Journal for ImmunoTherapy of Cancer

ISA101 and nivolumab for HPV-16$^+$ cancer: updated clinical efficacy and immune correlates of response

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To cite: Sousa LG, Rajapakshe K, Rodriguez Canales J, *et al*. ISA101 and nivolumab for HPV-16$^+$ cancer: updated clinical efficacy and immune correlates of response. *Journal for ImmunoTherapy of Cancer* 2022;**10**:e004232. doi:10.1136/jitc-2021-004232

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2021-004232).

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This article was presented at the Society for Immunotherapy of Cancer Annual Congress; November 9, 2018; Baltimore, Maryland, USA.

Accepted 08 January 2022

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ABSTRACT

Background The combination of ISA101, a human papilloma virus (HPV) 16 peptide vaccine, and nivolumab showed a promising response rate of 33% in patients with incurable HPV-16$^+$ cancer. Here we report long-term clinical outcomes and immune correlates of response.

Methods Patients with advanced HPV-16$^+$ cancer and less than two prior regimens for recurrence were enrolled to receive ISA101 (100 µg/peptide) on days 1, 22, and 50 and nivolumab 3 mg/kg every 2 weeks beginning day 8 for up to 1 year. Baseline tumor samples were stained with multiplex immunofluorescence for programmed death-ligand 1 (PD-L1), programmed cell death protein-1 (PD-1), CD3, CD8, CD68, and pan-cytokeratin in a single panel and scanned with the Vectra 3.0 multispectral microscope. Whole transcriptome analysis of baseline tumors was performed with Affymetrix Clariom D arrays. Differential gene expression analysis was performed on responders versus non-responders.

Results Twenty-four patients were followed for a median of 46.5 months (95% CI, 46.0 months to not reached (NR)). The median duration of response was 11.2 months (95% CI, 8.51 months to NR); three out of eight (38%) patients with objective response were without progression at 3 years. The median and 3-year overall survival were 15.3 months (95% CI, 10.6 months to 27.2 months) and 12.5% (95% CI, 4.3% to 36%), respectively. The scores for activated T cells ((CD3$^+$PD-1$^+$)+(CD3$^+$CD8$^+$PD-1$^+$)), activated cytotoxic T cells (CD3$^+$CD8$^+$PD-1$^+$), and total macrophage ((CD68$^+$PD-L1$^-$)+(CD68$^+$PD-L1$^+$)) in tumor were directly correlated with clinical response ($p<0.05$) and depth of response with the two complete response patients having the highest degree of CD8$^+$ T cells. Gene expression analysis revealed differential regulation of 357 genes (≥1.25 fold) in non-responders versus responders ($p<0.05$). Higher expression of immune response, inflammatory response and interferon-signaling pathway genes were correlated with clinical response ($p<0.05$).

Conclusions Efficacy of ISA101 and nivolumab remains promising in long-term follow-up. Increased infiltration by PD-1$^+$ T cells and macrophages was predictive of response. Enrichment in gene sets associated with interferon-γ response and immune infiltration strongly predicted response to therapy. A randomized trial is ongoing to test this strategy and to further explore correlates of immune response with combined nivolumab and ISA101, versus nivolumab alone.

Trial registration number NCT02426892.

BACKGROUND

Human papilloma virus driven (HPV$^+$) cancers are highly prevalent worldwide with the most common tumors being oropharyngeal and anogenital cancers. The E6 and E7 viral proteins play a critical role in HPV-induced carcinogenesis and have constitutive expression in HPV-associated tumors representing an ideal target for therapeutic vaccines.

The ISA101 vaccine consists of HPV-16 E6 and E7 synthetic long peptides which are efficiently processed by dendritic cells to activate CD4$^+$ and CD8$^+$ T-cells and drive HPV-16-specific antitumor immunity (online supplemental figure 1). ISA101 can eradicate HPV-16$^+$ pre-malignant vulvar lesions and lesion clearance is correlated with the strength of the T cell response.[1] However, despite increasing the HPV-16 immune response, ISA101 monotherapy has limited efficacy in the advanced cancer setting, likely due to dominant immunosuppressive signals within the tumor microenvironment (TME).[2]

Immune checkpoint inhibitors (ICI) have shown survival benefit in the treatment of incurable oropharyngeal squamous cell carcinoma (OPSCC), irrespective of HPV status, however, only 15%–20% of patients experience objective responses.[3–5] HPV$^+$ OPSCC has a significantly better prognosis in comparison with HPV$^-$ OPSCC and presents with higher T-cell infiltration.[6] The recognition of tumor-specific antigens by T-cells is integral to the success of cancer immunotherapy. In this context, HPV-16 vaccination with ICI could enhance tumor regression through

increasing the frequency of tumor-specific vaccine-activated T cells whose longevity and effector function is subsequently preserved and amplified by programmed cell death protein-1 (PD-1) blockade. This rationale supported our phase II trial with ISA101 and nivolumab for patients with advanced HPV-16$^+$ tumors. We observed an objective response rate (ORR) of 33%, durable duration of response, promising survival and favorable safety profile.[7]

Discovering a deeper understanding of the TME composition and metabolic pathways has become a fundamental step in discovering genetic and epigenetic adaptations in cancer, as well as in revealing distinct immunologic signatures and immune-resistance mechanisms, such as loss of interferon-γ (IFN-γ) signaling and T-cell exhaustion. Here we report long-term efficacy data and immune correlatives of response from this phase II study of ISA101 and nivolumab in patients with HPV-16$^+$ solid tumors.[7]

METHODS

Study design and patients

The design of this phase II trial was reported previously.[7] Eligible patients had incurable HPV-16$^+$ solid tumor with ≤1 regimen for recurrent disease, an Eastern Cooperative Oncology Group performance status of 0–1, and measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1.

Patients received ISA101 subcutaneously, 100 µg/peptide, for a total of three doses on days 1, 22, and 50 and nivolumab intravenously, 3 mg/kg starting on day 8 and administered every 2 weeks for 12 months or until progression of disease, toxic effects, or withdrawal of consent (online supplemental figure 2).[7] Tumors were assessed radiologically at baseline, prior to cycle six of nivolumab and then every 6 weeks according to RECIST. Tumor biopsies were mandatory pretreatment and planned at first restaging. The primary endpoint was ORR.

Multiplex immunofluorescence

Multiplex immunofluorescence (mIF) analysis was performed using previously validated methods.[8] Formalin-fixed paraffin-embedded 4 µm thick were stained with an automated system (BOND-MAX; Leica Microsystems) using antibodies against programmed death-ligand 1 (PD-L1), PD-1, CD3, CD8, CD68, and pan-cytokeratin AE1/AE3 in a single panel. Slides were imaged using the Vectra 3.0 spectral imaging system (PerkinElmer), and analyzed by the imaging inForm 2.3.1 software (PerkinElmer).

Transcriptomic analysis

The presence of tumor in baseline biopsies was confirmed by a pathologist, and total RNA was extracted using the RNeasy Mini Kit (Qiagen). RNA quality was assessed via bioanalyzer (Agilent) and whole transcriptome analysis was performed on Affymetrix Clariom D Pico microarray by Affymetrix using their standard protocol. The raw CEL files were imported into R statistical software for pre-processing and normalization with Robust Multichip Average algorithm under oligo package. Batches were further normalized and integrated using the ComBat algorithm implemented in inSilicoMerging package.[9] Differential gene expression analysis was performed on responders versus non-responders via Student's t-test in collaboration with the Baylor College of Medicine Multi-Omics Data Analysis Core. Genes were considered as differentially expressed if the p value was <0.05 and the absolute fold change was ≥1.25 (≤1/1.25). Differentially expressed genes were further evaluated for association with survival by comparing the bottom 50% and top 50% with log-rank test. Clustering and heatmaps were generated using Matplotlib, NumPy and SciPy libraries under python. Gene Set Enrichment Analysis (GSEA) using Molecular Signature Database (MSigDB)[10] was performed to find enriched pathways (q<0.25).

Responders and non-responders

We segregated patients based on clinical benefit for the mIF and gene expression analyses. Responders: patients with (complete response (CR), N=2), partial response (PR, N=3), or stable disease (SD, N=1) with progression free survival (PFS) ≥6 months, according to RECIST V.1.1. Non responders: patients who had progressive disease (PD, N=9) or SD with PFS <6 months (N=2). The patients with SD in the responders group had regression of targeted lesions that did not meet RECIST defined PR and PFS of 9.4 months. The two patients with SD in the non-responders group did not have tumor regression and had PFS of 3.8 and 5.3 months.

Statistical analysis

Continuous variables were summarized by median and range, while categorical variables were summarized by frequency and percentage; t-test or Mann-Whitney U test was performed to evaluate the difference in continuous variable between patient groups according to variable distribution. Fisher's exact test was used to evaluate the association between categorical variables. The Kaplan-Meier method was used to calculate PFS, overall survival (OS), and duration of response. The log-rank test was performed to assess the difference of time-to-event outcome among different groups. P values <0.05 are considered statistically significant. Statistical software SAS V.9.4 (SAS), S-Plus 8.2 (TIBCO Software), and R V.3.4.4 were used for all the analyses.

RESULTS

Long-term efficacy outcomes

Baseline characteristics of the 24 patients were described previously.[7] The median follow-up time was 46.5 months (95% CI, 46.0 to not reached (NR)) as of data lock July 31, 2020. The median duration of response was 11.2 months (95% CI, 8.51 to NR); At the 3-year follow-up, the three progression-free patients at 18 months remained

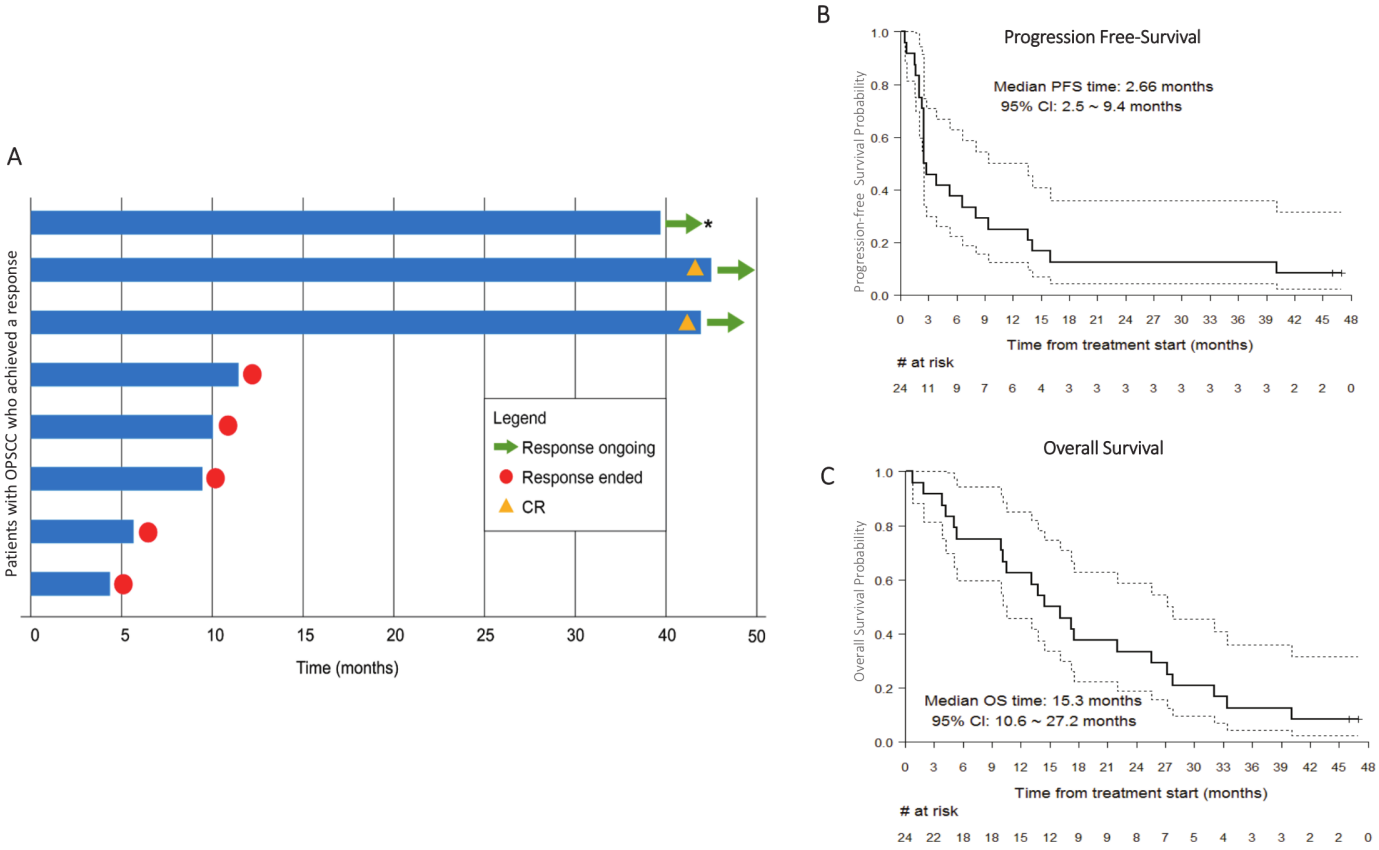


Figure 1 Treatment response and survival. (A) Duration of response for each patient (blue bars). The asterisk indicates a patient with response ongoing who died from a non-cancer related cause (intracranial hemorrhage). (B) and (C) are Kaplan-Meier curves for progression free-survival (PFS) and overall survival (OS), respectively. CR, complete response; OPSCC, oropharyngeal squamous cell carcinoma.

without progression; two patients, both with CR remain progression-free under surveillance at 46 and 47 months. One patient with PR expired from an intracranial hemorrhage, unrelated to cancer, 40 months from treatment start. Three days prior to his death CT imaging remained stable with one 2.1 cm pulmonary nodule (figure 1A).

The median PFS was 2.66 months (95% CI, 2.5 to 9.4 months, figure 1B) and PFS rates at 2 and 3 years were identical at 12.5% (95% CI, 4.3% to 36%). The median OS was 15.3 months (95% CI, 10.6 to 27.2 months) (figure 1C). The rates of OS at 2 and 3 years were 33% (95% CI, 18.9% to 58.7%) and 12.5% (95% CI, 4.3% to 36%), respectively.

The TME landscape

We evaluated the TME of HPV$^+$ tumors from 17 evaluable baseline biopsies, 6 (35%) of which were from responders and 11 (65%) from non-responders. Combining tumor and stromal compartments, scores for activated T cells ((CD3$^+$PD-1$^+$)+(CD3$^+$CD8$^+$PD-1$^+$)), activated cytotoxic T cells (CD3$^+$CD8$^+$PD-1$^+$), and total macrophages ((CD68$^+$PD-L1$^-$)+(CD68$^+$PD-1$^+$)) were significantly higher among responders ($p<0.05$) (figure 2A–C). The highest scores of both total and cytotoxic activated T cells were present in the tumors from the two patients with CR, indicating an association with depth of response. There was no correlation of PD-L1 expression in tumor, stroma or combined compartments with response, differing from the correlation we observed with immunohistochemistry.[7]

Differentially expressed genes and enriched biological pathways

Pre-therapy biopsies from 16/24 patients were evaluable for gene expression. Among the 16 specimens, 6 were from responders (37.5%) and 10 were from non-responders (62.5%). Gene expression analysis (figure 3A) revealed differential regulation of 357 genes between responders and non-responders ($p<0.05$). Of these, 237 genes were upregulated in responders, and 120 genes were downregulated (figure 3A).

GSEA (figure 3B) revealed that the five most highly enriched gene sets among responders were connected to cellular immune response, inflammation response and interferon signaling pathway, consistent with previous literature showing that immunogenic gene expression is correlated with benefit from ICI.[11 12] The most highly enriched gene sets in tumors from non-responders were involved in olfactory signaling pathway, which has been associated with tumor proliferation.[13] Notably, hypoxia-related genes were upregulated in tumors from responders (figure 3B) in contrast to other studies showing the relationship of hypoxia to immune resistance.[14] However,

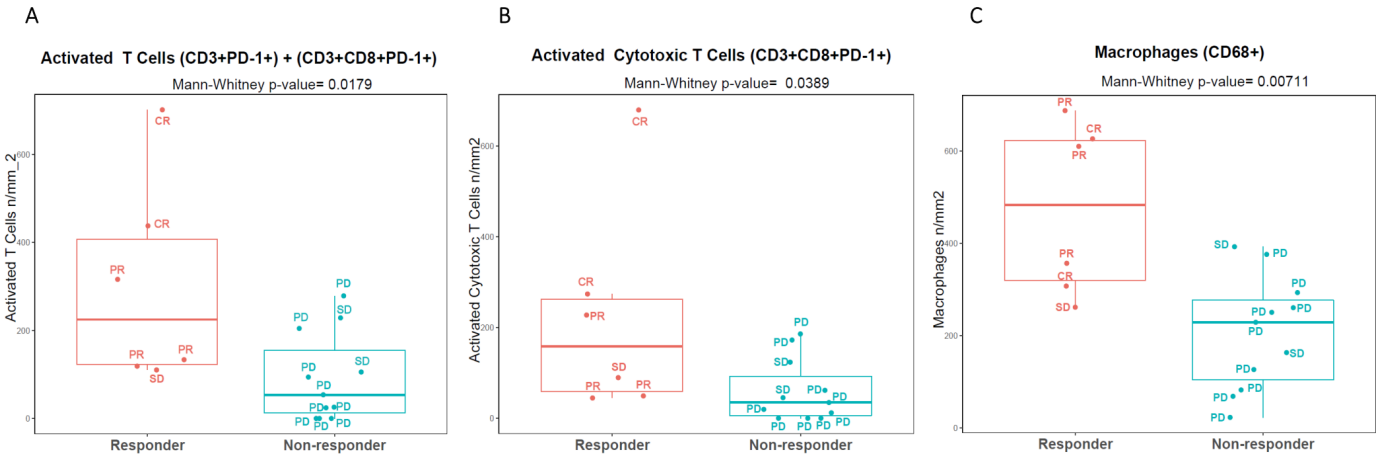
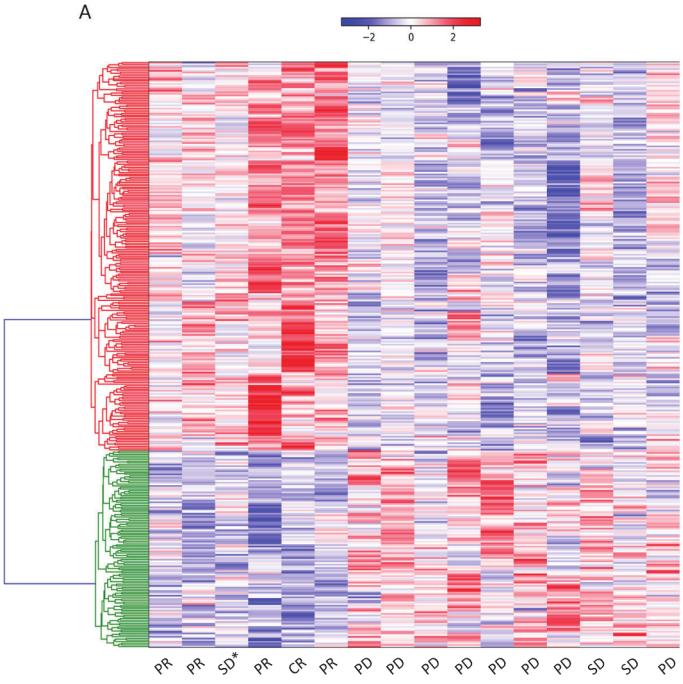


Figure 2 Tumor immune microenvironment. (A), (B) and (C): Comparison of immune cell-types quantities at baseline according to treatment response (responders (CR, PR and SD ≥6 months) versus non-responders (PD and SD <6 months)). Each dot represents a patient. CR, complete response; OPSCC, oropharyngeal squamous cell carcinoma; OS, overall survival; PD, progressive disease; PFS, progression free survival; PR, partial response; SD, stable disease.

the top seven upregulated genes of the hypoxia gene set set belong to the proteasome group. Proteasomes play a role in degrading proteins that are consequently exposed on the cellular surface and recognized by T cells and have been associated with IFN-γ pathway activation and response to ICI in patients with melanoma.[15] Notably, HIF1α, HIF2, and VHL, commonly upregulated in hypoxia, were not enriched in the responders.

The expression of CD68, a pan-macrophage marker, was associated with improved survival (p=0.0058, online supplemental figure 3). This is consistent with the observation that CD68 levels were significantly higher in responders than non-responders (figure 2C). A positive correlation with survival was similarly found for other genes involved in macrophage recruitment or activation or adaptive immune response (CHI3L1, ANGPTL2, TAP1, IL15RA) (online supplemental figure 3). In contrast, eight genes encoding zinc finger proteins (ZFPs) were associated with reduced survival (online supplemental table 1). ZFPs form one of the largest families



B

TOP 5 ENRICHED GENESETS	
RESPONDERS	NES
HM gene set	
Interferon Gamma Response	6.61
Interferon Alpha Response	5.78
Complement	4.28
Coagulation	4.50
Hypoxia	3.52
GO gene set	
Response to Biotics Stimulus	5.76
Immune Effector Process	5.43
Regulation of Immune System Process	5.42
Regulation of Cell Differentiation	5.40
Locomotion	5.37
Innate Immune Response	5.11
NON-RESPONDERS	NES
HM gene set	
None	
GO gene set	
Olfactory Transduction	6.16
Sensory Perception of Smell	5.92
Detection of Stimulus Involved in Sensory Perception	5.91
Olfactory Signaling Pathway	5.79
Sensory Perception of Chemical Stimulus	5.63

Figure 3 Gene expression analysis. (A) Gene Set Enrichment Analysis heatmap. Expression data set sort by correlation with treatment response. Criteria is p=0.05, log2 FC ≥1.25 and max intensity 1. Rows represent genes and columns represent samples. Red, upregulated genes; blue, downregulated genes; white, unchanged gene expression. (B) Five most enriched gene sets of non-responders and responders. CR, complete response; GO, Gene Ontology - biological process gene sets from Molecular Signatures Database (MSigDB); HM, hallmark gene sets from Molecular Signatures Database (MSigDB); NES, normalized enrichment score; PD, progressive disease; PR, partial response; SD, stable disease <6 months, SD*, SD ≥6 months.

of transcription factors and function in many biological processes. Recent studies have demonstrated that ZFPs are closely associated with different stages of cancer development, including signal transduction cascades, carcinogenesis, and cell proliferation.[16]

The TME composition was inferred by xCell, a computational tool to deconvolute cell type proportion using gene expression data. A total of 64 immune and stromal cells were deconvoluted. A significant correlation was found between M0-polarized and M1-polarized macrophage levels and response to treatment ($p<0.05$), whereas no significant difference was found in M2 macrophage levels between groups (online supplemental figure 4). These observations support the correlation between CD68 density and therapeutic benefit observed in our mIF panel. There were no significant differences of T cell, monocyte, or neutrophil levels between groups (online supplemental figure 4). Lastly, the cytolytic score, calculated from gene expression levels of granzyme A and perforin, positively correlated with response ($p=0.0282$, online supplemental figure 5).

DISCUSSION

In this updated analysis, the benefit of adding ISA101 to nivolumab remains promising among patients with recurrent or metastatic (R/M) HPV-16$^+$ cancer, with a median OS of 15.3 months and 2-year OS rate of 33%. These results are encouraging when compared with those from contemporaneously performed studies using ICI for HPV$^+$ cancers, especially HPV$^+$ OPSCC, although admittedly our patient population is more heterogeneous and totaled only 24 patients. Checkmate-141, a phase III trial using nivolumab monotherapy showed a median OS (mOS) of 9.1 months and 2-year OS rate of 16.9% among patients with HPV$^+$ OPSCC.[17] Although our study showed longer survival, only 79% of our patients were platinum-refractory versus 100% in Checkmate-141. Similarly, KEYNOTE-055, showed an mOS of 8 months in platin and cetuximab-refractory HPV$^+$ OPSCC.[18] Recently, preliminary results from a phase Ib/IIa trial with similar design to ours combining MEDI0457, an HPV vaccine plus IL-12, with durvalumab, an anti-PD-L1 antibody, in patients with HPV-16$^+$ or 18$^+$ R/M OPSCC were reported in abstract form.[19] Patients were mixed as regards platin-sensitivity and were required to have ≥1 line of prior therapy. ORR was 22% (6/27), and disease control rate at 24 weeks was 26%.[19] Finally, triple combination of PDS0101, a liposomal therapeutic vaccine targeting HPV-16 E6/E7, M9241, an immune-cytokine targeting DNA release of from necrotic tumor cells, and bintrafusp alfa, showed promising activity in patients with refractory HPV +solid tumors with an ORR of 71% (9/14), but at the cost of a higher rate of adverse events (grade 3–4=36%).[20]

CD8$^+$ cytotoxic T cells have a central role in targeting cancer and, thus, are the focus of cancer immune suppression. Elevated T cell density in the TME typical of 'hot' cancers has been linked to improved prognosis in many solid tumors, including HPV$^+$ OPSCC[21 22] and to higher frequencies of ICI responses.[22] We found that higher rates of PD-1$^+$ T cells and PD-1$^+$ cytotoxic T cells significantly correlated with treatment response.[23] This correlation between activated T cell infiltration and response to ISA101 plus nivolumab supports the hypothesis that this combination may enhance adaptive antitumor immunity via expansion of HPV-specific T cells in response the ISA101 vaccine coupled with anti-PD-1-mediated protection of these T cells from attenuation in the TME.[24]

Macrophages and their precursors constitute a heterogeneous and dynamic population of the stroma that can operate across a functional spectrum from pro-inflammatory antigen presentation to tumor-supportive immune suppression. They are classically divided in two main phenotypic poles: the inflammatory/classical M1, associated with Th1 response and anticancer effects, and the tumor-associated macrophage M2, associated with tumor progression through Th2-responses and inhibition of pro-inflammatory T cells.[25] Kim *et al* showed increased infiltration by M1 macrophages in HPV$^+$ OPSCC tumors that were immune rich based on gene expression clustering. Furthermore, these immune rich tumors were correlated with better survival and response to immunotherapy.[22] In our study, baseline expression of the pan-macrophage marker CD68 was directly correlated with clinical response and xCell analysis confirmed that the resting M0-macrophage and polarized-M1 macrophage fractions were higher among responders. We speculate that in an environment enriched with resting M0-macrophages, an HPV-16 vaccine may promote macrophage polarity towards the pro-inflammatory M1 phenotype, perhaps driven by pro-inflammatory cytokine production from active, infiltrating E6/E7 specific T cells, favoring tumor destruction.

Our transcriptional profiling of baseline tumor tissue revealed that 357 genes were differently expressed according to treatment response. Among responders, enrichment in gene sets associated with immune response, inflammation and interferon signaling pathway predicted response to treatment, as has been reported previously with ICI.[22 23] These data suggest that the putative impact from HPV vaccination may be to qualitatively and quantitatively improve T cell infiltration for the subset of tumors whose TMEs are susceptible to such, as evidenced by baseline inflammation. In contrast, genes associated with olfactory signaling were significantly upregulated among non-responders. Aberrant expression of olfactory-related genes has been reported in some types of solid cancer, such as breast, prostate, and colorectal, where it is associated with tumor development and progression.[13 26] For instance, OR7C1 has been shown to play an essential role in maintenance and proliferation of colorectal cells to the extent that it has been proposed as a potential target for colorectal cancer immunotherapy.[26]

Surprisingly, hypoxia gene sets were upregulated in tumors from responders, specifically the proteasome genes within the set. Constitutive proteasomes are

upregulated in several types of malignancy, reflecting their high metabolism and demand for protein degradation. By degrading proteins, proteasomes produce immunogenic peptides that may be presented to APCs through MHC class I. Indeed, overexpression of proteasomes has been correlated with the presence of IFN-γ-secreting tumor-infiltrating lymphocytes and with ICI response.[15] Moreover, important genes related to hypoxia, such as HIF1α, HIF2, and VHL, were not enriched in responders. Given the high complexity of gene expression with multiple functions being performed by a single gene, we believe our hypoxia result should be interpreted cautiously since it may be driven by an enrichment of genes playing a different role than hypoxia, such as immune-response and other metabolic pathways.

We are aware that our study has important limitations, mainly relying on the small sample size, the absence of post-treatment specimens and the single-arm design. A randomized phase II trial of ISA101 and PD-1 blocking antibody cemiplimab is ongoing to confirm these findings and further explore correlates of immune response (NCT03669718). If results are promising, ISA will file for Food and Drug Administration registration as accelerated approval.

In conclusion, with a median follow-up of nearly 4 years, ISA101 and nivolumab therapy resulted in durable responses and promising survival in patients with R/M HPV-16$^+$ cancer. The addition of ISA101 may potentially enhance antitumor immunity through generation of E6 and E7 targeted lymphocytes that home to tumor sites and act as a priming agent. Lastly, our data that immune and inflammatory responses were associated with response add to the growing literature regarding HPV$^+$ OPSCC immunoregulation.

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Acknowledgements We thank the research nurses, data managers, and regulatory personnel for their indispensable assistance. Nivolumab was supplied by Bristol Myers Squibb. ISA101 was supplied by ISA Pharmaceuticals.

Contributors Concept and design: BSG and MAC. Acquisition, analysis, or interpretation of data: LGdS, BSG, MAC, KR, JRC, RLC, QW, TZB, IW, JL, and JW. Critical revision of the manuscript for important intellectual content: LGdS, BSG, EM, WW, FMJ, RF, LF, IW, CJMM, MAC, BSG. Statistical analysis: KR, LF, QW, JL, JW. Obtained funding: BSG. Supervision: BSG. All authors take public responsibility for the content of the work submitted.

Funding This work was supported by University of Texas MD Anderson Cancer Center HPV-Related Cancers Moon Shot, University of Texas MD Anderson Cancer Center Oropharynx Program Stiefel Gift, Abell-Hangar Foundation Chair, and University of Texas MD Anderson Cancer Center Support Grant P30 CA016672. KR and CC were partially supported by NCI P30CA125123, CPRIT Proteomics & Metabolomics Core Facility Support Award RP170005, and NIEHS grants 1P30ES030285 and 1P42ES0327725.

Competing interests RF: reports personal fees from Regeneron-Sanofi, Ayala Pharmaceuticals, Klus Pharma, Bicara Therapeutics, Medscape, Carevive systems, G1 Therapeutics, Prelude Therapeutics, Merck, and Intellisphere in the past 36 months outside of the submitted work; and institutional fees from AstraZeneca, Merck, Genentech, Pfizer, Rakuten, Nanobiotix, and EMD Serono in the past 36 months. WW has received honoraria/speaker's fees and/or participated in advisory boards from Roche/Genentech, Bristol Myers Squibb, Eli Lilly, Merck, AstraZeneca, Pfizer, Takeda, Janssen, Boehringer Ingelheim, Novartis, Sanofi Aventis. CJMM is Chief Scientific Officer of ISA Pharmaceuticals, a biotech company aiming at registration of a long peptide vaccine very similar to the one studied in this manuscript. MAC reports grants and personal fees from ImmunoGenesis, Inc. and ImmunoMet, Inc., personal fees from Alligator Bioscience, Inc., ImmunOS, Inc., Oncoresponse, Inc., Pieris, Inc., Nurix, Inc., Aptevo, Inc., Servier, Inc., Kineta, Inc., Salarius, Inc., Xencor, Inc., Agenus, Inc., Mereo, Inc., Astrazeneca, Inc., Amunix, Inc., Adagene, Inc., outside the submitted work; In addition, Dr. Curran has a patent Methods and Composition for Localized Secretion of Anti-CTLA-4 Antibodies with royalties paid by multiple licensees, a patent Dual specificity antibodies which bind both PD-L1 and PD-L2 and prevent their binding to PD-1 with royalties paid by ImmunoGenesis, Inc. IIW reports consults fees from ROCHE, Bayer, BMS, Astra Zeneca, Pfizer, HTG molecular, Merck, ClaxoSmithKline, Guardant Health, Novartis, Flame, Sanofi, Janssen, Daiichi Sankyo, Oncocyte, Amgen, MSD, Medcape; honoraria from Medscape, Roche, Pfizer, AstraZeneca, Platform Health, Merck; and Grants from Genetech, HTG Molecular, Merck, BMS, Medimmune, Adaptive, Adaptimmune, EMD Serono, Pfizer, Takeda, Amgen, Karus, Johnson & Johnson, Bayer, Iovance, 4D, Novartis, Akova. JJL reports he received grant from Cancer Center Support Grant (CCSG) P30 CA016672 from National Cancer Institute. BG reports consult fees from Regeneron and grants from Pfizer Inc, ISA Pharmaceuticals, and Medimmune.

Patient consent for publication Not applicable.

Ethics approval The study protocol was approved by the University of Texas MD Anderson Cancer Center Institutional Review Board. ID 2014-1047. The trial was registered on ClinicalTrials.gov (NCT02426892). 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. The clinical data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1 van Poelgeest MIE, Welters MJP, Vermeij R, *et al*. Vaccination against oncoproteins of HPV16 for noninvasive Vulvar/Vaginal lesions: lesion clearance is related to the strength of the T-cell response. *Clin Cancer Res* 2016;22:2342–50.
2 Melief CJM, Welters MJP, Vergote I, *et al*. Strong vaccine responses during chemotherapy are associated with prolonged cancer survival. *Sci Transl Med* 2020;12:eaaz8235.
3 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. *The Lancet* 2019;393:156–67.
4 Mehra R, Seiwert TY, Gupta S, *et al*. Efficacy and safety of pembrolizumab in recurrent/metastatic head and neck squamous cell carcinoma: pooled analyses after long-term follow-up in KEYNOTE-012. *Br J Cancer* 2018;119:153–9.
5 Ferris RL, Blumenschein G, Fayette J, *et al*. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med* 2016;375:1856–67.
6 Lechien JR, Seminerio I, Descamps G, *et al*. Impact of HPV infection on the immune system in oropharyngeal and Non-Oropharyngeal squamous cell carcinoma: a systematic review. *Cells* 2019;8:1061.
7 Massarelli E, William W, Johnson F, *et al*. Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16–Related cancer. *JAMA Oncol* 2019;5:67–73.
8 Parra ER, Uraoka N, Jiang M, *et al*. Validation of multiplex immunofluorescence panels using multispectral microscopy for immune-profiling of formalin-fixed and paraffin-embedded human tumor tissues. *Sci Rep* 2017;7:13380.
9 Taminau J, Meganck S, Lazar C, *et al*. Unlocking the potential of publicly available microarray data using inSilicoDb and inSilicoMerging R/Bioconductor packages. *BMC Bioinformatics* 2012;13:335.
10 Subramanian A, Tamayo P, Mootha VK, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545–50.
11 Hwang S, Kwon A-Y, Jeong J-Y, *et al*. Immune gene signatures for predicting durable clinical benefit of anti-PD-1 immunotherapy in patients with non-small cell lung cancer. *Sci Rep* 2020;10:643.
12 Ayers M, Lunceford J, Nebozhyn M, *et al*. IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017;127:2930–40.
13 Sanz G, Leray I, Dewaele A, *et al*. Promotion of cancer cell invasiveness and metastasis emergence caused by olfactory receptor stimulation. *PLoS One* 2014;9:e85110.
14 Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 2010;29:625–34.
15 Kalaora S, Lee JS, Barnea E, *et al*. Immunoproteasome expression is associated with better prognosis and response to checkpoint therapies in melanoma. *Nat Commun* 2020;11:896.
16 Jen J, Wang Y-C. Zinc finger proteins in cancer progression. *J Biomed Sci* 2016;23:53.
17 Ferris RL, Blumenschein G, Fayette J, *et al*. Nivolumab vs investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression. *Oral Oncol* 2018;81:45–51.
18 Bauml J, Seiwert TY, Pfister DG, *et al*. Pembrolizumab for platinum- and Cetuximab-Refractory head and neck cancer: results from a single-arm, phase II study. *J Clin Oncol* 2017;35:1542–9.
19 Aggarwal NFS C, Algazi AP, Sukari A. 916MO - Safety and efficacy of MEDI0457 plus durvalumab in patients (pts) with human papillomavirus-associated recurrent/metastatic head and neck squamous cell carcinoma (HPV+ R/M HNSCC). *Ann Oncol* 2020.
20 Strauss J, Floudas CS, Abdul Sater H, *et al*. Phase II evaluation of the triple combination of PDS0101, M9241, and bintrafusp alfa in patients with HPV 16 positive malignancies. *J Clin Oncol* 2021;39:2501–01.
21 Nordfors C, Grün N, Tertipis N, *et al*. CD8+ and CD4+ tumour infiltrating lymphocytes in relation to human papillomavirus status and clinical outcome in tonsillar and base of tongue squamous cell carcinoma. *Eur J Cancer* 2013;49:2522–30.
22 Kim MH, Kim J-H, Lee JM, *et al*. Molecular subtypes of oropharyngeal cancer show distinct immune microenvironment related with immune checkpoint blockade response. *Br J Cancer* 2020;122:1649–60.
23 Mandal R, Şenbabaoğlu Y, Desrichard A, *et al*. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight* 2016;1:e89829.
24 Curran MA, Glisson BS. New hope for therapeutic cancer vaccines in the era of immune checkpoint modulation. *Annu Rev Med* 2019;70:409–24.
25 Vitale I, Manic G, Coussens LM, *et al*. Macrophages and metabolism in the tumor microenvironment. *Cell Metab* 2019;30:36–50.
26 Morita R, Hirohashi Y, Torigoe T, *et al*. Olfactory receptor family 7 subfamily C member 1 is a novel marker of colon cancer–initiating cells and is a potent target of immunotherapy. *Clin Cancer Res* 2016;22:3298–309.