such as oropharyngeal cancer, hypopharyngeal cancer, and laryngeal cancer. While survival outcomes may differ among these subtypes, the limited number of studies precludes subgroup analyses, necessitating cautious interpretation of the findings.

Despite these limitations, this study offers a thorough and comprehensive summary of randomized controlled trials on first- and second-line immunotherapy for R/M HNSCC.

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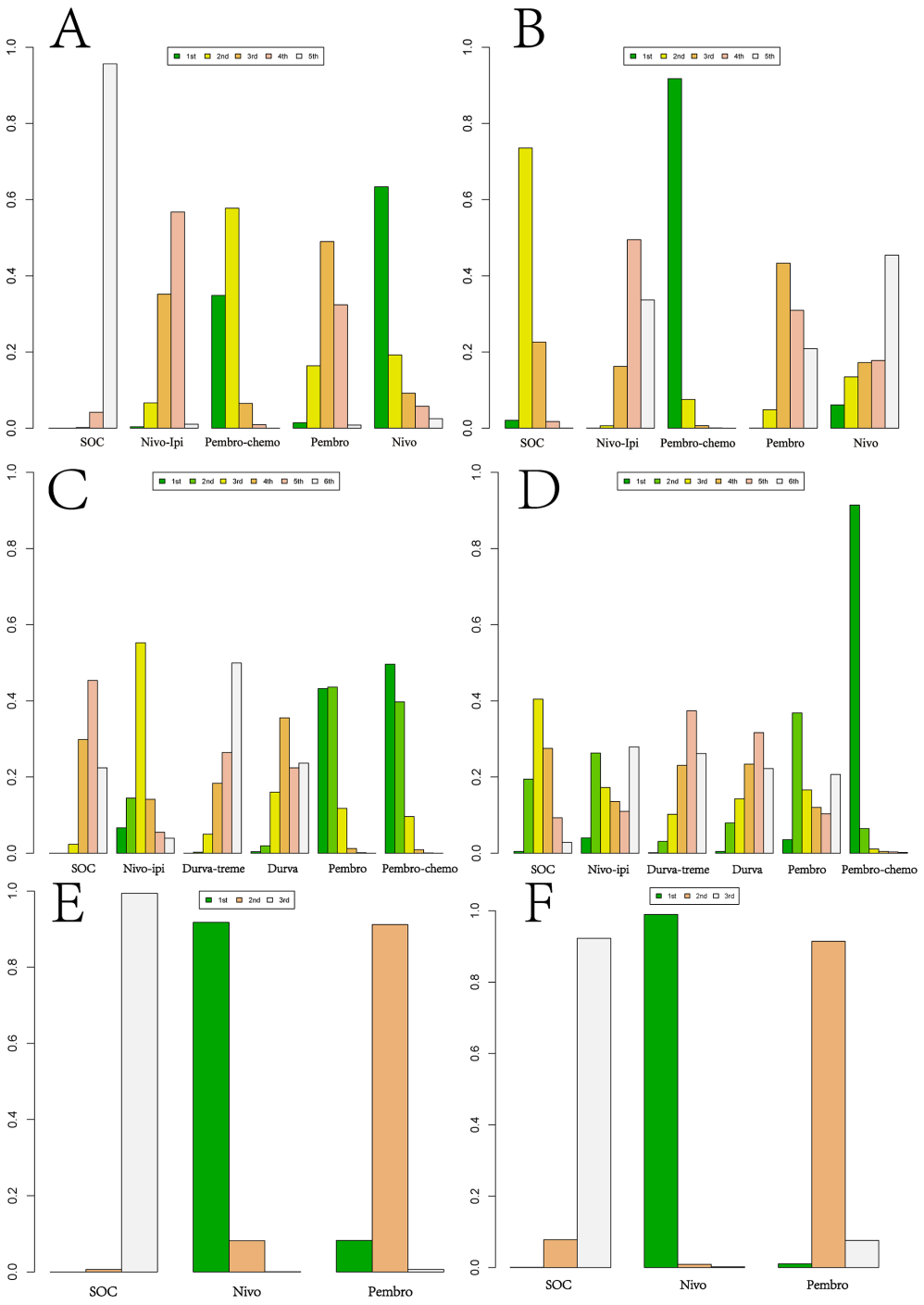

FIGURE 12
Bayesian Ranking Profiles for Efficacy and Safety of Various First-line or Second-line immunotherapy Regimens in R/M HNSCC Patients with Different PD-L1 Expression Levels. **(A)** First-line OS Ranking for PD-L1 Expression ≥1%. **(B)** First-line PFS Ranking for PD-L1 Expression ≥1%. **(C)** First-line OS Ranking for PD-L1 Expression ≥20%. **(D)** First-line PFS Ranking for PD-L1 Expression ≥20%. **(E)** Second-line OS Ranking for PD-L1 Expression ≥1%. **(F)** Second-line PFS Ranking for PD-L1 Expression ≥1%.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

WC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. QW: Conceptualization, Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. TX: Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing. JiL: Formal analysis, Project administration, Validation, Writing – original draft, Writing – review & editing. MH: Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. YM: Resources, Visualization, Writing – original draft, Writing – review & editing. LZ: Validation, Visualization, Writing – original draft, Writing – review & editing. WX: Resources, Visualization, Writing – original draft, Writing – review & editing. SL: Resources, Writing – original draft, Writing – review & editing. LS: Investigation, Software, Writing – original draft, Writing – review & editing. WL: Validation, Writing – original draft, Writing – review & editing. ZB: Visualization, Writing – original draft, Writing – review & editing. JuL: Resources, Writing – original draft, Writing – review & editing. ZL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1508885/full#supplementary-material

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A case of camrelizumab-induced anaphylaxis and successful rechallenge: a case report and literature review

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Immune checkpoint inhibitors have been extensively utilized in the treatment of various malignancies, with camrelizumab being one of the agents in this therapeutic class. In this study, we report for the first time a case of an allergic reaction to camrelizumab in a patient with nasopharyngeal carcinoma, who was successfully rechallenged after antiallergic treatment. The patient, a 62-year-old male, was diagnosed with advanced nasopharyngeal carcinoma, exhibiting cancer infiltration and multiple metastases. He underwent multiple cycles of therapy, tolerating camrelizumab, nab-paclitaxel, and nedaplatin (200 mg of camrelizumab every 3 weeks) without adverse reactions in the first four cycles. However, during the fifth cycle, after the intravenous infusion of camrelizumab, he experienced gradual onset of dizziness and chest tightness within 15 minutes (peripheral arterial oxygen saturation was approximately 94%, blood pressure was 76/42 mmHg, heart rate was 83 beats per minute, and respiratory rate was 15 breaths per minute). The camrelizumab infusion was immediately halted, and the patient was treated with intravenous dexamethasone (10 mg) combined with intramuscular diphenhydramine, calcium gluconate, and 500 ml of normal saline; his blood pressure gradually increased to 110/80 mmHg within 10 minutes, and pruritic erythematous macules appeared on his skin, particularly on the upper limbs. Subsequently, nab-paclitaxel was infused, and upon completion, the erythematous macules on the limbs faded. The patient was then rechallenged with a slow infusion of camrelizumab, which was well-tolerated without discomfort or a drop in blood pressure. The patient did not report significant discomfort. Although acute allergic reactions are relatively rare among immune-related adverse events, due to the widespread clinical application of camrelizumab, its potential for allergic reactions should be given high priority.

KEYWORDS

immune checkpoint inhibitors, camrelizumab, anaphylaxis reaction, case report, anaphylaxis shock

Introduction

With the continuous advancement of medical technology, the treatment of malignant tumors has evolved from traditional surgery, radiotherapy, and chemotherapy to more precise and personalized therapeutic approaches (1). In recent years, the emergence of immunotherapy has brought revolutionary changes to cancer treatment, particularly in certain types of tumors where it has become an integral part of the standard treatment regimen (2).

Immune checkpoint inhibitors (ICIs) reactivate the patient's own immune system to combat tumors by blocking mechanisms that allow tumor cells to evade immune surveillance (3). Camrelizumab (SHR-1210) is an immune checkpoint inhibitor, a monoclonal antibody targeting the PD-1 receptor (4). It enhances the body's antitumor immune response by blocking the interaction between PD-1 and its ligand PD-L1, thereby relieving the immunosuppressive effect of tumor cells on T-cells. Camrelizumab has been approved for the treatment of various malignancies, including esophageal squamous cell carcinoma and non-small cell lung cancer (5, 6).

Despite the significant potential of immune checkpoint inhibitors in oncology, they can also lead to adverse reactions, including immune-related adverse events. Anaphylactic shock is one of the severe adverse reactions, which, although rare, poses a threat to patients' life safety when it occurs (7). In this article, we report a case of acute allergic reaction induced by the infusion of camrelizumab, and the successful rechallenge with camrelizumab after antiallergic management.

Case presentation

General information

The patient is a 62-year-old male who was diagnosed with nasopharyngeal carcinoma six years ago and has had stable disease control after multiple cycles of chemotherapy combined with radiotherapy. The patient has a smoking history of over 20 years, with 20 cigarettes per day, and a history of moderate alcohol consumption.

Treatment course

The patient, a 62-year-old male, presented to our hospital's Otolaryngology Department in 2018 with nasal congestion and blood-tinged nasal discharge. A biopsy of a nasopharyngeal neoplasm indicated non-keratinizing carcinoma, leading to a diagnosis of nasopharyngeal carcinoma. He underwent chemoradiotherapy with three cycles of the TP regimen, specifically consisting of paclitaxel liposome (Lipusu) 210mg on day 1 and nedaplatin 130mg on day 1, supplemented with antiemetic, gastric protection, and fluid support treatments. The radiotherapy concluded on November 2, 2018. The patient tested negative for Epstein-Barr virus and had regular follow-ups. A nasopharyngeal MRI in June 2024 showed progression of bone destruction compared to previous scans. A whole-body PET/CT imaging on June 29, 2024, revealed: 1. Mildly increased glucose metabolism in the right posterior wall of the nasopharynx post-radiotherapy for nasopharyngeal carcinoma, suggesting possible inflammatory changes or viable tumor tissue, with recommendations for follow-up; destruction of the pterygoid and abnormally increased glucose metabolism in the clivus region of the occipital bone, indicating possible tumor involvement. On July 17, 2024, August 8, 2024, August 30, 2024, and October 5, 2024, the patient received nab-paclitaxel 400mg intravenous infusion and nedaplatin 135mg intravenous infusion chemotherapy along with camrelizumab 200mg intravenous infusion for immunotherapy. On October 28, 2024, the patient was readmitted with a blood pressure of 134/85 mmHg. Fifteen minutes after the infusion of camrelizumab, he gradually developed dizziness and chest tightness (peripheral arterial oxygen saturation was approximately 94%, blood pressure was 76/42 mmHg, heart rate was 83 beats per minute, and respiratory rate was 15 breaths per minute), with clear consciousness. Suspecting anaphylactic shock, camrelizumab was immediately discontinued, and the patient was treated with intravenous dexamethasone (10 mg) combined with intramuscular diphenhydramine, calcium gluconate, and 500 ml of normal saline; within 10 minutes, his blood pressure gradually increased to 110/80 mmHg, and pruritic erythematous macules appeared on his skin, especially on the upper limbs (as shown in Figure 1). Subsequently, nab-paclitaxel was infused, and after completion, the erythematous macules on the limbs faded. After thorough communication with the patient and his family, who considered the drug to be expensive but effective and wished to try the remaining medication again, the patient was informed of the potential risks of recurrent anaphylactic shock and other life-threatening risks. The patient acknowledged the risks and was willing to accept them. The patient was then rechallenged with a slow infusion of camrelizumab at a rate of 10 drops per minute, with close monitoring of blood pressure, oxygen saturation, and other vital signs. The infusion proceeded smoothly without discomfort or a drop in blood pressure. The infusion rate was gradually increased to 30 drops per minute half an hour later, and the entire process was uneventful without adverse reactions. On December 13, 2024, January 3, 2025 and January 25, 2025, the patient received tislelizumab (0.2 g, D1) as part of immunotherapy maintenance, accompanied by anti-allergic prophylaxis with promethazine (25 mg), dexamethasone (5 mg), and loratadine (8.8 mg). The treatment was well tolerated, with no significant adverse reactions reported. Subsequent follow-up nasopharyngeal MRI indicated a reduction in the size of the skull base lesion.

Literature search

The search terms "camrelizumab," "immune checkpoint inhibitors," and "allergic reactions" were used to retrieve relevant case reports from the Wanfang Data, Web of Science, and PubMed databases up to November 2024. An analysis and summary of the clinical characteristics and outcomes of the detected cases were conducted. As of November 2024, 4 similar case reports were identified from PubMed, and the summarized information is presented in Table 1.

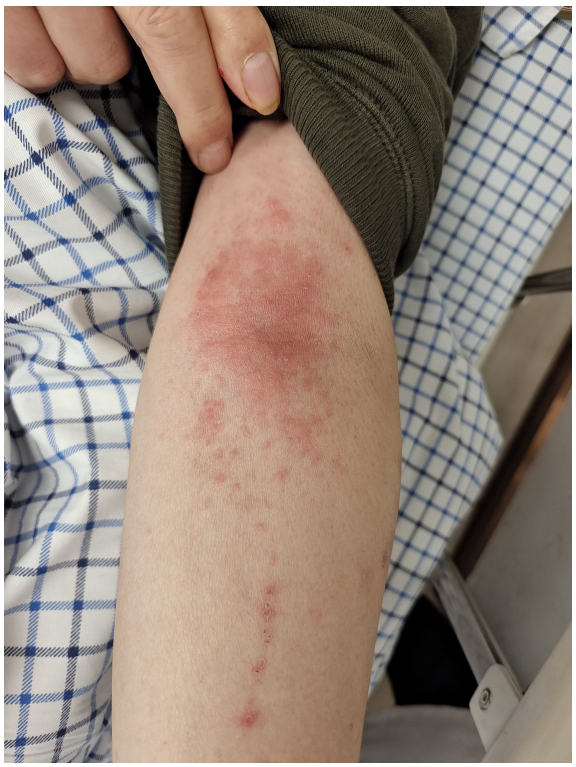

FIGURE 1
Allergic reaction following drug injection. The image shows erythematous rash and localized swelling on the forearm of the patient after drug administration, indicative of an allergic response.

Discussion

In the new era of immunotherapy, PD-1 inhibitors such as camrelizumab have demonstrated significant efficacy in the treatment of various tumors. However, the allergic reactions they cause, particularly anaphylactic shock, though rare, can pose a serious threat to patients' lives when they do occur (8). In this case, the patient experienced anaphylactic shock during the treatment of nasopharyngeal carcinoma with camrelizumab. After timely rescue and antiallergic treatment, the patient was successfully rechallenged with camrelizumab, continued the treatment, and achieved a good therapeutic effect.

According to the literature review, anaphylactic shock caused by monoclonal antibody biologics typically occurs in the early stages of treatment, especially in the first few cycles. These reactions are characterized by rapid onset, involving the skin, mucous membranes, or both, such as generalized urticaria, itching, or flushing, as well as respiratory impairment (e.g., difficulty breathing, asthma-bronchospasm, wheezing, reduced peak expiratory flow, and hypoxemia). Additionally, blood pressure drop or end-organ dysfunction (e.g., muscle rigidity, syncope, incontinence) are also common clinical manifestations (8–10).

In managing anaphylactic shock, rapid recognition and timely treatment are crucial. According to the guidelines of the European Academy of Allergy and Clinical Immunology (EAACI) Multidisciplinary Taskforce, any of the above symptoms, when present, should raise a high suspicion of an allergic reaction (11). In this case, the patient exhibited symptoms rapidly after receiving

TABLE 1 Literature review of infusion reaction/anaphylaxis caused by immune checkpoint inhibitor.

Author	Immune checkpoint inhibitor	Types of cancer	Adverse reaction	Occurrence time	Clinical treatment	
Choi B et al	Nivolumab	hepatocellular carcinoma	1. Facial flushing, shortness of breath, and low back pain 2. Allergy-like symptoms	1. 10 min after the second infusion 2. 11min after the third cycle of treatment	1. Discontinued and treated with diphenhydramine and hydrocortisone, followed by a slow infusion of the remaining nivolumab 2. Resolved with symptomatic treatment, switched to pembrolizumab for subsequent immunotherapy	Choi B, McBride A, Scott AJ. Treatment with pembrolizumab after hypersensitivity reaction to nivolumab in a patient with hepatocellular carcinoma. Am J Health-syst Pharm: Ajhp: Off J Am Soc Health-syst Pharm. 2019 Oct 15;76(21):1749–52.
Ogawara D et al	Nivolumab	lung squamous cell carcinoma	Skin itching and flushing which quickly spread all over the body, and the blood oxygen saturation decreased from 97% to 92	15 min after the second infusion	The infusion was stopped immediately and oxygen inhalation, chlorpheniramine, and methylprednisolone were given	Ogawara D, Soda H, Ikehara S, Sumiyoshi M, Iwasaki K, Okuno D, et al. Nivolumab infusion reaction manifesting as plantar erythema and pulmonary infiltrate in a lung cancer patient. Thorac Cancer. 2017 Nov;8 (6):706–9.
Mercedes Sáenz de Santa	Nivolumab	hepatocellular carcinoma	Facial flushing, rash, and eyelid edema	Near the end of the third cycle	The symptoms disappeared spontaneously	Sáenz de Santa María García M, Noguerado-Mellado B, Rojas-Pérez-Ezquerra P, Prieto-García

(Continued)

TABLE 1 Continued

Author	Immune checkpoint inhibitor	Types of cancer	Adverse reaction	Occurrence time	Clinical treatment	
María García et al					about 45 min after withdrawal	A, Bartolomé-Zavala B, Tornero P. First case of allergy to nivolumab. J Allergy Clin Immunol, Pract. 2017;5 (4):1140–1.
Liu K et al	Camrelizumab	Esophageal squamous cell carcinoma	Palpitation, dyspnea and a feeling of death; the pulse rate in the indoor air was 70 beats/min, the blood pressure was 69 centimeters of 24 mm mercury, the respiratory rate was 28 beats/min, and the pulse oxygen saturation was 86%	10 min after the second infusion	intravenous infusion, epinephrine, dexamethasone sodium phosphate, calcium gluconate and norepinephrine	Liu K, Bao JF, Wang T, Yang H, Xu BP. Camrelizumab-induced anaphylactic shock in an esophageal squamous cell carcinoma patient: a case report and review of literature. World J Clin Cases. 2022 Jun 26;10 (18):6198–204.
Yizhuo Zhao et al	atezolizumab	small cell lung cancer	anaphylactic shock, such as dyspnea, cold limbs, and loss of consciousness. A	three minutes after the second infusion	oxygen, epinephrine, dopamine, methylprednisolone,	Zhao Y, Peng W, Abbas M, Shi M, Tang Y, Wang L, et al. Anaphylactic shock in a small cell lung cancer patient receiving atezolizumab therapy: a rare but potentially fatal complication. Invest New Drugs. 2022 Feb;40 (1):209–14.
Ji Hyun Oh et al	atezolizumab	hepatocellular carcinoma	facial flushing and generalized itching and soon lost consciousness with hypotension and an oxygen saturation of 90%.	5 minutes after starting the first cycle of atezolizumab	dexamethasone, chlorpheniramine and norepinephrine	Oh JH, Seo KI, Kim HK, Choi GS. Successful desensitization to atezolizumab-induced near-fatal anaphylaxis in patients with hepatocellular carcinoma: a case report and literature review. Asia Pac Allergy. 2024 Aug;14 (3):139–42.
Weiting Liang et al	Cadonilimab	lung cancer	chest distress and shortness of breath.	15 min after the second infusion	diphenhydramine 20 mg, promethazine 25 mg, and compound sodium chloride 500 mL. Dexamethasone 5 mg	Hong DI, Madrigal-Burgaleta R, Banerji A, Castells M, Alvarez-Cuesta E. Controversies in allergy: chemotherapy reactions, desensitize, or delabel? J Allergy Clin Immunol, Pract. 2020 Oct;8 (9):2907-2915.e1.
Weiting Liang et al	Cadonilimab	nasopharyngeal cancer	multiple red skin bumps on the trunk and pruritus	during the fifth infusion	intravenous infusion of dexamethasone 10 mg and esomeprazole 40 mg,	Agrawal S, Statkevich P, Bajaj G, Feng Y, Saeger S, Desai DD, et al. Evaluation of immunogenicity of nivolumab monotherapy and its clinical relevance in patients with metastatic solid tumors. J Clin Pharmacol. 2017 Mar;57 (3):394–400.
Weiting Liang et al	Cadonilimab	squamous cell carcinoma of the cervix	chest distress, facial flushing, abdominal pain, vomiting, and profuse sweating	3 min after the first infusion	Dexamethasone 30 mg, diphenhydramine 20 mg, promethazine 50 mg, and compound sodium chloride 500 mL were administered	Isabwe GAC, de Las Vecillas Sanchez L, Castells M. Management of adverse reactions to biologic agents. Allergy Asthma Proc. 2017 Nov 1;38 (6):409–18.
Weiting Liang et al	Cadonilimab	hepatocellular carcinoma	sweating, low BP (64/42 mmHg), flaked red rash and pruritus appeared on the arm, neck, and buttocks	during the third infusion	intramuscular injection of butyryl 30 mg, diphenhydramine and dexamethasone	Ramírez-Cruz S, Lucena-Campillo MA, Vila-Albelda C, Garrido-Arévalo M, De Agustín-Sierra L, García-Díaz B. Desensitization protocol to nivolumab without corticosteroid use in a kidney cancer patient.

(Continued)

TABLE 1 Continued

Author	Immune checkpoint inhibitor	Types of cancer	Adverse reaction	Occurrence time	Clinical treatment	
						Farm Hosp: Organo Of Expr Cient Soc Esp Farm Hosp. 2020 Jul 1;44(4):182–3.
Weiting Liang et al	Cadonilimab	cervical cancer	redness and swelling in the face, numbness, and itching in the mouth, low BP (85/60 mmHg),	during the second infusion	Dexamethasone 10 mg, cimetidine 0.4 g, and promethazine 25 mg	
Weiting Liang et al	Cadonilimab	lung cancer*	systemic cold sweats and rapid breathing, BP was 92/ 61 mm Hg, and HR was 104 beats/min	during the second infusion	Dexamethasone 10 mg, cimetidine 0.2 g, diphenhydramine 20 mg	
Weiting Liang et al	Cadonilimab	adenoid cystic carcinoma	shivering with undetectable low BP	during the seventh infusion	dexamethasone 10 mg	

camrelizumab treatment, consistent with the clinical presentation of an allergic reaction. The patient also experienced a significant drop in blood pressure, which allowed for the diagnosis of anaphylactic shock.

The possibility of reusing the drug:

The question of whether to reuse the drug after an anaphylactic shock event is a complex one. Generally, once an anaphylactic shock has occurred, it is not recommended to reuse the same drug for safety reasons. However, each case requires an individualized assessment, taking into account factors such as the patient's tumor status, treatment response, and the severity of the allergic reaction. In this case, the patient gradually developed symptoms such as headache and chest tightness 15 minutes after the infusion, rather than an immediate rapid drop in blood pressure, and did not exhibit loss of consciousness. In the management of the symptoms, the drug was discontinued, and antiallergic medications were administered along with fluid resuscitation, after which the patient's blood pressure recovered, symptoms were alleviated, and no medications such as epinephrine were used. We considered that the patient's anaphylactic shock was not extremely urgent and rapidly progressive.

Subsequently, regarding the continuation of the remaining camrelizumab infusion, we communicated fully with the patient and their family. They believed that the drug was expensive but effective and wished to try the remaining medication again. We informed the patient and their family of the potential risks of recurrent anaphylactic shock and other life-threatening risks. The patient acknowledged the risks and was willing to accept them. The patient was then rechallenged with a slow infusion of camrelizumab at a rate of 10 drops per minute, with close monitoring of vital signs such as blood pressure and oxygen saturation. The infusion proceeded smoothly without discomfort or a drop in blood pressure. After half an hour, the infusion rate was gradually increased to 30 drops per minute, and the entire process was uneventful without adverse reactions.

In the new era of immunotherapy, PD-1 inhibitors such as camrelizumab have shown significant efficacy in the treatment of various tumors. However, allergic reactions they cause, particularly anaphylactic shock, though rare, can pose a serious threat to patients' lives. Beyond PD-1 inhibitors like camrelizumab, there is also a report concerning cadonilimab, a PD-1/CTLA-4 bispecific antibody developed by a Chinese company. This report introduced seven cases of infusion reactions caused by cadonilimab, with symptoms including chills, fever, and rash, even including blood pressure drop. After antiallergic treatment, three of these cases also underwent rechallenge with cadonilimab and successfully continued cadonilimab treatment without allergic reactions. Therefore, rechallenge after severe infusion reactions can also be attempted in some patients (12).

Additionally, there is a report of a patient with hepatocellular carcinoma treated with atezolizumab who experienced a severe allergic reaction, including blood pressure drop, oxygen saturation decrease, and loss of consciousness, only after being rescued in the ICU was the patient out of danger. Due to the good tumor treatment effect, the patient eventually chose to try the drug again. Through antihistamine, glucocorticoid, and other antiallergic drugs for pretreatment, and gradually increasing drug concentration and infusion rate for desensitization treatment, atezolizumab was eventually used again in the patient. In our report, the patient did not undergo desensitization treatment with gradually increasing drug concentration and infusion rate for subsequent treatments; if used in the future, such desensitization treatment may be safer.

In this case, we observed that the patient developed an allergic reaction after the fifth cycle of camrelizumab treatment(as shown in Figure 2, By Figdraw.). Based on the timing of the reaction, we believe that this allergic response may be a pseudoallergic reaction rather than a typical IgE-mediated allergic reaction. According to the study by McNeil et al., MrgprX2 is a receptor almost exclusively expressed on mast cells, and it has been shown to cause mast cell activation in

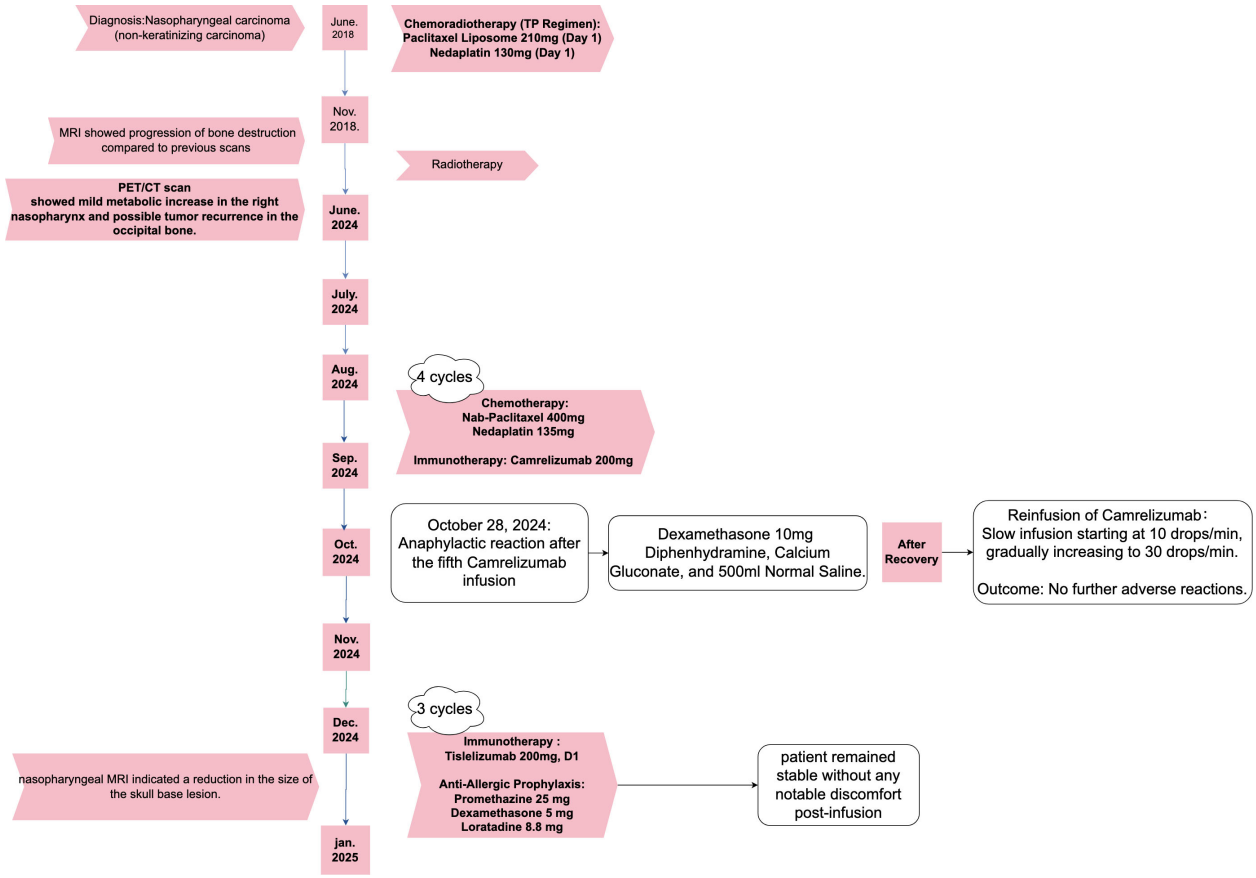

FIGURE 2
Timeline of the patient's treatment.

response to several chemotherapeutic agents (13, 14). The activation of MrgprX2 is not IgE-mediated but occurs through direct binding with the drug, leading to the release of mediators such as histamine from mast cells, thereby causing allergy-like symptoms. This finding is important for understanding allergic reactions induced by immunotherapeutic drugs, such as camrelizumab.

Furthermore, considering the low affinity characteristic of the MrgprX2 receptor, we hypothesize that the patient was able to tolerate rechallenge therapy by avoiding the rapid activation of the allergic threshold, thus successfully enduring the retreatment. Unlike IgE-mediated reactions, if the allergic reaction were IgE-mediated, rechallenge would typically not be successful, as IgE antibodies would quickly trigger a strong allergic response.

Therefore, considering the pseudoallergic reaction mechanism mediated by MrgprX2 is of significant value for the clinical application and management of future immunotherapies (15). With the development of MrgprX2 antagonists, therapeutic strategies targeting these non-IgE-mediated allergic reactions may offer more options for patients, especially for those who cannot tolerate conventional treatments.

In summary, although the incidence of acute allergic shock caused by camrelizumab is low, they seriously threaten patients' lives, interrupt the continuity of immunotherapy, and affect the prognosis of tumor patients. With the increasing application of immunotherapy in clinical practice, allergy history and other risk factors should be carefully considered to minimize the occurrence of adverse reactions (16). At the same time, identifying factors related to anaphylactic shock caused by ICIs, screening susceptible patients, and clinical skin testing to reduce the risk of anaphylactic shock are issues that deserve attention and in-depth research.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by The ethics committee of The Second Affiliated Hospital ZheJiang University School of Medicine. The studies were conducted in accordance with

the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article. We confirm that written informed consent was obtained from the participant(s)/patient(s) for the publication of this case report.

Author contributions

PS: Investigation, Writing – original draft, Methodology. YJ: Writing – original draft, Formal Analysis, Supervision, Writing – review & editing. LD: Writing – original draft, Conceptualization. LF: Writing – original draft, Data curation, Methodology, Supervision. YT: Data curation, Writing – original draft, Conceptualization, Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Neoadjuvant immunochemotherapy versus neoadjuvant immunoradiotherapy in locally advanced oral squamous cell carcinoma

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Objective: To juxtapose the efficacy and safety profiles of neoadjuvant immunochemotherapy (NAIC) and neoadjuvant immunoradiotherapy (NAIR) in the management of locally advanced oral squamous cell carcinoma (SCC).

Methods: A retrospective analysis of prospectively gathered data was conducted. The study evaluated the impact of NAIC versus NAIR on various parameters, including pathologic complete response (pCR), major pathologic response (mPR), clinical to pathological downstaging, surgical site infection, quality of life, pathologic adverse features, and prognostic outcomes.

Results: The study encompassed a total of 120 patients, with 73 undergoing NAIC. The pCR and mPR rates in the NAIR group were 25.5% and 63.8%, respectively, closely mirroring the 31.5% and 69.9% observed in the NAIC cohort. A propensity for clinical to pathological downstaging and a reduced incidence of pathologic adverse features was noted in the NAIC population. However, both groups exhibited similar distributions in surgical site infection rates, quality of life metrics, grade 3/4 adverse events, and overall survival. In the Cox proportional hazards model, patients receiving NAIC demonstrated a hazard ratio of 0.87 (95% confidence interval: 0.65-0.98) for 3-year locoregional control, relative to the NAIR group.

Conclusion: In the context of locally advanced oral SCC, both NAIC and NAIR exhibited robust efficacy and safety profiles. Nevertheless, NAIC provided superior locoregional control compared to NAIR, thereby emerging as the more favorable initial therapeutic option over NAIR.

KEYWORDS

neoadjuvant immunochemotherapy, neoadjuvant immunoradiotherapy, oral squamous cell carcinoma, pathologic complete response, quality of life

Introduction

Oral squamous cell carcinoma (SCC) ranks as the most prevalent malignant tumor among all head and neck cancers, with a majority presenting at an advanced stage upon initial diagnosis primarily due to lymph node metastasis (1). Current standard treatment consists of surgical intervention followed by adjuvant radiotherapy or chemoradiation; however, nearly half of these patients experience locoregional failure or distant metastasis (2). The lack of substantial improvement in prognosis underscores the pressing need for innovative treatment strategies for oral SCC.

In light of the encouraging survival advantages delineated by a seminal trial (3), immunotherapy has been sanctioned as the primary treatment modality for recurrent/metastatic SCC of the head and neck. A marked pivot towards exploring immunotherapy within the neoadjuvant context for untreated head and neck SCC has garnered considerable interest. A succession of clinical trials has demonstrated remarkable therapeutic efficacy and a paucity of adverse effects associated with neoadjuvant immunochemotherapy (NAIC) in head and neck SCC (4–6). In the Illuminate Trial (4), a cohort of twenty patients was enrolled. NAIC was found to be eminently tolerable, with a negligible incidence of grades 3-4 adverse events in but three patients. The rate of major pathological response (mPR) was 60%, encompassing a 30% pathological complete response (pCR). Throughout their median 23-month follow-up, disease-free survival was observed at 90%, with an overall survival (OS) rate of 95%. An additional phase II trial, involving 48 patients, yielded an objective response rate of 89.6%. Among the 27 patients who underwent surgical intervention, 17 (63.0%) achieved an mPR or pCR, with a pCR rate of 55.6%. Grade 3 or 4 treatment-related adverse events were reported in only two patients (5). A retrospective analysis of 21 patients (6) revealed an mPR of 66.7%, including 11 patients who attained a pCR. The overall response rate was 90.5%, and the rate of complete response was 28.6%. There were no grade 4 adverse events or instances of delayed surgery. Recently, a phase 1b trial concentrated on the efficacy of immunoradiotherapy (NAIR) in head and neck SCC (7), reporting mPR and pCR rates of 86% and 67% respectively. Clinical to pathological downstaging was observed in 90% of patients treated, with no delays in surgery. In another retrospective study (8), an analysis of 30 patients revealed no serious adverse events, with mPR, pCR, and clinical to pathological downstaging rates of 60.0%, 33.3%, and 83.3% respectively. Over a median follow-up period of 13.5 months, the disease-free survival and OS at 24 months were 70.4% and 76.4% respectively. Radiation oncologists are also keen to explore the synergistic potential of radiotherapy and immunotherapy in head and neck SCC (9). Current evidence suggests that both NAIC and NAIR demonstrate pronounced efficacy in head and neck SCC; however, the comparative effectiveness and safety profiles of these two modalities remain to be elucidated.

Thus, our objective is to compare the efficacy and safety profiles of NAIC and NAIR in the context of locally advanced oral SCC.

Patients and methods

Ethical approval

This study was approved by Our Hospital Institutional Research Committee, and written informed consent for medical research was obtained from all patients before starting the treatment. All methods were performed in accordance with the Declaration of Helsinki.

Study design

To fulfill our objective, a retrospective analysis was conducted on prospectively collected data. Between January 2020 and December 2021 a total of 140 consecutive patients diagnosed with resectable cT1/2N+ or cT3/4N_{any} oral SCC were enrolled at a tertiary cancer center, but 20 cases refused to take part in this research. Finally, 47 patients received NAIR, while the remaining underwent NAIC. All participants were requested to complete the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) using the validated Chinese translation prior to neoadjuvant therapy, prior to surgery, six months postoperatively, and one year post-surgery. Patient demographics, pathology, treatment details, and follow-up information were meticulously analyzed.

Study variables

All patients underwent contrast-enhanced MRI, CT, and PET/CT scans to assess the primary sites and the status of the neck. Tumor and neck stages were assessed according to the 8th edition of the AJCC system. All pathological specimens were reviewed by at least two experienced head and neck pathologists. The degree of pathological differentiation was classified into three categories: well-differentiated, moderately differentiated, and poorly differentiated. Lymphovascular invasion (LVI) was considered positive when tumor cells were detected within the lymphatic channels. Perineural invasion (PNI) was deemed present if tumor cells infiltrated nerve structures (10). mPR was defined as ≤ 10% residual viable tumor identified through pathological examination of the resected tissue, while pCR was characterized by the absence of residual malignant lesions (11). The combined positive score (CPS) served to evaluate the proportion of PD-L1-positive tumor cells and infiltrating immune cells relative to the total number of viable tumor cells.

The primary outcomes of interest included mPR and pCR. Secondary outcomes encompassed neoadjuvant therapy-related adverse events, clinical to pathological downstaging, quality of life (QoL), surgical site infection, adverse pathologic feature, 3-year locoregional control (LRC) and overall survival (OS). Locoregional control time was calculated from the date of surgery until the date of first locoregional recurrence or the last follow-up, while OS time was measured from the date of surgery to the date of death or the last follow-up. Radiologic responses were assessed in accordance with the Response Evaluation Criteria in Solid Tumors version 1.1, while adverse events were graded based on the NCI-CTCAE (version 4.0).

NAIC, NAIR, and surgery

In the NAIC group, the treatment regimen included docetaxel at a dose of 75 mg/m², cisplatin at 75 mg/m², and pembrolizumab or alternative PD-L1 inhibitors at 200 mg of each three-week cycle by intravenous injection for two to three cycles. Conversely, the NAIR group received intravenous administration of Pembrolizumab or Penpulimab or Tislelizumab at 200 mg every two weeks. A prescribed dose of 40 Gy was delivered, targeting primary tumors and all radiographically visible metastatic lymph nodes. The target lesions were delineated and confirmed by two radiation oncologists as the gross tumor volume, which was then uniformly expanded by an additional 2–3 mm to establish the planning target volume. Radiation therapy was prescribed to ensure 95% coverage of the planning target volume, administered at 1.8–2.0 Gy per fraction, with five fractions per week.

Surgery was scheduled within one to four weeks following the completion of the neoadjuvant regimen. Surgical plans and resection margins were predefined based on baseline evaluations conducted prior to neoadjuvant therapy and remained unchanged irrespective of therapeutic response. Subsequent adjuvant therapy was initiated within six weeks post-surgery, focusing on the tumor bed with a margin of 1-2 cm. Adjuvant chemotherapy was administered based on clinical judgment and pathological characteristics, typically encompassing cisplatin over a duration of 4-6 cycles at a dose of 75 mg/m².

EORTC QLQ-C30

The QLQ-C30 questionnaire has been transformed into five functional scales—physical, role, cognitive, emotional, and social—alongside three symptom scales that encompass fatigue, pain, and nausea/vomiting. Additionally, it includes a global health and QoL scale as well as six individual symptom measures. Patients were instructed to evaluate the presence of symptoms or functional limitations on a Likert scale ranging from one to four. A high score for the functioning scale and for the global QoL scale represents a better level of functioning, whereas higher levels in the symptom scales or the single-item scales denotes a high level of symptoms or problems.

Statistical analysis

Primary outcomes were compared between the two cohorts utilizing the Chi-square test or Fisher's exact test. LRC and OS was evaluated via univariate and Cox regression models, with results presented as hazard ratios (HR) and 95% confidence intervals (CI). Categorical secondary outcomes were analyzed using the Chi-square test or Fisher's exact test, while continuous secondary outcomes were compared employing the Mann-Whitney U test. All statistical analyses were conducted using R version 3.4.4, and a p-value of less than 0.05 was deemed statistically significant.

Results

Baseline data

A total of 120 patients were included in this study, with a mean age of 55 ± 12 years. The cohort comprised 75 males and 45 females. The ECOG performance status was recorded as 0 in 53 patients and 1 in 67 patients. Among the participants, 67 were identified as smokers and 57 as drinkers. The primary tumor sites included the tongue in 49 patients, the floor of the mouth in 30 patients, buccal mucosa in 23 patients, and gingiva in 18 patients. Clinical stages were distributed as stage III in 77 patients and stage IV in 43 patients. A total of 20 patients had a CPS of less than 1, while 36 patients had a CPS of 20 or greater. Pathological differentiation was classified as well in 32 patients, moderate in 63 patients, and poor in 25 patients. Resection status of R0, R1, and R2 were accomplished in 115 (95.8%), 4 (3.3%), and 1 (0.8%) patients, respectively.

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Seventy-three patients underwent NAIC, exhibiting a similar distribution across all variables compared to those receiving NAIR (all p > 0.05, Table 1). Of these 73 patients, 50 (72.6%) were administered two cycles of NAIC, while the remainder (27.4%) underwent three cycles of NAIC. Pembrolizumab, Penpulimab, and Tislelizumab were prescribed for 30 (41.1%), 20 (27.4%), and 23 (31.5%) patients, respectively.

Primary outcome

In the NAIR group, mPR was observed in 63.8% of the total population, with 12 cases (25.5%) achieving a pCR. In the NAIC cohort, 51 patients demonstrated mPR, and pCR was noted in 23 cases (31.5%), although this difference was not statistically significant (p = 0.482). Patients in the NAIC group were more likely to achieve pCR.

The association between radiologic and pathologic assessments is illustrated in Figure 1. A pCR was consistently accompanied by a complete radiologic response; however, for other radiologic responses, the pathologic status could not be accurately predicted.

Secondary outcome

Clinical to pathologic downstaging was obtained in 100 patients (Figure 2), which was achieved in 65 patients (89.0%) in the NAIC group, significantly higher than the 74.5% observed in the NAIR population (p = 0.046). Adverse pathological features, including LVI, PNI, or extranodal extension, were noted in 13.7% of the NAIC group, which was significantly lower than the 31.9% in the NAIR cohort, the difference was mainly attributed by LVI distribution (Table 2, p = 0.022). The incidence of surgical site infections was similar between the two groups (6.8% vs. 8.5%, p = 0.736) (Figure 3).

The completion rate of the questionnaire was 100% in both groups prior to neoadjuvant therapy and before surgery. In the NAIC group, 70 patients (95.9%) and 60 patients (82.2%) completed the questionnaire at six months and twelve months postoperatively, respectively. In the NAIR population, 41 patients (87.2%) and 40 patients (85.1%) completed the questionnaire at six months and twelve months postoperatively. Global QoL showed continuous improvement from the onset of therapy, maintaining a stable status at six months post-surgery. All five functional scales exhibited significant declines following the completion of neoadjuvant therapy but gradually returned to baseline levels or improved within six months post-surgery. Symptoms displayed dynamic alterations at various time points, with complaints of pain, constipation, and diarrhea consistently decreasing. No significant differences were observed across all domains between the two cohorts at the same time points (all p > 0.05, Figure 4).

Neoadjuvant therapy-related adverse events were prevalent, though most were graded as 1 or 2. The most common grade 3/4 event in both groups was mucositis, followed by rash and anemia,

TABLE 1 Demography and pathologic data between neoadjuvant immunochemotherapy (NAIC) and neoadjuvant immunoradiotherapy (NAIR) groups.

Variable	Total (n=120)	NAIC (n=73)	NAIR (n=47)	p*
Age				
≤55	60	33	27	
>55	60	40	20	0.190
Sex				
Male	75	45	30	
Female	45	28	17	0.809
ECOG PS[&]				
0	53	35	18	
1	67	38	29	0.299
Smoker				
No	53	31	22	
Yes	67	42	25	0.640
Drinker				
No	63	35	28	
Yes	57	38	19	0.213
Primary site				
Tongue	49	30	19	
Mouth floor	30	19	11	
Buccal	23	14	9	
Gingiva	18	10	8	0.961
Clinical stage				
III	77	45	32	
IV	43	28	15	0.473
CPS[#]				
<1	20	13	7	
1-20	64	36	28	
≥20	36	24	12	0.542
Differentiation				
Well	32	20	12	
Moderate	63	37	26	
Poor	25	16	9	0.878
Resection status				
R0	115	70	45	
R1	4	2	2	
R2	1	1	0	1.000

* refer to the comparison between NAIC and NAIR groups using the Chi-square test.
& ECOG, eastern cooperative oncology group performance status.
# CPS, Combined positive score.

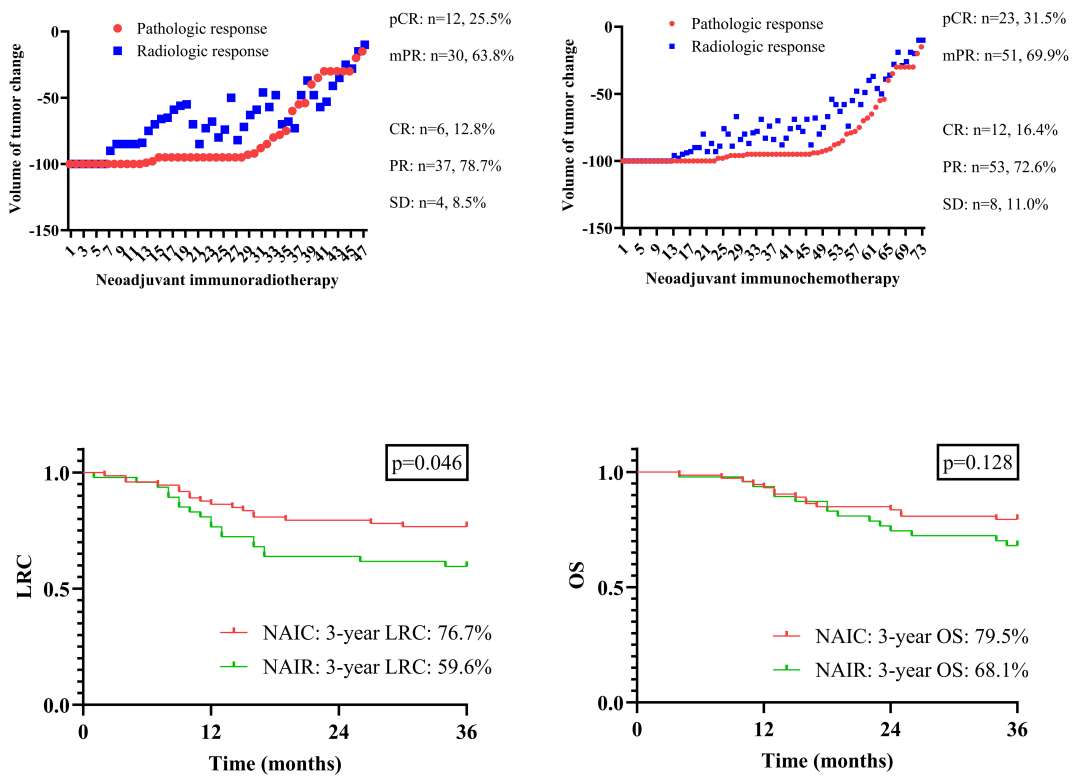

FIGURE 1
Radiologic and pathologic assessment in patients managed with neoadjuvant immunoradiotherapy and neoadjuvant immunochemotherapy; comparison of locoregional control (LRC) and overall survival (OS) in patients managed with neoadjuvant immunoradiotherapy and neoadjuvant immunochemotherapy.

with both cohorts exhibiting similar incidences of all grade 3/4 events (all $p > 0.05$, Table 3).

All patients were followed for at least three years, during which 36 locoregional recurrences and 30 deaths were documented. The three-year OS rates were 79.5% in the NAIC group and 68.1% in the NAIR group, although this difference was not statistically significant ($p = 0.128$, Figure 1). However, the NAIC cohort demonstrated a three-year LRC rate of 76.7%, which was significantly higher than the 59.6% observed in the NAIR group ($p = 0.046$, Figure 1).

To assess the independence of these findings, a Cox regression model was performed, incorporating neoadjuvant therapy and yp

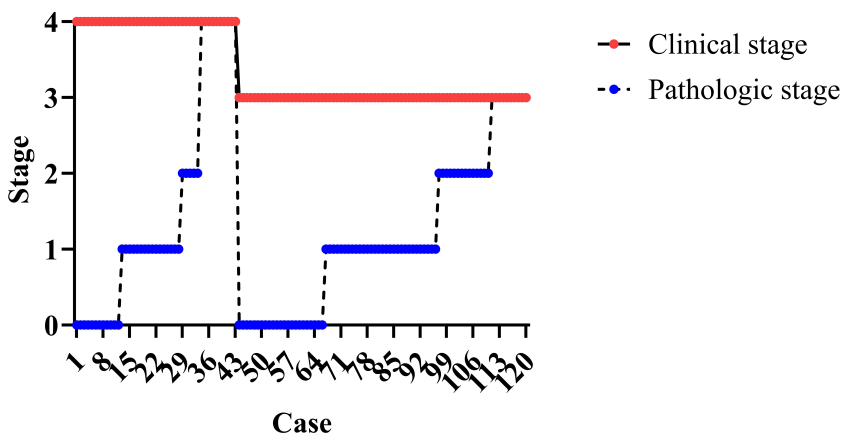

FIGURE 2
Detailed information of clinical to pathologic downstaging.

TABLE 2 Adverse pathologic features in patients treated by neoadjuvant immunochemotherapy (NAIC) or neoadjuvant immunoradiotherapy (NAIR).

Adverse pathologic feature	NAIC (n=73)	NAIR (n=47)	p
Lymphovascular invasion	5 (6.8%)	9 (19.1%)	0.040
Perineural invasion	4 (5.5%)	7 (14.9%)	0.107
Extranodal extension	3 (4.1%)	6 (12.8%)	0.152
Overall	10 (13.7%)	15 (31.9%)	0.022

stage as factors due to their significance in the univariate analysis. Compared to the NAIR group, patients receiving NAIC had a HR of 0.87 (95% CI: 0.65-0.98). When comparing patients with a yp T0N0 stage, those with yp stages I/II did not show an increased risk of locoregional failure. However, patients with yp stages III/IV exhibited a significantly higher risk, with an HR of 4.47 (95% CI: 2.10-12.45) (Table 4).

Discussion

Our paramount discovery entailed that in the context of locally advanced oral SCC, NAIC and NAIR exhibited comparable efficacy and safety, manifesting satisfactory rates of pCR and mPR, along with a low incidence of grade 3/4 adverse events. Nonetheless, NAIC not only afforded a superior three-year LRC but also yielded a greater likelihood of clinical to pathological downstaging and a reduced prevalence of adverse pathological features compared to NAIR. QoL was significantly affected by neoadjuvant therapy, yet nearly all scales experienced a recovery to baseline levels or achieved even better status. This investigation stands as the inaugural study to compare NAIC and NAIR in the treatment of locally advanced oral SCC, thereby elucidating a preference for NAIC as the more favorable treatment option over NAIR.

In light of the promising survival benefits associated with immunotherapy in the recurrent/metastatic setting of head and neck SCC (3), the potential of immunotherapy as a neoadjuvant treatment has garnered considerable interest, with a multitude of clinical investigations having been reported. A recent systematic review (12) collated data from 1092 patients across 24 studies, revealing an average objective response rate of 37%. Notably, immunochemotherapy demonstrated a superior objective response rate compared to immunotherapy alone in patients with untreated head and neck SCC. Therefore, the combination of immunotherapy with other therapeutic modalities tended to elicit a more efficacious response than immunotherapy administered in isolation. In a preceding phase 1b clinical trial (7), a cohort of twenty-one patients underwent treatment with NAIR at a cumulative dose of either 40 Gy administered in five fractions or 24 Gy in three fractions. All patients tolerated the treatment well, with no resultant delays in surgery. Within this collective study population, the rates of mPR and pCR were 86% and 67%, respectively. Clinical to pathological downstaging was observed in 90% of the treated patients. This outcome was particularly striking, as the majority achieved a pCR, which is indicative of a longer survival duration. However, a notably lower incidence of pCR was observed in the present study, with a potential explanation being that we exclusively enrolled patients with oral SCC, whereas the previous study comprised predominantly of patients with HPV-positive oropharyngeal SCC, a subset known to respond favorably to radiotherapy. Another retrospective investigation (9) delineated the outcomes of 30 oral SCC patients who received NAIR, with all cases demonstrating good tolerance to the neoadjuvant treatment, devoid of serious adverse events. The rates of complete response, partial response, and stable disease were 10.0%, 46.7%, and 43.3%, respectively. The rates of mPR, pCR, and clinical to pathological downstaging were 60.0%, 33.3%, and 83.3%, respectively. Over a median follow-up period of 13.5 months, 26 patients (86.7%) who had undergone surgical resection remained alive. The disease-free survival and OS at 24 months were 70.4% and 76.4%, respectively. These findings, in conjunction with our own depiction, collectively underscore the high efficacy and safety profile of NAIR in the treatment of oral SCC.

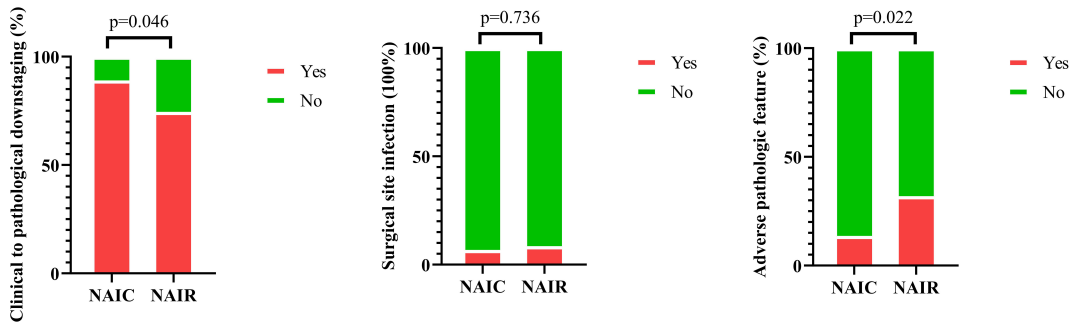

FIGURE 3
Comparison of incidences of clinical to pathologic downstaging, surgical site infection, and pathologic adverse features in patients managed with neoadjuvant immunoradiotherapy and neoadjuvant immunochemotherapy.

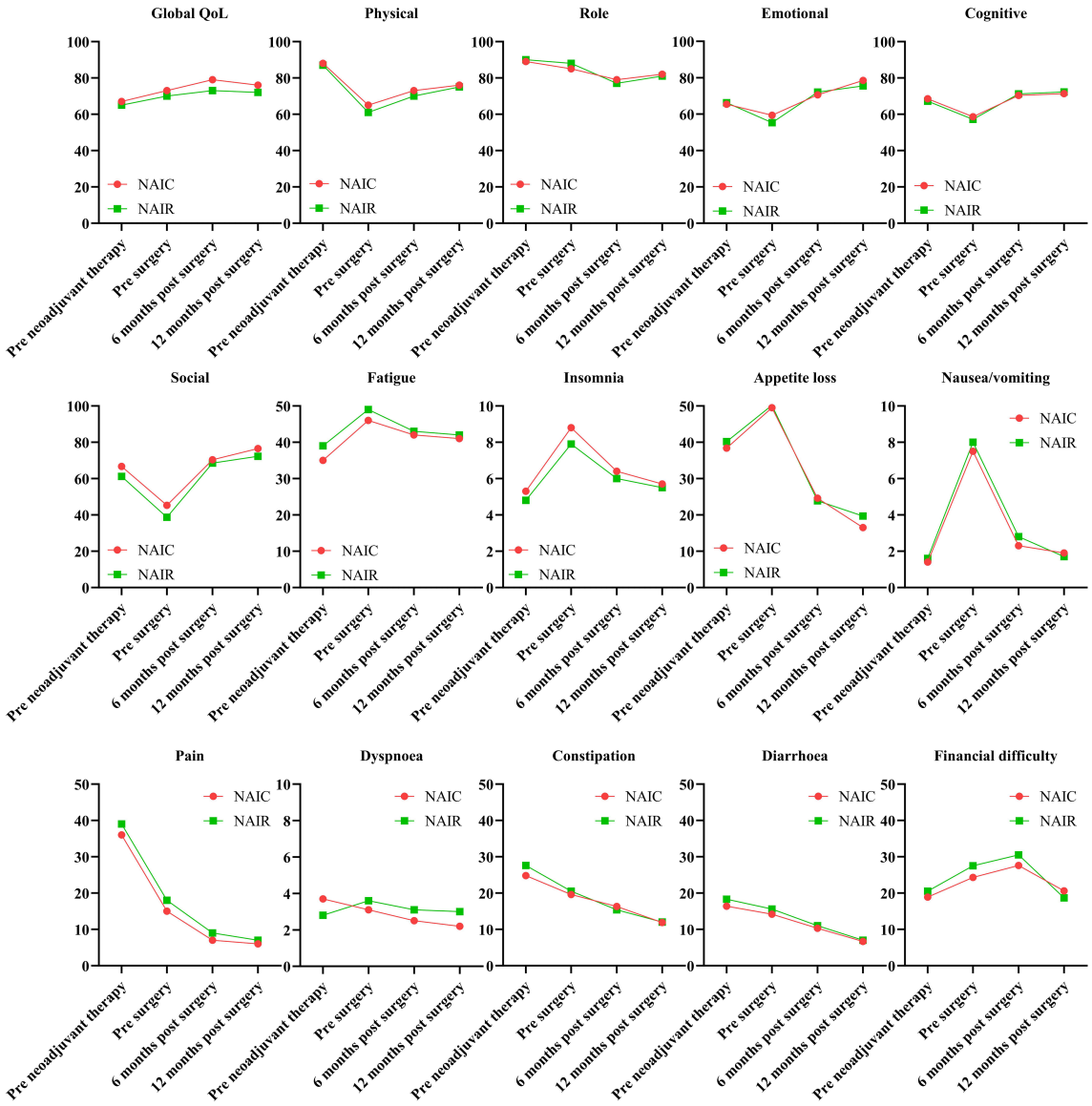

FIGURE 4
Quality of life in patients managed with neoadjuvant immunoradiotherapy and neoadjuvant immunochemotherapy.

Contemporary literature increasingly favors the concomitant use of immunotherapy and chemotherapy as a neoadjuvant treatment regimen. Huang et al. (4) enrolled 20 patients with locally advanced oral SCC, wherein NAIC was well-tolerated, with only three patients experiencing grades 3-4 adverse events. The completion rates for NAIC and subsequent R0 resection were uniformly 100%. The mPR rate stood at 60%, encompassing a 30% pCR. Over a median follow-up period of 23 months, disease-free survival and OS rates were 90% and 95%, respectively. Yao et al. (6) presented an analysis of 21 patients with head and neck SCC who underwent radical surgery and comprehensive cervical lymph node dissection following NAIC. The mPR rate was 66.7%, with 11 patients achieving a pCR. The overall response rate was 90.5%, and the complete response rate was 28.6%. The predominant adverse event was anemia, occurring in 61.9% of patients. No grade 4 adverse events or surgical delays were reported. Laryngeal preservation rates reached 90.9%, and all patients had negative surgical margins confirmed pathologically. In a separate cohort of 79 patients reported by Yan et al. (13), the R0 resection rate was an impressive 98.7%. Pathological assessment revealed that 53.1% of patients achieved either pCR or mPR. Following a median follow-up of 17.0 months, the 1-year disease-free survival and OS rates were 87.2% and 97.4%, respectively. Comparable findings were also corroborated by Chen et al. (14), Yu et al. (15), and our own analysis. Significantly, our study may be the first to address the question of whether there exists a discernible difference in efficacy and safety between NAIC and NAIR. On the one hand, both treatment arms demonstrated high pCR and mPR rates, with no substantial disparity in surgical site infection rates or overall survival. On the other hand, NAIR was associated with a less

TABLE 3 Neoadjuvant therapy related adverse events in neoadjuvant immunochemotherapy and neoadjuvant immunoradiotherapy groups.

Event	NAIC (n=73)		NAIR (n=47)		p*
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	
Mucositis	47 (64.4%)	4 (5.5%)	30 (63.8%)	3 (6.4%)	1.000
Vomiting	43 (58.9%)		30 (63.8%)		
Xerostomia	34 (46.6%)		25 (53.2%)		
Fatigue	30 (41.1%)		21 (44.7%)		
Rash	25 (34.2%)	3 (4.1%)	20 (42.6%)	2 (4.3%)	1.000
Pain	24 (32.9%)		19 (40.4%)		
Hypotension	19 (26.0%)		16 (34.0%)		
Anemia	13 (17.8%)	2 (2.7%)	11 (23.4%)	1 (2.1%)	1.000
Anorexia	11 (15.1%)		9 (19.1%)		
Hypothyroidism	11 (15.1%)		9 (19.1%)		
Leukopenia	11 (15.1%)	1 (1.4%)	8 (17.0%)	1 (2.1%)	1.000
Hypokalemia	9 (12.3%)		8 (17.0%)		
Transaminitis	7 (9.6%)	1 (1.4%)	7 (14.9%)	0	1.000
Fever	5 (6.8%)		6 (12.8%)		
Hyponatremia	4 (5.5%)		5 (10.6%)		
Pneumonia	2 (2.7%)		1 (2.1%)		

*refer to the comparison of grade 3/4 event incidence between the two groups using the Fisher test.

TABLE 4 Cox model analysis the impact of neoadjuvant immunochemotherapy (NAIC) versus neoadjuvant immunoradiotherapy (NAIR) on locoregional control.

Variable	p	HR [95%CI]
Neoadjuvant therapy (NAIC vs NAIR)	0.032	0.87 [0.65-0.98]
yp stage		
ypT0N0		ref
yp stage I/II	0.218	2.86 [0.56-7.59]
yp stage III/IV	0.011	4.47 [2.10-12.45]

favorable 3-year LRC, a finding that may be attributed to a reduced likelihood of clinical to pathological downstaging and a higher prevalence of adverse pathologic features in patients treated with NAIR.

QoL constitutes a pivotal consideration in the management of cancer (16), yet regrettably, it is seldom analyzed in the aftermath of neoadjuvant therapy. To the best of our knowledge, only a single pertinent study has been documented. In this study (11), 30 patients with oral SCC treated with NAIR were assessed. Regarding the functional scales, emotional, physical, social, role, and cognitive functioning demonstrated improvement at 1.5 and 2 years post-radiotherapy completion, with all functional scores equating to or surpassing baseline levels at the 2-year mark. All EORTC QLQ-C30 functioning and symptom scales, excluding nausea and vomiting, exhibited significant resolution at 2 years following the conclusion of radiotherapy. These findings align with those observed in our NAIR cohort, albeit we have conducted a comparative analysis between NAIC and NAIR. On the one hand, it was observed that the impact of both interventions on each QoL domain was analogous at corresponding time points. On the other hand, it was intriguing to note that while global QoL consistently recovered, other functional and symptom scales—except for pain, constipation, and diarrhea—experienced a minor deterioration following the completion of neoadjuvant therapy. This observation is reflective of the efficacy in cancer control exhibited by both NAIR and NAIC.

Limitation in current study must be acknowledged, first, there was lack of randomization, it increased our selective bias; second, our sample size was relatively small, it might decrease our statistic power; third, this was a single-center design limited by relatively short follow-up, further clarification on long-term toxicities, biomarker-driven stratification, and external validation was needed.

In conclusion, within the context of locally advanced oral SCC, both NAIC and NAIR demonstrated substantial efficacy and safety,

characterized by comparable rates of pCR and mPR, as well as analogous QoL and OS. However, NAIC conferred a superior LRC compared to NAIR, thereby positioning NAIC as the preferable initial therapeutic choice over NAIR.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by Henan cancer hospital Institutional Research Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GD: Writing – original draft, Writing – review & editing. WW: Writing – original draft, Writing – review & editing. QD: Writing – original draft, Writing – review & editing. YL: Writing – original draft, Writing – review & editing.

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Oncologic outcomes following neoadjuvant immunochemotherapy in locally advanced oral squamous cell carcinoma

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Background: To assess the oncologic outcomes in patients with oral squamous cell carcinoma (SCC) who underwent treatment with radiotherapy (RT) or chemoradiation therapy (CRT) following neoadjuvant immunochemotherapy and surgery.

Methods: Data from patients who underwent neoadjuvant immunochemotherapy, surgery, and adjuvant therapy were collected prospectively and analyzed retrospectively. The primary outcomes assessed were 3-year overall survival and locoregional control. Secondary endpoints included the objective response rate (ORR), rates of pathologic complete response (pCR) and major pathologic response (MPR), as well as safety.

Results: A total of 137 patients were included in the analysis. Neoadjuvant therapy yielded an ORR of 81.7%, with pCR and MPR achieved in 47 and 73 patients, respectively. Grade III and IV adverse events were rare, comprising only 1.6% of all events. The addition of adjuvant chemotherapy to RT did not show a significant reduction in the risk of locoregional recurrence. However, with regards to overall survival, the hazard ratios were 0.85 (95% CI: 0.73-0.96) for the MPR group and 0.66 (95% CI: 0.37-0.89) for the pCR group, both significantly higher than that in patients with incomplete pathologic response. The addition of adjuvant chemotherapy to RT was associated with a 5% reduction in the risk of mortality (95% CI: 1%-14%), the protective effect of CRT was the most obvious in patients with MPR.

Conclusion: Neoadjuvant immunochemotherapy demonstrated high safety and efficacy in oral SCC. CRT was superior to RT in terms of overall survival especially in patients with MPR when administered following neoadjuvant immunochemotherapy and surgery.

KEYWORDS

neoadjuvant immunochemotherapy, oral squamous cell carcinoma, chemoradiation, radiotherapy, safety

Background

Oral squamous cell carcinoma (SCC) represents the predominant histological subtype among head and neck malignancies, often presenting at an advanced local stage upon initial detection (1). The standard therapeutic approach typically involves a combination of surgical intervention and adjuvant radiotherapy (RT). However, despite advancements in reconstructive techniques utilizing regional and free flap procedures, the profound impact of vital organ resection on the quality of life remains a significant concern in clinical practice (2, 3).

While traditional neoadjuvant chemotherapy regimens centered around platinum agents have not shown a significant survival advantage in oral SCC (4), they have been linked to a substantial increase in the possibility of preserving the mandible by nearly 50% (5). With a deepening understanding of immune checkpoint pathways, immunotherapy has emerged as a superior alternative to traditional chemoradiotherapy, leading to prolonged overall survival in recurrent or metastatic head and neck SCC (6, 7). Nivolumab and pembrolizumab has been approved by FDA in SCC in head and neck (8). The integration of immunotherapy into neoadjuvant protocols has garnered considerable interest, with a series of clinical trials demonstrating that neoadjuvant immunotherapy, with or without chemotherapy, can achieve an impressive objective response rate (ORR) exceeding 95%. Moreover, pathologic complete response (pCR) rates of 30% or higher and major pathologic response (MPR) rates of approximately 70% have been observed (9, 10). These compelling outcomes prompt a reevaluation of the optimal management approach for oral SCC patients who achieve pCR or MPR following neoadjuvant immunotherapy.

Against this backdrop, the present study aims to assess the oncologic outcomes in oral SCC patients who have undergone treatment with radiotherapy (RT) or chemoradiation therapy (CRT) following neoadjuvant immunochemotherapy and surgery.

Patients and methods

Ethical approval

This study was approved by Henan Cancer Hospital Institutional Research Committee, and written informed consent for medical research was obtained from all patients before starting the treatment. All methods were performed in accordance with the relevant guidelines and regulations.

Study design

In pursuit of this objective, prospectively collected data was subjected to retrospective analysis. Commencing in January 2019, a regimen combining immunotherapy and chemotherapy was implemented in neoadjuvant management of locally advanced oral SCC following thorough elucidation of potential complications. Between January 2019 and December 2022, a total of 154 patients diagnosed with primary locally advanced oral SCC underwent neoadjuvant immunochemotherapy, with subsequent surgical intervention performed on 137 patients who constituted the final cohort for analysis; 17 patients were excluded due to lack of surgical intervention. Comprehensive data encompassing demographic profiles, pathological characteristics, treatments administered, and follow-up details for these patients were meticulously documented.

Variable definition

Assessment of all pathological sections was conducted by at least two specialized head and neck pathologists. Locally advanced disease staged as cT1-2N1-3 or cT3-4N0-3 was classified in alignment with the 8th edition of the AJCC system. Lymphovascular invasion (LVI) was deemed positive if cancer cells were detected within lymphatic vessels, while perineural invasion (PNI) was considered positive if cancer cells infiltrated a nerve. Extranodal extension (ENE) was indicative of cancer cells extending beyond the lymph node (LN) capsule. pCR denoted the absence of residual viable tumor cells in both the primary tumor and all resected lymph nodes, whereas MPR indicated ≤10% residual viable tumor cells in the resected tumor specimens. Incomplete pathological response (IPR) signified the presence of >10% viable tumor cells in resected tumor specimens. Immunohistochemical staining of PD-L1 expression was performed using the PD-L1 IHC 22C3 pharmDx assay with evaluation based on the combined positive score (CPS), determined by the number of PD-L1-staining cells divided by the total viable tumor count.

ORR was defined as the proportion of patients exhibiting a best response of complete or partial response as per RECIST 1.1 criteria before surgery (11). Clinical to pathological downstaging was characterized by a decline in T or N stage of pathologic staging relative to clinical staging (cTNM) according to the 8th edition of the AJCC cancer staging manual. Adverse events were graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (12).

Outcome variables

Primary outcome variables encompassed 3-year overall survival (OS) and locoregional control (LRC), with OS time calculated from the date of surgery to the date of death or last follow-up, and LRC time calculated from the date of surgery to the date of initial locoregional recurrence or last follow-up. Co-secondary endpoints included ORR, rates of pCR and MPR, and safety markers.

Treatment

Treatment protocols involved the administration of docetaxel at 75mg/m^2 on days 1 and 8, cisplatin at 75mg/m^2 on days 1 and 2, and pembrolizumab at 200mg on day 4 of each three-week cycle for two or three cycles. Surgery was scheduled within one to four weeks

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post completion of the six-week neoadjuvant regimen. Surgical plans and resection margins were predefined based on baseline evaluations preceding neoadjuvant therapy and remained unchanged irrespective of treatment response. Subsequent RT or CRT was initiated within six weeks post-surgery, targeting the tumor bed with a 1-2cm margin, and a prescribed dose of 60-66 Gy. Adjuvant chemotherapy was administered guided by clinical judgment and pathological characteristics, typically entailing cisplatin over 4-6 cycles at 75mg/m2.

Statistical analysis

For primary outcome variables, the impact of RT versus CRT on OS and LRC was assessed using univariate and multivariable Cox models, with outcomes presented as hazard ratios (HR) and 95% confidence intervals (CI). Secondary endpoints were descriptively outlined. Statistical analyses were conducted using R 3.4.4, with a significance level set at $p<0.05$.

Results

Baseline data

A total of 137 patients (90 males and 47 females) were enrolled for analysis, with a mean age of 50 ± 18 years. Among the cohort, 80 patients were active smokers, and 61 individuals reported alcohol consumption. Primary tumor sites were categorically distributed as follows: 61 cases in the tongue, 31 in the floor of the mouth, 25 in the buccal region, and 20 in the gingiva. Clinical tumor staging revealed T2 tumors in 14 patients, T3 in 85, and T4 in 38 cases. Notably, 87 patients presented with clinically positive lymph nodes, with 29 cases classified as N1, 40 as N2, and 18 as N3. Cancer staging indicated stage III disease in 39 patients and stage IV in 98 individuals. Assessment of PD-L1 expression demonstrated a CPS of less than 1 in 32 patients and 20 or higher in 38 patients. All patients achieved negative surgical margins. Two and three cycles of neoadjuvant therapy were administered to 100 and 37 patients, respectively. of Among the cohort, 77 patients underwent treatment with RT, while the remaining received treatment via CRT. Both treatment groups demonstrated a harmonious distribution across these parameters (Table 1, all $p>0.05$).

Efficacy

All patients completed the designated two cycles of neoadjuvant immunochemotherapy. Clinical evaluation revealed that 32 patients attained a complete response, 80 manifested a partial response, and 25 displayed stable disease, with no instances of disease progression. An impressive ORR of 81.7% was observed (Figure 1). Upon pathological assessment, 47 patients achieved pCR, 73 exhibited MPR, and IPR was observed in only 17 patients. Clinical to pathological downstaging was observed in 120 patients (87.6%).

Association analysis demonstrated that among patients achieving a clinical complete response, all achieved a pCR. Conversely, among patients without a clinical complete response, only 14.3% attained a pCR, signifying a significant distinction (Table 2, $p<0.001$).

TABLE 1 Baseline date of the 137 patients treated by neoadjuvant immunochemotherapy.

Variable	Total	RT (n=77)	CRT (n=60)	p*
Age				
≤50	81	47	34	
>50	56	30	26	0.606
Sex				
Male	90	50	40	
Female	47	27	20	0.832
Smoker				
Yes	80	40	40	
No	57	37	20	0.083
Drinker				
Yes	61	35	26	
No	76	42	34	0.804
Site				
Tongue	61	31	30	
Mouth floor	31	18	13	
Buccal	25	15	10	
Gingiva	20	13	7	0.674
cT				
T2	14	8	6	
T3	85	45	40	
T4	38	24	14	0.571
cN				
N0	50	30	20	
N1	29	17	12	
N2	40	22	18	
N3	18	8	10	0.706
Cancer stage				
III	39	24	15	
IV	98	53	45	0.427
CPS%				
<1	32	20	12	
1-19	67	37	30	
≥20	38	20	18	0.691

*Comparison between radiotherapy (RT) and chemoradiation (CRT) groups.
% CPS, combined positive score.

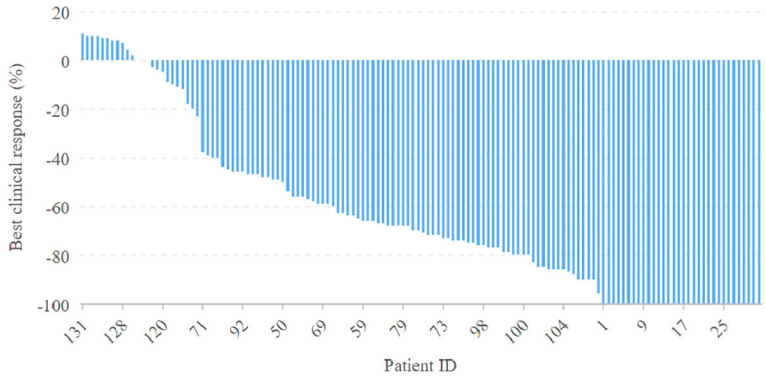

FIGURE 1
Clinical evaluation of neoadjuvant immunochemotherapy efficacy in the 137 patients with oral squamous cell carcinoma.

Safety

A total of 621 adverse events were documented, with an average of 4.5 events per patient, but there was no long term events. Severe grade III and IV adverse events were notably rare, accounting for merely 1.6% of all reported events and were observed in 10 patients. The most prevalent adverse reactions were alopecia (100%), nausea (65.0%), and leukopenia (54.7%), whereas anemia was the least frequent adverse event (n=3, 2.2%). Severe adverse symptoms were predominantly associated with leukopenia and thrombocytopenia (Table 3).

Survival

In univariate analysis, primary tumor site and treatment response significantly impacted prognosis for LRC (Figure 2). Subsequent multivariable analysis revealed that compared to patients with buccal or gingival tumors, those with tumors in the tongue or floor of the mouth had a hazard ratio (HR) of 2.16 (95% CI: 1.25-5.34), reflecting a significant difference (p=0.007). The HRs were 0.84 (95% CI: 0.75-0.95) for the MPR group and 0.66 (95% CI: 0.47-0.88) for the pCR classification, both significantly higher (p=0.025 and p=0.005, respectively) compared to patients with IPR. The inclusion of adjuvant chemotherapy alongside RT did not correlate with a reduced risk of locoregional recurrence (p=0.073, Figure 2) (Table 4).

TABLE 2 Efficacy of neoadjuvant immunochemotherapy in the 137 patients.

Clinical	Pathologic		
	Complete response	Major pathologic response	Incomplete pathological response
Complete response	32	0	0
Partital response	15	60	5
Stable disease	0	13	12

Regarding OS, primary tumor site, treatment response, and adjuvant therapy exhibited significant associations with prognosis in univariate analysis (Figure 2). Upon further multivariable analysis, patients with tumors in the tongue or floor of the mouth had a HR of 3.14 (95% CI: 1.53-7.36) compared to those with buccal or gingival tumors, representing a significant disparity (p=0.017). The HRs were 0.85 (95% CI: 0.73-0.96) for the MPR group and 0.66 (95% CI: 0.37-0.89) for the pCR classification, both significantly higher (p=0.018 and p<0.001, respectively) than those for patients with IPR. The addition of adjuvant chemotherapy to RT correlated with a 5% reduction in mortality risk (95% CI: 1%-14%) (Table 5).

Subgroup analysis

A subgroup analysis was conducted to assess the impact of RT versus CRT on prognosis among patients stratified by pathological treatment response (Table 6). Patients classified with MPR displayed a 24% decreased mortality risk when treated with CRT compared to RT alone, a statistically significant finding. However, in other subgroups, CRT and RT demonstrated comparable influences on OS and LRC (all p>0.05).

Discussion

Our paramount discovery underscored the remarkable safety profile of neoadjuvant immunochemotherapy in the management of locally advanced oral SCC, showcasing an outstanding ORR exceeding 80% and an impressive pCR rate of 34.3%. Noteworthy, all tumors were successfully subjected to R0 resection. Notably, when juxtaposed with RT alone, the adoption of CRT yielded a superior 5% increment in OS with a 95% CI ranging from 1% to 14%, as opposed to LRC. Furthermore, both MPR and pCR emerged as robust predictors for both OS and LRC outcomes. This study serves as a pioneering effort, furnishing the initial substantiation of enhanced survival benefits conferred by neoadjuvant immunochemotherapy, thereby potentially reshaping the clinical approach to addressing locally advanced oral SCC.

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TABLE 3 Grade of adverse events in neoadjuvant immunochemotherapy in the 137 patients.

Events	I/II	III/IV
Alopecia	137 (100%)	–
Nausea	89 (65.0%)	–
Leukopenia	70 (51.1%)	5 (3.6%)
Anorexia	70 (51.1%)	–
Fatigue	55 (40.1%)	–
Constipation	50 (36.5%)	–
Hypothyroidism	42 (30.7%)	–
Pain	30 (21.9%)	–
Thrombocytopenia	25 (18.2%)	5 (3.6%)
RCCEP*	14 (10.2%)	–
Fever	10 (7.3%)	–
Pneumonia	7 (5.1%)	–
Diarrhea	5 (3.6%)	–
Rash	4 (2.9%)	–
Anemia	3 (2.2%)	–

*RCCEP, reactive cutaneous capillary endothelial proliferation.

The phenomenon of immune evasion serves as a pivotal driver of tumor progression, catalyzing the emergence of immunotherapy as a vanguard in the realm of oncological treatment. Notably, Nivolumab has heralded a paradigm shift in cancer therapeutics, elevating the one-year survival rate in malignant melanoma from 42.1% with conventional chemotherapy regimens to an impressive 72.9% (13). The transformative impact of Nivolumab extends across diverse malignancies, including non-small cell lung cancer, renal cell carcinoma, and head and neck SCC (14, 15). In a pivotal CheckMate-141 trial, Nivolumab showcased its prowess in treating platinum-resistant recurrent/metastatic head and neck SCC, yielding a median survival of 7.7 months—a noteworthy 2.6-month enhancement compared to the standard treatment cohort's median survival of 5.1 months. Notably, the 2-year survival rates were substantially elevated at 16.9% for the Nivolumab group compared to 6% in the standard treatment arm, signifying a notable 32% reduction in mortality risk. These findings underscore the superiority of Nivolumab immunotherapy over conventional chemotherapy in addressing recurrent/metastatic head and neck SCC (16). Subsequent investigations such as the Keynote-040 study have corroborated these advancements, with Pembrolizumab demonstrating comparable efficacy to Nivolumab (17). This collective body of research has solidified the pivotal role of immunotherapy as a second-line therapeutic modality for managing recurrent/metastatic head and neck SCC. Moreover, landmark studies like Keynote-048 in the realm of head and neck SCC immunotherapy have shed light on the enduring benefits of Pembrolizumab monotherapy for individuals with high PD-L1 expression, showcasing noteworthy long-term survival efficacy compared to traditional treatment modalities (18). The paradigm shift towards immunotherapy, both as a first-line and second-line treatment, has been endorsed in various clinical guidelines, heralding a new era of improved outcomes and prognostic advancements in addressing recurrent/metastatic head and neck SCC. Overall, the advent of immunotherapy has revolutionized the therapeutic landscape for recurrent/metastatic head and neck SCC, presenting a compelling avenue for enhancing treatment efficacy and refining patient prognosis.

In light of the encouraging clinical outcomes witnessed with immunotherapy in recurrent/metastatic head and neck SCC, research endeavors have ventured into exploring its application in the neoadjuvant setting. Findings from a pioneering single-arm clinical study by Luginbuhl et al. (19) have illuminated the potential of neoadjuvant immunotherapy in synergy with chemotherapy. The incorporation of nivolumab alongside paclitaxel and carboplatin regimens revealed a striking pCR rate of 49%, with a combined pCR and MPR rate reaching a noteworthy 65% in locally advanced resectable head and neck SCC. Similarly, outcomes from a phase II study evaluating the neoadjuvant regimen of treprizumab combined with chemotherapy exhibited compelling advancements in pathological remission, mirroring the substantial effects observed in previous single-arm investigations combining similar regimens (20). Remarkably, the neoadjuvant therapy employing immunotherapy in conjunction with chemotherapy showcased pCR rates of 57.14% and 22.22%, respectively, with corresponding pCR+MPR rates of 92.85% and 22.22%, a tantalizing progression from prior results (21). This enhanced efficacy might be attributed to the utilization of albumin-bound paclitaxel and cisplatin regimens within the chemotherapy protocol. Studies have demonstrated the superior anti-tumor effects of protein-bound paclitaxel when combined with platinum, fluorouracil, and cetuximab in locally advanced head and neck SCC compared to conventional paclitaxel-based regimens. Notably, albumin-bound paclitaxel obviates the need for hormone pretreatment, circumventing the immunosuppressive effects of hormones and facilitating the optimal therapeutic impact of immunotherapy. This aspect may serve as a contributing factor to the enhanced rate of pathological remission observed in the neoadjuvant setting, pointing towards a promising avenue for improving treatment outcomes in this challenging clinical domain.

The side effects associated with preoperative neoadjuvant therapy incorporating immunotherapy alongside chemotherapy are predominantly manageable. Common adverse reactions encompass granulocyte deficiencies, electrolyte imbalances, nausea, and temporary hair loss. Vigilant monitoring of pertinent laboratory parameters throughout the treatment course proves instrumental in mitigating these adverse effects. Notably, in the context of this study, no instances of treatment-induced adverse reactions impeding subsequent therapeutic interventions surfaced during the neoadjuvant therapy regimen. It is noteworthy that immune-related adverse reactions, such as hyperthyroidism and hypothyroidism, manifested more frequently during the later phases of maintenance immunotherapy, underscoring the dynamic nature of immune modulation throughout the treatment continuum.

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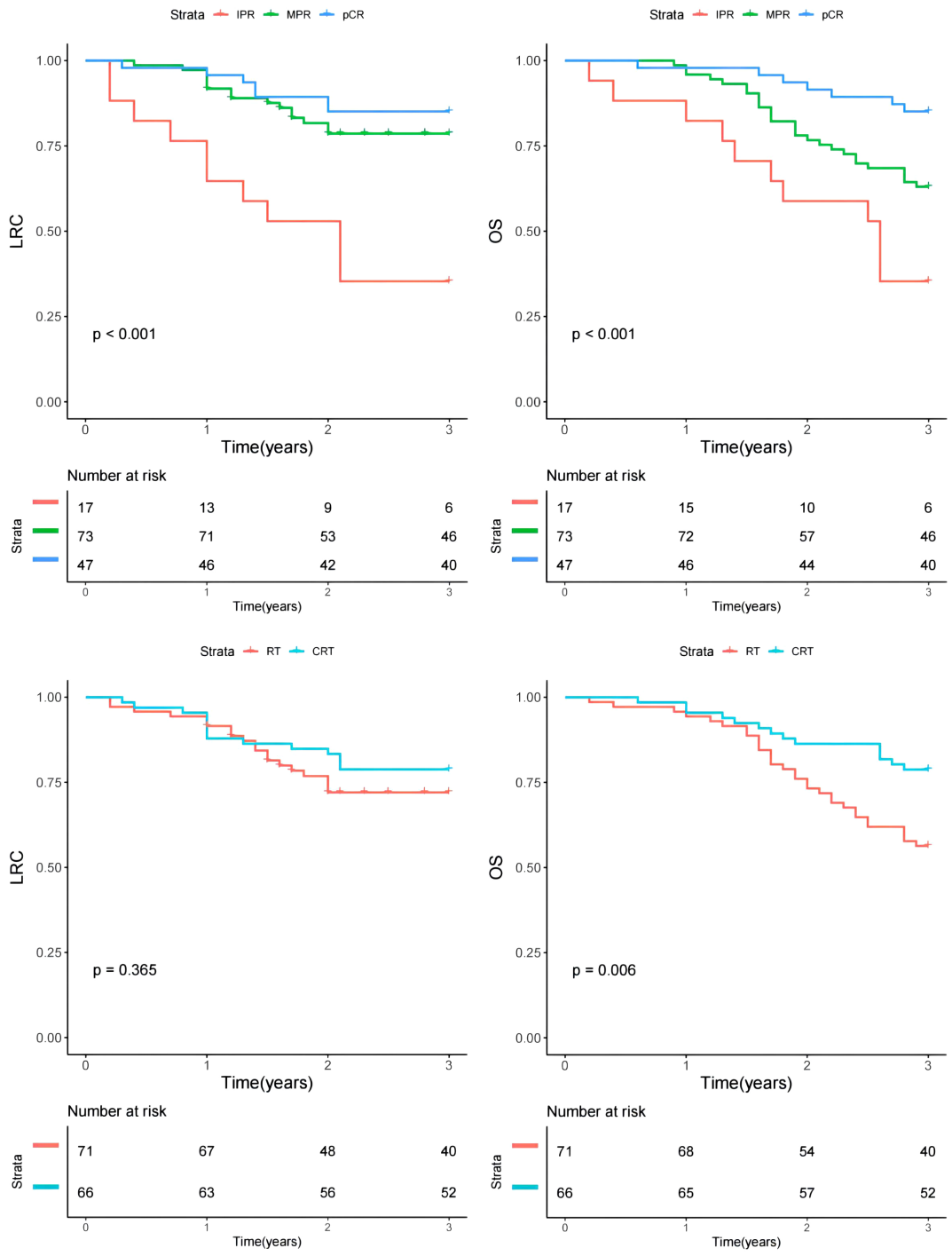

FIGURE 2
Comparison of overall survival (OS) and locoregional control (LRC) in patients with different features.

A crucial determinant in enhancing the effectiveness of immunotherapy lies in the precise identification of potential beneficiaries through robust screening methodologies. Unraveling the intricacies of biomarkers indicative of immunotherapy responsiveness stands at the forefront of research pursuits. The expression level of PD-L1 emerges as a pivotal gauge for prognosticating the efficacy of immunotherapy, with the CPS serving as a recommended predictor for immunotherapeutic outcomes in head and neck malignancies as per the NCCN guidelines (22). Notably, a CPS value equal to or exceeding 20 signifies a significant advantage in immune monotherapy efficacy, with an escalating CPS correlating with augmented prospects of responding favorably to immunotherapy. Previous investigations underscored the high incidence of PD-L1 expression in oral squamous cell carcinoma patients, with a positive rate reaching 87.88%, thus indicating the potential benefits of immunotherapy in

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TABLE 4 Univariate and multivariable analysis of predictors for locoregional control.

Variable	Univariate	Multivariable	
	p	p	HR [95%CI]
Age			
≤50			
>50	0.723		
Smoker			
Yes			
No	0.358		
Drinker			
Yes			
No	0.549		
Site			
Buccal/Gingiva			ref
Tongue/Mouth floor	<0.001	0.007	2.16 [1.25-5.34]
CPS[%]			
<1			
1-19			
≥20	0.322		
Treatment response*			
IPR			ref
MPR		0.025	0.84 [0.75-0.95]
pCR	<0.001	0.005	0.66 [0.47-0.88]
Perineural invasion			
No			
Yes	0.245		
LVI[&]			
No			
Yes	0.098		
Adjuvant therapy^			
RT			
CRT	0.073		

*IPR, Incomplete pathological response; MPR, major pathological response; pCR, pathologic complete response.
[&]LVI, Lymphovascular invasion.
^RT, radiotherapy; CRT, chemoradiation.

TABLE 5 Univariate and multivariable analysis of predictors for overall survival.

Variable	Univariate	Multivariable	
	p	p	HR [95%CI]
Age			
≤50			
>50	0.476		
Smoker			
Yes			
No	0.813		
Drinker			
Yes			
No	0.544		
Site			
Buccal/Gingiva			ref
Tongue/Mouth floor	<0.001	0.017	3.14 [1.53-7.36]
CPS[%]			
<1			
1-19			
≥20	0.315		
Treatment response*			
IPR			ref
MPR		0.018	0.85 [0.73-0.96]
pCR	<0.001	<0.001	0.66 [0.37-0.89]
Perineural invasion			
No			
Yes	0.543		
LVI[&]			
No			
Yes	0.209		
Adjuvant therapy^			
RT			ref
CRT	0.006	0.036	0.95 [0.86-0.99]

*IPR, Incomplete pathological response; MPR, major pathological response; pCR, pathologic complete response.
[&]LVI, Lymphovascular invasion.
^RT, radiotherapy; CRT, chemoradiation.

this patient subset (20). Encouragingly, all oral cancer patients with CPS ≥ 20 achieved a MPR post neoadjuvant therapy, suggesting a substantial therapeutic benefit conferred by neoadjuvant immunotherapy in conjunction with chemotherapy. This bodes well for augmenting the long-term survival rates among this cohort of patients. Moreover, despite a subset of our patients exhibiting a CPS below 1, the overall response rate exceeding 80% underscores the imperfect predictive utility of PD-L1 alone in determining treatment efficacy. Although widely utilized immune efficacy predictors such as tumor mutation burden and microsatellite instability in head and neck SCC have not entirely met clinical exigencies in terms of accuracy, the quest continues for more precise screening biomarkers pinpointing advantageous patient populations. Exploratory endeavors into tertiary lymphoid

TABLE 6 Subgroup analysis of the impact of adjuvant therapy on survival stratified by pathologic assessment.

Assessment*	Overall survival		Locoregional control	
	p	HR [95%CI]	p	HR [95%CI]
pCR				
RT		ref		ref
CRT	0.365	1.27 [0.83-4.29]	0.176	1.13 [0.75-6.32]
MPR				
RT		ref		ref
CRT	0.018	0.76 [0.54-0.88]	0.427	1.53 [0.82-5.43]
IPR				
RT		ref		ref
CRT	0.764	1.85 [0.36-8.90]	0.792	2.01 [0.35-9.17]

*pCR, pathologic complete response; MPR, major pathologic response; IPR, incomplete pathologic response; RT, radiotherapy; CRT, chemoradiation.

structures within lung cancer, liver and gallbladder cancers, and malignant melanoma have unveiled their potential as autonomous predictors of immunotherapeutic outcomes (23). However, the applicability of these markers in neoadjuvant immunotherapy for locally advanced oral cancer warrants further investigation, presenting an intriguing avenue for future research pursuits.

The prognosis of locally advanced oral cancer remains a pressing concern, necessitating concerted efforts to enhance patient survival rates and overall outcomes. While induction chemotherapy may not universally bolster long-term survival in individuals with head and neck SCC, meticulous stratified analyses have unveiled a compelling narrative. Notably, following induction chemotherapy, surgical interventions yielded a commendable 10-year survival rate of 76.2% for patients achieving a pCR, starkly contrasting with the 41.3% rate observed in those falling short of the coveted pCR milestone (24). Within the realm of neoadjuvant therapy, both pCR and MPR have emerged as internationally acclaimed prognostic markers, crucial for predicting overall survival post-treatment. Pathologic remission stands as an objective and insightful yardstick for assessing the efficacy of neoadjuvant therapy, concurrently offering pivotal insights into the long-term benefits conferred (25). Leveraging the pathologic remission benchmarks elucidated in this study, it is conjectured that the innovative adjuvant protocol integrating immunotherapy with chemotherapy holds significant promise for bolstering the overall survival rates of individuals battling oral SCC. Current study data signal a promising trajectory in augmenting OS and LRC through the integration of immunotherapy with neoadjuvant chemotherapy. Post-neoadjuvant immunochemotherapy and surgical interventions remain areas warranting further exploration, with lingering uncertainties persisting regarding the optimal management strategies for patients achieving pCR or MPR milestones. At our center, surgical excision scope adherence to the initial disease stage remains standard practice, underpinned by the need for a deeper comprehension of the tumor regression patterns post-treatment. Noteworthy observations include the potentiation of overall survival through adjuvant chemotherapy complementing radiotherapy, particularly accentuated in patients achieving MPR, pioneering a novel finding in the field. Despite conventional indicators advocating chemoradiotherapy for cases featuring extranodal extension or positive margins, our pathological analyses notably omitted these factors, possibly attributable to the protracted anti-cancer effects of immunotherapy coupled with the synergistic potential of adjuvant chemotherapy.

The contrasting efficacy evaluations based on the RECIST 1.1 criteria prelude to surgery vis-à-vis postoperative pathological assessments echo a recurrent disparity observed in prior immunoneoadjuvant therapies (26). While the RECIST 1.1 standard scrutinizes tumor dimensions to gauge efficacy, the postoperative pathological appraisal delves deeper, scrutinizing residual tumor cells, necrosis levels, inflammatory responses, and tissue reactions, thus offering a comprehensive portrayal of the treatment response. It is discernible that pathological evaluation eclipses the RECIST 1.1 standard in furnishing a more nuanced understanding of tumor responsiveness, thereby furnishing a robust framework for guiding subsequent adjuvant therapeutic interventions. Future research trajectories mandate a meticulous examination of imaging attributes, extraction of features correlated with pathological assessments, refinement of image evaluation for predictive pathological responses, and an overall enhancement of clinical diagnostic and therapeutic acumen.

Limitation in current study must be acknowledged, first, there was inherent bias within the retrospective study, second, our sample size was small, it might decrease our statistic power, third, only 3-year survival was reported, longer follow-up was required.

In summary, immunochemotherapy plays an important role in the neoadjuvant treatment stage of oral cancer, achieving synergistic effects, effectively improving pathological remission and controllable safety. Pathological evaluation can objectively and accurately evaluate the effectiveness of immunotherapy, providing reliable reference for formulating adjuvant treatment plans. CRT provides better OS than RT in cases with MPR.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by This study was approved by Henan Cancer Hospital Institutional Research Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your

previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

GL: Writing – original draft, Writing – review & editing. JW: Writing – original draft, Writing – review & editing. QF: Writing – original draft, Writing – review & editing. LD: Writing – original draft, Writing – review & editing. WD: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Machine learning-driven identification of exosome-related biomarkers in head and neck squamous cell carcinoma

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Background: Head and neck squamous cell carcinoma (HNSCC) is a common cancer associated with elevated mortality rates. Exosomes, diminutive extracellular vesicles, significantly contribute to tumour development, immunological evasion, and treatment resistance. Identifying exosome-associated biomarkers in HNSCC may improve early diagnosis, treatment targeting, and patient classification.

Methods: We acquired four publically accessible HNSCC gene expression datasets from the Gene Expression Omnibus (GEO) database and mitigated batch effects utilising the ComBat technique. Differential expression analysis and exosome-related gene screening found a collection of markedly exosome-associated differentially expressed genes (ERDEGs). Subsequently, 10 key exosome-related genes were further screened by combining three machine learning methods, LASSO regression, SVM-RFE and RF, and a clinical prediction model was constructed. Furthermore, we thoroughly investigated the biological roles of these genes in HNSCC and their prospective treatment implications via functional enrichment analysis, immune microenvironment assessment, and molecular docking confirmation.

Results: The study indicated that 10 pivotal exosome-related genes identified by the machine learning method had considerable differential expression in HNSCC. Clinical prediction models developed from these genes have shown high accuracy in prognostic evaluations of HNSCC patients. Analysis of the immunological microenvironment indicated varying immune cell infiltration in HNSCC, and the association with ERDEGs proposed a potential mechanism for immune evasion. Molecular docking validation indicated novel small molecule medicines targeting these genes, establishing a theoretical foundation for pharmacological therapy in HNSCC.

Conclusion: This research identifies new exosome-related indicators for HNSCC through machine learning methodologies. The suggested biomarkers, particularly ANGPTL1, exhibit significant promise for diagnostic and prognostic uses. The investigation of the immunological microenvironment yields insights into immune modulation in HNSCC, presenting novel avenues for therapeutic targeting.

KEYWORDS

head and neck squamous cell carcinoma, exosome biomarkers, machine learning, immune microenvironment, therapeutic target discovery

Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is among the most prevalent malignant neoplasms of the head and neck, with significant morbidity and mortality rates globally (1, 2). Notwithstanding advancements in the diagnosis and treatment of HNSCC in recent years, the prognosis for patients, particularly those in advanced stages, remains unfavourable, characterised by a low five-year survival rate (3, 4). Consequently, a thorough investigation of the molecular pathways of HNSCC, together with identifying novel biomarkers and prospective therapeutic targets, is crucial for enhancing the clinical management of patients.

In recent years, exosomes, as significant extracellular vesicles, have garnered considerable attention in tumour biology research. An exosome is a nanoscale vesicle released by cells, abundant in biomolecules, including proteins, RNA, DNA, and lipids, which can modulate the tumour microenvironment via intercellular communication and is pivotal in carcinogenesis, progression, metastasis, and medication resistance (5–7). Research indicates that exosomes play a role in tumour cell signalling and affect tumour immune evasion by modulating immune cell activity (8). Moreover, exosomes' particular molecular constituents (e.g., miRNAs, lncRNAs, and proteins) have demonstrated significant diagnostic and prognostic significance across various malignancies (9, 10). The precise functions of exosome-related genes in HNSCC and their potential as biomarkers have not been comprehensively examined.

Concurrently, machine learning (ML) is progressively employed as a potent data analysis instrument in the biomedical sector. Machine learning can extract essential elements from extensive datasets using algorithms, develop prediction models, and offer accurate disease diagnosis, classification, and therapy assistance (11–13). In tumour research, machine learning has been effectively utilised for analysing gene expression data, biomarker screening, and developing clinical prognostic models (14, 15). The integration of machine learning and exosome-associated gene study in HNSCC remains nascent, and its potential has yet to be thoroughly investigated.

This study systematically identified exosomal biomarkers in HNSCC by integrating multi-omics data and machine learning. We explored their roles in the tumour immune microenvironment and drug discovery. Four HNSCC gene expression datasets were obtained from the Gene Expression Omnibus (GEO) database, with batch effects mitigated via the ComBat technique to ensure uniformity. Through differential expression analysis, exosome-associated gene screening, and functional enrichment, we identified highly differentiated exosome-related genes (ERDEGs). Three machine learning approaches—Least absolute shrinkage and selection operator (LASSO) regression, Support Vector Machine Recursive Feature Elimination (SVM-RFE), and Random Forest (RF)—were integrated to pinpoint 10 core exosome-related genes, enabling the development of a clinical prediction model. Additionally, we analysed associations between these genes and the immunological microenvironment, while screening potential small-molecule drugs, thereby providing a theoretical basis for future translational research.

Materials and methods

Data acquisition and preprocessing

Four HNSCC gene expression datasets—GSE25099 (57 tumours vs. 22 normals from Taiwan, Affymetrix), GSE30784 (167 tumours vs. 45 normals from US, Affymetrix), GSE37991 (40 tumour-normal pairs from Taiwan, Illumina), and GSE127165 (57 laryngeal SCC-normal pairs from China, Illumina)—were retrieved from GEO and harmonised using ComBat batch correction (sva v3.46.0) to preserve biological variance while eliminating platform-specific technical artefacts. Raw microarray data underwent rigorous preprocessing: RMA background correction with quantile normalisation, log2 transformation, and filtering of genes expressed (CPM > 1) in ≥ 50% samples. Quality control retained samples with median intensity > 2 SDs above cohort mean and > 85% detection rate, alongside genes exhibiting > 0.2 coefficient of variation (CV). Missing values were imputed via k-nearest neighbours (k = 15), with batch effect removal efficacy confirmed through principal component analysis (PCA) clustering patterns and interquartile range consistency in boxplots.

Differential expression analysis

Differentially expressed genes (DEGs) were identified using the Limma pipeline, defined by statistical significance ($p < 0.05$) and absolute log2 fold change ($|log2FC| > 1$). Results were visualised through a heatmap (pheatmap R package) displaying hierarchical clustering of top DEGs across samples, and a volcano plot (ggplot2 R package) contrasting log2FC against—log10 (p-value), with significant DEGs highlighted.

Exosome-related gene screening

Exosome-related genes were extracted from the GeneCards database (Supplementary Table 1). Genes linked to exosomes were found using the search phrase "exosome" and filtered according to a relevance score > 2 to guarantee high-confidence relationships. The list of DEGs derived from the Limma pipeline was cross-referenced with the curated exosome-related gene list. The Venn diagram was created utilising the VennDiagram R tool, visually illustrating the intersection between the two gene sets. Genes located in the intersection were identified as exosome-related differentially expressed genes (ERDEGs).

Functional enrichment profiling

Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment studies were conducted utilising the clusterProfiler R package to investigate the biological activities and pathways related to the ERDEGs. The enrichment analysis was conducted using a significance threshold of adjusted p-value < 0.05, and the findings were illustrated using bar graphs and dot plots. Gene Set Enrichment Analysis (GSEA) was performed to further examine the functional characteristics of the ERDEGs at the gene set level. The Hallmark gene sets from the Molecular Signatures Database (MSigDB) served as the reference gene sets. GSEA was conducted utilising the fgsea R package, and enrichment scores were computed to ascertain gene sets significantly enriched in the ERDEGs. The results were illustrated by enrichment plots, with the foremost enriched gene sets given according to their normalised enrichment score (NES) and a false discovery rate (FDR) < 0.25.

Machine learning-based biomarker discovery

Three machine learning approaches were sequentially applied for feature selection: (1) LASSO regression (glmnet v4.1-6) performed dimensionality reduction via L1 regularisation, where the optimal λ value minimising prediction error was determined through 10-fold cross-validation, retaining genes with non-zero coefficients as candidate biomarkers; (2) SVM-RFE (e1071 v1.7-13) iteratively refined the feature subset by recursively eliminating lowest-weight features based on linear kernel SVM classifier performance until peak classification accuracy was achieved; (3) RF (randomForest v4.7-1.1) quantified feature importance via Gini impurity reduction across 500 decision trees, with final biomarker prioritisation based on descending importance scores, thereby establishing a robust multi-algorithm consensus for subsequent translational validation.

Clinical predictive model construction

Receiver Operating Characteristic (ROC) curve analysis was performed to assess the efficacy of the clinical predictive model. The ROC curve was produced with the pROC R package, which computes the area under the curve (AUC) to assess the model's discriminatory capacity. A nomogram was created with the Regression Modelling Strategies (RMS) R package to enhance the clinical implementation of the predictive model. The nomogram graphically illustrates the correlation between predictor variables and the anticipated outcome, enabling doctors to assess the likelihood of a specific clinical event for individual patients. The model's coefficients were utilised to allocate point values to each predictor, and the cumulative points were correlated with the projected likelihood. Calibration curves were constructed to evaluate the concordance between expected and observed outcomes, confirming the nomogram's reliability.

Immune microenvironment characterisation

Single-sample Gene Set Enrichment Analysis (ssGSEA) was conducted utilising the LM22 signature matrix, which encompasses gene expression profiles of 22 immune cell types to delineate the immune cell composition inside the tumour microenvironment. The Gene Set Variation Analysis (GSVA) R software was utilised to compute enrichment scores for each immune cell type in individual samples. Box plots illustrated the findings to emphasise discrepancies in immune cell prevalence among samples. The relationship between ERDEGs and immune cell infiltration was assessed using Spearman correlation, followed by visualisation of the results with the pheatmap software.

Drug sensitivity prediction

To forecast drug sensitivity based on the discovered ERDEGs, drug-gene connection data were sourced from the Drug Signatures Database (DSigDB). Drug enrichment analysis was conducted to find possible therapeutic agents that target ERDEGs. The fgsea R package was utilised for the study, wherein ERDEGs were evaluated for enrichment against the drug-gene sets derived from DSigDB. The enrichment scores were computed, and statistical significance was evaluated with an FDR < 0.25. The outcomes were prioritised according to the NES, and the most enriched pharmaceuticals were determined. The data were visualised through bar and enrichment plots, emphasising the most promising compounds for further examination.

Molecular docking validation

The three-dimensional structures of the target proteins were obtained from the AlphaFold Protein Structure Database to confirm the interactions between projected drug candidates and their target proteins. The three-dimensional structures of small-molecule compounds found by drug sensitivity prediction were obtained from the PubChem database. Molecular docking simulations were performed utilising AutoDock Vina, a prevalent method for forecasting ligand-protein interactions. The target protein and small-molecule compounds were formatted in PDBQT, and a grid box was established to surround the putative binding site. Docking simulations used an exhaustiveness parameter of 8 to guarantee comprehensive sampling of the binding conformations. The highest-ranking postures' binding affinities (measured in kcal/mol) were evaluated, and the findings were illustrated using PyMOL to investigate the molecular interactions.

Regulatory network analysis

The starBase database examined the relationships between RNA-binding proteins (RBPs) and their target transcripts. The outcomes were refined according to high-confidence connections (e.g., corroborated by numerous CLIP-seq datasets), and the RBP-target gene network was illustrated using Cytoscape to emphasise critical regulatory linkages. The Transcriptional Regulatory Relationships Unravelled by Sentence-based Text Mining (TRRUST) database was utilised to deduce transcription factor (TF) regulatory interactions. The interactions between transcription factors and target genes were extracted to form a regulatory network. The network was depicted using Cytoscape, with nodes symbolising transcription factors and target genes and edges denoting regulatory interactions.

Cell lines

Human Oral Keratinocytes (HOK) and human HNSCC cell lines, HN4, HN6, SCC9, and CAL27, were obtained from Wuhan Pricella Biotechnology Co. Ltd. DMEM medium was used for cultivation. The above medium was supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin. All cell lines were cultured in a cell incubator at 37°C with 5% CO2 concentration.

RNA extraction and quantitative real-time polymerase reaction

Total RNA was extracted using a silica-membrane column-based purification kit (Takara #9767), wherein the gDNA-Eraser column adsorbed genomic DNA while the RNA Pure column selectively bound RNA, yielding high-purity total RNA. Reverse transcription was performed with PrimeScript™ RT Reagent Kit (Takara #RR037A), followed by SYBR Green-based qPCR (Takara) on a Bio-Rad CFX96 system. Reactions were conducted in duplicate under two-step cycling: 95°C/30 sec denaturation, 40 cycles of 95°C/5 sec and 60°C/30 sec. GAPDH served as endogenous control, with relative gene expression quantified via $2-\Delta\Delta Ct$ method against normalised cycle threshold (Ct) values.

Plasmids design and transfection

Firstly, the primers of angiopoietin like 1 (ANGPTL1) gene were designed by Anhui General Gene Technology Co., Ltd. and amplified by PCR, and the ends of cDNA were digested using XbaI and BamHI restriction endonucleases, and the pcDNA3.1(+) empty vector was digested in the same way; then, DNA ligase was utilised to ligate the amplified target fragment with the vector, and pcDNA3.1(+)-ANGPTL1 (containing the target vector for the ANGPTL1 gene) was obtained, and the ligated product was transformed into the receptor cells. The ANGPTL1 plasmid construction was successful after shaking the bacteria, coating the plate, selecting the positive clones, sequencing, amplifying the bacterial solution, and carrying out plasmid extraction and purification.

Cell counting kit-8 proliferation assay

Transfected SCC9 and CAL27 cells were seeded in 96-well plates at a density of 2,000 cells per well (six replicates per group). Cell proliferation was assessed at 0, 24, 48, and 72 h. For CCK-8 assays, 10 μL of CCK-8 solution was added to each well containing 100 μL of culture medium. After incubation at 37°C for 2 h, absorbance at 450 nm was measured using a microplate reader to quantify proliferation differences between groups.

Colony formation assay

Transfected SCC9 and CAL27 cells were seeded in 6-well plates (200 cells/well) and cultured under standard conditions. The medium was refreshed every 3 days for 1–2 weeks until visible colonies formed. Cells were then washed with PBS, fixed with 4% paraformaldehyde for 30 min, and stained with 0.1% crystal violet for 10 min. After rinsing to remove excess stain, plates were air-dried at room temperature. Colony numbers were quantified using ImageJ software.

Wound healing assay

The transfected SCC9 and CAL27 cells were added to 6-well plates with 5×10^5 cells per well, respectively. Incubate in the incubator overnight. Two parallel lines were drawn in the 6-well plate with a 100 μL pipette tip the next day. Wash the cells with PBS solution and add serum-free medium. Continue to incubate in the incubator, and observe and photo record under the inverted microscope at 0 h and 24 h.

Transwell migration and invasion assays

Transfected SCC9 and CAL27 cells were resuspended at 1×10^5 cells/mL. 100 μL cell suspension (10,000 cells/well) was seeded into Transwell inserts, with 600 μL medium containing 30% FBS added to the lower chamber. After 24 h incubation, inserts were fixed with 4% paraformaldehyde (10 min), stained with 0.1% crystal violet (15 min), and washed with PBS. Non-migrated cells on the upper membrane surface were removed by cotton swab. Migrated cells were imaged under a light microscope and quantified using ImageJ. For the invasion test, the protocol matched the migration assay except that transwell membranes were pre-coated with Matrigel (BD Biosciences; 1:8 dilution in serum-free medium) for 1 h at 37°C before cell seeding.

Statistical analysis

Statistical analyses were performed using GraphPad 9.4.1. The Mann-Whitney U test was used to compare the means between the two groups, which are characterised by continuous measures that are not normally distributed. The t-test was used to analyse the comparison of means between the two groups, which are characterised by the need to conform to normally distributed measures. The chi-square test was used to analyse the difference between the two groups for count data.

Results

Identification of ERDEGs in HNSCC

We developed a comprehensive analytical framework by integrating four separate HNSCC datasets (GSE25099, GSE30784, GSE37991, and GSE127165), which included 321 tumour samples and 164 normal tissue samples. PCA indicated substantial batch effects among cohorts before normalisation (Figure 1A, Supplementary Figure 1B). After ComBat batch correction, the variation in batch effects on gene expression distribution was substantially eliminated across all cohorts (Figure 1B, Supplementary Figure 1B). Utilising the limma program ($p < 0.05$, $|\log2FC| > 1$), we found 514 consistently dysregulated genes across all datasets. The volcano plot identified 237 upregulated genes and 277 downregulated genes (Figure 1C). Hierarchical clustering of the top 50 differentially expressed genes distinctly separated tumour from normal tissues (Figure 1D). We curated 878 experimentally confirmed exosome-related genes from the GeneCards database (Relevance score > 2). The investigation of the intersection between DEGs and exosome genes identified 39 ERDEGs (Figure 1E).

Functional enrichment analysis of ERDEGs

GO enrichment study identified numerous considerably enriched biological processes, cellular components, and molecular functions. The most significant biological processes encompassed the positive regulation of neuroinflammatory responses and the

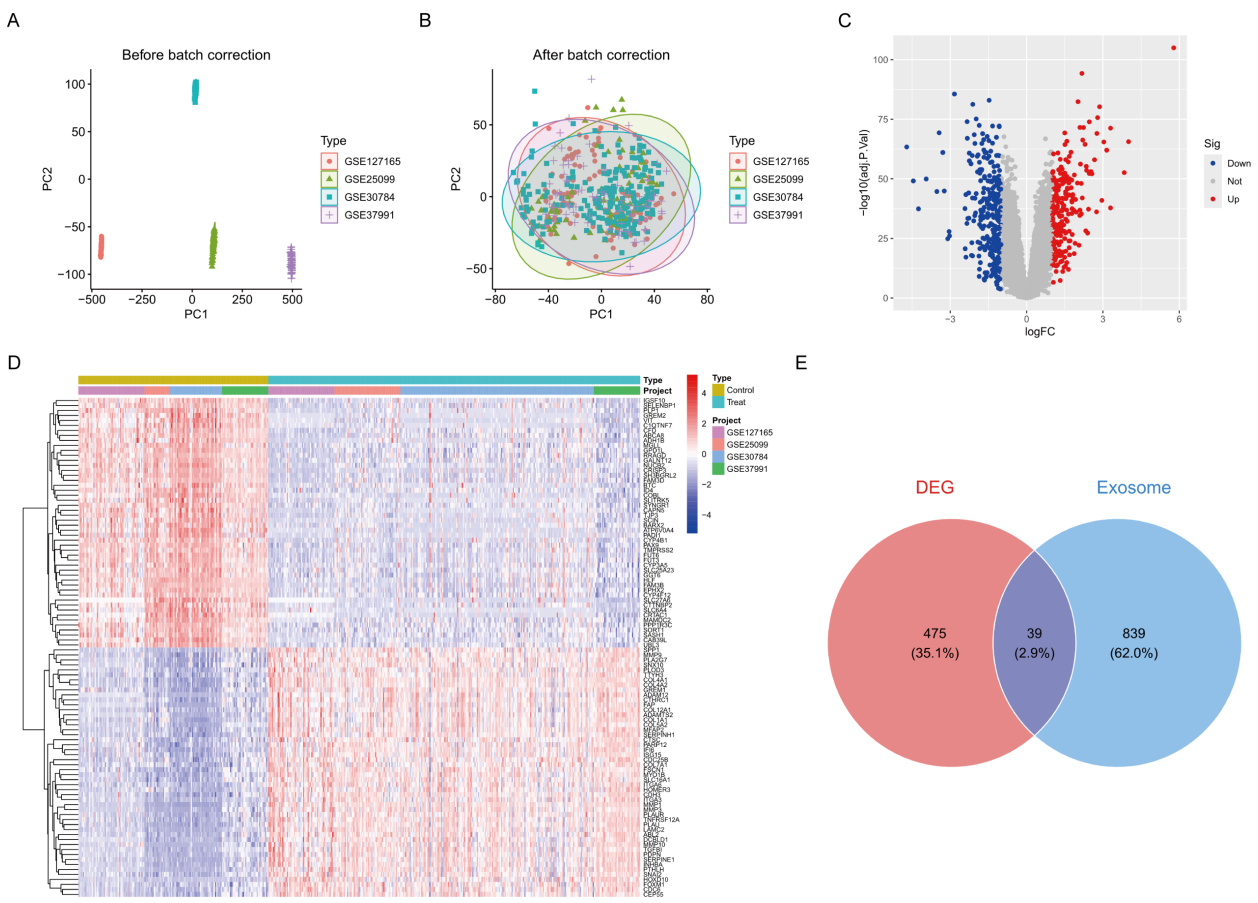

FIGURE 1
Differential expression analysis. **(A)** Pre-batch-corrected PCA plot. **(B)** Post-batch-corrected PCA plot. **(C)** Volcano plot of DEGs. **(D)** Heatmap of top 50 DEGs across cohorts. **(E)** Venn diagram of DEG-exosome gene intersection.

positive regulation of leukocyte activation, indicating the potential involvement of these genes in immunological responses and neuroinflammatory pathways. Enriching the vesicle lumen and secretory granule lumen indicates that these genes may participate in vesicular transit and secretion. The enrichment of cytokine receptor binding and protease inhibitory activity indicates a significant involvement of these genes in immunological signalling and protease regulation (Figure 2A). KEGG pathway analysis identified several pathways strongly linked to the genes, including fluid shear stress and atherosclerosis, graft-versus-host disease, and ferroptosis. Enriching the TNF signalling pathway and the rheumatoid arthritis pathway indicates that these genes may be pivotal in inflammatory responses and autoimmune disorders (Figure 2C). To enhance our comprehension of the association between genes, functions, and pathways, we further developed a gene-function network relationship map and a gene-pathway network relationship map. The gene-function network diagram illustrated the strong correlation between genes and essential functions, including immune response and neuroinflammation (Figure 2B). In contrast, the gene-pathway network diagram elucidated how these genes performed their biological roles by engaging in various significant signalling pathways (e.g., IL-17 signalling pathway, TNF signalling pathway, etc.) (Figure 2D). The network maps corroborated the aforementioned enrichment analysis findings and offered novel insights into the probable processes of genes in disease. Moreover, GSEA analysis corroborated the activation of numerous significant signalling pathways, including Cell Cycle and Cytokine Cytokine Receptor Interaction, which exhibited robust positive enrichment. The substantial enrichment of pathways, including ECM-receptor interaction and Cell Cycle, indicates their potential roles in cell adhesion and division, which may be linked to tissue remodelling and cancer progression (Figure 2E). Metabolic pathways, including Drug Metabolism Cytochrome P450, Metabolism of Xenobiotics by Cytochrome P450, and Tyrosine Metabolism, exhibited significant negative enrichment, indicating that these genes may be crucial in drug metabolism and the detoxification of exogenous compounds (Figure 2F).

Machine learning-based biomarker discovery

We conducted a one-way logistic regression analysis with a significance threshold of $p < 0.05$ to develop the HNSCC risk model, initially identifying 39 critical ERDEGs. This work employs three machine learning algorithms—LASSO, SVM-RFE, and RF—concurrently to improve the reliability of feature selection through comprehensive screening. LASSO regression effectively handles high-dimensional data by incorporating L1 regularisation, filtering out 17 essential ERDEGs while maintaining predictive efficacy. This method is particularly suited for datasets with many features, as it promotes sparsity in the model by selecting the most influential variables (Figures 3A, B). SVM-RFE iteratively eliminates less important features based on classifier accuracy, ultimately identifying 30 optimal candidate genes. This technique excels in selecting features that maximise classification performance, even in complex datasets (Figures 3C, D). Random Forest utilises out-of-bag error estimation and Gini importance scores to identify 17 hallmark genes with diagnostic significance. Its robust ensemble learning approach ensures that important features are consistently identified, even when faced with noisy or high-dimensional data (Figures 3E, F). By synthesising the outcomes from all three algorithms using a Venn diagram, we identified ten diagnostic ERDEGs, which were consistently highlighted across the different approaches (Figure 3G). This integrated feature selection strategy ensures the robustness and reliability of the final gene set for HNSCC risk modelling.

Clinical validation of diagnostic models

Boxplot analysis demonstrated significant differential expression of critical genes between control and treatment groups ($p < 0.001$ for all comparisons). Genes such as matrix metallopeptidase 9 (MMP9), ANGPTL1, bone marrow stromal cell antigen 2 (BST2), ubiquitin-like 3 (UBL3), baculoviral IAP repeat containing 5 (BIRC5), Thy-1 cell surface antigen (THY1), clusterin (CLU), myocilin (MYOC), profilin 2 (PFN2), and fibronectin 1 (FN1) demonstrated distinct expression profiles, with MMP9 and FN1 exhibiting the most significant upregulation in the treatment group (Figure 4A). Correlation analysis revealed intricate relationships among the genes. FN1 highly correlated with THY1 ($r = 0.74$, $p < 0.001$). BIRC5 had inverse correlations with ANGPTL1 ($r = -0.52$) and UBL3 ($r = -0.52$). CLU demonstrated moderate co-expression with ANGPTL1 ($r = 0.51$) (Figure 4B). The Circos plot analysis delineated critical genes to particular chromosomal regions. CLU (chromosome 8) and THY1 (chromosome 11) are in regions that regulate the extracellular matrix. UBL3 on chromosome 13 and BIRC5 on chromosome 17 are located in regions associated with apoptosis (Figure 4C). To evaluate the diagnostic efficacy of pivotal genes identified by the LASSO risk model for HNSCC, logistic regression diagnostic models and column line plots were employed to demonstrate the impact of the expression of 10 selected ERDEGs on HNSCC. ROC curve analysis designated UBL3 as the most potent single-gene biomarker (AUC = 0.927, 95% CI: 0.901–0.953), surpassing other possibilities such as ANGPTL1 (AUC = 0.895) and MMP9 (AUC = 0.885) (Figure 4E). The multivariate model encompassing all genes attained remarkable diagnostic accuracy (AUC = 0.983, 95% CI: 0.973–0.991), greatly above that of individual markers (Figure 4D). To rigorously evaluate model generalizability, we performed independent validation using the TCGA-HNSCC dataset ($n = 546$), which was completely independent from all prior training and feature selection procedures. The diagnostic model achieved near-perfect discrimination with an AUC of 0.999 (95% CI: 0.996–1.000) (Supplementary Figure 2A). Individual biomarkers demonstrated robust predictive capacity, including BIRC5 (AUC = 0.962), MMP9 (AUC = 0.951), and ANGPTL1 (AUC = 0.889), with all 10 genes

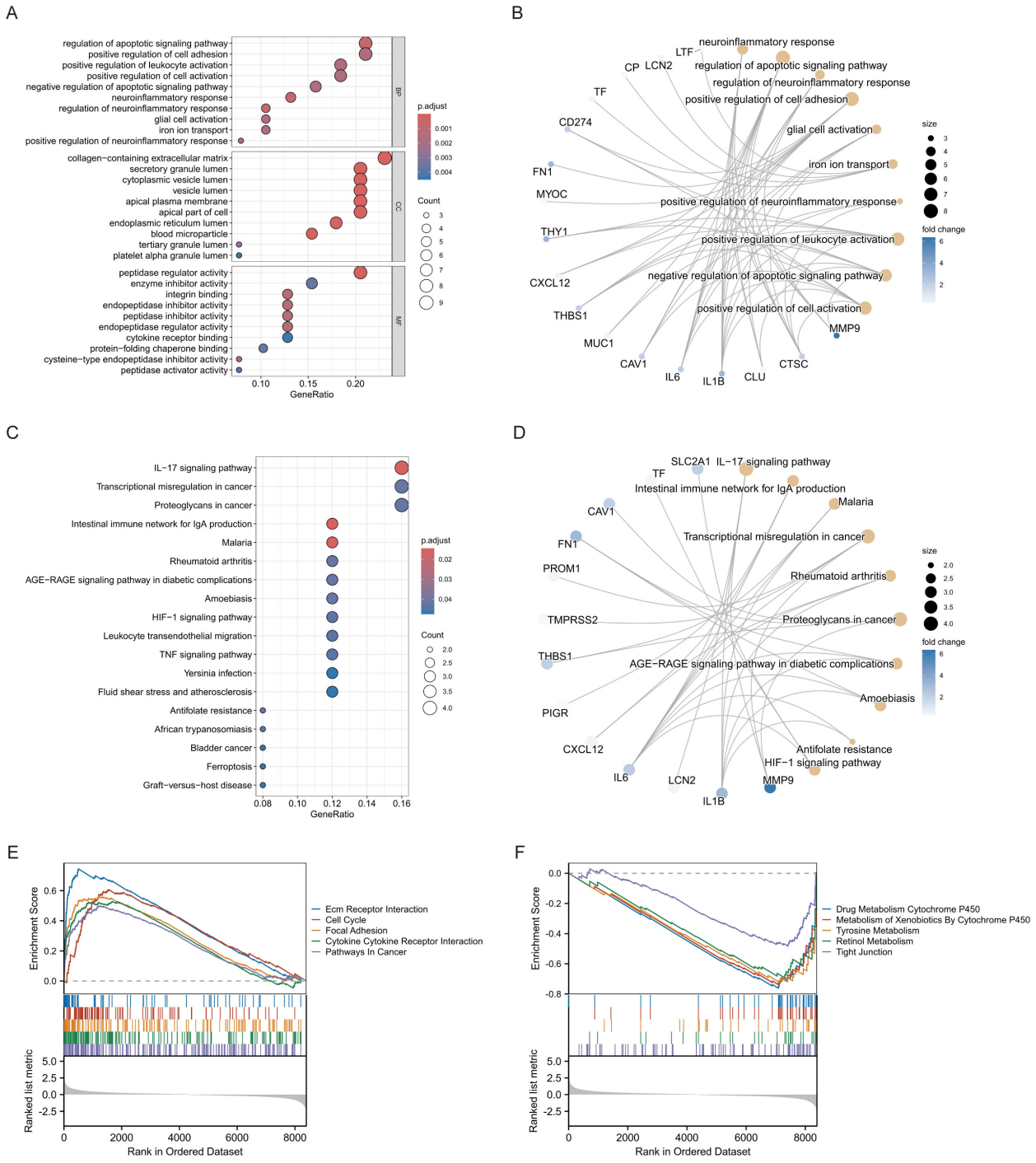

FIGURE 2
Enrichment analysis of ERDEGs. **(A)** GO enrichment analysis of ERDEGs. **(B)** Network diagram of ERDEGs with functional correlations. **(C)** KEGG enrichment analysis of ERDEGs. **(D)** Network diagram of ERDEGs related to pathway. **(E, F)** GSEA enrichment analysis of ERDEGs.

showing AUC > 0.75 (Supplementary Figure 2B). Calibration curves exhibited robust concordance between projected probabilities and actual outcomes (Brier score = 0.083), with negligible discrepancy between apparent and bias-corrected estimates (Figure 4F). The decision curve study validated clinical utility within 10–80% threshold probabilities, demonstrating enhanced net benefit relative to treat-all or treat-none approaches (Figure 4G). The nomogram assessed the contributions of individual genes to disease risk, with UBL3 (5.5–9.5 points) and FN1 (3–12 points) exhibiting the highest weightings. Total scores of 300 points or higher indicated a predicted risk exceeding 90%, facilitating accurate categorising of high-risk patients (Figure 4H).

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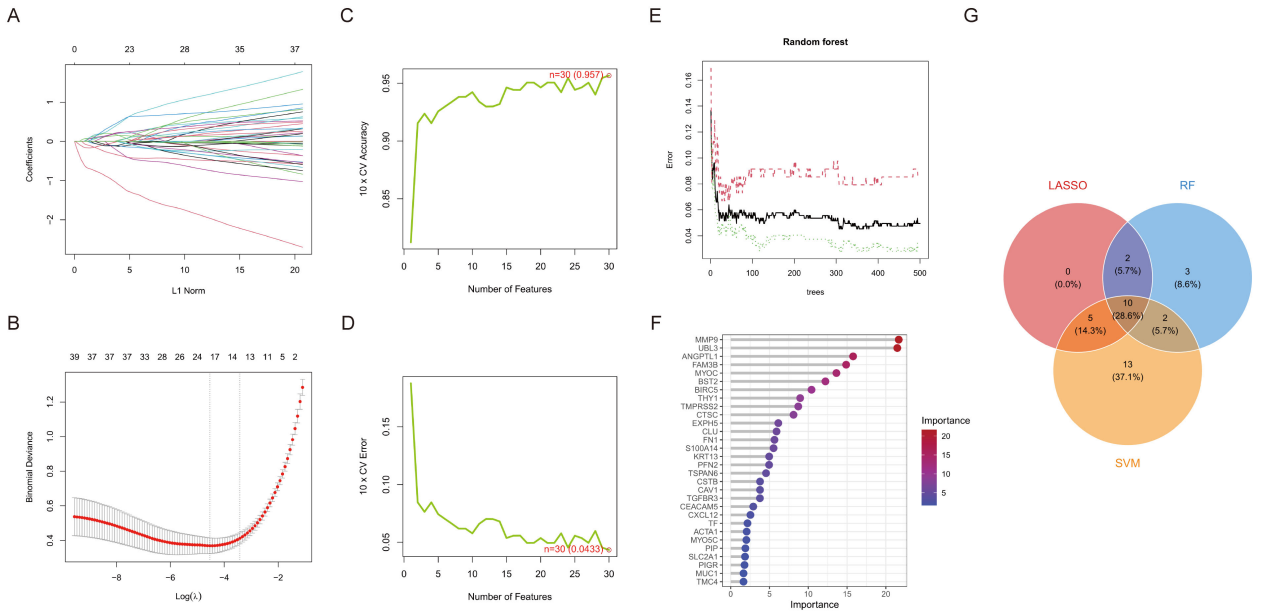

FIGURE 3
Machine learning screens for disease characterising genes. **(A)** Change in model bias under cross-validation. **(B)** LASSO regression coefficient L1 paradigm change. **(C)** Cross-validation accuracy and number of features change. **(D)** Cross-validation error and number of features change. **(E)** Plot of number of trees versus error rate in RF. **(F)** Ranking of importance of genetic variables in contributing to model prediction. **(G)** Venn diagram of LASSO, RF and SVM-RFE selected feature genes.

Immune microenvironment characterisation

An examination of immune infiltration was conducted using the CIBERSORT method to investigate the link between immunoreactivity and HNSCC, revealing the infiltration of 28 immune cell types, with 14 kinds exhibiting significant differences between the treatment and control groups. Neutrophils were more prevalent in HNSCC, but Natural Killer T cells, Activated CD4 T cells, Activated B cells, and Memory B cells were more prevalent in the control group (Figure 5A). The Spearman analysis demonstrated a link between immune cells and ERDEGs, as illustrated in Figure 5B. UBL3 was prevalent in activated CD8 T cells, gamma delta T cells, myeloid-derived suppressor cells (MDSCs), and natural killer cells, exhibiting a favourable correlation with inflammation-related signalling pathways, potentially contributing significantly to the control of innate immunity. BIRC5 exhibits a strong negative correlation in Immature B cells, Activated CD8 T cells, and Regulatory T cells, suggesting that these innate immune cells are inhibited during T cell proliferation. ANGPTL1 is prominently expressed in effector memory CD4 T cells and myeloid-derived suppressor cells (MDSCs), potentially contributing to immunosuppression and the regulation of the tumour microenvironment. MYOC is significantly expressed in effector memory CD4 T cells and type 2 T helper cells, indicating its potential influence on antigen presentation functionality.

Therapeutic target exploration

Small molecule medicines modulating hub gene expression were gathered from DSigDB on the Enrichr platform. The outcomes for prospective small molecules were produced using their P-values to signify the closeness between the small molecule and the gene. Figure 6A and Supplementary Table 2 illustrate the prospective small molecule therapeutics for the hub genes. To clarify the binding activity between the hub gene proteins and their respective medications, additional molecular docking of the HNSCC-related hub genes (BIRC5, MMP9, THY1, FN1, CLU) and the initial five small-molecule medicines was conducted. Consequently, receptor-ligand docking outcomes were acquired utilising the identical methodology. In molecular docking, intermolecular forces, primarily hydrogen bonding, were considered. Figures 6B–F depicts the docking configuration of small molecule pharmaceuticals and proteins.

Regulatory network analysis

This study established a regulatory network for RBPs, with green nodes denoting RBPs and orange nodes indicating target genes. Central to the network, genes, including BIRC5, FN1, CLU, MMP9, and UBL3, were co-regulated by various RBPs. BIRC5, an established anti-apoptotic gene integral to cell survival and carcinogenesis, is modulated by several RNA-binding proteins

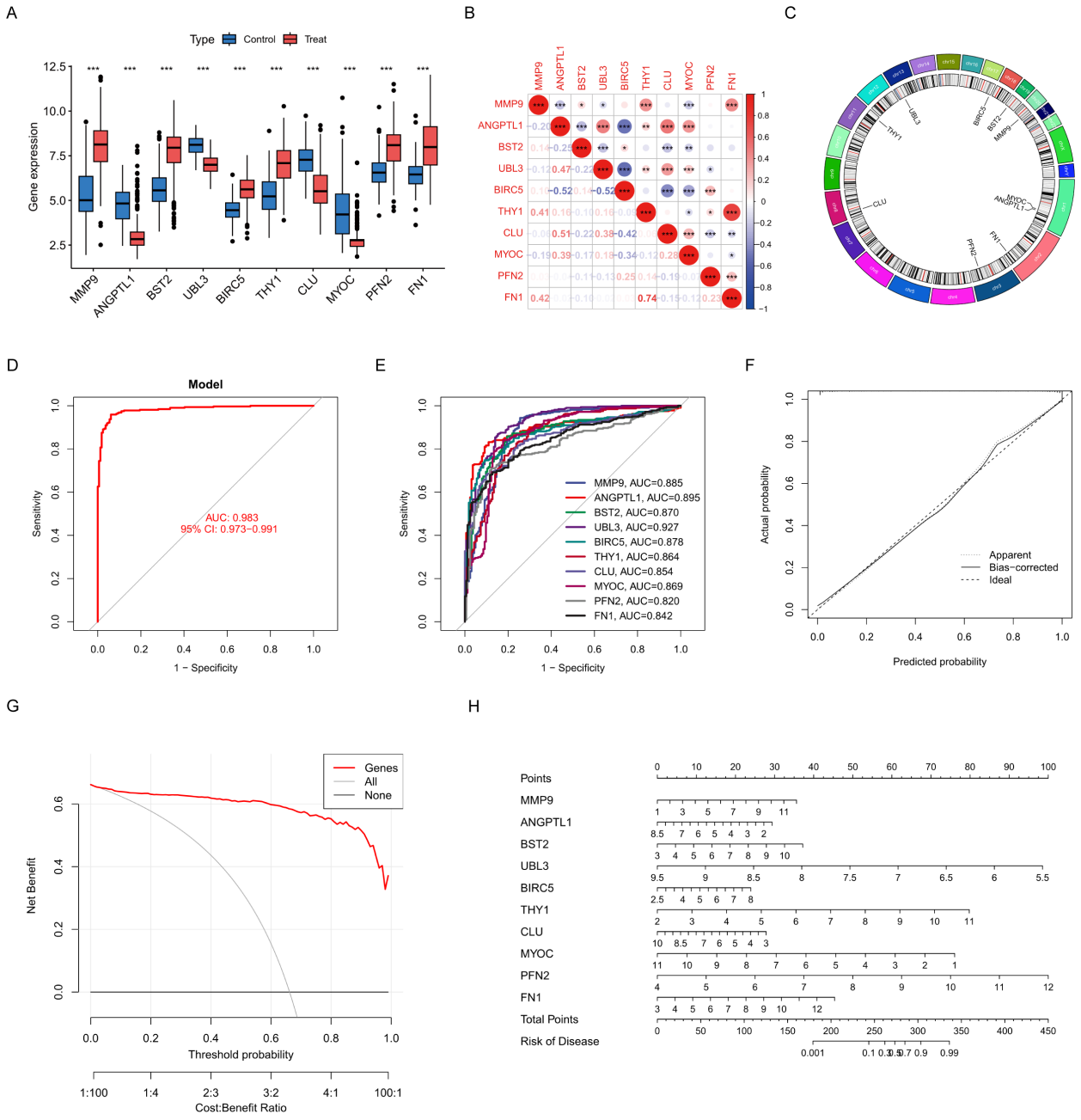

FIGURE 4
Construction and characterisation of characterisation genes. **(A)** Box line plot comparing gene expression in control and treated groups. **(B)** Correlation plots reveal expression relationships between genes. **(C)** Loop plots demonstrate the distribution and association of genes on chromosomes. **(D)** Model ROC plot to assess overall diagnostic performance. **(E)** ROC plot for each gene. **(F)** Calibration curve graph compares predicted probability with actual probability. **(G)** Decision curve plots measure the net benefit of clinical applications. **(H)** Column line graphs construct individualised risk prediction models. Data were showed as mean ± SD, *P < 0.05, **P < 0.01, ***P < 0.001.

and may be intricately regulated at the post-transcriptional level. FN1 is an extracellular matrix protein essential for cell adhesion, migration, and tissue repair, and its interactions with several RBPs indicate a sophisticated regulatory mechanism at the RNA level (Figure 7A). This study also established a TF regulatory network, wherein yellow nodes denote TFs and orange nodes signify target genes. Central to the network, genes including MMP9, BIRC5, CLU, BST2, and THY1 were co-regulated by various transcription factors. MMP9, a gene integral to extracellular matrix disintegration and cancer spread, is modulated by many transcription factors and may be meticulously regulated throughout cellular migration and tissue remodelling. BIRC5, an anti-apoptotic gene crucial for cell survival and carcinogenesis, is regulated by many transcription factors, indicating its modulation by different signalling pathways at the transcriptional level (Figure 7B).

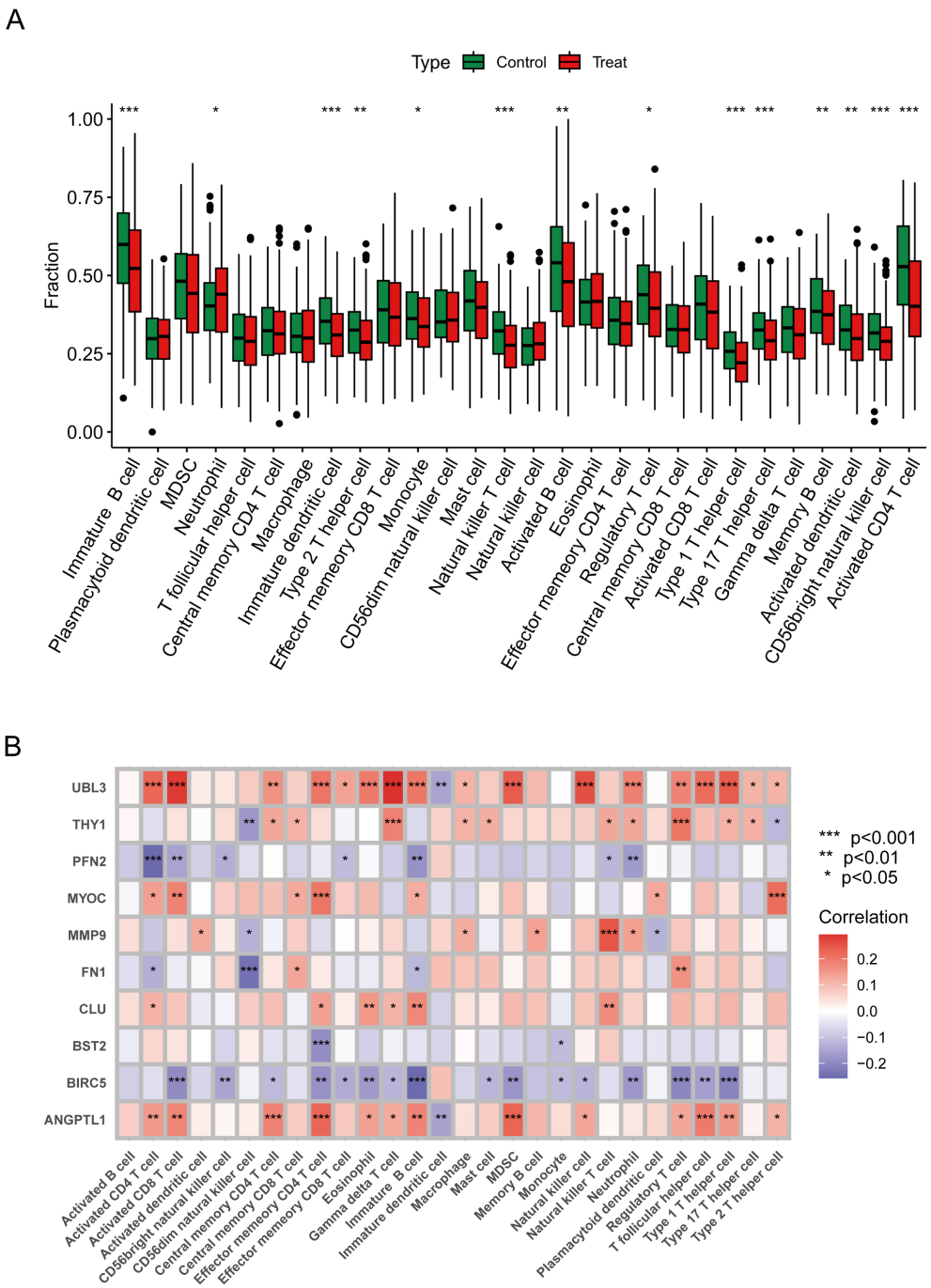

FIGURE 5
Immune properties of characterised genes. **(A)** Immune difference plot comparing the change in distribution of immune cells between control and treated groups. **(B)** Correlation heatmap demonstrating correlation and significant differences between genes and immune cells. Data were showed as mean ± SD, *P < 0.05, **P < 0.01, ***P < 0.001.

ANGPTL1 inhibited HNSCC cell proliferation, migration, and invasion

Using qRT-PCR to detect the differences in ANGPTL1 mRNA expression among different HNSCC cell lines, the ANGPTL1 mRNA expression levels in HNSCC cells were significantly lower than those in the HOK cell line (Figure 8A). HNSCC samples from the HPA database showed absent ANGPTL1 protein expression (staining intensity score = 1), while normal oral mucosa maintained moderate expression (score = 2) (Supplementary Figure 3A), and expression was further reduced in patients with TNM stage II-III (stage I/II vs stage III/IV: log2FC = 0.47, p = 0.0038) (Supplementary Figure 3B). Selected two head and neck squamous cell carcinoma cell lines, SCC9 and CAL27, with low ANGPTL1 expression as subjects for subsequent research. We created a model for overexpression of the ANGPTL1 gene and

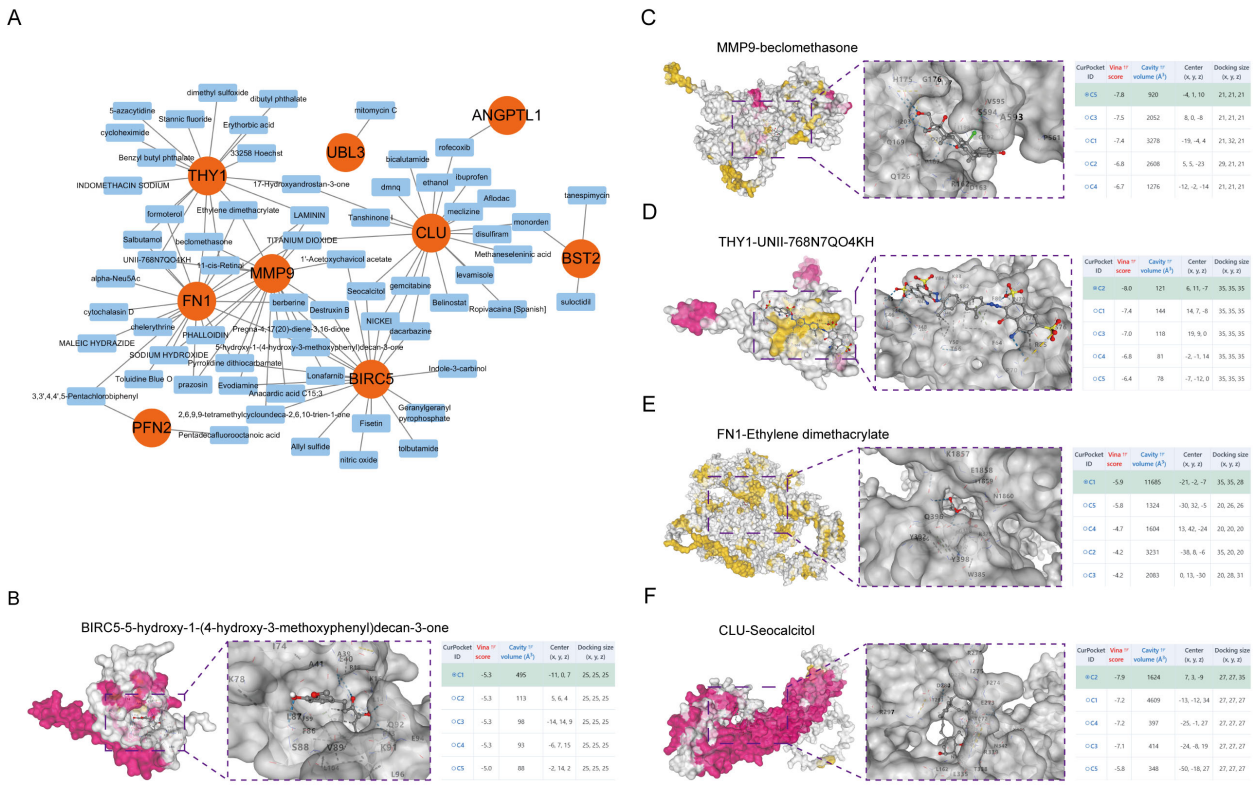

FIGURE 6
Molecular docking of small molecule drugs. **(A)** Drug Regulatory Networks. The 3D structure of molecular docking shows the results of molecular docking of BIRC5 with 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one **(B)**, MMP9 with beclomethasone **(C)**, THY1 with UNII-768N7QO4KH **(D)**, FN1 with Ethylene dimethacrylate **(E)**, and CLU with Seocalcitol **(F)**.

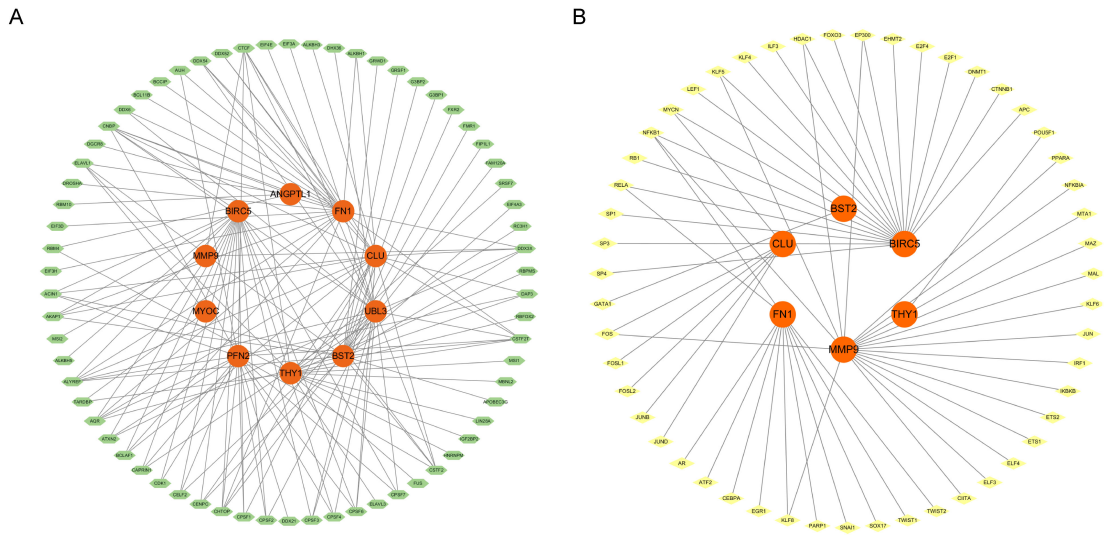

FIGURE 7
(A) RBP regulatory network diagram (RNA binding protein). **(B)** TF transcription factor regulatory network diagram.

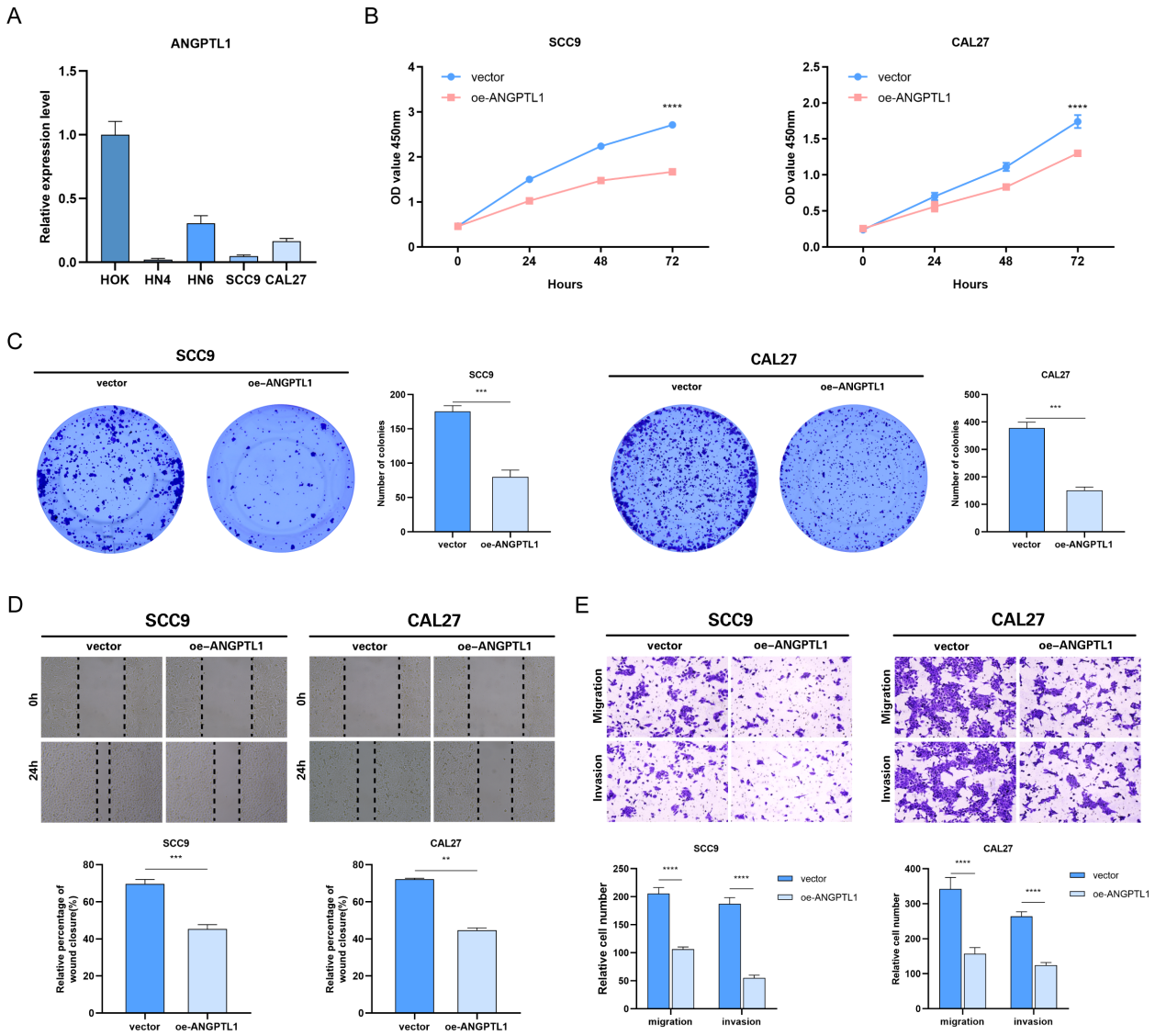

FIGURE 8
Effect of overexpression of ANGPTL1 on the functional phenotype of HNSCC cells. **(A)** Results of qRT-PCR assay of ANGPTL1 mRNA in various HNSCC cell lines. **(B)** CCK-8 proliferation assay of HNSCC cells affected by overexpression of ANGPTL1. **(C)** Clone formation assay to verify the effect of overexpression of ANGPTL1 on the proliferation of HNSCC cells. **(D)** The effect of overexpression of ANGPTL1 on the migration ability of HNSCC cells was verified by scratch assay. **(E)** Transwell assay was performed to verify the effect of overexpression of ANGPTL1 on the migration and invasion ability of HNSCC cells. Data were showed as mean ± SD, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

introduced vector-NC and oe-ANGPTL1 into HNSCC cells by transfection. The results of the CCK-8 experiment showed that the cell proliferation rate in the ANGPTL1 overexpression group of SCC9 and CAL27 cells was significantly lower than that in the control group cells (Figure 8B). The results of the colony formation assay showed that the cell cloning ability of the ANGPTL1 overexpression group in SCC9 and CAL27 cells was significantly inhibited (Figure 8C). To further verify the role of ANGPTL1 protein in the migration and invasion abilities of HNSCC cells, scratch assay results showed that in SCC9 and CAL27 cells, the cell migration ability in the ANGPTL1 overexpression group was significantly lower than that in the control group (Figure 8D). In the transwell experiment, after ANGPTL1 protein overexpression in HNSCC cells SCC9 and CAL27, a decrease in the transmembrane invasion ability of the cells was observed (Figure 8E).

Discussion

This study utilised sophisticated machine learning methods to systematically discover exosome-related indicators in HNSCC, a cancer marked by significant morbidity and death. We found 10 ERDEGs with substantial diagnostic and prognostic potential by integrating multi-omics data and analysing the immunological microenvironment. UBL3 was identified as a strong single-gene biomarker with an AUC of 0.927, whereas a combined model utilising all 10 ERDEGs had outstanding diagnostic accuracy

(AUC = 0.983). These findings underscore the effectiveness of machine learning in transforming intricate information into clinically applicable insights. The development of a nomogram facilitated accurate risk classification, with a total score beyond 300 points associated with over 90% disease risk, highlighting its effectiveness in individualised patient management. Genes such as THY1, FN1, and BIRC5 function as diagnostic markers and demonstrate significant correlations with immune cell infiltration and tumour growth, indicating their dual involvement in disease identification and therapeutic intervention.

MMP9 facilitates tumour invasion and metastasis through the degradation of the extracellular matrix (16). In HNSCC, elevated MMP9 expression was substantially correlated with lymph node metastases and unfavourable prognosis (17, 18). Exosomes transport MMP9 to distant tissues, altering the microenvironment to establish a pre-metastatic niche and increasing the invasiveness of HNSCC (19). ANGPTL1 functions as an anti-angiogenic agent and a tumour suppressor (20, 21). ANGPTL1 is downregulated in several malignancies, and multiple studies have evidenced its inhibitory function in tumour growth and metastasis (22, 23). Exosomal ANGPTL1 reprograms Kupffer cells and reduces their MMP9 expression, averting hepatic vascular leakage and impeding colorectal cancer liver metastases (24). BST2 participates in immunological modulation and viral suppression (25, 26). In HNSCC, the overexpression of BST2 may enhance tumour cell survival by activating the AKT/ERK1/2 pathway and is linked to immune evasion (27). UBL3 modulates the ubiquitin cascade process (28). Recently, UBL3 was identified as a post-translational modification that facilitates protein sorting into tiny extracellular vesicles (29). BIRC5 is an anti-apoptotic protein that significantly influences cell proliferation, differentiation, migration, and invasion (30–33); its elevated expression in HNSCC is associated with treatment resistance and unfavourable prognosis (34, 35). THY1 participates in the regulation of cell adhesion and migration (36, 37). Research indicates that THY1 on the surface of extracellular vesicles (EVs) or the receptor cell surface interacts with corresponding integrins to facilitate the binding, uptake, and distribution of EV contents (38). The function of THY1 in intracellular vesicles remains unclear; nevertheless, it has been identified in non-follicular vesicles and neuronal synaptic vesicles (39). CLU functions as a molecular chaperone that participates in stress response and the regulation of apoptosis (40, 41). In oral cancer cells, CLU overexpression enhances the activation of the AMPK/Akt/mTOR-mediated autophagy pathway, hence promoting cell survival (42). MYOC is mainly linked to glaucoma and has received limited research attention in the context of cancer (43). There is insufficient evidence to establish a direct involvement in HNSCC development or exosome function; nonetheless, it may indirectly influence tumour behaviour through the modulation of ECM hardness, warranting additional investigation. PFN2 modulates the reorganisation of the actin cytoskeleton (44). In HNSCC, PFN2 enhances tumour invasiveness via epithelial-mesenchymal transition (EMT) (45). PFN2 promotes tumour angiogenesis within the tumour microenvironment via cancer-derived exosomes (46). FN1 is an essential extracellular matrix element that facilitates tumour cell adhesion, motility, and metastasis (47–49). In HNSCC, elevated FN1 expression correlates with MDSC infiltration and an immunosuppressive microenvironment (50). Exosomal FN1 can stimulate fibroblasts through integrin signalling, facilitating pro-carcinogenic ECM remodelling and enhancing metastasis (51).

Functional enrichment analysis indicated that ERDEGs are primarily associated with pathways essential to HNSCC pathogenesis, including TNF signalling, IL-17 signalling, and ECM-receptor interactions. These pathways are pivotal to inflammation, immune evasion, and metastasis. FN1, a crucial extracellular matrix protein, promotes tumour cell adherence and migration, and its noted association with heightened infiltration of Activated CD8 T cells and MDSCs (52, 53). BIRC5, an anti-apoptotic gene, exhibited an inverse correlation with regulatory T cells, suggesting its role in inhibiting anti-tumour immune responses (54). *In vitro* experiments provided experimental support that ANGPTL1 plays an anti-cancer role, inhibiting the proliferation, migration, and invasion of HNSCC cells. These mechanistic findings highlight the diverse functions of exosome-related genes in influencing tumour biology via intracellular signalling and extracellular communication within the tumour microenvironment.

Examining immune infiltration patterns in HNSCC tissues indicated a tumour-promoting environment characterised by increased neutrophils and reduced natural killer T cells. ERDEGs such as UBL3 and ANGPTL1 displayed substantial connections with immunosuppressive cell types, including MDSCs, suggesting their involvement in immune evasion. Notably, UBL3 was associated with Activated CD8 T cells and pro-inflammatory pathways, highlighting its contradictory involvement in immune activation and tumour growth. MMP9 and FN1 were linked to extracellular matrix remodelling, a process essential for forming metastatic niches (55–57). Based on these findings, drug sensitivity estimates and molecular docking revealed prospective therapeutic drugs targeting essential ERDEGs. BIRC5 showed affinity for anti-mitotic agents such as berberine, aligning with its function in cellular survival (58), whereas THY1 and FN1 were anticipated to engage with immune checkpoint inhibitors, reinforcing their promise in combinatorial therapy designed to augment anti-tumour immunity.

Notwithstanding these gains, some limits merit attention. During the development of the model, we employed cross-validation as well as multiple feature selection methods to minimise the risk of overfitting. However, despite this, overfitting is still a concern, especially in the case of high-dimensional datasets. To reduce the risk of overfitting, we suggest that future studies should conduct further external validation and consider applying more stringent regularisation techniques to improve the reliability and generalisation of the model. Although *in vitro* investigations offered preliminary insights into ANGPTL1's functional significance, extensive *in vivo* studies are necessary to clarify the molecular contributions of other ERDEGs, including their role in immune regulation. Translational initiatives might also benefit

from experimental confirmation of anticipated medication interactions using patient-derived models, such as organoids or xenografts. Furthermore, future research should isolate tumour-specific exosomes to directly correlate ERDEGs expression with exosomal cargo and functional outcomes, thereby refining our understanding of exosome-mediated intercellular communication in HNSCC progression.

Conclusion

In conclusion, this work employed a machine learning methodology to uncover dependable exosome-related biomarkers for HNSCC. We conducted an extensive bioinformatics analysis to thoroughly investigate exosome-associated genes' expression patterns and functional roles in HNSCC, emphasising their significant contribution to tumour growth and immune modulation. A molecular docking study indicated distinct interactions between exosome-associated proteins and pharmacological targets. These findings highlight the significance of exosomes in cancer biology and offer new avenues for future translational research focused on enhancing the early diagnosis of HNSCC, personalised therapy approaches, and patient prognosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

YH: Conceptualization, Validation, Writing – original draft. YL: Data curation, Formal analysis, Writing – original draft. JT: Visualization, Writing – original draft. YW: Investigation, Writing – review & editing. ZZ: Methodology, Writing – review & editing. RL: Methodology, Writing – review & editing. ZY: Project administration, Writing – review & editing. HL: Funding acquisition, Supervision, Writing – review & editing. JW: Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1590331/full#supplementary-material

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Advances and challenges in immunotherapy in head and neck cancer

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Head and neck squamous cell carcinoma (HNSCC) remains a challenging malignancy with suboptimal survival outcomes despite advances in surgery, radiotherapy, and chemotherapy. Immunotherapy, particularly immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1), has transformed treatment paradigms, yet its full potential in HNSCC is still being explored. This review evaluates the current landscape of immunotherapy in both locally advanced (LA) and recurrent/metastatic (R/M) HNSCC, discussing key clinical trials, emerging biomarkers, and novel therapeutic strategies. For LA HNSCC, phase III trials such as KEYNOTE-412 and JAVELIN Head and Neck 100 failed to demonstrate survival benefits with ICI-chemoradiotherapy combinations in unselected populations, though *post hoc* analyses suggest efficacy in PD-L1–positive tumors. Recent studies, including KEYNOTE-689 and NIVOPOSTOP GORTEC 2018-01, indicate potential benefits of perioperative ICIs in resectable disease. In R/M HNSCC, ICIs have redefined the standard of care. KEYNOTE-040 and CheckMate 141 led to Food and Drug Administration (FDA) approvals of pembrolizumab and nivolumab, while KEYNOTE-048 established pembrolizumab monotherapy for PD-L1 combined positive score (CPS) ≥1 and pembrolizumab plus chemotherapy as first-line treatment. However, dual checkpoint blockade trials (KESTREL, CheckMate 651) have yielded mixed results, highlighting the complexity of immune resistance. Beyond ICIs, emerging strategies include oncolytic virotherapy, chimeric antigen receptor-T cell therapy (CAR-T), and cancer vaccines, with promising preclinical and early-phase clinical results. Biomarkers such as PD-L1 expression, tumor mutational burden (TMB), and Human Papillomavirus (HPV) status play a critical role in treatment selection, but further validation is needed. Despite advancements, challenges persist, including heterogeneous response rates, immune-related

toxicities, and optimal integration of immunotherapy in multimodal treatment regimens. Future research should focus on refining biomarker-driven treatment algorithms, developing rational immunotherapy combinations, and leveraging tumor microenvironment modifications to enhance therapeutic efficacy.

KEYWORDS

immune checkpoint inhibitors, immunotherapy, radiotherapy, chemoradiotherapy, head and neck squamous cell carcinoma, locally advanced, recurrent/metastatic

1 Introduction

HNSCC represents a heterogeneous group of malignancies arising from the mucosal epithelium of the oral cavity, pharynx, and larynx. Despite advancements in multimodal treatment strategies, including surgery, radiotherapy, and chemotherapy, the prognosis for patients with LA and R/M HNSCC remains suboptimal, particularly in the platinum-refractory setting (1). The emergence of immunotherapy has transformed the treatment landscape of HNSCC, with ICIs demonstrating clinical benefit in a subset of patients (2). However, significant challenges remain, including variable response rates, immune-related toxicities, and the need for predictive biomarkers to optimize patient selection (3).

Immune checkpoint blockade targeting PD-1 and its ligand PD-L1 has shown promise in the treatment of R/M HNSCC, leading to the approval of pembrolizumab and nivolumab based on the results of KEYNOTE-040 and CheckMate 141 (4, 5). These agents have extended survival in select patients, yet many still exhibit primary or acquired resistance, underscoring the need for further investigation into combination strategies, tumor microenvironment interactions, and alternative immunotherapeutic approaches (6).

In the locally advanced setting, multiple phase III clinical trials, including KEYNOTE-412 and JAVELIN Head and Neck 100, have explored the integration of ICIs with standard chemoradiotherapy (CRT) (7, 8). While these studies did not demonstrate significant survival benefits, emerging evidence suggests that a subset of patients, particularly those with PD-L1-positive tumors, may derive benefit from ICI-based regimens (9).

Beyond ICIs, novel immunotherapeutic strategies such as therapeutic cancer vaccines, oncolytic viruses, and CAR-T therapy are under investigation, aiming to enhance antitumor immunity in HNSCC (10). Additionally, a deeper understanding of the tumor microenvironment, immune evasion mechanisms, and the role of predictive biomarkers, including PD-L1 expression, TMB, and HPV status, is critical for advancing precision medicine in HNSCC (11).

This review provides a comprehensive analysis of recent advancements and ongoing challenges in immunotherapy for HNSCC. We discuss key clinical trials shaping current practice, emerging therapeutic modalities, the evolving role of biomarkers, and potential future directions to improve outcomes in this patient population. By synthesizing the latest evidence, we aim to offer a balanced perspective on the state of immunotherapy in HNSCC and highlight areas for further research and innovation. EMBASE and MEDLINE databases were systematically searched to identify the phase II and III randomized controlled trials utilizing ICIs in HNSCC. We performed a thorough review of all the identified studies, including their methods, patient population, treatment assignments, primary and secondary outcomes.

2 Tumor microenvironment (TME) and immune contexture in HNSCC

Although there have been significant advancements made in the treatment of HNSCC, the five-year survival rate remains at 50% (12). This is partially due to the fact that not all HNSCCs respond to immune checkpoint blockade therapy, which has recently become prolific in the use of various malignancies. Instead, focus has turned to the TME, a complex ecosystem consisting of various cells that surround tumors inside the body (13).

2.1 Cellular and molecular composition of the tumor microenvironment

The TME plays a pivotal role in the progression, immune evasion, and treatment response of HNSCC. The TME consists of cancer-associated fibroblasts (CAFs), immune cells, adipocytes, fibroblasts, vascular endothelial cells, and epithelial cells that interact with tumor cells, providing nutrients and space for expansion (13–15). The adaptive immune system is suppressed via an overproduction of cytokines which are released secondary to apoptosis of T-cells and changes made to the antigen processing machinery. Transforming growth factor-beta (TGF-β) plays a dual role in promoting epithelial-to-mesenchymal transition and activating CAFs. CAFs play a crucial role in tumor proliferation, invasion, and metastasis (14). In addition to cytokines and various cells, another crucial aspect of the TME is the hypoxic and inflammatory environment secondary to increased radical oxygen species (ROS) production due to genetic changes in malignant cells (specifically in the *TP53* and *NOTCH1* pathways) (14). This

hypoxic environment promotes angiogenesis and altered metabolism as HNSCC malignant cells utilize both glycolytic and oxidative processes through interactions between the cells and the TME to allow for tumorigenesis. These complex interactions and the specialized microenvironment support the idea that the long-held notion of "condensed mucosa" involves not just epithelial cells, but rather the entire tissue (14).

2.2 Tumor immune contexture in HNSCC

The tumor immune microenvironment (TIME) is a term used to describe the spatial organization as well as the density of immune infiltrate within the TME (15). The TIME has been used as a relative outcome and prognosis predictor for patients (Figure 1). For example, the presence of a large number of cluster of differentiation eight positive cytotoxic T cells (CD8+), type 1 helper T cells (Th1), and their associated cytokines inside the TIME corresponds to a robust immune system response that can inhibit tumor and tumor progression to some extent (15). CD8+ cells in particular are among the most powerful immune cells and serve as a central focus of successful cancer immunotherapies (16), these cells can kill cancer cells directly by releasing cytotoxic factors such as granulozyme and tumor necrosis factor-α (TNF-α). There are other cells that play a role in the immune response against cancer such as natural killer (NK) cells which can directly kill cancer cells similar to CD8+ and interact with other cells in TME to promote the anti-tumor effect, and dendritic cells which are one of the main antigen-presenting cells, however, the roles of both these cell types can be negatively impacted by the abnormal metabolic environment in TME (e.g., hypoxia) and inhibitory factors (e.g., TGF-β). Regulatory T cells (Treg) on the other hand can promote tumor survival through multiple different mechanisms such as enhancing tumor angiogenesis and inhibiting the anti-tumor immune response. Tumor-associated macrophages (TAMs) can have different roles depending on their type; they are differentiated into two main types, M1 and M2 which have anti-tumor and pro-tumor effects, respectively (17). Immune checkpoints are regulatory mechanisms that exist to act as an autoregulatory mechanism for T cells. Indeed, the high amount of immune checkpoints that exist within a TME promotes tumor growth by enabling cancer cell escape from the immune system (17). ICIs focus on targeting immune inhibitory modulators that typically regulate the immune response. By genetically modifying these receptors using chimeric antigen receptors, we are able to specify and enhance CD8+ efficacy (16).

One of the most prominent immune checkpoint pathways that promote progression of malignancy is the PD-1) and PD-L1 (18). When PD-1 on T cells interact with PD-L1 on tumor cells, T cell activation is inhibited (Figure 2); moreover, this interaction can also induce T cell apoptosis, reduce production of cytokines, and induce tolerance to the antigen which allows the tumor cell to escape immune surveillance and promotes malignant proliferation (18). By binding to either PD-1 or PD-L1, immune checkpoint inhibitors disrupt this interaction, restoring the recognition and killing mechanism of immune cells and compromising tumor cell escape (18). Therefore, the PD-1/PD-L1 axis has garnered substantial attention as a focus of targeted therapy within TME (15). Another immune checkpoint pathway that is especially prevalent in laryngeal and nasopharyngeal malignancies is the cytotoxic T lymphocyte antigen-4 (CTLA-4) that regulates the function of Treg cells to prevent the immune system from overreacting (17). CTLA-4 inhibitors work by blocking the CTLA-4 which enhances the immune response to fight the cancer cells.

2.3 Therapeutic implications

Given the relatively high amounts of somatic mutations and therefore neoantigens recognized by T cells, HNSCC is considered an immunogenic tumor (19). The tumor escape mechanisms discussed above allow HNSCC to develop at a significant rate despite the anti-tumor immune responses (19). The immunogenic nature of HNSCC does, however, make it susceptible to immunotherapy. Given the efficacy seen in trials with the use of anti-PD-1 blockers in the setting of R/M HNSCC, this approach has also been incorporated into LA HNSCC (19). Studies involving nivolumab, an IgG4 monoclonal antibody directed against PD-1 protein, revealed longer overall survival while pembrolizumab, another anti-PD-1 agent demonstrated a 19% decreased risk of death in the treatment arm versus standard of care (19). Two of the most common CTLA-4 ICIs are ipilimumab and tremelimumab which exert their effects by blocking CTLA-4 function — thereby decreasing Treg function — and subsequently increasing T cell function (17). Monoclonal antibody therapy, which targets tumor surface antigen, is also an essential component of HNSCC treatment (17). Of these, one of the most common is cetuximab, an epidermal growth factor receptor (EGFR) inhibitor that prevents tumor cell proliferation, promotes complement mediated cell lysis, and allows for tumor death via antibody-dependent cell-mediated cytotoxicity.

Given the complex TME of HNSCC, which involves a diverse network of cells, receptors, and signaling pathways, combination therapy may offer enhanced therapeutic benefits, particularly when compared to traditional monotherapy. Recent studies have shown that ICI therapy was more effective following radiotherapy/chemotherapy treatment as demonstrated by increased infiltrative activity of CD8+ cells, increased number of suppressor Treg cells, and increased number of PD-1 positive T cells (17). Other studies demonstrated improved outcomes when cetuximab is used in combination with chemoradiotherapy compared to chemotherapy alone. In addition, when cetuximab is used in combination with ICIs, there is a specific immune response towards the tumor by altering the immune checkpoint expression on tumor infiltrating lymphocytes (TILs) (17). Although these studies offer promising results, further studies are needed to appropriately assess safety

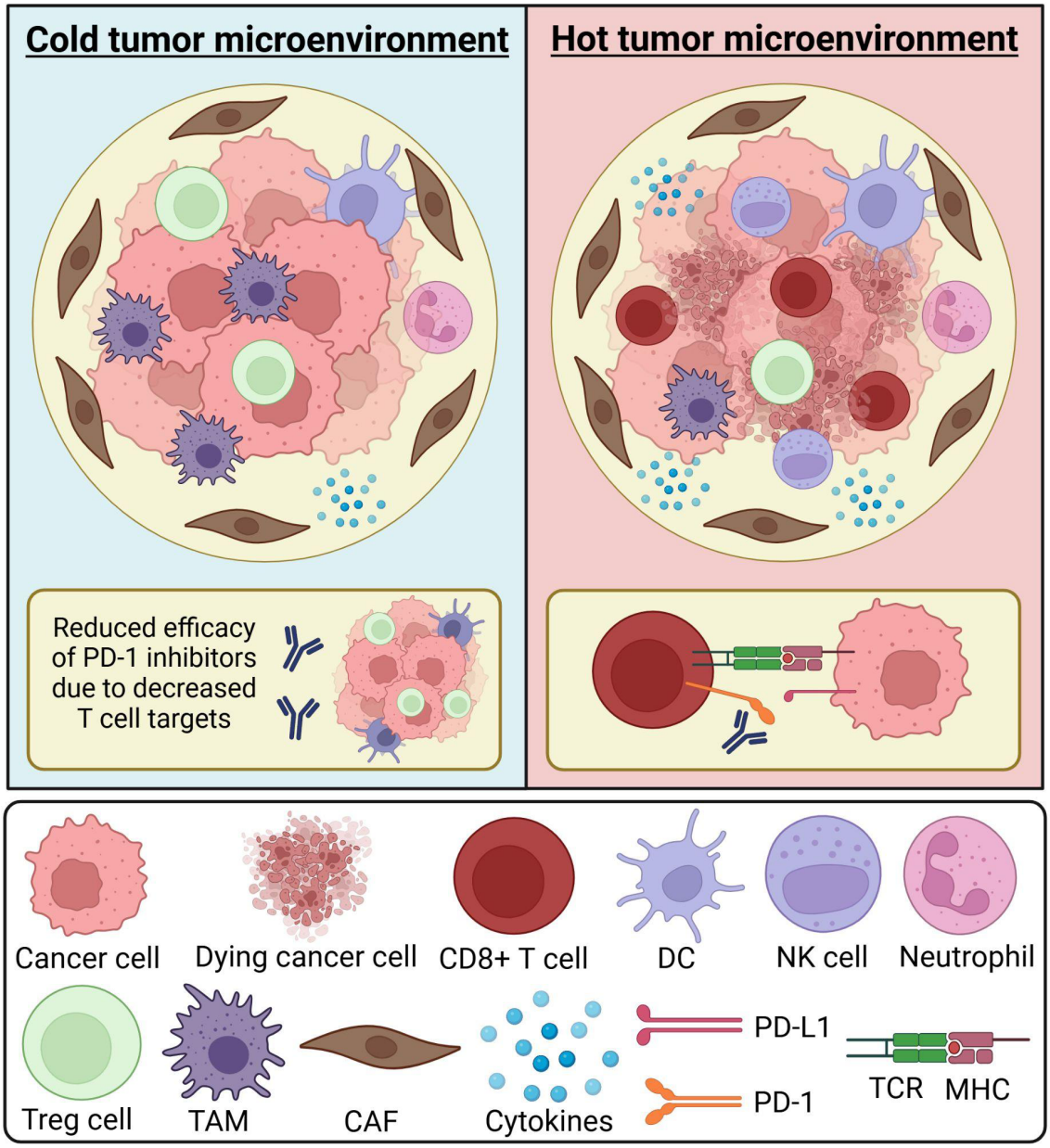

FIGURE 1
Composition of the tumor immune microenvironment. The tumor microenvironment (TME) consists of various immune cells, stromal cells, and cytokines. Immune cells within the TME may include neutrophils, CD8+ T cells, dendritic cells (DCs), and natural killer (NK) cells. The composition of the TME can influence biomarker expression and the efficacy of immune checkpoint inhibitors. A "cold" tumor microenvironment is characterized by low immune cell infiltration, often referred to as an immune desert or immunosuppressive environment. It may contain a higher proportion of immunosuppressive cells, such as tumor-associated macrophages (TAMs) and regulatory T (Treg) cells. The scarcity of T cell targets can reduce the effectiveness of immune checkpoint inhibitors, such as PD-1 inhibitors. A "hot" tumor microenvironment is enriched with immune cells, such as CD8+ T cells, increasing the availability of biomarkers like PD-1. In this setting, cytotoxic T cells can effectively recognize and eliminate cancer cells, enhancing antitumor immunity. Understanding the immune composition of the TME and its impact on therapeutic response is a critical aspect of oncology research. CAF, cancer-associated fibroblast; DC, dendritic cell; MHC, major histocompatibility complex; NK cell, natural killer cell; TAM, tumor-associated macrophage; TCR, T cell receptor; TME, tumor microenvironment; Treg cell, T regulatory cell. Created in BioRender. Thein, K. (2025) https://BioRender.com/t08e962.

profiles and optimal dosing before clinical implementation. The tumor microenvironment and immune contexture in LA HNSCC create a highly complex and immunosuppressive landscape that promotes tumor progression and resistance to therapy. The presence of inhibitory immune checkpoint pathways such as PD-1/PD-L1 and CTLA-4, along with a hypoxic and cytokine-rich environment, enables tumors to evade immune detection. While ICIs have revolutionized cancer treatment, not all HNSCC tumors respond effectively, highlighting the need for a deeper understanding of the interactions between the TME and the immune system. Recent advances in immunotherapy, particularly with ICIs like nivolumab and pembrolizumab, have demonstrated

improved survival outcomes, though challenges remain in achieving consistent and durable responses across all patient populations.

Given the multifaceted nature of the TME, combination therapies incorporating ICIs with radiation, chemotherapy, and monoclonal antibodies such as cetuximab offer a promising approach to enhancing anti-tumor immunity. These strategies not only improve immune infiltration but also help overcome immune resistance mechanisms. However, further research is needed to optimize dosing regimens and mitigate potential toxicities. Future studies should focus on personalized treatment approaches that integrate immune profiling and biomarker-driven strategies to refine therapeutic responses and improve overall survival rates in LA HNSCC.

3 Immune checkpoint inhibitors in locally advanced HNSCC

The current standard of care (SoC) treatment for LA HNSCC includes a combination of CRT or surgery followed by adjuvant radiotherapy with or without chemotherapy. High-dose cisplatin is considered the preferred agent, though there are multiple alternative chemo regimens for patients who are ineligible to receive cisplatin such as cetuximab or a combination of carboplatin plus 5-fluorouracil (20).

The remarkable success of pembrolizumab in the treatment of unresected R/M HNSCC (4, 21) has spurred a series of studies to evaluate the efficacy of ICIs in the locally advanced setting too. Over

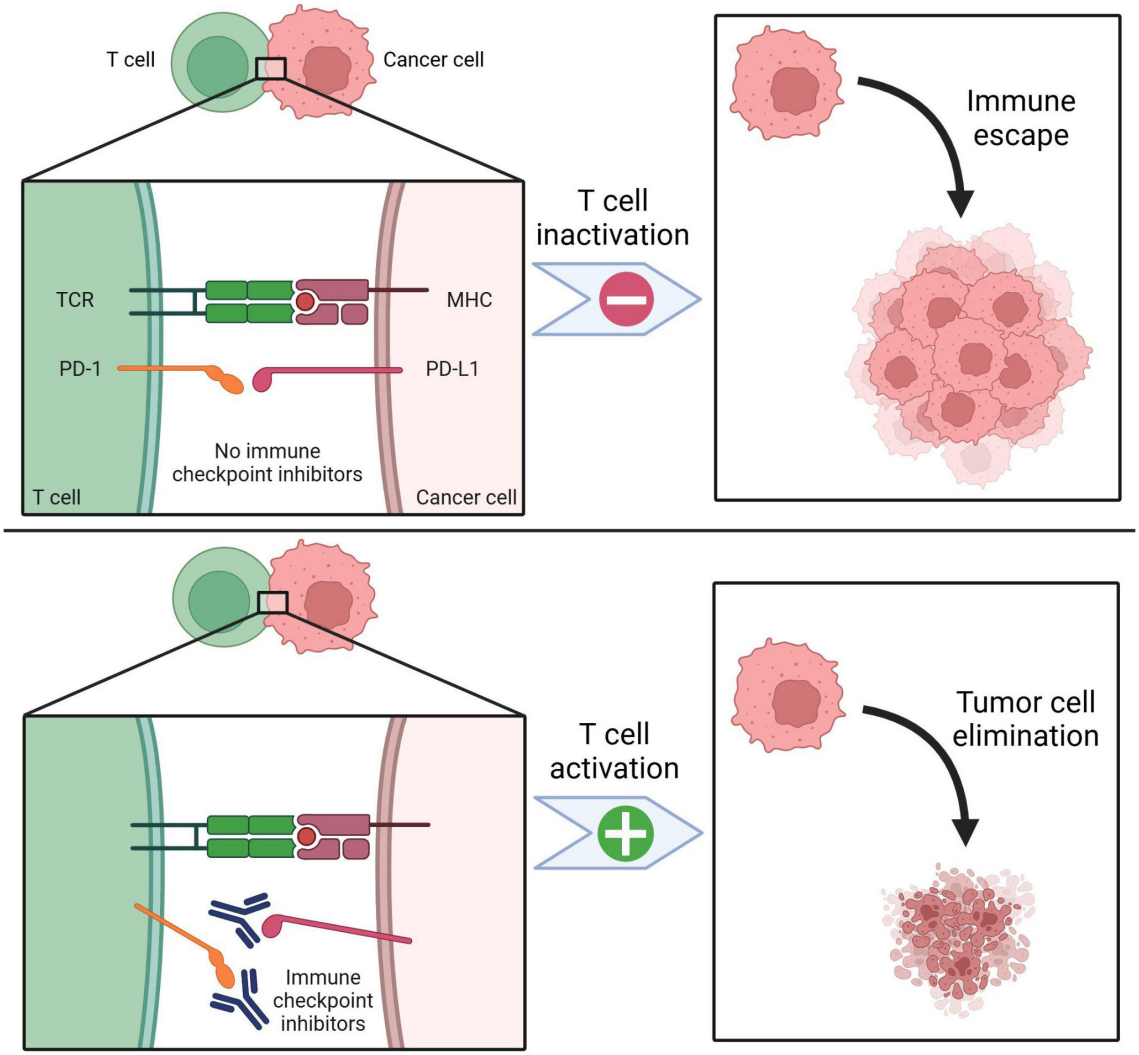

FIGURE 2
PD-1 / PD-L1 expression and immune checkpoint inhibition. PD-1 is a key biomarker for targeting head and neck cancers. PD-1 receptors are expressed on T cells, while PD-L1 receptors are found on cancer cells. Effective T cell activation also requires the presence of the T cell receptor (TCR) and the major histocompatibility complex (MHC). In the absence of immune checkpoint inhibition (top panel), PD-1 binds to PD-L1, leading to T cell inactivation. This suppresses immune surveillance, allowing cancer cells to evade detection and proliferate. With immune checkpoint inhibitors (bottom panel), such as antibodies targeting PD-1 or PD-L1, T cell inactivation is blocked. As a result, more T cells remain active, enhancing tumor cell recognition and elimination. Created in BioRender. Thein, K. (2025) https://BioRender.com/t08e962.

the past few years, multiple trials have evaluated the use of immunotherapy in LA HNSCC.

3.1 Immunotherapy plus standard-of-care chemoradiotherapy (definitive setting)

JAVELIN Head and Neck 100, the first phase 3 randomized controlled trial (RCT) investigating ICI plus CRT in general and the first phase 3 RCT to evaluate ICI in LA HNSCC, tested avelumab plus CRT followed by avelumab maintenance therapy versus standard CRT. Unfortunately, it failed to meet its primary endpoint of prolonging progression-free survival (PFS) and therefore was terminated at the time of preplanned interim analysis (8). KEYNOTE-412, another phase 3 study with a similar design, also failed to show a survival benefit of ICI in patients with newly diagnosed, high-risk, and previously untreated LA HNSCC. It evaluated pembrolizumab plus CRT followed by pembrolizumab maintenance versus placebo plus CRT followed by placebo maintenance (7). There are multiple theories surrounding the lack of efficacy observed in those trials. These include the concomitant administration of immunotherapy with high doses of radiation applied to lymph nodes which can affect its immune function, and subsequently the anti-tumor immune response, the enrollment of a PD-L1 unselected patients, and the incorporation of both p16-negative and p16-positive tumors, as p16-positive tumors tend to have higher sensitivity to CRT and better overall prognosis (7).

Despite the failure of both trials to show an overall survival benefit with ICI use, one remarkable finding was that both studies demonstrated a potential survival benefit with ICI plus CRT in the subgroup of patients with PD-L1 positive status (7, 8). We postulated that with a larger sample size the effect of PD-L1 status may become more apparent, and that a survival benefit would likely be observed in PD-L1 positive subgroups compared to a potential harmful effect in PD-L1 negative subgroups. This served as the rationale for our decision to perform a meta-analysis including these two trials (JAVELIN HEAD and Neck 100 + KEYNOTE-412). Our meta-analysis revealed an improved PFS in the ICI + CRT group compared to CRT alone in the PD-L1-positive cohort with a hazard ratio (HR) of 0.78 (95% confidence interval [CI]: 0.63-0.97; P=0.02), while it showed a potential harmful effect of ICI + CRT in the PD-L1-negative cohort compared to CRT alone (HR 1.31; 95% CI: 0.99-1.75; P=0.06). These impressive results open the door to future studies which may help guide the use of immunotherapy in the appropriate patient population. Of note, our study was accepted for poster presentation at the American Head and Neck Society (AHNS) annual meeting which was held in May 2025.

3.2 Immunotherapy in cisplatin-ineligible LA HNSCC

Standard-of-care chemotherapies — especially cisplatin — are known to have significant toxicities, and sometimes patients are not suitable candidates for high-dose cisplatin-based chemotherapy due to age, physical status, or other comorbidities. As such, multiple studies have investigated ICIs plus radiotherapy (RT) alone in cisplatin-ineligible patients with LA HNSCC, which is expected to have a favorable toxicity profile compared to SoC CRT (22, 23).

GORTEC 2015–01 PembroRad, the first randomized trial to evaluate ICI plus RT alone in LA HNSCC, tested pembrolizumab versus cetuximab with concurrent RT. The primary endpoint was locoregional control (LRC) at 15 months following the end of RT. The study did not meet its primary endpoint of improving LRC and pembrolizumab–RT combination did not show any survival benefit over cetuximab–RT (24). NRG-HN004, a recently published phase II/III RCT, is another trial that evaluated immunoradiotherapy combination in cisplatin-ineligible LA HNSCC. It investigated durvalumab vs cetuximab with concurrent and adjuvant RT with a primary endpoint of PFS. Similar to PembroRad, ICI plus RT combination has also failed to improve outcomes compared to cetuximab–RT (25).

The role of ICIs in cisplatin-ineligible LA HNSCC was further explored, but with a different combination regimen. GORTEC 2017–01 REACH, phase III RCT, evaluated the combination of avelumab–cetuximab–RT in two different patient cohorts, fit for cisplatin and unfit for cisplatin. In both cohorts, the experimental arm incorporated SoC RT plus cetuximab and avelumab during RT followed by avelumab for one year. On the other hand, the control arm included SoC RT plus cisplatin in the fit cohort and SoC RT plus cetuximab in the unfit cohort. The study showed that in cisplatin-unfit patients, the addition of avelumab to cetuximab–RT had a favorable effect on PFS and distant metastases but not overall survival (OS). Interestingly, in the cisplatin-fit patients the combination of avelumab–cetuximab–RT had a detrimental effect with lower rates of PFS and OS compared to SoC RT plus cisplatin (26). These findings raise further questions about the role of ICIs in cisplatin-eligible population and about the optimal combination regimen for cisplatin-ineligible LA HNSCC.

3.3 Recent updates in treatment of LA HNSCC

There are multiple approaches to investigate ICIs in LA HNSCC as discussed above, including in the neoadjuvant, concurrent, and adjuvant settings. Investigators of IMvoke010 (27), recently published in March 2025, decided to evaluate ICIs in LA HNSCC with a sequential approach. This was based on the results from a phase II trial evaluating pembrolizumab plus CRT in LA HNSCC that showed a longer 1- and 2-year PFS with the sequential approach compared to the concurrent approach (28). IMvoke010 evaluated atezolizumab, PD-L1 inhibitor, vs placebo in LA HNSCC following the completion of multimodal definitive therapy. Unfortunately atezolizumab failed to show any survival benefits in the overall study population and all subgroups. However, there was a trend towards longer event-free survival (EFS) in patients with tumors expressing PD-L1 ≥5%, similar to the findings from JAVELIN HEAD and Neck 100 and KEYNOTE-412. This finding

underscores the need for more studies to further evaluate this association, as it may help guide the selection of an appropriate subgroup of patients with LA HNSCC who might benefit from ICIs.

3.3.1 Immunotherapy in the perioperative setting

Despite the failure of previous trials evaluating ICIs to show a meaningful benefit in LA HNSCC, promising results have emerged from additional studies over the past few months that evaluated ICIs in the perioperative setting.

KEYNOTE-689 was noted to be the first phase III trial to show positive outcomes in patients with resected LA HNSCC. The study evaluated perioperative pembrolizumab, an anti-PD-1 antibody, in patients with newly diagnosed stage III or IVA resected LA HNSCC. Patients received pembrolizumab with standard RT (with or without cisplatin) followed by pembrolizumab maintenance, compared to adjuvant RT (with or without cisplatin) alone. There was a statistically significant and clinically meaningful improvement in the EFS for patients who received the pembrolizumab regimen. The study also revealed a statistically significant improvement in major pathologic response (mPR) in the pembrolizumab arm compared to adjuvant RT alone (29). The results of KEYNOTE-689 were presented at the American Association of Cancer Research (AACR) annual meeting 2025.

Earlier this year, another phase III trial, NIVOPOSTOP GORTEC 2018-01, reported meeting its primary endpoint of improving disease-free survival (DFS). It evaluated the addition of anti-PD-1, nivolumab, to SoC RT and cisplatin after surgery compared to SoC RT and cisplatin alone. A statistically significant and clinically meaningful improvement in DFS was observed for patients receiving nivolumab as a postoperative treatment for resected LA HNSCC with high risk of relapse (30). The remarkable findings from these trials have the potential to change clinical practice, underscoring the promising role of ICIs and the need for continued investigation into their efficacy in managing LA HNSCC. The results of NIVOPOSTOP will be presented at the upcoming American Society of Clinical Oncology annual meeting in May-June 2025. There are currently multiple other ongoing trials and the literature will only continue to grow. Table 1 provides a summary of the characteristics of the clinical trials evaluating ICIs in LA HNSCC. Figure 3 shows an overview of PFS/EFS data in some of the key clinical trials in LA HNSCC.

4 Immune checkpoint inhibitors in recurrent/metastatic HNSCC

4.1 Immunotherapy in platinum-refractory R/M HNSCC

For many years the standard of care for R/M HNSCC centered around chemotherapy combination regimens, using agents such as cisplatin, methotrexate, bleomycin, and fluorouracil. The advent of the EGFR inhibitor cetuximab ultimately led to a shift in the SoC to include platinum chemotherapy augmented by cetuximab. This was based on the results of the EXTREME clinical trial (NCT00122460), which demonstrated that the addition of cetuximab to platinum based chemotherapy with fluorouracil significantly increased median overall survival from 7.4 months in the chemotherapy group to 10.1 months in the cetuximab group (HR for death, 0.80; 95% CI, 0.64 to 0.99; P=0.04) (31). Despite this, R/M HNSCC remained a significant contributor to morbidity and mortality in head and neck cancer patients, and treatments were not without substantial toxicities.

The introduction of immunotherapies, specifically immune checkpoint inhibitors. ushered in a new era in the treatment of R/M HNSCC. The clinical trials KEYNOTE-040 and CheckMate 141 evaluated the anti-PD-1 antibodies pembrolizumab and nivolumab, respectively, in the treatment of R/M HNSCC and demonstrated remarkable results (4, 5). Specifically, KEYNOTE-040 was a randomized, open-label, phase III study that compared pembrolizumab to standard therapy using methotrexate, docetaxel, or cetuximab in patients with R/M HNSCC previously treated with a platinum-containing regimen. Pembrolizumab was associated with a significantly improved median OS of 8.4 months (95% CI 6.4–9.4) compared to 6.9 months (95% CI 5.9–8.0) in those treated with standard of care regimens. Treatment with pembrolizumab was also associated with far less grade 3 or worse treatment-related adverse events (33 [13%] of 246 vs 85 [36%] of 234).

Similarly, CheckMate 141 was also a randomized, open-label, phase III trial and compared nivolumab to SoC chemotherapy in patients with recurrent HNSCC whose disease had progressed within 6 months of treatment with a platinum-based regimen. Treatment with nivolumab resulted in an improved median OS of 7.5 months (95% CI, 5.5 to 9.1) compared to 5.1 months (95% CI, 4.0 to 6.0) in the group that received standard therapy. Compared to the control, the nivolumab intervention group had double the response rate (13% vs. 6%) and double the one-year overall survival (36% vs. 16.6%). Additionally, patients in the nivolumab arm experienced significantly less grade 3 or higher treatment-related adverse events (TRAEs) (13.1% vs. 35.1%). The success of these two trials ultimately led to the FDA approval of both pembrolizumab and nivolumab for the treatment of platinum-refractory R/M HNSCC in 2016.

In the years following, additional immunotherapeutic agents were also explored. In 2018, the HAWK study evaluated treatment with durvalumab in patients with PD-L1-high tumor cell expression who had platinum-refractory R/M HNSCC. The results were promising, with an objective response rate (ORR) of 16.2% (95% CI, 9.9-24.4), median PFS of 2.1 months (95% CI, 1.9-3.7), and median OS of 7.1 months (95% CI, 4.9-9.9) (32). This led to further exploration of durvalumab in additional regimens and populations. The CONDOR study was a phase II RCT that assessed durvalumab with or without tremelimumab in PD-L1 low/negative patients with R/M HNSCC. The findings of this trial indicated a manageable toxicity profile, with grade 3/4 TRAEs occurring in 15.8% of patients in the durvalumab plus tremelimumab combination arm, 12.3% of patients in the durvalumab monotherapy arm, and 16.9% in the tremelimumab monotherapy arm. ORR was 7.8% in the combination arm, 9.2% for durvalumab monotherapy, and 1.6% for

TABLE 1 Randomized clinical trials on immune checkpoint inhibitors in locally advanced HNSCC.

Study Name	Study Type	Study Population	Number of Patients (Experimental/ Control)	Treatment Intervention		Primary Endpoint
				Experimental Arm	Control Arm	
JAVELIN Head and Neck 100	Randomized, double-blind, placebo-controlled, phase III	Histologically diagnosed, high-risk, previously untreated LA HNSCC	350/347	Avelumab plus CRT (Cisplatin + RT) followed by avelumab maintenance for up to 12 months	Placebo plus CRT (Cisplatin + RT) followed by placebo maintenance for up to 12 months	PFS
KEYNOTE-412	Randomized, double-blind, phase III	Newly diagnosed, pathologically proven, high-risk LA HNSCC with no previous treatment	402/402	Pembrolizumab plus CRT (Cisplatin + RT) followed by pembrolizumab maintenance every 3 weeks for total of 14 doses	Placebo plus CRT (Cisplatin + RT) followed by placebo maintenance every 3 weeks for total of 14 doses	EFS
GORTEC 2015-01 PembroRad	Open-label, randomized, controlled, multicenter, phase II	Cisplatin-ineligible, histologically confirmed, non-operated LA HNSCC	67/66	Pembrolizumab every 3 weeks during RT	Cetuximab weekly during RT	LRC at 15 months after the end of RT
NRG-HN004	Open-label, multicenter, parallel-group, randomized, phase II/III	Cisplatin-ineligible LA HNSCC	123/63	RT plus durvalumab every 4 weeks for up to seven cycles	RT plus cetuximab weekly for up to eight cycles	PFS
GORTEC 2017-01 REACH	Randomized, controlled, phase III	2 cohorts (fit for cisplatin and unfit for cisplatin)	Unfit cohort: 275 patients total Fit cohort: 426 patients total	In both cohorts: RT plus cetuximab and avelumab followed by avelumab maintenance for 1 year	Fit cohort: cisplatin plus RT Unfit cohort: cetuximab plus RT	PFS
KEYNOTE-689	Randomized, active-controlled, open-label, phase 3	Newly diagnosed, stage III or IVA resected LA HNSCC	~ 704 patients total	Pembrolizumab every 3 weeks for 2 cycles prior to surgery followed by pembrolizumab (for 15 cycles) plus SOC RT with or without cisplatin after surgery	No neoadjuvant therapy prior to surgery followed by SOC RT with or without cisplatin after surgery	EFS
NIVOPOSTOP GORTEC 2018-01	Randomized controlled, open-label, phase 3	Resected LA HNSCC with high risk of relapse	680 patients total	Nivolumab plus SOC cisplatin–RT after surgery followed by 6 cycles of nivolumab	SOC cisplatin–RT after surgery	DFS
IMvoke010	Global, double-blind, phase 3	LA HNSCC without disease progression after completion of multimodal definitive treatment	203/203	Atezolizumab every 3 weeks up to 1 year	Placebo	EFS

CRT, chemoradiotherapy; RT, radiotherapy; PFS, progression-free survival; EFS, event-free survival; DFS, disease-free survival; LRC, locoregional control; SOC, standard-of-care.

tremelimumab monotherapy, suggesting that durvalumab monotherapy and combination regimen may result in clinical benefit (33).

The EAGLE study further evaluated both durvalumab monotherapy and combination therapy with tremelimumab, comparing treatment groups to SoC in a phase III RCT (34). However, no statistically significant improvements in OS were noted for durvalumab versus SoC [HR: 0.88; 95% CI, 0.72-1.08; P=0.20] or durvalumab plus tremelimumab versus SoC [HR: 1.04; 95% CI, 0.85-1.26; P=0.76] [6]. Despite the negative results in the EAGLE study, durvalumab remained a promising therapeutic agent, particularly due to its substantially better toxicity profile compared to SoC.

4.2 Checkpoint inhibitors in untreated locally incurable R/M HNSCC

For many years the first line treatment for R/M HNSCC centered around cetuximab with platinum based chemotherapy and fluorouracil, which is associated with a median OS of 10.1 months and significant toxicities as evidenced by the results of the EXTREME trial. This changed in 2019, with the landmark KEYNOTE-048 trial demonstrating beneficial survival effects from the use of pembrolizumab (21). KEYNOTE-048 was a multicenter, open-label, phase III RCT that compared pembrolizumab monotherapy and pembrolizumab with chemotherapy to the EXTREME regimen in patients with

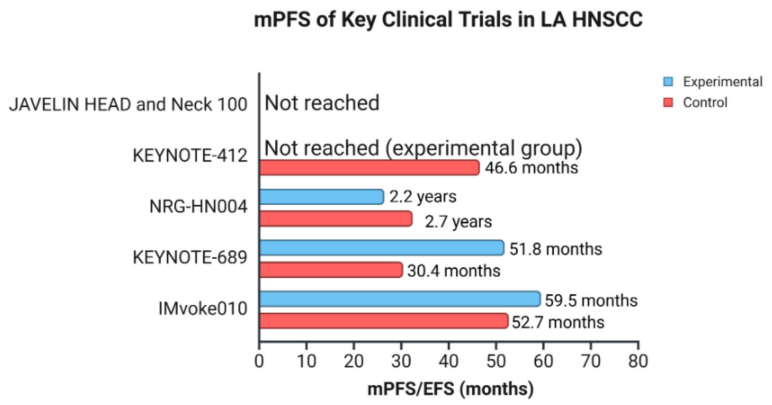

FIGURE 3
mPFS/EFS of key clinical trials in LA HNSCC. The graph represents an overview of mPFS/EFS among 5 clinical trials, with comparison between experimental and control groups in patients with LA HNSCC. The data for median overall survival were not represented as results were not reached. mPFS (median progression-free survival), EFS (event-free survival), LA HNSCC (locally advanced head and neck squamous cell carcinoma). Created in BioRender. Thein, K. (2025) https://BioRender.com/l8wlnx3.

untreated, incurable R/M HNSCC. The study found that pembrolizumab monotherapy improved OS compared to cetuximab with chemotherapy in patients with a CPS of 20 or more (HR 0.61; 95% CI 0.45–0.83; P=0.0007) and CPS of 1 or more (HR 0.78;95% CI 0.64–0.96; P=0.0086). Additionally, pembrolizumab plus chemotherapy improved OS compared to cetuximab with chemotherapy in the total population (HR 0.77;95% CI 0.63–0.93; P=0.0034) regardless of CPS score. Pembrolizumab monotherapy was also associated with significantly fewer adverse events (55% compared to 83% in the EXTREME regimen group). It is important to note, however, that neither pembrolizumab monotherapy nor pembrolizumab with chemotherapy improved progression free-survival compared to the EXTREME regimen. The remarkable results of KEYNOTE-048 ultimately led to the FDA approval of pembrolizumab plus chemotherapy for all populations and pembrolizumab monotherapy for patients with PD-L1 CPS ≥1 as first line treatment for R/M HNSCC in June of 2019. This drastically altered the treatment landscape for R/M HNSCC, and many new studies emerged evaluating immune checkpoint inhibitors as first line therapy as well as unique combination regimens. KEYNOTE-669 in particular hypothesized that the addition of epacadostat would enhance the activity of pembrolizumab. The study was a multi-site, open label phase III RCT that compared pembrolizumab plus epacadostat, pembrolizumab monotherapy, and the EXTREME regimen in patients with locally incurable, untreated, RM HNSCC (35).

KESTREL, also an open-label phase III RCT, evaluated the efficacy of durvalumab with and without tremelimumab compared to the EXTREME regimen in patients with R/M HNSCC (36). The study did not meet its primary endpoint of improved survival, finding that both durvalumab with and without tremelimumab were not superior to the EXTREME regimen with regards to OS (HR 0.96; 95% CI 0.69–1.32; P=0.787 and HR 1.05; 95% CI 0.80–1.39, respectively) as well as PFS (2.8 and 2.8 versus 5.4 months). Similarly, CheckMate 651 compared nivolumab plus ipilimumab against the EXTREME regimen and showed no statistically significant improvement in OS in all randomized or CPS ≥ 20 populations (37). However, there was a survival benefit observed in patients with CPS ≥ 1, as the median OS was 15.7 versus 13.2 months (HR 0.82; 95% CI, 0.69–0.97). The findings of these trials indicated a variable response to immunotherapy, with the efficacy of therapeutic agents being highly dependent on the combination regimen it is utilized in, patient characteristics such as biomarker expression, and the specific setting it is administered in. Nivolumab, however, was proven to be a promising agent when CheckMate 141 first demonstrated its superiority in platinum-refractory R/M HNSCC. Additionally, nivolumab plus ipilimumab had demonstrated long term, durable survival benefits for various other cancers, including non–small cell lung cancer (NSCLC), melanoma, renal cell carcinoma, esophageal squamous cell carcinoma, and malignant pleural mesothelioma (38–42). Thus, CheckMate 714 sought to further analyze the regimen utilized in CheckMate 651 by assessing the individual contributions of each agent, comparing nivolumab plus ipilimumab to nivolumab alone as first line therapy (43). The trial did not meet its primary endpoint of ORR benefit in the combination therapy arm compared to the nivolumab monotherapy arm in patients with platinum-refractory R/M HNSCC, finding an ORR of 13.2% (95% CI, 8.4–19.5%) and 18.3% (95% CI, 10.6–28.4%) respectively (odds ratio [OR], 0.68; 95.5% CI, 0.33-1.43; P=0.29).

4.3 Immunotherapy-cetuximab combination regimens

The interest in combining immunotherapeutic agents with other components of standard care grew substantially following the results of KEYNOTE-048. Two notable trials have evaluated the combination of ICIs with cetuximab in R/M HNSCC. The clinical trial NCT03370276 assessed nivolumab plus cetuximab in two cohorts: Cohort A consisting of those who had received any prior

systemic therapy for R/M HNSCC and Cohort B consisting of those that had not received prior systemic therapy (44). The study found that median OS in cohort A was 11.4 months, with a 1 year OS 50% (90% CI, 0.43–0.57) and in cohort B was 20.2 months, with a 1-year OS 66% (90% CI, 0.59–0.71), suggesting that cetuximab and nivolumab combination therapy is beneficial in patients with RM HNSCC regardless of prior treatment status. The second trial, NCT03082534, assessed pembrolizumab and cetuximab combination therapy and found that 6-month ORR was 45% (95% CI 28–62), suggesting that pembrolizumab with cetuximab may also prove to be a fruitful combination regimen (45). Characteristics of clinical trials evaluating ICIs in R/M HNSCC are summarized in Table 2. Figure 4 represents survival and response data for some of the key clinical trials in R/M HNSCC.

4.4 Future directions

While R/M HNSCC has seen drastic shifts in SoC, therapeutic regimens with immunotherapies (and specifically ICIs) are still evolving. As it stands, morbidity and mortality rates remain high, and there are pros and cons to each treatment regimen. For example, while monotherapies with ICIs are typically associated with much better toxicity profiles, they are often not as efficacious or as widely applicable as combination regimens. Further, much of the current research is limited in the sense that the assessed therapies are only beneficial for specific patient populations. Additional trials with both precise and informed choices for treatment regimens in appropriate patient populations, tailored to patient characteristics such as biomarker expression, are needed to address the gaps that currently exist.

5 Novel immunotherapies in head and neck cancer

Significant improvements in the treatment strategies of HNSCC have been made in the past decade; the five-year overall survival in these patients remains to be 30-65%, depending on healthcare resources and systems (46). Substantial research has been conducted in the past two decades, which has resulted in the introduction of newer therapeutic modalities for HNSCC. Cancer immunotherapy remains a successful modality, which is based on altering the complex host immune environment to mount a response against tumor cells and prevent the evasion of malignant cells from detection. The introduction of these novel immunotherapies has shifted the treatment for R/M HNSCC, improving clinical outcomes as evidenced by recent trials (47). Different ICIs targeting the PD-1/PD-L1 axis have been approved for various malignancies. Notably, findings from the KEYNOTE-048 trial have led to the approval of ICIs as a first-line therapy for recurrent/metastatic HNSCC (47). Additionally, many other promising immunotherapies, including CAR-T cell therapy, oncolytic virus therapy, and vaccines, are currently under investigation (Figure 5).

5.1 Oncolytic virotherapy

Cancer therapies using oncolytic viruses (OVs) are becoming an emerging area of research and therapeutics. In these therapies, a virus is designed to selectively target and lyse tumor cells without affecting host cells. The mechanism of action for OVs to mount an antitumor response primarily involves three aspects: 1) direct virus-mediated cytotoxicity, where the virus targets the tumor cells specifically and self-replicates, leading to infection and lysis of tumor cells; 2) viral infection that alters the tumor vascular system enhancing influx of neutrophils, causing vascular collapse and cell death; 3) virus-mediated release of cytokines and chemokines inducing immunogenic cell death resulting in the release of pathogen-associated molecular pattern molecules (PAMPs), damage-associated molecular pattern molecules (DAMPs), tumor-associated antigens (TAAs), and tumor-associated neoantigens (TANs), which activate the innate immune system and induce immunologic transformation from 'cold' tumors to 'hot' tumors (48).

Adenoviruses (AD) have received much attention for this purpose due to their ability to grow in high concentrations *in-vitro*, replicate in the episomal form, upregulate costimulatory molecules and induce chemokine and cytokine responses in cells (49). The first oncolytic adenovirus – Oncorine (H101) – was approved by the Chinese state FDA for head and neck malignancies in 2005; however, the first approved oncolytic virus by the US FDA was a genetically modified herpes simplex virus (HSV) named 'talimogene laherparepvec' in October 2015 (47, 49). Since then, multiple clinical trials have been underway to test this novel approach for treatment. OVs have been injected intratumorally (IT) and intravenously (IV) in combination with chemotherapy or immunotherapy in numerous clinical trials, showcasing excellent safety and efficacy profiles with promising results in response and survival (47, 50). The viruses currently being utilized in the clinical trials include DNA viruses such as AD, HSV, and vaccinia virus (VV), as well as RNA viruses such as reovirus (RV), vesicular stomatitis virus (VSV), and measles virus (MV) (48).

In the year 2000, the National Cancer Institute in the US started the phase I trial of the first-generation oncolytic AD, ONYX-015, for the treatment of head and neck cancers. In the phase II trial of the ONYX-015, a significant tumor regression (>50%) in 21% of patients was observed; however, due to funding issues, the phase III trial was terminated. Since then, multiple clinical trials involving AD have been underway. Recently, E10A - an AD with engineered insertion of human endostatin gene is currently being studied with a combination of paclitaxel and cisplatin for the treatment of HNSCC. AdAPT-001, another genetically engineered virus is also currently being investigated in the clinical trial known as BETA PRIME, both with and without immune checkpoint inhibitors. Multiple other clinical trials involving reovirus (reolysin), HSV virus (T-VEC), measles virus (MV-NIS), and vaccinia virus (Pexa-Vec) are currently under investigation with possible outcomes (47, 48).

The crucial challenge in the application of OVs is the pre-existing immunity against viruses due to previous infection or

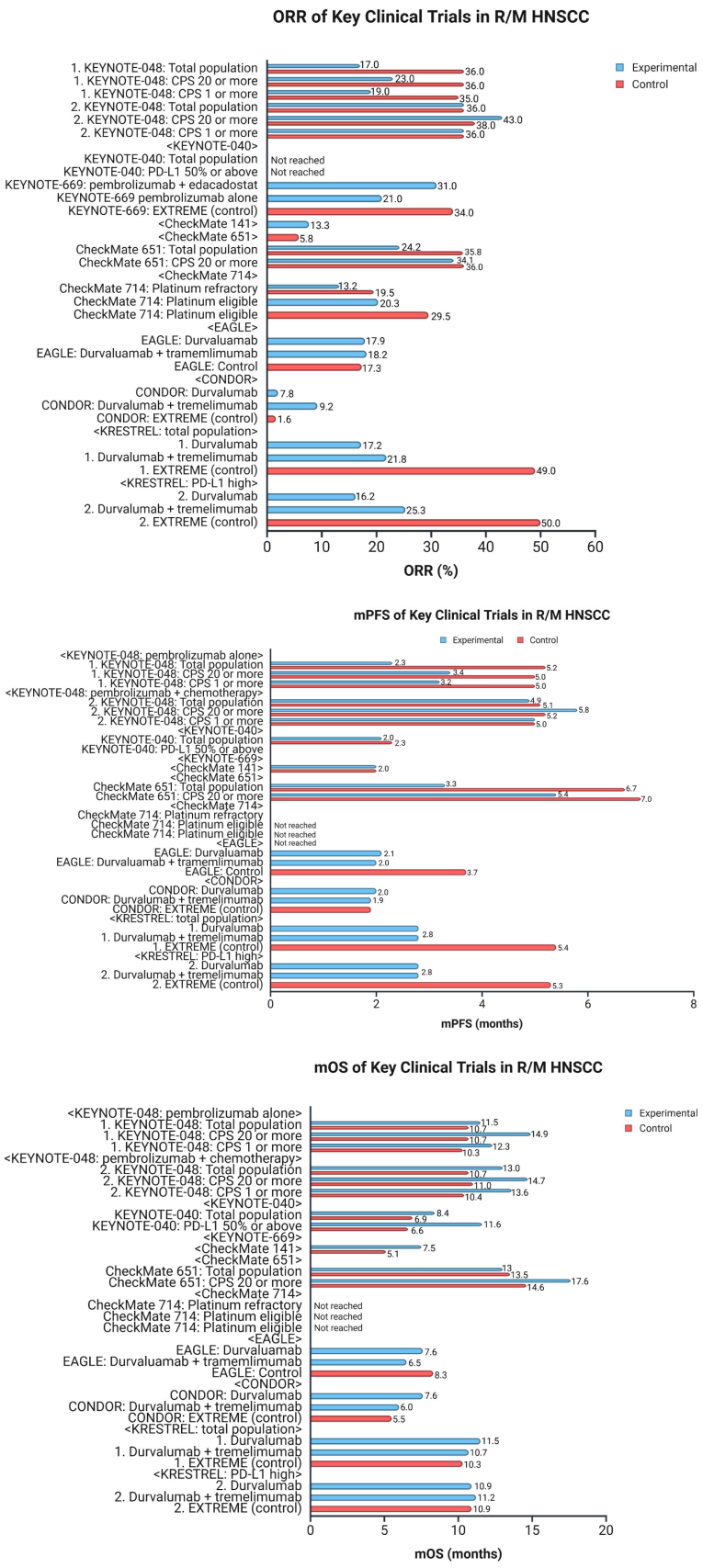

FIGURE 4
Data for clinical trials in R/M HNSCC. The figure represents key data for clinical trials in recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC). The top graph compares ORR across the different trials. The middle graph compares mPFS, and the bottom graph compares mOS. R/M HNSCC, recurrent/metastatic head and neck squamous cell carcinoma; ORR, objective response rate; mPFS, median progression-free survival; mOS, median overall survival; CPS, combined positive score; PD-L1, programmed cell death-ligand 1. Created in BioRender. Thein, K. (2025) https://BioRender.com/l8wlnx3.

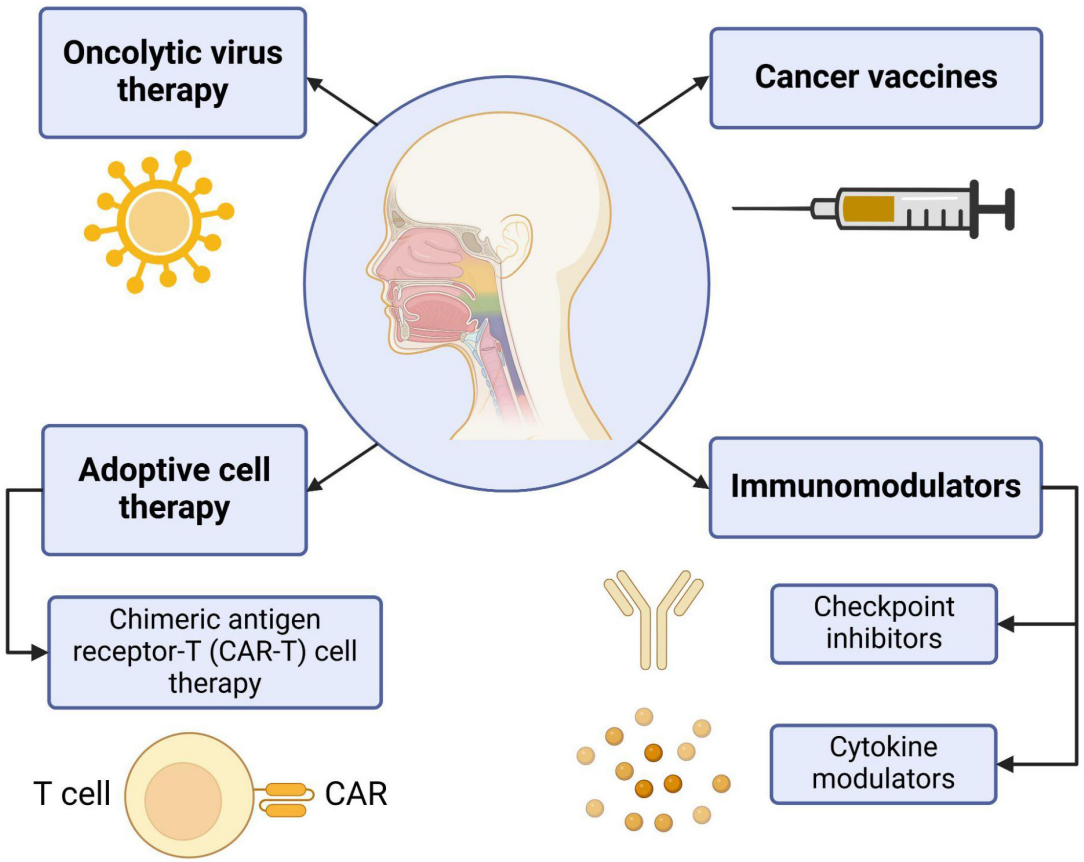

FIGURE 5
Novel immunotherapy agents for head and neck cancers. Several novel immunotherapy options are being explored for head and neck cancers. The main categories of these agents include oncolytic virus therapies, cancer vaccines, adoptive cell therapy, and immunomodulators. Adoptive cell therapy encompasses approaches such as chimeric antigen receptor T (CAR-T) cell therapy, which enhances T cell recognition of tumor cells. Immunomodulators include checkpoint inhibitors, such as PD-1 and PD-L1 inhibitors, as well as cytokine modulators that regulate immune responses. These emerging therapies offer promising strategies to improve immune system activation against head and neck cancers. Created in BioRender. Thein, K. (2025) https://BioRender.com/t08e962.

immunization, which can reduce OVs' efficacy. Intercellular junctions also act as a barrier against viral penetration, imposing resistance to OVs (46). Further work is required to optimize viral virulence and safety, improve target delivery and immune evasion, and, lastly, streamline mass production of the OVs (48).

5.2 Chimeric antigen receptor–T cell therapy

CAR-T cell therapy, a novel immunotherapy, was introduced in the 1980s and demonstrated significant anti-tumor efficacy in hematologic cancers. Briefly, In CAR-T cell therapy, T cells from the patient's body are genetically altered to express the antibodies that specifically recognize the tumor antigen in a non-major histocompatibility complex (MHC)-restricted manner (51). The successful utilization of this technique in the treatment of hematologic malignancies and its anti-tumor effect in solid tumors has prompted further research in this aspect of medicine (51, 52). Clinical studies on CAR-T cells for treating HNSCC are still in the preclinical stages, and the progression to clinical trials is still not optimistic (52).

NCT01818323 is the first clinical trial for patients diagnosed with locally advanced/recurrent HNSCC. In this trial, a retrovirus has been used to engineer T cells to coexpress two chimeric receptors: T1E28z and 4αβ. T1E28z is a chimeric antigen receptor that engages multiple ErbB dimers majorly expressed in HNSCC; on the other hand, 4αβ, a chimeric cytokine receptor, is designed to be inserted in the IL-4 incorporated T cell (T4) (51). The results from the trial were successful, demonstrating overall disease control of 69% after T4 immunotherapy without lymphodepletion, and the adverse effects were also ≤ grade 2, without dose-limiting toxicities (51, 52). Furthermore, it is worth noting that the results of trials on CAR-T cells as a treatment modality for HNSCC on the professional clinical trial registration website are not very abundant.

TABLE 2 Randomized clinical trials on immune checkpoint inhibitors in recurrent/metastatic HNSCC.

Study Name	Study Type	Study Population	Number of Patients (Experimental/ Control)	Treatment Intervention		Primary Endpoint
				Experimental Arm	Control Arm	
KEYNOTE-048	Phase III, Randomized, Open-label, Multi-center	Untreated, incurable R/M HNSCC	882 (301/300/281)	Pembrolizumab/ Pembrolizumab + Chemotherapy	EXTREME regimen	OS, PFS
KEYNOTE-040	Phase III, Randomized, Open-label, Multi-center	R/M HNSCC, post-platinum failure	495 (247/248)	Pembrolizumab	Investigator's choice (methotrexate, docetaxel, or cetuximab)	OS
KEYNOTE-669	Phase III, Randomized, Open-label, Multi-center	Untreated, incurable R/M HNSCC	89 (35/19/35)	Pembrolizumab + Epacadostat/ Pembrolizumab	EXTREME regimen	ORR
CheckMate 141	Phase III, Randomized, Open-label, Multi-center	R/M HNSCC, post-platinum failure	361 (240/121)	Nivolumab	Investigator's choice (methotrexate, docetaxel, or cetuximab)	OS
CheckMate 651	Phase III, Randomized, Open-label, Multi-center	Untreated, incurable R/M HNSCC	947 (469/478)	Nivolumab + Ipilimumab	EXTREME regimen	OS
CheckMate 714	Phase II, Randomized, Open-label, Multi-center	Untreated, incurable R/M HNSCC	425 (211/214)	Nivolumab + Ipilimumab	Nivolumab monotherapy	ORR, DOR
HAWK	Phase II, Non-randomized, Open-label, Multi-center	R/M HNSCC with PD-L1 ≥25%	112 (Single-arm)	Durvalumab	None (single-arm study)	ORR
EAGLE	Phase III, Randomized, Open-label, Multi-center	R/M HNSCC, post-platinum failure	736 (245/247/244)	Durvalumab/ Durvalumab + Tremelimumab	Investigator's choice (methotrexate, docetaxel, cetuximab)	OS
CONDOR	Phase II, Randomized, Open-label, Multi-center	R/M HNSCC with PD-L1 <25%	267 (92/91/84)	Durvalumab/ Durvalumab + Tremelimumab	Tremelimumab	ORR
KESTREL	Phase III, Randomized, Open-label, Multi-center	Untreated, incurable R/M HNSCC	823 (275/276/272)	Durvalumab/ Durvalumab + Tremelimumab	EXTREME regimen	OS
Pembro-Cetuximab (A Sacco et al.)	Phase II, Non-randomized, Open-label, Single-center	R/M HNSCC	33 (Single-arm)	Pembrolizumab + Cetuximab	None (single-arm study)	ORR*
Nivo-Cetuximab (C Chung et al.)	Phase II, Non-randomized, Open-label, Single-center	R/M HNSCC	46 (Single-arm)	Nivolumab + Cetuximab	None (single-arm study)	OS

OS, overall survival; PFS, progression-free survival; ORR, objective response rate; DOR, duration of response; ORR*, overall response rate.

Multiple targets have been identified as potential targets for CAR-T cell therapy in HNSCC, within which the *ErbB* family (also known as EGFR) is of significant importance (51). EGFR has been found to be overexpressed in hypopharyngeal carcinomas, which include 5% of the HNSCC (50). CD70 expression was also found in 19% of biopsy-proven HNSCC (51). Park et al. in their research demonstrated that anti-CD70 CAR-T cells can effectively eliminate HNSCC when compared to the non-treatment group. Similarly, CD70-targeted CAR-T has also shown success in patients with clear-cell carcinoma with a disease control rate of 76.9%. Mucin 1 (*MUC1*) also has a higher expression in HNSCC which also makes it a potential target for CAR-T cell therapy.

Although a significant amount of time and resources have been invested in the development of CAR-T cell therapy, this modality is still in its infancy (51). Five FDA-approved CAR-T cell products have produced promising results in hematologic malignancies; the clinical activity in solid tumors is modest, and potential toxicities are still a concern (52). Various barriers have been identified contributing to the slow advancement in CAR-T cells for the treatment of HNSCC (51). 1) Physical barriers, the stroma-rich solid tumors limit the penetration of T-cells in the tumor sites, producing lower anti-tumor activity. 2) Physiochemical barriers, the release of cytokines such as TGF-β and interleukin (IL) 10 by immunosuppressive cells reduces the efficacy of infused CAR-T cells. The acidic, hypoxic and low-nutrient tumor microenvironment also potentiates the effect. 3) Pathological barriers, which include intratumoral inhibitory factors, lack of chemokine receptors in some solid tumors, and tumor antigen

loss and heterogeneity, remain a primary obstacle to the success of CAR-T cell therapy in HNSCC (52, 53). Recent advances in engineering techniques, newer methods for target antigen spotting, and the combination of CAR-T cells with other treatment modalities have shown great potential in overcoming these challenges. However, further research is required before its effective use in the treatment of HNSCCs (51).

5.3 Vaccinations

Multiple FDA-approved prophylactic vaccines, including Cervarix, Gardasil®, and, more recently, Gardasil®9, have been established to protect against HPV infection and its associated diseases, such as genital warts and cancer (46). Although no relevant epidemiological studies are available, the prophylactic effect on head and neck cancers is assumed to be present (54). These vaccinations work by inducing neutralizing antibodies that are effective in preventing HPV- associated malignancies but are not useful in its treatment. Viral proteins E6 and E7 play a crucial role in the cancer pathology of the head and neck (HNCs) and, therefore, are considered to be good targets for vaccine development (46, 54). Multiple clinical trials are underway to assess the safety and efficacy of the vaccines against E6 and E7 proteins. For instance, a phase 1b/2 clinical trial of a DNA vaccine containing three plasmids expressing HPV16/18 E6 and E7 proteins with IL-12 in combination with durvalumab (NCT03162224). Another example is the listeria monocytogenes-derived live attenuated vaccine targeting HPV16 E7 (NCT02002182). Multiple vaccines against HPV antigen in combination with checkpoint inhibitors are also under study (46).

Other promising targets for vaccine design are TAAs (46, 54). TAAs are unmutated self-proteins on cancer cells, such as *MUC1* and carcinoembryonic antigen (CEA). *MUC1* is a glycoprotein on the surface of all epithelial cells; its abnormal expression is associated with a cancerous phenotype, making it an ideal target for developing a cancer vaccine (46, 54). In HNCs, phase I/II trials are ongoing, targeting *MUC1* combined with Tadalafil (NCT02544880), while trials testing CEA have been completed but have yet to report results (54).

6 Biomarkers

6.1 The role of predictive biomarkers in HNSCC and immunotherapy

The advent of ICIs targeting CTLA-4 and PD-1 has revolutionized the treatment HNSCC. These therapies have demonstrated survival benefits in both recurrent/metastatic and treatment-refractory cases (9). However, despite these advancements, up to 60% of patients fail to respond to PD-1/PD-L1 blockade, highlighting the urgent need for predictive biomarkers to better stratify candidates for immunotherapy (55). Since immune-related toxicities can be severe and ICIs are costly, optimizing patient selection based on validated biomarkers is crucial to enhance clinical efficacy while minimizing unnecessary risks and financial burden (56). The most widely studied biomarkers in HNSCC include PD-L1 expression, TMB, and HPV status, each of which offers insight into potential immunotherapy responsiveness.

6.2 PD-L1 expression as a predictive biomarker

PD-L1 expression is one of the most established biomarkers for response to ICIs (Figure 2), as PD-L1–positive tumors generally exhibit greater sensitivity to PD-1/PD-L1 blockade. Clinical trials, including KEYNOTE-040 and KEYNOTE-048, demonstrated that patients with PD-L1–positive tumors (CPS ≥1 or ≥20) had significantly better survival outcomes when treated with pembrolizumab compared to standard chemotherapy (4). However, PD-L1 expression alone is not an absolute predictor of response, as some PD-L1–negative tumors still respond to ICIs, while certain PD-L1–positive tumors remain resistant. Variability in testing methodologies, cutoff values, and intratumoral heterogeneity further complicates its reliability as a standalone biomarker (57).

6.3 HPV status and immunotherapy response

HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) is recognized as a distinct clinical and molecular entity with a better prognosis and greater sensitivity to chemoradiotherapy compared to HPV-negative HNSCC (58). The presence of HPV-derived oncoproteins, such as E6 and E7, promotes an immune-activated tumor microenvironment, leading to higher levels of tumor-infiltrating lymphocytes (TILs) and increased PD-L1 expression, suggesting a potential for enhanced response to ICIs (2). Early studies, such as KEYNOTE-012, indicated that HPV-positive tumors might be more responsive to pembrolizumab than HPV-negative tumors (5). However, subsequent trials, including KEYNOTE-040 and CheckMate-141, failed to confirm a significant difference in ICI response between HPV-positive and HPV-negative patients (59). This discrepancy suggests that while HPV status may contribute to tumor immunogenicity, it is not a definitive predictor of ICI efficacy on its own, and additional biomarkers are needed for accurate patient selection.

6.4 Tumor mutational burden and immune responsiveness

TMB, defined as the total number of somatic mutations per megabase of DNA, has been studied as a potential biomarker for predicting response to ICIs across multiple cancer types, including

HNSCC. Generally, HPV-negative tumors exhibit higher TMB than HPV-positive tumors, likely due to tobacco-induced mutagenesis. Retrospective analyses of clinical trials have suggested a correlation between high TMB and increased response to pembrolizumab, particularly in HPV-negative tumors (60). However, TMB has not demonstrated consistent predictive value in HPV-positive cancers, as these tumors may elicit immune responses based on viral antigen presentation rather than mutation-driven neoantigens (6). While TMB is a promising marker, standardized cutoffs and prospective validation are needed before it can be routinely used in clinical decision-making.

6.5 Why predictive biomarkers matter

The integration of predictive biomarkers into clinical practice is essential for advancing precision medicine in HNSCC. Identifying patients most likely to benefit from ICIs helps maximize therapeutic outcomes, reduce exposure to ineffective treatments, and minimize the risk of immune-related adverse effects. Furthermore, because ICIs are costly and resource-intensive, biomarker-driven treatment strategies improve cost-effectiveness by ensuring that only patients with a higher likelihood of response receive these therapies. Additionally, as resistance mechanisms to ICIs continue to emerge, biomarker research will be critical for guiding combination therapies that enhance treatment efficacy, such as pairing ICIs with chemotherapy, radiotherapy, or novel targeted agents (11).

Given the heterogeneous nature of HNSCC, no single biomarker is sufficient for predicting ICI response. A multi-biomarker approach that integrates PD-L1 expression, HPV status, and TMB—alongside emerging factors such as immune gene expression profiling, tumor microenvironment characteristics, and microbiome composition—may provide a more comprehensive framework for patient selection. Future research should focus on prospective validation and the development of robust biomarker algorithms to ensure more precise and personalized treatment strategies in HNSCC.

7 Challenges and future directions in immunotherapy for HNSCC

Despite the transformative impact of immunotherapy on the treatment landscape of HNSCC, significant challenges remain. While ICIs have provided meaningful survival benefits for a subset of patients, the reality is that many do not experience durable responses (5, 6). A deeper understanding of the mechanisms behind immune resistance, along with the refinement of patient selection through better biomarkers, is essential to optimizing the effectiveness of these therapies (18). Additionally, balancing efficacy with toxicity remains a crucial consideration, particularly as combination strategies are explored (4, 7). As research in this field continues to expand, overcoming these hurdles will be critical to ensuring that immunotherapy reaches its full potential in HNSCC management.

7.1 Heterogeneous response to ICIs and immune resistance mechanisms

One of the most pressing challenges in HNSCC immunotherapy is the highly variable response to ICIs. While some patients exhibit robust and sustained responses, many fail to benefit due to primary or acquired resistance. The complex interplay between tumor-intrinsic factors, such as defects in antigen presentation and oncogenic signaling pathways, and tumor-extrinsic factors, such as an immunosuppressive TME, contribute to these disparities (5, 6). Overcoming these resistance mechanisms requires innovative approaches, including dual checkpoint blockade (e.g., PD-1 plus CTLA-4 inhibitors) and novel immune-modulating agents targeting pathways such as TGF-β, indoleamine 2,3-dioxygenase (IDO), and lymphocyte activation gene 3 (*LAG-3*) (18). Additionally, the combination of ICIs with traditional therapies, such as radiotherapy and chemotherapy, has shown potential to enhance immune priming, but further optimization is needed to determine the most effective regimens (7).

7.2 Refining biomarker-driven patient selection

Currently, the selection of patients for ICI therapy is largely guided by PD-L1 expression, yet its predictive value remains inconsistent. Many PD-L1–negative tumors still respond to ICIs, while some PD-L1–positive tumors remain refractory (4). Other biomarkers, such as TMB and HPV status, have been explored but similarly lack definitive predictive utility (9). However, exploring further biomarker-driven approaches for patient selection remains highly important. A multi-modal approach that integrates genomic, transcriptomic, and immune profiling may offer a more precise way to identify those most likely to benefit from immunotherapy (56). The most recent national comprehensive cancer network (NCCN) guidelines for head and neck cancer recommend next generation sequencing (NGS) for biomarker identification (20). Performing multi-omic studies to different sites of the head and neck cancer (oral cavity, salivary gland, pharynx.etc) can help reveal potential differences in response to different therapies. HNSCC is known to have significant intratumoral heterogeneity resulting in variable responses to ICIs, applying a multi-omic approach for molecular subtyping has shown a potential benefit in patient stratification (61). It is highly important to consider employing these multi-modal biomarker evaluations to guide creating more precise personalized treatment plans. The gut microbiome has also emerged as a potential modulator of ICI response, warranting further exploration into how microbiome-targeted interventions might enhance treatment efficacy (62). Moving forward, a major focus of research should be on developing robust biomarker-driven

algorithms that allow for truly personalized treatment strategies in HNSCC.

7.3 Toxicity and immune-related adverse events

Although ICIs are generally better tolerated than cytotoxic chemotherapy, immune-related adverse events (irAEs) remain a significant concern. These toxicities can affect nearly every organ system, with complications such as pneumonitis, colitis, and endocrinopathies that can range from mild to life-threatening (63). The challenge is further compounded when ICIs are combined with other therapeutic modalities, as toxicity profiles can become more complex (64). Proactive monitoring and risk stratification are key to mitigating these adverse effects, as is the identification of biomarkers that predict susceptibility to irAEs (65). In HPV-positive HNSCC, where survival outcomes are already favorable, treatment de-escalation strategies that incorporate ICIs while minimizing toxicity are an area of growing interest (66).

7.4 The role of combination and novel immunotherapies

While single-agent ICIs have provided meaningful survival benefits in select patients, combination strategies may hold the key to improving outcomes more broadly. However, not all combinations are equally effective, and some may introduce unacceptable levels of toxicity. Ongoing studies are evaluating the synergy between ICIs and chemotherapy, radiotherapy, and targeted therapies, with the goal of identifying optimal regimens (31). Beyond checkpoint blockade, novel immunotherapies such as cancer vaccines, bispecific T-cell engagers (BiTEs), and adoptive cell therapies (including chimeric antigen receptor [CAR] T cells) represent exciting frontiers in HNSCC treatment (60). Although CAR-T cell therapy has revolutionized the management of hematologic malignancies, its application in solid tumors like HNSCC has been limited by the challenges of TME-mediated immunosuppression and antigen heterogeneity. Engineering CAR-T cells with enhanced tumor infiltration capabilities and resistance to immunosuppressive signals may help overcome these barriers (67).

7.5 The role of interdisciplinary collaboration and emerging technologies

Interdisciplinary collaboration between different specialists (e.g., immunologists, oncologists, bioinformaticians) is pivotal in advancing the immunotherapy research in HNSCC through combining expertise from various fields. Potential collaboration models can include establishing research groups to facilitate expertise and knowledge exchange between experts from different specialties, or opening interdisciplinary centers to promote cross-disciplinary research and training. A potential new area of research in the future can focus on bioinformatics-driven analysis of the genomic and transcriptomic data to help identify biomarkers for treatment response prediction and creation of more personalized treatment plans. AI-assisted drug design and gene editing are now considered promising tools for optimizing immunotherapy in HNSCC. AI can potentially be involved in all aspects of the HNSCC care from early detection and diagnosis to treatment planning, identification of mutations through genomic data analysis, development of targeted therapies, and finally monitoring and surveillance (68). Gene editing technologies such as CRISPR/Cas9 can be used to modify immune and cancer cells in the TME to help improve the efficacy of immunotherapy; it has been investigated in many types of cancer including HNSCC (69). For example, Zhou et al. were able through CRISPR to upregulate the expression of major histocompatibility complex (MHC) I and proliferation of CD8+ T cells in HNSCC which could improve the cancer cell response to PD-1 immunotherapy (70). Future research should further investigate the roles of AI and gene editing in HNSCC management and how to effectively apply them into clinical practice.

7.6 Unanswered questions

Despite the progress made in HNSCC immunotherapy, several unanswered questions remain. One of the most fundamental issues is how to accurately predict which patients will benefit from ICIs. While PD-L1, TMB, and HPV status have been explored as biomarkers, their reliability remains inconsistent. Future research must focus on refining predictive models through multi-omic integration, incorporating genomic, transcriptomic, and immune profiling to develop a more precise stratification system.

Another major area of uncertainty lies in optimizing combination strategies. While adding ICIs to chemotherapy, radiotherapy, or targeted agents has shown promise, the ideal sequencing, dosing, and patient selection criteria remain unclear. While many studies in the past have relied on evaluating immunotherapy in the adjuvant setting, promising results have emerged recently from studies such as KEYNOTE-689 and NIVOPOSTOP GORTEC 2018–01 which evaluated ICIs in LA HNSCC in the perioperative setting and successfully met their primary endpoint of improving survival, this would help shape future studies to identify the appropriate setting and sequencing to use ICIs. Further studies are also needed to understand how to maximize synergy while minimizing toxicity, particularly in the context of treatment de-escalation for HPV-positive disease, where excessive treatment intensity may be unnecessary.

Additionally, the potential of novel immunotherapies, such as tumor vaccines and adoptive cell therapies, is still being explored. While early trials have demonstrated promising results, questions remain regarding their long-term efficacy, the best way to integrate them into existing treatment paradigms, and the logistical challenges associated with their implementation.

Lastly, the role of the gut microbiome in modulating immune responses has emerged as an intriguing avenue for research. Studies in other malignancies suggest that specific microbial compositions may enhance or impair ICI efficacy, but how this applies to HNSCC remains unclear. Investigating whether microbiome-targeted interventions, such as probiotics or fecal microbiota transplantation, can improve immunotherapy outcomes represents an exciting frontier in cancer research.

8 Conclusions

While immunotherapy has undeniably revolutionized the treatment of HNSCC, significant challenges remain in optimizing its application. The variability in patient response underscores the need for better biomarkers, while the growing exploration of combination strategies necessitates a careful balance between efficacy and toxicity. Addressing immune resistance mechanisms, whether through novel checkpoint inhibitors, modulation of the tumor microenvironment, or emerging strategies such as microbiome-targeted interventions, will be crucial in improving outcomes.

As research progresses, the field of HNSCC immunotherapy is poised for continued evolution. By integrating precision medicine approaches, refining treatment de-escalation strategies, and exploring innovative therapeutic modalities, the next phase of immunotherapy development can bring more effective and personalized options to patients. The ultimate goal is to expand access to durable responses while minimizing adverse effects, ensuring that immunotherapy remains a cornerstone of HNSCC treatment in the years to come.

At the end, we would like to mention a few limitations of this review article, including searching only two databases to identify the key clinical trials evaluating ICIs in HNSCC, discussion of mainly phase II and III trials only, in addition to the unavailability of full data for some of the studies (e.g., KEYNOTE 689, NIVOPOSTOP GORTEC 2018-01).

Author contributions

HA: Conceptualization, Investigation, Project administration, Writing – review & editing, Writing – original draft. TK: Writing – review & editing, Conceptualization, Writing – original draft. AH: Writing – original draft. YM: Writing – review & editing, Software. RN: Writing – original draft. RS: Writing – original draft. KN: Writing – original draft. DJ: Writing – original draft. J-LB: Writing – review & editing, Supervision, Validation. KT: Supervision, Conceptualization, Writing – review & editing, Project administration, Validation.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Long-term immune checkpoint inhibitor therapy in a patient with metastatic nasopharyngeal carcinoma: a case report

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Background: Immunotherapy has revolutionized cancer treatment. However, the duration of treatment and the timing of discontinuation are major concerns. Current pivotal trials predominantly advocate for a fixed two-year regimen of immune checkpoint inhibitors (ICIs), exemplified by pembrolizumab and toripalimab, as first-line therapy for patients with advanced malignancies. Alternatively, for specific ICIs, including nivolumab, camrelizumab, and tislelizumab, continuous administration until disease progression has emerged as a favored approach. Nevertheless, whether to discontinue treatment after two years remains intensely debated within the medical community, underscoring the need for further research to clarify optimal treatment durations.

Case presentation: In November 2018, a 44-year-old male presented with a persistent headache. Following a positive nasopharyngeal mucosal biopsy, he was diagnosed with non-keratinizing undifferentiated carcinoma of the nasopharynx cT4N2M0. An Epstein-Barr Virus (EBV) DNA load of 800 copies/mL was detected. The patient completed two cycles of induction chemotherapy with liposomal paclitaxel and nedaplatin, followed by platinum-based concurrent chemoradiotherapy, resulting in a progression-free survival (PFS) of 23.6 months. The EBV DNA load dropped significantly to 190 copies/mL. However, during a routine examination in January 2021, metastases in the lung and mediastinal lymph nodes were detected, and the EBV DNA load was measured at 2200 copies/mL. Consequently, surgical intervention was performed, followed by radiotherapy and two years of ICI treatment. Throughout the ICI maintenance period, the EBV DNA level remained consistently below the limit of detection. Remarkably, three months after treatment discontinuation, the patient exhibited a rebound in EBV DNA (1620 copies/mL). Nevertheless, imaging scans revealed no evidence of tumor progression. Following an ICI rechallenge, the patient's EBV DNA load returned to undetectable levels. The patient continues the ICI therapy and has thus far achieved a PFS of 41.6 months.

Conclusion: EBV DNA levels could serve as an informative marker to predict the necessity of therapy discontinuation during immunotherapy maintenance. Notably, a post-discontinuation ICI rechallenge can still yield favorable outcomes potentially accredited to immune memory.

KEYWORDS

immune checkpoint inhibitor, immunotherapy, therapy discontinuation, treatment duration, EBV DNA

Introduction

Immunotherapy represents a pivotal advancement in the treatment of patients with advanced malignant tumors, significantly enhancing outcomes (1). The determination of the optimal duration for immunotherapy remains a contentious issue within the research community. Historically, clinical trials evaluated the efficacy of ICIs for a maximum of two years in responsive patients. For instance, the KEYNOTE-010 trial observed that of the 79 patients who completed a fixed two-year course of ICI therapy, 57.7% maintained PFS at the two-year mark (2). In a related study, the KEYNOTE-024 trial reported that among 39 patients administered pembrolizumab for two years, 82% were still alive after five years (3). The JUPITER-02, CAPTAIN-1st and RATIONALE-309 trials demonstrated that, for the first-line treatment of recurrent or metastatic nasopharyngeal carcinoma (NPC), patients who received ICIs in combination with chemotherapy experienced significantly prolonged PFS and overall survival (OS) compared to those treated with chemotherapy alone. In the JUPITER-02 trial, toripalimab was administered for up to two years. The median PFS was 21.4 months, while the median OS was not reached following a 36-month follow-up period in the toripalimab-based combination group (4). In contrast, in the CAPTAIN-1st and RATIONALE-309 trials, treatment with camrelizumab and tislelizumab persisted until radiographic progression or unacceptable toxicity manifested. In the CAPTAIN-1st trial, the camrelizumab group had a significantly longer PFS than the placebo group, with median PFS of 9.7 months and 6.9 months, respectively (5).Similarly, the RATIONALE-309 trial showed that the tislelizumab group exhibited a markedly longer PFS than the placebo group, with median PFS reaching 9.2 months and 7.4 months, respectively (6). These findings underscore the efficacy of these ICIs in enhancing PFS for the relevant patient populations. To optimize the effectiveness of ICIs, oncologists may prefer to maintain treatment until disease progression or toxicity arises. However, it is currently unclear whether prolonged ICI treatment results in longer survival times. Additionally, oncologists should also take into account the economic burden and adverse events associated with long-term treatment. Balancing the risks and benefits remains a challenging issue in clinical practice.

There is a notable scarcity of data regarding patients with advanced malignancies who have undergone ICI therapy for more than two years, as well as on drug-off criteria in real-world practice so far. Masatoshi Kudo suggested that continuous normalization of three tumor markers (AFP, AFP-L3, and PIVKA-II) for 12 to 24 weeks could serve as a criterion for discontinuing treatment in liver cancer patients with complete responses (7). Additionally, Zhang et al. noted the potential role of circulating tumor DNA (ctDNA) as a meaningful biomarker for assessing whether to continue treatment in advanced cancers (8). For recurrent or metastatic NPC, EBV DNA has been identified as a key prognostic biomarker, primarily serving to evaluate treatment efficacy and monitor disease progression (9–11). Wang et al. found that patients with a ≥ 50% decrease in plasma EBV DNA load at week 4 had an objective response rate of 48.3%, compared to 5.7% for those with a < 50% decrease (12). However, this study only analyzed the association between EBV DNA and treatment response, not treatment discontinuation criteria. Encouragingly, Liu et al. developed an initial prognostic risk stratification model integrating IL-6 and EBV DNA load to predict outcomes in recurrent or metastatic NPC patients treated with ICIs (13). This model may potentially inform future discontinuation criteria. Overall, the criteria for treatment discontinuation in patients with recurrent or metastatic NPC remain poorly validated, leaving a significant gap in real-world data on post-discontinuation relapse rates.

Case report

A 44-year-old male was referred to our institution with a persistent headache in November 2018. The patient had an Eastern Cooperative Oncology Group (ECOG) performance status of 0. Following diagnosis with non-keratinizing undifferentiated carcinoma of the nasopharynx, the patient was staged as cT4N2M0 according to the AJCC 8th edition guidelines. An EBV DNA load amounting to 800 copies/mL was detected. Subsequently, the patient received two cycles of induction chemotherapy with liposomal paclitaxel (135 mg/m^2 on day 1)

and nedaplatin (80 mg/m² on day 1, every 21 days), followed by intensity-modulated radiation therapy (IMRT) combined with concurrent chemotherapy (nedaplatin 100 mg/m² on day 1, every 21 days). The overall treatment course resulted in a PFS of 23.6 months. Radiation dosages administered included: 69.96 Gy in 33 fractions to the gross tumor volume (GTVnx) and to the positive neck lymph nodes (GTVnd), 60.06 Gy to the high-risk clinical target volume (CTV1), and 54.12 Gy to the low-risk clinical target volume (CTV2) (Figures 1A, B). The EBV DNA load demonstrated a significant reduction, falling to 190 copies/mL, a finding that underscores the observed efficacy of the treatment.

In January 2021, routine examination revealed metastases in the patient's lung and mediastinal lymph nodes (Figures 2A, B). Concurrently, the EBV DNA load was found to be 2200 copies/mL. Subsequently, he underwent wedge resection and lymph node biopsy. Immunohistochemistry showed the tumors were positive for PD-L1. First-line treatment was initiated with GP (gemcitabine 1000 mg/m² on days 1 and 8, cisplatin 75 mg/m² on day 1, every 21 days) and toripalimab (240 mg on day 1, every 21 days) for one course. During treatment, the patient experienced grade 4 neutropenia, leading to chemotherapy suspension due to intolerable toxicity. After recovery from myelosuppression, radiotherapy was administered, with prescription doses of 60 Gy in 30 fractions to the GTV and 54 Gy to the CTV via IMRT (Figures 2C, D). The patient received two years of toripalimab maintenance therapy after IMRT, which induced grade 2 hypothyroidism managed with thyroxine hormone replacement therapy. Throughout the ICI maintenance period, EBV DNA load remained persistently below the detectable threshold. Three months after treatment cessation, an EBV DNA rebound was documented, peaking at 1620 copies/mL. However, imaging scans revealed no tumor progression (Figure 2E, F). Following an ICI rechallenge, the patient's EBV DNA load returned to undetectable levels (Figure 3). The patient continues ICI treatment and has achieved a PFS of 41.6 months thus far.

Discussion

The optimal duration of immunotherapy, whether finite or continuing until progression, remains a prominent subject of debate. While the initial phase I trials proposed a 2-year limit to therapy (14, 15), several previous clinical trials have demonstrated that patients who completed two years of ICI treatment experienced long-term PFS and OS (4, 16, 17). For instance, the KEYNOTE-010 trial revealed that patients treated with pembrolizumab for two years exhibited favorable prognoses, with 1-year PFS and OS rates of 72.5% and 98.7%, respectively (16). Similarly, In the JUPITER-02 trial, patients with recurrent or metastatic NPC showed clinically significant PFS and OS benefits after two years of toripalimab treatment. ICI led to a remarkable extension of PFS, with a median PFS of 21.4 months for the toripalimab group versus 8.2 months for the placebo group. Furthermore, the median OS in the ICI group was not reached at the time of analysis, while it was 33.7 months in the placebo group, highlighting the substantial survival benefit of toripalimab-based combination therapy (4). The findings from

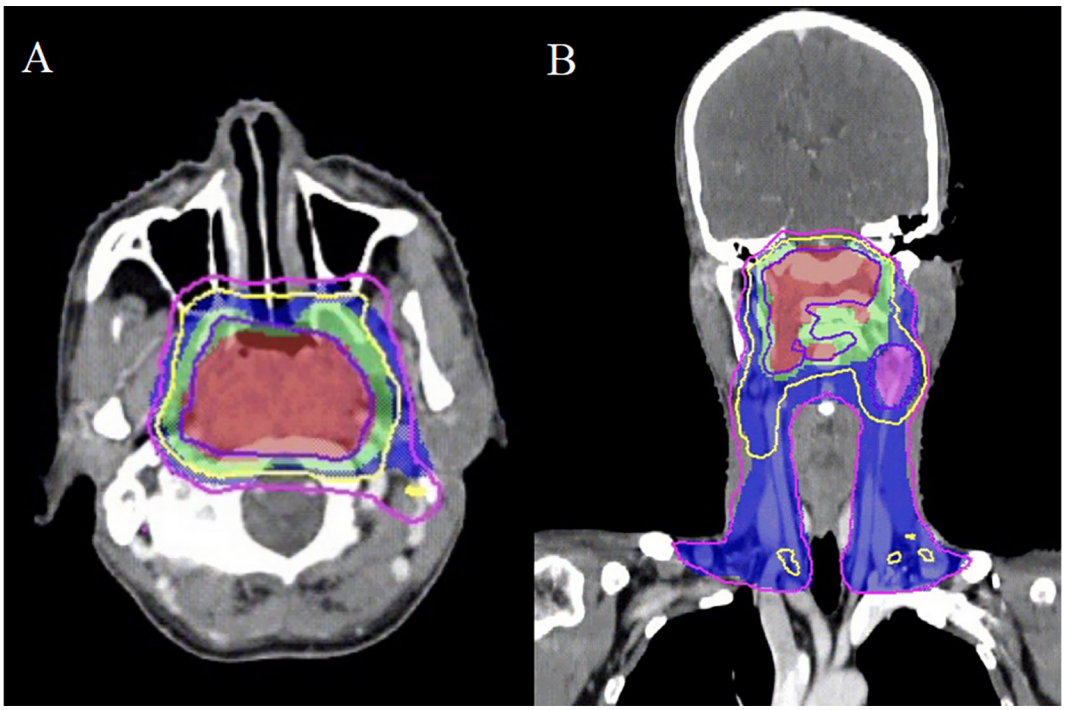

FIGURE 1
The intensity-modulated radiotherapy plan for nasopharyngeal carcinoma demonstrates three distinct isodose distributions: the gross tumor volume (GTVnx) is encompassed by the 69.96 Gy isodose curve (slate blue), while the clinical target volumes CTV1 and CTV2 are covered by the 60.06 Gy (yellow) and 54.12 Gy (purple) isodose lines, respectively. Transverse **(A)** and coronal **(B)** sections illustrate the treatment plan in these planes.

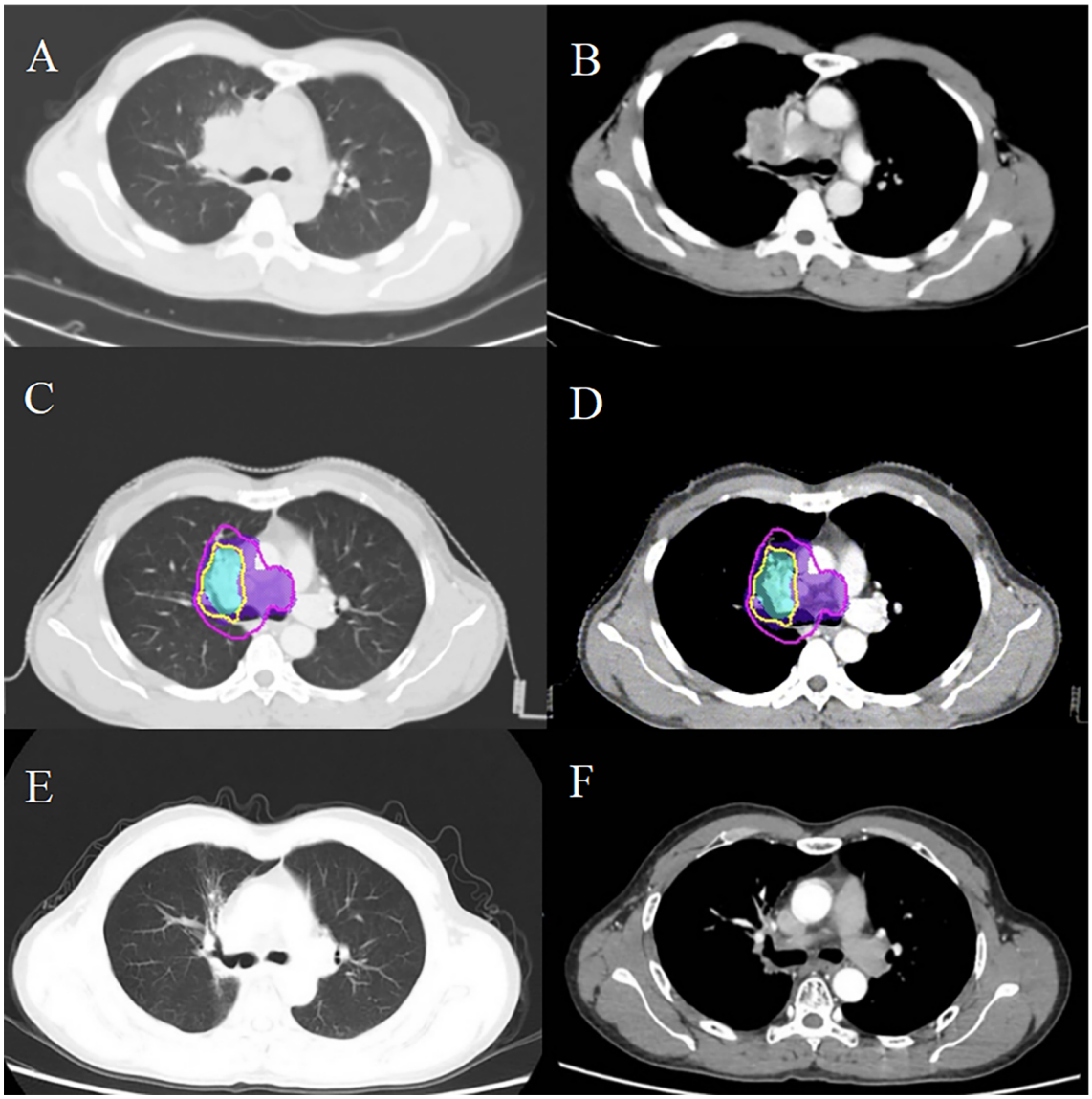

FIGURE 2
Comparative imaging analysis delineates post-radiotherapy metastatic progression in thoracic regions. Post-treatment surveillance CT identified metastatic lesions in the pulmonary parenchyma **(A)** and mediastinal nodal stations **(B)**. Dosimetric mapping demonstrates therapeutic coverage with the 60 Gy isodose contour (yellow) delineating the GTV, while the 54 Gy isodose (purple) demarcates the CTV in postoperative imaging series **(C, D)**. Serial follow-up imaging shows sustained locoregional control **(E, F)**, with no radiographic evidence of disease recurrence.

both trials provide substantial evidence in support of ICI as an effective first-line treatment strategy for recurrent or metastatic NPC.

A retrospective analysis of patients with advanced non-small cell lung cancer (NSCLC) treated with immunotherapy found no significant impact on patient OS after treatment discontinuation at two years (18). This analysis evaluated 706 patients and showed that discontinuing immunotherapy at two years versus continuous treatment was associated with OS rates of 79% and 81%, respectively, with no significant difference in mortality risk, indicating that discontinuation may be safe (18). In contrast, the CheckMate-153 trial demonstrated longer median PFS (24.7 months vs. 9.4 months) and OS (not reached vs. 28.8 months) in patients receiving continuous versus one-year fixed-duration treatment, suggesting that continuing immunotherapy may improve outcomes (19). Additionally, the KCSG LU20–11 study reported that the majority of patients who discontinued ICI after two years experienced disease progression within the first 12 months (20). Together, these findings highlight the dilemma faced by oncologists in balancing treatment continuation with clinical benefit. The prolonged ICI maintenance duration [24 months vs. the median 7–9 months reported in KEYNOTE-048 (21)] underscores the critical need for disease-specific treatment guidelines to inform optimal discontinuation strategies.

No definitive guidelines govern the discontinuation of immunotherapy in patients exhibiting an objective response. While Kudo's recommendations provide clinicians with a framework for individualized therapeutic decision-making regarding safe treatment discontinuation in hepatocellular carcinoma (7), scarce research has

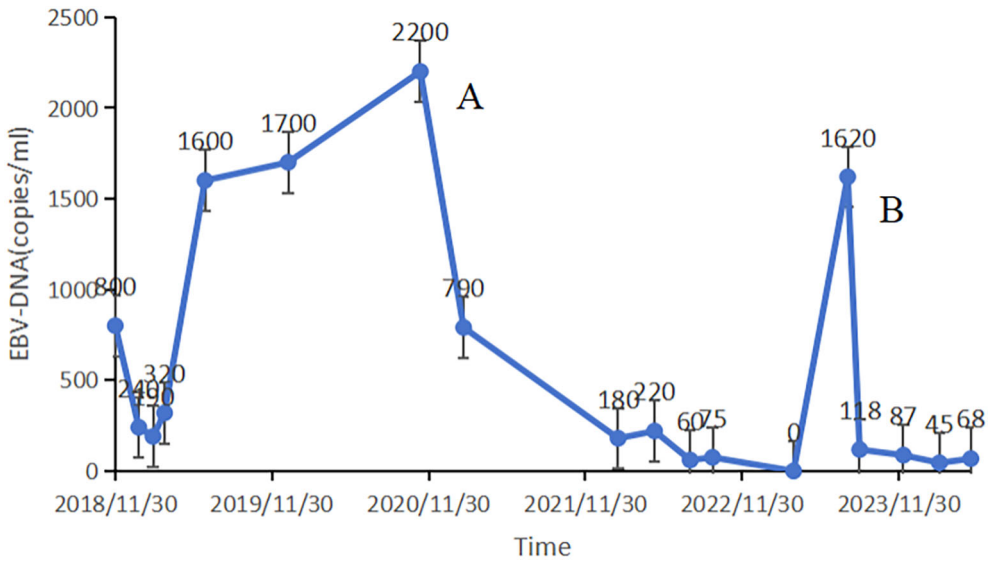

FIGURE 3
EBV DNA load demonstrates dynamic correlation with disease progression, serving both as a quantitative biomarker for therapeutic monitoring and a predictive indicator for treatment discontinuation criteria. The patient was found to have pulmonary and mediastinal lymph nodes metastases **(A)**. A rebound in EBV DNA levels was recorded three months after discontinuing the treatment **(B)**.

explored similar scenarios after immunotherapy discontinuation in head and neck squamous cell carcinoma (HNSCC). Although KEYNOTE-048 validated pembrolizumab in PD-L1-high HNSCC, our patient with EBV-driven NPC required a tailored approach, prioritizing EBV DNA monitoring over PD-L1 status. Monitoring EBV DNA load—a key biomarker for diagnosis, treatment response, and potential immunotherapy management in nasopharyngeal cancer—is pivotal. A retrospective analysis showed EBV DNA had 85.9% and 92.8% accuracy in detecting regional recurrence and distant metastasis, respectively (22). The POLARIS-02 study showed that 14 patients responding to toripalimab had at least a 100% increase in EBV DNA titer 3 months before radiographic disease progression (12). Moreover, an analysis demonstrated that 40% of patients with complete or partial response experienced significantly increased EBV DNA load during ICI maintenance, leading to disease progression. All patients with stable disease had significantly elevated EBV DNA load during this period, and 74.2% subsequently developed progression (23). These findings suggest that substantial EBV DNA load increases during ICI maintenance may serve as an early predictor of progression and a potential indicator for continuing treatment. In our case, EBV DNA load remained within normal ranges for two years during immunotherapy. Three months after ICI discontinuation, viral load rebounded, but returned to undetectable levels after therapy resumption. This may be indicative of a rapid clearance of antibodies, which could result in a relatively brief treatment duration in the local tumor environment (24). It is also possible that resistance mechanisms may develop when chronic PD-1 blockade is removed (25), underscoring the need for prolonged immunotherapy. Improved treatment outcomes generally justify continuing ICI administration.

The report has certain limitations. First, it does not explore biological mechanisms underlying EBV DNA rebound, such as clonal evolution or immune escape, nor validate rebound events through repeat biopsies. Second, the absence of comparisons to analogous cases in the literature hinders interpretation of whether the observed EBV DNA rebound represents a typical or exceptional clinical scenario.

Clinicians must carefully balance the benefits of prolonged ICI therapy against late toxicity risks while integrating multifaceted patient-specific factors. In our case, continuation of treatment despite EBV DNA rebound was justified by the patient's asymptomatic status and durable radiographic response, reflecting ongoing tumor control. However, broader considerations, including performance status, severity of side effects, financial burdens, and patient preference, are equally critical for tailoring optimal immunotherapy duration and aligning with the patient-centered goals.

Conclusion

Determining the optimal duration of immunotherapy should be informed by the risk-benefit profile of each individual. Current literature suggests that a two-year course of immunotherapy might be reasonable, but an extended course may also yield survival benefits. Notably, EBV DNA load may serve as a predictive biomarker for guiding therapy discontinuation during immunotherapy maintenance. As several prospective "stop or go" studies on immunotherapy are underway, we await further insights.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by People's Hospital of Guangxi Zhuang Autonomous Region. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DQ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. ZL: Conceptualization, Data curation, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. HL: Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A retrospective efficacy and safety study of pembrolizumab/cetuximab neoadjuvant therapy in locally advanced hypopharyngeal cancer

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Purpose: The primary objective of this study was to retrospectively assess the efficacy and safety profiles of two neoadjuvant regimens combining either pembrolizumab or cetuximab with paclitaxel and cisplatin in patients with locally advanced hypopharyngeal cancer (LAHPC).

Methods: LAHPC patients who received surgical resection at our hospital between August 2022 and February 2024 were enrolled in the study. All patients received neoadjuvant treatment before surgery and postoperative adjuvant therapy. They were categorized into two groups based on the neoadjuvant regimen: the paclitaxel + cisplatin + pembrolizumab (TP + PEMBRO) group and the paclitaxel + cisplatin + cetuximab (TP + CETUX) group. We evaluated various parameters including treatment response rate, adverse effects, surgical modalities, and survival outcomes for both groups.

Results: A total of 32 LAHPC patients were enrolled into the study, with 16 patients in each group. The TP + PEMBRO group demonstrated a significantly superior objective response rate (ORR) of neoadjuvant treatment compared to the TP + CETUX group (87.5% vs 68.75%, $P < 0.05$). In terms of surgical procedures, the TP + PEMBRO group exhibited a higher proportion of minimally invasive surgeries (87.5% vs 56.25%, $P < 0.05$), and both the tracheotomy rate and indwelling gastric tube rate were relatively lower in this group. Regarding patient prognosis, the 1-year overall survival (OS) rate in the TP + PEMBRO group was 100%, and the 1-year relapse-free survival (RFS) rate was 92.31%. In contrast, the TP + CETUX group had a 1-year OS rate of 93.75% and a 1-year RFS rate of 81.25%. There was no significant disparity in adverse events between the two groups, and no grade 3–4 severe adverse events occurred.

Conclusion: The neoadjuvant TP regimen integrating pembrolizumab or cetuximab was associated with higher transoral surgery (TOS) rates and

laryngeal preservation rates. Notably, the TP + PEMBRO regimen outperformed the TP + CETUX regimen in terms of treatment response rate and the proportion of minimally invasive surgeries, suggesting a novel and efficacious neoadjuvant treatment for LAHPC.

KEYWORDS

hypopharyngeal cancer, neoadjuvant therapy, pembrolizumab, cetuximab, minimally invasive surgery, organ preservation, safety, efficacy

1 Introduction

Hypopharyngeal cancer (HPC) represents a relatively uncommon head and neck malignancy, with 6,475 and 2,314 new cases emerging annually in China and the United States, respectively (1). The insidious nature of HPC results in over 80% of patients presenting at the locally advanced stage (LAHPC) upon initial diagnosis (2–4). The prognosis of LAHPC is poor, as the 5-year overall survival (OS) rate hovers around 22 - 30%, and there has been minimal improvement in patient prognosis over the past few decades. Additionally, the recurrence rate of LAHPC is relatively high, with nearly half of patients experiencing recurrence following multimodal treatment (5–7). Given its proximity to the larynx, the majority of LAHPC patients require total laryngectomy, leading to permanent loss of laryngeal function and severely compromising patients' quality of life. Therefore, identifying strategies to enhance the prognosis and quality of life of LAHPC patients is an urgent clinical need.

Preoperative neoadjuvant treatment has emerged as a crucial approach, as it can effectively reduce the tumor burden, facilitating preoperative tumor downstaging. This, in turn, can augment the local control rate and overall survival rate, also increasing the likelihood of organ function preservation during surgery. The landmark Veterans Affairs trial and EORTC 24891 trial have firmly established that in locally advanced laryngeal and hypopharyngeal cancers, induction chemotherapy and radiotherapy (RT) can enhance the laryngeal preservation rate without significantly compromising patient prognosis (8, 9). In 2008, the cetuximab-based platinum and fluorouracil regimen (EXTREME regimen) was approved as the first-line treatment for recurrent or metastatic (r/m) head and neck squamous cell carcinoma (HNSCC) (10). This regimen remained the standard of care for the subsequent decade until the KEYNOTE - 048 and CHECKMATE - 141 studies in 2018 demonstrated the efficacy of PD - 1 immune checkpoint inhibitors in r/m HNSCC (11).

In addition to the EXTREME regimen, paclitaxel combined with cisplatin (TP regimen) is also a commonly employed first-line treatment for LAHPC. Multiple studies have corroborated the safety and efficacy of the TP regimen combined with cetuximab (TP + CETUX) in patients with locally advanced head and neck cancer (LA - HNSCC) (12–14). Preliminary findings from clinical trials have also suggested that neoadjuvant treatment incorporating PD - 1 inhibitors with TP + CETUX can induce a high pathological tumor regression rate in LA - HNSCC (15–18). However, to date, no study has directly compared the efficacy of neoadjuvant regimens combining pembrolizumab or cetuximab with TP in LAHPC. This retrospective analysis of LAHPC cases in our center was designed to comprehensively compare the efficacy and safety of these two neoadjuvant regimens.

2 Materials and methods

2.1 Patient enrollment

This retrospective study encompassed LAHPC patients who visited the Department of Otorhinolaryngology Head and Neck Surgery of the First Affiliated Hospital of Fujian Medical University from August 2022 to February 2024. The inclusion criteria were as follows: 1) Histologically confirmed hypopharyngeal squamous cell carcinoma, with no prior history of anti - tumor treatment; 2) Clinical stage III - IV, and the imaging evaluation indicating a resectable tumor; 3) All patients received neoadjuvant treatment, surgical intervention, and postoperative RT at our center; 4) No distant metastasis detected at the time of initial visit; 5) Patients had regular postoperative follow - up with complete data records available. The exclusion criteria were: 1) A history of other malignancies within the previous 5 years, autoimmune diseases, a history of severe/uncontrolled heart disease, or interstitial lung disease; 2) Previous treatment with immune checkpoint inhibitors; 3) A history of severe infection up to 28 days prior to enrollment.

Patients were stratified into two groups based on the neoadjuvant treatment protocol. One group was administered the pembrolizumab + paclitaxel + cisplatin (TP + PEMBRO) regimen, while the other group received the cetuximab + paclitaxel + cisplatin (TP + CETUX) regimen. This study was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University.

2.2 Treatment protocols

The treatment strategies for all patients were formulated by a multidisciplinary team (MDT) comprising of medical oncologists,

radiation oncologists, pathologists, radiologists, and other specialists.

In the TP + PEMBRO group, patients underwent neoadjuvant treatment with pembrolizumab at a dose of 200 mg on day 1, cisplatin at 75 mg/m^2 on day 1, and paclitaxel at 175 mg/m^2 on day 1. Each treatment cycle spanned 21 days, with a maximum of 4 cycles. In the TP + CETUX group, patients received the combination of cisplatin at 75 mg/m^2 on day 1 and paclitaxel at 175 mg/m^2 day 1, along with cetuximab. The initial dose of cetuximab was 400 mg/m^2 administered via intravenous infusion over 2 hours, followed by a weekly dose of 250 mg/m^2 infused over 1 hour, for a maximum of 3 cycles.

Regardless of the response of the lesion to neoadjuvant treatment, all patients proceeded to surgical resection. The surgery was scheduled 3 weeks after the completion of the last neoadjuvant treatment cycle. The surgical plan and postoperative follow - up treatment were meticulously determined through MDT discussions. The surgical resection scope was delineated based on post - treatment imaging and electronic laryngoscopy findings. The surgical approach (minimally invasive surgery refers to transoral ablation of primary tumor [transoral surgery, TOS] and open surgery involved transcervical approaches with/without laryngectomy) was selected according to the extent of the lesion. All surgeries were performed by an experienced head and neck surgeon. Lymph node dissection was carried out on the ipsilateral or bilateral neck after neoadjuvant treatment, taking into account the initial metastasis range of LAHPC and the stage of the primary tumor. The margin status of the pathological specimens was analyzed, and histological data were collected to guide adjuvant treatment following MDT consultations.

2.3 Evaluation of treatment efficacy and adverse reactions

All patients underwent CT and MRI scan prior to treatment as baseline data on tumor size, 26 patients (11/16 in the TP+CETUX group and 15/16 in the TP+PEMBRO group) received PET-CT before treatment to ensure that the tumors were free of distant metastases. The efficacy of neoadjuvant treatment was evaluated using the Response Evaluation Criteria in Solid Tumors version 1.1. Specifically, by comparing the imaging results after treatment with those at the initial diagnosis, the response to neoadjuvant treatment was categorized as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD).

Postoperative pathological evaluation was conducted by examining the residual tumor cells in the resected samples. The evaluation of the primary tumor and cervical lymph node (LN) was performed separately. Pathological response was assessed by two blinded pathologists using whole-tumor sections. Pathological complete response (PCR) was defined as the absence of any residual tumor tissue in both the primary site and LN metastasis. Major pathological response (MPR) was defined as the presence of less than 10% of viable tumor cells in the primary lesion (19). Immune partial response (IPR) was defined as a ≥ 30% reduction in the sum of the maximum diameters of all target lesions in the patient, maintained for at least four weeks.

The assessment of adverse events was based on the Common Terminology Criteria for Adverse Events (CTCAE) V5.0. The monitoring and recording of adverse events encompassed the entire neoadjuvant treatment period and extended 30 days after the last neoadjuvant treatment.

2.4 Data collection and follow-up

The baseline variables collected in this study included age, gender, smoking history, drinking history, and TNM classification. The treatment - related variables included imaging objective response rate (ORR), surgical method, postoperative pathological complete response rate, tracheotomy, indwelling gastric tube rate and swallowing function, laryngeal preservation rate, short - term survival rate, and adverse events.

Patient follow - up was conducted in accordance with the NCCN guidelines. Specifically, patients were followed up every 2–3 months during the first year after surgery and every 4–6 months from the second year onwards. Data were collected through outpatient visits or telephone interviews. Additionally, patients underwent electronic laryngoscopy and CT/MR scans during follow - up. The survival outcomes of patients included overall survival (OS) and relapse - free survival (RFS).

2.5 Statistical analysis

In the statistical analyses, the chi-square test was utilized to compare the baseline characteristics of patients. The Student's t – test was employed to compare the means between the two groups. The Kaplan - Meier survival curve was used to analyze patient survival. The statistical and graphing software utilized were GraphPad Prism 9 and R 4.1.1. The statistical values were reported as mean ± standard error, and the survival rates were described as 95% confidence intervals. Two-tailed p value < 0.05 were defined as statistically significant.

3 Results

3.1 Patient characteristics

A total of 32 patients were included in this study. The mean age of the patients was 60.41 years (ranging from 49 to 78 years). A total of 18 patients had T1–2 stage primary tumors (56.25%), including 9 each in the TP+CETUX and TP+PEMBRO groups. There was no statistically significant difference in the primary tumor stage between the two groups (P=0.70). There were 11 (34.38%) stage III patients and 21 (65.62%) stage IV patients. Nineteen (59.38%) patients had a smoking or drinking history. No significant differences were observed in clinical characteristics such as age,

gender, smoking history, drinking history, anatomic site of primary tumor and tumor stage between the two treatment groups (Table 1).

All patients received 2 to 4 cycles of neoadjuvant treatment. Due to suboptimal response to neoadjuvant treatment, 3 patients in the TP + CETUX group and 2 patients in the TP + PEMBRO group received 3 cycles of neoadjuvant treatment, and 1 patient in the TP + PEMBRO group received 4 cycles of neoadjuvant treatment (P=1).

3.2 Efficacy of neoadjuvant treatment and adverse events

All patients underwent PET-CT or MRI examinations before and after neoadjuvant treatment to precisely measure the size of the primary tumor and cervical LN and to evaluate treatment efficacy. The overall ORR of all patients was 78.13% (25/32), and no patient exhibited disease progression (PD) (Figure 1A). Notably, in the TP + PEMBRO group, the tumor shrinkage rate was significantly higher than that in the TP + CETUX group (-75.06 ± 27.56% vs -40.72 ± 28.35%, P=0.0016). The ORR proportion in the TP + PEMBRO group was also higher than in the TP + CETUX group (87.5% vs 68.75%, P =0.043, Figure 1B). Table 2 provides a detailed record of the efficacy evaluation of patients.

During neoadjuvant treatment, grade 1–2 adverse events were noted among both treatment groups, while no grade 3–4 adverse events occurred. The most prevalent adverse events included anemia (62.50%, 20/32), fatigue (56.25%, 18/32), and neutropenia (50.00%, 16/32), among others (Table 3, Figure 2). Throughout the treatment process, no treatment - related adverse events that led to drug discontinuation, dose reduction, or death were observed, nor were any serious immune - related adverse events detected. There was no significant difference in the incidence of adverse events between the two groups treatment groups.

3.3 Surgical outcomes and postoperative pathological evaluation

All patients underwent resection of the primary tumor and neck lymph node dissection subsequent to neoadjuvant treatment. Twenty - three patients underwent minimally invasive transoral plasma surgery, while the remaining patients underwent open surgery, with 3 patients undergoing total laryngectomy. The minimally invasive surgery rate in the TP + PEMBRO group was higher than in the TP + CETUX group (87.50% vs 56.25%, P=0.049), and there was no significant difference in the choice of open surgical methods between the two groups (Table 1).

During the perioperative period, 13 patients underwent tracheotomy, including 4 patients in the TP + PEMBRO group and 9 patients in the TP + CETUX group (Figure 3A). Excluding the patients who underwent total laryngectomy, the average indwelling time of the tracheotomy cannula in the TP + PEMBRO group was 85 ± 30.41 days, while in the TP + CETUX group, it was 171.5 ± 34.10 days (P=0.15, Figure 3B). When compared by surgical method, the average indwelling time of the tracheotomy cannula in the minimally invasive surgery group was 90 ± 34.64 days and 169 ± 34.51 days in the open surgery group (P=0.20, Figure 3C).

A total of 14 patients had indwelling gastric tubes for postoperative feeding, with 5 patients in the TP + PEMBRO group and 9 patients in the TP + CETUX group (Figure 3A). The indwelling time of the gastric tube in the TP + PEMBRO group was 22 ± 9.59 days and 59 ± 27.70 days in the TP + CETUX group (P=0.38, Figure 3D). Similarly, when compared by surgical method, the average indwelling time of the gastric tube in the minimally invasive surgery group was 11 ± 1.51 days, and in the open surgery group, it was 70.56 ± 29.48 days (P=0.13, Figure 3E).

All pathological specimens were evaluated by experienced pathologists after surgery. Overall, 10 patients achieved PCR (31.25%), 13 patients achieved MPR (40.63%), and the remaining 9 patients displayed IPR (28.13%). There was no significant statistical difference in the overall pathological evaluation between the treatment groups (P = 0.13, Table 2).

However, when the primary tumor and LN were evaluated separately, significant differences emerged. In the primary tumor PCR, the rate was 46.88% (15/32), with 68.75% (11/16) in the TP + PEMBRO group and 25.00% (4/16) in the TP + CETUX group; the MPR rate was 25.00% (8/32), with 18.75% (3/16) in the TP + PEMBRO group and 31.25% (5/16) in the TP + CETUX group; the IPR rate was 28.13% (9/32), with 12.50% (2/16) in the TP + PEMBRO group and 43.75% (7/16) in the TP + CETUX group (P=0.04).

In lymph node PCR, the rate was 46.88% (15/32), with 62.50% (10/16) in the TP + PEMBRO group and 31.25% (5/16) in the TP + CETUX group; the MPR rate was 28.13% (9/32), with 37.50% (6/16) in the TP + PEMBRO group and 18.75% (3/16) in the TP + CETUX group; the IPR rate was 25.00% (9/32), all exhibited in the TP + CETUX group (P=0.0042) (Table 2).

3.4 Postoperative adjuvant treatment and follow-up

All patients received RT after surgery, with the dose ranging from 50 to 66 Gy. The median follow-up time of patients was 14.63 ± 3.22 months. In terms of patient prognosis, the 1-year OS rate in the TP + PEMBRO group was 100%, and the 1-year RFS rate was 92.31% (95% CI: 56.64 - 98.88%). In the TP + CETUX group, the 1-year OS rate was 93.75% (95% CI: 63.24 - 99.10%), and the 1-year RFS rate was 81.25% (95% CI: 52.46 - 93.54%). There was no significant difference in 1-year OS (P=0.44) and RFS (P=0.30) between the two groups of patients (Figures 4A, B).

Figure 4C details the main events during the treatment process of all patients. In the TP + PEMBRO group, one patient who passed away had tumor recurrence at the anastomosis 9 months after total laryngectomy and succumbed 13 months after surgery. The postoperative pathological evaluation of this patient was IPR. In the TP + CETUX group, one patient had tumor recurrence 8

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TABLE 1 Clinical Characteristics of HPSCC patients.

Characteristics	TP+CETUX (n=16)	TP+PEMBRO (n=16)	Total (n=32)	P value
Sex				N/A
Male	16 (100.00%)	16 (100.00%)	32 (100.00%)	
Age (Year)				0.56
Mean ± SD	59.56 ± 6.78	61.25 ± 9.18	60.41 ± 7.98	
Smoking				0.47
No	5 (31.25%)	8 (50.00%)	13 (40.63%)	
Yes	11 (68.75%)	8 (50.00%)	19 (59.38%)	
Alcohol				1
No	6 (37.50%)	7 (21.88%)	13 (40.63%)	
Yes	10 (62.50%)	9 (28.13%)	19 (59.38%)	
Subsite of primary tumor				0.34
Pyriform sinus	12 (75%)	11 (68.25%)	23 (71.87%)	
Postcricoid	3 (18.75%)	1 (6.25%)	4 (12.50%)	
Posterior wall of pharynx	1 (6.25%)	2 (12.50%)	3 (9.38%)	
≥ (subsites	0 (0.00%)	2 (12.50%)	2 (6.25%)	
T stage [a]				0.70
T1	3 (18.75%)	2 (12.50%)	5 (15.63%)	
T2	6 (37.50%)	7 (43.75%)	13 (40.63%)	
T3	4 (25.00%)	2 (12.50%)	6 (18.75%)	
T4	3 (18.75%)	5 (31.25%)	8 (25.00%)	
N stage [a]				0.18
N0	0 (0.00%)	2 (6.25%)	2 (6.25%)	
N1	8 (50.00%)	4 (12.50%)	12 (37.50%)	
N2	7 (43.75%)	10 (62.50%)	17 (53.12%)	
N3	1 (6.25%)	0 (0.00%)	1 (3.13%)	
TNM Stage [a]			0.26	
III	7 (43.75%)	4 (25.00%)	11 (34.38%)	
IV	9 (56.25%)	12 (75.00%)	21 (65.63%)	
Treatment cycle			1	
2	13 (81.25%)	13 (81.25%)	26 (81.25%)	
3	3 (18.75%)	2 (12.50%)	5 (15.63%)	
4	0 (0.00%)	1 (6.25%)	1 (3.13%)	
Treatment to surgery (months)			0.48	
Mean ± SD	2.13 ± 0.39	2.25 ± 0.58	2.19 ± 0.49	
Surgical procedure				0.14
TOS [b]	9 (56.25%)	14 (87.50%)	23 (71.88%)	
Larynx-preserving hypopharyngectomy	5 (31.25%)	1 (6.25%)	6 (18.75%)	
Total laryng- and hypopharyngectomy	2 (12.50%)	1 (6.25%)	3 (9.38%)	

(Continued)

TABLE 1 Continued

Characteristics	TP+CETUX (n=16)	TP+PEMBRO (n=16)	Total (n=32)	P value
Neck dissection			0.39	
Unilateral	14 (87.50%)	11 (68.75%)	25 (78.13%)	
Bilateral	2 (12.50%)	5 (31.25%)	7 (21.88%)	
Tracheostomy				0.11
No	7 (43.75%)	12 (75.00%)	19 (59.38%)	
Pre-treatment	2 (12.50%)	1 (6.25%)	3 (9.38%)	
Pre-surgery	7 (43.75%)	2 (12.50%)	9 (28.13%)	
Post-surgery	0 (0.00%)	1 (6.25%)	1 (3.13%)	
Nasogastric tube placement				0.29
No	7 (43.75%)	11 (68.75%)	18 (56.25%)	
Yes	9 (56.25%)	5 (31.25%)	14 (43.75%)	
Adjuvant radiotherapy dose (Gy)				0.31
Mean ± SD	62.00 ± 4.38	60.75 ± 2.05	61.38 ± 3.42	
Follow-up time (months)				0.39
Mean ± SD	14.13 ± 3.10	15.13 ± 3.36	14.63 ± 3.22	
Survival status				1
Live	14 (87.50%)	15 (93.75%)	29 (90.63%)	
Death	2 (12.50%)	1 (6.25%)	3 (9.38%)	
Recurrence			0.6	
No	13 (81.25%)	15 (93.75%)	28 (87.50%)	
Yes	3 (18.75%)	1 (6.25%)	4 (12.50%)	

[a]According to 8th edition AJCC staging manual.
[b]TOS, transoral robotic surgery.

months after minimally invasive surgery, with a postoperative pathological evaluation of PCR. This patient underwent total laryngectomy and is still alive as of this writing. Additionally, two patients who underwent open surgery had a postoperative pathological evaluation of IPR and experienced tumor recurrence 7 months after surgery. They died 8 months and 12 months after surgery, respectively.

3.5 A representative case

A representative case was one adult patient with hypopharyngeal malignant tumor (T4aN2cM0, IVA) (Figures 5A, B). After two cycles of neoadjuvant treatment with TP + PEMBRO, the tumor regression achieved CR (Figures 5C, D). Subsequently, TOS and bilateral lymph node dissection were performed (Figure 6A). Notably, tracheotomy was not required during the operation. The postoperative pathology confirmed a pathological complete response (PCR). After the operation, the patient received RT. To date, during the 12-month follow-up, the patient has maintained good swallowing and voice functions, and no tumor recurrence has been detected (Figures 6B, C).

4 Discussion

The overarching goal in the management of LAHPC is to optimize function preservation and enhance the quality of life of patients while ensuring survival. Neoadjuvant chemotherapy plays a pivotal role in this context by reducing the tumor burden prior to surgery, thereby potentially increasing the function preservation rate and minimizing the extent of tumor resection (20). Currently, the conventional neoadjuvant chemotherapy regimens for LAHPC include the TPF regimen and the TP + CETUX regimen. In previous clinical studies, the ORR of these regimens was 63.3% and 74.5%, respectively. However, despite their application, there has been no substantial improvement in patient prognosis (12, 14, 21, 22). The advent of immunotherapy has introduced novel treatment alternatives for LAHPC patients (23). In this retrospective study, we analyzed LAHPC patients who received either the neoadjuvant TP + CETUX or TP + PEMBRO regimens and underwent surgical resection at our center. The results demonstrated that the TP + PEMBRO regimen was superior to the TP + CETUX regimen in terms of response rate and minimally invasive surgery rate; it also exhibited a favorable safety profile.

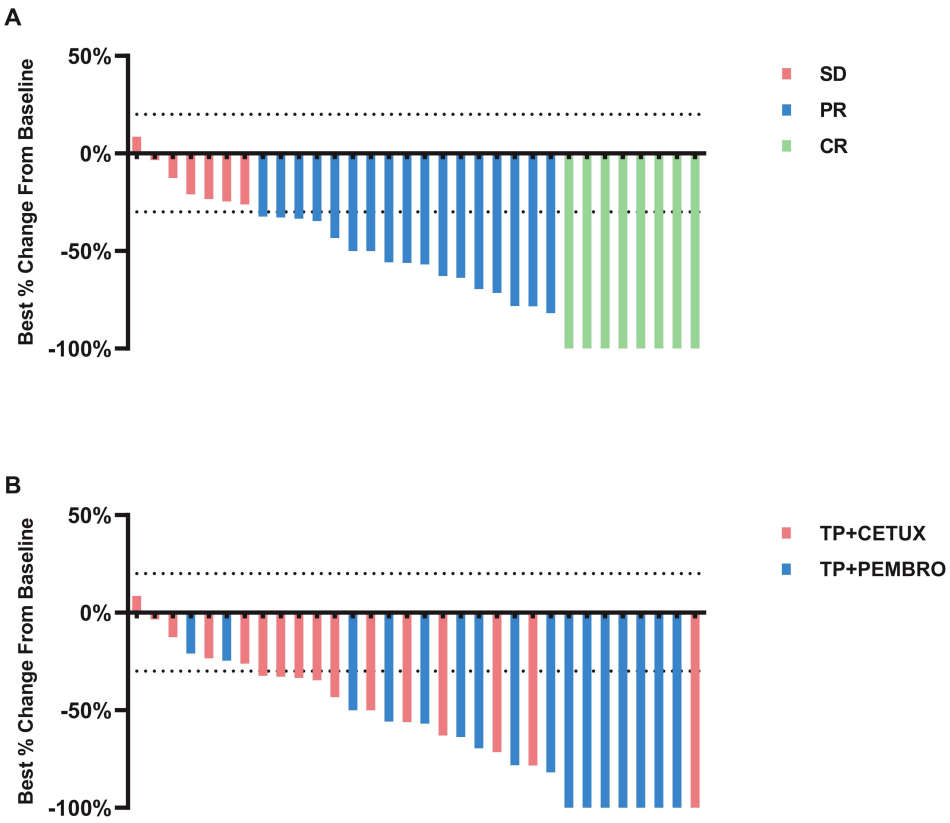

FIGURE 1
The response of tumors to neoadjuvant therapy. **(A)** Percentage reduction of tumor diameter compared with baseline with RECIST 1.1 criteria. **(B)** Tumor regression of patients received different neoadjuvant treatment regimens.

Among the 32 patients included in this study, the overall ORR was 78.13% (25/32). Specifically, the ORR rate in the TP + PEMBRO group was 87.50% (14/16), while in the TP + CETUX group, it was 68.75% (11/16). The response rate of the TP + PEMBRO group was significantly better than that of the TP + CETUX regimen ($P < 0.05$). No grade 3 or higher adverse events were observed in this study, and the incidence of adverse events in the different treatment groups was comparable, indicating the safety of the neoadjuvant treatment regimens. Previous studies have shown that neoadjuvant chemotherapy combined with PD - 1 inhibitors has demonstrated high pathological remission rates with acceptable safety in LAHNSCC patients (16, 24). The ORR of the TP + CETUX group in this study was consistent with that of previous studies, while the ORR of the TP + PEMBRO group was relatively higher. This discrepancy may be attributed to the significant heterogeneity of HNSCC with different primary sites included in previous studies, as well as differences in drug regimens. These factors make it challenging to accurately evaluate the response and efficacy of neoadjuvant chemotherapy combined with immunotherapy in the treatment of LAHPC. In contrast, all patients in our study were pathologically confirmed LAHPC patients, and the treatment drugs and regimens were relatively uniform, enabling us to precisely assess the response of different treatment regimens.

Reducing the tumor burden, minimizing the tumor resection range, and maximizing the function preservation rate are of utmost importance in neoadjuvant chemotherapy for LAHPC. In this study, 71.88% of patients (23/32) underwent minimally invasive transoral surgery after neoadjuvant treatment. Notably, although TP+PEMBRO group had more patients with T4 tumors (31.3% vs. 18.8%), 87.50% (14/16) of patients in the TP + PEMBRO group underwent minimally invasive surgery, while 56.25% (9/16) patients in the TP + CETUX group received TOS, which is in line with previous studies (2) ($P < 0.05$). This suggests that patients treated with TP+PEMBRO may have a higher percentage of tumor regression The overall laryngeal preservation rate of all patients reached 90.62% (29/32), surpassing the function preservation rates observed in landmark trials of induction chemotherapy combined with radiotherapy (8, 9). A recent prospective study involving 15 LAHPC patients who received NAC combined with PD - 1 monoclonal antibody treatment reported a total laryngeal preservation rate of 86.6% (25), similar to the laryngeal preservation rate in our cohort. Concurrently, in terms of tracheotomy and indwelling gastric tube, 40.62% (13/32) of patients underwent tracheotomy, and 43.75% (14/32) of patients had indwelling gastric tubes during surgery. Although there was no significant difference in the proportion of tracheotomy and indwelling gastric tube and the duration of indwelling between the two groups, the number of cases and the duration of indwelling of tracheotomy and indwelling gastric tube in the TP + CETUX group were generally higher than those in the TP + PEMBRO group. This aligns with prior studies of TP + cetuximab regimens, which reported tracheotomy rates of 53% (12), compared to 25% in our TP + PEMBRO cohort. Similarly, TPF-based regimen

TABLE 2 Response to neoadjuvant chemotherapy.

Characteristics	TP+CETUX (n=16)	TP+PEMBRO (n=16)	Total (n=32)	P value
Tumor shrinkage (%)				0.0016*
Mean ± SD	-40.72 ± 28.35	-75.06 ± 27.56	-57.89 ± 32.57	
Clinical evaluation [a]				0.04*
CR	1(6.25%)	7(43.75%)	8(25.00%)	
PR	10(62.50%)	7(43.75%)	17(53.13%)	
SD	5(31.25%)	2(12.50%)	7(21.88%)	
Total pathologic response [b]				0.13
IPR	7(43.75%)	2(12.50%)	9(28.13%)	
MPR	6(37.50%)	7(43.75%)	13(40.63%)	
PCR	3(18.75%)	7(43.75%)	10(31.25%)	
Pathologic response (primary tumor)				0.04*
IPR	7(43.75%)	2(12.50%)	9(28.13%)	
MPR	5(31.25%)	3(18.75%)	8(25.00%)	
PCR	4(25.00%)	11(68.75%)	15(46.88%)	
Pathologic response (LN)				0.0042*
IPR	8(50.00%)	0(0.00%)	8(25.00%)	
MPR	3(18.75%)	6(37.50%)	9(28.13%)	
PCR	5(31.25%)	10(62.50%)	15(46.88%)	

[a]Evaluated based on RECIST 1.1 criteria. CR, complete response; PR, partial response; SD, stable disease.
[b]Assessed by postoperative pathology. IPR, immune partial response; MPR, major pathologic response; PCR, pathologic complete response.

historically documented tracheotomy rates of 45-55% (21), underscoring the potential advantages of pembrolizumab-based therapy. Considering the relatively high proportion of stage IV patients in our cohort (65.62%, 21/32), induction treatment holds significant potential in reducing tumor size, preserving organ functions, and minimizing perioperative complications.

This study also conducted a comprehensive pathological evaluation of patient tumor specimens after surgery. The results revealed that the overall PCR rate was 31.25% (10/32) and the MPR rate was 40.63% (13/32). In the TP + PEMBRO group, the PCR rate was 43.75% (7/16), and the MPR rate was 43.75% (7/16); in the TP + CETUX group, the PCR rate was 18.75% (3/16), and the MPR rate was 37.50% (6/16). Although there was no significant statistical difference in the overall pathological evaluation between the two groups, the number and proportion of PCR and MPR cases in the TP + PEMBRO group were higher than those in the TP + CETUX group. This finding is consistent with previous observations in other tumors, suggesting that immunotherapy combined with neoadjuvant chemotherapy can potentially achieve higher PCR rates than neoadjuvant chemotherapy alone. Additionally, when we evaluated the primary tumor and LN separately, we noted that the pathological remission patterns differed. Fang et al. reported that in locally advanced laryngeal and hypopharyngeal cancers, the response rate of neck lymph node metastases to neoadjuvant chemotherapy combined with immunotherapy was relatively low (26). However, the results of the CIAO study indicated that the pathological remission effect of lymph node metastases to immune checkpoint inhibitors was better than that of the primary tumor (27). These findings suggest that there may be heterogeneity in the microenvironment of the primary tumor and lymph node metastases in HNSCC patients. Larger sample clinical studies or multi - omics studies are warranted to further evaluate the correlation and differences in the composition of the microenvironment between different lesions.

For LAHPC patients, current guidelines recommend postoperative adjuvant treatment, which typically includes RT or chemoradiotherapy (28, 29). In this study, all patients received postoperative RT with a dose ranging from 50 to 66 Gy. We also conducted regular follow-up of all patients. The 1-year OS and RFS rates in the TP + PEMBRO group were 100% and 92.31%, respectively; in the TP + CETUX group, the 1-year OS and RFS rates were 93.75% and 81.25%, respectively. During the follow-up process, a total of 4 recurrences were observed, with 3 of these patients eventually dying. The postoperative pathological evaluation of the deceased patients was IPR, while the postoperative evaluation of the surviving patient after recurrence was MPR. This patient remained alive after salvage total laryngectomy. A previous study demonstrated that the 2-year progression - free survival rate of patients who achieved MPR after neoadjuvant immunotherapy was 100%, significantly better than that of IPR patients (30). However, some studies have also suggested that in HPC, neoadjuvant treatment can improve the laryngeal preservation rate but may not have a

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TABLE 3 Adverse effects during neoadjuvant treatment.

Characteristics	TP+CETUX (n=16)	TP+PEMBRO (n=16)	Total (n=32)	P value
Hematologic				0.96
Leukopenia	8 (50.00%)	6 (37.50%)	14 (43.75%)	
Neutropenia	9 (56.25%)	7 (43.75%)	16 (50.00%)	
Anemia	9 (56.25%)	11 (68.75%)	20 (62.50%)	
Thrombocytopenia	1 (6.25%)	1 (6.25%)	2 (6.25%)	
Lymphocytopenia	1 (6.25%)	1 (6.25%)	2 (6.25%)	
Nonhematologic				0.97
Hepatotoxicity	3 (18.75%)	3 (18.75%)	6 (18.75%)	
Nephrotoxicity	2 (12.50%)	1 (6.25%)	3 (9.38%)	
Mucositis	1 (6.25%)	0 (0.00%)	1 (3.13%)	
Nausea	6 (37.50%)	5 (31.25%)	11 (34.38%)	
Vomiting	5 (31.25%)	4 (25.00%)	9 (28.13%)	
Rash	7 (43.75%)	0 (0.00%)	7 (21.88%)	
Fatigue	10 (62.50%)	8 (50.00%)	18 (56.25%)	
Neurotoxicity	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Diarrhea	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Thyroid dysfunction	1 (6.25%)	2 (12.50%)	3 (9.38%)	
Pruritus	7 (43.75%)	4 (25.00%)	11 (34.38%)	
Reactive cutaneous capillary	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Endothelial proliferation	0 (0.00%)	0 (0.00%)	0 (0.00%)	

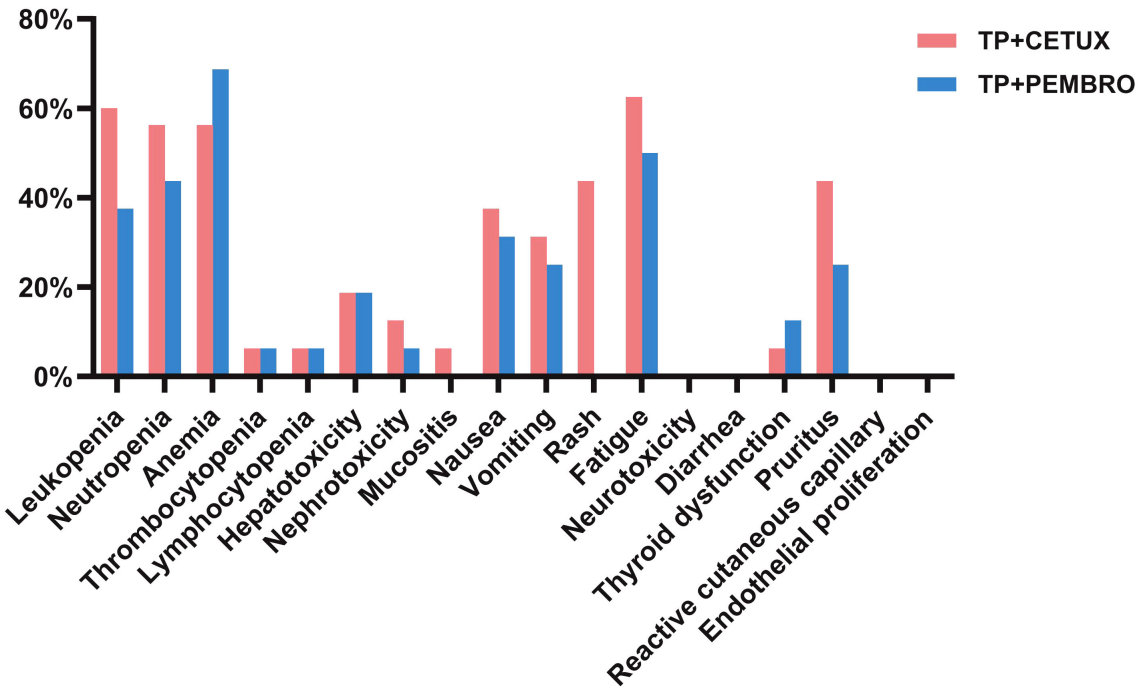

FIGURE 2
The adverse effects of different neoadjuvant treatment regimens.

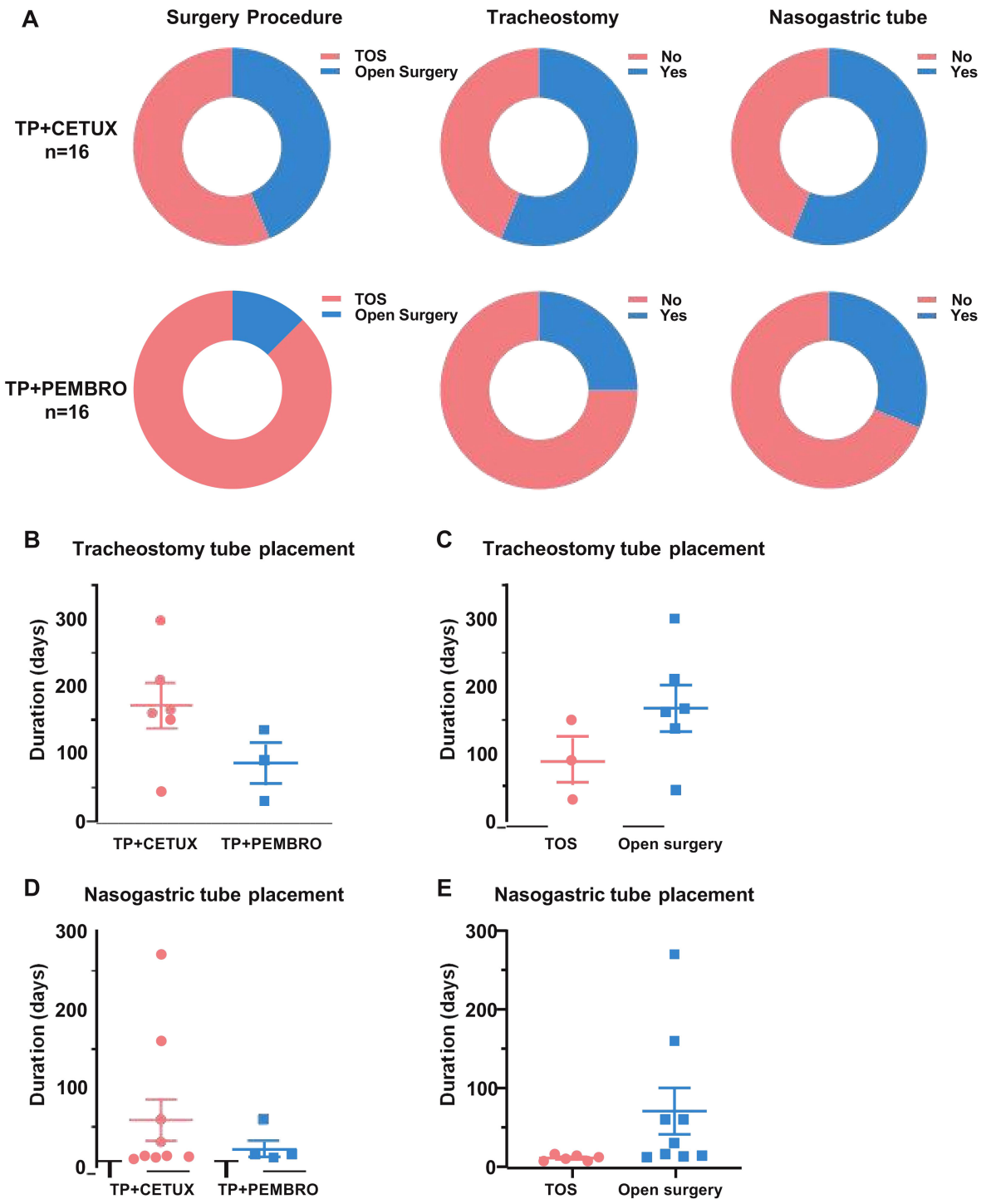

FIGURE 3
The surgical modalities of patients. **(A)** The percentage of surgical approach, tracheotomy and indwelling gastric tube in patients received different neoadjuvant regimens. **(B, C)** The indwelling time of tracheotomy cannula under different neoadjuvant treatment regimens **(B)** and surgical approaches **(C)**. **(D, E)** The indwelling time of nasogastric tube under different neoadjuvant treatment regimens **(D)** and surgical approaches **(E)**.

significant impact on OS (31, 32). Some researchers have even argued that the current data regarding the impact of tumor response after neoadjuvant immunotherapy on survival remains inconclusive (33). Although the follow-up period of this study was relatively short, the short - term survival rate of the TP + PEMBRO group was more favorable than that of the TP + CETUX group. Hence, larger sample sizes and longer follow-up durations are essential to further clarify this finding.

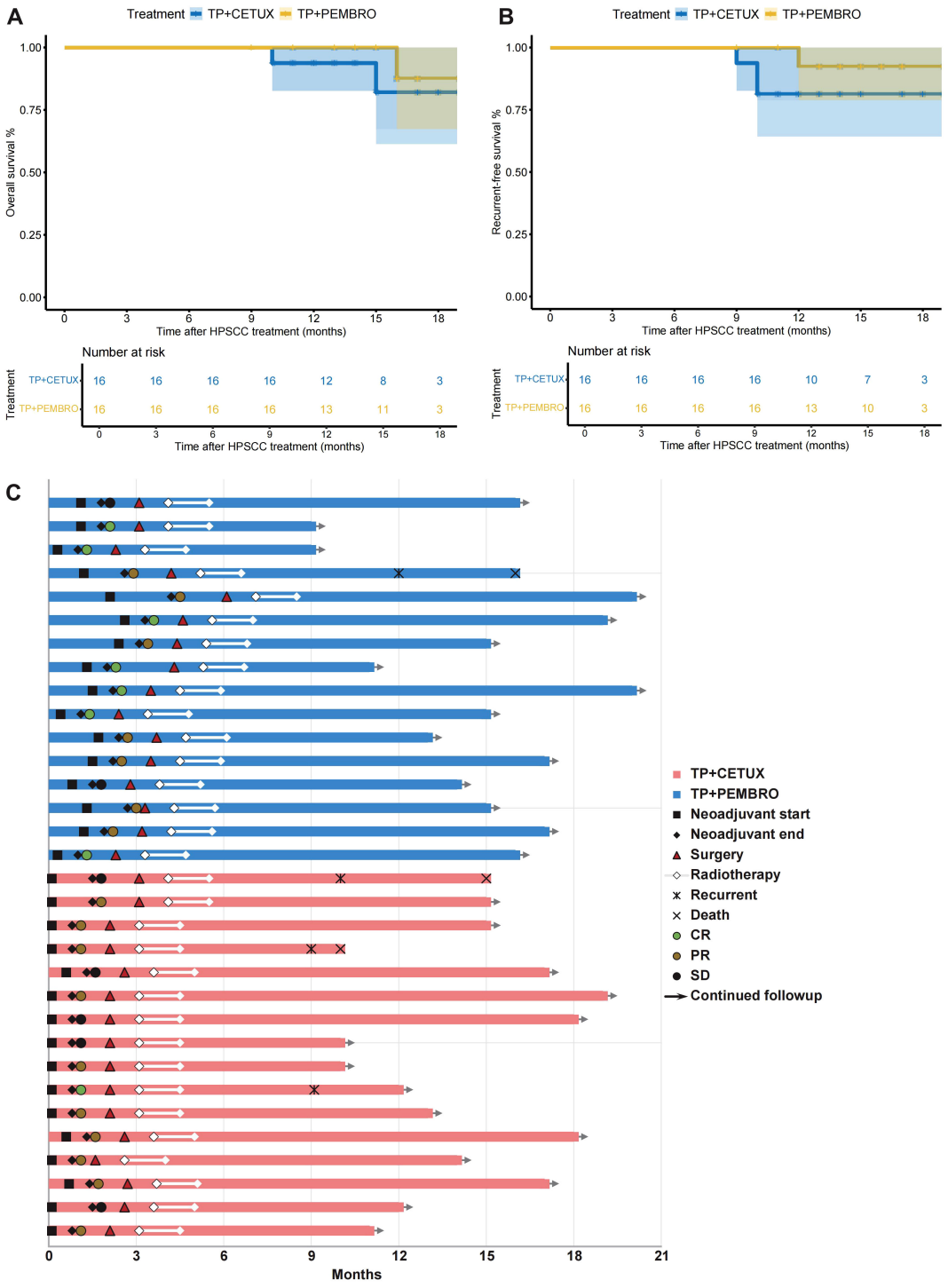

FIGURE 4
Survival and treatment exposure of patients. **(A, B)** The overall survival (OS) **(A)** and relapse-free survival (RFS) **(B)** of patients. **(C)** The swimmer plot revealed the treatment exposure and response of neoadjuvant treatment, surgery, and adjuvant therapy in 32 LAHPC patients.

Several limitations of the present study must be acknowledged. As a retrospective analysis from a single institution, the small sample size and selection bias cannot be discounted. Additionally, the relatively short follow-up time of the included patients precludes effective evaluation of the long - term efficacy of the treatment. Therefore, future studies should aim to include larger sample sizes and longer follow-up durations to more precisely elucidate the efficacy of different regimens. Furthermore, prospective controlled studies are necessary to r validate the results of this study. Nevertheless, this study has provided preliminary evidence suggesting that the neoadjuvant treatment regimen of TP + PEMBRO may offer greater advantages over the TP + CETUX regimen in LAHPC patients.

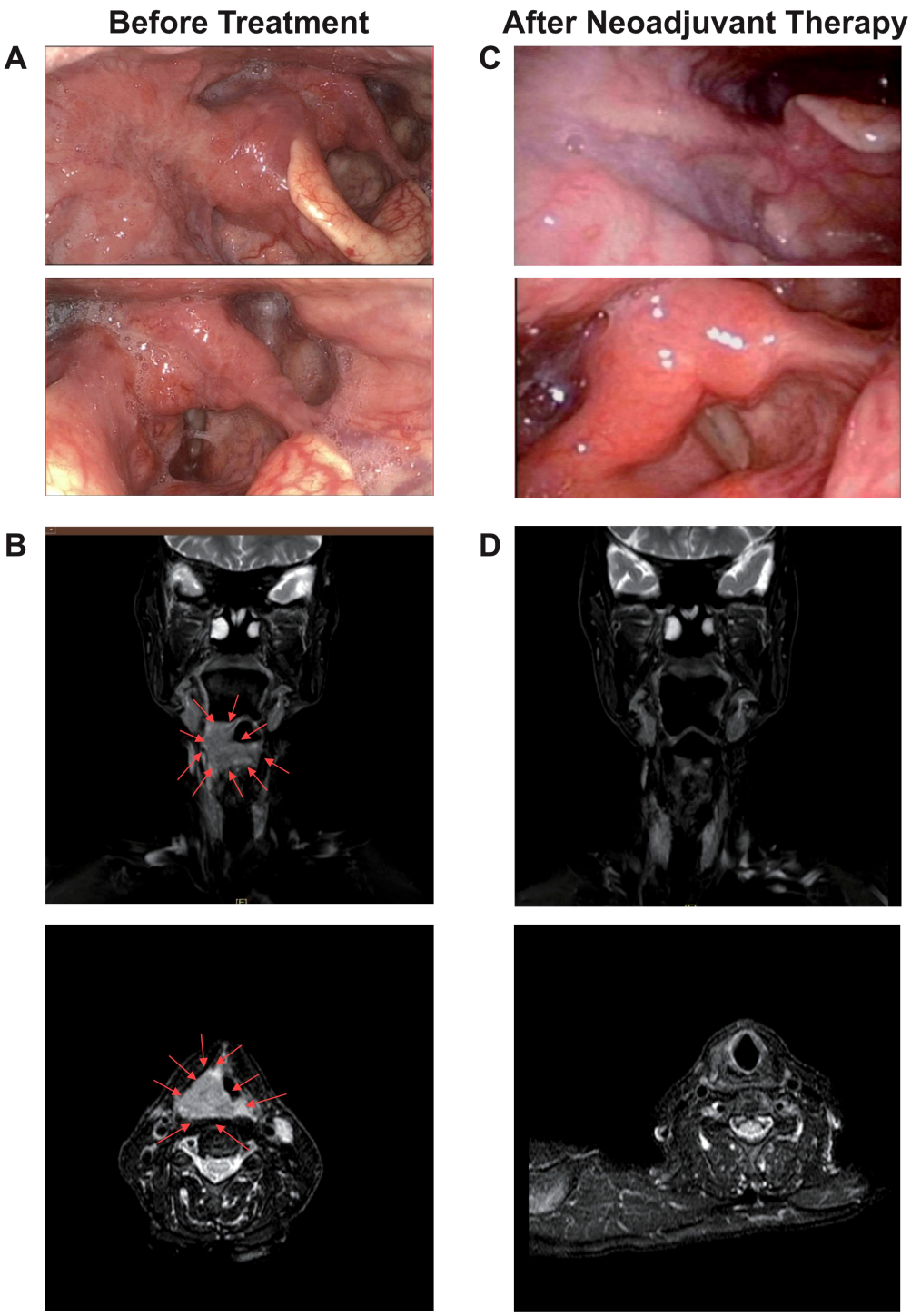

FIGURE 5
A representative case of tumor regression after neoadjuvant TP+PEMBRO regimen. **(A)**The scope of the tumor under electronic laryngoscope before neoadjuvant treatment: involving the right lateral wall of the oropharynx, the base of the tongue, the right pyriform sinus, the postcricoid area. **(B)** The MRI before neoadjuvant treatment. **(C)** No tumor was found under electronic laryngoscope after neoadjuvant treatment. **(D)** No tumor was found under MRI after neoadjuvant treatment. The direction of the red arrow indicates the tumor.

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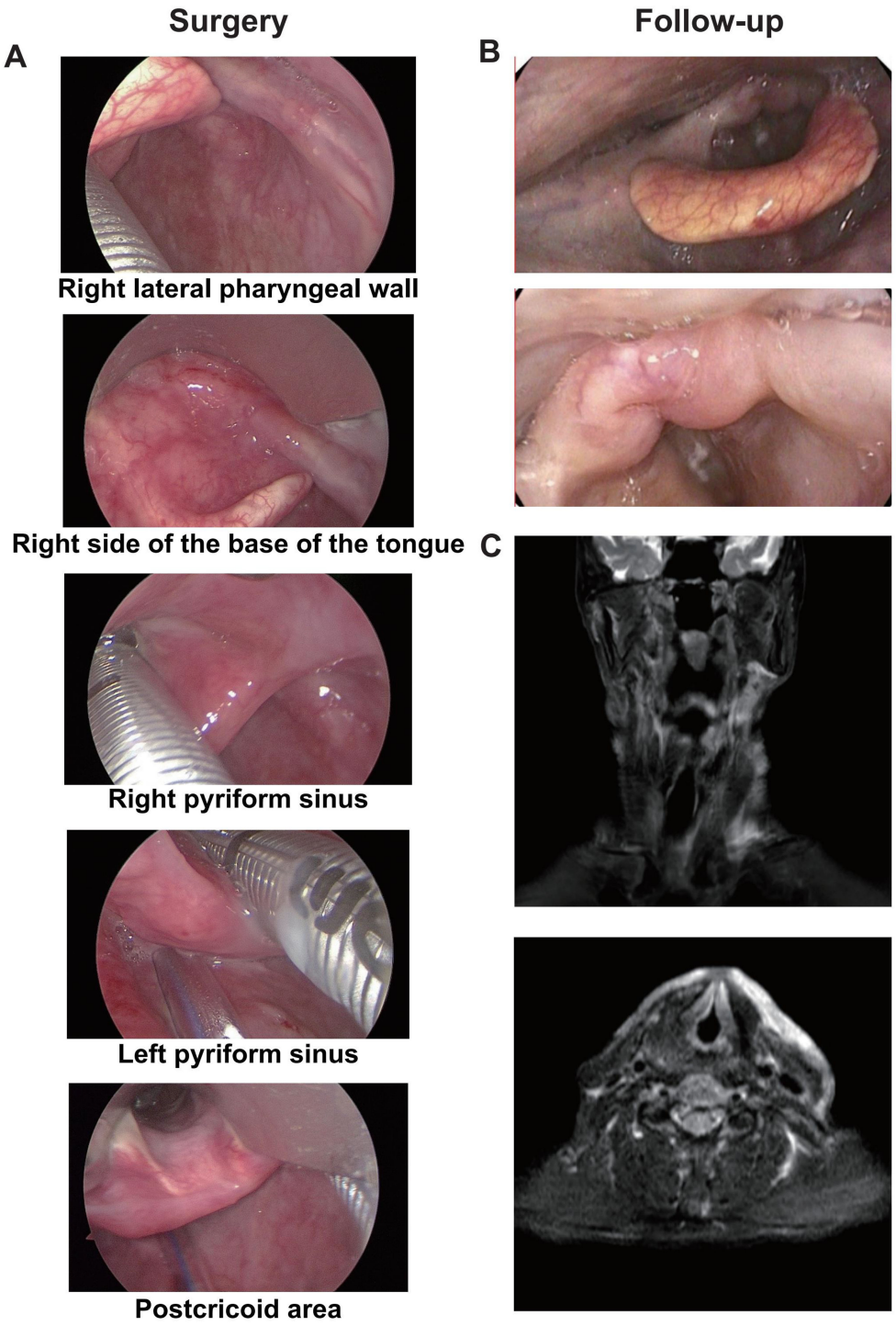

FIGURE 6
The Intraoperative image and postoperative follow-up of the representative case. **(A)** The scope of the tumor under suspension laryngoscope endoscopy after neoadjuvant treatment: No tumor was found on the right lateral wall of the oropharynx, the base of the tongue, the right pyriform sinus or the postcricoid area. **(B)** No tumor was found in the reexamination by electronic laryngoscope one year after the operation. **(C)** No tumor was found in the reexamination by MRI one year after the operation.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by The Review and Ethics Committee of The First Affiliated Hospital of Fujian Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

GY: Formal analysis, Writing – original draft, Conceptualization, Investigation. XW: Formal analysis, Investigation, Writing – original draft, Conceptualization. HL: Conceptualization, Formal analysis, Investigation, Writing – original draft. ZC: Data curation, Software, Writing – original draft. CL: Software, Supervision, Visualization, Writing – review & editing. GL: Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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