INTRODUCTION

The burgeoning field of cancer immunotherapy has made significant progress with the application of immune checkpoint inhibitors in the treatment of solid tumours at different sites across the body. Such therapeutic strategies seek to harness the body's own immune system and tip the balance in favour of antitumour immunity. The first clinically validated immune checkpoint therapy targeting cytotoxic
T-lymphocyte-associated antigen (CTLA-4) mediated tumour regression and increased overall survival in melanoma patients, but was associated with frequent immune-related adverse events.\(^{1,5}\) Programmed death 1 (PD-1) protein and its ligand PD-L1 was subsequently discovered\(^{6,7}\) and shown to have good safety and efficacy in inducing durable tumour regression and prolonged stable disease in patients with advanced cancers including non-small cell lung cancer (NSCLC), melanoma, renal cell, ovarian, colorectal, pancreatic, gastric and breast cancer.\(^{8}\) However, the vast majority of head and neck cancer patients, about 80%, remain unresponsive to immune checkpoint inhibitor therapy, highlighting the need for more effective immunotherapies and predictive biomarkers.\(^{9}\) IDO1 inhibitors for melanoma, glioblastoma, NSCLC, pancreatic and breast cancer are under investigation by pharmaceutical companies and sponsors.\(^{10}\) To date, IDO inhibitors for head and neck cancer have been tested in only several published clinical studies.\(^{11-18}\)

Head and neck squamous cell carcinoma (HNSCC) is the sixth leading cause of cancer worldwide and is diagnosed in 8000 new patients annually in the UK.\(^{19}\) HNSCCs are divided into two clinically, genomically and immunologically distinct subgroups based on their association with human papillomavirus (HPV) infection: (a) the majority of HNSCCs are HPV-negative and tend to present in older patients, usually with a history of smoking and alcohol use; their tumours are often characterised by p53 mutations and have poor 5-year survival ranging from 33.8% to 66.8% depending on subsite, whilst (b) HPV-positive HNSCCs arise mainly in younger, Caucasian, non-smokers and their tumours are characterised by integration of viral genome and the expression of E6 and E7 viral oncoproteins which result in the inactivation of p53 and retinoblastoma (Rb) protein, and subsequent overexpression of p16, but better prognosis and overall survival as their tumours are often radiosensitive.\(^{20}\) Current surgical and non-surgical treatments for HNSCC have devastating functional and cosmetic consequences. Survival has improved little in the past four decades, that is most less than 50%.\(^{21}\) The head and neck tumour microenvironment (TME) is a site of intense immunological activity, driving a recent emergence in immunotherapy being applied to HNSCC.

Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme which plays a critical role in the immunity of the TME via tryptophan metabolism. Its activity is increased in the TME of many cancers and its expression was found to be a negative prognostic indicator in melanoma,\(^{22}\) ovarian,\(^{23}\) colorectal\(^{24}\) and lung cancer.\(^{25,26}\) IDO inhibits natural and therapy-induced antitumour immunity as it catabolises the amino acid tryptophan to generate kynurenine and other immunosuppressive catabolites which activate Foxp3 regulatory T cells and attenuate effector T-cell responses to inhibit immune-mediated killing of tumour cells. Due to its important role in TME immunity, IDO is an immune checkpoint which can be potentially exploited to improve treatment outcomes. However, the immunotherapeutic role of IDO in HNSCC requires further exploration.

Our review aims to systematically assess the current literature for pre-clinical and clinical evidence on the immunotherapeutic role of IDO in HNSCC. Our objectives were to (a) identify all studies which investigated IDO in HNSCC, (b) identify studies which investigated IDO activity and/or expression in HNSCC, (c) evaluate the effectiveness of IDO inhibitors at improving the outcomes of patients with HNSCC, (d) compare the use of IDO inhibitors alone and in combination with other treatments for HNSCC and (e) evaluate the potential for immunotherapeutic strategies involving the IDO pathway in the treatment of HNSCC.

### Keypoints

- IDO is integral to TME immunity in HNSCC particularly in HPV-positive cancers.
- IDO can be used to modulate existing therapies and has applications in combinatorial immunotherapy.
- Retrospective studies have shown its presence in the TME and suggest a link to HNSCC treatment outcome.
- However, the exact mechanism of IDO-driven immune modulation in the HNSCC TME remains unclear.
- We now require prospective longitudinal studies to track IDO activity and expression throughout HNSCC treatment, thence optimise IDO-based immunotherapy.

### 2 | MATERIALS AND METHODS

#### 2.1 | Data sources and literature search

A systematic literature search was conducted in Ovid MEDLINE, Ovid Embase, Scopus, Web of Science, Cochrane library and ClinicalTrials.gov databases from inception until present day. The PRISMA guidelines for study selection were followed.\(^{27}\) All studies that evaluated the involvement of IDO in HNSCC were systematically retrieved. The following search terms and strategy was used: ("indoleamine 2,3-dioxygenase" OR "IDO" OR "IDO1" OR "IDO-1" OR "IDO2" OR "IDO-2") AND ("squamous cell carcinoma" OR "squamous cell cancer" OR "SCC"). The titles and abstracts from the initial search results were screened independently by two authors DJL and JCKN. To ensure inclusion of all studies related to HNSCC, DJL and JCKN manually screened the studies with squamous cell carcinoma to include those involving the head and neck region.

#### 2.2 | Study selection

In the initial screening, the following criteria were required for inclusion: (a) HNSCC from any head and neck subsite (oral, oropharynx, nasopharynx, larynx and hypopharynx), (b) study of IDO expression or activity, (c) all study types (prospective or retrospective, observational or experimental, pre-clinical or clinical), (d) published in English language only and (e) original articles and conference abstracts.
Duplicates, correspondence, review articles and studies without data on IDO in the context of HNSCC were excluded.

2.3 | Data extraction and analysis

Following the generation of a list of articles meeting the inclusion criteria, DJL and JCKN each performed an in-depth review of the studies and extracted data for comparison. Similar studies were grouped together for qualitative analysis.

3 | RESULTS

3.1 | Included studies

A total of 273 studies were identified from databases and seven studies from additional sources, and 146 were screened after removal of 134 duplicates. A total of 100 studies were excluded with reasons described in the PRISMA flow diagram in Figure 1. After full-text review, 40 studies were included in the final analysis.

Evidence from pre-clinical and clinical studies involving IDO was extracted. A total of 22 full-text articles, 17 conference abstracts and seven clinical trials without results were evaluated. Of those seven studies involved cell lines, eight assessed tumour immunohistochemistry (IHC), 10 were IDO gene transcription studies, and 15 others reported on clinical trials (eight published, seven registered without results). All seven cell line studies used different HNSCC cell lines. The prognostic studies involving IHC investigated IDO expression in HNSCC at different subsites, defined as; lower lip, oral cavity, tongue and larynx. The clinical trials compared survival with combination therapies involving IDO1 inhibitor vs monotherapy using PD-1 inhibitor Pembrolizumab, and assessed IDO expression value as a predictive biomarker for response to PD-L1 therapy. Additionally, 13 conference abstracts were identified and summarised in Table S1.

3.2 | Cell line studies on IDO in HNSCC

The seven studies which investigated IDO in HNSCC cell lines, each is an immortalised cell culture developed from a single human HNSCC tumour, are summarised in Table 1. Of those, the majority investigated IDO in oral and oropharyngeal HNSCC cell lines. The studies were heterogeneous in the method of cell line analysis, which ranged from enzyme-linked immunosorbent assay (ELISA), 2D/3D cell culture, computational simulation model, enzymatic IDO activity assay, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and IFNγ stimulation followed by

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**FIGURE 1** PRISMA flow diagram for study selection. Abbreviations: IDO, indoleamine 2,3-dioxygenase; HNSCC, head and neck squamous cell carcinoma
| First author, year, country | Journal | Cell line (subsite) | TNM stage | Assay method | Results interpretation |
|----------------------------|---------|---------------------|-----------|--------------|------------------------|
| Riess, 2020 (Germany)     | Frontiers in Immunology | Hypopharyngeal: FADU | T2 | HNSCC cell lines were cultured and treated with IFNγ for 24 h and 72 h, then treated with cytostatic drugs including 5-fluorouracil (5-Fu), Cisplatin, Gemcitabine and Cetuximab. IDO1 immunofluorescence was performed on the treated cells and kynurenine pathway (KP) metabolites in the cell culture supematant was quantified by liquid chromatography tandem mass spectrometry | IDO1 expression was low, but inducible upon IFNγ treatment of HNSCC cells. Upon treatment with 5-Fu, Gemcitabine and Cetuximab, IDO1 and additional genes of the KP (KYAT1, KYAT2 and KMO) were induced. Cyclin-dependent kinase inhibitor Dinaciclib suppressed the KP, whilst conventional chemotherapeutics tend to activate the KP |
| Al-Samadi, 2019 (Finland) | Experimental Cell Research | Oral cavity: tongue: HSC-3 | T2 | In vitro 3D microfluidic chip assay. HSC-3 was embedded in human tumour-derived matrix along with patients' serum, cancer and immune cells, which were then loaded with anti-PD-L1 and IDO1 inhibitors. Immune cell migration and cancer cell proliferation rates were evaluated | IDO1 inhibitor induced immune cell migration towards cancer cells in HSC-3 and two HNSCC patient samples, which could change the tumour from "cold" to "hot" and enhance the efficacy of other immunotherapeutic drugs in combination. This in vitro 3D microfluidic chip assay could be used to further test immunotherapeutic drugs against patient samples |
| Bates, 2018 (USA)         | Translational Cancer Research | Oral cavity: tongue: SCC4, SCC15, SCC25 | T3N0M0 | ELISA to determine the concentration of IDO in cell lysates | SCC15 produced significantly more IDO than any of the six other cell lines. HNSCC cell lines from different hosts can have varying amounts of biomarkers. These differences could be due to the stage of disease, site of tumour, tissue type or genomic differences between patients. These results support personalised medicine in treating HNSCC |
| Subramanian, 2018 (USA)   | Cancer Research | Cell line not specified | T2 | HNSCC cell lines grown in 2D culture. Kynurenine levels measured by MS. IDO1 levels in tissue measured by Western Blot | High levels of kynurenine in HNSCC cell lines shown through metabolic profiling via MS. Checkpoint inhibition of IDO1 leads to an upregulation of genes in glycolysis (ACLY, G6PD, COX5A, LPL and PFKL) and apoptosis (CASP7, CASP9, BCL2L11) in vitro |
| Bates, 2017 (USA)         | Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology | Oral cavity: tongue: SCC4, SCC15, SCC25 | T2 | Cell line-specific predictive computational simulation models used to predict expression of IDO1 | Predicted IDO expression in SCC4 (17.29%), SCC15 (2.75%) and SCC25 (4.97%) with respect to controls. In the simulation model, SSC4 was classified as a non-responder whilst SCC15 and SCC25 were classed as responders to PD-L1 immunotherapy |

(Continues)
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3.3 | Tumour immunohistochemistry studies on IDO in HNSCC

### Table 1 (Continued)

| First author, year (country) | Journal | Cell line (subsite) | TNM stage | Assay method | Results interpretation |
|-----------------------------|---------|---------------------|-----------|--------------|------------------------|
| El Jamal, 2016 (USA)        | Cell Division | HNSCC Mouth: CLS-354, SCC nasal septum: RPMI 2650 | — | Enzymatic IDO activity assay, absorbance at 490 nm. Immunoblot with anti-IDO antibody 1:1500 (BioGenes, Berlin, Germany). Western Blot | Described central role of IDO in IFNγ-induced apoptosis of HNSCC cells by the suppression of HO-1 leading to the accumulation of ROS and activation of apoptotic pathways |
| Liang, 2015 (China)         | Biochimica et Biophysica Acta - Molecular Basis of Disease | Oral SCC: HSC-3, SCC-4, Normal human keratinocyte: HaCaT | — | IDO mRNA isolation by qRT-PCR | IDO expression was significantly induced in HaCaT, HSC-3 and SCC4 by STING activation. Suggests the establishment of HNSCC TME by immunosuppressive cytokines such as IDO could be promoted by 2′-3′ cGAMP a activation of STING |

Abbreviations: ELISA, enzyme-linked immunosorbent assay; TNM, Tumour Node Metastasis; SCC, squamous cell carcinoma; MS, mass spectrometry; HO-1, haem oxygenase-1; ROS, reactive oxygen species; STING, stimulator of interferon genes; qRT-PCR, quantitative reverse transcription polymerase chain reaction; cGAMP, 2′-5′,3′-5′ cyclic AMP-GMP.
## TABLE 2  Tumour immunohistochemistry studies on IDO in HNSCC

| First author, year (country) | Tumour site/stage | Source/preparation | Primary antibody, manufacturer (clone), dilution | Cases (n) | Method of analysis | Results interpretation | Compliance with REMARK checklist |
|-----------------------------|-------------------|--------------------|-----------------------------------------------|----------|-------------------|----------------------|-----------------------------|
| Wang, 38 2019 (China)       | Oral SCC (OSCC)   | FFPE tissue sections collected before and after 4-wk treatment with six cycles of Nimotuzumab | Anti-IDO1 (Abcam, ab211017), 1:1500              | 36        | Retrospective. IHC slides were assessed by 3 independent pathologists blindly and a staining score was given | Nimotuzumab therapy increased the expression of IDO in the TME of OSCC patients compared with baseline pre-treatment \( P = .0053 \), suggesting IDO as a marker of immune status | Checklist items 6 and 9 not fulfilled |
| Succaria, 78 2018 (USA)     | HNSCC, unspecified | Archived HNSCC specimen | Unspecified                                      | 27        | Retrospective. IDO expression by tumour cells and infiltrating immune cells | >500 IDO+expressing cells/mm² in 17/27 HNSCC specimens, IDO expressed by tumour cells and infiltrating immune cells in 12/27 (44%) cases (range 5%-95% tumour cells+) | — |
| Venkata, 79 2017 (India)    | HNSCC, unspecified | FFPE                | —                                              | 50        | Retrospective. Stained slides analysed manually and by digital algorithms | Increased expression of IDO in tumour cells correlated with FOXP3-positive immune cells. Overall percentage of IDO and CD8-positive immune cells were higher than PD-L1 and FOXP3-positive immune cells | — |
| Seppälä, 35 2016 (Finland) | Tongue SCC (58) and lymph node samples (32), control group (30) with tongue squamous cell hyperplasia | FFPE                | Anti-IDO monoclonal antibody, (MAB5412) 10.1, 1:200 | 108       | Retrospective. Semi-quantitative light microscopic evaluation by two observers. IDO proportion and IDO staining intensity scores were calculated | IDO expression was higher in tongue hyperplasia than SCC. In tumour stage T2-T4 and tumours with strong inflammation at the invasive front, IDO expression correlated with poor survival | Fulfilled all items |
| Ye, 36 2013 (China)         | Laryngeal SCC: Glottic (92), Subglottic (65); Stage: Early I-II (78), Late III-IV (109) | FFPE surgical specimen, tissue block with tumour cells and non-neoplastic laryngeal tissue was selected | IDO, Chemicon (AB5968) 10.1, 1:300             | 187       | Retrospective. IDO staining intensity in the tissue and tumour-infiltrating lymphocytes. Correlation with survival analysis | Tumour IDO expression not significantly correlated with histology, clinical/nodal stage or tumour differentiation, but positively associated with density of FOXP3+ TILs \( P = .028 \). High IDO expression was an independent predictor of poor DFS (HR = 3.973, \( P = .026 \)) and OS (HR = 3.258, \( P = .029 \)) | Fulfilled all items |

(Continues)
## Table 2 (Continued)

| First author, year (country) | Tumour site/stage | Source/preparation | Primary antibody, manufacturer (clone), dilution | Cases (n) | Method of analysis | Results interpretation | Compliance with REMARK checklist |
|-----------------------------|-------------------|--------------------|-----------------------------------------------|----------|-------------------|------------------------|-------------------------------|
| Kuales, 2011 (Germany)      | Lower lip SCC     | Lesional biopsies, FFPE | Anti-IDO monoclonal antibody, Millipore (10.1), 1:150 | 47       | Retrospective. Density of inflammatory infiltrate at the invasive front of each tumour was calculated | IDO expression correlated with moderate to intense inflammatory infiltrate and was found in myeloid CD11c+ S100+ DCs along the border of invasive tumour cells where Foxp3 regulatory T cells were also present | — |
| Laimer, 2011 (Austria)      | Oral SCC          | Paraffin blocks, deparaffinised and rehydrated sections mounted on slides | Anti-IDO human antibody, Chemicon, Millipore (ab9252) sheep polyclonal, 1:500 | 88       | Retrospective. IDO expression was evaluated and total expression score given. Cox proportional hazard model for the relationship of IDO expression with survival time | IDO expression, staging, tumour grade 3 were prognostic for poorer overall survival. IDO was a prognostic factor in patients who received adjuvant (radio) chemotherapy, but had no impact in patients without adjuvant therapy | Checklist items 6 and 9 not fulfilled |
| Ferdinande, 2008 (Belgium)  | Tonsil SCC (26), Tongue SCC (12) | Unspecified | — | 38 | Inflammatory infiltrate evaluated and scored (semi)quantitatively | 73% of tonsil SCC and 92% of tongue SCC showed IDO expression in tumour cells, focally at invasive front, and no association was found with TNM stage. IDO was present mostly in DCs | — |

Abbreviations: REMARK, Reporting Recommendations for Tumour Marker Prognostic Studies; SCC, squamous cell carcinoma; FFPE, formalin-fixed paraffin-embedded; IHC, immunohistochemistry; TIL, tumour-infiltrating lymphocytes; DFS, disease-free survival; DC, dendritic cell.
increase in IDO expression was seen in clinical non-responders to Nimotuzumab (anti-epidermal growth factor receptor) therapy, suggesting that IDO may be a biomarker of immune status in the TME during therapy in oral SCC patients.38

3.4 | IDO gene transcription studies in HNSCC

We included 10 studies which investigated IDO gene transcription, summarised in Table 3. A variety of sources including tissue and blood specimen, 3D tumour microspheres, The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were used to acquire transcription data for analysis. Methods of analysis included single-sample gene set enrichment analysis (ssGSEA), NanoString analysis, MassARRAY, quantitative polymerase chain reaction (qPCR) and gene expression analysis in peripheral blood mononuclear cells (PBMCs). IDO was strongly expressed in human papillomavirus (HPV) positive HNSCCs and correlated with E7 HPV antigen expression.39 IDO1 was overexpressed in tumours from never-smokers and never-drinkers; and gene expression profiles showed that IDO1 together with PD-L1 were co-overexpressed in HNSCCs30-42 compared to IDO1 presence in normal head and neck tissue.42 Recent studies of methylation of CpG sites suggest that IDO1 expression levels are epigenetically regulated by DNA methylation and hypermethylation of IDO1 is associated with poor overall survival.43,44 Measuring expression during treatment, a significant (3.6-fold) increase was seen in IDO expressed in PBMCs during radiotherapy for patients with stage III-IV HNSCC.45 Whereas after chemoradiation treatment, IDO1 mRNA levels correlated with worse overall survival, and a combined decrease in expression of PD-L1 and IDO1 post-treatment was associated with better progression-free and overall survival.46

3.5 | Clinical trials of IDO inhibitors in HNSCC

Clinical trials with published results on IDO in HNSCC are all in early phase (I-II) and assessed IDO1 inhibitor in combination with a PD-1/PD-L1 inhibitor (Table 4). All HNSCC patients enrolled in the published trials had advanced metastatic or recurrent disease. Pembrolizumab12,14,15,17 (PD-1 inhibitor) was most commonly combined with IDO inhibitors, followed by Nivolumab16 (PD-1 inhibitor), Durvalumab13 and Atezolizumab11 (both PD-L1 inhibitors). The trials showed (a) objective responses (34%-55%) and disease control rates (62%-70%) for Epacadostat (IDO1 inhibitor) in combination with a PD-1 inhibitor;12,14,16 (b) safety profile of IDO1 and PD-L1 inhibitor combination therapy was consistent with previous reports of each checkpoint inhibitor as monotherapies15 and (c) IDO gene expression as a predictive biomarker for response to PD-L1 therapy.18 The most common treatment-related adverse event associated with the immune checkpoint trials was fatigue (22%-32%).11,13,15 Existing clinical trials testing IDO inhibitors in HNSCC, registered on ClinicalTrials.gov without published results are summarised in Table S2.

4 | DISCUSSION

4.1 | Summary of main results

Existing evidence suggests that IDO is integral to TME immunity in HNSCC in the context of positive HPV status, modulating existing therapies and application in combinatorial immunotherapy. The differential expression of IDO and predicted response to immunotherapy based on simulation models as shown by cell line studies suggests that differences in IDO as a biomarker may be related to the stage of disease, site of tumour, tissue type or genomic differences between patients. These findings further support a personalised approach to future HNSCC therapies. To date, only four retrospective studies have investigated IDO expression in formalin-fixed paraffin-embedded tumour specimen as a prognostic biomarker for HNSCC.35-38 Interestingly, Laimer37 and colleagues showed that IDO was a prognostic factor in patients who received adjuvant (radio) chemotherapy, but had no impact in patients without adjuvant therapy. Saâda-Bouzid47 showed that IDO1 rs3739319 (A/G or A/A) was associated with a longer progression-free survival and overall survival. They suggest that the prognostic and predictive value of IDO polymorphism should be tested prospectively in the context of immune checkpoint inhibitor era. Wirth and colleagues41 showed an increase in IDO1 in PD-L1+ HPV+HNSCCs and proposed that IDO1 is an adaptive immune resistance pathway to anti-PD-1 monotherapy. These results support the rationale for future combinatorial therapies involving IDO1 and PD-1. Liang et al40 showed that STING activation, as indicated by staining, was greatest around the nucleus in a majority (16 of 25) of HPV-positive tongue SCC samples and was present in the whole cytoplasm in 22 of 25 HPV-negative samples. They suggest that HPV hijacks and activates STING by DNA sensing which induces an immunosuppressive microenvironment through IDO expression and recruitment of regulatory T cells allowing the establishment of tumourigenesis in tongue SCC. The central role of IDO expression and activation by STING is also consistent with previous work on DNA sensing via STING by Huang,48 Lemos49 and colleagues.

4.2 | IDO inhibitors commonly applied in pre-clinical and clinical studies

IDO can be expressed in tumour cells but multiple TME cell types may also express IDO including dendritic cells, macrophages, fibroblasts, endothelial/epithelial cells and PBMCs,50,51 though lymphoid cells (eg T cells and tumour-infiltrating lymphocytes) rarely express IDO. It is important to distinguish between IDO protein abundance in the TME (detected by IHC) and IDO enzyme activity (measured peripherally in the blood and in situ using homogenised tissues), as multiple factors impact IDO activity including enzyme cofactors and natural inhibitors such as hemin and nitric oxide, respectively. As IDO inhibits innate and adaptive immunity, IDO inhibitors (IDOi) have been tested as drugs to potentiate antitumour immunity in the TME. A recent review by Lemos and colleagues summarised the IDOi under pre-clinical
**TABLE 3** IDO gene transcription studies in HNSCC

| First author, year (country) | Cases (n), source | Method of analysis | Results interpretation |
|------------------------------|-------------------|-------------------|-----------------------|
| Economidou, 2020 (Greece) | 113 locally advanced HNSCC patients who underwent cisplatin chemotherapy, peripheral blood collected at baseline and 1 wk after end of treatment | Expression of IDO1 in the EpCAM+CTC fraction before and after cisplatin chemoradiation. Multivariate Cox regression analysis was used to assess the prognostic value of PD-L1 and IDO1 expression | IDO1 was significantly overexpressed at baseline compared to post-treatment (P = .007). IDO1 mRNA expression at baseline was associated with better PFS (HR = 0.19, P = .017). Post-treatment IDO1 mRNA levels correlated with worse OS (HR = 3.27, P = .008). Patients with combined decreased expression of PD-L1 and IDO1 post-treatment had better PFS (P = .043) and OS (P = .021) |
| Sailer, 2019 (Germany) | 528 HNSCC patients, TCGA; 138 HNSCC patients as a validation cohort from the University Hospital Bonn | Methylhyal of 3 CpG sites was correlated with mRNA expression, immune cell infiltration, mutational burden, HPV status and OS | IDO1 methylation and IDO1 mRNA expression were inversely correlated in the promoter and promoter flank region. IDO1 promoter flank hypermethylation was associated with poor OS (P < .001). These suggest that IDO1 expression levels are epigenetically regulated by DNA methylation |
| Lecerf, 2019 (France) | 96 HNSCC patients who underwent primary surgery | Real-time polymerase chain reaction was used to assess the expression of 46 immune-related genes | IDO1 (75%) was among the most significantly overexpressed immune-related genes and had significantly higher mRNA expression level in HNSCC compared to normal head and neck tissue (P < .0001) |
| Chen, 2019 (China) | 167 oral SCC gene expression data set (GSE30784) and 54 oral SCC DNA methylation data set (GSE75537), obtained from the GEO | Correlations between methylation level of CpG sites and OS of oral SCC patients were assessed by univariate Cox regression analysis followed by robust likelihood-based survival analysis | A two-CpG-based prognostic signature for OSCC OS prediction was obtained, which included the sites cg17892178 and cg17378966 that are located in NID2 and IDO1, respectively |
| Krishna, 2018 (USA) | 119 HNSCC transcriptomes | Epitope mapping from HPV+HNSCC PBMCs using Elispot, flow cytometry immune cell phenotyping, ssGSEA of HPV and immune gene signatures | IDO was strongly expressed in HPV+ vs HPV− HNSCC (P = .001). Its expression correlated with E7 HPV antigen expression (R² = 0.84, P = .033). Combined inhibition of PD-1 and IDO-1 can sensitise HPV+HNSCCs to CD8+ cytotoxic T-lymphocyte-mediated cytotoxicity |
| Page, 2018 (USA) | 3D-EX platform, 3D tumour microspheres were produced from fresh HNSCCs | NanoString analysis for expression of genes including IDO1 | Increased expression of IDO1 gene in HNSCC which were responsive to checkpoint inhibitor treatment ex vivo |
| Fox, 2017 (France) | 212 oral SCC who were NSND, HPV+ samples were excluded. 4 cohorts: TCGA, GEO1, GEO2, CLB | Gene expression profiles generated using microarrays and targeted-RNA sequencing. Functional pathway analysis performed using ssGSEA and STRING | IDO1 was overexpressed in tumours from NSND vs SD (P = .0046). Elderly female patients were more common in NSND and harboured less gene mutations (P = .0006). PD-L1 and IDO1 were co-overexpressed, suggesting a higher potential benefit of combination therapy involving both |
| Saâda-Bouzid, 2017 (France) | 36 recurrent metastatic HNSCC treated with anti-PD-1 or anti-PD-L1 or in combination with a second checkpoint inhibitor | Extraction of blood DNA and genotyped by MassARRAY and multivariate analysis with PFS and OS | A genotype of IDO1 rs3739319 (A/G or A/A) was associated with a longer PFS and OS, (HR = 8.4, P = .002) and (HR = 6.2, CI95% 1.5-25.9, P = .01), respectively. Allelic variation of IDO1 rs3739319G>A, implicated in transcription and regulation was associated with longer survival in patients treated with anti-PD-1 therapy |
| Wirth, 2017 (USA) | Validation cohort of 25 HPV+HNSCC patients | Microarray of 59 immune-related genes to compare expression profiles in HPV+HNSCCs. qPCR and protein expression assay used in validation cohort | There was a 65-fold increase in IDO1 in 10 PD-L1+ vs 5 PD-L1− HPV+HNSCCs (P = .004). IDO1 expression was upregulated and co-localised in the TME of the validation cohort. IDO1 expression increased and correlated with disease progression in anti-PD-1 treated HNSCC patients |
| Won, 2017 (USA) | 15 patients with stage III-IV HNSCC, blood specimen collected before, during and after RT | Gene expression analysis in patients’ PBMCs | A 3.6-fold (P = 1) increase seen in IDO expression in patients’ PBMCs during RT along with other protein mediators that promote immune suppression, suggesting RT could induce tolerogenic effects in HNSCC which can be targeted with checkpoint inhibitor therapy |

Abbreviations: HPV, human papillomavirus; PBMCs, peripheral blood mononuclear cells; ssGSEA, single-sample gene set enrichment analysis; SCC, squamous cell carcinoma; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; CLB, Centre Léon Bérard cancer centre, Lyon, France; NSND, never-smokers and never-drinkers; SD, smokers drinkers; STRING, search tool for the retrieval of interacting genes/proteins; qPCR, quantitative polymerase chain reaction; PFS, progression-free survival; OS, overall survival; RT, radiotherapy.
and clinical evaluation.\textsuperscript{52} In total there are seven IDOi drugs under evaluation in Phase I-III clinical trials and four that are applied in preclinical studies. The two front-running drugs in development are the non-selective IDOi indoximod (also known as D-1MT or NLG-8186) and the tryptophan competitive IDOi epacadostat (INCB024360). Drugs being tested in clinical trials include the non-selective IDO and TDO (tryptophan 2,3-dioxygenase) inhibitor navoximod (NLG-919),\textsuperscript{53,54} which is approximately tenfold more selective for IDO1 than TDO2; the selective IDO1 inhibitor linrodostat (BMS-986205)\textsuperscript{55} and IDO1 and TDO2 inhibitor PF-06840003\textsuperscript{56} which are both more than 100-fold selective for IDO1 than TDO2. Indoximod, epacadostat and linrodostat are also being evaluated in combination drug trials given 100-fold selective for IDO1 than TDO2. Indoximod, epacadostat and linrodostat are also being evaluated in combination drug trials given

\begin{table}[h]
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\begin{tabular}{|l|l|l|l|l|l|}
\hline
First author, year (country) & Trial name ID & Phase & Design & Disease & Eligibility \\
\hline
Jung,\textsuperscript{11} 2019 (South Korea) & NCT02471846 & I & Open-label, multicentre, dose-escalation and expansion trial & Locally advanced, recurrent or metastatic incurable solid malignancy & Progression following at least one standard therapy \\
\hline
Mitchell,\textsuperscript{12} 2018 (USA) & ECHO-202/KEYNOTE-037 NCT02178722 & I/II & Multicentre, non-randomised, open-label trial & Advanced solid tumours: stage IIIB, IV or recurrent NSCLC, melanoma, RCC, EA, UC, TNBC or HNSCC & All patients with one or more previous therapy, or no available curative treatment \\
\hline
Naing,\textsuperscript{13} 2018 (USA) & ECHO-203 NCT02318277 & I/II & Dose-escalation, open-label trial & Advanced solid tumours: PC, melanoma, NSCLC, HNSCC & Failed at least 1 prior treatment, intolerant to or refused standard treatment \\
\hline
Hamid,\textsuperscript{14} 2017 (USA) & ECHO-202/KEYNOTE-037 NCT02178722 & I/II & Multicentre, non-randomised, open-label trial Reporting of HNSCC results & HNSCC & Metastatic HNSCC with ≥1 prior CT regimen \\
\hline
Hamid,\textsuperscript{15} 2017 (USA) & ECHO-202/KEYNOTE-037 NCT02178722 & II & Multicentre, non-randomised, open-label trial Reporting of phase II safety & Advanced or recurrent NSCLC, melanoma, RCC, EA, UC, TNBC or HNSCC & All patients with one or more previous therapy, or no available curative treatment \\
\hline
Perez,\textsuperscript{16} 2017 (USA) & ECHO-204 NCT02327078 & I/II & Non-randomised, open-label trial & Advanced cancers: melanoma, NCSCLC, HNSCC, CRC, OVC, GBM, B-cell NHL & All adult patients with pathologically confirmed disease \\
\hline
Gangadhar,\textsuperscript{17} 2016 (USA) & ECHO-202/KEYNOTE-037 NCT02178722 & I & Multicentre, non-randomised, open-label trial & Advanced melanoma and select solid tumours & All patients with one or more previous therapy, or no available curative treatment \\
\hline
Seiwert,\textsuperscript{18} 2016 (USA) & KEYNOTE-012 NCT01848834 & Ib & Non-randomised, open-label, multicentre trial & PD-L1 positive recurrent or metastatic HNSCC & Pathologically confirmed disease, any number of prior treatment regimens \\
\hline
\end{tabular}
\caption{Clinical trials of IDO inhibitors in HNSCC with published results}
\end{table}

Abbreviations: NSCLC, non-small-cell lung cancer; RCC, renal cell cancer; EA, endometrial adenocarcinoma; UC, urothelial carcinoma; TNBC, triple-negative breast cancer; DLT, dose-limiting toxicities; ORR, overall response rate; TRAE, treatment-related adverse event; CR, complete response; PR, partial response; SD, stable disease; PC, pancreatic cancer; CT, chemotherapy; DCR, disease control rate; CRC, colorectal cancer; OVC, ovarian cancer; GBM, glioblastoma; NHL, non-Hodgkin lymphoma; PFS, progression-free survival; OS, overall survival; AE, adverse event.

In vitro studies of IDOi drugs in cancer include those investigating indoximod\textsuperscript{58} and linrodostat.\textsuperscript{59} Applied as monotherapy to patient-derived colorectal cancer cell lines, indoximod exhibited rather low direct cytotoxic activity, whereas coculturing the cell lines in an allogeneic setting using naïve, “unprimed” lymphocytes from healthy volunteers generally boosted the antitumoural effect of indoximod.\textsuperscript{56} However, this was not tested in an autologous setting using partially exhausted lymphocytes from cancer patients. Interestingly, low IDO expressing cells responded better to indoximod monotherapy, suggesting that indoximod likely targets additional pathways, although the precise mechanism of action is yet to be elucidated. It is important to stress that IDOi drugs may promote antitumour effects by targeting tumour accessory cells in malignant lesions and/or in tumour-draining lymphoid tissues, and not necessarily by targeting tumour cells per se. Nonetheless, multiple studies support the use of non-selective IDOi drugs to counteract tumour-induced immunosuppression and increase antitumour efficacy in combination with other therapeutics.
4.3 | Other combination immunotherapeutic strategies

Apart from immune checkpoint blockade alone, other combination immunotherapeutic strategies have been studied. Rational combinations have been tested based on the knowledge that activating STING in the TME of mice stimulated protective antitumour immunity; however, preliminary outcomes from a clinical trial reveal little benefit of STING agonist monotherapy. To overcome this therapy resistance, Lemos and colleagues showed that in mice bearing established Lewis lung carcinoma (LLC) tumours, intratumoral treatment with STING agonist, synthetic cyclic diadenyl monophosphate (CDA) and co-treatment with selective COX2 inhibitor celecoxib eliminated the primary tumour burden, prevented metastasis and induced durable protective antitumour immunity. Co-treatment with IDOi drugs indoximod, navoximod and linrodostat also enhanced antitumour responses to CDA, especially in co-treatment with linrodostat which induced rapid tumour regression and increased survival, however, did not eliminate the primary tumours. Interestingly, inhibiting COX2 also significantly reduced IDO activity, which may contribute to greater antitumour activity elicited by celecoxib in combination with CDA. Another strategy to enhance antitumour immunity is to deliver recombinant enzymes that act downstream of IDO inhibitors or cancer vaccine reduced Kyn levels in the TME, attenuated immune suppression and promoted tumour control in vivo. However, the effectiveness of this strategy in promoting clinical responses in patients is yet to be proven. Although speculative at present, combining IDOi drugs with radiotherapy treatment may modulate the TME in favour of antitumour activity and help overcome treatment resistance in cases where the disease is less radiosensitive, for instance in HPV-negative HNSCCs where radiotherapy controls less than 50% of disease cases that have concurrent nodal metastases. The hypothesised role of IDO in the immune microenvironment is summarised in Figure 2.
4.4 | Strengths, limitations and potential bias of evidence

This narrative systematic review synthesises the existing evidence on studies involving IDO in HNSCC. Due to the heterogeneity among the studies, a meta-analysis was not possible and a qualitative analysis was therefore performed on the groupings of study types. Although existing HNSCC cell line studies showed IDO involvement in certain cell lines (SCC4, SCC15) and influenced by

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**FIGURE 2** IDO immune microenvironment hypothesis. (Chemo)radiotherapy and CDA treatments activate STING to incite antitumour immunity but also boost immune regulation to enhance therapy resistance. Multiple STING-responsive pathways involving chronic inflammation and tumour progression and associated with immune checkpoints (PD-1/L, CTLA-4, IDO) result in therapy resistance. Blocking these pathways modulate the TME in favour of antitumour immunity. Immune, inflammatory and metabolic biomarkers in blood reflect changes in the TME caused by treatments and therapy resistance. Abbreviations: CDA, cyclic diadenyl monophosphate; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; IDO, indoleamine 2,3-dioxygenase; IFN-1, interferon-1; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells

**FIGURE 3** Expression of IDO1 in HNSCC based on HPV status. Box and whisker plots of IDO1 expression in HPV-positive, HPV-negative and normal adjacent tissue generated from TCGA data. This comparison shows significant differences in IDO1 expression when comparing: HPV-positive vs normal tissue \(P = .00002\), HPV-positive vs HPV-negative \(P = .00112\) and HPV-negative vs normal tissue \(P < .00001\)
STING activation, conclusions cannot be drawn from these studies on the influence of the whole immune system or local TME dendritic cell response on the IDO pathway. Considering current published clinical trials evidence, all trials have tested an IDO1 inhibitor in combination with either a PD-1 or PD-L1 inhibitor. Potential reasons for a limited response seen with IDO inhibitors in existing trials could be (a) poor IDO inhibitor with a short half-life, (b) ineffective dosing regimen and (c) redundant mechanisms and/or no synergy between PD-1/PD-L1 and IDO pathways. Furthermore, there has been no report of IDO immune-based therapy or IDO inhibitor therapy in comparison with current standard of care treatments for HNSCC. In addition to IDO there are also other enzymes which have an influence on tryptophan metabolism. Apart from IDO, tryptophan 2,3-dioxygenase (TDO2) is another rate-limiting enzyme of the kynurenine pathway (KP). TDO2 is mainly expressed in the liver; however, it has also been shown to be overexpressed in tumours as a means of immune evasion. Although TDO2 and IDO activity cannot be distinguished based on peripheral blood analysis of KP activity, Riess et al showed that glioblastoma multiforme (GBM) tumours had higher TDO2 expression whilst HNSCC tumours mainly presented with IDO1. While IDO is responsive to inflammatory signals (eg interferons), stress-related signals such as glucocorticoids induce TDO2 expression, highlighting the radical differences in upstream pathways that induce KP metabolic activity. In their series an HPV-positive HNSCC case showed the highest abundance of IDO1, reflecting its higher relative immunogenicity. In the current literature, there are sparse evidence of the influence of TDO2 in HNSCC; however, it is possible for TDO to have an effect on the immune TME given its role in metabolic KP activity.

4.5 | Implications for future clinical practice and research

Although there exists a spectrum of immune cell infiltrates in HNSCC, it is now recognised that HPV-positive and HPV-negative HNSCCs have distinctly different immunophenotypes characterised by increased T-cell infiltrate, more immune cells expressing PD-L1 and increased presence of markers of immune activation (eg granzyme and perforin) in HPV-positive tumours whilst HPV-negative tumours tend to have less abundant immune cell infiltrates including T cells and Tregs. The immunophenotype of HNSCCs including the presence of immune cell infiltration and immune checkpoints within the TME of HPV-positive and HPV-negative tumour types may be important predictive biomarkers that will help guide personalised immunotherapeutic approaches in the future. A comparison of IDO1 expression data from TCGA shows that HPV-positive HNSCCs have significantly higher IDO1 expression when compared to HPV-negative HNSCCs (P = .001) and adjacent normal tissue (P < .001) (Figure 3) although the IDO expression is also elevated in HPV-negative HNSCC when compared to normal tissue. Furthermore, studies in various solid cancers investigating the differences between primary tumour and corresponding lymph node metastases have shown that strong tumoural IDO expression is associated with metastatic disease. It has been observed in both colorectal and breast cancer that IDO expression pattern is consistent between the primary tumour and metastatic sites. This suggests IDO expression as a modulator of cancer inflammation and immune evasion in both primary and metastatic tumour progression. As more evidence mounts in favour of the involvement of IDO in the TME of HNSCC and a better understanding of its mechanistic role in HNSCC immune modulation emerges, IDO-based therapies are likely to be translated to clinical practice to improve outcomes for HNSCC patients. An increasing number of new IDO inhibitors are currently being discovered. An example is DN-016 a highly potent, selective, orally available IDO1 inhibitor with good absorption, distribution, metabolism and excretion and safety profile which was presented at ASCO 2018. Also, HTI 1090 a dual inhibitor of IDO1 and hepatic enzyme tryptophan 2,3-dioxygenase (TDO) is currently in phase I trial for advanced solid tumours including HNSCC. Considered “best in class” IDO1 inhibitor, BMS-986205 was recently halted from phase III trial but earlier-phase combination studies are still ongoing. IDO inhibitor utility as a single agent or in combination with other checkpoint inhibitors is still under clinical evaluation and recently published results show promising overall response rates in HNSCC. Existing evidence suggests an increasing role for IDO-based therapies in the context of PD-L1 or HPV-positive HNSCCs; however, this observation requires more mechanistic evidence to elucidate the relationship.

Future research on IDO in HNSCC should seek to address the following questions:

1. Is the IDO pathway regulated by treatment (eg radiotherapy)?
2. Can IDO immune status be used as a predictive biomarker of treatment outcome?
3. What is the relationship between the IDO pathway and HPV or PD-L1 status in HNSCC?

Improved understanding of the mechanistic role of IDO in modulating local TME immunity will inform the targeting of the IDO pathway to optimise HNSCC therapy. The ability to measure IDO activity in the peripheral blood of cancer patients allows researchers to prospectively map and characterise potential groups of patients who may benefit from modified treatment doses (eg radiotherapy) based on IDO immune status. The correlation of IDO activity and expression at the tumour, draining lymph nodes, and in peripheral blood can potentially lead to less invasive sentinel lymph node and liquid biopsies to inform stratified, personalised HNSCC immune-based therapy.

5 | CONCLUSIONS

Current evidence shows the presence of IDO in the TME and suggests a link to prognosis and prediction of HNSCC treatment outcome. However, the exact mechanism of immune modulation by the
IDO pathway in the TME of HNSCC remains unclear. Future translational studies need to prospectively map the activity and expression of IDO throughout HNSCC treatment to achieve a mechanistic understanding of its involvement in TME immunity and to inform the design of precision, stratified immunotherapeutic approaches involving IDO.

CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analysed in this study.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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