Head and neck squamous cell carcinoma (HNSCC) is significantly under-represented in worldwide cancer research, yet survival rates for the disease have remained static for over 50 years. Distant metastasis is often present at the time of diagnosis, and is the primary cause of death in cancer patients. In the absence of routine effective targeted therapies, the standard of care treatment remains chemoradiation in combination with (often disfiguring) surgery. A defining characteristic of HNSCC is the amplification of a region of chromosome 3 (3q26-29), which is consistently associated with poorer patient outcome. This review provides an overview of the role the 3q26-29 region plays in HNSCC, in terms of both known and as yet undiscovered processes, which may have potential clinical relevance.
**Introduction**

Head and neck cancer is the 6th most common cancer in the world [1] with over 11 000 new cases in the UK in 2014 [2]. Most of these tumours (> 90%) are squamous cell carcinomas [head and neck squamous cell carcinoma (HNSCC)] [3], occurring in the oral cavity, oropharynx, larynx and hypopharynx. On average, almost half of all HNSCC diagnoses occur in the over 65 age group. HNSCC is typically regarded as a disease associated with poor socio-economic status, characterised by environmental and lifestyle factors such as smoking tobacco and consuming alcohol which cooperatively exacerbate risk [4]. The incidence of HNSCCs has seen an alarming rise over the last decade [2], notably in the atypical, younger age groups (< 45 years) [5], where exposure to the established risk factors is minimal, if evident at all. More recently, a causative association with human papilloma virus (HPV) infection was recognised [6], but HPV is reported to be tumourigenic in a low number of patients and occurs within specific subsites of the oral cavity, namely tonsil, base of tongue and lateral wall of the oropharynx [7]. In the USA, the increased incidence of HPV and oral cancer in younger patients appear to correlate with incidence rates recorded in the UK [8]. Moreover, it has a distinct aetiology and a better prognosis [9]. HPV-negative disease represents the majority of cases, and is the focus of this review.

The prognosis associated with early stage presentation of HNSCC is generally favourable, following treatment with surgery or radiation. However, HNSCCs are highly aggressive, and prone to metastasis and rapid recurrence [10]: as such, patients often present with locally advanced disease requiring invasive and disfiguring treatment with multiple modalities. The 5-year survival has remained stagnant for decades, at approximately 40–50% [11] which drops sharply to 10–26% [12,13] in patients with prognostic markers of metastatic disease such as nodal involvement and extracapsular spread [14,15]. Standard of care treatment comprises a chemoradiation approach (generally coupling radiation with cisplatin, docetaxel or 5-FU chemotherapy) and this therapeutic strategy remains the mainstay treatment for locoregionally advanced HNSCC in combination with surgery.

Recent years have seen a drive to design personalised strategies to target specific tumour cell populations in patients with advanced cancers. Indeed, genomic characterisation has helped improve the survival rates of patients diagnosed with frequently presenting cancer types, with the classification of disease based on genetic and molecular constituents opening the door for innovative therapeutic strategies. HNSCC, which is characterised by high levels of intratumoural heterogeneity [16], has not, to date, profited from such approaches. Intratumoural heterogeneity represents a complicating factor in the successful treatment of various cancer types, with genotypically distinct clonal populations contributing to both drug resistance and immune modulation [17]. Furthermore, while inhibition of a signalling pathway may be effective in one subpopulation, it may paradoxically drive the proliferation of another [18]. Intratumoural heterogeneity, coupled with the occurrence of distinct driver mutations has led to a need to better understand these processes, with the aim of exploiting them therapeutically [19]. Multiple studies in patient cohorts have been conducted to identify novel therapeutic targets and predictive biomarkers of cancer [20]. This has led to the identification of mutations in key tumour suppressor genes (e.g. TP53, CDK2NA) [21,22] and known oncogenes (e.g. PIK3CA, EGFR) [23,24]. Additional in vitro studies have focused on genomic analyses under hypoxia [25], as HNSCCs are recognised to develop hypoxic niches, which are associated with a poor prognosis. Moreover, genetic and epigenetic alterations in DNA repair pathway components (BRCA1, BRCA2 and FANCD2) [26–28], epigenetic modifiers (NOTCH, HOXA9, H3S3T2, NPY, EZH2) [29–33] and genes involved in hereditary cancers (VHL, MLH1, RB1) [34–36] are also present, although it is important to note that next-generation sequencing approaches may also detect mutations in these genes which are present in nontransformed cells and do not provide any information about how such mutations contribute to HNSCC.

Targeted agents to some of the key genetic aberrations outlined above have been tested in HNSCC clinical trials. One such target is the epithelial growth factor receptor (EGFR), where a clear oncogenic role has been established. Overexpression of EGFR is known to occur in > 90% of HNSCCs, and is predictive of poor prognosis [37]. The EGFR-targeting antibody cetuximab was licensed for use in HNSCC by the FDA in 2006 [38]. However, the overall improvement in patient survival using cetuximab in combination with radiotherapy and platinum/5-FU chemotherapy has been poor given the extent of alteration in patient tumours [39–41]. The significantly higher cost of personalised therapies should be noted here, especially given that many are currently unaffordable. Despite much effort, cetuximab remains the first and (at the time of writing) only targeted treatment for HNSCC. In August 2016, pembrolizumab (Keytruda; Merck & Co. Inc, Kenilworth, NJ, USA)
was approved for use in recurrent or metastatic HNSCC [42] and is the first example of an approved immunotherapy agent for cancer, where it targets programmed cell death protein 1 (PD-1) on the surface of T cells, stimulating them to target tumour cells. The agent was previously approved for use in unresectable or metastatic melanoma [43] and nonsmall cell lung cancer (NSCLC) [44], where it prolongs progression free and overall survival. The approval of pembrolizumab in HNSCC was based on the KEYNOTE-012 trial conducted on 174 HNSCC patients, resulting in an overall response rate of 16%, with tumour recession lasting ≥ 6 months in 82% of responsive patients [45]. A second PD-1 inhibitor, nivolumab (Opviodo), was approved in November 2016 for the same population as pembrolizumab, following on from the truncation of the phase 3 CheckMate-141 trial after it was determined to have met its primary end-point. [46]. While numerous ongoing phase 3 trials bring promise for immunotherapy in HNSCC, additional work is required to fulfil its application potential. There is therefore an urgent need for novel, targeted therapies that proportionally reflect the heterogeneous complexity of the disease.

Our understanding of HNSCC aetiology and pathogenesis continues to improve, owing largely to advances in genomic characterisation. Projects such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) have been instrumental in uncovering prevalent genetic alterations, and these data have facilitated identification of therapeutic targets. Of the numerous genomic aberrations identified, particular changes are present in a higher than average percentage of patients, and the resulting impact on gene expression levels may hold previously unexplored therapeutic candidates. This review examines the findings from genomic analyses, including our own, focusing on the chromosomal region 3q26-29, a defining feature of squamous cell carcinomas, estimated to occur in approximately 75% of HNSCCs [47] and a similar percentage of lung SCCs (LSCC). Several genes within the amplicon have been shown to exhibit synchronised amplification in LSCC, namely FXR1, ECT2 and PRKCI, which suggests a degree of cooperation within the amplicon [48]. We provide an evaluation of the genes within 3q26-29 and their potential clinical relevance in HNSCC.

**Identification of pro-oncogenic factors in 3q26-29**

Chromosome 3 is the third largest chromosome with ~200 million base pairs, and encodes 1078 protein-coding genes [49]. Analysis of copy number alterations from genomic identification of significant targets in cancer analysis of the TCGA HNSCC data set (http://cancergenome.nih.gov) reveals a differential between the two arms of chromosome 3, with 3p demonstrating high rates of deletion and 3q presenting with copy number amplification, most prevalent in the 3q26-29 region. Gains in this region are common [50], and contain known oncogenic drivers, yet appear to be late events in tumour progression [51]. Moreover, the presence of amplifications within this region has been reported to correlate with tumour progression and tumour recurrence [52]. Table 1 provides a comprehensive summary of 3q26-29 copy number gains, as found in the literature. The 3q26-29 amplicon is spread over four major cytogenetic bands with 10 subdivisions, and contains 188 protein-encoding genes with associated TCGA genomic data: 62% of these genes also have increased gene expression (z-score > 2 in > 10% patients, E. J. Shanks, M. A. Davidson & M. Neilson, unpublished results) [53]. Figure 1 provides current genomic alteration data (copy number amplification, mRNA upregulation and mutation status) for genes known to arbitrate key oncogenic driver events in HNSCC, including PIK3CA (which encodes the p110 catalytic subunit of PI3K) and TERC, the telomerase RNA component gene [54], as well as novel genes revealed in our analysis. However, it is unlikely that all of the genes within the amplicon will be relevant to disease, thus necessitating the implementation of other characteristics such as survival and mutation status. Using a stepwise process, we have interrogated gene copy number and gene expression data from the HNSCC data set, coupled with patient survival statistics to support identification of potential oncogenic targets with clinical relevance. A total of 10 genes (AHSG, EIF5A2, FXR1, IGF2BP2, PSMD2, SEC62, ALG3, DNAJC19, IL1RAP and NMD3) were identified using this approach, which have a significant negative impact on overall survival when amplified (P ≤ 0.05, Fig. 2). Most of these are spread throughout the amplicon with no obvious clustering. Within this set, six genes exert a greater negative impact on survival when altered simultaneously rather than individually (AHSG, EIF5A2, FXR1, IGF2BP2, PSMD2 and SEC62) with the difference in overall survival being highly significant (overall survival P = 2.78 × 10⁻⁰⁶, disease-free survival P = 1.63 × 10⁻⁰³, Fig. 3).

Strikingly, four of these genes (AHSG, EIF5A2, FXR1 and IGF2BP2) encode RNA-binding proteins (RBPs), which regulate the translation of numerous
Table 1. Summary of 3q26-29 amplification events found in different cancer types based on studies in the current literature. Forty studies are presented in total. Findings are presented at the level of detail given in the original study, in addition to study size. Pan-cancer studies include multiple cancer types within the same analysis.

| Title                                                                 | Author(s), year | Method                          | Key gene(s)              | Region       | % Amplified (A); % gain (G); total (#) | References |
|---------------------------------------------------------------------|----------------|---------------------------------|--------------------------|--------------|---------------------------------------|------------|
| Pan-cancer                                                         |                |                                 |                          |              |                                       |            |
| Comparative genomic hybridization and chromosomal instability in solid tumors | Rooney et al. 1999 | Meta-analysis                    | –                        | q arm        | G 16.4% (2210) | [216]     |
| Systematic Interrogation of 3q26 Identifies TLOC1 and SKIL as Cancer Driver | Hagerstrand et al. 2013 | Meta-analysis (Tumorscape, TCGA) | TLOC1, SKIL             | 26           | Tumorscape: A 22% (3131) | [214]     |
| Emerging landscape of oncogenic signatures across human cancers     | Ciriello et al. 2013 | Meta-analysis (TCGA)             | –                        | 26           | Subclass C3 tumours A 64% (163)       | [217]     |
| The chromosome 3q26 OnCassette: A multigenic driver of human cancer | Fields et al. 2016 | Meta-analysis (TCGA)             | SOX2, ECT2, PRKCI, PIK3CA | 26           | > 50% G: LSCC 85% Oesophageal 85% Ovarian serous 85% Cervical 78% HNSCC 75% Bladder 60% | [47]      |
| Head and neck squamous cell carcinoma                              |                |                                 |                          |              |                                       |            |
| Comparative genomic hybridization detects novel deletions and amplifications in head and neck squamous cell carcinomas | Speicher et al. 1995 | CGH                             | –                        | q arm        | G 77% (13) + nodal involvement 5/9 + metastasis 3/3 | [218]     |
| Frequent novel DNA copy number increase in squamous cell head and neck tumors | Brzoska et al. 1995 | CGH                             | –                        | 26-27        | G 50% (10) | [219]     |
| Distinct patterns of chromosomal alterations in high- and low-grade head and neck squamous cell carcinomas | Bockmühl et al. 1996 | CGH                             | –                        | q arm        | G 87% (30) | [220]     |
| Recurrent chromosomal imbalances detected in biopsy material from oral premalignant and malignant lesions by combined tissue microdissection, universal DNA amplification, and comparative genomic hybridization | Weber et al. 1998 | CGH                             | –                        | q arm        | G 43% (14) + metastasis 3/3 | [221]     |
| Comparative genomic hybridization analysis reveals 3q gain resulting in genetic alteration in 3q in advanced oral squamous cell carcinoma | Oga et al. 2001 | CGH                             | –                        | q arm        | G 41% (17) + nodal involvement 5/9 + metastasis 1/1 | [222]     |
Table 1. (Continued).

| Title                                                                 | Author(s), year      | Method       | Key gene(s) | Region | % Amplified (A); % gain (G); total (#) | References |
|-----------------------------------------------------------------------|----------------------|--------------|-------------|--------|----------------------------------------|------------|
| A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains | Redon et al. 2001    | CGH/FISH     | PIK3CA      | q arm  | 67% (21)                              | [223]      |
| Molecular cytogenetic characterization of head and neck squamous cell carcinoma and refinement of 3q amplification | Singh et al. 2001    | CGH, SKY, FISH | –           | 26–27  | 42% (12)                              | [224]      |
| Amplification of 3q26 approximately qter correlates with tumor progression in head and neck squamous cell carcinomas | Hashimoto et al. 2001 | CGH          | –           | 26–29  | 91% (32)                              | [51]       |
| Amplification of the 3q26.3 locus is associated with progression to invasive cancer and is a negative prognostic factor in head and neck squamous cell carcinomas | Singh et al. 2002    | FISH         | –           | q arm  | 56% (50)                              | [52]       |
| Genomic gain of PIK3CA and increased expression of p110alpha are associated with progression of dysplasia into invasive squamous cell carcinoma | Woenckhaus et al. 2002 | FISH      | PIK3CA      | 26     | 55% (11) G 100%                       | [225]      |
| Comparative genomic hybridization reveals genetic progression of oral squamous cell carcinoma from dysplasia via two different tumorigenic pathways | Noutomi et al. 2006  | CGH          | –           | 26–29  | 83% (35)                              | [226]      |
| Lung or oesophageal DNA amplification on chromosome 3q26.1-q26.3 in squamous cell carcinoma of the lung detected by reverse chromosome painting | Brass et al. 1996    | FISH         | –           | 26.1–26.3 | 33% (9)                              | [227]     |
| Title | Author(s), year       | Method | Key gene(s) | Region | % Amplified (A); % gain (G); total (#) | References |
|-------|-----------------------|--------|-------------|--------|----------------------------------------|------------|
| Patterns of chromosomal imbalances in adenocarcinoma and squamous cell carcinoma of the lung | Petersen et al. 1997 | CGH | – | q arm | LSCC G 80% (25) LAC G 20% (25) | [228] |
| DNA gains in 3q occur frequently in squamous cell carcinoma of the lung, but not in adenocarcinoma | Bjorkgvsit et al. 1998 | CGH | – | q arm | G 43% (25) | [229] |
| Comparative genomic hybridization analysis detected frequent overrepresentation of chromosome 3q in squamous cell carcinoma of the lung | Chujo et al. 2002 | CGH | – | q arm | G 71% (41) | [230] |
| Significance of p63 amplification and overexpression in lung cancer development and prognosis | Massion et al. 2003 | FISH | TP63 | 28 | A 88% (217) | [231] |
| SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas | Bass et al. 2009 | SNP Array | SOX2 | 26.33 | Lung: A 23% (47) Oesophageal: A 15% (40) | [232] |
| Molecular cytogenetic fingerprinting of esophageal squamous cell carcinoma by comparative genomic hybridization reveals a consistent pattern of chromosomal alterations | Pack et al. 1999 | CGH | – | q arm | G 50% (17) | [233] |
| Study of abnormal chromosome regions in esophageal squamous cell carcinoma by comparative genomic hybridization: relationship of lymph node metastasis and distant metastasis to selected abnormal regions | Sakai et al. 2010 | CGH | – | 29 | q29 A 82.4% (51) q28 A 78.4% q27 A 74.5% q26.3 A 68.6% +local spread > 6 lymph nodes q24-26 11/12 | [234] |
| Comparative genomics of esophageal adenocarcinoma and squamous cell carcinoma | Bandla et al. 2012 | CGH | – | q arm | SCC G 60% (70) EAC 15% (189) | [235] |
| Title                                                                 | Author(s), year | Method | Key gene(s) | Region | % Amplified (A); % gain (G); total (#) | References |
|----------------------------------------------------------------------|-----------------|--------|-------------|--------|----------------------------------------|------------|
| Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization | Ried et al. 1994 | CGH    | --          | q arm  | G 77% (13)                             | [236]      |
| Comparative genomic hybridization analysis detects frequent, often high-level, overrepresentation of DNA sequences at 3q, 5p, 7p, and 8q in human non-small cell lung carcinomas | Balsara et al. 1997 | CGH    | --          | q arm  | G 85% (20)                             | [237]      |
| Prostate                                                             |                  |        |             |        |                                        |            |
| Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping | Cher et al. 1996 | CGH    | --          | q arm  | G 45% (20)                             | [238]      |
| Comparative genomic hybridization reveals DNA copy number gains to frequently occur in human prostate cancer                      | Sattler et al. 1999 | CGH    | --          | q arm  | G 56% (16)                             | [239]      |
| Novel amplification unit at chromosome 3q25-q27 in human prostate cancer                                            | Sattler et al. 2000 | CGH    | IL12A, MDS1, SLC2A2, SOX2 | 25–27 | G 30% (10)                             | [240]      |
| Genomic and expression analysis of the 3q25-q26 amplification unit reveals TLOC1/SEC62 as a probable target gene in prostate cancer | Jung et al. 2006 | FISH   | TLOC1, SEC62 | 25–26 | G 36% (22)                             | [210]      |
| Cervical                                                             |                  |        |             |        |                                        |            |
| Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix           | Heselmeyer et al. 1996 | CGH    | --          | q arm  | G 90% (10)                             | [241]      |
| Advanced-stage cervical carcinomas are defined by a recurrent pattern of chromosomal aberrations revealing high genetic instability and a consistent gain of chromosome arm 3q | Heselmeyer et al. 1997 | CGH    | --          | q arm  | G 77% (30)                             | [242]      |
genes throughout the cell, and as such are involved in a wide range of functions. Pathway analysis of these genes has uncovered interactions with established, HNSCC-associated, oncogenic driver genes that are commonly found to be coamplified and coexpressed in squamous cell carcinomas [47] (Fig. 3), including \textit{PIK3CA}, which is part of the most frequently altered oncogenic pathway in HNSCC, with its constituent genes exhibiting mutations or copy number variations in approximately 30–40% of HNSCC tumour samples [55]. One such network member, protein kinase C iota (\textit{PRKCI}) is frequently coamplified with \textit{PIK3CA}; and has been implicated in invasion, chemoresistance and survival of tumours [56]. Furthermore, PRKCI has been shown to drive cancer cell survival via regulation of PIK3CA/AKT-mTOR signalling [57]. Other

Table 1. (Continued).

| Title | Author(s), year | Method | Key gene(s) | Region | % Amplified (A); % gain (G); total (#) | References |
|-------|----------------|--------|-------------|--------|--------------------------------------|------------|
| Comparative genomic hybridization detects genetic alterations during early stages of cervical cancer progression | Umayahara et al. 2002 | CGH | – | 26.1–28 | Invasive carcinoma: G 67% (12) | [243] |
| Ovarian | | | | | | |
| Genetic analysis of benign, low-grade, and high-grade ovarian tumors | Iwabuchi et al. 1995 | CGH | – | 25–26 | High grade G 50% (26) Low grade G 28% (18) | [244] |
| Overrepresentation of 3q and 8q material and loss of 18q material are recurrent findings in advanced human ovarian cancer | Arnold et al. 1996 | CGH | – | q arm | G 51% (47%) | [245] |
| Comparative genomic hybridization detects frequent overrepresentation of chromosomal material from 3q26, 8q24, and 20q13 in human ovarian carcinomas | Sonoda et al. 1997 | CGH | – | 26.3–29 | G/A 36% (25) High grade 6/10 | [246] |
| Other cancer types | | | | | | |
| Distinct chromosomal imbalances in uterine serous and endometrioid carcinomas | Pere et al. 1998 | CGH | – | 26.1–29 | Serous G 50% (24) Endometrial G 8% (24) | [247] |
| A recurrent pattern of chromosomal aberrations and immunophenotypic appearance defines anal squamous cell carcinomas | Heselmeyer et al. 1997 | CGH | – | q arm | G 30% (23) | [248] |
| High-level DNA amplifications are common genetic aberrations in B-cell neoplasms | Werner et al. 1997 | CGH | – | 26–29 | A 20% (5) G 40% | [249] |
| Breast cancer in young women (≤ 35 years): genomic aberrations detected by comparative genomic hybridization | Weber-Mangal et al. 2003 | CGH | – | q arm | 21% (88) | [250] |
network proteins include SOX2, which has a central role in the maintenance of tumour cell stemness [58] and the cytokinesis regulator ECT2, which can act activate Rho signalling pathways and drive transformation [59].

Interestingly, this complements recent work carried out by Fields et al. [47], who proposed a similar model in LSCC where identified genes cooperate within a defined signalling network to drive an aggressive stem-like phenotype. Large, inclusive studies such as this have an advantage in the search for novel therapeutics because they capture important interactions between known players as well as identifying previously unrecognised genes which may be targetable. For this reason, repurposing of existing therapeutics for applications in cancer has gained credence through demonstrable example, and is now considered an important tenet in the drug discovery process [60]. We will focus on the six genes identified in our HNSCC analysis, and discuss the mechanisms by which they may contribute to HNSCC tumour recurrence and metastasis. Opportunities for therapeutic intervention will be highlighted.

**Fragile X-related gene 1 (FXR1, 3q26.33)**

FXR1 is a member of a small family of RBPs comprised of Fragile X mental retardation 1 (FMR1, the cause of the inherited condition fragile X syndrome [61]) and fragile X-related 2 (FXR2) [62,63]. RBPs are essential mediators of RNA stability and post-transcriptional processing in mammalian cells (there are approximately 422 predicted in the human genome [64]), and thus can influence multiple signalling pathways through transcript binding [65]. In this regard, their deregulation can affect a number of their target genes [53] and play a major role in the progression of cancer [66–68]. HNSCC cancers, in particular, are highly enriched for RBPs [69].
Increased levels of FXR1 mRNA were significantly associated with poor overall survival and metastasis-free survival in HNSCC [48]. FXR1 regulates the expression of epithelial cell transforming sequence 2 (ECT2) and protein kinase C, iota (PRKCI) in lung SCC, both of which are encoded within 3q26-29. Furthermore, suppression of FXR1 impairs tumour growth in vivo [48]. It is tempting to speculate that a similar network might exist in HNSCC, as ECT2 has been shown to drive cellular proliferation [70]. The FXR1 gene also exhibits a significant tendency towards coamplification with SOX2 in HNSCC [71], and the amplification of both have independently been reported to predict poor prognosis in cancer [48,72].

RBPs can influence the fate of tumour cells through processes including, but not limited to, apoptosis and cell cycle arrest [73–75]. Cellular senescence induced for example by cyclin-dependent kinase inhibitors (CDKi) presents an alternative approach to conventional chemotherapy. Senescence occurs in response to the p53-mediated activation of oncogenes during the premalignant phases of HNSCC [76]. However, TP53 is frequently mutated in the disease, allowing tumour cells to escape cell cycle arrest and continue proliferating [77], which can also lead to increased resistance to chemo- and radiotherapy [78]. FXR1 may support this evasion of arrest through negative regulation and/or destabilisation of p21 [79,80] and stabilisation of telomerase RNA component (TERC) activity in vitro [81].

In the light of this, FXR1 inhibition could be a valuable asset in overcoming cisplatin resistance in HNSCC. Rapidly dividing HNSCC cells are more sensitive than normal cells to DNA-damaging agents such as cisplatin, and decreased sensitivity to cisplatin is common in HNSCCs with mutant TP53 owing to their inability to undergo senescence [82]. Tumours with high-risk mutations of TP53 are more reliant on mitotic checkpoints to temporarily halt cell cycle processes and repair damaged DNA [83]. Abrogation of the G2/S checkpoint leads to the accumulation of cells with extensive DNA damage and subsequent mitotic arrest [84,85]. Inhibitors of the G2 checkpoint tyrosine kinase Wee-1, such as MK-1775, sensitisise mutant TP53 HNSCC to cisplatin treatment by abrogating G2/S cell arrest and promoting cellular senescence [86,87]. At the time of writing, there are two clinical trials recruiting for pilot studies of MK-1775 in combination with cisplatin in advanced, high-risk HNSCC, and clinical trials in solid tumours have shown successful proof of concept [88].

Research into cancer immunotherapy has gained significant momentum within the last 5 years, and represents a promising therapeutic strategy in HNSCC, especially with regard to prevention of tumour recurrence [89]. This is exemplified by the recent approval of pembrolizumab. FXR1 may have a putative role in the innate immune response through positive regulation of the proinflammatory cytokines C-C motif-chemokine ligand 2 (CCL2) and interleukin 1 beta (IL1β) in monocytes [79]. CCL2 mRNA is significantly upregulated in HNSCC compared to normal human keratinocytes and is secreted by HNSCC tumour cells to recruit peripheral monocytes and macrophages. This phenomenon, known as cancer-related inflammation,
endorses tumour cell survival and encourages local invasion and metastasis [90]. A role for cancer-associated fibroblasts (CAFs) may also be evident where CCL2 levels are high and antibodies targeting CCL2 can reduce CAF migration [91]. Interestingly, CCL2 is detectable in the serum of HNSCC patients, but the predictive power as a prognostic biomarker remains inconclusive [92–96]. IL-1β is a critical mediator of inflammation and was identified as part of a set of salivary biomarkers (OAZ, SAT, IL8 and IL-1β) which collectively detected oral cancer with high accuracy [97]. Higher levels of IL-1β are associated with advanced stage HNSCC [98] and interactions with a number of oncogenes, including TGFBI, PTGS2, CCL2 and MMP9/13, have been reported through bioinformatics modelling [99]. Additionally, treatment with IL-1β promotes a more invasive morphology in HNSCC cells through induction of epithelial to mesenchymal transition (EMT) markers such as Snail, Slug and Vimentin and activation of the oncogenic signalling pathways, AKT and ERK [100–102]. Recent reports suggest that EMT is dispensable for invasion in some cancer types, but has an important role in chemoresistance [103,104].

FXR1 was found to be frequently amplified in SCC of the lung (LSCC) [105], where elevated blood plasma levels were associated with inferior patient outcome [106], indicating FXR1 as a potential prognostic biomarker for LSCC. The level of FXR1 in HNSCC patient blood samples has not yet been studied, leaving its role as a biomarker in the disease yet to be explored. The capacity of FXR1 to influence such a wide range of cellular processes, including the modulation of critical inflammatory mediators such as CCL2...
and IL-1β, appears to confer a critical survival advantage for HNSCC cells. Its role as an RNA-binding protein empowers FXR1 with a substantial, tumour-specific regulatory potential thus making it a promising target for therapeutic intervention in HNSCC.

Eukaryotic translation initiation factor 5A2 (EIF5A2, 3q26.3)

EIF5A2 encodes a small (17 kDa) mRNA-binding protein which has a highly homologous sequence identity with its only isoform, EIF5A1 [107]. EIF5A2 is overexpressed in various cancers [108–110]. The EIF5A proteins are notable because they are the only known proteins containing hypusine, a rare amino acid synthesised during post-translational modification of a specific lysine residue [111]. Hypusination requires two sequentially acting enzymes, deoxyhypusine synthase and deoxyhypusine hydroxylase, and is essential for sustained proliferation in mammalian cells [112], potentially through enhanced translation of RhoA [113]. Proliferative dependence on hypusination has been previously demonstrated in HNSCC [114].

Hypusinated EIF5A2 is essential for HIF-1α activation in hypoxia [115]. Hypoxia is a defining characteristic of HNSCC [116,117], and arises where solid malignant tumours outgrow their blood supply leading to extrinsic areas of nutrient and oxygen deprivation in the tumour microenvironment [118,119]. Hypoxia exerts a strong selective pressure which promotes genomic instability, giving rise to more aggressive tumours, with resistance to apoptosis, greater invasive potential and enhanced angiogenic capacity [119,120]. Importantly, intratumoural regions of hypoxia are refractory to chemotherapy, where lack of infiltration by blood vessels may preclude exposure to an effective dose [121], and to radiation, where low O2 conditions reduces sensitivity [122]. Therapeutically, pertinent avenues are being explored with enthusiasm in a hypoxic setting. Of note, the anti-VEGF inhibitor, bevacizumab, is being trialled in combination with other chemotherapeutics and has shown promise in phase II clinical trials with overall response rates of 30% [123–125]. EIF5A2 is upregulated in response to hypoxia in oesophageal SCC concurrently with HIF-1α [126], and contributes to metastasis and angiogenesis within this setting. As such, EIF5A2 is likely to play a role in the mediation of hypoxia-related effects.

EIF5A2 may also constitute a component of the sonic hedgehog (Hh) signalling pathway, through GL11-dependent upregulation, as demonstrated in pancreatic cancer cells [127]. Hh signalling oversees a multitude of cellular processes, including stem cell maintenance and cellular differentiation. The importance of Hh signalling in HNSCC is evident through the association of high Gli1 expression with poor survival in patients undergoing radiotherapy [128] and by its implication in anti-EGFR therapeutic resistance. Many patients undergoing cetuximab treatment exhibit a refractory response, which may be due, in part, to increased expression of Gli1 [129]. Concordant in vivo administration of cetuximab and the Hh inhibitor IPI-926 reported promising results with regard to delayed tumour progression, regression and sensitisation to radiation [129,130]. A pilot clinical study of cetuximab and IPI-926 in recurrent/metastatic HNSCC showed indications of antitumour activity [131], and further clinical trials will be of great interest.

Through its proven interrelatedness with well-known oncogenic pathways such as Hh and the consequences of post-translational modifications such as hypusination, it is probable that EIF5A2 acts as an oncogenic driver in HNSCC. While mechanistic studies directly linking EIF5A2 to HNSCC tumour progression have yet to emerge, there is a strong supportive evidence to suggest that the protein may be a significant therapeutic target.

Insulin-like growth factor 2-binding protein 2 (IGF2BP2, 3q27.2)

Amplification of IGF2BP2 carries the highest independent survival significance in our analysis. It belongs to a conserved family of mRNA-binding proteins [IGF2BP1, IGF2BP2 (also called IMP, VICKZ and ZBP) and IGF2BP3] which influence a variety of cellular processes through interactions with their target transcripts [132]. IGF2BPs are expressed predominantly in the embryo, but are re-expressed in some aggressive cancers [133]. Interestingly, IGF2BP2 is the most divergent member of the family, with expression being maintained in several adult tissues [134].

Structurally, IGF2BPs contain two N-terminal RNA recognition motifs (RRMS) and four K-homology (KH) domains in the C terminus. RNA binding is mainly facilitated via KH domains [135], although the RRMs domains may act to stabilise interactions with target RNA [136]. The IGF2BP family execute predominantly post-transcriptional processes, although IGF2BP1 was shown to be involved in nuclear export, supporting observations that IGF2BPs can act at the site of mRNA transcription [137,138]. IGF2BP2 forms large, granular complexes called ribonucleoproteins (RNPs) which are responsible for maintaining the integrity of mRNA during its transport throughout
the cell. Two alternate isoforms of IGF2BP2 are generated by alternative translational initiation [139], giving rise to proteins of 62 and 66 kDa.

Comprehensive regulatory analysis of IGF2BP2 expression has yet to be conducted, but recent studies suggest that the high-mobility-group A2 (HMGA2) transcription factor plays a prominent regulatory role. HMGA2 has a remarkably similar expression pattern to IGF2BP2 and is also significantly overexpressed in HNSCC cancer in correlation with decreased overall survival [140,141]. Moreover, embryonic HMGA2 was shown to regulate the level of IGF2BP2 mRNA [142] promoting its transcription in cooperation with NFKB1 [143]. Conversely, IGF2BP2 was found to be the most significantly downregulated gene in Hmg2 knockout mice, which present with impaired muscle development and reduced myoblast proliferation. This effect, which could be rescued by re-expression of IGF2BP2 [144], provides strong support for direct interplay between IGF2BP2 and HMGA2.

The therapeutic relevance of IGF2BP2 in IGF signalling has been recognised, especially with regard to its role in EGFR-related chemoresistance in HNSCC, mediated by crosstalk of overlapping receptor tyrosine kinases [145–148]. Intrinsic and acquired resistance are commonplace, through alterations of pathways to facilitate the circumvention of EGFR inhibition and prevent a full response to cetuximab [149,150]. Cotargeting of these pathways represents a promising solution to overcome resistance.

It has been shown that IGF2BP2 binds to and stabilises IGF2 mRNA within the cell, positively regulating its translation. IGF2 acts primarily through IGF1 receptor (IGF1R) to exert its effect on cellular signalling, particularly within a PI3K/AKT axis [151]. In addition to its regulation of IGF2, IGF2BP2 can also directly influence the translation of IGF1 receptor (IGF1R) [152]. Activation of IGF1R can overcome EGFR inhibition by gefitinib in HNSCC through compensatory activation of downstream AKT and ERK [153]. Consistent with this, inhibition of IGF1R signalling using small molecule inhibitors such as PQIP has been shown to improve response to anti-EGFR therapy [154,155]. This combinatorial approach is being tested in phase 2 clinical trials using (i) cetuximab and IMC-A12, an anti-IGF1R monoclonal antibody, and (ii) cetuximab and OSI-906, a dual IGF1R/IR inhibitor, although this study for use in platinum-refractory, recurrent/metastatic HNSCC, was withdrawn for strategic reasons.

Many HNSCCs originate from a subpopulation of stem-like cells which are capable of initiating tumour growth and maintaining its progression and targeting of such populations could enhance the efficacy of current therapies [156]. IGF2BP2 expression itself was determined to be regulated directly by let-7 [157], a characteristically downregulated miRNA family in HNSCC. Let-7 is repressed by Lin28a, which promotes the maintenance of a stem-like phenotype in HNSCC through HMGA2-mediated modulation of POUSF1 (Oct4) and SOX2 expression [158], supporting previous observations that let-7 negatively modulates expression of stemness genes [159]. Moreover, overexpression of Lin28b, IGF2BP2 and IGF2 is associated with increased risk of recurrence in HNSCC [157]. IGF2BP2 has been shown to promote a stem-like glioblastoma multiforme (GBM) phenotype by preventing let-7-mediated repression of genes, including but not limited to HMGA2 [160,161], thus promoting tumourigenesis. This implies that a reciprocal feedback mechanism exists between let-7 and IGF2BP2. A novel metabolic role for IGF2BP2 came from a finding that it regulates oxidative phosphorylation (OXPHOS) in GBM by binding and stabilising mRNA components of the mitochondrial respiratory chain complex [162] including two complex I proteins, NDUFS3 and NDUFS7. Maintenance of OXPHOS by IGF2BP2 is critical for survival of GBM spheroids and progression [162]. Similarly, IGF2BP2 can inhibit the translation of the mitochondrial transport protein, uncoupling protein-1 (UCP1), a protein which has was shown to bring about autophagy and cell death in a breast cancer model [163]. Elevated levels of IGF2BP2 are also linked to increased rates of metastasis and poor survival in other cancers, including oesophageal and colorectal carcinomas [164,165].

Given the comprehensive interactions of IGF2BP2 within the cellular environment, it is unsurprisingly a candidate for cancer therapy. Despite little direct evidence for a causal role in HNSCC progression, its prevalence in the general cancer setting provides a strong rationale for further study. Should IGF2BP2 preferentially act through the IGF2/PI3K axis, pre-existing approved therapeutic options may be repurposed to drastically improve patient outcome. At the time of writing, there are over 10 clinical trials using PI3K inhibitor as a mono or combination therapy in HNSCC which are in various stages of completion. However, despite a solid biological rationale for testing PI3K inhibitors in HNSCC patients, some agents have not translated into meaningful treatment strategies. A PI3K inhibitor, PX-866, failed to show any enhanced benefit in unselected HNSCC patients when combined with doxetaxel or cetuximab [166].
Alpha-2-HS-glycoprotein (AHSG, 3q27)

AHSG (also known as fetuin-A) encodes a glycoprotein which is synthesised in and secreted from the liver [167]. AHSG is a member of the type 3 cystatin family of plasma proteins and has a wide range of physiological functions including inhibition of insulin receptor (IR) tyrosine kinase activity and regulation of calcium homeostasis. It is located on 3q27, which has been proposed as a susceptibility locus for type 2 diabetes (T2D) [168]. A link between T2D and cancer is becoming increasingly apparent, although the association between T2D and HNSCC risk appears to be modest [169–171]. However, metformin (which targets AMPK/mTORC1) is the first-line therapeutic for T2D and has been found to improve the overall survival of HNSCC patients [172]. Interestingly, HNSCC cells are unique in that they produce endogenous AHSG [173], a process also evident in nontumour tissue.

Region 3q27 also contains IGF2BP2 (as detailed above), in which small nuclear polymorphisms have been correlated with T2D: this could provide a functional connection between two T2D susceptibility genes which both converge on the insulin pathway [174,175]. As discussed, IGF2BP2 stimulates IR and IGFRI activation via enhanced translation of IGF2 and subsequent activation of IGFR1 [176]. Conversely, AHSG inhibits insulin-stimulated tyrosine phosphorylation of IR and insulin receptor substrate (IRS-1) [177]. Furthermore, in vivo studies have shown that Ahsg knockout mice have increased sensitivity to insulin, enhanced glucose clearance and are resistant to weight gain when fed a high-fat diet [178]. It is conceivable that IGF2BP2 overexpression in HNSCC provides a compensation mechanism to complement enhanced AHSG-mediated repression of insulin signalling.

A role in HNSCC is further supported by the observation that AHSG is important for cellular adhesion. Reduced AHSG expression results in a diminished capacity for migration and invasion [173], possibly by protecting the metalloproteinase MMP-9 from autolytic degradation [179]. Furthermore, loss of AHSG has been shown to delay tumourigenesis and progression of breast cancer, where AHSG−/−/PyMT mice had dramatically reduced tumour incidence, with the majority of null mice being tumour free at completion of the study [180,181]. However, the role of AHSG in cancer is not clear cut, particularly with regard to the regulation of transforming growth factor β (TGFβ), which is known to have roles as both a tumour suppressor and tumour promoter (reviewed by Inman [182]).

AHSG has also been proposed as a tumour antigen in several types of cancer, including breast, glioblastoma, pancreatic and most recently prostate [183], indicating potential utilisation as a novel biomarker. It may be that the AHSG plays different roles in different tumour types, but evidence to date would suggest that it is a significant prognostic factor for HNSCC progression.

Proteasome 26S subunit, non-ATPase 2 (PSMD2, 3q27.1)

PSMD2 (Rpn1) encodes a non-ATPase subunit of the 19S regulatory component of the 26S proteasome complex. This complex is responsible for proteolysis of ubiquitin-tagged proteins. Highly proliferative tumour cells exhibit higher than normal rates of protein turnover and have an increased dependence on proteasomal machinery [184,185]: indeed, increased rates of proteasome activity have been well documented in cancer [186]. Chemical inhibitors of the proteasome are a powerful tool in cancer therapy, and have been in widespread use in multiple cancers since the FDA’s approval of bortezomib (Velcade; Millennium Pharmaceuticals Inc., Takeda Oncology, Cambridge, MA, USA) in 2003 for the treatment of multiple myeloma [187].

Bortezomib, and second-generation proteasomal inhibitors such as carfilzomib, specifically inhibit the chymotrypsin-like activity of the 20S proteasomal core. Targeting proteasomal activity has been shown to stimulate apoptosis in tumour cells through induction of proapoptotic factors (such as Bim and Bak) [188] and cell cycle arrest [187,189]. In HPV-driven HNSCC, bortezomib treatment results in the upregulation of functional p53 [190]. Bortezomib can also enhance endoplasmic reticulum stress in multiple human cancers and inhibit the prosurvival NF-κB signalling pathway [191]. There are multiple subunits of the proteasomal complex, but it is evident that individual subunits can play distinct roles in cancer: in breast cancer, for example, PSMD9 expression was shown to predict radioresistance [192] and PSMD3 was coamplified with HER2 where coexpression of the two genes led to a greater inhibition of cell growth and induced apoptosis [193]. PSMD2 was identified as part of a gene signature associated with metastatic lung cancer [194] (in which increased 3q26-29 copy number is also evident in a large percentage of patients [195]) and poor prognosis. Reduced expression of PSMD2 resulted in decreased proteasomal activity in lung adenocarcinoma cells resulting in growth inhibition and induction of apoptosis [196] through activation of p21.
Conversely, vulnerabilities in cancer cells may present through genomic loss of certain genes [197], including proteasomal genes. PSMC2 (also part of the 19S regulatory subunit) exists in a complex with PSMC1, PSMD2 and PSMD5. Tumour cells harbouring partial copy number loss of the PSMC2 gene are more vulnerable to its suppression than nontumour cells containing normal copies [197]. Collectively, these interesting observations highlight the dynamic role of the proteasome and its component subunits in tumourigenesis and cancer progression.

Proteasomal inhibition is a recognised therapeutic strategy for solid tumours [198]. Bortezomib has been incorporated into clinical trials for HNSCC in combination with existing chemotherapeutics, but the outcomes have, to date, been somewhat disappointing, with patient response rates as low as 3% when used as a single agent [199–201]. Additionally, as with the majority of therapies, the emergence of resistance [202] and drug-associated off-target toxicities [203] are driving the development of second- and third-generation proteasome inhibitors [204]. While proteasomal inhibition is by no means a novel anticancer therapy, the potentially sensitising effect of subunit suppression may prove a useful strategy for overcoming resistance in various types of cancer.

**SEC62 homology, preprotein translocation factor (SEC62, 3q27.1)**

SEC62 is involved in the targeting and translocation of small presecretory proteins into the ER. Ribosome-derived precursor proteins destined for secretion must pass through a translocation pore in the ER, which is comprised of the heterotrimeric Sec61 complex (Sec61α, Sec61β, Sec61γ) [205]. It is estimated that around 30% of the cellular proteome passes through this complex [206]. The significance of this pathway in cancer is not well characterised. Functionally, Sec62 has been shown to regulate the ER stress response [207], which is pronounced in cancer cells due to altered protein homeostasis [208]. Disruption of ER Ca$^{2+}$ levels can trigger a build-up of unfolded proteins, which is resolved through the unfolded protein response including the heat shock and chaperonin protein families, the expression of which is also amplified in many tumour types, and results in apoptosis [209].

Sec62 has, however, been implicated in several cancers after it was initially indicated as potential target in prostate cancer [210]. SEC62 knockdown reduces migration and invasion in a number of cancer cell lines, including PC-3 (prostate), HT1080 (fibrosarcoma), TX3868 (glioblastoma), A549 (lung carcinoma) and H1299 (NSCLC) [211]. In NSCLC, high Sec62 expression was associated with lymph node metastasis [212] and correlated with reduced survival in NSCLC patients [212]. Use of small molecule inhibitors of the Ca$^{2+}$-binding protein, calmodulin, resulted in a similar phenotype to SEC62-silenced cells, with decreased migration and increased sensitivity to ER stress [213].

Interestingly, a gain of function of SEC62, in conjunction with another 3q26-29 amplified gene SK1 like proto-oncogene (SKIL, 3q26.2) conferred a more oncogenic phenotype [214]. Simultaneous overexpression of the two genes induces a significant increase in anchorage-independent growth, a key marker of transformation. Notably, both proteins were found to act independently of PIK3CA and SOX2 in this study, offering further support to the potential significance of lesser studied genes located within the 3q26-29 amplicon.

**Concluding remarks**

In this review, we have discussed the potential importance/role of six highly overexpressed genes, which collectively have a significant impact on HNSCC patient survival. In some cases, these have roles in known processes for which clinical testing is underway, as is the case for IGF2BP2 and its modulation of downstream insulin pathway signalling, and the role of PSMD2 in proteasomal function. Others represent novel strategies with a strong rationale for further exploration as innovative therapeutics and/or predictive biomarkers of disease progression. These are not limited to HNSCC, and may hold potential in targeting SCCs originating at other anatomical sites. The evidence presented here points to IGF2BP2 as being the most significant candidate gene for HNSCC in terms of both its correlation with survival, and its recognised involvement in oncogenic processes.

HNSCC is genomically heterogeneous and its progression is driven by a diverse range of intracellular signalling pathways. Despite the identification and characterisation of many tumour initiating and promoting genes, effective targeted treatments have been scarce to date. Comprehensive genomic characterisation is rapidly changing how cancer is defined, evidenced by a move to classify/stratify tumours based on their molecular subtype [215], in addition to anatomical site. In recent years, a discernible shift towards an integrated approach to identifying novel therapies has occurred, where large experimental...
‘omics’ studies and clinical data sets are multiplexed to determine the most promising molecular targets which have the potential to benefit a substantial proportion of the patient population. This has generated new insights into the relationship between the enrichment and functional implication of genetic aberrations and the associated clinical response and has paved the way for promising new treatment strategies.

While investment in refining existing treatments could deliver much needed improvements in their efficacy, emerging and innate resistance to therapeutics will continue present a challenge, resulting in the incomplete elimination of the tumour and/or persistence of residual tumour niches leading to swift recurrence, often with metastasis. As such, pursuing new therapeutic strategies is essential. Failure to fuel the drug discovery target pipeline will result in a continued reliance on these established therapies, and protraction of the poor survival rates associated with this disease, which have remained unchanged despite our efforts to deliver better treatments. Even the most efficacious drug is unlikely to deliver as a single agent and is unlikely to be beneficial to every patient; therefore, maximising the use of the vast data resources available to identify targets which have a key functional role in supporting tumourigenesis and progression may prove extremely useful in finding a new therapeutic avenue and the patients it will benefit.

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Author contributions

MAD planned the structure of the manuscript and wrote the first draft. MAD and EJS prepared the figures and worked together to produce the final version of the manuscript.

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M. A. Davidson and E. J. Shanks

Review of established and prospective oncogenes

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