Head and Neck Cancer (JL Geiger, Section Editor)

Precision and Immunoprevention Strategies for Tobacco-Related Head and Neck Cancer Chemoprevention

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Opinion statement

To date, there is no FDA-approved chemoprevention approach for tobacco-related HNSCC. Effective chemoprevention approaches validated in sufficiently powered randomized trials are needed to reduce the incidence and improve survival. In this review, we recap the challenges encountered in past chemoprevention trials and discuss emerging approaches, with major focus on green chemoprevention, precision prevention, and immunoprevention. As our current depth of knowledge expands in the arena of cancer immunotherapy, the field of immunoprevention is primed for new discoveries and successes in cancer prevention.

Introduction

Globally head and neck cancer squamous cell carcinoma (HNSCC) is the 6th most common cancer and accounts for over 650,000 cases and 330,000 deaths annually. In the USA, HNSCC accounts for 3% of all cancers resulting in approximately 53,000 new cases and 10,800 deaths annually [1]. The two major etiologies for HNSCC are environmental carcinogenesis, where dominant risk factors include tobacco and alcohol use, and viral carcinogenesis secondary to oral infection with human papillomavirus (HPV) 16. Although epidemic in the USA and Western Europe, HPV-positive cancers represent less than 15% of HNSCC cases worldwide. Despite advances in cancer detection and treatments including surgery, radiation, chemotherapy, and immunotherapy, the overall survival rate for HPV-negative HNSCC has been stagnant at 40–60% for three decades [2, 3]. Even after curative treatment, people with a first tobacco-related HNSCC have a 3–6% yearly rate of second primary tumor (SPT) formation. While HPV vaccination has the potential for herd immunity and preventing HPV-related HNSCC within a generation, no similar medical approach exists for the prevention of HPV-negative cancers. Tolerable and effective chemoprevention approaches are critically needed to supplement the field of smoking cessation to achieve a greater impact and reach across this vulnerable population.

Two key biological concepts underlie chemoprevention strategies for environmentally induced HNSCC: field cancerization and multistep carcinogenesis. The term field cancerization was introduced by Danely Slaughter in 1953 when he observed that grossly normal epithelium adjacent to resected HNSCC exhibited multicentric histologic abnormalities including dysplasia or carcinoma in situ [4]. The resulting hypothesis was that the entire oral mucosal field was condemned by carcinogenic alterations. Even microscopically normal epithelium surrounding HNSCC tumors often harbors molecular alterations driven by carcinogen exposure [4, 5]. The second concept is multistep carcinogenesis, which describes the molecular process by which initiation, promotion, and progression occur in a stepwise manner, with DNA damage and genomic instability ultimately resulting in malignant transformation of the oral epithelium. Important studies characterizing chromosomal abnormalities including loss of heterozygosity (LOH) have informed a model of HNSCC transformation, with common early events including LOH in 9p21 leading to p16 *CDKN2A* inactivation followed by the loss of 3p21 and 17p13 (the locus of *TP53*) [6]. Subsequent LOH events have been linked to cyclin D1 amplification and *PTEN* inactivation [6]. The biological features of field cancerization and multistep carcinogenesis in HNSCC present rational targets for chemoprevention approaches.

Treatment

Chemoprevention is the active process of applying natural or synthetic chemicals for the reversal, suppression, or prevention of invasive carcinoma. Chemoprevention strategies can be classified as primary or secondary. Primary prevention is aimed at high-risk populations who are otherwise healthy without precursor lesions or cancer. Primary prevention of tobacco-related HNSCC involves interventions aimed at reducing the incidence of disease in otherwise healthy current or heavy smokers, or other tobacco users (chew or snuff). Secondary prevention targets populations that have oral premalignant lesions (OPLs) or invasive carcinomas, aiming to prevent malignant transformation or an SPT. OPLs can include leukoplakia, erythroplakia, dysplasia, or carcinoma in situ. Risk of malignant transformation of a specific OPL is unclear; however, certain biomarkers are associated with increased risk, specifically LOH at critical sites. Chemoprevention agents that revert histological and clinical features of OPLs do not clearly equate to a long-term reduction in risk of transformation or

SPT, although this is a common clinical trial model to investigate the biologic activity of candidate agents [7–9]. Over the last decade, chemoprevention strategies for environmentally driven HNSCC have evolved including the use of micronutrients, whole food supplements, precision, and immunoprevention approaches targeting each of these at-risk populations. Table 1 summarizes all recently completed and ongoing chemoprevention trials.

Diet and lifestyle

Tobacco cessation

Tobacco cessation is the single most important prevention tool against HNSCC and exemplifies a primary prevention approach. Smoking cessation has been shown to reduce the risk of HNSCC development by 30% after being smoke free for 1–4 years [10]. In the USA, public policy efforts to reduce tobacco consumption include anti-tobacco advertising, limits in tobacco industry advertisements, increasing cigarette taxes, public smoking bans, provisions for tobacco cessation treatment, restrictions in youth access, and prevention education [11]. In addition, the FDA has approved seven first-line pharmacotherapies, including five nicotine replacement therapies and two non-nicotine-based oral medications (bupropion and vareninline) [12], which may be used alone or as part of an evidence-based intervention program.

Plant-rich diet

Multiple epidemiological studies have shown that diets heavy in fruits and vegetables, particularly those rich in micronutrients such as vitamin A or β-carotene, are associated with reduced risk of tobacco-related cancers as well as SPTs [13–16]. Given failure of single micronutrient studies in the prevention of lung or HNSCC, due to efficacy or tolerability, the chemoprotective effects from plant-rich diets are hypothesized to require whole foods delivering a combination of beneficial micronutrients and phytochemicals. The U.S. National Cancer Institute's recommendation to include 5 servings of fruits and vegetables in the daily diet has significant merit in current or former smokers. Due to the symptom burden following HNSCC treatment, including dysphagia, strategies to include whole fruits and vegetables include purees and supplements.

Micronutrients and supplements

Micronutrients are essential dietary elements required in very small quantities, such as zinc, selenium, and vitamin A. Original micronutrient studies in HNSCC prevention focused on antioxidants including vitamin A and β-carotene. Previously, two landmark studies showed the vitamin A analogue 13-cis-retinoic acid (isotretinoin) induced regression of OPLs and reduced the incidence of second primary tumors in patients with previously treated HNSCC compared to placebo [17–19]. However, chronic treatment with high-dose isotretinoin was intolerable and incidence of SPTs returned to

Table 1. Summary of recently completed and ongoing chemoprevention trials (2016–2020)

Trial	Intervention	Type	Status	Clinical trial ID
Nutritional or green chemoprevention				
A phase I, randomized, double-blind, placebo-controlled, dose-escalation study to evaluate the safety and pharmacokinetics of SBS-101 after intraoral application in patients with oral premalignant lesions (OPL)	SBS-101	Secondary	Not yet	NCT03939364
Evaluation of effect of topical melatonin in treatment of oral leukoplakia	Topical melatonin	Secondary	Recruiting	NCT04251845
Clinical study of Avmacol® for detoxification of tobacco carcinogens in heavy smokers	Avmacol®	Primary	Active, not recruiting	NCT0342230
Broccoli sprout extract in preventing recurrence in patient with tobacco-related head neck squamous cell cancer	Avmacol®	Secondary	Active, not recruiting	NCT03182959
Effect of Avmacol® in the oral mucosa of patients following curative treatment for tobacco-related head and neck cancer	Avmacol®	Secondary	Recruiting	NCT03268993
Effect of oral black raspberry administration on oral cell DNA adducts in smokers	Black raspberry lozenges	Primary	Not yet recruiting	NCT04372914
A pilot phase I study of the use of functional confections in promoting oral health in men and women	Strawberry gummy	Primary	Active, not recruiting	NCT01514552
Precision prevention				
A pilot multi-center international double-blind placebo-controlled randomized study of Sulindac, a Pan-Cox inhibitor, in oral premalignant lesions	Sulindac	Secondary	Completed, has results*	NCT00299195
Phase IIB randomized, placebo-controlled trial of pioglitazone for oral premalignant lesions an inter-consortium collaborative study	Pioglitazone hydrochloride	Secondary	Terminated early, has results	NCT00951379
M4OC-prevent: metformin for oral cancer prevention	Metformin hydrochloride	Secondary	Active, not recruiting, has results	NCT02581137
Chemoprevention of head and neck squamous cell carcinoma (HNSCC) with valproic acid (GAMA)	Valproic acid	Secondary	Completed	NCT02608736
Immunoprevention				
Safety and efficacy of nivolumab in treating oral proliferative verrucous leukoplakia	Nivolumab (Opdivo)	Secondary	Recruiting	NCT03692325
PD-1 immune checkpoint inhibition for the reversal of squamous dysplasia in high-risk current and former smokers with or without a history of lung cancer	Nivolumab (Opdivo)	Secondary	Recruiting	NCT03347838
A phase II open label, single arm study to evaluate the efficacy of pembrolizumab for luekoplakia	Pembrolizumab (Keytruda)	Secondary	Recruiting	NCT03603223

*Primary outcome results not yet posted

baseline 3 years after discontinuation [20]. Subsequent studies evaluated lower doses of isotretinoin, which proved tolerable but did not reduce SPT occurrence [21, 22]. Likewise, early successes in β-carotene chemoprevention trials were ultimately followed by disappointment when the CARET trial revealed an increased risk of lung cancer and death from cardiovascular disease were associated with β-carotene treatment [23].

- SB-101. A new application of isotretinoin as a topical oral-adhesive film (SBS-101) is ongoing. In this phase 1, randomized double-blind placebo-controlled dose-escalation study, the safety and pharmacokinetics of SB-101 will be evaluated (NCT03939364). Patients with oral leukoplakia or erythroplakia will receive intraoral applications of 0.1%, 0.2%, or 0.3% SB-101 and monitored for both overall response and adverse reaction (AE).

- Topical melatonin. Melatonin is a naturally occurring hormone produced by the pineal gland, which regulates circadian sleep cycles. Melatonin also possesses intriguing biologic properties relevant to chemoprevention including potent antioxidant function, induction of apoptosis, and inhibition of telomerase activity. In this placebo-controlled trial, participants with tobacco-associated leukoplakia will be treated with a topical 3% formula containing 15 mg of melatonin once daily for 6 weeks then followed for 3 months to determine change in lesion size and degree of dysplasia.

Green chemoprevention

Green chemoprevention describes the use of whole foods or their simple extracts for cancer prevention. Such interventions tend to be more cost-effective and are generally well tolerated raising the promise of global dissemination even in developing nations [24]. The chemoprotective effects of diets rich in fruits and vegetables may benefit from synergistic activity among micronutrients and phytochemicals. Phytochemicals such as isothiocyanates from crucifers, organosulfides from garlic and onions, and polyphenols from berries and teas possess anticarcinogenic, antimutagenic, and anti-inflammatory properties [24–26]. Phytochemicals exert their effects through multiple mechanisms including inhibition of oncogenic signaling pathways, such as PKC/RAS/MAPK, or PI3-kinase/AKT; suppression of pro-proliferative or anti-apoptotic transcription factors, NF-κB or AP-1; and upregulation of NRF2 target genes within the antioxidant response element [27•]. Green chemoprevention agents are currently being evaluated in primary and secondary HNSCC prevention.

- Broccoli seed and sprout extract (Avmacol®). Avmacol® is a nutraceutical containing a blend of sulforaphane glucosinolate (glucoraphanin) and myrosinase derived from broccoli sprout and seed extracts. Sulforaphane is thought to promote detoxification via the NRF2 pathway and support immune function, making it an appealing candidate in tobacco-related prevention efforts. Avmacol® is under study in otherwise healthy active/current smokers (NCT034022) to determine whether it increases detoxification of tobacco carcinogens, and in current or former tobacco users following curative-intent therapy for a first tobacco-related OPL or HNSCC of any stage (NCT03182959, NCT03268993) to observe if NRF2 target

genes are upregulated in the so-called condemned epithelium.

- Black raspberry (BRB) lozenges. A dietary supplement containing whole black raspberries in lozenge form is currently under examination in a primary prevention population of current smokers. Each BRB lozenge contains 1 g of freeze-dried black raspberry powder (approximately 5 black raspberries), in the form of a dissolvable slow-release lozenge (NCT04372914). Primary outcome will be measured as the change of HB-releasing adducts detected in buccal cells from baseline to end of treatment at 8 weeks, as a measure of DNA damage caused by tobacco smoke-specific nitrosamines.
- Strawberry gummy. Gummy supplements containing freeze-dried whole strawberries are being evaluated in current or non-smokers, testing hypotheses concerning compliance and toxicity (NCT01514552). Each 6-g gummy contains 45% freeze-dried fruit equivalent to 1 cup of strawberries. Participants will be asked to consume 2 gummies four times daily.

Pharmacologic treatment

Precision prevention

Precision prevention integrates precision medicine approaches with an individual's unique risk profile, defined by both genomic and lifestyle risk factors [28, 29]. Prolonged incubation times for OPL lesions to develop into malignancies, even after exposure to known carcinogens such as tobacco, may uniquely leverage precision chemoprevention. Hanahan and Weinberg first outlined the hallmarks of cancer in 2000, which suggested that an accumulation of genomic changes in 6 functionally distinct categories was necessary for tumorigenesis to occur [30]. These areas are applicable to HNSCC chemoprevention and include self-sufficiency in growth signals, evasion of apoptosis, insensitivity to anti-growth signals, sustained angiogenesis, limitless replicative potential, and tissue invasion and metastasis. The hallmarks of cancer have since been expanded to include deregulated cellular energetics, immune evasion, genomic instability, and tumor-promoting inflammation [31]. Clinical and experimental data combined with new technologies such as next-generation sequencing (NGS) have elucidated novel molecular targets for precision prevention. Commonly mutated genes in HPV-negative HNSCC include many tumor suppressor genes including *TP53*, *CDKN2A*, *FAT1*, *NOTCH1*, *KMT2D*, *NSD1*, and *TGFBR2*, and the oncogene *PIK3CA* [32]. Overexpression of EGFR is also common, occurring in 80–90% of HNSCC tumors and is associated with poor survival [33, 34]. Cyclooxygenase-2 (COX2), IL-6 and IL-6R, cyclin D1, MMPs, HIF1α, WNT-β-catenin, and STAT3 signaling are also dysregulated at various stages in HNSCC oncogenesis [35••]. Of note, COX2 expression is significantly upregulated in the oral mucosa of smokers and is driven by EGFR signaling [36]. Early precision chemoprevention studies have focused on molecular inhibition of EGFR and cyclooxygenase 2, while more recent studies focus on other NSAIDS, anti-diabetics, and epigenetic modifiers.

- Erlotinib. Erlotinib is an EGFR protein tyrosine kinase inhibitor approved clinically for the treatment of locally advanced or metastatic non-small cell

lung cancer. Although preclinical studies showed promising results supporting the use of erlotinib in HNSCC prevention, EPOC, a large phase II study of erlotinib in patients with high-risk OPL possessing LOH at 9p and 3p failed to reduce oral cancer-free survival [37]. Efficacy may have been limited by dose-limiting toxicities, requiring dose reductions in half of study participants. Although erlotinib was not proven to be effective, LOH was confirmed as a significant molecular biomarker of oral cancer risk.

- Celecoxib. Celecoxib is a non-steroidal anti-inflammatory (NSAID) specific for COX2, which converts arachidonic acid to prostaglandin E2 (PGE_2), the major prostanoid responsible for chronic inflammation [38]. Greater than 80% of OPLs or HNSCC tumors display COX2 overexpression [39–42]. Although animal studies showed that nonselective COX2 inhibition delayed carcinogenesis and suppression of tumor cell growth and invasion of OSCCs [38, 43], human studies yielded mixed results and were finally halted when chronic celecoxib use was associated with increased cardiovascular toxicity [44–47]. Low-dose [48], topical [49], and combination [50] approaches were also evaluated and ultimately abandoned due to toxicity or limited efficacy, reviewed in [51]. A phase I dual precision approach utilizing both erlotinib and celecoxib evaluated 12 participants with oral leukoplakia, mild, moderate, or severe dysplasia, or carcinoma in situ, who were treated with celecoxib 400 mg daily with escalating doses of erlotinib, 50 mg, 75 mg, and 100 mg once daily for 6 months. The study showed that the maximum tolerated dose of erlotinib with celecoxib in this population was only 50 mg, one-third of the FDA-approved therapeutic dosage. A positive overall histologic response rate was observed in 63% of participants as well as downregulation of EGFR and p-ERK in paired biopsies, which correlated with response to treatment [50]. Larger randomized trials using this combination have not been attempted, likely because chemoprevention mandates agents with long-term tolerability to be administered continuously for effective prevention by delay.
- Sulindac. Sulindac is a pan-COX2 inhibitor and is among the latest in this class of NSAIDS to be tested. In a double-blind placebo-controlled randomized study of 63 subjects, the clinical safety and molecular effects of sulindac (150 mg bid for 24 weeks) against OPLs were examined. All 63 enrolled patients completed the study; however, the primary endpoint has not yet been reported. Notably, patients on active treatment did not display increased rates of all-cause mortality or serious AEs compared to those on placebo.
- Pioglitazone. Pioglitazone, a thiazolidinedione-type drug, is traditionally used to control blood sugar; however, many drugs in this class have also been shown to upregulate peroxisome proliferation-activated receptor-gamma (PPARγ). Pioglitazone is a potent and highly selective agonist for PPARγ, which acts as a tumor suppressor, downregulating genes involved in cell proliferation and angiogenesis through its engagement with the retinoid X receptor. A phase IIB double-blind randomized placebo-controlled trial evaluated clinical and histological response of OPLs to 24 weeks of pioglitazone (NCT00951379). The trial was terminated for slow accrual after a total of 52 participants were enrolled, including 27

randomized to the active arm. There were a total of 12 responders (46%) in the treatment arm compared to 8 (32%) in the placebo arm; biomarker data and statistical parameters have not been reported.

- Metformin. Metformin is a widely used anti-diabetic drug. In a phase IIA trial, clinical and histological response of OPLs to 3 months of metformin was evaluated (NCT02581137). The study enrolled 26 participants and 23 completed and observed a 17.4% clinical response and 60.9% histological response rate. Interestingly, both pioglitazone and metformin have been shown to possess immunomodulatory properties. Pioglitazone inhibits macrophage and monocyte activation, metformin impacts macrophage polarization, and both may possess anti-inflammatory properties [52, 53].

- Valproic acid (VA). VA is a modifier of epigenetic events which specifically acts as a histone deacetylase inhibitor and promotes DNA methyltransferase degradation. This class of anti-cancer drugs modulates multiple pathways related to initiation and progression. VA acts to promote histone acetylation when orally administered at doses of 20–40 mg/kg or 1000–1500 mg per day. In a phase 0 randomized placebo-controlled trial, patients with a prior HPV-negative HNSCC were treated with VA 1500 mg per day for 3 months. Primary outcome measures will consist of changes in protein or histone acetylation measured in salivary samples, pre- and post-treatment.

Immunoprevention

Immunoprevention is the prevention of cancer through the use of immunomodulatory approaches including but not limited to vaccines, immunostimulators, and antibodies. Immunoprevention represents a growing field of interest for prevention of HNSCC carcinogenesis. A subset of tobacco-induced mutations may generate neoantigens that trigger an immune response [54, 55]. As a result, the immune system may eliminate neoantigen-containing oral epithelial cells and prevent oral cancer development. Indeed, the higher risk of oral cancer observed in immunosuppressed organ and bone marrow transplant recipients supports a protective role for the immune system against HNSCC development [56–58]. The phenomenon is recognized as immunosurveillance and was first outlined by Thomas and Burnet in the 1950s, and later replaced by the theory of immunoediting coined by Dunn and Schreiber to reflect the increasingly apparent dual role of immunity not only in preventing but also in sculpting the tumoral process [59]. Immunoediting is comprised of three phases, elimination, equilibrium, and escape. A closer look at key players at each of these stages in HNSCC development offers a unique opportunity for successful immunoprevention approaches to modulate immune responses to prevent, halt, or reverse precancerous conditions or cancer development (Fig. 1). A simplified representation of the primary immune microenvironment induced in the "normal mucosa" of smokers is depicted in Fig. 1. In this stage, both chronic inflammation and dysfunctional or altered immune cells co-exist. The resulting immune milieu has been
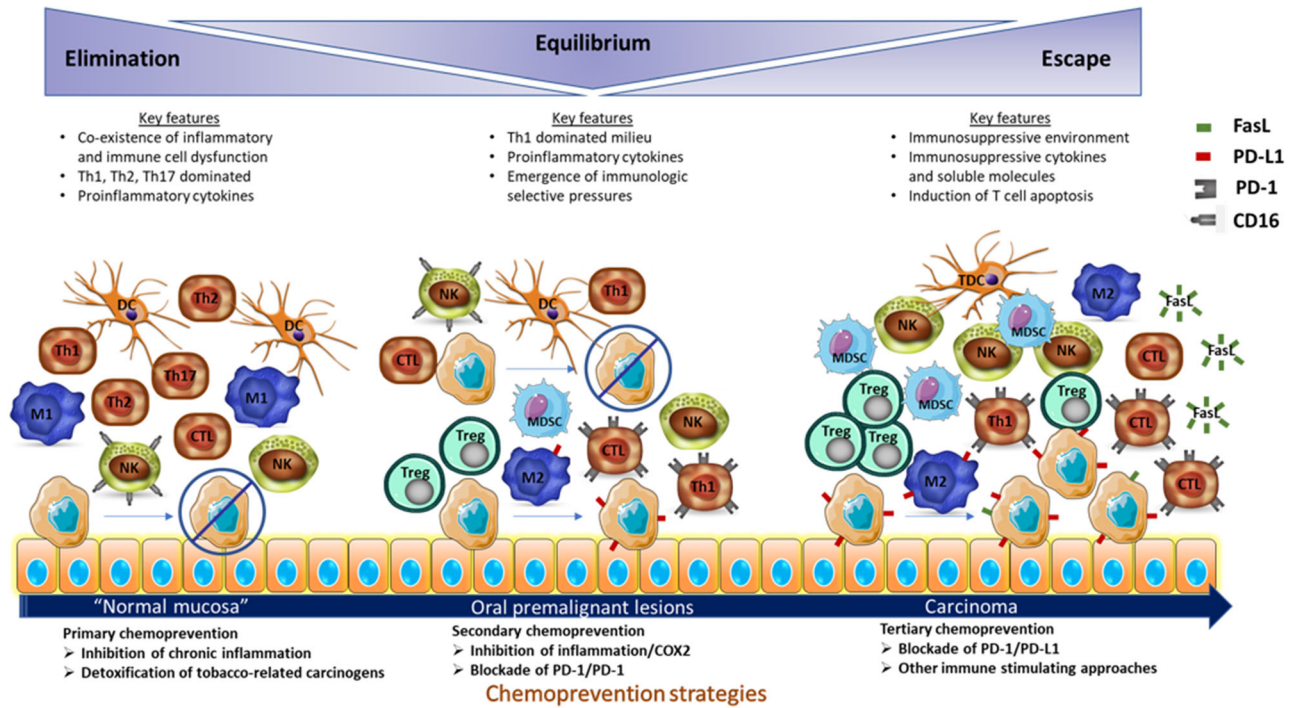
Fig. 1. Model of tobacco-related turmorigenesis and immunoediting. The figure is a simplified model of the evolution of immunoediting as it occurs throughout the process of tobacco-related HNSCC malignant transformation. Elimination in this stage "normal" tobacco-exposed mucosa is capable of identifying and eliminating tumor cells. The microenvironment typically is dominated by Th1, Th2, and Th17 T cells. M1-inflammatory macrophages, NK cells (both functional and dysfunctional), and dendritic cells (with tradition or altered phenotypes) can also be observed. Primary strategies for immunoprevention at this stage may serve to reduce chronic inflammation, or detoxify tobacco-related carcinogens. Equilibrium in this stage the emergence of immunosuppressive features imposes selective pressures on abnormal premalignant cells. Some premalignant lesions may spontaneously regress while others transform into malignancies. These lesions are characterized by the infiltration of Th1-dominated T cells, proinflammatory mediators, and presence of regulatory T cells (Treg), M2-immunosuppressive macrophages (M2), and myeloid-derived suppressors cells (MDSC). PD-L1 expression is also apparent at this stage. Key chemoprevention targets at this stage include blockade of chronic inflammation via COX2 inhibition, and PD-1/PD-L1 checkpoint inhibition. Escape-HNSCC tumors are consider to be largely immunosuppressive in nature. HNSCC tumors are characterized by the presence of multiple immunoevasive features including expansion of immunosuppressive M2 macrophages, MDSCs, Tregs, and tolerogenic dendritic cells (TDC), upregulation of checkpoint molecules PD-1/PDL1 and TIM-3, and immune evasive mechanisms such as expression of soluble and membrane-bound Fas ligand (FasL)

characterized and Th1-, Th2-, and Th17-type inflammation [60]. Other prominent immune cells observed in the oral mucosa of tobacco users include dendritic cells (DC), M1-proinflammatory macrophages, and NK cells. Characterization of dendritic cells with impaired activation, diminished T cell stimulatory capacity, and increased production of Th2-promoting cytokine have been described in this environment [61–64]. Similarly, NK cells from smokers also have been shown to produce significantly less proinflammatory cytokines IFNγ and TNFα, and have reduced cytotoxic functions [65]. Cumulatively, the resulting mucosa can be described as state of permissive chronic inflammation characterized by

impaired antimicrobial functions and dysregulated innate immune responses. Although outside of the scope of this review, specific effects of smoking on individual immune cell subsets have also been characterized (reviewed in [66••]).

The emergence of OPLs is the first observable indication that immunosurveillance may be impaired. Key features at this stage of tumorigenesis include the emergence of immunosuppressive cell mechanisms of immune evasion. Among these are regulatory T cells (Treg), M2-immunosuppressive macrophages, and myeloid-derived suppressor cells [67, 68]. The presence of M2 macrophages observed in OPLs at this stage has been associated with progression to HNSCC [67]. The emergence of checkpoint molecules such as PD-L1 and PD-L2 also becomes apparent at this stage and is associated with risk of malignant transformation, suggesting that PD-L1 inhibition may be an important secondary prevention strategy in OPLs [69]. Th-1 helper T cells are thought to be the predominant T helper cell population within OPLs [70], although Th2 and Th17 cells may also be present [71]. Significantly, the proinflammatory cytokines G-CSF, RANTES, MCP-1, and PGE$_2$ are detectable in OPLs at much higher levels than observed in HNSCC cells [71]. The extent of immunosuppressive cells/features in equilibrium with anti-tumorigenic immunity observable at this stage seems to be a key determining feature of transformation and should be further characterized to identify potential immunoprevention targets.

Once OPLs have progressed to carcinomas, the immune microenvironment is largely immunosuppressive and has acquired permissive features that allow for tumor cell escape [72••]. Increased immunosuppressive mechanisms such as expression of soluble and membrane-bound Fas ligand (FasL) [73, 74], upregulation of immune checkpoint molecules including PD-L1 on tumor cells, antigen-presenting cells, or stromal cells [75], and PD-1 and TIM-3 on T cell subsets [76], combined with influxes of immunosuppressive cell populations including Treg, MDSC, M2-macrophages cancer-associated fibroblasts (CAFs), and tolerogenic DC all contribute to immune escape in HNSCC [67, 75, 77–81]. Although anti-tumoral immune cell subsets such as CD8 and NK cells can also be detected, impaired cytotoxic activity is commonly observed [77, 82]. Immunosuppressive cytokines and other molecules including IL-6, TGF-β, arginase 1, iNOS, and idoleamine 2,3-dioxygenase (IDO), also add to the immunosuppressive milieu. Not surprisingly, patients with higher levels of immunosuppressive features have been shown to have a poorer prognosis [67, 75, 77]. Immunoprevention trials currently ongoing include:

• Anti-PD1 Immune Checkpoint Inhibitors. Immune checkpoint activation, a mechanism evolved to prevent immune attack to normal tissue, is often observed in HNSCC, suppressing anti-tumor immunity [83, 84]. This critical mechanism of maintaining self-tolerance has been exploited for cancer therapy using blocking antibodies for

immune checkpoint receptors such as PD-1. Multiple clinical trials have shown that PD-1 blockade improves survival in patients with recurrent/metastatic HNSCC [85–88]. These observations prompt the question of whether PD-1 inhibitors could also be effective during early stages of HNSCC development and prevent the progression of OPLs. In mouse models, PD-1 blockade has been shown to prevent oral cancer development induced by the tobacco-surrogate 4-nitro-quinoline 1-oxide (4NQO), a carcinogen that induces oral dysplastic lesions that may progress to carcinomas following a stepwise process that resembles human oral cancer progression [89–93]. PD-1 blockade prevented the development and malignant progression of OPLs, associated with recruitment and activation of T cells, and induction of apoptosis in epithelial cells of the oral lesions [89]. These preclinical studies suggest that PD-1 inhibitors could confer preventive benefits to patients with OPLs. A limiting factor to implementing immunoprevention strategies in the clinic is the potential side effects associated with checkpoint inhibitors, which may detract from their use in a relatively healthy patient population. As our understanding of checkpoint inhibitors-related adverse effects increases, their management and prevention are expected to improve, bringing immunoprevention for oral cancer prevention closer to fruition, especially for patients with high-risk OPLs. Currently, there are three ongoing clinical studies in secondary HNSCC populations utilizing nivolumab or pembrolizumab, monoclonal antibodies targeting the PD-1 checkpoint. These studies will evaluate safety and efficacy (NCT03692325), and clinical response (NCT03347838, NCT03603223).

Emerging therapies

- To overcome the potential limitations of immunoprevention strategies based on checkpoint inhibitors, an attractive approach is to use inhibitors of oncogenic pathways that promote immunosuppression, such CDK4/6 or PI3K [94••, 95–98]. Inactivation of these pathways may promote anti-tumor immunity, in addition to the direct anti-tumor effects. As genetic alterations that result in CDK4/6 or PI3K activation have been observed in OPLs, this strategy may be effective in oral cancer prevention.
- Importantly, immunomodulatory effects of agents including NSAIDs and anti-diabetics could be considered an immunoprevention approach. Cruciferous vegetables and other green chemoprevention agents have been shown to reduced inflammation. When evaluating these agents, researchers should consider important off-target effects on the immune system.
- As the field of immunoprevention grows, characterizing the immunomodulatory effects of experimental interventions and matching them with key targets for immunoprevention will be important. This begins with more comprehensive mapping of immune responses across the spectrum up malignant transformation and correlating these phenotypes with clinical outcome and risk. Finally,

it will demand the systematic characterization of immunomodulatory properties of new and approved agents. This dual approach for comprehensive immune profiling for both determination of risk and immune targets combined with creation of a database of available agents would constitute the next generation of precision immunoprevention.

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Declaration

Conflict of Interest
Sara M. Centuori declares that she has no conflict of interest. Carlos Caulin declares that he has no conflict of interest. Julie E. Bauman declares that she has no conflict of interest.

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- • Of importance
- •• Of major importance

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