

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Head Neck Squamous Cell Cancer Genomics: Oncogenes, Tumor Suppressor Genes and Clinical Implications

Anand B. Pathak and Satyam Satyarthi

Abstract

Head Neck Squamous Cell Cancer is genomically heterogenous. Common somatic mutations involve TP53, CDKN2A, FAT1, NOTCH1, PIK3CA, KMT2D and NSD1, less frequently others. Epigenetic changes also contribute to HNSCC biology. Alterations in tumor suppressor genes is a major oncogenic event in HNSCC. Genomic heterogeneity exists between different subsites within head neck region and also between the primary and metastatic disease. Intratumor heterogeneity has also been recognized. Based on key genomic alterations, four major molecular subtypes have been identified. Multi-omics analysis has provided further insights into HNSCC biology and shed light on EGFR pathway and immunogenomics. Corelative genomics of tumor cells, stromal cells and immune cells have led to emergence of distinct immune molecular subtypes of HNSCC. Major tumor suppressor genes and oncogenes have a correlation with prognosis, survival and treatment resistance. EGFR pathway is in focus for renewed understanding of resistance to EGFR targeted treatments and novel ways to target EGFR pathways. Increasingly genomic data is being leveraged towards clinical use including HNSCC prevention, prediction of metastases, survival and prognostication, fine tuning use of surgery, chemotherapy and radiation therapy, identifying patients for using immunotherapy, predicting drug resistance and gaining new information from radiological studies. Several novel targeted therapies are being pursued in clinical trials. Molecular co targeting strategies are being developed. Understanding the way tumor suppressor genes and oncogenes shape HNSCC biology and clinical behavior is bringing the much-needed therapeutic breakthrough in this tough to treat disease.

Keywords: Head Neck, Squamous, genomics, clinical, profiling, applications

1. Introduction

Head neck squamous cell carcinomas (HNSCC) include cancers arising in the mucosa of oral cavity, pharynx, larynx, hypopharynx. According to GLOBOCAN 2020 report, worldwide head neck cancer statistics indicate that there are 1,518,133 cases of head neck cancers per year, resulting in approximately 510,771 deaths per year. In Asia there are 944,946 cases of head neck cancers per year, resulting in approximately 347,870 deaths per year. High incidence rates have

been reported from developing countries including India, Pakistan, Bangladesh, Taiwan, and Sri Lanka [1].

Treatment of HNSCC is guided uniformly by anatomic location, tumor size, presence or absence of nodal and distant metastases. Oral cavity cancers are primarily treated with surgery followed by adjuvant radiation or chemo-radiation based on pathological features. Cancers in the oropharynx, larynx and hypopharynx are primarily treated with chemo-radiation with function preservation as the main goal of therapy. Neo-adjuvant chemotherapy is used in locally advanced tumors to improve resectability. EGFR targeting drugs afatinib, Cetuximab and immune check point inhibitors pembrolizumab, nivolumab are the only FDA approved biological treatments today.

Clinicians managing HNSCC face number of challenges today. Some of these include.

- High mortality in spite of optimal use of currently existing therapeutics.
- Lack of clinically meaningful biological classifier of HNSCC other than HPV status.
- Continued emergence of treatment resistance.
- Great variability in clinical outcome despite uniformity in approach.
- Continued reliance on anatomical factors (TNM) to guide treatment.
- High morbidity and poor quality of life after conventional treatments
- Lack of robust biomarkers to select EGFR targeted therapy which seems to be the only existing targeted therapy for HNSCC.
- Lack of effective systemic adjuvant systemic therapies in high-risk patients.
- Lack of genomically directed therapies similar to other oncogene addicted cancers.
- Lack of effective later lines of therapies
- Low response rates to currently approved immune check point inhibitors
- Lack of robust biomarkers to predict nodal, distant metastases and recurrence
- And even lack of predictive biomarkers for selection of conventional treatments, not to mention lack of robust biomarkers for prognosis.

Considerable work has been done on deciphering HNSCC at genomic level. Major alterations in tumor suppressor genes and oncogenes in HNSCC have been identified. Multi-omics studies have shed considerable light on how genomic alterations shape HNSCC biology and clinical behavior. Number of studies are addressing how knowledge about HNSCC genomics/multi-omics can be leveraged to address some of the challenges faced by clinicians managing HNSCC. The need to break the ground in HNSCC prevention and therapy has never been so urgent.

This chapter attempts to review key alterations in tumor suppressor genes and oncogenes in HPV negative HNSCC and the potential clinical implications of these

alterations. Key insights gained from multi-omics studies will also be highlighted. This review also quotes some of the novel targeting therapies and novel strategies. Specifically, insights gained in EGFR targeting and immune therapies will also be discussed in the context of genomics. Since the amount of literature being published is so large, it is beyond the scope of this review to provide exhaustive coverage on each aspect of head neck cancer genomics. Hence few indicative studies are quoted to elaborate each point to give the reader a basic orientation. This review will focus on HPV- HNSCC.

2. Head neck squamous cell cancer (HNSCC): oncogenes and tumor suppressor genes

The Cancer Genome Atlas (TCGA) provided landscape of somatic genomic alterations by profiling 279 head neck squamous cell carcinomas. Tobacco related head neck squamous cell cancers showed loss of function mutations of TP53, CDKN2A inactivation, Copy number alterations of 3q26/28, 11q13/22. Few subgroups showed alterations in NSD1, WNT pathway genes AJUBA and FAT1, NFE2L2 [2]. HPV positive cases showed mutations of PIK3CA, loss of TRAF3 and amplification of cell cycle gene E2F1. Whole exome sequencing and microarray data showed unstable HNSCC genome showing high copy number alterations including copy number loss and copy number gains. Co amplifications of CCND1, FADD and CTTN and BIRC2 and YAP1 were found. Focal deletions were found in NSD1 and tumor suppressor genes including FAT1, NOTCH1, SMAD4, CDKN2A. Focal amplifications were found in receptor tyrosine kinases (RTKs) like EGFR, ERBB2, FGFR1. There was a small subset of oral cavity cancer characterized by activating mutations in HRAS, inactivating mutations in CASP8 and wild type TP53. This subset has been labeled as 'M' class which is driven by mutations rather than copy number alterations with tumorigenesis involving RAS, cell death pathway and NFkB. Fusion oncogenes like ALK, ROS or RET were not observed. MET exon 14 skipping mutation was uncommon. Loss of tumor suppressor function was more common than protein coding fusion events.

TCGA identified genes which can be grouped into (1) genes responsible for cell survival and proliferation (TP53, HRAS, EGFR, PIK3CA), (2) cell cycle control genes (CDKN2A AND CCND1), (3) cellular differentiation (NOTCH1) and (4) adhesion and invasion signaling (FAT1). Out of the most commonly mutated genes, TP53, CDKN2A, CASP8 AND NSD1 are differentially mutated across anatomic sites in the head neck region.

Frequency wise the common mutations in HNSCC are listed in **Table 1**.

More than 70% of HNSCC harbor mutations in the tumor suppressor p53 (TP53). TP53 mutations have been characterized in several ways. These mutations could be somatic or missense mutations, functional, partially functional or non-functional, and based on the alteration of DNA binding, as disruptive and non-disruptive.

TP53 mutations influence cell cycle, genomic integrity causing aberrant proliferation, disrupted apoptosis and defective DNA repair. TP53 mutation is probably the main actor in HNSCC pathogenesis and occur early in carcinoma. These mutations are also very high in metastatic HNSCC. Mutation rates of TP53 vary across different subsites in head neck. Larynx and hypopharynx have the highest TP53 mutation rate (83.5%), oral cavity and tongue 75.6%. oropharynx including tonsils and base of tongue have the lowest mutation rate 28.6% [3].

CDKN2A is the second most commonly altered gene in HNSCC CDKN2A encodes a CDK4/CDK6 kinase inhibitor which constrains cells from progressing

Mutations	Percentage
TP53	72
CDKN2A	22
FAT1	23
NOTCH1	19
PIK3CA	21
KMT2D	18
NSD1	10
CASP8	9
NFE2L2	6
FBXW7	5
TGFBR2	4
HRAS	4
CUL3	4
RB1	3
HLA-A	3
PTEN	2
TRAF3	1

Table 1.
Somatic mutations (TCGA findings).

through G1 restriction point. CDKN2A mutations are rare in HPV+ HNSCC [3]. Mutations involving NOTCH gene are third most common in HNSCC [3, 4]. NOTCH family members are transmembrane proteins (NOTCH 1-4) and two family of ligands the Jagged and the Delta-like proteins, involved in cell to cell communication and regulations of squamous differentiation.

CCND1 encodes cyclin D1 and regulates G1-to-S phase transition by formation of complexes with cyclin dependent kinases like CDK4 and CDK6. CCND1 is amplified in 30-40% of HNSCC with cyclin D1 overexpression [3]. AJUBA regulates cell division, vertebrate ciliogenesis and left–right axis determination. NSD1 is a tumor suppressor gene. Mutations in KMT2D and HLA-A contribute to a defective immunosurveillance. EGFR is commonly overexpressed in HNSCC and has been explored as a therapeutic target. PIK3CA alterations are common in HNSCC especially in HPV+ cancers. PIK3CA are seen in patients with advanced HNSCC harboring multiple PI3K pathway mutations [3]. MET is a Hepatocyte Growth Factor (HGF) receptor which regulates cancer cell plasticity through reversible programming of epithelia-mesenchymal transition (EMT) [3]. MET overexpression leads to MET/ HGF pathway activation and correlates with worse outcome.

2.1 Epigenetics in HNSCC

Epigenetic changes such as DNA methylation, histone acetylation and expression of small non coding RNAs affect gene expression. There is some evidence of importance of epigenetic changes in HNSCC. Global hypomethylation has been linked to poor prognosis. Epigenetic changes is one major method for tumor resistance. Many tumor suppressor genes like CDKN2A, CDH1, MGME, RASSF1A show promoter hypermethylation [5].

2.2 Key oncogenic events in HNSCC

In terms of key driver oncogenic events in HNSCC can be summarized as follows; (**Table 2**).

In the TCGA dataset, most of the tumors that were sequenced were from early-stage surgical samples. The genomic profile of recurrent/metastatic HNSCC could be different. The American Association for Cancer Research has undertaken a project Genomic Evidence Neoplasia Information Exchange (GENIE) which is an international data sharing project allowing multiple international institutions to share their data of cancer sequencing. This combined dataset includes 700 patients with HNSCC, 40% representing patients with metastases. The frequency of common mutations in HNSCC in the three datasets TCGA, AACR GENIE and COSMIC are found comparable and has paved the way for developing targeted therapies [6].

2.3 Genomic heterogeneity of HNSCC at different subsites and between primary and recurrent metastatic tumor

In addition to HPV status as one important biological differential, different subsites of HNSCC seem to harbor differences at genomic level. TP53 mutations are most frequent in Laryngeal/hypopharyngeal sites followed by oral cavity followed by oropharynx [3]. David Vossena et al. did DNA sequencing on 111 HPV negative HNSCC, 55 oral and 56 laryngeal/pharyngeal cancers and identified somatic point

Function	Gene	Event
Tumor Suppressor	TP53	Loss of function mutation
Tumor suppressor	CDKN2A	Mutation, homozygous deletion, protein down regulation
Tumor suppressor	NF1	Mutation, amplification
PI(3)K	PIK3CA	Amplification, mutation
PI(3)K	PTEN	Mutation, protein downregulation
PI(3)K	PIK3R1	mutation
Oncogenes	CCND1	amplification
Oncogenes	MYC	Amplification
Oncogenes	HRAS	Mutation
Receptor Tyrosine Kinases (RTKs)	EGFR	Amplification, mutation, protein up regulation
RTKs	FGFR1	Mostly amplification, rarely mutation
RTKs	ERBB2	Amplification, protein up regulation, mutation
RTKs	IGF1R	Amplification, mutation
RTKs	EPHA2	Mutation
RTKs	DDR2	Amplification, mutation
RTKs	FGFR2	Amplification, mutation
RTKs	FGFR3	Amplification, mutation
RTKs	MET	Amplification, exon 14 skipping mutation

Table 2.
Oncogenic events in HNSCC.

mutations and copy number alterations. They also included sample data from TCGA to expand analysis. Mutational profiles of oral and laryngeal pharyngeal squamous cell carcinoma showed many similarities. However, oral squamous cell carcinoma was significantly enriched for CASP8 and HRAS mutations. Laryngeal/pharyngeal squamous cell cancers were enriched in LAMA and NSD1. Overall, oral squamous cell carcinoma had fewer somatic point mutations and copy number alterations. Laryngeal/pharyngeal squamous cancer scored higher on mutational and genomic scar signatures associated with homologous recombination DNA repair defects explaining differential response to chemoradiation [7].

Recurrent and metastatic HNSCC do share driver mutations with their primaries in addition to accumulating new mutations. High rates of TERT promoter mutations are found in recurrent or metastatic HPV- HNSCC. HPV+ HNSCC may also start exhibiting mutational landscape of HPV- negative tumors after recurrence and metastases. Recurrent HPV+ positive tumors may get enriched in TP53 mutations and lack PIK3CA mutations as compared to primary HPV+ primary tumors [8].

As noted earlier, head neck mucosal squamous cell carcinoma occurs at several subsites. Clinical behavior heterogeneity in terms of response to therapy, metastatic rate is commonly observed. Clinical heterogeneity is observed even within a single subsite. Tumor cells are known to accumulate genetic alterations over time. Some of these are driver mutations and some are passenger mutations. Heterogenic cell clones undergo selection leading to development of aggressive clones with growth advantage. This is one main reason for development of resistance to chemotherapy and radiation therapy. High degree of intratumor heterogeneity leads to tumor progression, inferior treatment outcome and reduced survival. Whole genome analysis of 74 cases of HNSCC used to calculate Mutant Allele Tumor Heterogeneity (MATH) can be a genetic biomarker of high-risk disease. High MATH has been found to have shorter overall survival [9, 10]. Targeted monotherapies are unlikely to be major breakthrough in HNSCC. Rational combination of two or several therapies or effective co-targeting seems to be the way forward.

2.4 Molecular subtypes of HNSCC cancers based on gene expression profiles

Chung et al. and Walter et al. described four distinct molecular classes in HNSCC based on gene expression patterns: basal, mesenchymal, atypical, and classical (**Table 3**) [11, 12]. The basal subtype is characterized by over-expression of genes functioning in cell adhesion including COL17A1, and growth factor and receptor TGFA and EGFR, high expression of transcription factor TP63. The mesenchymal subtype shows over expression of genes involved in immune response and genes associated with epithelial to mesenchymal transition including vimentin, desmin, TWIST1, and HGF. The classical subtype is shows over-expression of genes related to oxidative stress response and xenobiotic metabolism. The atypical subtype shows elevated expression of CDKN2A, LIG1, and RPA2, low expression of EGFR.

2.5 Multi-omics analysis of HNSCC and novel insights

Huang et al. did proteogenomic study on 108 HPV negative HNSCCs in order to gain biological insights and novel treatment strategies [13]. They found correlation between 263 proteins, 173 phosphoproteins and overall survival. Poor prognosis associated proteins/phosphoproteins were enriched in pathways for somatic copy number alteration drivers, DNA replication, cell cycle and RNA processing. They

Molecular subtype	Key features
Classical	TP53 mutation CDKN2A loss 3q amplification Alterations in oxidative stress genes like KEAP1, NFE2L2, CUL3, Smoking history Laryngeal subsite
Basal	NOTCH1 inactivation Decreased SOX2 expression HRAS-CASP8 co-mutation Co-amplified 11q13/q22
Atypical	Lack of chromosome 7 amplification Activating exon 9 mutations (PIK3CA domain)
Mesenchymal	Alterations in innate immunity genes, High expression of CD56 Low frequency of HLA class I mutations

Table 3.
Molecular subtypes of HNSCC and key features.

also found poor prognosis associated with FAT1 truncation or 11q13.3 amplification. Analysis of Rb pathway showed interesting observations. CDKN2A and CCND1 alterations do not always result in increased CCND1 protein and CDK4/6 activity. Rb status was found more effective indicator of CDK4/6 dependent cell cycle activity than genomic or transcriptomic markers.

Similarly, novel insight was obtained in EGFR pathway. EGFR amplification activates EGFR in a ligand independent manner. The EGFR monoclonal antibody works by binding to the extracellular domain of EGFR to prevent ligand induced activity. Therefore, EGFR ligand abundance is more important to activity of anti EGFR moAbs than EGFR amplification or overexpression.

Immune-proteogenomic analysis revealed immunosuppressive somatic copy number alterations. Higher immune cell infiltration was linked to low clinical stage, less smoking and better prognosis. Immune hot tumors showed both cytotoxic immune enzymes and immunosuppressive proteins. This explains why the response to immune check point inhibitors in PD L1 positive HNSCC patients is modest. In immune cold tumors, the low immune infiltration was not driven by lack of tumor antigen sources but deficient Antigen Presentation Machinery (APM) pathway.

Further Huang et al. divided HNSCC tumors into three clusters using multi-omics data. Cluster I was associated with laryngeal site, strong smoking and high chromosome instability (CIN). Proteomic data suggested linkage between aberrant epigenetic activity, smoking and high CIN. This cluster had the worst prognosis. Cluster II showed elevation of several basal factors and high translational activity. Cluster III showed tumors with weak smoking history, higher immune scores and higher stromal scores. So, cluster I, II and III were CIN, Basal and Immune subtypes respectively. In terms of treatment selection, CIN subtype was associated with frequent aberrations of CCND1, CDKN2A and Rb hyperphosphorylation indicating potential for CDK 4/6 inhibitors. Basal subtype was associated with high EGFR ligand activity suggesting a potential role for anti-EGFR mAbs. The immune subtype could be appropriate for immune checkpoint blockade. Frequency of high level of biomarkers were 32% in CIN tumors, 62% in Basal tumors and 83% in Immune tumors emphasizing the tremendous potential to select appropriate therapy.

New targets for therapy were also identified including KIT, ECER1G, PLAU, SERPINE1, TOP2A, MMPs, several cell cycle and DNA damage related kinases. Multiple C/T and neoantigens were also found in their analysis which could be potential immunotherapy targets.

2.6 Immunogenomics

Thorsson et al. and Tamborero et al. did extensive immunogenomic analysis of many tumors and came out with six molecular immune subtypes: wound healing (C1), IFN gamma dominant (C2), inflammatory (C3), lymphocyte depleted (C4), immunologically quiet (C5) and TGF-beta dominant (C6) [14, 15]. In the TCGA HNSCC cohort, most tumors were C1 with elevated expression of angiogenic genes, high proliferation rate and a Th2 cell bias to the adaptive immune infiltrate or C2 with the highest M1/M2 macrophage polarization, a strong CD8 signal and prominent TCR diversity.

Genomic and neoantigen evolution from primary to first metastases was studied by Charles Schutt et al. between 23 paired primary and recurrent HNSCC tumors [16]. They found 6 genes which predicted neoantigens in 4 or more patients. Neoantigens in shared genes had increased CD3+ and CD8+ T cell infiltration and duration of survival with disease.

Yao Yao et al. in a study involving 5 HNSCC tumors and normal tissue found four immune related genes, PVR, TNFRSF12A, IL21R, SOCS1 to be significantly associated with overall survival [17]. They tried to integrate these four genes with pathological N stage to better predict overall survival. High expression of PVR AND TNFRSF12A indicated poor overall survival whereas high expression of IL21R and SOCS1 indicated better overall survival.

Chen et al. characterized the immune landscape of HNSC by their tumor and stromal compartments to identify novel immune molecular subgroups [18]. In their study, a training cohort of 522 HNSC samples from the Cancer Genome Atlas profiled by RNA sequencing was analyzed. Gene patterns from tumor, stromal, and immune cell genes were separated. Correlations were studied between the expression patterns with a set of immune-related gene signatures, potential immune biomarkers, and clinicopathological features. Validation was done with six independent datasets containing 838 HNSC samples.

Approximately 40% of HNSCs were labeled as immune class based on enriched inflammatory response, enhanced cytolytic activity, and active interferon- γ signaling. Within this, some samples had markers of exhausted immune response and some had markers of active immune response. The Exhausted Immune Class was characterized by enrichment of activated stroma and anti-inflammatory M2 macrophage signatures, WNT/transforming growth factor- β signaling pathway activation and poor survival. Active immune class showed enriched proinflammatory M1 macrophage signature, enhanced cytolytic activity, abundant tumor-infiltrating lymphocytes, high human papillomavirus (HPV) infection, and favorable prognosis. Such a subgrouping might help in tailoring immune therapies to appropriate subsets of patients.

Several genomic features may influence response to immune check point inhibitors [19]. High tumor mutational burden is associated with neoepitope presentation and immune hot phenotype leading to enhanced benefit with immune check point inhibitors. NSD1 inactivating mutations, global DNA hypomethylation, aneuploidy, may lead to impaired chemokine signaling and immune effector response leading to an immune cold phenotype and low benefit from immune checkpoint inhibitors. Groups led by Many HNSCC specific studies tried to subtype patients as immune molecular subtypes. Considerable work is also being done

to understand immune events occurring in the areas of field cancerization around an oral premalignant lesion raising the hope for using immunotherapy as immunoprevention. Integrated omics studies are also being pursued to understand occurrence of immune related adverse events and development of immune resistance.

3. Head neck cancer genomics: clinical implications

3.1 Clinical and therapeutic implications of major tumor suppressor genes and oncogenes in HNSCC

TP53 mutation which is common in HNSCC has predictive value for disease free and overall survival. There is a correlation between TP53 mutations and resistance to chemotherapy drugs like cisplatin, doxorubicin, paclitaxel also leading to lower rates of pathological complete responses to neoadjuvant chemotherapy. Absence of TP53 mutated DNA in the surgical margins has been found to improve local recurrence free survival. Patients with no TP53 mutated DNA in the surgical margins may be spared post-operative radiation therapy. Disruptive TP53 mutations predict locoregional recurrence.

Mutant TP53 could be targeted in several ways; (1) introduction of wild type TP53 inside the cancer cells, (2) reactivation of some function of wild type TP53 in mutant cancer cells, (3) degradation of mutant TP53, or (4) targeting coexisting genetic alterations such as CDKN2A deletions or PIK3CA activation to induce synthetic lethality.

CDKN2A It is associated with worse survival in recurrent metastatic HNSCC. Frequent alterations of PI3K-AKT- m TOR pathway has raised the hope for therapeutic targets. However, the results with PI3K/AKT/m TOR pathway targeting have been inconsistent.

There could be a scope for combination of PI3K inhibition with chemotherapy and/or radiation. Currently there are trials underway combining buparlisib, copanlisib and alpelisib in combination with radiation, cisplatin and/or cetuximab. mTOR inhibitors sirolimus, everolimus and temsirolimus have limited efficacy in HNSCC. Further work is needed in this area to develop effective strategies. Activated PI3K/Akt also confers resistance to MET inhibition. Therefore, combining MET/PI3K inhibition might be a good strategy. CCND1 amplification has been associated with recurrence and metastases. It may also confer resistance to cisplatin and EGFR inhibitors. CDK4/6 inhibitors abemaciclib and palbociclib are being tested in combination with cetuximab and IMRT in locally advanced HNSCC. Oral squamous cell carcinoma patients with NOTCH pathway mutations are three times more likely to die with recurrent disease. NOTCH1 mutation may serve as biomarker for identification of HNSCC with higher sensitivity to radiotherapy and chemotherapy. Activated NOTCH1 also contributes to resistance of PI3K inhibitors. NOTCH1 inhibition may enhance efficacy of conventional chemotherapeutic agents by targeting head neck cancer stem cells. Exposure to chemotherapeutic agents may lead to selection of recurrent tumors enriched in cancer stem cells. NOTCH1 inhibition may attenuate such an effect [4].

EGFR is commonly overexpressed in HNSCC [20]. It is associated with resistance to radiation therapy and chemotherapy and worse locoregional and disease-free survival. Two agents Cetuximab a monoclonal antibody binding to the extracellular domain of EGFR and Afatinib a small anti molecule tyrosine kinase inhibitor have been approved by FDA [21, 22]. However, currently there is no biomarker to select patients for these drugs. Considerable work has been done to understand resistance mechanisms to anti-EGFR monoclonal antibodies and -EGFR

tyrosine kinase inhibitors. These include (1) Metabolic pathways, (2) cross talk with other signaling pathways, (3) dysregulation of EGFR pathway, (4) epithelial mesenchymal transition and nuclear translocation of EGFR. This understanding will help in overcoming EGFR resistance.

There could be several ways to augment EGFR targeting such as (1) combination of EGFR Mab and EGFR TKi, (2) Horizontal targeting multiple HER receptors, (3) Vertical targeting with inhibition of EGFR and other RTKs involved in nuclear translocation of EGFR.

MET alterations are low but important in terms of serving as a target for therapy. Several drugs are available such as tivantinib, cabozantinib, crizotinib to target MET. MET mutations are also associated with EGFR inhibitor resistance and reduced sensitivity to VEGFR TKIs. Dual VEGFR/c-MET inhibition or dual blockade of MET/EGFR could enhance efficacy.

3.2 Genomic data to improve head neck cancer prevention

We know that progression from normal epithelium to fully developed squamous cell cancer occurs through a multistep process often involving a stage of pre-malignant lesions. It has been found that these stages of normal epithelium, pre-malignant lesions and malignant lesions are not only different histologically, but are different in terms of genomics. Some earlier studies using Affymetrix Gene Chips found that progression from normal epithelium to pre-malignant lesions are associated with more transcriptional alterations than progression from pre-malignant lesions to malignant lesions. Moreover, the normal, pre-malignant and malignant lesions cluster differently. Based on this, there could be potential to classify pre-malignant lesions into low risk and high risk with appropriate treatment approach of aggressive treatment of high- risk lesions to prevent occurrence of HNSCC [23].

3.3 Prediction of metastases based on genomic profiling

Currently, presence or absence of neck nodal metastases is the only robust predictor of recurrence and metastases. Therefore, in most cases clinical N0 necks are addressed with surgical neck dissection. This often means treating a great majority of patients with unnecessary surgery. Currently, there is no single gene mutation or genomic profile which can predict recurrence and metastases as effectively as neck nodal status. This could be due to the fact that occurrence of metastases involves multiple genetic, molecular and metabolic pathways in addition to influence of host immune system. Genomic changes necessary for metastases may exist in majority of primary tumor at diagnosis paving the way to develop a robust metastatic gene signature.

Cromer et al. studied patients with hypopharyngeal squamous cell cancers using gene expression and found metastatic prediction accuracy of 92% using 168 gene targets [24].

Roepman et al. studied expression profiles of 82 primary oral cavity and oropharynx squamous cell cancers using 102 genes as predictors and observed predictive accuracy of 86% in comparison to clinical staging accuracy of 68% [25].

In view of the different lymphatic drainage patterns of different anatomical subsites, probably each anatomic subsite will need different genetic signature to predict nodal metastases.

Karpothiou et al. studied 18 HNSCC and corresponding node metastases and non-neoplastic tissue for RT-qPCR for EGFR, VEGF, Claudin7, Maspin, Survivin and SCCA [26]. They found differential gene expression levels in node metastases compared to the primary tumor and some correlation with prognosis.

Zevallos et al. did a retrospective study applying four molecular subtypes of HNSCC namely Basal (BA), Mesenchymal (MS), Atypical (AT) and Classical (CL) to oral cavity and laryngeal squamous cell cancers [27]. They found that early-stage oral cavity cancer with MS subtype was associated with high risk of nodal metastases. In laryngeal cancer, CL subtype was associated with worse overall survival. Oral cavity squamous cell cancers were predominantly BA and MS whereas laryngeal cancers were predominantly CL and AT subtype.

Ribeiro et al. used array comparative genomic hybridization data from HNSCC patients to develop a model to predict HNSCC recurrence/metastasis [28]. In their study of 104 HNSCC patients, this predictive model showed a good accuracy (>80%). Validation was done in an independent population from TCGA data portal. The genomic model included chromosomal regions from 5p, 6p, 8p, 9p, 11q, 12q, 15q and 17p, containing many upstream and downstream signaling pathways associated with cell proliferation and invasion. This model will need further large-scale study and has the potential to individualize clinical management and also identify potential therapeutic targets.

3.4 Survival and prognostication

There is considerable heterogeneity in the outcome of HNSCC patients with similar TNM stage. Number of studies are addressing this question. Investigators from China came out with a six gene signature (PEX11A, NLRP2, SERPINE1, UPK, CTTN, D2HGDH) using bioinformatics analysis of TCGA dataset, as a new prognostic marker for predicting survival of HNSCC patients [29]. They also did Gene Set Enrichment Analysis and found some pathways significantly enriched between high risk and low risk groups. Clinical trials testing such signature will be helpful.

Reddy et al. in a meta-analysis approach identified respective differentials (tongue: 3508, laryngopharynx: 4893, oropharynx: 2386) [30]; validation in TCGA revealed markers with high incidence (altered in >10% of patients) in tongue (n = 331), laryngopharynx (n = 701) and oropharynx (n = 404). Assessment of these genes in clinical sub-cohorts of TCGA indicated that early stage tongue (MTFR1, C8ORF33, OTUD6B) and laryngeal cancers (TWISTNB, KLHL13 and UBE2Q1) were defined by distinct prognosticators. Similarly, correlation with perineural/angio-lymphatic invasion, identified discrete marker panels with survival impact (tongue: NUDCD1, PRKC1; laryngopharynx: SLC4A1AP, PIK3CA, AP2M1). Alterations in ANO1, NUDCD1, PIK3CA defined survival in tongue cancer patients with nodal metastasis (node+ ECS-), while EPS8 is a significant differential in node+ ECS- laryngopharyngeal cancers.

3.5 Genomics and surgery in HNSCC

Goal of head neck cancer surgery includes wide resection of the primary tumor, neck dissection in clinically selected patients with the goal of obtaining adequate negative margins and acceptable functional outcome. Adjuvant therapy depends upon presence or absence of certain histopathological findings like positive margins, angiolymphatic space invasion, perineural invasion, nodal metastases, nodal metastatic burden, extracapsular extension in the involved nodes. How can HNSCC genomics help in precision surgery [31]?

1. Refining indications for surgery: There is often a dilemma in early - stage oral cavity cancers that are clinically N0, whether to do elective neck node dissection. Here genomic characterization of the primary tumor might help in prediction of nodal metastases and help in selection of patients for neck

dissection [32]. Similarly, negative predictive value of transcriptomic signature in early - stage oral cavity cancer might help in avoiding unnecessary neck dissection [33].

2. Surgery for pre-malignant lesions.

Some premalignant lesions progress to malignant lesions. Molecular genomic studies might help to identify such lesions so that they can be resected immediately [34].

3. Some patients with oligometastatic cancer with indolent behavior might be surgical candidates. Genomic studies on tumor dormancy might help identify such patients who could benefit by metastasectomy [35].

4. Genomic prediction of radiosensitivity (discussed below) might help avoid surgery in such patients.

5. Markers of aggressiveness: Genomics might predict for occurrence of extracapsular spread in involved and hence help allocate patients for adjuvant chemoradiation [36].

6. Perineural invasion is a known pathological marker of aggressiveness. Genomic expression profile of perineural invasion indicating aggressiveness might help triage patients for appropriate adjuvant therapies after surgery [37].

7. Genomic analysis of surgical margins.

In-spite of clear surgical margins about 15% patients do recur after surgery. Molecular analysis of the surgical margins might identify such patients and improve surgical resectability [38–41].

8. Many oral cavity cancers involve mandible. Mandibular resections add considerable morbidity and impair quality of life. Genomic studies might help in deciding extent of mandibular resections based on tumor tropism to involve bone [42].

9. Neoadjuvant immunotherapy is being increasingly pursued in clinical trials with its potential to real down stage the tumor and prevent metastases. This might redefine approach to surgery in near future [43–46].

10. Follow up of patients after cancer surgery: Functional genomics might help in optimizing follow of patients after curative surgical resection by identifying markers of aggressiveness. Genomic profile identification of perineural invasion might help in enhanced surveillance of such patients [47]. Patients with intratumor heterogeneity might be at risk of recurrence. Such patients can be identified prospectively [48]. Genomics may also help in prediction of loco-regional relapse. Group led by Davide Gissi analyzed DNA methylation for the following genes: ZAP70, ITGA4, KIF1A, PARP15, EPHX3, NTM, LRRTM1, FLI1, MIR193, LINC00599, MIR296, TERT, and GP1BB in the brushings from the tumor area at diagnosis and from the regenerating area 6 months after surgery in 49 consecutive patients [49]. As per a predefined cut-off value, sample was labeled as positive or negative. At diagnosis 47 out of 49 specimens were found positive. 16 out of 49 patients had positive scores

at six months after resection. 7 patients relapsed and out of these 6 patients had a positive score in the regenerative area after surgery. The presence of a positive score after oral cancer treatment was the most powerful variable related to the appearance of locoregional relapse. The authors concluded that 13-gene DNA methylation analysis by oral brushing may have a clinical application as a prognostic non-invasive tool in the follow-up of patients surgically treated for oral cavity squamous cancers.

3.6 Genomics to help using radiation therapy in HNSCC

Radiation therapy is mainstay of therapy in majority of HNSCC either as an adjuvant after surgery in oral cavity cancers, as principal treatment with or without chemotherapy in non-oral cavity cancers, as palliative or salvage therapy. Currently, radiation therapy strategies are same across anatomic sites based purely on TNM stage. There are no robust biomarkers of prediction of response, resistance and outcome in HPV- HNSCC.

Genomics have the potential to guide radiation response/resistance and predict toxicities. SF2, survival fraction at 2Gy in cell lines was published by Torres-Roca et al [50].

Pramana et al. also found potential to use gene expression profiling to predict outcome after chemo-radiation in head neck cancer [51].

Radiosensitivity index has been shown to predict clinical outcome in HNSCC patients treated with chemo-radiation in clinical trials, with 2 year survival of 86% in radiation sensitive signature versus 61% in resistant signature [52].

Concept of GARD (Genomic adjusted radiation dose) was developed by Jacob Scott et al., using a gene expression-based radiation-sensitivity index and linear quadratic model to derive GARD. GARD based clinical module potentially can allow individualization of radiation therapy and guide new design for genomically guided clinical trials [53].

Tumor hypoxia is known to lead to radiation resistance. Work has been to develop genomic signature to predict tumor hypoxia so that appropriate intervention strategies targeting tumor hypoxia can be developed [54].

Along the same lines immunogenomics might be used to predict outcome of radiation and immune therapies given in different combinations. Biology based radiation adaptation trials are already going in HPV positive HNSCC.

Gene alterations can also predict radiation induced toxicity and identify patients who are super sensitive to radiation therapy. Whitney Sumner et al. analyzed 37 HNSCC patients and found that genetic alterations in BRCA2, ERBB3, NOTCH1, and CCND1 were associated with higher mean grad radiation toxicity [55]. Alterations in TNFAIP3, HNF1A, SPTA1 and CASP8 were found in radiation supersensitive patients. Such an approach will help in improving therapeutic index of radiation therapy in HNSCC.

Overexpression of FOXC2, MDR1, MRP2, ERCC1, PDGF-C, NRG1, survivin are linked to treatment resistance. Amongst the miRNAs, overexpression of miR-371a-p, miR-34c-50, miR-1323 and downregulation of miR-324-3p, miR-93-3p, miR-4501 has been linked to radio-resistance in nasopharyngeal carcinoma [56].

3.7 Genomics and chemotherapy in HNSCC

Cisplatin remains the most common chemotherapy drug used in HNSCC. However, resistance to cisplatin is common. Number of genomic correlates of cisplatin response/resistance have been identified. Sanne et al. employed an array of 21,121 pools of siRNAs targeting unique human genes in the NCBI RefSeq database

and performed in vitro genome-wide functional genetic screen to identify genes that influence the response to cisplatin in HNSCC cells [57]. By siRNA-mediated knockdown, Fanconi anemia/BRCA pathway emerged as the predominant pathway for cisplatin response in HNSCC cells. Goretti Duran et al. investigated thirty-six selected single nucleotide polymorphisms (SNPs) in 29 genes in 110 patients treated with cisplatin-based chemoradiotherapy and found that genetic polymorphisms with activity in intracellular detoxification (GSTP1), DNA repair (ERCC1, ERCC4, ERCC5, RAD51), and multidrug resistance-associated protein (ABCB1, ABCC1, ABCC2) affect drug toxicity in patients with head and neck who received platinum-based CRT [58]. Gene variants and haplotypes of ERCC1 were associated with the risk of developing hematologic toxicity.

Hiroyuki Shimomura et al. examined Non-SMC Condensin I Complex Subunit H (NCAPH) expression in OSCC and performed a functional analysis of human Oral Squamous Carcinoma Cells (OSCC) and found that resistance to cisplatin, carboplatin, and nedaplatin was enhanced by NCAPH in OSCC cells. NCAPH silencing combined with platinum decreased multidrug resistance [59]. There was no association between NCAPH and resistance to paclitaxel, docetaxel, and 5-fluorouracil.

Lot of studies are looking at potential of using circulating tumor cells and circulating tumor DNA to monitor for recurrence and evolution of treatment resistance.

3.8 Genomics and immunotherapies in HNSCC

Immunotherapy is a promising approach and seems to have added a new paradigm to several cancers including HNSCC. However, with currently available immune checkpoint inhibitors, the response rate is low, very few patients derive benefit, many patients fail to respond, some patients develop hyper-progressive disease and patients may develop immune related adverse events in an unpredicted fashion.

In 2016, FDA approved anti PD1 antibodies pembrolizumab and nivolumab [60, 61]. With establishment of nivolumab and pembrolizumab in the treatment of recurrent metastatic HNSCC, there are several studies looking at different ways to combine them with established treatments like surgery, radiation therapy and chemotherapy including cetuximab. These molecules are being tested in the neoadjuvant, concurrent and adjuvant therapeutic spaces in HNSCC. Ipilimumab an anti-CTLA-4 antibody which works well has been shown to reverse resistance to treatment in HNSCC. Ipilimumab given after cetuximab has been shown to reverse resistance to cetuximab. It has been observed that there is increased infiltration with Treg cells following exposure to cetuximab. Ipilimumab eliminates these Treg cells. Several trials are underway looking at combinations of ipilimumab, radiation and nivolumab.

Several genomic features may influence response to immune check point inhibitors [19]. High tumor mutational burden is associated with neoepitope presentation and immune hot phenotype leading to enhanced benefit with immune check point inhibitors. NSD1 inactivating mutations, global DNA hypomethylation, aneuploidy, may lead to impaired chemokine signaling and immune effector response leading to an immune cold phenotype and low benefit from immune checkpoint inhibitors.

3.9 Genomics and drug resistance

3.9.1 Co-relation between genomic alterations and drug resistance

Several studies have found association of drug resistance and genomic alterations listed below. This knowledge might help in selecting appropriate patients for chemotherapy/drugs including targeted drugs and avoiding un-necessary treatment in those who may not benefit from it [3].

Genomic marker	Therapy resistance
TP53	Cisplatin resistance
EGFR	Radiation resistance
CCND1	Gefitinib resistance
NOTCH1	PI3K inhibitor
MET	Resistance to Cetuximab, Erlotinib
PIK3CA	Bio-radiation with cetuximab, PI3K inhibitors MET inhibitors

3.10 Genomics and radiomics

Imaging including contrast enhanced CT scan, MRI scans and recently PET scans are commonly used to accurately stage the patient at diagnosis and also to monitor response and recurrence. Radiomics based on image texture analysis has the potential to provide valuable real time information about tumor biology and response/resistance to treatment. Studies are looking at correlation between radiomics and genomics. Group led by Kerstin Zwirner at Eberhard Karls university in Germany looked at genetic tumor profiles and radiomic features in 20 HNSCC patients treated with primary radio-chemotherapy [62]. They did NGS of the tumor and corresponding normal tissue and analyzed 327 genes. TP53, FAT1 and KMT2D were the most frequently mutated driver genes in their cohort. They found good correlation between reduced radiomic intra-tumor heterogeneity and somatic mutations in FAT1 with small tumor volumes. Radiomic features of heterogeneity did not correlate with somatic mutations in TP53 or KMT2D. Radiomics and genomics remain work under progress.

4. Head neck cancer genomics and newer targets, drugs and strategies in HNSCC

4.1 New targets, ways and drugs in HNSCC

In addition to focusing on common mutations, there are rare mutations with druggable targets worth exploring [6].

1. Rearrangement of Neutrotrophic Tropomyosin Receptor Kinase (NTRK) gene. NTRK 1,2 and 3 fusions are found in 3%, 1.6% and 3% of HNSCC in the AACR GENIE data set. Pan TRK inhibitor Larotrectinib is being tested in these patients.

2. HRAS

HRAS is a farnesyl transferase substrate depending exclusively on farnesylation. HRAS mutations have been found in 4% of HNSCC patients in the GENIE data set. Tipifarnib which is highly selective inhibitor of farnesyl transferase is being tested in HRAS mutated HNSCC.

3. Antibody Drug Conjugate (ADC) are monoclonal antibodies conjugated to cytotoxic agents. Antibody targets a particular cell surface protein and the drug payload is delivered inside the cell. Several ADCs are being tested in HNSCC including ABBV-221, AVID100 which target EGFR, BAY1129980

targeting C4.4a, IMMU-132 which targets TROP-2 antigen and tisotumab vedotin targeting human tissue factor.

4. DNA damage repair. DNA damage response (DDR) pathway is a druggable target. The most glaring example is PARP inhibitors in BRCA1/2 mutated cancers. About 8% of HNSCC cases have alterations in the DDR related genes. There are several DDR pathway inhibitors targeting DNA damage signaling proteins like ATM, ATR, DNA-PK, WEE1, CHK1 and 2.
5. Tumor Mutational Burden (TMB). High tumor mutational burden is associated with increased expression of tumor specific antigens on the cancer cell surface making the cancer more immunogenic. About 25% HNSCC patients have high TMB having >20 mutations per mega-base of DNA making them susceptible to immunotherapy.
6. Dynamic Monitoring of tumor using ctDNA. As the cancer clinically evolves, it's genomic and molecular landscape changes. ctDNA are short fragments of double stranded DNA shed in the blood by the tumor undergoing necrosis and apoptosis. ctDNA may have mutational profile not seen in the normal cells and could represent the changing genomic landscape of tumor in vivo. Serial monitoring of ctDNA could help in detecting early relapse and help appropriately matched therapies to be delivered in real time.
7. FGFR2 and FGFR3 fusion occurs in 1-3% of HNSCC. These patients might benefit by FGFR inhibitors.

Several targeted drugs are being tested in clinical trials. Exhaustive review of these are beyond the scope of this chapter.

4.2 Molecular co-targeting strategies

HNSCC are genetically highly heterogenous. Monotherapies with targeted therapies yield modest benefit with eventual development of resistance. So, combining two or more molecularly targeted agents might emerge as effective therapy.

There could be several ways to do this; (1) targeting molecules within convergent signaling pathways, (2) targeting molecules with non-overlapping mechanisms of action, (3) targeting anti-tumorigenic molecules working synergistically with conventional chemotherapy or radiation therapy. Several clinical trials evaluating this strategy are listed below (**Table 4**) [5].

4.3 Molecular tumor boards in HNSCC

Most HNSCC will have complex genomes making it difficult to select therapy. This might not be correlating with traditional risk factors. There could be multiple driver mutations in a case or part of a tumor. E. g. a patient might be NOTCH1/PIK3CA double mutant. The question could be should this patient receive a WNT pathway inhibitor or PIK3CA inhibitor or both? The treating Head neck cancer clinician will have to document the genomic data, use of targeted drugs and record longitudinal follow up of each case to further develop use of NGS data in the clinic.

Multidisciplinary involvement of head neck surgeons, geneticists, medical oncologists, radiation oncologists, translational biologists will be integral to formulate personalized treatment approaches in head neck cancers.

Clinical trial	Intervention
NCT02124850	Cetuximab + motolimod + Nivolumab
NCT01218048	cetuximab + surgical resection + adjuvant cisplatin, carboplatin, radiation
NCT02277197	ficlatuzumab + Cetuximab
NCT 0957853	Cetuximab + anti IgG1 antibody + surgical resection
NCT 3153982	Ruxolitinib + Surgical resection
NCT02035527	Raf inhibitor + Doectaxel + cisplatin
NCT01051791	Everolimus
NCT01588431	Bevacizumab + Cetuximab + Docetaxel + Cisplatin followed by radiation/surgical resection
NCT02769520	Pembrolizumab
NCT01316757	Erlotinib + Cetuximab + paclitaxel + carboplatin
NCT01016769	Temsirolimus + Paclitaxel + Carboplatin
NCT02741570	nivolumab + ipilimumab + cetuximab + cisplatin + carboplatin +5fluracil
NCT02952586	Avelumab + cisplatin + radiation
NCT02499120	Cetuximab + Palbociclib

Table 4.
Clinical trials evaluating molecular co-targeting strategies.

European Society for Medical Oncology (ESMO) has a designed a scale (ESCAT) to guide the clinician to select a novel targeted drug with highest potential of efficacy in an HNSCC patient. The most Accordingly, compelling actionable molecular alterations in HNSCC included HRAS activating mutations (tipifarnib, farnesyl transferase inhibitor), MSI, high TMB (for immune check point inhibitors), NTRK fusions (TRK tyrosine kinase inhibitors), CDKN2A inactivating alterations (CDK 4/6 inhibitors) and EGFR amplification (afatinib) [63].

4.4 Big data in HNSCC

Big data approach is being explored in head neck oncology integrating data generated from genomic studies, radiomic data generated from CT, MRI and PET scans, data generated from clinical evaluation and optical imaging, data generated from radiation therapy response and toxicity and integration of all other non-genetic data such as epidemiology, diet, habits, stress, socioeconomic factors etc. There is a multicentric study BD2Decide (Big Data Models for personalized head neck cancer decision support) going on to explore Big Data approach. Three main goals for Big Data in HNSCC will be 1) support and augment clinical decisions, 2) generate new knowledge, 3) develop guidelines for HNSCC prevention and management [64].

5. Conclusions

HNSCC is genomically unstable. TCGA has identified key alterations in tumor suppressor genes and oncogenes in HNSCC. TP53 alteration is a key player in HNSCC tumorigenesis and biology. Principally loss of tumor suppressor drives tumorigenesis than oncogene addiction in HNSCC, however, a small subset of oral

cavity cancer may be driven by mutations rather than loss of tumor suppressor gene function. Tumor heterogeneity is prominent in HNSCC and is a major challenge in developing effective therapies. Biologic classifier of HNSCC remains to be implemented in the clinic. Clustering of HNSCC according to multi-omics studies may be more clinically meaningful. Immune therapy is a major treatment paradigm in oncology in general including HNSCC. Corelative genomics and immune contexture will help realize the full potential of this approach. Sufficient indication exists linking major genomic alterations in HNSCC and clinical behavior including performance of conventional treatments. Opportunity exists to leverage this knowledge to fine tune currently existing surgical, radiation and chemotherapeutic approaches. EGFR targeting remains important in HNSCC in spite of lack of predictive biomarker and eventual treatment resistance. Mutli-omics studies have shed light on resistance to EGFR targeting and novel ways to target EGFR axis. Several studies are addressing genomically targeted monotherapies, molecular co targeting strategies and ways to escalate and deescalate treatment intensity based on biology. Time has come to implement molecular tumor boards in HNSCC regularly. BIG data approach will certainly help design multi-pronged approach to control HNSCC globally. Tissue repositories, participation in clinical trials and multi-institutional collaboration remains critical to further progress.

Conflict of interest

None.

Author details

Anand B. Pathak* and Satyam Satyarthi

Department of Medical Oncology, National Cancer Institute, Nagpur, India

*Address all correspondence to: cancercarengp@gmail.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Hyuna Sung, Jacques Ferlay, Rebecca L Siegel: Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021 May;71(3):209-249. doi: 10.3322/caac.21660
- [2] Nature vol 517, 29 Jan 2015, 576-581
- [3] Xiaohua Jiang, Jing Ye, Zhihui Dong : Novel genetic alterations and their impact on target therapy response in head and neck squamous cell carcinoma. *Cancer Management and Research* 2019;11 1321-1336. Doi:10.2147/CMAR.5187780
- [4] Zhi-Li Zhao, Lu Zhang, Cong-Fa Huang: NOTCH1 inhibition enhances the efficacy of conventional chemotherapeutic agents by targeting head neck cancer stem cell *Scientific Reports* volume 6, Article number: 24704 (2016)
- [5] (J D Kemmer, D E Johnson, J R Grandis. Leveraging Genomics for head and neck cancer treatment. *Journal of Dental Research* 2018, vol 97(6) 603-613). Doi:10.1177/0022034518756352
- [6] Eoghan Malone and Lillian L Siu : Precision Medicine in Head and Neck Cancer: Myth or Reality? *Clinical Medicine Insights: Oncology* Volume 12: 1-7. DOI: 10.1177/117955491877958
- [7] David M. Vossena, Caroline V.M. Verhagena, Marcel Verheij : Comparative Genomic Analysis of oral versus laryngeal and pharyngeal cancer. *Oral Oncology* 81(2018) 35-44
- [8] Luc G T Morris, Raghu Chandramohan, Lyndsay West : The Molecular Landscape of recurrent and metastatic head neck cancers: Insights from a precision oncology sequencing platform. *JAMA Oncol* 3(2): 244-255
- [9] Serge J. Smeets, Ruud H. Brakenhoff, Bauke Ylstra : Genetic classification of oral and oropharyngeal carcinomas identifies subgroups with a different prognosis *Cell Oncol* 2009;31(4): 291-300
- [10] Swanton C.: Intratumor heterogeneity through space and time. *Cancer Res* 2012;73(19):4875-4882
- [11] Chung CH, Parker JS, Karaca G : Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell.* 2004; 5(5):489-500
- [12] Walter V, Yin X, Wilkerson MD : Molecular subtypes in head and neck cancer exhibit distinct patterns of chromosomal gain and loss of canonical cancer genes. *PLoS One.* 2013; 8(2): e56823
- [13] Chen Huang, Lijun Chen, Sara R Savage : Proteogenomic Insights into the biology and treatment of HPV negative head and neck squamous cell carcinoma. 2021. *Cancer Cell* 361-379. Doi:10.1016/j.ccell.2020.12.007
- [14] Thorsson, V.; Gibbs, D.L.; Brown, S.D : The Immune Landscape of Cancer. *Immunity* 2018, 48, 812-830 e814
- [15] Tamborero, D.; Rubio-Perez, C.; Muinos, F. : A Pan-cancer Landscape of Interactions between Solid Tumors and Infiltrating Immune Cell Populations. *Clin. Cancer Res.* 2018, 24, 3717-3728
- [16] Charles Schutt, Hua Sun, Jaya Sarin Pradhan : Genomic and neoantigen evolution from primary tumor to first metastases in head and neck squamous cell carcinoma. *Oncotarget*, 2021, vol.12(No.6) pp:534-548
- [17] Yao Yao, Zhongyi Yan, Senlin Lian: Prognostic Value of novel immune related genomic biomarkers identified

in head and neck squamous cell carcinoma. *J Immunother Cancer* 2020;8:e000444. Doi:10.1136/jitc-2019-000444

[18] Y.-P. Chen, Y.-Q. Wang, J.-W. Lv : Identification and validation of novel microenvironment-based immune molecular subgroups of head and neck squamous cell carcinoma: implications for immunotherapy. *Annals of Oncology* 30: 68-75, 2019 doi:10.1093/annonc/mdy470

[19] Bohai Feng and Jochen Hess : Immune-related mutational landscape and gene signatures: Prognostic value and therapeutic impact for head and neck cancer. *Cancers*2021,18,1162. DOI.org/10.3390/cancers13051162

[20] Hyung Kwon Byeon, Minhee Ku, Jaemoon Yang : Beyond EGFR Inhibition: multilateral combat strategies to stop the progression of head and neck cancer. *Experimental & Molecular Medicine* 2019, 51:8 doi: 10.1038/s12276-018-0202-2

[21] James A. Bonner, Paul M. Harari, Jordi Giralt: Radiotherapy plus Cetuximab for Squamous-Cell Carcinoma of the Head and Neck *N Engl J Med* 2006; 354:567-578. DOI: 10.1056/NEJMoa053422) 10.1056/NEJMoa053422)

[22] Jean-Pascal H Machiels, Robert I Haddad, Jérôme Fayette : Afatinib versus methotrexate as second-line treatment in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck progressing on or after platinum-based therapy (LUX-Head & Neck 1): an open-label, randomised phase 3 trial. *Lancet Oncol* 2015; 16: 583-94. doi.org/10.1016/S1470-2045(15)70124-5

[23] Ha PK, Benoit NE, Yochem R : A transcriptional progression model for head and neck cancer. *Clin Cancer Res* 2003;9:3058-3064

[24] Cromer A, Carles A, Millon R : Identification of genes associated with tumorigenesis and metastatic potential of hypopharyngeal cancer by microarray analysis. *Oncogene* 2004;23: 2484-2498

[25] Roepman P, Wessels LF, Kettelarij N: An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nat Genet* 2005;37:182-186.

[26] Georgia Karpothiou, Marie-Laurie Stachowitz, Jean Marc Dumollard: Gene Expression comparison between the primary tumor and it's lymph node metastases in Head Neck Squamous cell carcinoma, a pilot Study. *Cancer Genomics and Proteomics* 16:155-161 (2019)

[27] Jose P. Zevallos, Angela L. Mazul Vonn Walter: Gene Expression Subtype predicts nodal metastases and survival in HPV negative head neck cancer. *Laryngoscope* 2019 January; 129(1): 154-161

[28] Ilda Patrícia Ribeiro, Francisco Caramelo, Luísa Esteves : Genomic predictive model for recurrence and metastasis development in head and neck squamous cell carcinoma patients *Scientific reports* | 7: 13897 | DOI:10.1038/s41598-017-14377-x

[29] Juncheng Wang, Xun Chen, Yuxi Tian : Six-gene signature for predicting survival in patients with head and neck squamous cell carcinoma January 2020 *Aging* 12(1) DOI:10.18632/aging.102655

[30] Ram Bhupal Reddy, Samanta S. Khora, Amritha Suresh : Molecular prognosticators in clinically and pathologically distinct cohorts of head and neck squamous cell carcinoma—A meta-analysis approach *PLoS ONE* 14(7): e0218989

[31] Antoine Galmiche, Zuzana Saidak, Jebrane Bouaoud : Genomics and

Precision Surgery for head neck squamous cell carcinoma. *CanLet* 481: 1July2020, 45-54

[32] Biswas NK, Das C, Das S, Maitra A, Nair S, Gupta T : Lymph node metastasis in oral cancer is strongly associated with chromosomal instability and DNA repair defects. *Int J Cancer* 2019;145: 2568-2579.

[33] van Hooff SR, Leusink FK, Roepman P : Validation of a gene expression signature for assessment of lymph node metastasis in oral squamous cell carcinoma. *J Clin Oncol.* 2012;30: 4104-4110.

[34] Zhang L, Poh CF, Williams M, Laronde DM : Loss of heterozygosity (LOH) profiles--validated risk predictors for progression to oral cancer. *Cancer Prev Res (Phil)*. 2012;5: 1081-1089

[35] Goddard ET, Bozic I, Riddell SR, Ghajar CM. Dormant tumour cells, their niches and the influence of immunity. *Nat Cell Biol.* 2018;20:1240-1249.

[36] Wang W, Lim WK, Leong HS : An eleven gene molecular signature for extra-capsular spread in oral squamous cell carcinoma serves as a prognosticator of outcome in patients without nodal metastases. *Oral Oncol.* 2015;51:355-362

[37] Saidak Z, Clatot F, Chatelain D, Galmiche A : A gene expression profile associated with perineural invasion identifies a subset of HNSCC at risk of post-surgical recurrence. *Oral Oncol.* 2018;86:53-60.

[38] Saidak Z, Pascual C, Bouaoud J : A three-gene expression signature associated with positive surgical margins in tongue squamous cell carcinomas: Predicting surgical resectability from tumour biology? *Oral Oncol.* 2019;94:115-120

[39] Hayashi M, Wu G, Roh JL, Chang X : Correlation of gene methylation in

surgical margin imprints with locoregional recurrence in head and neck squamous cell carcinoma. *Cancer* 2015;121:1957-1965.

[40] Liu SA, Wang CC, Jiang RS, Wang WY, Lin JC : Genetic analysis of surgical margins in oral cavity cancer. *Br J Surg.* 2018;105:e142-e149

[41] Ogbureke KU, Weinberger PM, Looney SW, Li L, Fisher LW : Expressions of matrix metalloproteinase-9 (MMP-9), dentin sialophosphoprotein (DSPP), and osteopontin (OPN) at histologically negative surgical margins may predict recurrence of oral squamous cell carcinoma. *Oncotarget* 2012;3:286-298

[42] Park J, Kim HJ, Kim KR, Lee SK, Kim H, Park KK, Chung WY : Loss of RUNX3 expression inhibits bone invasion of oral squamous cell carcinoma. *Oncotarget* 2017;8: 9079-9092

[43] Hanna GJ, Adkins DR, Zolkind P, Uppaluri R : Rationale for neoadjuvant immunotherapy in head and neck squamous cell carcinoma. *Oral Oncol.* 2017;73:65-69

[44] Uppaluri R, Zolkind P, Lin T, Nussenbaum B, Jackson RS : Neoadjuvant pembrolizumab in surgically resectable, locally advanced HPV negative head and neck squamous cell carcinoma (HNSCC). *J Clin Oncol* 2017;35(suppl.):6012.

[45] Horton JD, Knochelmann H, Kent Armeson : Neoadjuvant presurgical PD-1 inhibition in oral cavity squamous cell carcinoma. *J Clin Oncol* 2019; 37(suppl):2574.

[46] Zuur CL, Elbers JBW, Vos JL : Feasibility and toxicity of neoadjuvant nivolumab with or without ipilimumab prior to extensive (salvage) surgery in patients with advanced head and neck cancer (the IMCISION trial,

- NCT03003637). *J Clin Oncol* 2019;37 (Suppl):2575.
- [47] Saidak Z, Clatot F, Chatelain D, Galmiche A : A gene expression profile associated with perineural invasion identifies a subset of HNSCC at risk of post-surgical recurrence. *Oral Oncol.* 2018;86:53-60.
- [48] Mroz EA, Patel KB, Rocco JW : Intratumor heterogeneity could inform the use and type of postoperative adjuvant therapy in patients with head and neck squamous cell carcinoma. *Cancer* 2020
- [49] Davide B. Gissi, Achille Tarsitano, Andrea Gabusi : 13-gene DNA Methylation Analysis from Oral Brushing: A Promising Non Invasive Tool in the Follow-up of Oral Cancer Patients. *J. Clin. Med.* 2019, 8, 2107; doi:10.3390/jcm8122107
- [50] Javier F. Torres-Roca, Steven Eschrich Haiyan Zhao : Prediction of Radiation Sensitivity using a gene expression classifier. *Cancer Res* 2005; 65: (16). August 15, 2005.
- [51] Jimmy Pramana, Michiel W M Van Den Brekel, Marie-Louise F van Velthuysen : Gene expression profiling to predict outcome after chemoradiation in head and neck cancer. *Inj J Radiat Oncol Biol Phys* 2007 Dec 1;69(5):1544-1552 Doi: 10.1016/j.ijrobp.2007.08.032.
- [52] Esrich SA, Praana J, Zhang H et : A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. *Int J Radiat Oncol Biol Phys* 2009; 75: 489-496. PubMed: 19735873
- [53] Jacob G Scott, Anders Berglund, Michael J Schell : A genome-based model for adjusting radiotherapy dose(GARD): a retrospective, cohort-based study. *Lancet Oncol.* 2017 Feb: 18(2): 202-211. Doi: 10.1016/S1470-2045 (16)30648-9
- [54] Toustrup K, Sorensen BS, Nordmark M, et al : Development of a hypoxia gene expression classifier with predictive impact for hypoxic modification of radiotherapy in head and neck cancer. *Cancer Res* 2011; 71: 5923-5931. PubMed: 21846821
- [55] Whitney Sumner, Xenia Ray, Leisa Sutton : Gene Alterations as predictors of radiation-induced toxicity in head and neck squamous cell carcinoma. *J Transl Med* 2021; 19: 212. Doi.10.1186/s12967-021 -02876-5
- [56] M. Giefing, M. Wierzbicka, K. Szyfter : Moving towards personalised therapy in head and neck squamous cell carcinoma through analysis of next generation sequencing data. *European Journal of Cancer* 55 (2016) 147e157. <http://dx.doi.org/10.1016/j.ejca.2015.10.0700>
- [57] Sanne R. Martens-de Kemp, Arjen Brink, Ida H. van der Meulen : FA/BRCA pathway identified as the major predictor of cisplatin response in head neck cancer by functional genomics. *Mol Cancer Ther*; 16(3) March 2017
- [58] Goretti Duran, Santiago Aguin, Raquel Cruz : Association of GSTP1 and ERCC1 polymorphisms with toxicity in locally advanced head and neck cancer platinum-based chemoradiotherapy treatment *Head & Neck.* 2019;1-12 DOI: 10.1002/hed.25754
- [59] Hiroyuki Shimomura, Tomonori Sasahira, Chie Nakashima : Non-SMC Condensin I Complex Subunit H (NCAPH) Is Associated with Lymphangiogenesis and Drug Resistance in Oral Squamous Cell Carcinoma. *J. Clin. Med.* 2020, 9, 72; doi:10.3390/jcm9010072
- [60] Barbara Burtneß¹, Kevin J Harrington², Richard Greil:

Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet* 2019 Nov 23;394(10212):1915-1928. doi: 10.1016/S0140-6736(19)32591-7

[61] R.L. Ferris, G. Blumenschein, Jr., J. Fayette: Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. *N Engl J Med.* 2016 Nov 10; 375(19): 1856-1867. PMID: 27718784

[62] Kerstin Zwirner, Franz J. Hilke, German Demidov: Radiogenomics in head and neck cancer: correlation of radiomic heterogeneity and somatic mutations in TP53, FAT1 and KMT2D 2. *Strahlentherapie und Onkologie* volume 195, pages771-779 (2019)

[63] Grégoire Marret, Ivan Bièche, Célia Dupain : Genomic Alterations in Head and Neck Squamous Cell Carcinoma: Level of Evidence According to ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) *JCO Precision Oncology* no. 5 (2021) 215-226.

[64] Carlo Restoghini, Analisa Trama, Elio Borgonovi.: Big Data in head and neck cancer. *Curr. Treat. Options in Oncol* (2018) 19:62