Article

Immunotherapy with PD-1 Inhibitor Nivolumab in Recurrent/Metastatic Platinum Refractory Head and Neck Cancers: The Early Experience of Two Academic Center

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Abstract: The prognosis of patients with recurrent or metastatic of the head and neck squamous-cell carcinoma (HNSCC) refractory to platinum-based chemotherapy is severe and, consequently, the identification of therapeutic options for this category of patients is a priority. Nivolumab, an anti- programmed cell death protein 1 (anti-PD-1) monoclonal antibody, has been approved for the treatment of recurrent or metastatic HNSCC after platinum-based therapy progressing. We present the early experience of two academic center including diagnostic, clinical, biological, therapeutic and outcomes characteristics of head and neck cancer (HNC) patients treated with Nivolumab. The purpose of the study is to identify certain peculiarities and to report them compared to the data from the literature in order to generate some hypotheses that coud constitute criteria for the selection of cases with maximum benefit to immunotherapy. Analyzing the data obtained from 18 patients treated in Emergency County Hospital Craiova, "Saint Nectarie" Oncological Hospital Craiova and Euroclinic Oncological Center Iasi January 2020 and March 2023 it could be hypothesized that lower nadir values of neutrophil to lymphocyte ratio (<3.5) and the adition of intensive regimens of chemotherapy and radiotherapy could justify a favorable response in terms of progression free survival (PFS) during immunotherapy with Nivolumab. To our knowledge, the study included the first two cases of second primary malignancy (SPM) in the head neck region treated with Nivolumab. The reported data support a possible benefit of the sequential administration of radiotherapy, immunotherapy for SPM cases. Higher positive dynamic of neutrophil to lymphocyt ratio (NLR) could be associated with worst outcome during immunotherapy. All study observations including the role of infections and antibiotic treatment as well as the possible biomarker value of NLR variation during immunotherapy should be investigated in multicenter clinical trials.

Keywords: Nivolumab; immunotherapy; head and neck cancers; HNSCC; chemotherapy; neutrophil to lymphocyt ratio; NLR; second primary malignancy; Cetuximab

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is associated with a poor prognosis and lower survival rates in the advanced stages of the disease even if a multimodal treatment is administered according to the current standards. The improvement of diagnostic and therapeutic performances brought minimal benefits in the HNSCC prognosis. As confirmed by preclinical data, immunosuppression and the ability to escape from immunosurveillance by the accumulation of genetic mutations during carcinogenesis or by disease progression are responsible for the therapeutic failure of HNSCC. In this context, the identification of some strategies to restore the antitumor immune response could bring benefit in term of treatment response with longer survival rates. Nivolumab, an anti–programmed death 1 (PD-1) monoclonal antibody, brought long-term overall survival (OS) benefits in platinum-refractory HNSCC cases compared to single agent chemotherapy treatment in the phase III CheckMate 141 trial. Scientific efforts are focused on identication of predictive biomarkers for favorable response to immunotherapy, head and neck tumors being associated with a tumor microenvironment (TME) considered highly immunosuppressive. Programmed death ligand-1 (PD-L1), combined positive score (CPS), tumor mutation burden (TMB), neutrophil to lymphocyte ratio (NLR), but also more recently radiomics biomarkers based on artificial intelligence applied to medical imaging are evaluated in order to identify cases that will have a long term response to immune checkpoint inhibitors (ICI) [1-8].

The aim of the study was to present the early experience of two academic center with the PD-1 inhibitor Nivolumab in relapsed/metastatic head and neck cancers (HNC) refractory to platinum agents.

2. Materials and methods

The cases of locoregional repalpse or metastatic HNC treated with Nivolumab were identified from the database of the Oncology Clinic of the Craiova County Emergency Hospital, "Saint Nectarie" Oncological Hospital and Euroclinic Oncological Center Iași. Biological, clinical and therapeutic data of the patients as well as outcomes were analyzed. The follow-up since initiation of Nivolumab therapy has varied between 3 months and 25 months.

3. Results

18 cases of locoreginal relapsed or metastatic HNC refractory to platinum-based chemotherapy treated with Nivolumab between January 2020 and March 2023 were identified. 17 (94,4%) of the 18 cases are squamous cell carcinomas and 1 (5,5%) case is an adenoid cystic carcinoma. 5 cases (27,5%) were anatomically located in the oropharynx (tonsils and tongue base), 5 cases (27,5%) were treated for oral cavity (tongue and floor of the mouth) cancers, 3 (16,5%) and 1 (5,5%) cases were treated for laryngeal respectively hipopharynx cancer. 2 cases were treated with Nivolumab for salivary gland - 1 case (5.55) respectively sinonasal cancer - 1 case (5.5%). 14 cases (77%) were initially staged - 8 cases (44%) stage III, 5 cases (27,5%) stage IVA and 1 case (5,5%) stage IVB (lung metastases) . Only 2 case (11%) received curative surgical treatment, 1 patient (5,5%) was treated with salvage surgery after relapsed and 1 case (5,5%) was treated with surgery for residual disease after a response to immunotherapy. The median age of the patients included in the study was 58,9 years (52 to 65 years) and 16 cases (85.7%) being male. 15 cased (82,5%) benefited from radiotherapy, 2 case (11%) for adjuvant purpose and 11 cases (60,5%) as definitive treatment, 2 cases (11%) were treted with re-irradiation (11%) and 1 case (5,5%) were treated with palliative purpose a in total doses ranging between 30Gy in 10 fractions and 70Gy in 35 or 33 fractions (simultaneous integrated bost). Poly-chemotherapy or single agent chemotherapy was also administered in all cases, the number of cycles varying between 1 and 10. Chemotherapy regimens included platinum base doublet or triple combination including Cisplatin, Carboplatin, 5-Fluorouracil, Docetaxel. Single agent chemotherapy with Capecitabine and Metotrexat and Carboplatin was also used. The median duration of immunotherapy treatment was 5.6 months (1 to 14 months) and 4 patient (22%) died up to 3 months after initiation of Nivolumab therapy. The median DFS was 34 months (0 to 84 months). Most of the failures were loco-regional recurrences, but in 4 cases (16.5%) the failure was due to lung metastasis

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(2 cases), mediastinal lymph node metastases (1 case) and both locoregional and lung reccurence (1 case) (Table 1, Table 2) 2. Infectious episodes were reported in 3 cases (16,5%) during treatment, Klebsiella species, Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus (MRSA) being mentioned as pathogens. Only one case was diagnosed with metachronous pancreatic cancer. Two of the cases meet the criteria of second primary malignancy (SPM). In both cases the initial disease free survival (DFS) was longer than 10 years. For the case that benefited from re-irradiation with a dose of 50 Gy in 25 daily fraction (both initial treatment and re-irradiation being delivered with an Rokus-M40 *former Soviet* Union Cobalt-60 machine without image guided treatment capability), OS after the initiation of Nivolumab was 16 months. The other SPM case treated with radiotherapy only for the first malignancy, but heavily treated with chemotherapy and Cetuximab had an OS of 3 months after the initiation of immunotherapy. It should be mentioned that this case required the administration of corticosteroids and antibiotics after the initiation of Nivolumab. No immune related adverse events (AEs) ≥ grade 3 were recorded. One death associated with admission to the emergency room for respiratory failure was not related to immune-mediated pneumonitis. Median nadir neutrophil to lymphocyte ratio (NLR) was 2.72 (1.43 to 8.27) and median NLR during the treatment was 6.01 (2.91 to 16.08). Much higher values of NLR during treatment are observed in patients who died early during immunotherapy. The median monthly delta-NLR variation was calculated in these cases, a positive dynamic of 13.07 per month being identified.

Table 1. Patient and disease characteristics.

Characteristics	N (total =18)	%
Age at the time of diagnostic		
Median (range)	58,9 (52-65) years	-
Histology		
squamous cell carcinomas (SCC)	17	94,4
adenoid cystic carcinoma	1	5,5
Anatomical tumor site		
oropharynx (tonsils and tongue base)	5	27,5
oral cavity (tongue and floor of the mouth)	3	16,5
larynx	3	16,5
hypopharynx	1	5,5
salivary gland	1	5,5
sinonasal	1	5,5
TNM stage at diagnostic		
Stage III	8	44
Stage IVA	4	27,5
Stage IVB	1	5,5

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Table 2. Multidisciplinary treatment and patient outcome characteiristics.

Characteristics	**N (total =18)**	%
Surgery		
curative intent	2	11
salvage	1	5,5
Radiotherapy		
adjuvant	2	11
definitive	11	60,5
re-irradiation	2	11
paliative	1	5,5
Immunotherapy treatment		
median duration (range)	5,6 (1-14)months	-
DFS		
Median (range)	34 (0-84) months	
Pattern of failure		
Loco-regional reccurence	12	66,6
Lung metastases	4	16,5
mediastinal lymph node metastases	1	5,5
loco-regional and lung metastases	1	5,5

4. Discussions

4.1. Immunotherapy in HNC - the challenges of a revolution

Locally-advanced HNSCC is characterized by a recurrence rate >50% within 3 years, survival in relapsed/metastatic cases refractory to platinum being generally less than 6 months, with limited therapeutic options in this context. Considering that disease progression, invasion and metastasis are associated with programmed death ligands (PD-L1 and PD-L2) and with Programmed cell death Protein 1 (PD-1) expressed on the surface of T lymphocytes, the use of immune checkpoint inhibitors was evaluated in multiple preclinical and clinical studies. PD-1 inhibitor Nivolumab was evaluated at a dose of 3 mg per kilogram every 2 weeks in comparison with a single agent of systemic therapy, including cases treated with Methotrexate, Docetaxel, or Cetuximab. The study that included 361 patients had OS as its main objective. Progression free survival (PFS), objective response rate (ORR), quality of life and safety profile were also assessed. 7.5 months versus 5.1 months were the median OS rates in the Nivolumab respectively monotherapy group (investigator choice). The treatment response rate was 13.3% versus 5.8% in favor of the patient lot treated with immune checkpoint inhibitors (ICI), with a significantly better toxicity profile (adverse events of grade 3 or 4) for Nivolumab (13.1% versus 35.1%). [9-11].

In a subgroup analysis, Gillison and collaborators demonstrate long-term outcomes in patients with recurrent/metastatic HNSCC who received Nivolumab in the first line. The authors mention a median overall survival (OS) at 2 years for patients who received first-line immunotherapy of 20.4%, in contrast to only 3.8% in the group that received the treatment chosen by the investigator. Thus, a clear benefit for immunotherapy in the first line for recurrent or metastatic HNSCC is highlighted. EXTREME regimen including Cetuximab, platinum and 5-Fluorouracil and, later, TPEx regimen including Cetuximab, platinum salts and Docetaxel were the preferred therapeutic options before ICI therapy entered the therapeutic spectrum of recurrent/metastatic HNSCC [12].

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Combined positive score (CPS)>1 was proposed to stratify patients between Pembrolizumab monotherapy (for CPS ≥ 1) and Pembrolizmumab in combination with platinum or fluorouracil. The double association between a Cytotoxic T-Lymphocyte-Associated protein-4 (CTLA-4) inhibitor, Ipilimumab and Nivolumab did not demonstrate a benefit in OS compared to the EXTREME regimen for cases with CPS>1 [1,9,13].

The KEYNOTE-040 trial, a phase III trial that included 247 patients from 97 medical centers (20 countries) demonstrated a benefit of 1.5 months in OS in the intention-to-treat population compared to standard treatment based on Methotrexate, Docetaxel, or Cetuximab in metastatic/recurring HNSCC when platinum-based chemotherapy was associated with Pembrolizumab. The use of immunotherapy in the first line of treatment for patients with high expression of PD-L1 was anticipated as early as 2019 by Saada-Bouzid et al. which mentions the prospects of immunotherapy with Pemrolizumab to become the first-line treatment in recurrent and metastatic HNSCC as a unique treatment for patients with high PD-L1 levels [14].

A meta-analysis and systematic review aims to investigate the efficacy of anti-PD-1- vs anti-PD-L1-based therapy in patients with relapsed or metastatic HNSCC, including data from the Keynote 040, Keynote 048, Eagle, Condor, Checkmate 141 including 3001 patients. The study compares PD-L1-based, PD-1-based and chemotherapy treatments. The data analysis did not highlight major differences between the studied groups in terms of treatment results, but anti-PD-1 administration was associated with better results in smokers and in HPV negative patients. Anti-PD-L1 was associated with favorable response in female patients and smokers. The results of the meta-analysis could help to stratify patients for the choice of the type of immune checkpoint inhibitors (ICI) [15].

A post hoc analysis that evaluated patients included in the CheckMate 141 trial evaluated Nivolumab in recurrent or metastatic HNSCC for cases treated beyond first RECIST-defined progression (TBP). Among the 60 patients who fulfilled this criterion, 25% of the cases associated a reduction in the size of the target lesions. 5% of these cases, were associated with a >30% reduction in tumor size. Haddad et al. also mentions the need to identify biomarkers of response beyond disease progression [16].

Salvage chemotherapy (SCT) after Nivolumab is considered a therapeutic option for recurrent/metastatic HNSCC. ORR and disease control rate were evaluated on a group of 21 cases that received SCT after Nivolumab. PFS and median OS were also evaluated. Values of 52.4% and 81.0% for ORR and disease control rate, as well as a median OS of 12.9 months and a median PFS of 5.4 months advocate for the possibility of using salvage chemotherapy in cases of relapsed and metastatic HNSCC after ICI treatment. Positive PD-L1 expression could be considered a prognostic factor of response to SCT [17].

The efficacy and safety of Nivolumab for the category of patients with relapsed and metastatic HNSCC sensitive to platinum was evaluated in a single institution prospective study in Japan. Cases were included based on the criterion of relapse or metastasis at least 6 months after therapy or chemoradiotherapy based on platinum salts. In the group of 22 platinum sensitive patients, the median OS was 17.4 months and the survival at 1 year was 73%. With an ORR of 36% and no adverse events (AEs) of grade 4 and 5 related to treatment, the regimen is considered safe and feasible. The retrospective study proposed by Okada and collaborators demonstrates on a group of 88 patients (60 platinum refractory cases), similar results of treatment with Nivolumab in metastatic or relapsed HNSCC cases, both in platinum sensitive and refractory cases [18].

Gavrielatou et al. analyzes the response of NNSCC to ICI treatment, evaluating and classifying biomarkers in 3 categories: tumor related biomarkers, host related biomarkers and other biomarkers related to the tumor microenvironment (TME). In the first category, PD-L1 or CPS, Interferon gamma (IFN-γ) signature genes and TMB are mentioned. An "inflamed" microenvironment is an immune environment including increased values of CD3 and CD8 lymphocytes, forkhead box P3 (FOXP3), T cell clonality, M1 macrophages and tertiary lymphoid structures (TLS). HPV positive status and microbiome including Akkermansia muciniphila and Enterococcus hirae are associated with positive response to immunotherapy [19].

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4.2. Neutrophil to lymphocytes ratio (NLR) – towards a new biomarker

NLR as a hematological marker of inflammation and the modified Glasgow prognostic score (mGPS) were evaluated for their predictive potential of response to treatment with Nivolumab was evaluated in HNSCC in the Japanese multi-institutional study that included 88 eligible patients. The values increased pre-treatment NLR and mGPS (=2) were associated with reduced survival. Thus, the increased NLR value was correlated with OS at 1 year and OS at one year: 45.3% vs 16.3% for cases with reduced NLR. Also mGPS low (=0) and mGPS higher (=2) was correlated with OS at 1 year of 37.4% and 26.1%, respectively. Another Japanese cohort study identifies high NLR values and a cut-off value of 5 for NLR as predictive for discontinuation of immunotherapy and treatment switch to "best supportive care" but also for unfavorable response to treatment. The authors propose the use of NLR as a biomarker for evaluating the effectiveness of immunotherapy with Nivolumab [5,6,20,21].

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The association of neutrophils with cancer progression and treatment outcomes is controversial. The association of NLR or tumor-associated neutrophils (TAN) and cancer prognosis was evaluated in a systematic reviews and meta-analyses including observational studies published in the MEDLINE, Embase and Cochrane databases. 204 meta-analyses from 86 studies met the inclusion criteria. 29% of these meta-analyses presented consistent evidence supporting the correlation between NLR and TAN and an unfavorable cancer prognosis, however, it is necessary to mention the heterogeneity of the studies including the inclusion of some cases treated with immunotherapy [25].

Immune dysfunction in relation to NLR is evaluated not only in cancer but also in space flight, a low gravity medium being associated with increased NLR values and lymphocyte dysfunctions. The study demonstrated that increased NLR values were associated with oxidative stress and the release of inflammatory cytokines triggered by simulated microgravity in laboratory mice [26].

A higher NLR ratio was significantly correlated with an unfavorable response to treatment overall survival and reduced progression-free survival in patients treated with ICI for various types of cancer. A combination of NLR with tumor mutational burden (TMB) can increase the predictive power. Thus, low NLR and high TMB associate a favorable response to ICI, while an increased NLR and a low TMB can be a biomarker for immunotherapy failure [27].The prognostic role of NLR in esophageal cancer patients treated with anti-PD-1 agents is evaluated on a group of 140 patients with unresectable or metastatic tumors treated with immunotherapy. In these cases, using Kaplan-Meier and the log-rank test, OS and progression free survival (PFS) were evaluated between patient groups. The multivariate Cox method was used to test the prognostic value of NLR. The study proposed a follow-up period of 20 months and a threshold value of NLR of 5. Pretreatment values of NLR ≥5 were associated with overall response rate (ORR), PFS and higher OS compared to cases with NLR<5 , of 46.7%, 10 months and 22 months respectively 12.1%, 3.5 months and 4.9 months. The study demonstrates that the pretreatment value of NLR can be used as a predictive biomarker of treatment response and prognosis in unresectable and metastatic esophageal cancer treated with anti-PD-1 immunotherapy [28].The association of NLR with the outcome of treatment with Nivolumab for recurrent or metastatic oral squamous cell carcinoma (OSCC) was evaluated on 13 cases in an observational study. The complete and partial response rates were 38.5% and 0%, respectively, and the rates of stable disease and progressive disease in non-responders were 77% and 53.8%, respectively. The study demonstrated a decrease in NLR in responders from a median value of 4.1 to

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3.3. In the case of non-responders, the median NLR increased from 5.6 pre-treatment to 9.4 during treatment. Also, a post-treatment value of NLR >10 was associated with an unfavorable response to post-Nivolumab salvage chemotherapy. And the cuttof value of 5 was identified as significant for unfavorable OS in the case of post-Nivoluamb NLR>5 values. The authors note the possibility of using NLR as a predictor of salvage chemotherapy post-Nivolumab [29].

The predictive and prognostic power of NLR, platelet-lymphocyte ratio (PLR), but also the dynamics of these biomarkers during treatment with programmed death-ligand 1 (PD-L1) inhibitors, as second-line treatment for non-small cell lung cancer (NSCLC) was analyzed in a study that identified the NLR value =5 as the cutoff for the PFS and OS endpoints. A PFS of 6.86 months vs 18.82 months was identified in cases with pretreatment NLR > 5 vs NLR ≤ 5. In a multivariate analysis NLR > 5 before chemotherapy and the presence of bone metastases were associated with reduced OS. The authors consider an NLR <5 as a prognostic biomarker of survival in NSCLC that benefits from Pembrolizumab immunotherapy in the second line [30].

NLR dynamics in the first 21 days after the start of immunotherapy was evaluated on a group of 509 patients with advanced cancers. And in this case, the value of 5 for NLR was identified as significant for predicting OS. The moderate decrease of NLR in the first weeks after the initiation of immunotherapy was associated with the longest OS (median of 27.8 months). Rapid variations, whether they were decreases or increases in NLr, were associated with shorter OS (median of 11.4 months). The non-linear change in NLR and the correlation with survival is reported for the first time by this study. An early decrease in NLR in the first 6 weeks of treatment is associated with a good prognosis in the case of patients with metastatic renal cancer treated with immunotherapy. At the same time, a relative change by ≥25% from baseline NLR value is associated with reduced ORR and OS in the same study [31,32].

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Table 3. NLR as a possible biomarker for different cancer types treated with ICI.

Main objective	Anatomical site of cancer	Number of case	Results/conclusion	Reference
Evaluation of NLR and TMB as biomarkers for response to ICI	Pan-cancer analysis; 16 cancer types	1714	NLR low/TMB high patient group benefit from ICI therapy	
Pretreatment NLR as a predictor biomarker of response to anti-PD-1 agents	Unresectable or metastatic esophageal cancer	140	Higher ORR and longer PFS for NLR <5; NLR ≥5 independently and significantly risc of disease progression, lower OS and poorer responise to immunotherapy	Gao et al, 2022
Prediction of response to second line Pembrolizaumab therapy using NLR and PLR	non-small cell lung cancer	119	NLR > 5 before immunotherapy showed significantly shorter mean PFS; NLR > 5 and higher PLR is correlated with poor OS	Petrova et al., 2020
Biomarker value of NLR dynamic in the first weeks of ICI treatment	advanced cancers	509	non-linear change in NLR is correlated with OS	Li et al., 2019
Pre-treatment and post-treatment NLR evaluation as predictor of outcomes	Lung cancer	2068	Higher NLR, both pre-treatment and post-treatment could predict PFS and OS for cases treated with immunotherapy	Jin et al., 2020
Correlation of delta variation of NLR during nivolumab monotherapy with OS	Gastric cancer	98	ΔNLR60 ≧ 2 in fisrt 60 days of immunothrepay is correlated with lower OS. A cutoff value of 3 was identified as being correlated with the prognosis for cases	Ota et al., 2020

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			treated with Nivolumab in monotherapy.	

4.3. Immunotherapy and HNC beyond HNSCC (focus on other histological types and second primary malignancy)

Certain histological subtypes or primary sites of HNC were not included in the CheckMate-141 trial. The study by Ueda et al. included 97 cases with these subtypes in order to evaluate the benefit of Nivolumab. Among these categories we mention nasopharyngeal squamous carcinoma and other adenoid cystic carcinoma. If for nasopharyngeal cancer the results of the study agree with those of the NCI-9742 trial, the benefit of Nivolumab being comparable to historical data, in the case of rare head and neck adenoid cystic and neuroendocrine tumors only 1 out of 14 cases was associated with a favorable response, the indication to administer immunotherapy in this case being considered premature by the authors. However, in undifferentiated tumors, Nivolumab was associated with benefit [36].

Patil et al. evaluated the benefit of a low dose of immunotherapy added to triple metronomic chemotherapy (TMC), based on data that support the OS benefit provided by metronomic chemotherapy. The phase III superiority study included relapsed or newly diagnosed patients with palliative treatment indication Eastern Cooperative Oncology Group performance status of 0-1. The TMC regimen included oral Methotrexate 9 mg/m2 once a week, Celecoxib 200 mg twice a day and Erlotinib 150 mg once a day, 20 mg Nivolumab being added every 3 weeks in the study arm. Having OS as an objective at 1 year, the results of the trial highlighted a benefit of the TMC + immunotherapy regimen (TMC-I), the median OS being 6.7 months and 10.1 months in the TMC arm and TMC-I, respectively. It should be noted that the rate of AEs is higher by 3.9% in the TMC arm. The study is proposed as paving the way for a new standard for cases with palliative indication that do not benefit from standard dose immunotherapy for various reasons [37].

SPM can be associated with radiotherapy treatment of HNC. SPM is considered an invasive cancer at a non-contiguous site diagnosed at least 6 months after the completion of radiotherapy. Analyzing the incidence of PMS in the head and neck region, Ng et al. identified a risk of 9% analyzing a group of 1512 patients, the majority of oropharyngeal cancer cases. Growth rates of 4, 10, and 25% were associated with time intervals of 5, 10, and 15 years at completion of treatment, with an estimated 5-year overall survival of 44%. Smoking is considered a risk factor not only of HNC, but also of PMS in the head and neck region. However, in the case of hypopharyngeal cancer, it is considered that SPM does not decrease OS. This observation is associated by Guo with the unfavorable prognosis of this subtype of HNC or with a shorter follow-up period of these cases. Intensity modulated radiotherapy (IMRT) is recommended as a technique for re-irradiation, taking into account the limits imposed by possible treatment related toxicities and the need to reduce the dose to organs at risk (OARs). The risk of PMS is considered proportional to the radiation dose, oral cancer and thyroid cancer are among the subtypes of PMS mentioned in the neck and neck region. If radiotherapy is associated with an increased risk of PMS, the new data report a reduction of this risk in cases treated with ICI for the first cancer. In the case of nasopharyngeal cancer, the use of intensity-modulated irradiation techniques is associated with a significantly lower risk of PMS compared to conventional radiotherapy. An irradiation dose >60 Gy is considered necessary to achieve tumor control, grade ≥3 late toxicity being estimated at 23.8%. If radiotherapy is associated with an increased risk of PMS, the new data report a reduction of this risk in cases treated with ICI for the first cancer [38-40].

4.4. Radiotherapy and immunotherapy in HNC – an alliance with perspectives

The subject of a synergistic association between immunotherapy and radiotherapy already has both the support of fundamental and clinical research. If, in the case of higher doses per fraction, the release of tumor neoantigens is the main pathophysiological substrate associated with the

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augmentation of the immune response, in the case of irradiation with the standard fractionation regimen, the reprogramming of the tumor microenvironment (TME) and the conversion of a "cold" immune phenotype into a "imflamed" phenotype could be associated with increasing response rates to immunotherapy. It is well known that in HNC the abundance of tumor infiltrating lymphocytes (TIL) has been associated with a favorable prognosis. The tumor/stroma ratio seems to have an essential role in establishing the prognosis, this proportion being dependent on the anatomical location but also on the involvement of viral etiology. The presence of CD8+ T lymphocytes, although reduced in tumors not associated with HPV, is considered prognostic. The presence of regulatory T lymphocytes (Treg) has an immunosuppressive role, and an association of a reduced proportion of CD8+ with an abundance of Treg could induce immunosuppression and reduce the response rates to immunotherapy. The exception are high Foxp3 Tregs whose high expression associated with CD8 lymphocytes and PD-L1 levels was correlated with the favorable response to the combination of radiotherapy - chemotherapy with Cisplatin or to the combination of radiotherapy - biological therapy with Cetuximab. The presence of natural killer (NK) cells is also correlated with a favorable prognosis, but is reduced in HPV negative HNC. Tumor-associated macrophages (TAM) have been associated with progression, metastasis, and the risk of recurrence after treatment. M2 macrophages are generally associated with the evolution of the disease, while M1 type macrophages have an antitumor role. For this reason, TME reprogramming with the help of radiotherapy or chemotherapy with the promotion of M1 macrophages can be an effective strategy to induce tumor regression [41-46].

The synergistic effects of immunotherapy and radiotherapy in HNC were summarized by Wong et al. in 6 main conclusions. The effects of radiotherapy and immunotherapy are shown to be synergistic by preclinical data, there is a potential for exploitation of the immune landscape characteristic of HNC cancers induced by viruses including Epstein-Barr Virus (EBV) in nasopharyngeal cancer and Human Papilloma Virus (HPV) in oropharyngeal cancer. Currently, the association between immunotherapy and radiotherapy is intensively analyzed in clinical studies, but until now there is no consensus and conclusive clinical evidence to demonstrate additional benefits brought by irradiation to immunotherapy. The sequence, dose and optimal administration time of each treatment must be identified in order to obtain a maximum synergistic potential [47].

However, the dual immunosuppressive and immunostimulatory effect of irradiation, coexisting in a fragile balance, makes it necessary to evaluate in studies the optimal therapeutic sequence. Immunosuppression induced by concurrent radio-chemotherapy-immunotherapy, but also the immunosupresion asoociated with elective irradiation of lymph nodes could be the cause of failures in clinical trials such as JAVELIN Head and Neck 100 (NCT02952586). Even if it refers to another ICI, Pembrolizumab, a sequential combination is considered optimal in the case of delivering radio-chemotherapy and immunotherapy [48-51]. Until now, at least 9 phase III trials have evaluated or are evaluating different immunotherapy agents in different sequences and associations in HNC. However, it is worth mentioning CHECKMATE 577 and PACIFIC trials, both trials with favorable results include the therapeutic sequence in which immunotherapy is administered as maintenance after radiochemotherapy and radiochemotherapy plus surgery respectively in lung and esophagus cancers. The IMvoke 010 trial (NCT03452137) addresses the concept of Atezolizumab in maintenance after definitive treatment of any type of HNC with a high risk of recurrence [48,52]. Nivolumab in combination with immunotherapy in different sequences and regimes, even if it is induction settings, concurrent setting or adjuvant/maintenance treatment associated with definitive radiotherapy in standard fractionation, SBRT or re-irradiation is evaluated for a possible synergistic association in clinical trials [Table 4][52-62].

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Table 4. Nivolumab and radiotherapy association in clinical trials.

Trial/Phase	Inclusion population	ICI treatment sequence	Status/Reason	Results
NRG HN005 NCT03952585/Phase II/III	HPV+ Oropharynx early stage, non-smokers	Concurrent Maintenance after any definitive therapy	Suspended (Scheduled Interim Monitoring)	Not reported
NCT03539198	Recurrent/metastatic HNSCC with ≥2 metastatic sites	Proton Stereotactic Body Radiotherapy (SBRT) concurrent with ICI	Terminated (Failure to accrue.)	Not reported
NCT02684253/Phase II	Recurrent/metastatic HNSCC with ≥2 metastatic sites	Stereotactic Body Radiotherapy (SBRT) concurrent with ICI	Completed	No evidence of ab-scopla effect and no superior response for SBRT + ICI patient group
NCT02764593 RTOG 3504/Phase I	Intermediate or high risk locally advanvced HNSCC	Concurrent, induction and adjuvant associated with definitive standard fractionation radiotherapy	Completed	Not reported
NCT03317327 REPORT/Phase I/II	Reccutrent HNSCC after radiotherapy and SPM	Concurrent with re-irradiation and maintenance	Recruiting	Not reported
NCT03247712/Phase I/II	Resecable locally advanced HNSCC	Neoadjuvant, SBRT, surgery and adjuvant ICI	Active, not recruiting	Not reported
NCT03576417 Nivo PostOp/Phase III	Locally advanced SCCHN with extra capsular extension and/or positive margins	Concurrent, Maintenance ICI with chemo-radiotherapy	Recruiting	Not reported

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5. Conclusions

The reported data must be interpreted within the limits generated by the small group of patients included, presenting an early experience of two academic center with Nivolumab immunotherapy in platinum-refractory HNC. It should be noted a median PFS during immunotherapy (including even adenoid cystic carcinoma) that exceeds the data from the literature, a possible explanation being the possible benefit of Nivolumab for cases with nadir NLR values <3.5 and the intensive chemotherapy and radiotherapy treatment delivered in most of the cases. The administration of radiotherapy and chemotherapy during the evolution of the disease could be a potentiation factor that augment the immune response by modulation of TME. To our knowledge, these are the first two cases of PMS in the head neck region treated with Nivolumab. The inaccessibility of a more conformal irradiation technique limits the irradiation with maximum doses and also the association of concurrent chemotherapy. The reported data support a possible benefit of the sequential administration of radiotherapy, immunotherapy and SPM. Higher positive dynamic of NLR could be associated with worst outcome during immunotherapy. All study observations including the role of infections and antibiotic treatment as well as the possible biomarker value of NLR variation during immunotherapy should be investigated in multicenter clinical trials to be validated.

References

1. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, Geese WJ, Kopit J, Shaw JW, Gillison ML. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016 Nov 10;375(19):1856-1867. doi: 10.1056/NEJMoa1602252.
2. Mireștean CC, Iancu RI, Iancu DPT. Micro-RNAs, the Cornerstones of the Future of Radiobiology in Head and Neck Cancers? Curr Oncol. 2022 Feb 2;29(2):816-833. doi: 10.3390/curroncol29020069.
3. Economopoulou P, Perisanidis C, Giotakis EI, Psyrri A. The emerging role of immunotherapy in head and neck squamous cell carcinoma (HNSCC): anti-tumor immunity and clinical applications. Ann Transl Med. 2016 May;4(9):173. doi: 10.21037/atm.2016.03.34.
4. Iancu RI, Zara AD, Mirestean CC, Iancu DPT. Radiomics in Head and Neck Cancers Radiotherapy. Promises and Challenges. Maedica (Bucur). 2021 Sep;16(3):482-488. doi: 10.26574/maedica.2020.16.3.482.
5. Hsieh JC, Wang HM, Wu MH, Chang KP, Chang PH, Liao CT, Liau CT. Review of emerging biomarkers in head and neck squamous cell carcinoma in the era of immunotherapy and targeted therapy. Head Neck. 2019 Oct;41 Suppl 1:19-45. doi: 10.1002/hed.25932.
6. Affolter A, Kern J, Bieback K, Scherl C, Rotter N, Lammert A. Biomarkers and 3D models predicting response to immune checkpoint blockade in head and neck cancer (Review). Int J Oncol. 2022 Jul;61(1):88. doi: 10.3892/ijo.2022.5378.
7. Iancu, D. T., Iancu, Roxana Irina. Romanian Journal of Functional & Clinical, Macro- & Microscopical Anatomy & of Anthropology / Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie . 2015, Vol. 14 Issue 4, p610-618. 9p.
8. Trivedi S, Sun L, Aggarwal C. Immunotherapy for Head and Neck Cancer. Hematol Oncol Clin North Am. 2021 Oct;35(5):1021-1037. doi: 10.1016/j.hoc.2021.05.010.
9. Gillison ML, Blumenschein G, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington KJ, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Jayaprakash V, Wei L, Ferris RL. Long-term Outcomes with Nivolumab as First-line Treatment in Recurrent or Metastatic Head and Neck Cancer: Subgroup Analysis of CheckMate 141. Oncologist. 2022 Mar 4;27(2):e194-e198. doi: 10.1093/oncolo/oyab036.
10. Pignon JP, le Maître A, Maillard E, Bourhis J. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiother Oncol 2009;92:4-14
11. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, Geese WJ, Kopit J, Shaw JW, Gillison ML. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016 Nov 10;375(19):1856-1867. doi: 10.1056/NEJMoa1602252.
12. Guigay J, Aupérin A, Fayette J, Saada-Bouzid E, Lafond C, Taberna M, Geoffrois L, Martin L, Capitain O, Cupissol D, Castanie H, Vansteene D, Schafhausen P, Johnson A, Even C, Sire C, Duplomb S, Evrard C, Delord JP, Laguerre B, Zanetta S, Chevassus-Clément C, Fraslin A, Louat F, Sinigaglia L, Keilholz U,

Bourhis J, Mesia R; GORTEC; AIO; TTCC, and UniCancer Head and Neck groups. Cetuximab, docetaxel, and cisplatin versus platinum, fluorouracil, and cetuximab as first-line treatment in patients with recurrent or metastatic head and neck squamous-cell carcinoma (GORTEC 2014-01 TPExtreme): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol. 2021 Apr;22(4):463-475. doi: 10.1016/S1470-2045(20)30755-5.

13. Borel C, Jung AC, Burgy M. Immunotherapy Breakthroughs in the Treatment of Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma. Cancers (Basel). 2020 Sep 21;12(9):2691. doi: 10.3390/cancers12092691.
14. Cohen EEW, Soulières D, Le Tourneau C, Dinis J, Licitra L, Ahn MJ, Soria A, Machiels JP, Mach N, Mehra R, Burtness B, Zhang P, Cheng J, Swaby RF, Harrington KJ; KEYNOTE-040 investigators. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet. 2019 Jan 12;393(10167):156-167. doi: 10.1016/S0140-6736(18)31999-8.
15. Botticelli A, Cirillo A, Strigari L, Valentini F, Cerbelli B, Scagnoli S, Cerbelli E, Zizzari IG, Rocca CD, D'Amati G, Polimeni A, Nuti M, Merlano MC, Mezi S, Marchetti P. Anti-PD-1 and Anti-PD-L1 in Head and Neck Cancer: A Network Meta-Analysis. Front Immunol. 2021 Aug 9;12:705096. doi: 10.3389/fimmu.2021.705096.
16. Haddad R, Concha-Benavente F, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Kasper S, Vokes EE, Worden F, Saba NF, Tahara M, Jayaprakash V, Lynch M, Li L, Gillison ML, Harrington KJ, Ferris RL. Nivolumab treatment beyond RECIST-defined progression in recurrent or metastatic squamous cell carcinoma of the head and neck in CheckMate 141: A subgroup analysis of a randomized phase 3 clinical trial. Cancer. 2019 Sep 15;125(18):3208-3218. doi: 10.1002/cncr.32190.
17. Ueki Y, Takahashi T, Ota H, Shodo R, Yamazaki K, Horii A. Role of programmed death-ligand 1 in predicting the treatment outcome of salvage chemotherapy after nivolumab in recurrent/metastatic head and neck squamous cell carcinoma. Head Neck. 2020 Nov;42(11):3275-3281. doi: 10.1002/hed.26374.
18. Okamoto I, Tsukahara K, Sato H. Single-center prospective study on the efficacy of nivolumab against platinum-sensitive recurrent or metastatic head and neck squamous cell carcinoma. Sci Rep. 2022 Feb 7;12(1):2025. doi: 10.1038/s41598-022-06084-z.
19. Gavrielatou N, Doumas S, Economopoulou P, Foukas PG, Psyrri A. Biomarkers for immunotherapy response in head and neck cancer. Cancer Treat Rev. 2020 Mar;84:101977. doi: 10.1016/j.ctrv.2020.101977.
20. Matsuki T, Okamoto I, Fushimi C, Sawabe M, Kawakita D, Sato H, Tsukahara K, Kondo T, Okada T, Tada Y, Miura K, Omura G, Yamashita T. Hematological predictive markers for recurrent or metastatic squamous cell carcinomas of the head and neck treated with nivolumab: A multicenter study of 88 patients. Cancer Med. 2020 Jul;9(14):5015-5024. doi: 10.1002/cam4.3124.
21. Ueda T, Chikuie N, Takumida M, Furuie H, Kono T, Taruya T, Hamamoto T, Hattori M, Ishino T, Takeno S. Baseline neutrophil-to-lymphocyte ratio (NLR) is associated with clinical outcome in recurrent or metastatic head and neck cancer patients treated with nivolumab. Acta Otolaryngol. 2020 Feb;140(2):181-187. doi: 10.1080/00016489.2019.1699250.
22. Yasumatsu R, Wakasaki T, Hashimoto K, Nakashima K, Manako T, Taura M, Matsuo M, Nakagawa T. Monitoring the neutrophil-to-lymphocyte ratio may be useful for predicting the anticancer effect of nivolumab in recurrent or metastatic head and neck cancer. Head Neck. 2019 Aug;41(8):2610-2618. doi: 10.1002/hed.25737.
23. Tanoue K, Tamura S, Kusaba H, Shinohara Y, Ito M, Tsuchihashi K, Shirakawa T, Otsuka T, Ohmura H, Isobe T, Ariyama H, Koreishi S, Matsushita Y, Shimokawa H, Tanaka R, Mitsugi K, Akashi K, Baba E. Predictive impact of C-reactive protein to albumin ratio for recurrent or metastatic head and neck squamous cell carcinoma receiving nivolumab. Sci Rep. 2021 Feb 2;11(1):2741. doi: 10.1038/s41598-021-82448-1.
24. Lee YG, Chang H, Keam B, Chun SH, Park J, Park KU, Shin SH, An HJ, Lee KE, Lee KW, Kim HR, Kim SB, Ahn MJ, Hwang IG. Outcomes and Biomarkers of Immune Checkpoint Inhibitor Therapy in Patients with Refractory Head and Neck Squamous Cell Carcinoma: KCSG HN18-12. Cancer Res Treat. 2021 Jul;53(3):671-677. doi: 10.4143/crt.2020.824
25. Cupp MA, Cariolou M, Tzoulaki I, Aune D, Evangelou E, Berlanga-Taylor AJ. Neutrophil to lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. BMC Med. 2020 Nov 20;18(1):360. doi: 10.1186/s12916-020-01817-1.

26. Paul AM, Mhatre SD, Cekanaviciute E, Schreurs AS, Tahimic CGT, Globus RK, Anand S, Crucian BE, Bhattacharya S. Neutrophil-to-Lymphocyte Ratio: A Biomarker to Monitor the Immune Status of Astronauts. Front Immunol. 2020 Nov 2;11:564950. doi: 10.3389/fimmu.2020.564950.
27. Valero C, Lee M, Hoen D, Weiss K, Kelly DW, Adusumilli PS, Paik PK, Plitas G, Ladanyi M, Postow MA, Ariyan CE, Shoushtari AN, Balachandran VP, Hakimi AA, Crago AM, Long Roche KC, Smith JJ, Ganly I, Wong RJ, Patel SG, Shah JP, Lee NY, Riaz N, Wang J, Zehir A, Berger MF, Chan TA, Seshan VE, Morris LGT. Pretreatment neutrophil-to-lymphocyte ratio and mutational burden as biomarkers of tumor response to immune checkpoint inhibitors. Nat Commun. 2021 Feb 1;12(1):729. doi: 10.1038/s41467-021-20935-9.
28. Gao Y, Zhang Z, Li Y, Chen S, Lu J, Wu L, Ma Z, Hu Y, Zhang G. Pretreatment Neutrophil-to-Lymphocyte Ratio as a Prognostic Biomarker in Unresectable or Metastatic Esophageal Cancer Patients With Anti-PD-1 Therapy. Front Oncol. 2022 Apr 13;12:834564. doi: 10.3389/fonc.2022.834564.
29. Tachinami H, Tomihara K, Ikeda A, Sekido K, Sakurai K, Imaue S, Fujiwara K, Hayashi R, Noguchi M. [Neutrophil-to-Lymphocyte Ratio(NLR)as a Predictive Indicator of the Response to Nivolumab in Patients with Oral Squamous Cell Carcinoma]. Gan To Kagaku Ryoho. 2021 Dec;48(12):1485-1490. Japanese.
30. Petrova MP, Eneva MI, Arabadjiev JI, Conev NV, Dimitrova EG, Koynov KD, Karanikolova TS, Valev SS, Gencheva RB, Zhbantov GA, Ivanova AI, Sarbianova II, Timcheva CV, Donev IS. Neutrophil to lymphocyte ratio as a potential predictive marker for treatment with pembrolizumab as a second line treatment in patients with non-small cell lung cancer. Biosci Trends. 2020 Mar 16;14(1):48-55. doi: 10.5582/bst.2019.01279.
31. Li M, Spakowicz D, Burkart J, Patel S, Husain M, He K, Bertino EM, Shields PG, Carbone DP, Verschraegen CF, Presley CJ, Otterson GA, Kendra K, Owen DH. Change in neutrophil to lymphocyte ratio during immunotherapy treatment is a non-linear predictor of patient outcomes in advanced cancers. J Cancer Res Clin Oncol. 2019 Oct;145(10):2541-2546. doi: 10.1007/s00432-019-02982-4.
32. Lalani AA, Xie W, Martini DJ, Steinharter JA, Norton CK, Krajewski KM, Duquette A, Bossé D, Bellmunt J, Van Allen EM, McGregor BA, Creighton CJ, Harshman LC, Choueiri TK. Change in Neutrophil-to-lymphocyte ratio (NLR) in response to immune checkpoint blockade for metastatic renal cell carcinoma. J Immunother Cancer. 2018 Jan 22;6(1):5. doi: 10.1186/s40425-018-0315-0.
33. Jin J, Yang L, Liu D, Li W. Association of the neutrophil to lymphocyte ratio and clinical outcomes in patients with lung cancer receiving immunotherapy: a meta-analysis. BMJ Open. 2020 Jun 3;10(6):e035031. doi: 10.1136/bmjopen-2019-035031.
34. Ota Y, Takahari D, Suzuki T, Osumi H, Nakayama I, Oki A, Wakatsuki T, Ichimura T, Ogura M, Shinozaki E, Suenaga M, Chin K, Yamaguchi K. Changes in the neutrophil-to-lymphocyte ratio during nivolumab monotherapy are associated with gastric cancer survival. Cancer Chemother Pharmacol. 2020 Feb;85(2):265-272. doi: 10.1007/s00280-019-04023-w.
35. Yamada T, Hayashi T, Inokuchi Y, Hayashi K, Watanabe H, Komori K, Kano K, Shimoda Y, Fujikawa H, Shiozawa M, Morinaga S, Rino Y, Masuda M, Ogata T, Oshima T. Impact of the Neutrophil-to-Lymphocyte Ratio on the Survival of Patients with Gastric Cancer Treated with Nivolumab Monotherapy. Target Oncol. 2020 Jun;15(3):317-325. doi: 10.1007/s11523-020-00716-y.
36. Sato Y, Fukuda N, Wang X, Urasaki T, Ohmoto A, Nakano K, Yunokawa M, Ono M, Sato Y, Mitani H, Tomomatsu J, Takahashi S. Efficacy of Nivolumab for Head and Neck Cancer Patients with Primary Sites and Histological Subtypes Excluded from the CheckMate-141 Trial. Cancer Manag Res. 2020 Jun 3;12:4161-4168. doi: 10.2147/CMAR.S249393.
37. Patil VM, Noronha V, Menon N, Rai R, Bhattacharjee A, Singh A, Nawale K, Jogdhankar S, Tambe R, Dhumal S, Sawant R, Alone M, Karla D, Peelay Z, Pathak S, Balaji A, Kumar S, Purandare N, Agarwal A, Puranik A, Mahajan A, Janu A, Kumar Singh G, Mittal N, Yadav S, Banavali S, Prabhash K. Low-Dose Immunotherapy in Head and Neck Cancer: A Randomized Study. J Clin Oncol. 2022 Oct 20:JCO2201015. doi: 10.1200/JCO.22.01015.
38. Lee HI, Kim JH, Ahn SH, Chung EJ, Keam B, Eom KY, Jeong WJ, Kim JW, Wee CW, Wu HG. Re-irradiation for recurrent or second primary head and neck cancer. Radiat Oncol J. 2021 Dec;39(4):279-287. doi: 10.3857/roj.2021.00640.
39. Zhao W, Lei H, Zhu X, Li L, Qu S, Liang X, Wang X. The clinical characteristics of secondary primary tumors in patients with nasopharyngeal carcinoma after intensity-modulated radiotherapy: A retrospective analysis. Medicine (Baltimore). 2016 Nov;95(45):e5364. doi: 10.1097/MD.0000000000005364.
40. Ng SP, Pollard C 3rd, Kamal M, Ayoub Z, Garden AS, Bahig H, Gunn GB, Frank SJ, Skinner HD, Phan J, Berends J, Morrison WH, Johnson JM, Ferrarotto R, Sturgis EM, Mohamed ASR, Lai SY, Fuller CD,

Rosenthal DI. Risk of second primary malignancies in head and neck cancer patients treated with definitive radiotherapy. NPJ Precis Oncol. 2019 Sep 27;3:22. doi: 10.1038/s41698-019-0097-y.

41. Manukian G, Bar-Ad V, Lu B, Argiris A, Johnson JM. Combining Radiation and Immune Checkpoint Blockade in the Treatment of Head and Neck Squamous Cell Carcinoma. Front Oncol. 2019 Mar 6;9:122. doi: 10.3389/fonc.2019.00122.
42. Biau J, Bourhis J. Combining immunotherapy and radiotherapy in head and neck squamous cell cancers: which perspectives? Curr Opin Oncol. 2020 May;32(3):196-202. doi: 10.1097/CCO.0000000000000628.
43. Ou D, Adam J, Garberis I, Blanchard P, Nguyen F, Levy A, Casiraghi O, Gorphe P, Breuskin I, Janot F, Temam S, Scoazec JY, Deutsch E, Tao Y. Clinical relevance of tumor infiltrating lymphocytes, PD-L1 expression and correlation with HPV/p16 in head and neck cancer treated with bio- or chemo-radiotherapy. Oncoimmunology. 2017 Jul 5;6(9):e1341030. doi: 10.1080/2162402X.2017.1341030.
44. Mireştean CC, Crişan A, Buzea C, Iancu RI, Iancu DT. Synergies Radiotherapy-Immunotherapy in Head and Neck Cancers. A New Concept for Radiotherapy Target Volumes-"Immunological Dose Painting". Medicina (Kaunas). 2020 Dec 23;57(1):6. doi: 10.3390/medicina57010006.
45. Genard G, Lucas S, Michiels C. Reprogramming of Tumor-Associated Macrophages with Anticancer Therapies: Radiotherapy versus Chemo- and Immunotherapies. Front Immunol. 2017 Jul 14;8:828. doi: 10.3389/fimmu.2017.00828.
46. Mandal R, Şenbabaoğlu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, Lee KW, Ganly I, Hakimi AA, Chan TA, Morris LG. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight. 2016 Oct 20;1(17):e89829. doi: 10.1172/jci.insight.89829.
47. Wong KCW, Johnson D, Hui EP, Lam RCT, Ma BBY, Chan ATC. Opportunities and challenges in combining immunotherapy and radiotherapy in head and neck cancers. Cancer Treat Rev. 2022 Apr;105:102361. doi: 10.1016/j.ctrv.2022.102361.
48. Lee NY, Ferris RL, Psyrri A, Haddad RI, Tahara M, Bourhis J, Harrington K, Chang PM, Lin JC, Razaq MA, Teixeira MM, Lövey J, Chamois J, Rueda A, Hu C, Dunn LA, Dvorkin MV, De Beukelaer S, Pavlov D, Thurm H, Cohen E. Avelumab plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck: a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. Lancet Oncol. 2021 Apr;22(4):450-462. doi: 10.1016/S1470-2045(20)30737-3.
49. https://www.healio.com/news/hematology-oncology/20220609/sequential-pembrolizumab-may-be-optimal-timing-with-chemoradiotherapy-in-headneck-cancer Accesed on March 26, 2023
50. Qian JM, Schoenfeld JD. Radiotherapy and Immunotherapy for Head and Neck Cancer: Current Evidence and Challenges. Front Oncol. 2021 Feb 3;10:608772. doi: 10.3389/fonc.2020.608772.
51. Mireștean CC, Iancu RI, Iancu DT. Immunotherapy and Radiotherapy as an Antitumoral Long-Range Weapon-A Partnership with Unsolved Challenges: Dose, Fractionation, Volumes, Therapeutic Sequence. Curr Oncol. 2022 Oct 2;29(10):7388-7395. doi: 10.3390/curroncol29100580.
52. https://clinicaltrials.gov/ct2/show/NCT03452137 Accesed on 2023 April 10
53. https://clinicaltrials.gov/ct2/show/NCT03952585 Accesed on 2023 April 10
54. https://clinicaltrials.gov/ct2/show/NCT03539198 Accesed on 2023 April 10
55. https://clinicaltrials.gov/ct2/show/results/NCT02684253 Accesed on 2023 April 10
56. McBride S, Sherman E, Tsai CJ, Baxi S, Aghalar J, Eng J, Zhi WI, McFarland D, Michel LS, Young R, Lefkowitz R, Spielsinger D, Zhang Z, Flynn J, Dunn L, Ho A, Riaz N, Pfister D, Lee N. Randomized Phase II Trial of Nivolumab With Stereotactic Body Radiotherapy Versus Nivolumab Alone in Metastatic Head and Neck Squamous Cell Carcinoma. J Clin Oncol. 2021 Jan 1;39(1):30-37. doi: 10.1200/JCO.20.00290.
57. https://clinicaltrials.gov/ct2/show/NCT02764593 Accesed on 2023 April 10
58. https://clinicaltrials.gov/ct2/show/NCT03317327 Accesed on 2023 April 10
59. https://clinicaltrials.gov/ct2/show/NCT03247712 Accesed on 2023 April 10
60. https://clinicaltrials.gov/ct2/show/NCT03576417 Accesed on 2023 April 10
61. Qian JM, Schoenfeld JD. Radiotherapy and Immunotherapy for Head and Neck Cancer: Current Evidence and Challenges. Front Oncol. 2021 Feb 3;10:608772. doi: 10.3389/fonc.2020.608772.
62. Yu Y, Zakeri K, Lee N. Javelin Head Neck 100: Should we combine immunotherapy with radiation therapy? Oncotarget. 2021 Oct 12;12(21):2223-2226. doi: 10.18632/oncotarget.27987.

 doi:10.20944/preprints202305.0561.v1