Prognostic factors, predictive markers and cancer biology: the triad for successful oral cancer chemoprevention

Jose Augusto Monteiro de Oliveira Novaes¹ & William N William Jr^{*1}

Oral squamous cell carcinomas represent a significant cancer burden worldwide. Unfortunately, chemoprevention strategies investigated to date have failed to produce an agent considered standard of care to prevent oral cancers. Nonetheless, recent advances in clinical trial design may streamline drug development in this setting. In this manuscript, we review some of these improvements, including risk prediction tools based on molecular markers that help select patients most suitable for chemoprevention. We also discuss the opportunities that novel preclinical models and modern molecular profiling techniques will bring to the prevention field in the near future, and propose a clinical trials framework that incorporates molecular prognostic factors, predictive markers and cancer biology as a roadmap to improve chemoprevention strategies for oral cancers.

First draft submitted: 1 April 2016; Accepted for publication: 7 June 2016; Published online: 22 June 2016

A major global health concern, oral squamous cell carcinoma (OSCC) is currently the 13th most common cancer worldwide, with an estimate of 300,000 new cases, and 145,000 deaths each year [1]. Tobacco, alcohol and/or betel nut exposure are well-established risk factors for OSCC. Interruption of carcinogen use is an obvious key intervention to reduce oral cancer incidence ('primary' prevention). Nonetheless, OSCC risk remains elevated even after alcohol and tobacco cessation and may take up to 20 years to reach baseline levels [2] illustrating the need for development of approaches that can interrupt/reverse the process of carcinogenesis after initiation, but before full malignant transformation. Furthermore, an increase in the incidence of oral cancer in women with no clear etiologic factors has been observed [3], supporting the need for improved understanding of molecular pathways dysregulation that lead to cancer and how they could be targeted for OSCC prevention.

Oral potentially malignant lesions (OPMLs) are often clinically characterized as leukoplakia and/or erythroplakia. Histologically, these lesions frequently exhibit hyperplasia, hyperkeratosis and/or varying degrees of dysplasia (i.e., intraepithelial neoplasias [IEN]). OPMLs are not obligate precursors of oral cancers, but they are estimated to precede development of at least 7% of OSCC [4]. OPMLs are in and of themselves risk factors for oral cancer, but have a variable frequency of malignant transformation, depending on other clinical, demographic, etiologic, histological and/or molecular features [5]. The anatomic accessibility of OPMLs for examination and biopsies provides an opportunity for clinical and translational studies of oral carcinogenesis. In this review, we will summarize the recent advances and future perspectives of molecularly focused strategies of oral cavity cancers chemoprevention, many of which stem from OPML-based research. We will specifically

KEYWORDS

• biomarkers
• chemoprevention
• head and neck cancer
• leukoplakia • loss of heterozygosity • oral cancer
• oral premalignant lesions
• predictive markers • risk factor

¹Department of Thoracic/Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 432, Houston, TX 77030, USA

*Author for correspondence: Tel.: +1 713 792 6363; Fax: +1 713 792 1220; wnwillia@mdanderson.org

discuss the interplay between prognostic factors, predictive markers and cancer biology, and their possible role as tools to develop clinical strategies to mitigate oral cancer.

Oral cancer chemoprevention

Chemoprevention refers to the use of compounds to prevent, arrest, or reverse the process of malignant transformation. The use of systemic agents is particularly favored to address field cancerization, one of the hallmarks of carcinogen-induced upper aerodigestive tract malignancies. Histologic IEN has been identified in visually normal/healthy tissues geographically separated from OPMLs [6]. Moreover, molecular aberrations associated with oral IEN have been found even in histologically normal appearing mucosa anatomically distant from the index OPML [7], or in histologically negative margins of a resected oral cancer [8,9]. These observations support the existence of ‘molecular IEN’, a term introduced by the American Association for Cancer Research Task Force on Cancer Prevention [10] that recognizes early molecular changes that can occur in the epithelium and place it at risk for cancer development. This ‘hidden’ population of cells can give rise to malignancies after complete resection of an oral cancer, supporting the potential role for ‘tertiary’ chemoprevention to reduce the risk of second primary tumors. Similarly, and equally necessary, is a strategy of ‘secondary’ chemoprevention that addresses OPMLs and distant premalignant clones before they turn into invasive tumors, particularly if deemed high risk for malignant transformation – surgical resection of OPMLs appears to have a limited role in reducing long-term oral cancer risk [11].

Chemoprevention studies in the oral cavity gained traction in the early 1980s with the benchmark randomized trials of retinoids by Hong, Lippman and colleagues and continue to this day using a diverse array of agents [12–17]. None of these studies led to development of a standard-of-care drug for head and neck cancer prevention, either because of lack of (sustained) efficacy, toxicities, or a combination thereof. Most of these clinical trials utilized leukoplakia response rates as the primary efficacy measurement, an end point that can be read out short-term (thus reducing trial complexity, duration and scale), but nowadays considered imperfect, given its weak association with long-term cancer risk [18]. However, in this 35-year period, the

science of clinical trial design has improved, and we are now entering an era of chemoprevention studies focusing on better-defined high-risk groups, in which more clinically meaningful end points can be assessed (such as development of invasive oral cancer). By using such streamlined approaches, it has become more feasible to evaluate agents with activity in advanced disease in the context of premalignancies, thus paving the way for molecular-based chemoprevention.

Trial design

The challenges of chemoprevention clinical trial design have been thoroughly reviewed elsewhere [19]. Central to this aspect is the need to recruit high-risk groups, as this strategy may greatly improve the chances of success of any given study, by optimizing the therapeutic index of the tested drug (i.e., reduction in the risk of cancer versus risk for therapy-related adverse events). Moreover, focusing eligibility on high-risk individuals also allows for a reduction in the sample size and duration of the study, especially if cancer incidence is chosen as the primary end point, because of the higher frequency of expected events.

Specifically in the context of OPML-based chemoprevention trials, it has been recognized that in order to identify these high-risk groups more precisely, we must advance the technology beyond the current methods of stratification of OPML based on histopathology. The presence of dysplasia of moderate to severe degrees is commonly used in risk assessment and as an indication for surgical resection of leukoplakias and erythroplakias. Approaching the stratification of these lesions in this superficial way is flawed at multiple levels: the dogmatic progression of dysplasia to carcinoma *in situ* and then to subsequent invasive cancer is at best inconsistent [20]. One of the reasons for that is the variation between classifications of dysplastic lesions among pathologists [21]. Additionally, histological assessment alone does not provide insights into the molecular pathway dysregulations that drives the carcinogenic process and may confer a malignant phenotype without necessarily causing stepwise ‘pre-malignant’ morphologic changes. In recognition of these limitations, an improvement in risk assessment tools by integrating molecular prognostic markers to histologic and clinical features has been proposed [22]. Loss of heterozygosity (LOH) at specific chromosomal sites in OPML lesions has

been the most robust molecular marker of oral cancer risk identified to date, and the first one to be prospectively tested and validated within the context of a chemoprevention study – the EPOC trial [17]. EPOC was the first precision medicine trial in cancer prevention based on molecular risk assessment and serves a design framework to be improved upon in future prevention studies in OPMLs and premalignant lesions of other sites.

LOH as a prognostic marker of oral cancer risk

Mao *et al.* first recognized LOH at certain chromosomal sites harboring tumor suppressor genes (primarily 3p14 and 9p21) as a marker of cancer risk in a small cohort of 37 patients with OPMLs [23]. Subsequently, two retrospective studies involving 116 and 68 OPML patients confirmed LOH as a prognostic marker of malignant transformation in individuals without [11] and with [24] a prior history of oral cancers, respectively. In an analysis of prospectively collected oral tissue samples of patients with OPMLs, the findings were again confirmed, with LOH-positive patients exhibiting a relative risk of oral cancer of 11.6 compared with LOH-negative individuals [25].

Building on these consistent findings from retrospective analyses, the EPOC clinical trial was designed. In EPOC, OPML patients were first evaluated for LOH, and only those considered to have high-risk profiles by this molecular criterion were randomized to receive the EGFR inhibitor erlotinib versus placebo for 1 year, with a primary end point of oral cancer-free survival [17]. While erlotinib did not reduce the incidence of oral cancer in the randomized population, EPOC demonstrated the feasibility of incorporating molecular risk assessment in chemoprevention trials, and prospectively validated the role of LOH as a prognostic marker in OPML – at 3 years, oral cancer-free survival was 76% for patients with LOH-positive lesions versus 87% for the LOH-negative group (hazard ratio [HR]: 2.19; $p = 0.01$).

Prognostic markers beyond LOH

Several other biomarkers in OPMLs have been investigated and found to be associated with higher cancer risk. These include (but are not limited to) podoplanin [26], deltaNp63 [17], cyclin D1 genotypes [27], specific mRNA expression [28] and DNA methylation [29]

profiles, miRNA-31 [30], human telomerase RNA component (*hTERC*) gene copy number [31], ABCG2 and BMI-1 expression [32], and EGFR protein, mRNA expression and gene copy number gains [33]. Most of these studies, however, consisted of retrospective analyses, including varying (but often limited) number of specimens, without validation on independent datasets.

Of particular interest is the role of EGFR in oral carcinogenesis, since this is the only of the aforementioned markers that has been shown to be a validated therapeutic target in advanced oral cancers [34]. In OPML patients enrolled in a retinoid/beta-carotene chemoprevention trial, EGFR protein overexpression and gene copy number gain was associated with increased risk of development of OSCC [33]. Likewise, Poh *et al.* observed that *EGFR* gene copy number gain occurred more frequently in lesions that subsequently progressed to cancer versus not [35]. In the EPOC trial, *EGFR* gene copy number gain was evaluated by FISH in a post-hoc analysis, and a novel association between LOH high-risk profiles and *EGFR* gene copy number gain was identified [17]. Individuals that were LOH-positive/*EGFR* FISH-negative or LOH-negative/*EGFR* FISH-positive were at intermediate risk for oral cancer (HR: 1.8) when compared with LOH-negative/*EGFR* FISH-negative groups; patients that were both LOH positive and *EGFR* FISH positive were at the highest risk of oral cancer (HR: 2.9). Nonetheless, the latter group still did not benefit from erlotinib, indicating that *EGFR* gene copy number was a prognostic marker of cancer risk, but not a predictive marker of activity of EGFR inhibitors within the context of OPMLs.

Predictive vs prognostic markers

The EPOC clinical trial serves as a case study to illustrate that chemoprevention strategies based solely on high-risk populations may not be sufficient to reduce the incidence of invasive cancers. Although the association between LOH positivity (prognostic marker and eligibility criterion) and *EGFR* gene copy number gain (possible predictive marker) provided further rationale for the use of erlotinib, the lack of efficacy of this drug even in the *EGFR* FISH-positive group clearly demonstrates the need for a better understanding of the molecular pathways that are pivotal for malignant transformation, as well as determination of the degree of dependency of premalignant

clones on these pathways. Likewise, strategies focused on predictive markers alone may miss the opportunity of treating the population in greatest need for chemoprevention, especially if there is a dissociation between risk and prevalence of the predictive marker – for example, a specific marker may confer sensitivity to a given drug, but the incidence of cancer among carriers of such biomarker may be so low that a trial in this marker-defined subgroup may not be feasible or relevant.

Identification of predictive biomarkers carry unique challenges that may explain their paucity in the prevention literature. Predictive biomarkers of benefit from pharmacologic agents can most reliably be identified in randomized clinical trials containing a placebo/no treatment group. Oftentimes, even in randomized trials, the power for detecting such biomarkers may be severely limited by the study's sample size. An understanding of the biology of candidate predictive biomarkers can also assist in evaluating their true value in forecasting drug efficacy.

To our knowledge, no robust predictive biomarkers of benefit from any pharmacologic agents have been identified in OPMLs. Notwithstanding, data from other organ sites demonstrate the feasibility of identifying predictive markers from chemopreventive interventions. In colorectal adenomas, high urinary prostaglandin E-M was found to be a biomarker predictive of benefit from aspirin [36]; single nucleotide polymorphisms at chromosomes 12 and 15 were associated with a benefit from aspirin/NSAIDs in preventing colorectal cancers [37]; benefits from tamoxifen in reducing breast cancer risk after ductal carcinoma *in situ* resection (lumpectomy) and radiation were confined to estrogen receptor positive groups [38].

Two aspects of translational research may be pivotal to advance identification of novel targets for chemoprevention and associated predictive markers within the context of OPMLs: improvements of preclinical models specific to premalignancies that can interrogate the role of key molecular pathways that may drive oral carcinogenesis and how their targeting can arrest or reverse this process; and comprehensive molecular profiling of OPMLs in clinically annotated datasets that would allow us to focus clinical and translational efforts in events that can be pharmacologically addressed and are most relevant to OSCC development in humans.

Supsavhad *et al.* recently reviewed available animal models of head and neck squamous cell carcinomas [39]. Of note is the development of oral premalignant lesions in carcinogen-induced models that can be leveraged for preclinical chemoprevention studies. Differences in biological behavior compared with human OSCC, and distinct molecular profiling of some of these models need to be taken into account when evaluating experimental data. Nonetheless, striking similarities in the genetic make-up of mouse oral carcinomas to human counterparts has been identified, for example, in the mouse oral cancer (MOC) syngeneic models [40]. Coupled with novel gene engineering techniques [41], these can become powerful tools to investigate biomarkers and pathways relevant to oral cancer prevention.

A progression model of carcinogenesis in human OPMLs has been proposed, initially based on chromosomal alterations harboring tumor suppressor genes and oncogenes [42], and subsequently based on transcriptomic changes [43]. The association of these models with clinical outcomes of OPMLs has never been comprehensively investigated. Moreover, a thorough characterization of the genetic landscape of OPMLs has never been performed. Again, novel molecular profiling techniques and bioinformatic approaches for integrative analysis of large-scale, clinically annotated data will certainly contribute to our understanding of the driver events of malignant transformation and how they can potentially be targeted, in analogy to the advances that The Cancer Genome Atlas (TCGA) Project has brought to management of invasive malignancies.

The case for an oral Pre-Cancer Genome Atlas

At least four groups have completed next-generation sequencing of invasive head and neck squamous cell carcinomas/OSCC [44–47]. TCGA represents the most comprehensive of these analyses and also includes proteomic and RNA profiling of specimens, as well as limited clinical data. These efforts have clearly demonstrated that: head and neck cancers can be classified in different subtypes, according to their molecular profiling, with implications as regards to outcomes and possibly, response to therapy [46]; a subset of head and neck cancers exhibit targetable alterations that can now be explored for therapeutic approaches [48]; and differences in molecular profiles have been observed according to

etiologic factors (e.g., viral versus non-viral) [48] and geographic origin of the patients (Indian vs Caucasian populations, and possibly use of smokeless tobacco vs not) [47], illustrating the need to identify pathways that are common and unique to specific subpopulations and may play a role in determining the best pharmacologic management.

Unfortunately, a similar effort in precancerous lesions has not been completed. The rationale for a Pre-Cancer Genome Atlas has recently been articulated by Campbell *et al.* [49]. Specific to OPMLs, the recent identification of *NOTCH-1* gain of function mutations in oral lesions of Chinese patients illustrates the potential implications of large-scale genomic analysis for cancer prevention [50]. Contrary to invasive head and neck cancers in Caucasian populations, these *NOTCH-1* mutations seem to be activating and could well represent the first actionable gene point mutation identified in OPMLs. It remains to be determined if and why these mutations occur at higher frequency in individuals from Han Chinese descent, their possible association with high content alcoholic beverage consumption (which is very prevalent in that part of the world), and if they are true drivers of malignant transformation. Notch-1 protein signaling acts on a broad spectrum of functions in cell cycle homeostasis, playing the role of either an oncogene or a tumor suppressor gene [51]. Elucidating the phenotypic consequences of these mutations in OPMLs could support or discourage investigations of *NOTCH-1* pathway inhibitors to prevent oral cancers, specifically in patients harboring a *NOTCH-1* mutation, thus illustrating how Pre-Cancer Genome Atlas could contribute to development of precision medicine in the prevention setting. One expects that additional targetable molecular events will be discovered upon completion of a comprehensive molecular profiling effort of OPMLs, coupled with information on their associations with cancer risk. Nonetheless, it is clear that the presence of gene mutations alone will not be enough to determine the therapeutic potential of such findings. As an example, all areas of benign melanocytic neoplasms surveyed in a cohort of 37 patients with histological evidence of melanoma and distinct precursors exhibited *B-RAF* V600E as the only pathogenic mutation, but intermediate lesions had a broader spectrum of initiating oncogene mutations (e.g., *B-RAF*V600K or K601E and *N-RAS* mutations). Invasive melanomas had distinct

patterns of mutations whether they arose from benign nevi (mostly *B-RAF* V600E plus additional mutations) or intermediate lesions (mostly *B-RAF*V600K or K601E and *N-RAS* mutations, plus additional mutations) [52]. Because not all benign melanocytic neoplasms transform into invasive cancer, understanding the sequence in which these mutations occur and how they influence cancer progression will be crucial to determine if, and when they should be targeted at the premalignancy stage. Likewise, gene sequencing of sun-exposed normal eyelid skin identified positively selected mutations in 18–32% of the samples, and included most of the key drivers of squamous cell carcinomas [53], illustrating the challenges of pinpointing which, if any of these mutation-harboring clones will progress to invasive carcinoma and how to arrest such process. We envision that evaluation of the biologic consequences of such mutations will be important to inform which molecular events should be targeted in the clinic for prevention. The same concepts can be applied to the study of OPMLs.

Future perspective

Oral cancer chemoprevention is now entering a new area of molecular-based approaches and we predict that, in the next decade, significant advances will be achieved as regards to our understanding of the molecular processes of oral carcinogenesis. The success of oral cancer chemoprevention will be largely determined by or ability to leverage this knowledge to improve clinical trial design and selection of agents to be tested in this setting. We have already demonstrated the feasibility of incorporating molecular risk assessment into prevention trials eligibility criteria. The next step will likely include identification of markers of cancer risk that can also act as predictive markers of benefit from any given drug, especially if they serve as pharmacologic targets and actively participate in the process of carcinogenesis. We propose that the highest priority for clinical development should be assigned to biomarker/drug combos that fulfill all of the following criteria: biomarkers that are associated with cancer risk; drugs that have improved activity in biomarker positive subpopulations; and biomarkers that have been validated in independent clinical datasets and/or preclinical models of oral carcinogenesis. The strategy of integrating prognostic factors, predictive markers and cancer biology will hopefully lead to the development of precision medicine approaches

that will become new standards of care for oral cancer chemoprevention.

Financial & competing interests disclosure

This manuscript received funding from the following grants: P30 CA016672 from the National Cancer Institute – NIH; RP140464 from the Cancer Prevention and

Research Institute of Texas. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

- Oral squamous cell carcinomas are often (but not always) associated with tobacco, alcohol and betel nut exposure, and represent a significant cancer burden worldwide.

Oral cancer chemoprevention

- Chemoprevention of oral cancers has been studied for more than three decades and so far, these investigations have failed to produce a pharmacologic strategy considered standard of care.

Trial design

- Focusing chemoprevention trial designs to enroll high risk populations may streamline drug development in this setting.

Prognostic markers of oral cancer risk

- Loss of heterozygosity profiles have been the most robust prognostic molecular marker of oral cancer risk identified to date, and the only one to be successfully incorporated as molecular eligibility criteria in an oral cancer chemoprevention clinical trial.

Predictive vs prognostic markers

- Chemoprevention strategies solely based on prognostic biomarkers may be insufficient to result in significant oral cancer risk reduction; incorporation of predictive markers of benefit into clinical trial may be needed, identified in preclinical models and/or integrative analyses of molecular profiling and clinical data.

The case for an oral Pre-Cancer Genome Atlas

- Comprehensive gene sequencing and RNA and DNA profiling of oral premalignant lesions may identify pathways key to the carcinogenic process, and novel targets for cancer prevention.

Future perspective

- The next generation of oral cancer chemoprevention trials will likely succeed if their design fully integrates the triad of molecular prognostic factors, predictive markers and cancer biology.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

- Ferlay J, Soerjomataram I, Dikshit R *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* 136(5), E359–386 (2015).
- Marron M, Boffetta P, Zhang ZF *et al.* Cessation of alcohol drinking, tobacco smoking and the reversal of head and neck cancer risk. *Int. J. Epidemiol.* 39(1), 182–196 (2010).
- Patel SC, Carpenter WR, Tyree S *et al.* Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. *J. Clin. Oncol.* 29(11), 1488–1494 (2011).
- Yanik EL, Katki HA, Silverberg MJ, Manos MM, Engels EA, Chaturvedi AK. Leukoplakia, oral cavity cancer risk, and cancer survival in the U.S. elderly. *Cancer Prev. Res. (Phila.)* 8(9), 857–863 (2015).
- William WN Jr. Oral premalignant lesions: any progress with systemic therapies? *Curr. Opin. Oncol.* 24(3), 205–210 (2012).
- Warnakulasuriya KA, Johnson NW. Sensitivity and specificity of OraScan (R) toluidine blue mouthrinse in the detection of oral cancer and precancer. *J. Oral Pathol. Med.* 25(3), 97–103 (1996).
- Pentenero M, Donadini A, Di Nallo E *et al.* Field effect in oral precancer as assessed by DNA flow cytometry and array-CGH. *J. Oral Pathol. Med.* 41(2), 119–123 (2012).
- Voravud N, Shin DM, Ro JY, Lee JS, Hong WK, Hittelman WN. Increased polysomies of chromosomes 7 and 17 during head and neck multistage tumorigenesis. *Cancer Res.* 53(12), 2874–2883 (1993).
- Tabor MP, Brakenhoff RH, Van Houten VM *et al.* Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. *Clin. Cancer Res.* 7(6), 1523–1532 (2001).
- Kelloff GJ, Lippman SM, Dannenberg AJ *et al.* Progress in chemoprevention drug development: the promise of molecular biomarkers for prevention of intraepithelial neoplasia and cancer – a plan to move forward. *Clin. Cancer Res.* 12(12), 3661–3697 (2006).

- 11 Rosin MP, Cheng X, Poh C *et al.* Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin. Cancer Res.* 6(2), 357–362 (2000).
- 12 Hong WK, Endicott J, Itri LM *et al.* 13-cis-retinoic acid in the treatment of oral leukoplakia. *N. Engl. J. Med.* 315(24), 1501–1505 (1986).
- **Seminal retinoid clinical trial in leukoplakias that paved the way for subsequent chemoprevention studies.**
- 13 Hong WK, Lippman SM, Itri LM *et al.* Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 323(12), 795–801 (1990).
- 14 Lippman SM, Batsakis JG, Toth BB *et al.* Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. *N. Engl. J. Med.* 328(1), 15–20 (1993).
- 15 Papadimitrakopoulou VA, William WN Jr, Dannenberg AJ *et al.* Pilot randomized Phase II study of celecoxib in oral premalignant lesions. *Clin. Cancer Res.* 14(7), 2095–2101 (2008).
- 16 Armstrong WB, Taylor TH, Kennedy AR *et al.* Bowman birk inhibitor concentrate and oral leukoplakia: a randomized Phase IIb trial. *Cancer Prev. Res. (Phila.)* 6(5), 410–418 (2013).
- 17 William WN Jr, Papadimitrakopoulou V, Lee JJ *et al.* Erlotinib and the risk of oral cancer: the Erlotinib Prevention of Oral Cancer (EPOC) randomized clinical trial. *JAMA Oncol.* 2(2), 209–216 (2016).
- **First large-scale, molecular-based precision medicine trial in cancer prevention, and the first study in oral premalignant lesions to use cancer as the primary end point.**
- 18 Papadimitrakopoulou VA, Lee JJ *et al.*, William WN Jr Randomized trial of 13-cis retinoic acid compared with retinyl palmitate with or without beta-carotene in oral premalignancy. *J. Clin. Oncol.* 27(4), 599–604 (2009).
- 19 William WN Jr, Heymach JV, Kim ES, Lippman SM. Molecular targets for cancer chemoprevention. *Nat. Rev. Drug Discov.* 8(3), 213–225 (2009).
- **Comprehensive discussion of chemoprevention clinical trial designs.**
- 20 Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. *J. Oral Pathol. Med.* 37(3), 127–133 (2008).
- 21 Kujan O, Khattab A, Oliver RJ, Roberts SA, Thakker N, Sloan P. Why oral histopathology suffers inter-observer variability on grading oral epithelial dysplasia: an attempt to understand the sources of variation. *Oral Oncol.* 43(3), 224–231 (2007).
- 22 Lee JJ, Hong WK, Hittelman WN *et al.* Predicting cancer development in oral leukoplakia: ten years of translational research. *Clin. Cancer Res.* 6(5), 1702–1710 (2000).
- 23 Mao L, Lee JS, Fan YH *et al.* Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nat. Med.* 2(6), 682–685 (1996).
- **Pivotal study identifying loss of heterozygosity profiles as a molecular marker of cancer risk in oral leukoplakia.**
- 24 Rosin MP, Lam WL, Poh C *et al.* 3p14 and 9p21 loss is a simple tool for predicting second oral malignancy at previously treated oral cancer sites. *Cancer Res.* 62(22), 6447–6450 (2002).
- 25 Zhang L, Poh CF, Williams M *et al.* Loss of heterozygosity (LOH) profiles – validated risk predictors for progression to oral cancer. *Cancer Prev. Res. (Phila.)* 5(9), 1081–1089 (2012).
- 26 Kawaguchi H, El-Naggar AK, Papadimitrakopoulou V *et al.* Podoplanin: a novel marker for oral cancer risk in patients with oral premalignancy. *J. Clin. Oncol.* 26(3), 354–360 (2008).
- 27 Izzo JG, Papadimitrakopoulou VA, Liu DD *et al.* Cyclin D1 genotype, response to biochemoprevention, and progression rate to upper aerodigestive tract cancer. *J. Natl Cancer Inst.* 95(3), 198–205 (2003).
- 28 Saintigny P, Zhang L, Fan YH *et al.* Gene expression profiling predicts the development of oral cancer. *Cancer Prev. Res. (Phila.)* 4(2), 218–229 (2011).
- 29 Foy JP, Pickering CR, Papadimitrakopoulou VA *et al.* New DNA methylation markers and global DNA hypomethylation are associated with oral cancer development. *Cancer Prev. Res. (Phila.)* 8(11), 1027–1035 (2015).
- 30 Maimaiti A, Abudoukeremu K, Tie L, Pan Y, Li X. MicroRNA expression profiling and functional annotation analysis of their targets associated with the malignant transformation of oral leukoplakia. *Gene* 558(2), 271–277 (2015).
- 31 Dorji T, Monti V, Fellegara G *et al.* Gain of hTERT: a genetic marker of malignancy in oral potentially malignant lesions. *Hum. Pathol.* 46(9), 1275–1281 (2015).
- 32 Liu W, Feng JQ, Shen XM, Wang HY, Liu Y, Zhou ZT. Two stem cell markers, ATP-binding cassette, G2 subfamily (ABCG2) and BMI-1, predict the transformation of oral leukoplakia to cancer: a long-term follow-up study. *Cancer* 118(6), 1693–1700 (2012).
- 33 Taoudi Benchekroun M, Saintigny P, Thomas SM *et al.* Epidermal growth factor receptor expression and gene copy number in the risk of oral cancer. *Cancer Prev. Res. (Phila.)* 3(7), 800–809 (2010).
- 34 Vermorken JB, Mesia R, Rivera F *et al.* Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N. Engl. J. Med.* 359(11), 1116–1127 (2008).
- 35 Tsui IF, Poh CF, Garnis C, Rosin MP, Zhang L, Lam WL. Multiple pathways in the FGF signaling network are frequently deregulated by gene amplification in oral dysplasias. *Int. J. Cancer* 125(9), 2219–2228 (2009).
- 36 Bezawada N, Song M, Wu K *et al.* Urinary PGE-M levels are associated with risk of colorectal adenomas and chemopreventive response to anti-inflammatory drugs. *Cancer Prev. Res. (Phila.)* 7(7), 758–765 (2014).
- 37 Nan H, Hutter CM, Lin Y *et al.* Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. *JAMA* 313(11), 1133–1142 (2015).
- 38 Allred DC, Anderson SJ, Paik S *et al.* Adjuvant tamoxifen reduces subsequent breast cancer in women with estrogen receptor-positive ductal carcinoma *in situ*: a study based on NSABP protocol B-24. *J. Clin. Oncol.* 30(12), 1268–1273 (2012).
- 39 Supsavhad W, Dirksen WP, Martin CK, Rosol TJ. Animal models of head and neck squamous cell carcinoma. *Vet J.* 210, 7–16 (2016).
- 40 Onken MD, Winkler AE, Kanchi KL *et al.* A surprising cross-species conservation in the genomic landscape of mouse and human oral cancer identifies a transcriptional signature predicting metastatic disease. *Clin. Cancer Res.* 20(11), 2873–2884 (2014).
- 41 Mou H, Kennedy Z, Anderson DG, Yin H, Xue W. Precision cancer mouse models through genome editing with CRISPR-Cas9. *Genome Med.* 7(1), 53 (2015).
- 42 Califano J, Van Der Riet P, Westra W *et al.* Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res.* 56(11), 2488–2492 (1996).
- **Proposes a molecular model for head and neck carcinogenesis, taking into account the field cancerization effect.**
- 43 Ha PK, Benoit NE, Yochem R *et al.* A transcriptional progression model for head

- and neck cancer. *Clin. Cancer Res.* 9(8), 3058–3064 (2003).
- 44 Agrawal N, Frederick MJ, Pickering CR *et al.* Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science* 333(6046), 1154–1157 (2011).
- 45 Stransky N, Egloff AM, Tward AD *et al.* The mutational landscape of head and neck squamous cell carcinoma. *Science* 333(6046), 1157–1160 (2011).
- 46 Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 517(7536), 576–582 (2015).
- **Comprehensive evaluation of somatic genomic alterations in invasive head and neck squamous cell carcinomas.**
- 47 India Project Team of the International Cancer Genome Consortium. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. *Nat. Commun.* 4, 2873 (2013).
- 48 Pickering CR, Zhang J, Yoo SY *et al.* Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov.* 3(7), 770–781 (2013).
- 49 Campbell JD, Mazzilli SA, Reid ME *et al.* The case for a Pre-Cancer Genome Atlas (PCGA). *Cancer Prev. Res. (Phila.)* 9(2), 119–124 (2016).
- 50 Izumchenko E, Sun K, Jones S *et al.* Notch1 mutations are drivers of oral tumorigenesis. *Cancer Prev. Res. (Phila.)* 8(4), 277–286 (2015).
- 51 Yap LF, Lee D, Khairuddin A *et al.* The opposing roles of NOTCH signalling in head and neck cancer: a mini review. *Oral Dis.* 21(7), 850–857 (2015).
- 52 Shain AH, Yeh I, Kovalyshyn I *et al.* The genetic evolution of melanoma from precursor lesions. *N. Engl. J. Med.* 373(20), 1926–1936 (2015).
- **Example of genetic characterization of premalignant lesions that can give insights into the process of molecular carcinogenesis.**
- 53 Martincorena I, Roshan A, Gerstung M *et al.* Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348(6237), 880–886 (2015).