Genomics and Advances Towards Precision Medicine for Head and Neck Squamous Cell Carcinoma

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Objective: To provide a review of emerging knowledge from genomics and related basic science, preclinical, and clinical precision medicine studies in head and neck squamous cell carcinoma (HNSCC).

Data Sources: The Cancer Genome Atlas Network (TCGA) publications, PubMed-based literature review, and ClinicalTrials.gov.

Review Methods: TCGA publications, PubMed, and ClinicalTrials.gov were queried for genomics and related basic science, preclinical, and developmental clinical precision medicine studies in HNSCC.

Results: TCGA reported comprehensive genomic analyses of 279 HNSCC, defining the landscape and frequency of chromosomal copy number alterations, mutations, and expressed genes that contribute to pathogenesis, prognosis, and resistance to therapy. This provides a road map for basic science and preclinical studies to identify key pathways in cancer and cells of the tumor microenvironment affected by these alterations, and candidate targets for new small molecule and biologic therapies.

Conclusion: Recurrent chromosomal abnormalities, mutations, and expression of genes affecting HNSCC subsets are associated with differences in prognosis, and define molecules, pathways, and deregulated immune responses as candidates for therapy. Activity of molecularly targeted agents appears to be enhanced by rational combinations of these agents and standard therapies targeting the complex alterations that affect multiple pathways and mechanisms in HNSCC.

Key Words: Clinical Trials, Genomics, Head and Neck Cancer, Molecular Targeted Therapy.

Level of Evidence: NA.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the most prevalent cancer arising from the epithelium of the upper aerodigestive tract, including the oral cavity, pharynx and larynx, and their anatomic subsites. Each year, HNSCC is diagnosed in over 50,000 Americans and 600,000 people worldwide.[1] Nearly half die of their disease, and survivors often suffer significant morbidity affecting voice, speech, taste, dental health, and swallowing. Tobacco, alcohol, betel nut, and other chemical carcinogens are implicated in the etiology of a majority of these cancers, while increasing incidence of human papilloma virus positive (HPV+) HNSCC with a better prognosis has been observed in developing countries. Additionally, a small but important subset of HNSCC arise in younger patients due to hereditary disorders affecting DNA repair.

The Cancer Genome Atlas and other large-scale genomics studies made possible by recent advances in technology have defined the broader landscape and frequency of chromosomal alterations, mutations, and expressed genes that contribute to pathogenesis, prognosis and resistance of HNSCC to therapy.[2–7] These findings and technologies are increasingly being incorporated in preclinical and clinical studies together with a rapidly expanding armamentarium of molecularly targeted small molecules and biologics such as humanized antibodies. In this review, we summarize salient findings from recent TCGA and other large-scale genomics studies, and selected preclinical and clinical studies that are exploring the potential of these discoveries to improve therapy of HNSCC. Frequent events affect cell cycle, death, and growth pathways in major subsets. Among important questions being raised, what are the implications and potential of these genomic alterations to define prognosis and strategies for prevention and therapy? Do cancers with gene mutations versus differences in gene dosage (copy number) and expression of a druggable target differ in therapeutic sensitivity? What other concurrent alterations promote resistance, and can combined therapies overcome these mechanisms of resistance?

MATERIALS AND METHODS

Study Methods

TCGA publications, PubMed, and ClinicalTrials.gov were queried for large-scale genomics and commonly altered target-related basic science, preclinical, and developmental clinical precision medicine studies in HNSCC. As most clinical studies

are phase I safety and pharmacology studies and few were found have reached the stage of phase II/III clinical trial identified through evidence based search methodology using PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses),[8] we performed a TCGA publication and PubMed review of the literature for large-scale genomics studies of HNSCC; preclinical studies targeting corresponding molecules or pathways; and ClinicalTrials.gov review of phase I-III studies available. Participants, Interventions, Comparisons, Outcomes, and Study design (PICOS) criteria were utilized in weighing studies to include. Studies were prioritized where the population included patients with tumors with histologically confirmed HNSCC, and preclinical studies using genotype confirmed HNSCC-derived genotyped cell lines, xenografts or patient-derived tumor xenografts. Genomic tissue and cell lines are predominantly derived from studies where interventions are biased towards surgical patients from whom sufficient tumor was available for macro dissection for enrichment of malignant tumor tissue for genomic studies or cell culture. As a result, TCGA and other studies are enriched and biased towards sampling of HPV(-) cases from oral cavity and laryngeal sites, relative to HPV-enriched cases from oropharynx, or nasopharynx often treated by chemoradiotherapy. Comparison for mutations in coding regions of genes, or copy number and expression is possible for studies that included whole exome sequencing, comparative genomic hybridization (CGH), and expression profiling. Statistics-driven bioinformatic analyses for significant genomic mutations and copy alterations, expression data, and multi-platform comparisons and integrative analyses of these platforms are limited to TCGA and a smaller number of large-scale HNSCC studies utilizing common tools. Outcomes data including overall, progression free, or disease-specific survival for genomics studies is of variable quality, but is included where analyses and statistically significant or near-significant trends are highlighted.

Search Methods

Articles for TCGA and large-scale genomic studies, reviews comparing TCGA and prior genomics studies, and related preclinical and clinical studies for HNSCC from January 1, 2010 to April 10, 2017 was performed using PubMed and public databases. Key words included head, neck squamous, genomics, RNA expression, proteomics studies including more than 30 tumors; related terms for basic, preclinical and clinical studies of components or multiple components of pathways altered in ≥5% of tumors.

RESULTS

The Cancer Genome Atlas and Other Large-Scale Genomics Studies Identify Genes and Chromosomal Regions Linked to Pathogenesis of HNSCC

The advent and improvements in massively parallel (next generation) sequencing and other array based methods during the past decade have made possible the comprehensive multi-platform TCGA and several large-scale studies defining the landscape of genomic and epigenetic alterations in HNSCC.[2–6,9] Globally, most HNSCC harbor complex chromosomal rearrangements, among which there are common and less frequent recurrent alterations that have been associated with HPV status, malignant phenotype, outcome, and certain gene(s) that are of potential clinical significance. More common

recurrent chromosomal alterations characterized by both classical karyotyping studies and TCGA, and possible mechanisms for their occurrence and selection during carcinogenesis have been recently reviewed.[10] Overall, HNSCC also display relatively frequent DNA mutations, similar to other cancers associated with more mutagenic carcinogens and/or defects in DNA repair.[2] Higher mutation frequency is considered a factor in generation of neo-antigens necessary for immune recognition and response to new immune checkpoint therapies. In addition to CpG transversions commonly attributed to tobacco carcinogen DNA adducts expected in HPV(-) tumors, HPV (-) and (+) HNSCC also display mutations within TpC dinucleotides linked to deregulation of APOBEC cytosine deaminases that repair altered bases.[3] These mutations affect certain motifs of relevance to recurrent mutation "hotspots" that can enhance function and potential sensitivity to therapeutics targeting particular oncogenic signal kinases. Rare hereditary mutations or deletions that affect genes involved in DNA repair of chromosomal breakage (Fanconi Anemia-BRCA pathway genes), telomerase maintenance of chromosomal ends (*TERC*, *TERT* genes), and mitotic cell division (*MYH9*) have been linked to increased risk of HNSCC with chromosomal derangements in younger patients.[11–13]

Key to understanding the similarities and differences in pathogenesis between HPV(+) and HPV(-) HNSCC are the different ways the cell cycle is deregulated to lead to uncontrolled proliferation, accumulation of chromosomal rearrangements and mutations, and malignant progression.[3] In HPV(+) HNSCC, early genes *E6* and *E7* encode oncoproteins that promote degradation of tumor suppressors TP53 and RB1, respectively, unleashing their braking effect that keeps cells from progressing from the quiescent G0 state into G1 and later phases of the cell cycle. HPV(+) cancers also exhibit amplification of *E2F1*, the transcription factor that promotes G1 cell cycle genes. In HPV(-) HNSCC, TCGA studies reveal that ∼84% of cancers have mutations of *TP53*, and a variety of upstream alterations that cumulatively can inhibit RB1 to accomplish a similar result. Essential among these are inactivation of the cyclin dependent kinase *CDKN2A*, which occurs by chromosome 9p21 deletion, gene mutation, or methylation in nearly all HPV(-) HNSCC. In contrast to HPV(-) tumors, HPV(+) HNSCC overexpress the *CDKN2A* encoded 16 kDa protein p16, which has been found to be a sensitive and relatively specific clinical-pathologic immunohistochemical marker for HPV status and better prognosis.[14] Also common are amplifications or transcriptional activation of cyclin *CCND1*, *CDK6* and *MYC* that promote proliferation. Thus, virtually 100% of HNSCC have viral or critical endogenous gene alterations affecting the cell cycle.

In addition to proliferation and clonal expansion of cells initiated by these alterations, the inactivation of TP53 by mutation or HPV affects its role in repair of DNA damage and as guardian of genomic integrity. TCGA revealed that most HNSCC harbor complex genomic alterations of varying severity that alone or together with other copy alterations and mutations are emerging as subtypes of potential prognostic and therapeutic significance. Dominating these are concurrent chromosome 3p arm deletions

and 3q arm amplifications, which are linked with worse prognosis, and respectively harbor several candidate tumor suppressor and oncogenes.[3] Among these, the 3q amplicon includes PI3-Kinase Catalytic subunit Alpha gene, *PIK3CA*, which is also the most frequently mutated oncogene in HNSCC. *PIK3CA* is co-amplified with and has been linked to enhancing the expression of 3q stemness gene *SOX2*.[15] PI3K also promotes preferential expression of an oncogenic ΔNp63 isoform of *TP63* encoded on 3q, involved in squamous differentiation.[16] Together, *PIK3CA* is amplified or mutated in ~34% of HPV(-) and 56% of HPV(+) TCGA HNSCC tumors, implicating the PI3K pathway in promoting growth factor dependent or independent growth, and common resistance to EGFR therapies. Consistent with this, smaller subsets harbor mutations or decreased expression of PI3K suppressors (*PTEN*, *PIK3R1*), or amplifications or mutations of growth factor receptor tyrosine kinases known to activate PI3K signaling, including *EGFR* (15%), *FGFR1* (10%), *ERBB2* (5%), *IGF1R* (4%), *EPHA2* (4%), *FGFR2*, 3 (2% each), *MET* (2%).[3] Among these, the 8p11 focal amplification harboring *FGFR1* also contains *WHSC1L1*, recently characterized to encode methylase NSD3 that modulates EGFR function and proliferation in HNSCC.[17] Overall, over 60% of HPV(-/+) HNSCC harbored alterations in growth factor receptor and PI3K signaling, making this pathway an important target for developmental therapeutics.

TCGA revealed that ~30% of HPV(-) HNSCC display amplification of 11q13, associated with a worse prognosis subtype, that has a median survival of less than 2 years.[3] This amplification incorporates several genes of potential biologic and therapeutic relevance, including cyclin D1 (*CCND1*) and Fas Associated Death Domain (*FADD*). *CCND1* has long been implicated in promoting deregulated G1 cell cycle progression and assumed to be the driver oncogene within this amplicon.[18] Consistent with this, most tumors with this amplification lack other alterations in upstream growth factor receptor, RAS or PI3K kinases,[3] although some with both may help explain resistance to agents targeting these upstream pathways. Interestingly, while FADD protein was originally shown to mediate cell death as part of Tumor Necrosis Factor Receptor (TNFR) complex, it has also recently been shown to play another role in promoting cell proliferation during G2/M cell division.[19] Further, its death function may be blocked by Inhibitor of Apoptosis (IAP) proteins, which are encoded by *BIRC2/3* genes, located in an adjacent co-amplification of 11q22, seen in ~8% of HNSCC. TCGA and other studies also uncovered inactivating mutations of another TNFR complex cell death mediator, caspase-8 (*CASP8*). Mutant *CASP8* is found in ~11% of HPV(-) tumors, mutually exclusive of amplification of *FADD*. Tumors with *CASP8* mutations often have activating mutations of *HRAS* or *PIK3CA*, but few other copy alterations, or *TP53* mutations. As such, these tumors appear to represent rarer but predominantly mutation-type driven subset of HNSCC. Intriguingly, a subset of ~22% of HPV(+) tumors have 14q32.32 deletions or inactivating mutations of TNFR Associated Factor (*TRAF3*), implicated in suppressing survival of myeloid cancers and cells

infected with other DNA viruses. Recently, the HPV(+) HNSCC subset with loss of *TRAF3* or *CYLD* has been associated with episomal HPV infection and better prognosis, distinguishing these from the subset with predominantly *PIK3CA* alterations and HPV integration.[20] Together, *FADD*, *BIRC2/3*, *CASP8*, *TRAF3*, and *CYLD* alter pathways that can lead to activation of proto-oncogene transcription factors Nuclear Factor-kappaB/REL that promote genes involved in cell survival, proliferation, angiogenesis, and aberrant inflammation and immunity.[21] Overall, ~44% of HPV(-) and 31% of HPV(+) HNSCC revealed alterations in cell death/survival and NF-κB pathways in immune recognition determinants HLA-A/B an B2M were also seen ~10% of HNSCC, consistent with mechanisms implicated in escape from immune-mediated cell death.[3]

Early exome sequencing and TCGA studies highlighted novel mutations predicted to inactivate *NOTCH1*,[3-5] encoding a transmembrane signal receptor whose ligand binding and cleavage produces a nuclear transcription factor that promotes a program of genes important in squamous differentiation. Mechanistic studies suggest the overexpression of ΔNp63 enhanced by PI3K on 3q may also suppress NOTCH expression and tumor suppressor function.[22,23] Tumors with loss of *NOTCH1* are more poorly differentiated and have been associated with worse prognosis.[24] Inactivation of *NOTCH* and genes *FAT1* and *AJUBA* may also converge on WNT-β-catenin signaling to affect cell differentiation. Overall, ~64% of HPV(-) and 44% of HPV(+) HNSCC have alterations in differentiation signaling pathway components. Finally, ~22% of HPV(-) tumors displayed defects in the KEAP1-CUL3-NFE2L2 components of the oxidative stress and damage pathway.

Preclinical and Clinical Studies Elucidating Potential Therapeutic Significance of Genomic Alterations in HNSCC

These studies underscored and clarified the extent to which HPV(-) and (+) cancers differ in mutational and chromosomal alterations that relate to their pathogenesis, and potential for new therapeutic approaches. Major subsets with alterations in key pathways and frequent mutations have raised interest in the potential of targeting PI3K-mTOR, MEK-MAPK, cell cycle, and BIRC/IAPs, in HNSCC (Fig. 1).

PI3K-AKT-mTOR

The PI3K-AKT-mTOR signaling pathway is a vital pathway for growth, survival, and metabolism for which there is evidence of frequent functional signal activation in HNSCC cell lines and tumors.[25-29] Genetic aberrations such as CNAs, mutations, and dysregulation of mRNA are prevalent. A number of first generation PI3K and mTOR inhibitors (Rapamycin, Temsorlimus, Everolimus) have shown activity in in vivo xenograft models.[30,31] Rapamycin has been shown to prevent tumorigenesis and suppress SCC-15 cells by inhibiting mTOR phosphorylation.[32] In a recent study, cell lines with *PIK3CA* mutations were sensitive to PI3K pathway inhibitors, whereas

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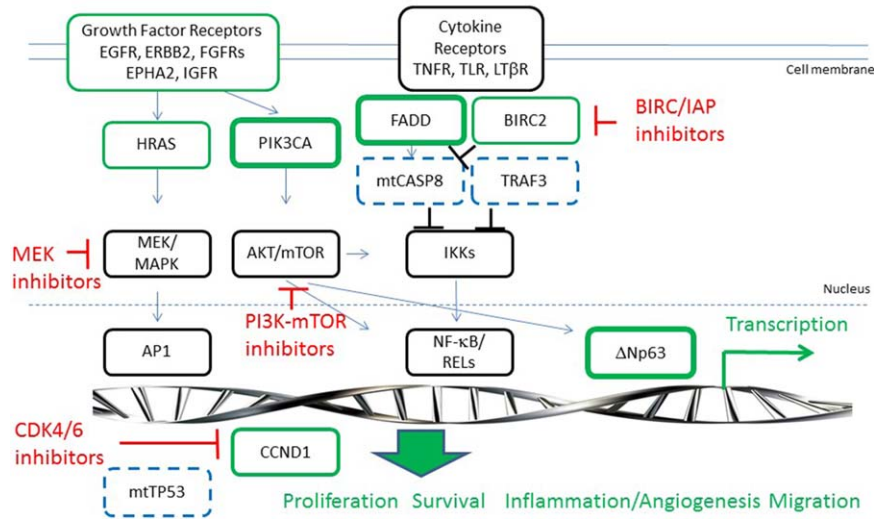
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Fig. 1. Frequent genomic alterations, pathways and targeted therapeutics under investigation in HNSCC. Genomic amplification or activating mutations of oncogenes (green) and inactivating alterations affecting tumor suppressors (blue dashed) affect key signal pathways and transcription factors that regulate the cancer gene program and phenotype. MEK/MAPK, PI3K-mTOR, FADD/BIRC and CDK4/6-CCND1 represent potential targets for therapy.

amplification status or proteomic profiling did not predict sensitivity.[29] Our studies showed that cell lines with *PIK3CA* amplifications or mutations were sensitive to dual PI3K-mTOR inhibitors, in association with inducible TP53, or AKT phosphorylation.[33,34] Evidence of pharmacodynamic inhibition of signaling and clinical activity was reported in a phase I study of Temsirolimus in newly diagnosed advanced-stage HNSCC.[35] The TEMHEAD trial was a single arm multicenter phase 2 trial of Temsirolimus that looked at refractory/recurrent metastatic squamous cell carcinoma in 40 patients which found that inhibition of the PI3K-AKT-mTOR axis was a putative novel treatment paradigm for SCCHN.[36] In 33 evaluable patients, disease stabilization occurred in 57.6%, and tumor shrinkage in 39.4%. Neither *PIK3CA* mutations nor HPV status were predictive for success with Temsirolimus treatment. Fatigue (47.5%), anemia (25.0%), nausea (20.0%), and pneumonia (20.0%) were the most common adverse events. A phase 2 trial evaluating the clinical benefit rate (CBR) of Everolimus in 9 patients was disappointing, with 3 patients discontinued due to toxicity and CBR of 28% showing that Everolimus was inactive as monotherapy in unselected patients with HNSCC.[37] The AKT inhibitor MK2206 (Merck, USA) in a phase 2 trial evaluating MK2206 as a single agent in HNSCC has recently been completed and currently awaiting publication of results. Table 1 summarizes trials of PI3K, mTOR and AKT inhibitors and combinations registered in ClinicalTrials.gov.

MEK/MAPK Pathway

The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway is activated in HNSCC by upstream growth factor receptors and *HRAS*, and may be inhibited by antagonists of these receptors and the intermediate kinase MEK that phosphorylates and activates MAPK/ERK.[25,38,39] TCGA has

identified amplifications of several growth factor receptors including *EGFR*, *ERBB2*, *IGF1R*, *FGFR1*, *3*, *EPHA2*, *MET*, and rarer mutations of *HRAS*, as possible genetic drivers upstream of MAPK/ERK that support MEK kinase as a key signaling intermediate and potential therapeutic target.[3] Whilst upstream growth factor receptors have been widely studied, on an individual basis they appear to drive oncogenic signaling and demonstrate clinical activity when targeted in relatively minor HNSCC subsets. However, convergence of these signals and activation of the MEK and ERK effectors of the MAPK pathway is prevalent and has been targeted in preclinical and early clinical studies. Biomarker and clinical responses to MEK inhibitor Trametinib were recently reported in HNSCC in a window of opportunity trial.[40] MEK inhibitor PD-0325901 has shown single-agent activity and ability to overcome resistance and enhance antitumor effects in vivo with the dual PI3K/mTOR inhibitor PF-384.[34] Disappointingly, phase I studies of MEK and PI3K-mTOR inhibitors demonstrated enhanced toxicity and narrow therapeutic window of the combination, and eventual progression on or soon after cessation of treatment. Studies in animal models suggest the direct anti-tumor activity of MEK inhibitors may be offset by suppression of beneficial immune responses elicited by mTOR inhibition or immune checkpoint therapy.[41] There are no registered MEK inhibitors in active clinical trial for HNSCC. Table 1 summarizes trials of MEK inhibitor combination registered in ClinicalTrials.gov.

Cell Cycle

The TCGA findings highlighted the potential for investigation of newer CDK4/6 inhibitors due to the near universality of cell cycle dysregulation in HNSCC.[3] Consistent with the prevalence of these alterations downstream of growth factor signaling, CDK4/6 blockade was found to enhance the efficacy of EGFR inhibition in

TABLE 1.
Trials with Small Molecule Inhibitors Targeting Genomic Pathway Alterations.

Drug	Combination	Status	Clinical Trial ID
PI3K Inhibitors			
BKM120(Novartis, USA)	Cetuximab	Phase I/II	NCT01816984
	Single agent	Phase II	NCT01737450
	Cisplatin	Phase I	NCT02113878
PX-866(Oncothyreon, USA)	Cetuximab	Phase I/II	NCT01252628
	Docetaxel	Phase I/II	NCT01204099
BYL719(Novartis, USA)	Cetuximab	Phase I/II	NCT01602315
	Single agent	Phase II	NCT02145312
	Paclitaxel	Phase I	NCT02051751
	Cisplatin	Phase I	NCT02537223
	Single agent	Phase II	NCT02145312
Copanlisib	Cetuximab	Phase I/II	NCT02822482
SF1126	Single agent	Phase II	NCT02644122
mTOR inhibitors			
Everolimus(Novartis)	Single agent	Phase II	NCT01051791
	Single agent	Phase II	NCT01133678
	Carboplatin, Cetuximab	Phase I/II	NCT01283334
	Carboplatin, paclitaxel	Phase I/II	NCT01333085
	Cetuximab	Phase I	NCT01637194
	Erlotinib	Phase II	NCT00942734
Rapamycin(pfizer,USA)	Single agent	Phase I/II	NCT01195922
Temsirolimus(Pfizer)	Single agent	Phase II	NCT01172769
	Cetuximab	Phase II	NCT01256385
	Carboplatin, paclitaxel	Phase I/II	NCT01016769
	Cetuximab	Phase I	NCT02215720
PI3K/mTOR Dual Inhibitors			
BEZ235(Novartis,USA)	Everolimus	Phase I	NCT01508104
PF04691502(pfizer,USA)	Single agent	Phase I	NCT00927823
PF05212384(Pfizer,USA)	Docetaxel, Cisplatin, Dacomitinib	Phase I	NCT01920061
	PD-901, Irinotecan	Phase I	NCT01347866
SAR245409(sanofi, USA)	Pimasertib	Phase I	NCT01390818
PQR309	Single agent	Phase I	NCT02483858
Akt Inhibitors			
MK2206(Merck, USA)	Single agent	Phase II	NCT01349933
MEK inhibitors			
PD-0325901	PF-05212384	Phase I	NCT01347866
CDK Inhibitor			
Palbociclib	Cetuximab	Phase I/II	NCT03024489
	Gedatolisib	Phase I	NCT03065062
P276–00	Single agent	Phase I/II	NCT00899054
	Single agent	Phase II	NCT00824343
Smac Mimetics/IAP antagonists			
Debio1143	Cisplatin	Phase I/II	NCT02022098
	Single agent	Phase I	NCT01078649
GDC0917	Single agent	Phase I	NCT01908413
	Single agent	Phase I	NCT01226277
LCL-161	Paclitaxel	Phase I	NCT01968915
	Paclitaxel	Phase I	NCT01240655
	Single agent	Phase I	NCT01098838
GDC0152	Single agent	Phase I/II	NCT00977067
Birinapant	Chemotherapy	Phase I/II	NCT01188499
	Single agent	Phase I	NCT00993239
HG5–1029	Single agent	Phase I	NCT00708006
ASTX-660	Single agent	Phase I/II	NCT02503423

SCC.[42] There is increasing data that suggests that HPV(+) HNSCC may benefit from CDK inhibition; low-dose Roscovitine, (CDK inhibitor) significantly inhibited the growth of HPV-associated xenograft tumors in mice without causing any detectable side effects.[43] Of recent interest, high phosphorylation of CDK4/6 target Threonine-356 on cell cycle regulator RB1 was found to predict survival in HPV(-) HNSCC, providing a rationale for investigation of these agents.[44] In HPV(-) HNSCC, the CDK4/6 inhibitor LY283519 has been found to be active alone and potent in combination with mTOR inhibitor in HNSCC models in vivo and in vitro.[45] A phase 1 study (NCT03065062) is currently recruiting participants looking at Palbociclib in combination with the PI3K/mTOR inhibitor Gedatosilib. Palbociclib(CDK4/6 inhibitor) is also currently being investigated as a single agent in a phase 1 dose escalation study to determine the maximum tolerated dose (MTD) and toxicity (NTC03024489). The MONARCH study was a phase 2 trial assessing the efficacy of a CDK inhibitor P276–00 in HNSCC, which was completed in 2013 but still awaiting publication. With increasing numbers of CDK inhibitors being developed this is an area of future promise.[46] Table 1 summarizes trials of CDK inhibitor and combinations registered in ClinicalTrials.gov.

Cell cycle checkpoints allow cells time for DNA repair and maintain genomic integrity before the cell undergoes cell division. Certain signaling cascades due to DNA damage result in the phosphorylation of Wee1 Tyrosine Kinase which is involved in the inhibitory phosphorylation of Cdk1 and Cdk2.[47] A number of preclinical studies have elaborated on the function of Wee1 Inhibitors to allow premature entry into cell division with unrepaired DNA resulting in cell death.[48,49] A phase I study (NCT01748825) administered oral 2.5g of AZD1775 (Wee1 inhibitor) for up to 2 weeks in adult patients with refractory tumors, found that partial responses were observed in one patient with head and neck cancer and one patient with ovarian cancer.[50]

SMAC Mimetics and BIRC/IAP Inhibition

FADD and IAP proteins are amplified and over-expressed in ∼40% of HNSCC, and can inversely modulate programmed cell death.[3,51] Second Mitochondrial Activator of Caspases (SMAC) mimetics are small molecule inhibitors that mimic smac, an endogenous IAP antagonist. This is a potential therapeutic target that has shown efficacy in preclinical models. Birinapant is a SMAC mimetic that demonstrated activity in FADD and IAP amplified tumor models in combination with death factors TNF and TRAIL, and radiation.[52] Seven patients with HNSCC out of a cohort of 50 patients were treated with the bivalent SMAC mimetic with dose range of 0.18 to 63 mg/m3 in a 3 + 3 dose escalation design once weekly, defining a phase II dose.[53] Debio 1143 is SMAC mimetic that antagonizes IAP activity (XIAP, cIAP-1, 2, and ML-IAP) and promotes apoptosis. Debio 1143 radiosensitizing action has also been shown in several in vitro and in vivo HNSCC preclinical models.[54] The results of the first-in-human oral Debio 1143 trial where 31 patients received oral disease ranging from 5 to 900 mg once daily

are encouraging. Optimal treatment response of stable disease was achieved in 5 of the patients and the authors concluded that Debio 1143 should be incorporated with other treatment modalities and sub-population screening.[55] An ongoing Phase I/II trial investigating Debio 1143 is still in the recruitment phase (NCT02022098) that is randomizing 94 participants comparing Debio 1143 to placebo, both with concomitant CRT (cisplatin, radiotherapy). A phase 1 study (NCT01098838) investigating LCL-161 (SMAC mimetic) had an undisclosed number of HNSCC and showed no objective response to once daily dose ranging of 10 to 3,000 mg, however they concluded that further development of LCL-161 is warranted given the favorable tolerability and pharmacodynamics activity.[56] The use of SMAC mimetics in the treatment of HNSCC is still in its infancy, however the positive results of these early preclinical and phase trials herald hope in the future. Table 1 summarizes trials of CDK inhibitor and combinations registered in ClinicalTrials.gov.

Immunotherapy

Recent exciting advancements of the management of HNSCC have been in the field of immunotherapy. Immune surveillance is an important mechanism to prevent progression of HNSCC. The cancer cells can evade the immune system through multiple mechanisms including T-cell tolerance, and inhibition of T cell-related pathways via co-receptors commonly known as immune checkpoints.[57] These checkpoints are negative co-stimulatory ligands expressed on tumor and infiltrating cells that bind receptors expressed on tumor infiltrating T-cells that functionally suppress T-cell function and induce T-cell apoptosis.[58] CTLA-4 and PD-1 normally mediate immunological homeostasis by acting as down-regulators of T-cell activity after elimination of pathogens, but their expression in HNSCC prematurely stunts effector T-cell immunity. Checkpoint inhibition primarily via CTLA-4 and PD-1 inhibitors is being investigated in a number of phase I/II clinical trials.

Nivolumab is an IgG4 mAb targeting the PD-1 receptor.[59] Since tumors expressing PD-L1 show the best response to Nivolumab, it is important to understand the proportion of HNSCC that express the PD-L1 ligand.[60] The CheckMate 141 trial (NCT02105636) randomized 361 patients with recurrent HNSCC in a 2:1 ratio to receive Nivolumab every 2 weeks or standard, single-agent systemic therapy (Ethotrexate, Docetaxel, or Cetuximab).[61] In this phase 3 trial, the median overall survival was 7.5 months in the Nivolumab group versus 5.1 months in the standard therapy. The median progression-free survival (2 months vs 2.3 months) and the response rate (13.3% vs 5.8%) were also better in the Nivolumab group in comparison to the standard therapy. Most significantly perhaps was that treatment-related adverse events in the Nivolumab group was significantly lower (13.1% vs 35.1%) supporting a more favorable safety profile and quality-of-life benefit of immunotherapy. A phase 2 trial (NCT02919683) is specifically looking at Nivolumab in HNSCC of the oral cavity. There is also an ongoing phase 1 study (NCT02124850) assessing whether modulation of biomarkers can predict tumor

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TABLE 2.
Trials with Immune Checkpoint Inhibitors.

Drug	Combination	Status	Clinical Trial ID
Nivolumab	Single agent	Phase III	NCT02105636
	Varlilumab	Phase I/II	NCT02335918
	INCB24360	Phase I/II	NCT02327078
	PLX3397	Phase I	NCT02526017
	Single agent	Phase I/II	NCT02488759
	Standard therapy	Phase I	NCT02764593
	Ipilimumab	Phase I/II	NCT03003637
	Ipilimumab	Phase II	NCT02823574
	Ipilimumab	Phase III	NCT02741570
	Single agent/ipilumamb	Phase II	NCT02919683
	Epacadostat	Phase I/II	NCT02327078
	Single agent vs Combination(Ipilumamb, BMS-986016, Daratumamb)	Phase I/II	NCT02488759
	IPI549	Phase I	NCT02637531
	TAK659	Phase I	NCT02834247
	Enadenotucirev	Phase I	NCT02636036
	Ipilimumab	Phase II	NCT03097939
Pembrolizumab	ACP-196	Phase II	NCT0454179
	Single agent	Phase III	NCT02252042
	Single agent	Phase III	NCT02358031
	INCB24360	Phase II	NCT02178722
	PLX3397	Phase I/II	NCT02452424
	Single agent	Phase II	NCT02296684
	MGA271	Phase I	NCT02475213
	Single agent	Phase II	NCT02255097
	Single agent	Phase II	NCT02769520
	Talimogene Laherparepvec	Phase I	NCT02626000
	Cisplatin	Phase II	NCT02641093
	Single agent	Phase II	NCT02289209
	Cisplatin	Phase II	NCT02777385
	Single agent	Phase II	NCT02609503
	Acalabrutinib	Phase II	NCT02454179
	Cisplatin	Phase III	NCT03040999
	Single agent	Phase II	NCT02841748
	Single agent/cisplatin/carboplatin/5-FU	Phase III	NCT02358031
	Docetaxel	Phase I/II	NCT02718820
	SD-101	Phase I/II	NCT02521870
	Single agent	Phase II	NCT03057613
	Chemoradiotherapy	Phase I	NCT02819752
	Single agent	Phase III	NCT02252042
	Single agent	Phase II	NCT02892201
	Cisplatin, IMRT	Phase I	NCT02775812
	Docetaxel, 5-FU, Cisplatin	Phase II	NCT03114280
	Single agent	Phase II	NCT03085719
	Vorinostat	Phase I/II	NCT02538510
	Single agent	Phase II	NCT02296684
	Single agent	Phase I	NCT02318771
	Single agent	Phase II	NCT02707588
	Cetuximab	Phase II	NCT03082534
	Cisplatin	Phase I/II	NCT02759575
	RecombinantEphB4-HSA fusion protein	Phase II	NCT03049618

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TABLE 2.
(Continued)

Drug	Combination	Status	Clinical Trial ID
	Levatinib	Phase I/II	NCT02501096
	PLX3397	Phase I/II	NCT02452424
	Levatinib	Phase I	NCT03006887
	Single agent	Phase I	NCT01848834
	Single agent	Phase II	NCT02644369
Atezoluzmab	Obintuzumab	Phase I	NCT02174172
	Single agent	Phase I	NCT01375842
PF-0502566	Pembrolizumab	Phase I	NCT02179918
Urelumab	Cetuximab	Phase I	NCT02110082
MEDI4736	Tremelimumab	Phase III	NCT02369874
	AZD9150/AZD5069	Phase I/II	NCT02499328
	Single agent	Phase II	NCT02207530
	ADXS 11–001	Phase I/II	NCT02291055
	Mogamulizumab	Phase I	NCT02301130
Tremelimumab	Mogamulizumab	Phase I	NCT02301130
Ipilumamab	Cetuximab and XRT	Phase I	NCT01935921
	MGA271	Phase I	NCT02381314
PF-4518600	Single agent	Phase I	NCT02315066
AZD17751(Wee1 Inhibitor)	Single agent	Phase I	NCT01748825

response in patients administered either Cetuximab and Motolimod (Cohort 1), or Cetuximab, Motolimod and Nivolumab (Cohort 2).

Ipilumumab is an IgG1 mAb targeting the CTLA-4 receptor.[60] Binding to the CTLA-4 receptors frees the B7 ligand on APCs to bind to the stimulatory CD28 receptor on T cells, resulting in activation.[60] Both Nivolumab and Ipilimumab play a role in regulation of T-cells albeit via different mechanisms and may act synergistically together. A number of trials are still in the recruitment stages with the phase I/2 IMCISION trial (NCT03003637), the phase 2 trial Checkmate 714 (NCT02823574), and the phase 3 Checkmate 651 (NCT02741570) investigating the benefits of immune combination with Ipilimumab and Nivolumab.

Pembrolizumab is a PD-1 inhibitor that has established safety profile in head and neck cancer patients.[7] KEYNOTE-012 (NCT01848834) is a multicenter phase 1 trial where 104 patients with recurrent or metastatic HNSCC received 10 mg/kg Pembrolizumab intravenously every 2 weeks.[7] Of the 104 patients, Pembroluzimab was well tolerated, with 10 (17%) of 60 PDL-1 positive patients having grade 3 or 4 drug-related adverse events, the most common of which were increases in the transaminases and hyponatraemia, with 1 patient developing a grade 3 drug-related rash.[7] The study is still under follow-up analysis but no longer recruiting patients. KEYNOTE-055 (NCT02255097) is a single-arm phase 2 study that showed that the response rate was 16% in 171 patients with recurrent/metastic HNSCC who received 200 mg of Pembroluzimab every 3 weeks.[62] The study is still ongoing but at the time of analysis 109 (64%) patients reported drug-related adverse events, with 26 patients (15%) experiencing a grade >3 event.[62]

Currently, most of the checkpoint inhibitor trials are phase I/II, however there are 8 phase III trials that are currently investigating a checkpoint inhibitor in head and neck cancer: 2 Nivolumab trials (NCT02105636 and NCT02741570); 5 Pembrolizumab (NCT02252042, NCT02358031, NCT03040999, NCT02358031, and NCT02252042); and 1 Tremelimumab (NCT02369874). Table 2 summarizes trials of immune checkpoint and combinations registered in ClinicalTrials.gov.

DISCUSSION AND CONCLUSIONS

TCGA and other large-scale studies have identified alterations affecting components of several key pathways and functions that provide a roadmap for investigation of developmental therapeutics with potential for precision medicine. The most common include amplifications or mutations of *PIK3CA* and several growth-factor receptors, that regulate cell growth and metabolism; a variety of alterations converging on CDK4/6-RB1-E2F1, *CCND1*, and *TP53*, affecting the cell cycle; *FADD* and *BIRC* (IAPs), modulating cell death and survival; and deregulation of immune recognition or activation. New therapeutics targeting these molecules or pathways have shown dramatic single-agent activity in preclinical models and a few patients, but have mostly demonstrated partial responses or stable disease in clinical trials. This increasingly appears to be due to the fact that most HNSCC tumors harbor complex chromosomal derangements and mutations altering multiple genes and pathways that contribute to the malignant phenotype, whereas relatively fewer are driven primarily by *CASP8* with *HRAS* or HPV with *PIK3CA* mutations with fewer chromosomal alterations. Recognition of this complexity

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suggests that investigation of rational combinations targeting several of these key pathways and their interaction with major genomic alterations may reveal wider activity and insight than prematurely focusing on sequence-based selection of the few patients with predicted activating mutations for single-agent trials. This includes the potential of marrying treatments that potentiate cell death using chemoradiation or immune checkpoint therapies, inhibitors of PI3K and FADD-IAP pro-survival pathways, and MAPK-cell cycle targets. The number of important genes already found on chromosomes 3 (*PIK3CA*, *SOX2*, *TP63*) and 11 (*FADD*, *BIRC2,3*), and others that are implicated in HNSCC pathogenesis and resistance underscore the continuing potential to identify new targets or agents for precision-medicine-based investigations.

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