Thomas Jefferson University

Jefferson Digital Commons

Department of Medical Oncology Faculty Papers

Department of Medical Oncology

8-1-2023

Nivolumab and ipilimumab in combination with radiotherapy in patients with high-risk locally advanced squamous cell carcinoma of the head and neck.

Jennifer Johnson

Ioannis A. Vathiotis

Larry Harshyne

Ayesha Ali

Voichita Bar-Ad

See next page for additional authors

Follow this and additional works at: https://jdc.jefferson.edu/medoncfp

 Part of the Immunotherapy Commons, Neoplasms Commons, and the Oncology Commons

Let us know how access to this document benefits you

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's Center for Teaching and Learning (CTL). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Medical Oncology Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Jennifer Johnson, Ioannis A. Vathiotis, Larry Harshyne, Ayesha Ali, Voichita Bar-Ad, Rita S. Axelrod, Emily Lorber, Joseph Curry, David Cognetti, Adam J. Luginbuhl, Madalina Tuluc, Scott W Keith, M.G. Mahoney, and Athanassios Argiris

Journal for ImmunoTherapy of Cancer

Nivolumab and ipilimumab in combination with radiotherapy in patients with high-risk locally advanced squamous cell carcinoma of the head and neck

Jennifer M Johnson,[1,2] Ioannis A Vathiotis ,[1] Larry A Harshyne,[1,3] Ayesha Ali,[4] Voichita Bar Ad,[4] Rita Axelrod,[1] Emily Lorber,[1] Joseph Curry,[2] David M Cognetti,[2] Adam J Luginbuhl,[2] Madalina Tuluc,[5] Scott Keith,[6] Mỹ G Mahoney,[2,7] Athanassios Argiris[1]

To cite: Johnson JM, Vathiotis IA, Harshyne LA, *et al*. Nivolumab and ipilimumab in combination with radiotherapy in patients with high-risk locally advanced squamous cell carcinoma of the head and neck. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e007141. doi:10.1136/jitc-2023-007141

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2023-007141).

Accepted 16 July 2023

Check for updates

© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Dr. Jennifer M Johnson; Jennifer.M.Johnson@Jefferson.edu

ABSTRACT

Background The combination of nivolumab and ipilimumab has been approved for the treatment of multiple solid tumors. This was a phase I study investigating definitive radioimmunotherapy (RIT) with nivolumab and ipilimumab for the treatment of locally advanced (LA) squamous cell carcinoma of the head and neck (SCCHN).

Methods Patients with newly diagnosed, stage IVA–IVB SCCHN eligible for cisplatin-based chemotherapy received nivolumab (3 mg/kg every 2 weeks for a total of 17 doses) and ipilimumab (1 mg/kg every 6 weeks for a total of 6 doses) starting 2 weeks prior to radiotherapy. The primary endpoint was safety of definitive RIT. Secondary endpoints included progression-free survival (PFS) and overall survival (OS). Exploratory endpoints included the association of baseline programmed death-ligand 1 (PD-L1) expression as well as on-treatment changes in immune bias with treatment outcomes.

Results Twenty-four patients were enrolled. With a median follow-up of 36.1 months, grade 3 or higher treatment-related adverse events were reported in 21 individuals (88%); 5 individuals developed in-field soft tissue ulceration during consolidation immunotherapy, resulting in one fatality. The 3-year PFS and OS rates were 74% (95% CI 58% to 94%) and 96% (95% CI 88% to 100%), respectively. PD-L1 combined positive score (CPS) did not correlate with death or disease progression. Decreases in extracellular vesicle PD-L1 within the concurrent RIT phase were associated with prolonged PFS (p=0.006). Also, interval decreases in circulating interleukin (IL)4, IL9, IL12, and IL17a during concurrent RIT were associated with subsequent ulceration.

Conclusions Definitive RIT with nivolumab and ipilimumab has sufficient clinical activity to support further development. Early changes in circulating biomarkers appear able to predict treatment outcomes as well as ensuing in-field soft tissue ulceration.

Trial registration number NCT03162731.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Definitive chemoradiotherapy with cisplatin results in suboptimal efficacy with substantial toxicity in patients with locally advanced squamous cell carcinoma of the head and neck (LA SCCHN).

WHAT THIS STUDY ADDS

⇒ This is the first reported study that assessed concurrent radioimmunotherapy (RIT) with nivolumab and ipilimumab as definitive treatment in LA SCCHN. Definitive RIT was safe overall and demonstrated clinical efficacy with excellent locoregional control; rates of soft tissue ulceration were higher than usual, representing a late in-field toxicity. Biomarker analysis revealed associations with efficacy and safety outcomes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provided evidence that supports further development of definitive RIT with nivolumab and ipilimumab in patients with LA SCCHN.

BACKGROUND

More than 60% of patients with squamous cell carcinoma of the head and neck (SCCHN) present with locally advanced (LA) disease, marked by local extension and/or metastasis to regional lymph nodes.[1] Multimodality treatment employing surgery, radiotherapy, and/or systemic therapy represents the current standard approach for LA SCCHN with the choice of therapy depending on the primary tumor site, stage, patient factors and local expertise.[2] Despite definitive treatment 5-year survival rates are below 50%, with prognosis being worse for patients with high-risk human papillomavirus (HPV)-negative tumors.[3–6] In addition, treatment with standard cisplatin-based regimens results in substantial toxicity that may compromise patient function and quality of life.[7–10] All the above underlines an unmet need to improve patient outcomes and, at the same time, reduce toxicity.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

The successful implementation of programmed cell death protein 1 (PD-1) inhibitors in recurrent or metastatic (R/M) SCCHN has mirrored the development of immunotherapy in other tumor types. Overarching directions in ongoing research have been to evaluate the potential role of PD-1 inhibitors in less advanced disease, that is, in the curative setting, and to improve efficacy through combination of PD-1 inhibitors with other agents and/or treatment modalities. The anti-PD-1 antibody nivolumab and the anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibody ipilimumab have complementary mechanisms of action and their combination is supported by preclinical observations. The combination of nivolumab and ipilimumab has proven benefit in many solid tumors.[11 12] In SCCHN it showed numerical improvement in overall survival versus the ErbituX in first-line Treatment of REcurrent or MEtatatic head and neck cancer (EXTREME) regimen in previously untreated patients with R/M SCCHN (CheckMate 651) and produced promising results in the preoperative setting.[13–16] Addition of radiotherapy appears to further shape the antitumor immune response through enhanced antigen presentation and adjuvant signaling, release of cytokines and/or chemokines, and upregulation of immune checkpoints.[17] Twyman Saint-Victor *et al* suggested that the increased efficacy of triple therapy could be explained by non-redundant mechanisms of action for each treatment; while CTLA4 blockade primarily decreases regulatory T cells and programmed death-ligand 1 (PD-L1) blockade essentially reverses exhaustion of CD8-positive T cells, radiation therapy diversifies the T-cell receptor repertoire within the tumor microenvironment.[18]

Based on this rationale, we performed a phase I pilot study to evaluate definitive treatment with nivolumab, ipilimumab and radiation therapy in cisplatin-eligible patients with LA SCCHN. We leveraged concurrent radiotherapy as an immune adjuvant to flip the immunosuppressive status of the tumor microenvironment. Furthermore, using combination immunotherapy, we endeavored to minimize the risks associated with platinum-based chemotherapy, without blunting antitumor efficacy.

METHODS

Study design and participants

We conducted a single-institution, single-arm, phase I pilot study at Thomas Jefferson University, Philadelphia, Pennsylvania, USA. The study protocol is included in online supplemental materials. Eligible patients were ≥18 years old and had pathologically confirmed, newly diagnosed clinical stage III–IVB (American Joint Committee on Cancer (AJCC), Eighth Edition) HPV-negative (oral cavity, hypopharynx, larynx or p16-negative oropharynx) or clinical stage II–III (AJCC, Eighth Edition) high-risk (either N2 or if T3N0 or T3N1 with ≥20 pack-years smoking history) HPV-positive (p16-positive oropharynx) SCCHN, tumor samples available for PD-L1 testing, measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1, Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-1, and adequate marrow and organ function (white blood cell count ≥ 2×10^9/L, absolute neutrophil count ≥ 1.5×10^9/L, platelet count ≥ 100×10^9/L, hemoglobin ≥ 9 g/L, bilirubin ≤1.5× upper limit of normal (ULN), aspartate aminotransferase (AST) and alanine transaminase (ALT) ≤3× ULN, glomerular filtration rate ≥ 40 mL/min or serum creatinine ≤ 1.5 ULN). Key exclusion criteria included diagnosis of primary nasopharyngeal carcinoma, presence of brain metastases, prior radiation therapy to the neck, known contraindications to radiation therapy, prior immunotherapy, immunodeficiency or concurrent use of systemic steroids greater than or equal to the equivalent of 10 mg of prednisone daily, HIV, hepatitis B or C, concurrent malignancies, active autoimmune disease, and interstitial lung disease or non-infectious pneumonitis.

Study procedures

Study participants received nivolumab (Bristol Myers Squibb) 3 mg per kg of body weight intravenously one time every 2 weeks plus ipilimumab (Bristol Myers Squibb) 1 mg per kg of body weight intravenously one time every 6 weeks (figure 1). Immunotherapy started 2 weeks prior to radiotherapy, continued concurrently with radiotherapy, and as consolidation therapy for a total of 17 doses of nivolumab and 6 doses of ipilimumab. Radiotherapy consisted of simultaneous-integrated boost intensity-modulated radiation therapy (SIB-IMRT), or volumetric modulated arc therapy delivered in 2 Gy daily fractions, 5 days per week, to a total of 70 Gy over 7 weeks.

Tumor assessments by 18F-Fludeoxyglucose (FDG) positron emission tomography (PET)/CT were done at baseline (up to 28 days before the date of the first dose

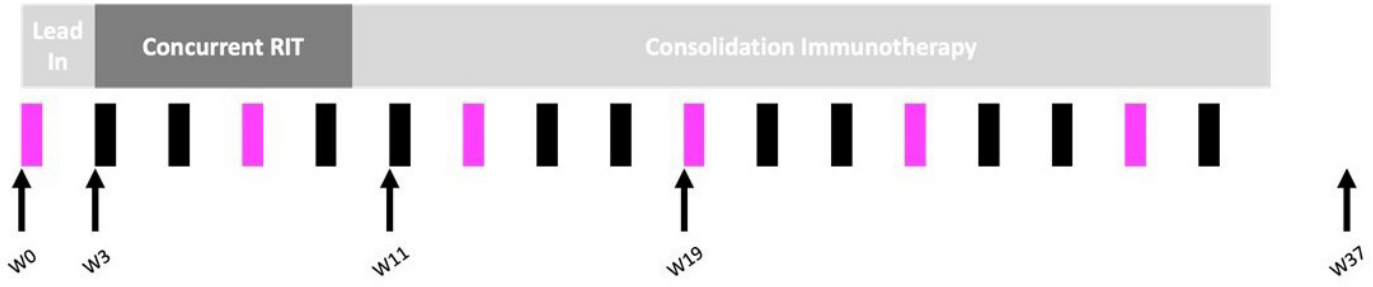

Figure 1 Study design. Pink bars represent doses of ipilimumab and nivolumab and black bars represent nivolumab alone. RIT, radioimmunotherapy; W0, week 0; W3, week 3; W11, week 11; W19, week 19; W37, week 37.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

of study drug) and 12 weeks after the completion of radiation therapy. Tumor response was investigator assessed according to RECIST, V.1.1. Patients were followed every 3 months for 6 months after study completion and evaluated for treatment-related adverse events (TRAEs), disease recurrence, and survival.

All TRAEs were collected prospectively throughout the study and during follow-up and graded according to the Common Terminology Criteria for Adverse Events V.5.0. The study was designed in two stages with safety stopping rules in place for grade 4–5 in-field toxicities occurring during and up to 2 weeks after radiation therapy. The first stage cohort included 12 patients; enrollment was suspended until the last patient had completed radiotherapy and toxicities had been assessed 2 weeks after radiotherapy completion. Twelve additional patients comprised the second stage (expansion) cohort in order to further assess safety and evaluate treatment efficacy. The limit for unacceptable toxicity was set at 25% (three instances) among the first 12 patients and at 21% (five instances) among the total of 24 patients. There was no dose escalation or de-escalation planned in this study. Written informed consent was obtained from all study participants per institutional guidelines. The study was conducted in accordance with the Declaration of Helsinki, Belmont Report, and the US Common Rule.

Outcomes

The primary endpoint of the study was safety of the combination of nivolumab and ipilimumab with radiation therapy as definitive treatment for patients with LA SCCHN, evaluated by the incidence of TRAEs of any grade occurring up to 6 months after the last dose of study drug. Secondary endpoints were objective response rate, 1-year progression-free survival (PFS), and 1-year overall survival (OS). Exploratory endpoints included the predictive value of PD-L1 CPS for treatment response as well as changes in immune bias, cell subset frequencies and circulating extracellular vesicles after combination therapy and their association with outcome.

Biomarker studies

Tumor PD-L1 expression was evaluated in recent archival or fresh pretreatment biopsies using automated immunohistochemistry (IHC; PD-L1 IHC 28–8 pharmDx assay; Dako, Agilent Technologies, Santa Clara, California, USA). PD-L1 expression by tumor cells and/or tumor-associated immune cells was determined using a combined positive score (CPS; number of PD-L1-positive cells (tumor cells, lymphocytes, macrophages)/total number of viable tumor cells ×100).

Whole blood samples for correlative analysis were obtained and processed at baseline and weeks 3 (preradiation), 11 (completion of radiation), 19 (consolidation therapy), and 37 (end of treatment) or in case of premature discontinuation. MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A (Millipore, HCYTA-60K) was used to identify cytokines present in sera collected from trial patients. Samples were analyzed in triplicate by a FLEXMAP 3D (Luminex). Peripheral blood mononuclear cells were stained with fluorescent-conjugated monoclonal antibodies specific for human CD3 (SK7, BioLegend), CD4 (RPA-T4, BD Biosciences), CD8 (REA734, Miltenyi Biotec), CD14 (M5E2), CD19 (SJ25C1), CD45 (H130), CD56 (B159, all from BD Biosciences), CD163 (215927, R&D Systems), and CD204 (REA460, Miltenyi Biotec) and analyzed by flow cytometry in a Fortessa (BD Biosciences). Finally, for the extracellular vesicle (EV) analysis, plasma from trial patients were stained with monoclonal antibodies specific for CD63 (H5C6), CD71 (M-A172), CD81 (1D6), and PD-L1 (MIH1) and a fluorescent dye to label lipid membranes (3,3′-Dioctadecyloxacarbocyanine perchlorate, Thermo Fisher); samples were immediately analyzed by flow cytometry in a Fortessa (BD Biosciences; online supplemental figure 1).

Statistical analysis

Descriptive statistics were used for demographic, safety, and treatment response analysis. Kaplan-Meier estimates of PFS and OS with 95% CIs were determined and differences between categories assessed using the logrank test. PFS was defined as time (days) from the day of the first dose of study drug to first disease progression event (new primary, recurrence, distant metastasis, or death from disease) or death from any cause; OS was defined as time (days) from the day of the first dose of study drug to death.

All biomarker analyses were exploratory. Univariate Cox proportional hazards regression modeling was used to evaluate the association of circulating cytokines, immune cells, and EVs with PFS. Welch's t-test was used to compare the mean levels of circulating cytokines, immune cells, and EVs of those with to those without soft tissue ulceration.

All hypothesis testing was performed at a two-sided significance level of α=0.05 with adjustment for multiple testing (Benjamini-Hochberg method, false discovery rate <0.05) where applicable.[19] Statistical analyses were carried out using the software package R V.3.6.1 (R Foundation for Statistical Computing) and GraphPad Prism software (V.9; GraphPad Software) based on the database as of August 1, 2022.

RESULTS

Between July 13, 2017, and July 11, 2019, 24 patients provided consent and were enrolled in the study. At data cut-off, the median follow-up was 41.0 months. The median age of the study cohort was 61 years (range 48–77). Most study participants were men (83%) with ECOG PS 0 (58%). Sixteen patients had a primary tumor localized in the oropharynx (14 HPV-positive and 2 HPV-negative), 6 in the larynx, and 2 in the hypopharynx. Eight patients had stage II, 6 had stage III and 10 had stage IVA disease (AJCC, Eighth Edition). Among patients with quantifiable

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

Table 1 Patient characteristics

Characteristic	N (%)
Total	24 (100)
Age	
Median (range)	60.5 (48–77)
Gender	
Male	20 (83)
Female	4 (17)
ECOG performance status	
0	14 (58)
1	10 (42)
Location of primary tumor	
Oropharynx	16 (66)
p16+	14 (88)
p16–	2 (12)
Larynx	6 (25)
Hypopharynx	2 (8)
Stage (AJCC Seventh Edition)	
IVA	23 (96)
IVB	1 (4)
T stage	
T1	1 (4)
T2	8 (33)
T3	9 (38)
T4	6 (25)
N stage	
N0	1 (4)
N1	1 (4)
N2	21 (88)
N3	1 (4)
Stage (AJCC Eighth Edition)	
II	8 (33)
III	6 (25)
IVA	10 (42)
PD-L1 CPS	
<1	5 (21)
1–19	8 (33)
≥20	9 (38)
Not available	2 (8)

AJCC, American Joint Committee on Cancer; CPS, combined positive score; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed death-ligand 1.

PD-L1 IHC data, 17 of 22 (77%) had CPS ≥1. Baseline patient characteristics are summarized in table 1.

No grade 4–5 acute in-field toxicities were reported in the preplanned safety analysis in the first 12 patients, thus enrollment continued to a total of 24 patients. There were no delays to initiate definitive therapy. All 24 patients received radiation therapy per protocol to a dose of 70 Gy over a median of 49 days (range 46–52). The mean number of administered nivolumab doses was 15.7 (range 9–17) with 17 patients (71%) receiving all 17 doses; the mean number of administered ipilimumab doses was 5.5 (range 3–6) with 18 patients (75%) receiving all 6 doses.

Table 2 Treatment-related adverse events. In-field toxicities including both acute and delayed effects are summarized along with selected immune-related adverse events

Event	Any grade, N (%)	Grade ≥3, N (%)
In-field		
Mucositis	24 (100)	10 (42)
Dermatitis—radiation	24 (100)	6 (25)
Dysphagia	23 (96)	7 (29)
Odynophagia	21 (88)	3 (13)
Dysgeusia	21 (88)	0 (0)
Xerostomia	20 (83)	1 (4)
Edema	13 (54)	1 (4)
Pain	10 (42)	1 (4)
Soft tissue ulceration	5 (21)	3 (13)
Hemorrhage—upper airway	4 (17)	1 (4)
Hypothyroidism	4 (17)	0 (0)
Laryngeal stenosis	2 (8)	2 (8)
Immune-related		
Dermatitis—drug	7 (29)	2 (8)
Pruritus	7 (29)	0 (0)
Thyroid dysfunction	4 (17)	0 (0)
Colitis	1 (4)	1 (4)
Elevated lipase	1 (4)	1 (4)
Hyperglycemia	1 (4)	1 (4)

Safety

All 24 treated patients were assessed for safety. Grade 3 or higher TRAEs were reported in 21 individuals (88%). Thirteen patients had treatment-related serious adverse events. TRAEs that led to treatment discontinuation were reported in seven cases (29%). One patient developed grade 3 colitis requiring discontinuation of study drugs and corticosteroid administration with subsequent full resolution of symptoms. One patient terminated therapy due to persistent mucositis without ulceration. Four additional patients discontinued therapy due to in-field necrosis resulting in ulceration. One patient experienced in-field erosion into the carotid artery 98 days post radiotherapy completion and died of exsanguination after denying medical therapy, with no sign of active cancer. Grade 3–4 select (immune-related) TRAEs occurred in five patients (21%). Table 2 summarizes in-field and immune-related adverse events; all other TRAEs are shown in online supplemental table 1.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

Sixteen of 24 patients (67%) experienced at least one grade 3 acute in-field toxicity during concurrent radioimmunotherapy (RIT) or within 2 weeks after the last fraction of radiation therapy; 7 patients (29%) experienced more than one grade 3 TRAE. No grade 4 or 5 TRAEs were recorded in this window. Soft tissue ulceration occurred in five individuals (21%). All ulcers occurred at the primary disease site and median time from radiotherapy completion to ulcer detection was 91 days (range 50–107). At data cut-off, four of the five patients (80%) had healed with a median of 237 days (range 148–330) until ulcer healing; two underwent hyperbaric oxygen therapy with complete resolution of the events. Eleven patients (46%) required percutaneous endoscopic gastrostomy (PEG)-tube placement, including three prior to and eight during radiation therapy. Four patients were PEG-dependent at 1 year post treatment initiation but only one at database lock. Median time to PEG removal was 291.5 days (range 116–1063).

Efficacy

Of the 24 patients evaluable for efficacy outcomes, none had progressive disease. Nineteen patients (79%) had a partial response (PR) and five patients (21%) had a complete response at the primary tumor and lymph node sites by RECIST, V.1.1. It should be noted that 9 of 19 patients who achieved a PR were considered to have residual inflammatory or reactive postradiation changes at 18F-FDG PET/CT performed 12 weeks after radiotherapy completion with no disease observed on physical examination and marked improvement in FDG avidity which resolved on subsequent evaluation. Median PFS for the study cohort was not reached; PFS at 1 year was 92% (95% CI 81% to 100%), PFS at 2 years was 88% (95% CI 75% to 100%) and PFS at 3 years was 74% (95% CI 58% to 94%; figure 2A). Locoregional control rate was 100% (95% CI 86% to 100%) at 1 and 2 years and 95% (95% CI 85% to 100%) at 3 years (figure 2B); no patient had failure at the primary site. Median OS for the study cohort was not reached; OS was the same at 1, 2, and 3 years, equal to 96% (95% CI 88% to 100%; figure 2C). Five patients had distant recurrence (including three with disease in

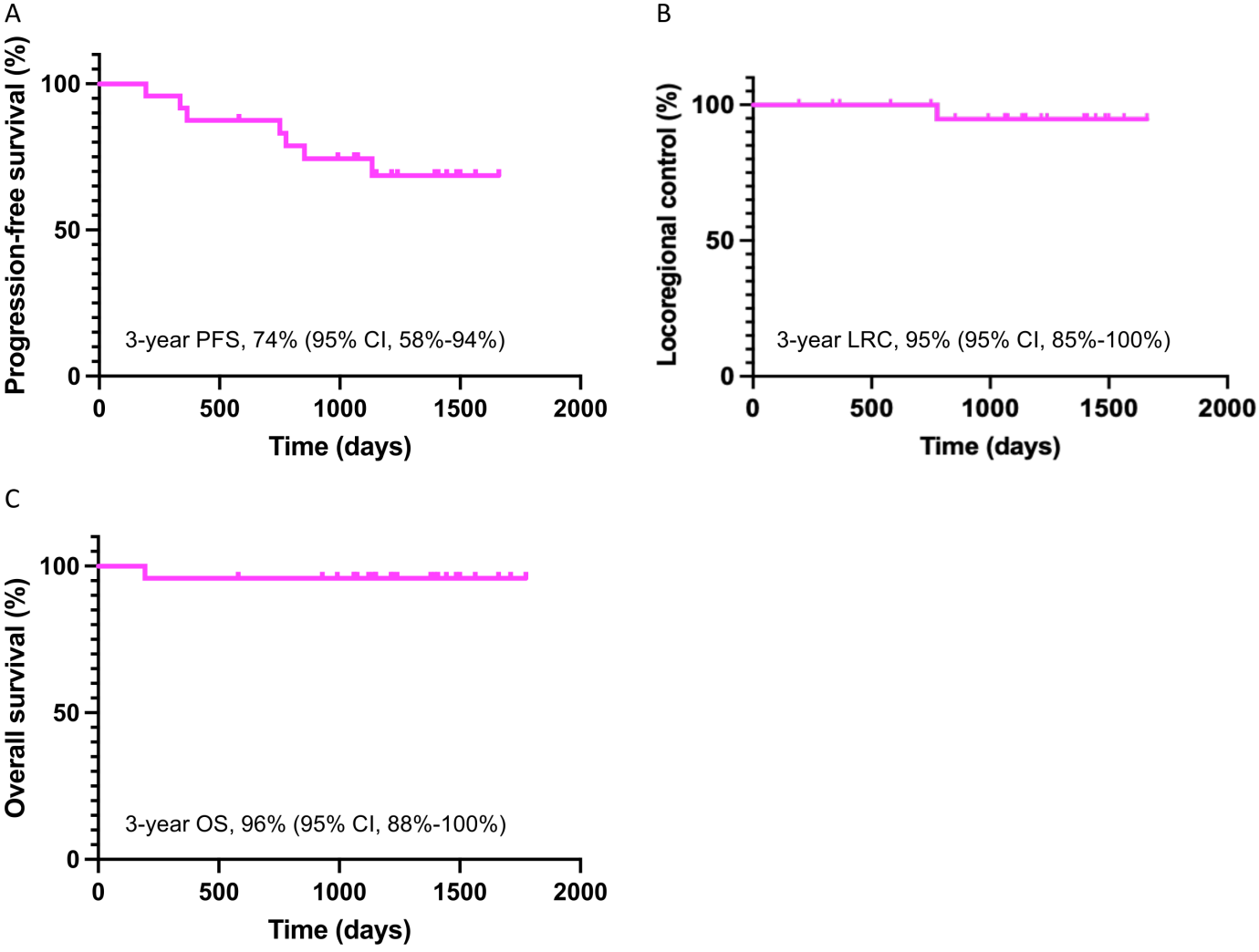

Figure 2 Kaplan-Meier estimates of progression-free survival (PFS), locoregional control (LRC) and overall survival (OS). Kaplan-Meier estimates of PFS (A), LRC (B) and OS (C) of the entire study cohort. Tick marks indicate censored observations.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

lung, two of whom were concerning for new primary, one with hilar lymphadenopathy and one with disease in both the lung and mediastinal lymph nodes), one had recurrence in regional lymph nodes and one died without disease recurrence. All patients with distant recurrence received local treatment with curative intent; three of these patients were alive and free of disease progression at data cut-off.

Biomarker analysis

Baseline PD-L1 status was prospectively analyzed; tissue was available in 22 treated patients. There were no correlations detected between PD-L1 status and CPS, disease progression, or death.

Univariate analysis revealed several cytokines associated with PFS (online supplemental table 2). For example, decreased peripheral interferon (IFN)-γ levels were associated with prolonged PFS at week 3 (p=0.043). As far as soft tissue ulceration status is concerned, changes in some cytokines within the concurrent RIT phase appeared to correlate with outcome (week 11/week 3 ratio; online supplemental table 3). In particular, peripheral decreases in interleukin (IL)4, IL9, IL12p70 as well as IL17a were associated with subsequent ulcer formation; notably, these associations remained significant after adjusting for multiple testing (p_{adj}=0.019, p_{adj}=0.019, p_{adj}=0.025 and p_{adj}=0.030, respectively; figure 3A–D).

Using multicolor flow cytometry, we also found that decreases in PD-L1 mean fluorescence intensity (MFI) in peripheral monocytes (CD14+) during consolidation immunotherapy (week 19/week 11 ratio) were associated with soft tissue ulceration (p_{adj}=0.003; online supplemental tables 4, 5). At week 19, patients with soft tissue ulceration had significantly decreased PD-L1, CD163 and CD204 MFI in peripheral monocytes (p_{adj}=0.011 for all three markers; figure 3E–G).

Finally, on-treatment changes in PD-L1 in EVs showed significant associations with both PFS and soft tissue ulceration (online supplemental tables 6,7). Decreases within

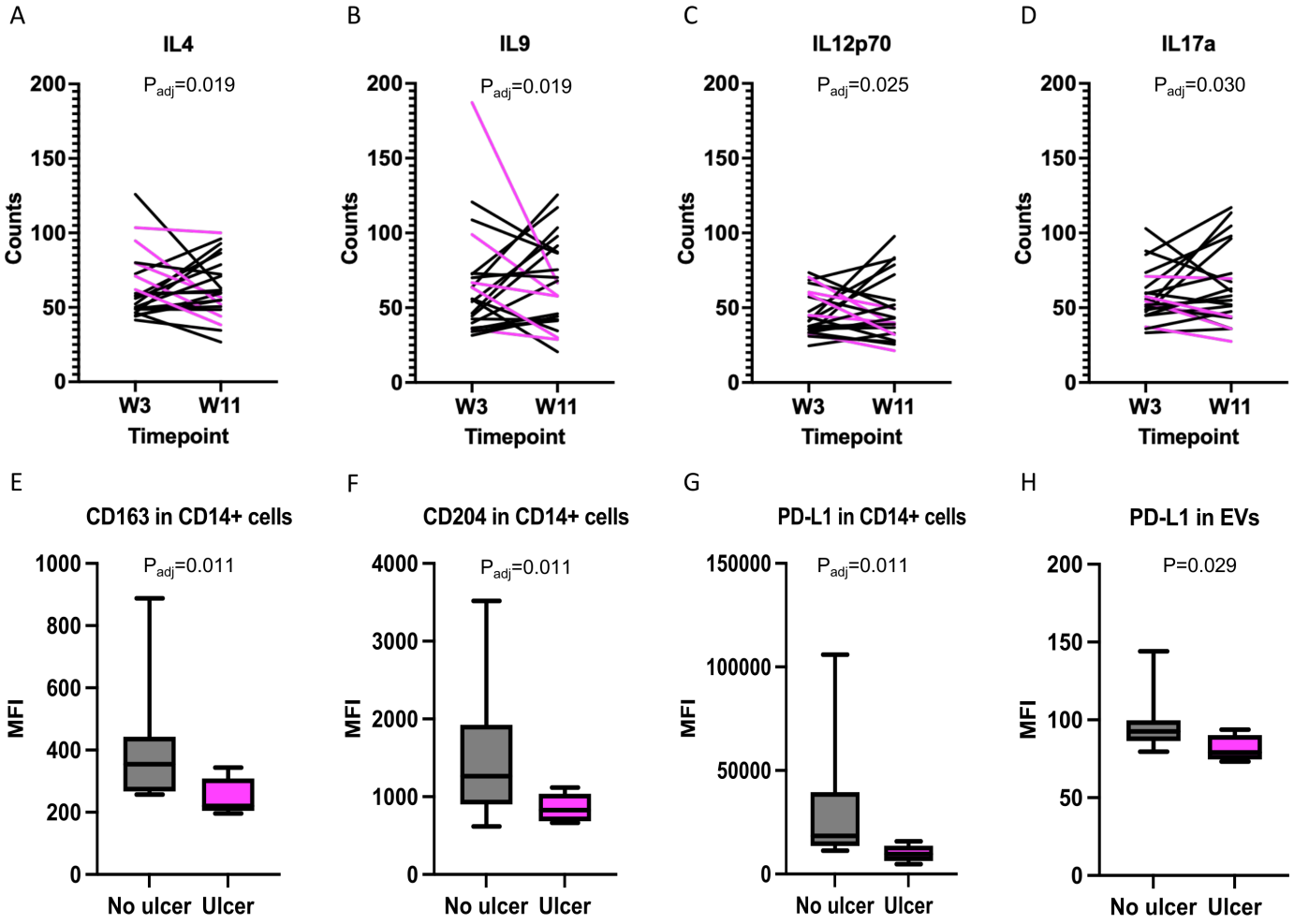

Figure 3 Peripheral blood parameters associated with soft tissue ulceration. Decreases in circulating levels of IL4 (A), IL9 (B), IL12p70 (C) and IL17a (D) within the concurrent radioimmunotherapy phase (week 11/week 3 ratio) are associated with soft tissue ulceration; magenta lines represent patients who subsequently developed soft tissue ulceration. At week 19, patients with soft tissue ulceration have significantly decreased expression of CD163 (E), CD204 (F), PD-L1 (G) in circulating monocytes as well as PD-L1 in circulating extracellular vesicles (H) compared with patients without soft tissue ulceration. EVs, extracellular vesicles; IL, interleukin; MFI, mean fluorescence intensity; PD-L1, programmed death-ligand 1; W3, week 3; W11, week 11.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

the concurrent RIT phase (week 11/week 3 ratio) were associated with prolonged PFS (p=0.006). In addition, decreased PD-L1 expression in EVs was associated with ulcer formation at week 19 (p=0.029; figure 3H).

DISCUSSION

Integration of PD-1 inhibitors in the treatment paradigm of R/M SCCHN has justified efforts to introduce immunotherapy earlier in the course of the disease, as an intensification strategy in patients at high risk for recurrence.[20–22] For patients with LA SCCHN, concurrent chemoradiotherapy confers an absolute survival benefit of 6.5% and 3.6% at 5 and 10 years, respectively, compared with locoregional therapy alone.[23] However, limited tolerability of high-dose cisplatin, representing the current standard of care, oftentimes poses therapeutic challenges and has precluded its use in elderly or frail individuals. To our knowledge, this is the first study combining nivolumab and ipilimumab with radiation therapy in the definitive setting for cisplatin-eligible patients with LA disease. According to the study schedule, a single, lead-in dose of nivolumab and ipilimumab was followed by concurrent RIT and subsequent consolidation immunotherapy using both agents, over a total period of 32 weeks.

Although rates of grade 3 or higher toxicities (88%) were comparable to historical controls with standard platinum-based chemoradiotherapy, we observed different toxicity profiles.[24] As expected, the use of a chemotherapy-free regimen reduced the risk of grade 3 or higher nausea and/or vomiting as well as cytopenias of any grade.[9] However, a concern was the development of acute in-field toxicity. We observed rates of grade 3 or higher radiation dermatitis and mucositis equal to 25% and 42%, respectively. While rates of mucositis are similar to those reported with standard-of-care cisplatin, rates of radiation dermatitis appear substantially higher compared with 8% with the latter.[9] In the CheckRad-CD8 study, concurrent RIT with durvalumab and tremelimumab resulted in grade 3 or higher radiation dermatitis and mucositis in 9% and 14% of the cases, respectively.[25 26] Notably, concurrent RIT with nivolumab and ipilimumab resulted in higher rates of grade ≥3 radiation dermatitis and mucositis compared with single-agent PD-(L)1 blockade administered concurrently with standard-of-care chemoradiotherapy as seen in both JAVELIN Head and Neck 100 and KEYNOTE-412.[27 28]

Soft tissue ulceration at the primary site occurred in 21% of the cases at a median of about 3 months post completion of radiotherapy, representing a late in-field toxicity. This toxicity was fatal in one case, where the patient declined any medical intervention. Furthermore, persistent mucosal inflammation was noted in two cases; this led to early discontinuation in one patient. The incidence of soft tissue ulceration in our study was higher compared with a cohort of patients with similar eligibility criteria treated with standard-of-care cisplatin in the same institution during the same period of time (9%), concurrent chemoradiation with anti-PD(L)1, as well as RIT with durvalumab and tremelimumab that was assessed in the CheckRad-CD8 study (5%).[25–29] Strategies to reduce the incidence of soft tissue ulceration may include treatment de-escalation with reductions in the dose of radiotherapy, potentially starting with HPV-positive disease (at the example of NRG-HN005, NCT03952585), as well as the use of predictive biomarkers. Interestingly, we observed that cytokine changes within the concurrent RIT phase correlated with subsequent ulcer formation. Decreases in circulating levels of IL4, IL9, IL12p70 and IL17a were all associated with soft tissue ulceration. Predominantly produced by T-helper 17 (Th17) cells, IL-9 has been shown to act through the STAT3 and STAT5 signaling pathways to support both Th17 cell differentiation in an autocrine manner and regulatory T cell (Treg) function; absence of IL-9 signaling weakens the suppressive activity of Tregs in vivo, tipping the balance towards inflammation.[30] Moreover, IL-4 is a major Th2 cytokine that suppresses the pro-inflammatory milieu and drives alternative (M2) macrophage activation.[31 32] The latter supports the ensuing decrease in circulating M2 macrophages within the consolidation phase (week 19/week 11 ratio) that we found to correlate with soft tissue ulceration as well.

Any grade as well as grade 3 or higher immune-mediated dermatologic adverse events were recorded in 29% and 8% of the patients, respectively, which is higher than 9% and 3%, respectively, reported in the CheckMate 651 study, suggesting a potential additive or synergistic effect of radiation therapy with dual immune checkpoint blockade.[14]

With PFS rates of 74% and OS rates of 96% at 3 years, definitive RIT with nivolumab and ipilimumab compared favorably to standard chemoradiotherapy using high-dose cisplatin.[7 9 24] Although PD-L1 CPS did not predict outcome, decreases in EV PD-L1 again during concurrent RIT (week 11/week 3 ratio) were associated with prolonged PFS, consistent with current literature.[33 34] Notably, definitive RIT resulted in excellent locoregional control of the disease, with only one regional failure and no local failure in 24 patients at high risk for recurrence. The latter may be explained by a growing in-field inflammatory reaction caused by the synergy of radiotherapy with dual immune checkpoint blockade; the significantly decreased expression of PD-L1 in both circulating monocytes and EVs seen at week 19 in patients who developed ulcers may serve as an indication of ongoing treatment response resulting in local accumulation of immune cells and excessive tissue damage.

A limitation of this study is the small sample size. Additionally, the fact that 14 of 24 study participants had HPV-positive oropharyngeal SCCHN and no patient had stage IVB disease (AJCC Eighth Edition) raise the concern for selection bias, which may impact efficacy outcomes. Combined with the lack of a comparator arm, which is inherent to the phase I study design, all the above preclude definitive conclusions regarding treatment efficacy.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

Given the negative results of PembroRad and discouraging data from phase III studies that incorporated chemotherapy with PD-(L)1 inhibitors (avelumab, pembrolizumab), our approach combining PD-1 and CTLA4 blockade appears reasonable.[27 28] The combination of nivolumab and ipilimumab with IMRT is currently being investigated in the context of a phase II study that is estimated to enroll 180 patients with LA, HPV-positive oropharyngeal SCCHN (NCT03799445); this study schedule differs in that one full cycle of dual immune checkpoint inhibition will be administered before radiotherapy initiation and no consolidation immunotherapy will be prescribed.

In summary, definitive RIT with nivolumab and ipilimumab showed promising efficacy with outstanding locoregional control and was generally safe but raised the concern of higher than usual in-field soft tissue ulceration. Future studies will need to interrogate the efficacy of concurrent RIT with dual immune checkpoint blockade across patient subgroups and identify biomarkers of response, with simultaneous incorporation of strategies to prevent soft tissue ulceration. Based on our results, this approach may be relevant for both cisplatin-eligible and cisplatin-ineligible patients.

Author affiliations
[1]Department of Medical Oncology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
[2]Department of Otolaryngology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
[3]Department of Cancer Biology, Thomas Jefferson University, Philadelphia, PA, USA
[4]Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
[5]Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
[6]Department of Pharmacology, Physiology, and Cancer Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
[7]Department of Dermatology and Cuaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Twitter Ioannis A Vathiotis @IVathiotis

Contributors All authors were involved in the trial conception/design, or the acquisition, analysis, or interpretation of data. All authors contributed to the drafting of the manuscript and approved the final version. JMJ is the study guarantor.

Funding This work was supported by Bristol-Myers Squibb. Grant # CA209-931. The Flow Cytometry and Human Immune Monitoring Core is supported by the Cancer Center Support Grant 5P30CA056036- 20.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study protocol was approved by the Thomas Jefferson Sidney Kimmel Cancer Center institutional review board (Protocol IRB# 17P.082). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iD
Ioannis A Vathiotis http://orcid.org/0000-0002-1772-5986

REFERENCES

1 Longo DL, Chow LQM. Head and neck cancer. *N Engl J Med* 2020;382:60–72.
2 Argiris A, Karamouzis MV, Raben D, *et al*. Head and neck cancer. *Lancet* 2008;371:1695–709.
3 Braakhuis BJM, Brakenhoff RH, Leemans CR. Treatment choice for locally advanced head and neck cancers on the basis of risk factors: biological risk factors. *Ann Oncol* 2012;23 Suppl 10:x173–7.
4 Cooper JS, Pajak TF, Forastiere AA, *et al*. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med* 2004;350:1937–44.
5 Licitra L, Perrone F, Bossi P, *et al*. High-risk human Papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2006;24:5630–6.
6 Cooper JS, Zhang Q, Pajak TF, *et al*. Long-term follow-up of the RTOG 9501/Intergroup phase III trial: postoperative concurrent radiation therapy and chemotherapy in high-risk squamous cell carcinoma of the head and neck. *Int J Radiat Oncol Biol Phys* 2012;84:1198–205.
7 Denis F, Garaud P, Bardet E, *et al*. Final results of the 94-01 French head and neck oncology and radiotherapy group randomized trial comparing radiotherapy alone with concomitant Radiochemotherapy in advanced-stage oropharynx carcinoma. *J Clin Oncol* 2004;22:69–76.
8 Tao Y, Auperin A, Sire C, *et al*. Improved outcome by adding concurrent chemotherapy to Cetuximab and radiotherapy for locally advanced head and neck Carcinomas: results of the GORTEC 2007-01 phase III randomized trial. *J Clin Oncol* 2018:JCO2017762518.
9 Gillison ML, Trotti AM, Harris J, *et al*. Radiotherapy plus Cetuximab or cisplatin in human Papillomavirus-positive oropharyngeal cancer (NRG oncology RTOG 1016): a randomised, Multicentre, non-inferiority trial. *Lancet* 2019;393:40–50.
10 Mehanna H, Robinson M, Hartley A, *et al*. Radiotherapy plus cisplatin or Cetuximab in low-risk human Papillomavirus-positive oropharyngeal cancer (de-escalate HPV): an open-label randomised controlled phase 3 trial. *Lancet* 2019;393:51–60.
11 Larkin J, Chiarion-Sileni V, Gonzalez R, *et al*. Five-year survival with combined Nivolumab and Ipilimumab in advanced Melanoma. *N Engl J Med* 2019;381:1535–46.
12 Peters S, Scherpereel A, Cornelissen R, *et al*. First-line Nivolumab plus Ipilimumab versus chemotherapy in patients with Unresectable malignant pleural Mesothelioma: 3-year outcomes from Checkmate 743. *Ann Oncol* 2022;33:488–99.
13 Vathiotis IA, Johnson JM, Argiris A. Enhancing programmed cell death protein 1 axis inhibition in head and neck squamous cell carcinoma: combination Immunotherapy. *Cancer Treat Rev* 2021;97:102192.
14 Argiris A, Harrington K, Tahara M, *et al*. Lba36 Nivolumab (N) + Ipilimumab (I) vs EXTREME as first-line (1L) treatment (TX) for recurrent/metastatic squamous cell carcinoma of the head and neck (R/M SCCHN): final results of Checkmate 651. *Annals of Oncology* 2021;32:S1310–1.
15 Schoenfeld JD, Hanna GJ, Jo VY, *et al*. Neoadjuvant Nivolumab or Nivolumab plus Ipilimumab in untreated oral cavity squamous cell carcinoma: A phase 2 open-label randomized clinical trial. *JAMA Oncol* 2020;6:1563–70.
16 Vos JL, Elbers JBW, Krijgsman O, *et al*. Neoadjuvant Immunotherapy with Nivolumab and Ipilimumab induces major pathological responses in patients with head and neck squamous cell carcinoma. *Nat Commun* 2021;12:7348.
17 Manukian G, Bar-Ad V, Lu B, *et al*. Combining radiation and immune Checkpoint blockade in the treatment of head and neck squamous cell carcinoma. *Front Oncol* 2019;9:122.
18 Twyman-Saint Victor C, Rech AJ, Maity A, *et al*. Radiation and dual Checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015;520:373–7.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.

19 Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 1995;57:289–300.
20 Ferris RL, Blumenschein G Jr, Fayette J, *et al*. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med* 2016;375:1856–67.
21 Cohen EEW, Soulières D, Le Tourneau C, *et al*. 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;393:156–67.
22 Burtness B, Harrington KJ, Greil R, *et al*. Pembrolizumab alone or with chemotherapy versus Cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet* 2019;394:1915–28.
23 Lacas B, Carmel A, Landais C, *et al*. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 107 randomized trials and 19,805 patients, on behalf of MACH-NC group. *Radiother Oncol* 2021;156:281–93.
24 Fietkau R, Hecht M, Hofner B, *et al*. Randomized phase-III-trial of concurrent Chemoradiation for locally advanced head and neck cancer comparing dose reduced radiotherapy with paclitaxel/ cisplatin to standard radiotherapy with fluorouracil/cisplatin: the Paccis-trial. *Radiother Oncol* 2020;144:209–17.
25 Hecht M, Gostian AO, Eckstein M, *et al*. Safety and efficacy of single cycle induction treatment with cisplatin/Docetaxel/ Durvalumab/ Tremelimumab in locally advanced HNSCC: first results of Checkrad-Cd8. *J Immunother Cancer* 2020;8:e001378.
26 Hecht M, Eckstein M, Rutzner S, *et al*. Induction Chemoimmunotherapy followed by Cd8+ immune cell-based patient selection for chemotherapy-free Radioimmunotherapy in locally advanced head and neck cancer. *J Immunother Cancer* 2022;10:e003747.
27 Lee NY, Ferris RL, Psyrri A, *et al*. 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;22:450–62.
28 Machiels J-P, Tao Y, Burtness B, *et al*. Lba5 - primary results of the phase III KEYNOTE-412 study: Pembrolizumab (Pembro) with Chemoradiation therapy (CRT) vs placebo plus CRT for locally advanced (LA) head and neck squamous cell carcinoma (HNSCC). *Annals of Oncology* 2022;33:S1399.
29 Ali AS, Manukian G, Johnson JM, *et al*. In-field toxicity analysis of a phase 1 clinical trial of Nivolumab and Ipilimumab with definitive radiation therapy in locally advanced squamous cell carcinoma of the head and neck. *Int J Radiat Oncol Biol Phys* 2023.
30 Elyaman W, Bradshaw EM, Uyttenhove C, *et al*. IL-9 induces differentiation of Th17 cells and enhances function of Foxp3+ natural regulatory T cells. *Proc Natl Acad Sci U S A* 2009;106:12885–90.
31 May RD, Fung M. Strategies targeting the IL-4/IL-13 axes in disease. *Cytokine* 2015;75:89–116.
32 Gordon S, Martinez FO. Alternative activation of Macrophages: mechanism and functions. *Immunity* 2010;32:593–604.
33 Poggio M, Hu T, Pai C-C, *et al*. Suppression of Exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell* 2019;177:414–27.
34 Yin Z, Yu M, Ma T, *et al*. Mechanisms underlying low-clinical responses to PD-1/PD-L1 blocking antibodies in Immunotherapy of cancer: a key role of Exosomal PD-L1. *J Immunother Cancer* 2021;9:e001698.

J Immunother Cancer: first published as 10.1136/jitc-2023-007141 on 3 August 2023. Downloaded from http://jitc.bmj.com/ on August 29, 2023 at Thomas Jefferson University. Protected by copyright.