Novel prognostic clinical factors and biomarkers for outcome prediction in head and neck cancer: a systematic review

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Current algorithms for the clinical management of patients with squamous cell carcinoma of the head and neck (HNSCC) are based on a stage-dependent strategy where all patients at the same TNM stage receive the same treatment. Patient outcomes might be substantially improved by biomarker-guided treatment selection based on individual differences in the genetic and biological characteristics of tumours. Rapid technical advances enabling fast and affordable comprehensive molecular characterisation of tumours have led to increased knowledge of the molecular pathways involved in neoplastic transformation and disease progression in HNSCC. Despite notable successes in other tumour entities, the exploitation of molecular data for the improvement of tumour staging, prognosis, and individual treatment selection for patients with HNSCC has not yet become clinical routine. In this Review, we discuss and merge existing and new information on prognostic biomarkers for HNSCC, with the potential to improve clinical management of patients in the near future.

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Introduction

Squamous cell carcinoma of the head and neck (HNSCC) contributes to the global cancer burden. Annually, about 140 000 new cases are diagnosed in Europe;[1] this number corresponds to about 4% of all malignant diseases in adults (6% in men; 2% in women),[1] and covers a wide range of geographically dispersed incidences.[1] The prognosis of this highly malignant disease is still poor—even with combined treatment involving surgery, radiotherapy, and chemotherapy, 5-year survival rates are unsatisfactory, ranging from approximately 60% in laryngeal carcinoma to only roughly 25% in hypopharyngeal carcinoma.[1] Besides the especially poor outcomes of distinct anatomical subgroups, disease-specific survival in HNSCC is also negatively affected by the tendency of these tumours to locally invade surrounding normal tissue and to metastasise to cervical lymph nodes. More than 50% of all patients with HNSCC are initially diagnosed at a locally advanced stage.[1] For patients with regional disease, 5-year survival is significantly worse than those with localised disease.[1] Stage-dependent differences in outcomes have consistently been observed over the past two decades, despite the development of risk-adapted curative treatment strategies. Novel strategies are thus urgently needed that change strategies from uniform treatment for all patients with the same clinical and histological features to biomarker-guided treatment selection based on individual differences in the genetic and biological behaviour of tumours.

Within the past 10 years, knowledge on the molecular alterations that drive neoplastic transformation and tumour progression in HNSCC has rapidly been growing. This substantial gain in knowledge has been achieved by major technical advancement allowing high-throughput molecular analysis of a large series of patient samples. Exploratory studies like those done in The Cancer Genome Atlas (TCGA) project[2] revealed a multitude of molecular changes at the genome, transcriptome, and proteome level in HNSCC that could be exploited as potential biomarkers. However, very few biomarkers are currently used in clinical practice or have proceeded towards validation for routine use.

In this Review, we describe the portfolio of prognostic tools for HNSCC that are available for routine patient care and briefly discuss their limitations with respect to clinical value. We then explain which novel prognostic biomarkers could offer complementary information beyond what is provided by current clinical practice, especially in the field of prognosis and treatment.

Data collection

Search strategy and selection criteria

For the purpose of this Review, we systematically searched the medical literature on both liquid-based and tissue-based biomarkers in HNSCC. We only selected studies including 50 or more patients with HNSCC. We particularly focused on prognostic and predictive biomarkers in the curative treatment setting for which a consistent association with clinical outcome was shown in at least two independent studies. We excluded from our search those biomarkers for which, despite evidence of a potential diagnostic value, a prognostic effect has not been shown. By use of this approach, we aimed to identify which are the most promising prognostic biomarkers in HNSCC and are thus candidates for further evaluation and validation studies. We searched the MEDLINE electronic database via PubMed for reports published in English between Jan 1, 2013, and Dec 31, 2018. Search terms included "prognosis", "oral", "oropharynx", "hypopharynx", "larynx", and "squamous cell carcinoma". Further details on search terms and pre-specified eligibility criteria are provided in the appendix. All titles and abstracts were examined by one author (IT) to exclude irrelevant studies. We assessed the full-text papers for selection of studies reporting on prognostic factors in patients with squamous cell carcinoma of the hypopharynx, larynx, oropharynx, or oral cavity who received surgery, radiotherapy, or chemoradiation as part of their disease-specific treatment. Finally, we did a

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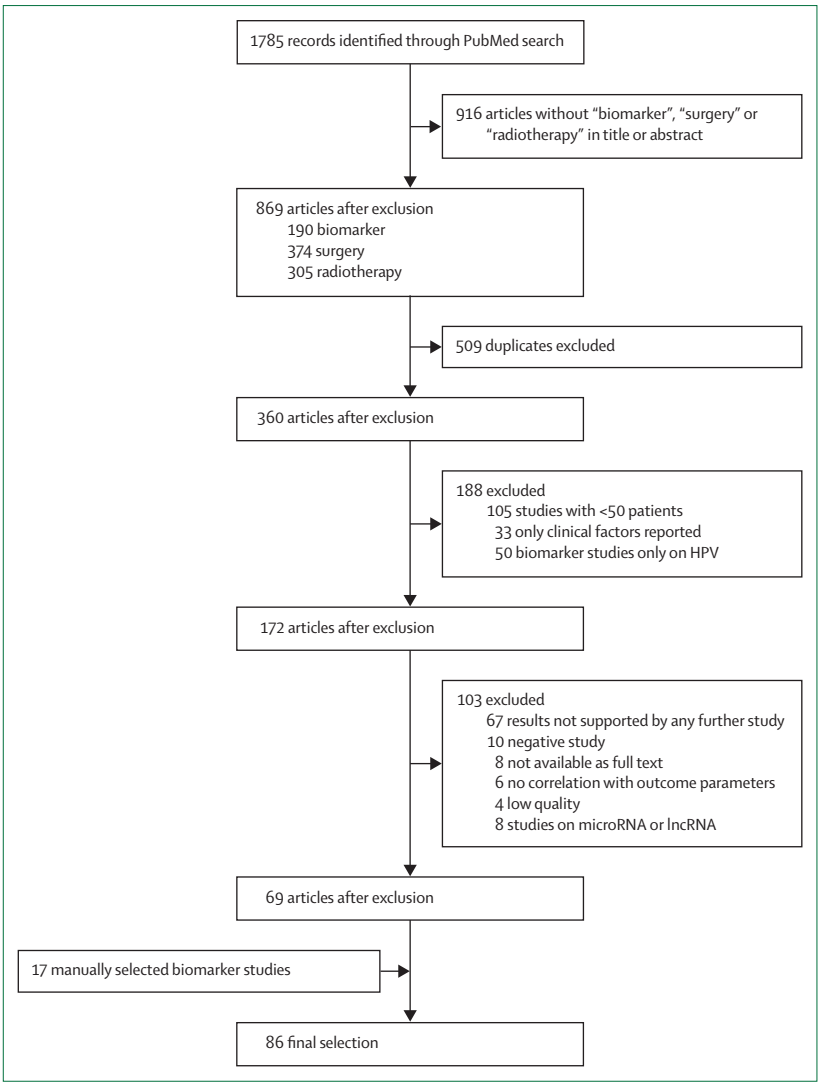

Figure 1: **Flowchart of article selection process**
HPV=human papillomavirus. LncRNA=long non-coding RNA.

manual search in key journals and the reference lists of the selected papers to find any other relevant citations that were missed by the electronic search.

Findings

In total, 86 studies reporting 78 different prognostic biomarkers were identified, which were classified according to our selection criteria (figure 1). 11 studies analysed biomarkers derived from blood samples (table 1), and 75 studies were done in tumour tissue or derivative cellular components (tables 2–4). A portion of studies were based on mixed cohorts of patients (23 [27%] of 86 studies). The majority of the 86 studies on selected anatomical subsites were done on tumours of the oral cavity (44 [51%]), whereas only 13 (15%) studies assessed the role of prognostic biomarkers in pure laryngeal carcinoma cohorts, four (5%) in oropharyngeal carcinoma cohorts, and two (1%) in hypopharyngeal carcinoma cohorts. This Review is divided into two parts—the first on classical prognostic factors including the prognostic and predictive role of human papillomavirus (HPV) and imaging-based biomarkers; and the second on novel prognostic factors, including liquid biopsy biomarkers and tissue-based biomarkers. Of the 78 biomarkers that we identified (figure 2), a consistent association existed between biomarker presence and specific clinical outcomes. For most biomarkers, higher expression or a gain in copy numbers (tables 1–4) was associated with poor prognosis. Collectively, this finding suggests that most biomarkers identified not only represent novel tools for the prediction of patient outcomes, but also serve as potential selection markers for molecular-driven treatment strategies.

Classical clinical prognostic factors

Treatment indication of HNSCC is based on a standardised diagnostic work-up including physical examinations, diagnostic imaging (eg, ultrasound, CT, MRI, and PET) and histopathological classification. Following publication of international guidelines for the interdisciplinary treatment of HNSCC, this diagnostic work-up ultimately results in treatment recommendations based on classical prognostic factors such as tumour subsite and size, nodal involvement, distant metastases, and tumour grading. Nowadays, surgery still has a prominent role in multimodal treatment of HNSCC and can also serve as a prognostic factor derived from surgical resection margins, since incompletely resected or inoperable tumours carry a worse prognosis. Therefore, the goal of surgery is complete microscopic tumour resection with adequate safety margins; otherwise, adjuvant radiotherapy has to be supplemented with additional chemotherapy.[87] However, additional treatment can jeopardise functional outcomes for patients with HNSCC. Before recognition of the strong prognostic effect of HPV status, three categories were routinely used for risk stratification of patients treated with upfront surgery. A postoperative high-risk situation was defined by resection margin involvement (R1) at the primary site or extracapsular spread at the different levels of lymph node, which are both independent prognostic factors for overall survival for HNSCC (level IIa evidence) according to the 7th edition TNM (TNM-7).[88] These categories had to be adapted for HPV-driven oropharyngeal carcinomas, where high-risk patient populations are now defined by five or more neck node metastases, a T stage of 3 or 4, and positive tumour margins, whereas extracapsular spread and an advanced nodal stage are not predictive for local recurrence.[89] These alterations in risk classification for HPV-positive HNSCC represent a relevant change in treatment paradigm. For postoperative intermediate and low-risk situations, data are only available from retrospective series (level III evidence) and allow either a reduction of the dose to the target volume, or, in some

	Expression or ratio	Potential clinical use	Number of independent studies reporting consistent association*	Example study			
				Study	Studied biomarkers	Subsite	Patients (n)
CTCs	Increased	Poor prognosis	≥3	Tinhofer et al (2014)[3]	CTCs	Mixed	144
CTCs	Increased	Poor prognosis	≥3	Grobe et al (2014)[4]	CTCs	Oropharyngeal carcinoma	80
IL1A 3′UTR SNP	Increased	Progression or transformation	2	Wang et al (2016)[5]	*IL1A* 3′UTR SNP	Oropharyngeal carcinoma	1008
MTHFR C677T and *A1298C*	Decreased	Reduced disease-specific survival after radiotherapy	2	Anders et al (2015)[6]	*MTHFR* SNP (PCR-RFLP)	Mixed	306
Neutrophil count	Increased	Poor prognosis	≥3	Sumner et al (2017)[7]	Neutrophil count	Mixed	196
NLR	Increased	Poor prognosis	≥3	Nakahira et al (2016)[8]	NLR	Hypopharyngeal carcinoma	118
NLR	Increased	Poor prognosis	≥3	Nakashima et al (2016)[9]	NLR	Oropharyngeal carcinoma	124
NLR	Increased	Poor prognosis	≥3	Charles et al (2016)[10]	NLR	Mixed	145
NLR	Increased	Poor prognosis	≥3	Ozturk et al (2016)[11]	NLR	Oropharyngeal carcinoma	57
NLR, CRP	Increased	Poor prognosis	≥3	Fang et al (2013)[12]	NLR, CRP	Oropharyngeal carcinoma	226
Red cell distribution width	Increased	Poor prognosis	≥3	Kara et al (2017)[13]	Red cell distribution width	Laryngeal carcinoma	81

CTCs=circulating tumour cells. UTR=untranslated region. SNP=single nucleotide polymorphism. RFLP=restriction fragment length polymorphism. NLR=neutrophil to lymphocyte ratio. CRP=C-reactive protein. *Between biomarker expression and outcome.

Table 1: **Blood-based biomarkers in squamous cell carcinoma of the head and neck**

instances, allow the waiving of concurrent chemotherapy. During the past 10 years, transoral robotic surgery has been evaluated in retrospective studies for oral cavity carcinomas and oropharyngeal carcinomas.[90–92] The largest of those studies was a retrospective review of records from 410 patients with laryngeal and pharyngeal cancer treated by transoral robotic surgery alone (47·3%), adjuvant radiotherapy alone (31·4%) and chemoradiation (21·3%).[90] The 2-year oncological outcome in terms of locoregional control, disease-specific, and overall survival was above 90%, which is similar to results for early-stage T1 or T2 oropharyngeal carcinomas after high-dose intensity-modulated radiotherapy. Transoral robotic surgery was done in 47·3% of all patients and 70% of neck-dissected patients.[93] Long-term functional and quality of life outcomes after robotic surgery in oropharyngeal carcinoma were superior with respect to weight loss, and according to the Performance Status Scale for Head and Neck Cancer Patients (a validated tool to quantify functional status), and the Eating Assessment Tool (EAT-10; a validated ten-item general measure of swallowing difficulty).[90,91] Thus, the type of surgery used to treat patients with HNSCC has an important prognostic role in HNSCC outcomes.

Prognostic and predictive role of HPV

During the past decade, HPV-driven oropharyngeal carcinoma has been established as a distinct subgroup in HNSCC that carries an independent survival prognosis. In the landmark RTOG 0129 trial,[94] a 58% reduction in the risk of death (hazard ratio [HR] 0·42; 95% CI 0·27–0·66) was observed for HPV-positive (compared with HPV-negative) patients with HNSCC. Recursive partitioning analysis revealed HPV status, number of pack years, and nodal status as better predictors of overall survival than T stage alone in HPV-negative tumours.[94] The excellent prognosis of HPV-positive HNSCC, preferentially at the oropharyngeal site, irrespective of the treatment modality, was confirmed by the following studies. In a 2018 population-based retrospective analysis of HNSCC cases from the US National Cancer Database, Li and colleagues[95] found that in 41950 out of 175 223 patients, HPV status was the strongest prognostic indicator for overall survival.

The International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S) did a trial in 1907 patients with non-metastasised HPV-positive oropharyngeal carcinoma from seven centres in the USA, Canada, the Netherlands, and Denmark.[96] When classifying patients with oropharyngeal cancer according to TNM-7,[88] investigators found that 5-year overall survival for stage I (88% [95% CI 74–100]), stage II (82% [71–95]), stage III (84% [79–89]), and stage VIA (81% [79–83]) was similar (global p=0·25), but lower for stage IVB (60% [53–68]; p<0·0001). 5-year overall survival also did not differ between stages N0 (80% [95% CI 73–87]), N1–N2a (87% [83–90]), and N2b (83% [80–86]), but was lower for stage N3 (59% [51–69]; p<0·0001). When using adjusted HRs, a new stage classification system specific to HPV-positive oropharyngeal cancer

	Expression	Potential clinical use	Number of independent studies reporting consistent association*	Example study			
				Study	Studied biomarkers	Subsite	Patients (n)
Proliferation and cell cycle (5 biomarkers; all upregulated in high-risk group)							
CCND1	Increased	Poor prognosis	≥3	Zhong et al (2013)[14]	CCND1	Oral cavity carcinoma	232
CCND1	Increased	Presence of occult LNM	≥3	Noorlag et al (2017)[15]	CCND1	Oral cavity carcinoma	158
EGFR	Decreased	Potential to bnefit from hypoxia-modifying drugs	≥3	Nijkamp et al (2013)[16]	EGFR	Laryngeal carcinoma	272
EGFR	Increased	Poor prognosis	≥3	Kontic et al (2015)[17]	EGFR	Laryngeal carcinoma	185
EGFR	Increased	Poor prognosis	≥3	Mehta et al (2015)[18]	EGFR	Oral cavity carcinoma	60
Ki67	Increased	Poor prognosis	2	Rademakers et al (2015)[19]	Ki67, CAIX	Laryngeal carcinoma	255
Ki67, MMP2, VEGF-C	Increased	Poor prognosis	≥3	Gontarz et al (2016)[20]	Ki-67, MMP-2, MMP-9, VEGF-C, VEGF-D	Oral cavity carcinoma	60
Cell survival (5 biomarkers; all upregulated in high-risk group)							
IGF1R-α	Increased	Poor prognosis	≥3	Mountzios et al (2013)[21]	IGF1R-α, IGF1R-β, IGF2R	Laryngeal carcinoma	285
p-AKT^{Ser473}	Increased	Poor prognosis	≥3	Lyu et al (2016)[22]	PTEN, p-AKT^{Ser473}	Oral cavity carcinoma	50
p-AKT^{Ser473}	Increased	Poor prognosis	≥3	Freudlsperger et al (2015)[23]	p-AKT$^{Thr308/Ser473}$	Mixed	120
p-AKT^{Thr308}	Increased	Poor prognosis	≥3	Li et al (2013)[24]	p-AKT^{Thr308}	Oral cavity carcinoma	191
PLCγ1	Increased	Poor prognosis	2	Ma et al (2013)[25]	PLCγ1	Oral cavity carcinoma	60
PLCγ1	Increased	Poor prognosis	2	Zhu et al (2014)[26]	PLCγ1	Oral cavity carcinoma	232
Survivin	Increased	Poor prognosis	≥3	Tiefenbock-Hansson et al (2017)[27]	WRAP53β, survivin	Laryngeal carcinoma	149
Hypoxia (2 biomarkers; both upregulated in high-risk group)							
CAIX	Increased	Poor prognosis	≥3	Bernstein et al (2015)[28]	CAIX, HIF-1α	Laryngeal carcinoma	114
HIF-2α	Increased	Poor prognosis	2	Lim et al (2017)[29]	HIF-2α	Oral cavity carcinoma	58
EMT and metastasis (23 biomarkers; 20 upregulated in high-risk group)							
Axin2, Snail	Increased	Progression or transformation	≥3	Zhang et al (2017)[30]	Axin2, Snail	Oral cavity carcinoma	154
CDH1	Decreased	Distant metastasis	≥3	Rodrigo et al (2014)[31]	CDH1, ANXA2, CTTN, FAK, EGFR, TP53, p-AKT	Mixed	88
CDH1, EZH2	Decreased CDH1, increased EZH2	Poor prognosis	≥3	Wang et al (2013)[32]	CDH1 , EZH2	Oral cavity carcinoma	67
CDH1, PDPN	Decreased CDH1, increased PDPN	Presence of LNM	≥3	Foschini et al (2013)[33]	CDH1, podoplanin	Oral cavity carcinoma	102
CK-17	Decreased	Poor prognosis	2	Coelho et al (2015)[34]	Cytokeratin-17, cytokeratin-19	Oral cavity carcinoma	67
CXCR4	Increased	Locoregional control	≥3	De-Colle et al (2018)[35]	SDF-1, CXCR4	Mixed	141
ELMO3	Increased	Poor prognosis	2	Kadletz et al (2017)[36]	ELMO3	Mixed	125
EpCAM	Increased	Poor prognosis	2	Sen and Carnelio (2016)[37]	EpCAM	Oral cavity carcinoma	60
EpCAM	Increased	Local recurrence after radiotherapy	2	Murakami et al (2014)[38]	EpCAM	Mixed	118
EZH2	Increased	Poor prognosis	≥3	Chang et al (2016)[39]	EZH2	Mixed	90
Ezrin	Increased	Poor prognosis	2	Chang et al (2016)[39]	Ezrin	Oral cavity carcinoma	80
Keratinisation	Increased	Poor prognosis	≥3	Cooper et al (2015)[40]	Keratinisation	Oropharyngeal carcinoma	208
Maspin, nm23-H1, ANG, CD105	Decreased MASPIN and nm23-H1, increased ANG and CD105	Local recurrence	≥3	Marioni et al (2014)[41]	Maspin, angiogenin, CD105, nm23-H1	Laryngeal carcinoma	76
PXN	Increased	Poor prognosis	≥3	Li et al (2015)[42]	Paxillin	Laryngeal carcinoma	84
PAI-1, SMA	Increased	Poor prognosis	≥3	Dhanda et al (2014)[43]	Serpin E1, SMA	Oral cavity carcinoma	102

(Table 2 continues on next page)

	Expression	Potential clinical use	Number of independent studies reporting consistent association*	Example study			
				Study	Studied biomarkers	Subsite	Patients (n)
(Continued from previous page)							
Tumour budding	Increased	Poor prognosis	≥3	Attramadal et al (2015)[44]	Tumour budding	Oral cavity carcinoma	62
Tumour budding	Increased	Poor prognosis	≥3	Shimizu et al (2018)[45]	Tumour budding	Oral cavity carcinoma	91
uPAR, PAI-1	Increased	Poor prognosis	2	Shimizu et al (2018)[45]	uPAR, uPA, PAI-1, Ki-67	Mixed	115
WISP1	Increased	Poor prognosis	≥3	Clausen et al (2016)[46]	WISP1	Oral cavity carcinoma	204
Stemness and detoxification (13 biomarkers; 12 upregulated in high-risk group)							
AKR1B10	Increased	Poor prognosis	2	Ko et al (2017)[47]	AKR1B10	Oral cavity carcinoma	77
Bmi1	Increased	Poor prognosis	≥3	Li et al (2014)[48]	Bmi1	Oral cavity carcinoma	52
CD133	Increased	Progression or transformation	≥3	Sun et al (2013)[49]	CD133	Oral cavity carcinoma	110
CD44, ABCG2	Increased	Poor prognosis	≥3	Yanamoto et al (2014)[50]	CD44v6 , ABCG2	Oral cavity carcinoma	89
GPX1	Increased	Poor prognosis	2	Lee et al (2017)[51]	GPX1, GPX4, TrxR1	Oral cavity carcinoma	233
HMGA2	Increased	Poor prognosis	2	Fang et al (2017)[52]	HMGA2	Oral cavity carcinoma	148
IMP3	Increased	Poor prognosis	2	Tarsitano et al (2016)[53]	IMP3, Laminin-5	Oral cavity carcinoma	64
Oct4, Myc	Increased	Poor prognosis	2	Sawant et al (2016)[54]	Oct4, c-Myc	Oral cavity carcinoma	87
P-gp	Increased	Poor prognosis	2	Kim et al (2016)[55]	Glucosylceramide synthase, Pgp	Oral cavity carcinoma	186
S100A7	Increased	Progression or transformation	≥3	Kaur et al (2014)[56]	S100A7, PTMA, 14-3-3κ, 14-3-3σ, hnRNPK	Oral cavity carcinoma	110
SOX2	Decreased	Recurrence risk	≥3	Attramadal et al (2015)[57]	SOX2	Oral cavity carcinoma	62
Inflammation and immunity (7 biomarkers; 4 upregulated in high-risk group)							
ANXA1	Increased	Poor prognosis	2	Zhu et al (2013)[58]	ANXA1	Oral cavity carcinoma	232
CD8 TIL	Increased	Good prognosis	≥3	De Meulenaere et al (2017)[59]	CD70, CD3, CD8, FoxP3	Oropharyngeal carcinoma	78
CD8 TIL	Increased	Good prognosis	≥3	Ono et al (2017)[60]	PD-L1, HLA class I, CD8 TIL	Hypopharyngeal carcinoma	83
IDO1	Increased	Poor prognosis	2	Seppala et al (2016)[61]	IDO1	Oral cavity carcinoma	58
Mage-A9	Increased	Poor prognosis	2	Han et al (2014)[62]	Mage-A9	Laryngeal carcinoma	123
PD-L1	Increased	Good prognosis	≥3	Balermpas et al (2017)[63]	PD-L1, PD-1, CD8 TIL	Mixed	161
PD-L1	Increased	Poor prognosis	≥3	Muller et al (2017)[64]	PD-L1 , PD-L2	Mixed	293
PD-L1, CD8 TIL, FoxP3 Treg	Increased	Good prognosis	≥3	Kim et al (2016)[65]	PD-L1, PD-1, LAG3, ICOS, FoxP3, CD3 TIL, CD8 TIL	Mixed	402
TLR4	Increased	Progression or transformation	2	Ilmarinen et al (2014)[66]	TLR-2, TLR-4, TLR-9	Laryngeal carcinoma	324
Other (5 biomarkers; four upregulated in high-risk group)							
Beclin-1	Decreased	Poor prognosis	2	Huang et al (2013)[67]	Beclin-1	Laryngeal carcinoma	82
HERG1	Increased	Poor prognosis	2	Fernandez-Valle et al (2016)[68]	HERG1	Oral cavity carcinoma	100
LGALS7	Increased	Radioresistance or chemoresistance	2	Matsukawa et al (2014)[69]	LGALS7	Oral cavity carcinoma	86
TP53	Increased	Poor prognosis	≥3	Solomon et al (2016)[70]	EGFR, TP53, cyclin D1, BCL-2	Oral cavity carcinoma	178
uPAR	Increased	Poor prognosis	2	Christensen et al (2017)[71]	uPAR, tissue factor, EGFR	Oral cavity carcinoma	191

LNM=lymph node metastasis. EMT=epithelial to mesenchymal transition. TIL=tumour-infiltrating lymphocytes. Treg=regulatory T cells. TP53=cellular tumor antigen p53. *Between biomarker expression and outcome.

Table 2: **Tissue-based immunohistochemistry biomarkers in squamous cell carcinoma of the head and neck**

(the ICON-S staging system), which was based on survival rates at 5 years, was proposed: stage I (T1–T2N0–N1), stage II (T1–T2N2 or T3N0–N2), stage III (T4 or N3) and stage IV (M1).[96] From a training cohort of 702 patients, lower neck lymph node involvement in stages I and II, and less than five versus five or more lymph nodes, were not considered independent prognostic factors for overall survival.[96]

The ICON-S initiative was the basis for the new 8th edition TNM (TNM-8) for head and neck cancer,

	Risk genotype	Potential clinical use	Number of independent studies reporting consistent association*	Example study				
				Study	Studied biomarkers	Method	Subsite	Patients (n)
Copy number alterations (6 biomarkers; 4 amplifications in high-risk group)								
CCND1	Increased	Presence of LNM	≥3	Noorlag et al (2017)[15]	*CCND1*	FISH	Oral cavity carcinoma	158
Copy-number alterations	Patterns	Poor prognosis	2	Rhie et al (2015)[72]	Various	Array CGH	Oropharyngeal carcinoma	58
EGFR	Increased	Progression or transformation	2	Bates et al (2016)[73]	*EGFR*	ISH	Oral cavity carcinoma	92
RB1, *PIK3CA*, *FGFR1*	*RB1* decreased; *PI3K* or *FGFR1* increased	Poor prognosis	≥3	Peng et al (2015)[74]	10 target genes	tNGS	Oral cavity carcinoma	310
Promotor methylation (3 biomarkers; 3 hypermethylated)								
GAL, *GALR1* or *GALR2*	Increased	Poor prognosis	≥3	Misawa et al (2017)[75]	*GAL*, *GALR1* or *GALR2*	Q-MSP	Mixed	202
GAL or *SSTR1*	Increased	Presence of LNM	≥3	Misawa et al (2015)[76]	*GAL*, *GALR1* or *GALR2*, *SST*, *SSTR1*, *TAC1*, *TACR1*	Q-MSP	Mixed	100
Single nucleotide variants (3 biomarkers; two loss-of-functions)								
NOTCH1	Increased	Poor prognosis	≥3	Liu et al (2016)[77]	*NOTCH1* mutations	Sanger	Mixed	128
TP53	Increased	Poor prognosis	2	Lapke et al (2016)[78]	*TP53* mutations	tNGS	Oral cavity carcinoma	345
TP53	Increased	Poor prognosis	≥3	Omura et al (2017)[79]	*TP53* mutations	Sanger	Mixed	57
TP53, *NOTCH1*, *KDR*	Increased	Poor prognosis	≥3	Tinhofer et al (2016)[80]	45-gene panel	tNGS	Mixed	97

LNM=lymph node metastasis. FISH= fluorescence in-situ hybridisation. CGH=comparative genomic hybridisation. ISH=in-situ hybridisation. tNGS= targeted next-generation sequencing. Q-MSP=quantitative methylation-specific PCR. Sanger=Sanger sequencing. *Between biomarker expression and outcome.

Table 3: **Tissue-based DNA biomarkers in squamous cell carcinoma of the head and neck**

	Expression	Potential clinical use	Number of independent studies reporting consistent association*	Example study				
				Study	Studied biomarkers	Method	Subsite	Patients (n)
Hypoxia signature, *CD44*, *SLC3A2*	Increased	Poor prognosis	≥3	Linge et al (2016)[81]	Hypoxia signature, *CD44*, *SLC3A2*, *MET*	nCounter system (NanoString; Seattle, WA, USA)	Mixed	158
BAX to *BCL2* ratio	Decreased	Poor prognosis	2	Giotakis et al (2016)[82]	*BAX* to *BCL2* ratio	RT PCR	Laryngeal carcinoma	105
HMGA2	Increased	Poor prognosis	≥3	Gunther et al (2017)[83]	*HMGA2*	RT PCR	Mixed	202
MAGEA1–6	Increased	Poor prognosis	2	Noh et al (2016)[84]	*MAGEA1–6*	RT PCR	Mixed	53
VEGF-A	Increased	Poor prognosis	2	Ko et al (2015)[85]	*VEGF-A*	RT PCR	Oral cavity carcinoma	60
EFNB2	Increased	Poor prognosis	2	Oweida et al (2017)[86]	*EFNB2*	RNA sequencing	Mixed	519

RT PCR=reverse transcription PCR. *Between biomarker expression and outcome.

Table 4: **Tissue-based mRNA biomarkers in squamous cell carcinoma of the head and neck**

which has a focus on HPV-positive tumours.[97] In a retrospective validation of 15 116 patients from the US National Cancer Database who were restaged from TNM-7 guidelines according to TNM-8 guidelines, clinical staging changed in 93·9% of patients and pathological staging changed in 91·7% of patients towards lower stages.[98] The reclassification of oropharyngeal cancer will have a considerable effect on multidisciplinary treatment approaches, and therefore should be further explored in clinical trials. HPV-positive HNSCC has unique epidemiological and clinical management features, which have been reviewed previously by Maxwell and colleagues.[99] Since, so far, highly aggressive radiotherapy and chemoradiotherapy regimens have been associated with substantial acute and long-term morbidities, a de-escalation of treatment intensity to reduce side-effects is warranted. For this purpose, identification of robust markers of HPV-driven disease with excellent prognosis (ie, HPV E6 or E7 mRNA indicating transcriptionally active HPV infection) is of utmost clinical importance.

Assessed by immunohistochemistry, p16 (CDKN2A) expression can be used as a surrogate marker for HPV-driven oropharyngeal carcinoma. However, the combination of HPV DNA positivity and p16 over-expression is more accurate than HPV or p16 status alone in predicting recurrence-free survival.[100] p16 or HPV status remains a relevant and independent predictor after disease progression.[101] HPV oncoproteins, proteins E6 and E7, found in blood serum from patients with HPV-positive tumours are also currently under evaluation. In addition, protein E6 or E7 serology is a highly reliable diagnostic marker for detection of HPV-driven oropharyngeal carcinoma,[102,103] and might also be a helpful tool for identifying patient subgroups carrying a high risk of recurrent disease.[104] Thus, protein E6 or E7 serology might be potentially valuable biomarkers in HPV-positive HNSCC. Also, quantitative measurement of HPV 16 DNA in salivary oral rinses has promise as a screening tool for patients with HPV 16-positive oropharyngeal carcinoma.[105–107] The analysis of persistent HPV 16 DNA in post-treatment oral rinses after primary treatment might also allow for early detection of recurrence in patients with HPV 16-positive oropharyngeal carcinoma.[105,106] Accuracy of HPV DNA detection might even increase with the combined analysis of saliva and plasma.[105] However, whether this non-invasive surveillance tool can identify local or regional recurrence at a timepoint amenable to surgical salvage remains uncertain.[108]

Bersani and colleagues[109] showed that the excellent prognosis of HPV-positive tumours is further improved when additional favourable features are present. The authors analysed data from 258 patients with oropharyngeal carcinoma, two-thirds of whom were randomly selected to a training cohort and the remaining third for a validation set for the investigation of several prognostic factors. By least absolute shrinkage and selection operator (known as LASSO) regression, the authors found that patients younger than 60 years, with a T stage lower than 3, displaying CD8-positive tumour-infiltrating lymphocytes above the median, and mRNA expression of the viral protein E2 antigen, had improved progression-free survival at 3 years and a risk of death below 3%.[109] Nauta and colleagues[110] analysed the prognostic power of the TNM-8 versus TNM-7 in HPV-driven oropharyngeal carcinoma. Overall survival differed between groups according to TNM-7 stage (stage I–II *vs* stage III, p=0·036; stage III *vs* IV, p=0·031). However, 5-year overall survival was lower for stage I and II disease than for stage III and IV disease, which shows the limitation of the TNM-7 classification system in this patient population.[110] When classified according to TNM-8, 5-year overall survival rates were 81% for stage I, 77% for stage II, and 48% for stage III. Subgroup analysis confirmed previous evidence of inferior overall survival in p16-positive, HPV DNA-negative patients compared with p16-positive, HPV DNA-positive patients with oropharyngeal carcinoma.[110] Again, this finding underlines the necessity for additional HPV DNA testing in all p16-positive patients who could potentially be selected for de-escalation trials.[110] Currently, several prospective clinical trials (eg, RTOG 1016 [NCT01302834], De-ESCALATE HPV [NCT01874171], TROG [NCT018455451], NRG JHN-002 [NCT02254278], NCT01530997, ECOG 1308 [NCT01084083], and The Quarterback Trial [NCT01706939]) are underway with novel de-escalation approaches for this subgroup of patients.[111]

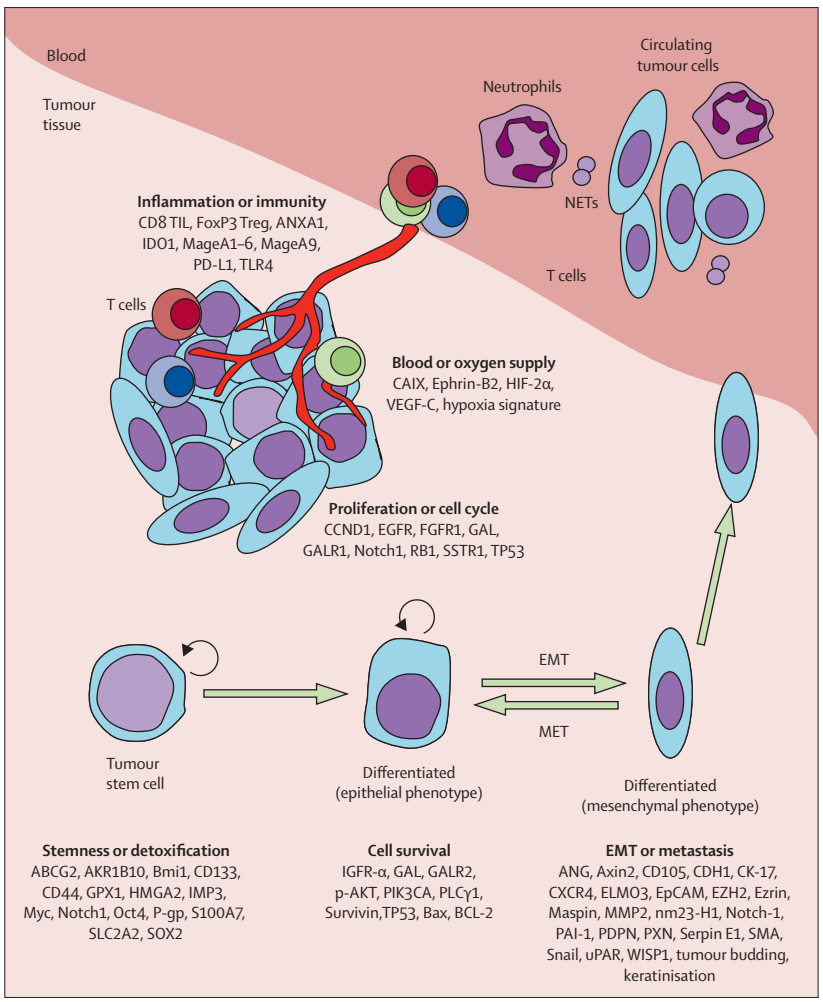

Figure 2: **Prognostic biomarkers in squamous cell carcinoma of the head and neck**
EMT=epithelial to mesenchymal transition. MET=mesenchymal to epithelial transition.NETs=neutrophil extracellular traps.TIL=tumour-infiltrating lymphocytes. TP53=cellular tumor antigen p53. Treg=regulatory T cells.

Imaging-based prognostic factors

Clinically established prognostic factors are supplemented by additional non-genetic factors, the majority of which still need to be validated prospectively. The broad use of non-genetic prognostic factors are not yet justified in clinical routine since, in many instances, they are not able to outperform classical prognostic factors and are not cost-effective. Tumour extension, as mirrored by the TNM

classification, is a major prognostic factor. For head and neck surgeons, non-invasive preoperative diagnostic measures would be extremely important; for example, information about the exact tumour spread could help surgeons to avoid classical unilateral or bilateral neck dissections, and their associated morbidities. The new promising CD206 receptor (MMR)-targeted radiopharmaceutical technetium-99m predicts lymphatic neck node involvement with a 98·8% overall accuracy rate, and might have the potential to replace neck dissections and their morbidities by the introduction of a minimally invasive sentinel node procedure.[112] Accordingly, a large phase 3 trial done in 564 patients, the majority of whom had tumours located at the oropharynx (82%; 460 of 564) displaying N2b stage (61%; 344 of 564) and p16-positive disease (75%; 335 of 446) showed non-inferiority for PET or CT-guided surveillance 3 months after chemoradiotherapy versus a planned neck dissection.[113] Such a patient-selection algorithm can reduce extensive surgery in favour of minimally invasive procedures, which could become the new standard of care.

Radiomics, defined as specific features from diagnostic imaging methods like CT, MRI, and PET, might also be valid non-genetic prognostic factors for HNSCC. Based on abundant anatomical and structural data, and functional aspects of tumour metabolism, new perspectives relevant for prognosis could be established. From a retrospective study in 80 patients, metabolic volume of the primary tumour was identified as an independent prognostic factor for patients receiving surgery as initial treatment.[114] An increase of 17·5 ml was associated with a 12·5-fold risk of disease recurrence and death. In a validation study published 2 years later, Zhang and colleagues[115] confirmed their earlier observation in terms of risk for disease recurrence and death, but not for regional lymph node metastases. In another study, which included 778 patients with oropharyngeal carcinoma, mathematical modelling of 902 radiomic features showed that a radiomic signature derived from standard CT imaging was predictive for HPV and p16 status.[116] Furthermore, a study on fluorine-18-fluorodeoxyglucose uptake at the tumour site after 10–20 Gy total dose was successful in tumour response monitoring.[117] A rapid decrease in standardised uptake value correlated with improved locoregional control and overall survival. In a retrospective analysis of data from 120 patients, of whom 84% had oral cavity carcinoma, radiomic features of primary tumours extracted from CT-guided radiotherapy planning were used for the establishment of a 24-feature prognostically relevant radiomic signature, which remained an independent predictor of overall survival and progression-free survival in a multivariate hazard regression model.[118] By combining the radiomic signature with p16 status, the prognostic performance could further be improved. Patients with a high signature score benefited from chemoradiotherapy in contrast to patients displaying low signature scores.[118]

Novel prognostic biomarkers

Many clinical trials aimed at informing the optimisation of multimodal HNSCC treatment have shown only moderate improvements in patient outcomes by sole modifications of type, dose, or sequence. This outcome suggests that tailored treatment might be more suitable than a one-size-fits-all approach to cancer radiotherapy. Novel strategies are thus aiming to improve treatment selection on the basis of individual biomarkers. This approach will assist the identification of patient groups that are likely to get no or only minor anti-tumour effects from current standard treatments and in which the largest benefit from novel molecular approaches can be expected.

Liquid biopsy biomarkers

Liquid biopsy is a minimally invasive detection method for molecular biomarkers in body fluids, which could serve as a novel tools in the clinical management of patients with HNSCC. 11 studies (table 1)[3–13] in which the potential of blood-derived biomarkers was evaluated satisfied our selection criteria. Seven of these papers assessed the prognostic performance of haematological parameters available from routine blood testing,[7–13] and two studies investigated the role of circulating tumour cells (CTCs).[3,4] An increased interest in haematological markers, such as neutrophil, lymphocyte, and platelet counts, along with inflammatory proteins, was raised by the observation of an association between chronic inflammation and cancer progression. A substantial effect of the neutrophil to lymphocyte ratio on prognosis was first described in nasopharyngeal cancer[119] and was subsequently shown for other major subsites such as the oral cavity,[9] the oropharynx,[10] and the hypopharynx.[8] A 2018 meta-analysis, including 24 studies and a total of over 6000 patients with HNSCC, confirmed the consistently increased HRs for death in patients with an elevated neutrophil to lymphocyte ratio.[120] A proposed pathophysiological mechanism to explain poor outcomes in these patients is the systemic activation of extracellular DNA aggregates associated with cytotoxic and proteolytic enzymes, which are produced by neutrophils.[121,122] These so-called neutrophil extracellular traps seem to contribute to increased metastasis to the lung and liver by sequestering CTCs at neutrophil extracellular trap deposits in chronically inflamed tissue.[121,122] As shown in an experimental mouse model of sustained inflammation caused by tobacco smoke exposure, neutrophil extracellular traps might also contribute to converting dormant cancer cells to aggressively growing metastases.[123] The awakening of these cancer cells was associated with neutrophil extracellular trap-mediated remodelling of the extracellular matrix, and could be prevented by an antibody against the remodelled version of a matrix protein called laminin-111.[123] Thus, therapies targeting neutrophil extracellular traps and their downstream effectors might represent a promising approach for

patients with an elevated neutrophil to lymphocyte ratio to reduce the risk of cancer recurrence.

Though a causal link between neutrophils, CTCs, and formation of aggressive metastases has yet to be shown in HNSCC, evidence of a potential role of CTCs in disease progression has been provided by independent studies done over the past 30 years, which have been discussed previously.[124,125] In line with a potentially prognostic role of CTCs, their detection has been correlated with increased risk of local or distant recurrence and progression-free and overall survival. A review of these studies would be beyond the scope of this paper. In one of the studies identified by our search strategy, CTCs were detected by the semi-automated CTC detection system CellSearch (Menarini-Silicon Biosystems, Bologna, Italy),[4] while PCR-based detection of transcripts of the EGFR were used as surrogate markers for CTCs in the second study.[3] In line with previous reports, both studies showed a significant association between the presence of CTCs and disease progression.[3,4] Overall, the wide variety of different enrichment procedures, a low sensitivity and specificity of the CTC-detection assays used in some previous studies, the use of different definitions of CTC-positive samples, and the inclusion of small heterogeneous patient cohorts, suggests caution is needed when describing the prognostic value of CTCs in HNSCC. The implementation of improved CTC enrichment strategies, which capture a greater population of CTCs, including CTC clusters with potentially enhanced metastasis-forming capacity,[126] and extended phenotyping rather than mere enumeration of CTCs,[127] might be necessary for improving their diagnostic value. For example, a CTC assay allowing the assessment of PD-L1 expression[127] might serve as a valuable companion diagnostic tool to predict the efficacy of immune checkpoint blockade treatments. Further prospective studies in homogeneous patient populations are warranted to implement CTC analysis in clinical routine.

The major advantage of the neutrophil to lymphocyte ratio biomarker is its easy determination from routine blood count assessment. By contrast, the CellSearch platform, although approved for clinical use by regulatory agencies, is not yet broadly used in HNSCC diagnostics because of the shortage of prospective studies confirming its value. The prognostic value of neutrophil to lymphocyte ratio and CTCs are uncertain because of the use of different cutoffs for the definition of a high neutrophil to lymphocyte group and CTC-positive blood samples, and the inclusion of heterogeneously treated patient cohorts in previous studies. Certainly, further investigations will be necessary for definite conclusions on the clinical value of these promising liquid biomarkers.

Tissue-based prognostic biomarkers

The vast majority (75 [87%] of 86) of selected biomarker studies investigated distinct tissue-based biomarkers using various techniques. 60 (80%) of these 75 studies assessed protein biomarkers by immunohistochemistry (table 2), which seemingly still represents the standard assay in studies aiming to establish prognostic biomarkers for clinical use. Categorisation of 60 molecular biomarkers according to their biological functions revealed various genes (23 [39%]) involved in epithelial to mesenchymal transition, cell migration, invasion, and metastasis,[32–46,128,129] and genes with important roles in stem-cell biology and detoxification (13 [22%]).[47–57] Further categories included genes involved in inflammation and immunity (7 [12%]),[58–66] cell proliferation and cell cycle control (5 [8%]),[14–20] cell survival (5 [8%]),[21–27] hypoxia (2 [2%]),[28,29] or other diverse biological processes (5 [8%]).[67–71] The remaining biomarker studies in tumour tissue were based on genomic DNA (table 3) or mRNA (table 4). Here, the prognostic value of copy number alterations (n=4 studies),[15,72–74] the promotor methylation status of distant gene loci (n=2),[75,76] somatic or germline single nucleotide variants (n=4),[77–80] and mRNA expression (n=6) of single genes[83,85,86] or gene sets[81,82,84] were evaluated.

Among the promising biomarkers identified by our systematic literature search was PD-L1, the expression of which can be measured by immunohistochemistry. This biomarker has gained much attention when expressed in tumour cells, or the tumour microenvironment, and is associated with anti-PD1 drug response in various tumour types.[130] Certain cancers, including HNSCC, frequently express PD-L1 on the surface of tumour cells and on infiltrating immune cells. Constitutive expression by tumour cells seems to be driven by dysregulated signalling pathways (eg, activation of the PI3K/AKT (PKB) pathway).[131] By contrast, adaptive expression of PD-L1 by tumour cells and immune cells can occur at the interface of tumour cell nests, where infiltrated immune cells secrete proinflammatory factors such as interferon-γ.[132] It seems likely that the type of mechanism driving PD-L1 expression in tumour tissue can also affect its prognostic value. This assumption is supported by the observation that inflammation-associated PD-L1 expression in immune cells is associated with a good prognosis,[63,65] whereas high expression of PD-L1 in tumour cells is associated with poor outcomes.[64,65]

Overexpression of EGFR and amplification of *CCND1* associated with overexpression of cyclin D1 are further biomarkers of high clinical potential. EGFR is expressed in more than 90% of HNSCC cases[133] and is responsible for constitutive activation of mitogenic signalling pathways. CCND1 (BCL-1) functions as a regulatory subunit of CDK4 and CDK6, the activities of which are required for the translation of mitogenic signalling into cell cycle progression. Thus, combined targeting of EGFR-dependent and CCND1-dependent pathways should be highly effective in HNSCC. The first clinical trial of the CDK4 and CDK6 inhibitor palbociclib in combination with cetuximab in recurrent or metastatic HNSCC showed impressive tumour response rates (overall

response rate 35%) even in cetuximab-resistant or platin-resistant disease.[134] The efficacy of such an approach might even be increased by biomarker-driven patient selection, an approach currently tested in the European Organisation for Research and Treatment of Cancer HNCG 1559 trial (UPSTREAM [NCT03088059]).[135]

Improving translation of biomarkers from bench to bedside

Although the biomarker studies selected fulfilled our selection criteria, we repeatedly observed substantial shortcomings in study design and statistical power. Inherent limitations to most of the identified studies include the retrospective approach of data collection, the combination of multiple HNSCC subsites with insufficient statistical power for subgroup analysis, heterogeneous therapy, and the potential for unknown confounders to affect patient outcomes. Furthermore, the use of tissue microarrays used in some of the immunohistochemistry studies might not be representative of results obtained from larger tissue sections. These shortcomings might have contributed to both false-positive and false-negative findings. However, we consider our validation approach for promising candidate biomarkers (requiring that two or more independent cohorts were studied) as a useful strategy to minimise this potential bias. Nonetheless, further prospective validation of candidate biomarkers in studies of uniformly treated patient cohorts will be required.[136]

Among the identified tissue-based biomarkers, only EGFR represents a target for an approved molecular drug (cetuximab), and few other candidates (PD-L1, CCND1, PI3K, AKT, and FGFR) are targets for clinical development. This situation clearly underlines the unsatisfying association between the large increase in our knowledge of the molecular characteristics of HNSCC tumours from comprehensive studies like the TCGA projects[2] and the continuingly low numbers of established molecular targets in this disease. Functional studies in appropriate preclinical models will be necessary for determining the biological function of genetic variants and altered gene expression in HNSCC, thereby accelerating insights into the molecular mechanisms of disease progression and treatment resistance.

Although the identification of molecular subgroups and the development of optimal subgroup-specific treatment regimens will certainly represent milestones in HNSCC, some clinical researchers envision individualised rather than subgroup-specific treatment. The concept of a phenotype-driven precision oncology approach is based on the notion that biological responses to chemical or genetic perturbations in patient individualised ex-vivo models can serve as predictive biomarkers for therapeutic response in the clinic.[137] First proof of the feasibility of such strategy in HNSCC was presented by Chia and colleagues[137] in 2017. In this study, drug screening in patient-derived primary cultures reproducibly predicted treatment response in matched patient-derived xenograft models. Besides drug sensitivity profiles, comprehensive omics strategies that interrogated these ex-vivo cultures was used to guide real-time therapeutic decisions and to predict tumour progression in n=1 co-clinical trials.[137] Future studies in larger patient cohorts are needed to assess the potential and feasibility of such an approach for routine clinical management.

Additional caveats for biomarker interpretations

The association of biomarkers with outcome can also be affected by the genetic background of patients and their exposure to environmental factors. This knowledge can explain why strong associations with survival, reported from well controlled biomarker studies of large patient cohorts, are sometimes not detected by others, despite comparable patient selection, identical molecular tests, and sufficient statistical power. For example, in a 2017 omics-based study in a Taiwanese cohort of patients with oral cavity carcinoma, an increase in mRNA expression levels of the cytidine deaminase APOBEC3A was established as an independent prognostic biomarker for overall and disease-specific survival.[138] Notably, in the same study, the authors failed to find any significant association of APOBEC3A expression with overall survival in the TCGA dataset of 312 cases.[138] As discussed by the authors, this difference might be due to the effect of genetic variations specific to the Taiwanese (mainly Han Chinese) patients that were studied, in comparison to patients of other ethnicities included in the TCGA dataset. Potential interference of genetic background and environmental factors on the prognostic value of biomarkers should be kept in mind when interpreting these results. Demographic analysis revealed a substantial imbalance in geographical origin among selected biomarker studies focused on HNSCC anatomical subsites. Of the 44 biomarker studies of oral cavity carcinoma, 21 (48%) were based on Asian patient populations. By contrast, 10 (77%) of 13 studies in laryngeal carcinoma were based on European patient populations who had mainly Caucasian genetic backgrounds. Ethnic background and potential differences in lifestyle and environmental exposure should not only be considered when interpreting the results from patient cohort studies, but also during clinical decision making and therapy, as a key practice of precision medicine.[138]

Conclusion

Different types of omics studies (genomics, transcriptomics, proteomics, and radiomics) in high-throughput molecular analyses of large patient cohorts, have led to a considerable gain in knowledge in the biological processes involved in neoplastic transformation and progression of HNSCC. These studies form a valuable basis for the development of biomarkers that can predict outcomes for a single patient. Despite increased numbers of studies based on training and matched validation cohorts, and

advanced bioinformatics modelling with supporting evidence from mechanistic preclinical studies, biomarkers that could outperform classical prognostic markers such as TNM stage, HPV status, or patient factors, have not reached clinical practice. Currently planned clinical trials hope to unveil and select those molecular biomarkers that do not only add to classical prognostic factors at the expense of higher costs, but also have the potential for facilitating personalised treatment of HNSCC. Given the high number of biomarkers identified, whether for quantitative reasons all of these will be validated in large clinical trials remains uncertain. Ultimately, a substantial risk of missing important biomarkers by chance will remain. For HPV-positive tumours, which are more frequently driven by activating mutations, we await results from large randomised clinical trials evaluating subtype-specific novel treatment regimens during the next few years. These outcomes will hopefully elucidate and strengthen the new TNM-8 classification system for HPV-driven tumours, and provide further insights into the potential of de-escalating treatment regimens to reduce late morbidity for patients.

The utmost question for future guideline-based HNSCC treatments is whether we will arrive at the end of this evolutionary process with a new paradigm for standard of care for HNSCC treatment. This model could be phenotype-driven and biomarker-based—in other words, a high-precision medicine approach. In this new treatment model, the question of whether the use of classical prognostic factors and data from prospective randomised clinical trials will still be used is debatable. To answer this question effectively, effort is required for clarification, mainly from translational research at the bench and also at the bedside. In the end, we might have a chance of squaring the circle by improving the therapeutic ratio with less morbidity for our patients, thereby also reducing the health-related economic burden.

Contributors

VB and IT did the literature search and analysed and interpreted data. VB wrote the section on classical and novel clinical prognostic factors. IT prepared the tables and figures and wrote the section on novel biomarkers. We both reviewed and approved the final manuscript before submission.

Declaration of interests

VB reports financial support as principal investigator for a clinical trial in HNSCC from Merck Serono and Sanofi, compensations from Merck Serono for chairing educational activities, and personal fees from Bristol-Myers Squibb for advisory board activities, outside the submitted work. IT reports compensation from Merck Serono for advisory board activity.

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