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1 **TITLE PAGE**

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50 **Simple Summary**

51 Oral cavity is the most common site of head and neck cancer which is ranked as the 8th most common
52 cancer worldwide. Oral cancer treatment is often associated with significant morbidity and is sometimes
53 ineffective. These cancers, mainly due to tobacco and alcohol consumption, can develop from oral
54 potentially malignant disorders, the most common of which is oral leukoplakia. Some of these oral
55 potentially malignant disorders disappear, while others will transform to oral cancer. Patients may also
56 develop cancer in the field of cancerization. Unfortunately, except for the surgical excision of lesions
57 with dysplasia, there is no effective intervention to effectively prevent transformation or cancer
58 development in the field of cancerization. Moreover, no standardized biomarker has been clearly
59 identified as sufficient to predict malignant transformation. In this article, several experts discuss the
60 main challenges in oral cancer prevention, in particular the need (i) to define new a new classification
61 system integrating cellular and molecular features aiming (ii) at better identifying patients at high risk
62 of malignant transformation, and (iii) at developing treatment strategies to prevent their malignant
63 transformation of oral potentially malignant disorders.

64

65 **Abstract**

66 Oral potentially malignant disorders (OPMD) may precede oral squamous cell carcinoma (OSCC).
67 Reported rate of malignant transformation of OPMD ranged from 3 to 50%. While some clinical,
68 histological, and molecular factors have been associated with a high-risk OPMD, they are to date
69 insufficiently accurate for treatment decision making. Moreover, this range highlights differences in
70 clinical definition of OPMD, variation in follow-up periods, and molecular and biological heterogeneity
71 of OPMD. Finally, while treatment of OPMD may improve outcome, standard therapy has been shown
72 to be ineffective to prevent OSCC development in patients with OPMD. In this perspective paper,
73 several experts discuss the main challenges in oral cancer prevention, in particular the needs (i) to define
74 an OPMD classification system by integrating new pathological and molecular characteristics aiming

75 (ii) to better identify OPMD at high risk of malignant transformation, and (iii) to develop treatment
76 strategies to eradicate OPMD or prevent malignant transformation.

77

78 **Key words (MeSH):**

79 Oral premalignant disorders, oral preneoplasia, oral cancer, prevention, diagnosis

80 INTRODUCTION

81 Oral cavity is the most common site of Head and Neck Squamous Cell Carcinoma (HNSCC) which is
82 ranked as the 8th most common cancer worldwide [1]. Oral SCC (OSCC) is a major cause of morbidity
83 and mortality [2,3]. OSCC are preceded by mucosal precancerous changes that might be visible as white
84 (leukoplakia) or red (erythroplakia) lesions, but are mostly not macroscopically visible, which explains
85 that most OSCC seem to develop *de novo*. However, the preceding precancerous changes can present
86 under the microscope as abnormal mucosal epithelium, also indicated as dysplasia, graded as mild
87 moderate and severe, or they can be identified by genetic markers. In 2017, the World Health
88 Organization (WHO) has defined oral potentially malignant disorders (OPMD) as “clinical
89 presentations that carry a risk of cancer development in the oral cavity, whether in a clinically definable
90 precursor lesion or in clinically normal mucosa” [4]. Thus OPMD may precede OSCC, and may be
91 visible or not [5]. While it is traditionally assumed that OPMD and OSCC are associated with similar
92 risk factors (*e.g.*, alcohol, tobacco, betel quid), a proportion of OPMD and OSCC cases occur in the
93 complete absence of any identifiable risk factor, particularly in young patients never drinkers/smokers
94 [6–9]. The overall worldwide prevalence of OPMD is about 4.5% [10]. The main risk factors of malignant
95 transformation of OPMD described to date are patient related, clinical (*e.g.*, female, >50 years; non-
96 smoker with a nonhomogeneous red lesion of the tongue and floor of mouth >200 mm² and existing for
97 several years; history of previous OSCC; diabetes mellitus), tumor related, histological (*i.e.*, severe
98 dysplasia), and molecular factors (*i.e.*, aneuploidy, loss of heterozygosity [LOH]). The reported
99 malignant transformation rates range from 3 to 66%, indicating that variable definitions may be used,
100 data with different follow-up periods have been collected and the existence of histological and in
101 particular molecular heterogeneity of OPMD [11–15]. For OPMDs that are visible standard policy is to
102 take multiple, repeated, and deep incision biopsies to check for invasive growth and dysplasia.
103 Treatment of the OPMD may prevent malignant transformation and improve outcome [6,11]. The
104 surgical excision of OPMD can decrease the risk of malignant transformation at the same site, but it
105 does not eliminate the risk of subsequent development of SCC at other sites [16]. To date, no standard

106 therapy has been shown to be effective in patients with OPMD to prevent OSCC development in the
107 entire field of cancerization [17].

108 Main challenges are (i) to define an OPMD classification system integrating new pathological and
109 molecular characteristics aiming (ii) to better identify OPMD at high risk of malignant transformation,
110 and (iii) to develop prevention strategies that would treat both the visible lesion and the entire field of
111 cancerization [18,19]. Large longitudinal studies of OPMD case with malignant transformation, as the
112 most relevant clinical outcome, are required.

113 Pathological perspective

114 As defined in the recent OPMD WHO classification, OPMD include fifteen disorders affecting the oral
115 mucosa (*e.g.* leukoplakia, erythroplakia, proliferative verrucous leukoplakia, oral submucous fibrosis
116 ...) and which are either secondary to genetic aberrations, exposure to exogenous factors such as tobacco
117 and/or immune-mediated disorders or related to rare inherited diseases [4,20]. The different histologic
118 features, especially those usually used to grade dysplasia (architectural and cytologic changes...) have
119 been reviewed elsewhere [21].

120 The histopathological diagnosis and grading of dysplasia are the gold standard in guiding OPMD
121 management. Unfortunately, especially in the oral cavity, it is challenging because of the high degree of
122 inter and intra-observer variability, resulting in limited value of grading of dysplasia as a predictive
123 factor for OPMD malignant transformation [22,23]. The WHO classification postulates that the more
124 advanced the degree of dysplasia, the higher the likelihood of developing oral squamous cell carcinoma
125 (OSCC). However, literature reports that OSCC may also arise from seemingly non-dysplastic
126 epithelium. The histology of these lesions is subtle and easily underdiagnosed. In particular, by
127 studying the abnormalities in the mucosa surrounding OSCC, it was recently shown that the dysplastic
128 changes are most commonly subtle (70%, with the features of so-called differentiated dysplasia) and
129 therefore may easily be undervalued by the pathologist [24]. To improve the dysplasia diagnosis,
130 authors proposed refined histopathological criteria, and have shown that immunohistochemistry with
131 antibodies against cytokeratin 13, cytokeratin 17, and Ki67 is a useful diagnostic adjunct. It has been
132 shown that compared to the classic histologic criteria (Who 2017), differentiated dysplasia improves the
133 prediction of oral leukoplakia at increased risk of malignant progression [25]. To address the issues in
134 histological diagnosis and grading of dysplasia, we should develop refined and standardized
135 histopathological criteria encompassing the various histological appearances for reliable diagnosis of
136 OPMD and implement validated immunohistochemical and molecular biomarkers.

137 In addition, Artificial Intelligence methods is becoming a powerful diagnostic adjunct [26]. In particular,
138 machine learning and deep learning algorithms are promising for diagnostic support (enhance

139 laboratory efficiency & quality assurance), as disruptive technology to standard biomarkers, and to
140 derive patterns not achievable by a human observer [27]. Although this field is rapidly evolving,
141 currently very few algorithms have reached clinical implementation [28].

142

143 Biomarkers, prospective high-risk cohorts with embedded trials

144 Besides clinical and histological characteristics of OPMD [4], several biomarkers have been proposed to
145 identify patients with OPMD at high risk of OSCC development [29]. LOH at specific chromosomal
146 sites (3p14 or 9p21) has been validated prospectively [30]. LOH was also found to be a biomarker
147 predicting the development of second oral malignancies in patients with an OPMD subsequent to the
148 treatment of a OSCC [31,32]. Prospective cohorts with long-term follow-up of patients with OPMD are
149 needed to identify other predictive biomarkers that may be used for clinical practice.

150

151 Biology of precancerous changes

152 In 1953 Slaughter *et al.*, concluded from histopathological studies of oral cancer specimen: '*From the*
153 *foregoing observations it would appear that epidermoid carcinoma of the oral stratified squamous epithelium*
154 *originates in a process of "field cancerization," in which an area of epithelium has been preconditioned by an as-*
155 *yet-unknown carcinogenic agent. Such a carcinogenic influence if operative enough in time and intense enough*
156 *in exposure produces an irreversible change in cells and cell groups in the given area, so that change of the process*
157 *toward cancer becomes inevitable.*' [33]. It is remarkable that this model was already reported before
158 tobacco and alcohol were identified as the major culprits of OSCC, and before the scientific world had
159 any clue on molecular carcinogenesis and the role of mutated cancer genes. At present we know that
160 cancer arises by the accumulation of genetic and epigenetic changes, causing a changed circuitry of
161 many signal transduction routes and invoking the acquired capabilities of cancer cells characterized as
162 the 'hallmarks of cancer' [34]. Hence, the onset and driving force of carcinogenesis is the accumulation
163 of genetic changes, albeit stroma interactions likely play a role in parallel. The genetic changes occurring
164 during oral carcinogenesis are now well defined [33,35–38]. Typical chromosomal changes such as loss

165 of 3p, 9p, and 17p that are frequently found in invasive HNSCC, are also found in precancerous changes,
166 and are in fact the most accurate predictors of malignant transformation of the OPMD as discussed
167 above [30].

168 Given the causal role of genetic changes in carcinogenesis, the upper aerodigestive tract field
169 cancerization may be explained, at least partially, by the accumulation of genetic changes in the mucosal
170 keratinocytes. There are no specific markers of stem cells in the mucosa, but we may assume that these
171 exist in the basal layer of the mucosal epithelium. The stemness of such cells is not intrinsic and fixed,
172 but most likely the result of a dynamic process as it is in the intestine [39]. These stem cells form the
173 basis of the mucosal units of transit, amplifying cells and differentiating cells in areas of approximately
174 200 cells wide, which together make up the mucosal epithelium. This clonal unit was demonstrated in
175 mouse epidermis using Axin2 lineage tracing experiments [40]. A somatic mutation in such a cell with
176 stemness properties will give rise to a mutated clonal unit as first described in 2002 using *TP53*
177 mutations as molecular marker [41]. These rare somatic mutations in cells have since then been shown
178 in numerous tissues and are studied using next generation sequencing approaches [42,43]. The mutated
179 cells compete with the wild type cells. In the skin, UV-induced cell death of normal cells supports the
180 extension of the preneoplastic cells [44]. In the esophagus, oxidative stress has been identified as a
181 potential factor that supports the proliferation of TP53-mutated cells over the wild type cells [45]. When
182 applying N-acetylcysteine (NAC) as oxidative stress reducing agent, the balance was shifted in
183 advantage of wild type cells. However, no effect of NAC to prevent recurrent cancer or second primary
184 tumors in both lung and head and neck cancer patients was seen in the EUROSCAN trial [46].

185 Besides environmental factors that may favor growth of genetically damaged cells, accumulation of
186 subsequent genetic alterations may induce a growth advantage and change the balance between normal
187 cells and genetically damaged cells, the latter displacing the normal mucosa by so far unresolved
188 mechanisms. It is likely not related to proliferation rate as normal keratinocytes, precancer and cancer
189 cells may have comparable cell division times, at least *in vitro* [47].

190

191 Field of cancerization

192 A field should be defined as group of cells with tumor-associated somatic genetic alterations.
193 Irrespective of the underlying biology and cellular interaction, the preneoplastic fields will develop in
194 time and can reach dimensions greater than 10 cm in diameter. As explained above, the minority is
195 clinically visible as asymptomatic persistent white or red lesion that cannot be rubbed off [20]. The
196 clinical aspect is poorly specific of OPMD given that not all lesions harbor histologically proven
197 dysplasia [25]. Hence, the visible lesions form the tip of the iceberg. Indeed, some normal surgical
198 margins of oral cancer specimen showed genetic changes, indicating that not all precancerous fields are
199 recognized by histology, and that we must rely on genetic markers to identify all potentially malignant
200 fields. However, with the introduction of differentiated dysplasia as novel morphological entity [24,25],
201 this may change soon. Whether they are visible or not, these potentially malignant changes may
202 transform into invasive cancers. The tumors are diagnosed and treated, but particularly when these
203 fields are not visible to the naked eye, they may stay behind and cause local relapses clinically diagnosed
204 either as local recurrence or second primary tumor depending on the distance (2 cm and/or different
205 subsite) and the time interval (3 years) [35,36].

206 In vitro cultures of visible lesions were reported in 2002 [48]. More recently, 98 2D cultures from normal
207 appearing mucosa of the surgical margins of patients with primary HNSCC were generated and
208 characterized for their molecular alterations and the number of population doublings (PDs) [47].
209 Cultures with more than 20 PDs and a random selection of nine other cultures with a normal life span
210 (<20 PDs) were analyzed for copy number changes and for mutations of the ten key HNSCC driver
211 genes using target-enrichment sequencing. Irrespective of the lifespan of < or > 20 PD, in 50% of the
212 cultures somatic genetic changes were identified with a large variety in type and number. Despite many
213 genetic alterations in some cultures and an apparent immortal lifespan, none formed tumors in
214 immunodeficient mice, demonstrating the lack of invasive capacity and confirming the precancerous
215 state [48]. This support that acquisition of immortality is an earlier event during OSCC progression than
216 acquisition of invasive properties. Most frequently mutated genes were *TP53*, *NOTCH1* and *FAT1*,

217 whereas *CDKN2A* showed frequent copy number losses. Most intriguingly, in four cultures copy
218 number changes were found but no mutations in key driver genes, suggestive that carcinogenesis may
219 start with copy number changes, although such precancerous cells may never transform.

220 In summary, field cancerization has been well characterized in genetic terms, the cells can be cultured
221 and even used for therapeutic target screening [49,50]. A field should be defined as group of cells with
222 tumor-associated somatic genetic alterations. A field should be larger than the clonal unit and
223 consequently larger than at least 200 cells wide and can reach dimensions of up to 10 cm in diameter.
224 Some fields present as dysplasia under the microscope, and some are macroscopically visible as a non-
225 specific persistent white or red lesion. These fields contain a variety of genetic changes, but typically
226 also mutations in the cancer driver genes of head and neck cancer. They develop by a process of somatic
227 mutation in relation to aging and carcinogen exposure. Why the normal epithelium is displaced remains
228 an enigma. Enhanced proliferation seems logical but is likely not the cause, and biological processes
229 perhaps stimulated by environmental cues, may be more likely.

230

231 The OPMD Immune microenvironment (IME)

232 The interplay between OPMD and IME has been poorly explored while it appears as a promising and
233 actionable target [51,52]. Briefly, compared to OPMD that transformed into OSCC, patients with
234 dysplastic OPMD and no subsequent malignant transformation had significantly more infiltrating
235 CD3+, CD4+ and CD8+ T-cells and decreased T-regulatory cells [53–56]. Furthermore, the progression
236 from OPMD to OSCC has shown increased number of CD163+ cells (M2 Macrophages), PD-L1
237 expression and decreased number of CD8+ cells [52,53,56–59]. More recently, the Saintigny Team (JB,
238 PS) studied the dynamic of the IME in the 4-NQO murine model of oral carcinogenesis [60], an accepted
239 model for the human disease in particular at early steps of tumorigenesis [61]. They found that changes
240 in the composition of immune infiltrate (T-cells, B-cells, M1/M2 macrophages) can already be observed
241 in histologically proven premalignant stages. Transcriptomic changes revealed activation of immune
242 related processes at early steps of oral carcinogenesis. On the other hand, when the gene expression

243 data of 86 patients with OPMD were challenged with transcriptomic features coming from HNSCC
244 patients, the lesions could be stratified in several clusters, and the OPMD from the mesenchymal,
245 hypoxia and classical molecular subgroups showed a higher risk of malignant transformation in
246 comparison with the immune-related ones [62].

247 It is tempting to speculate on OPMD within the concept of “immunoediting”, hypothesizing that these
248 lesions are in the equilibrium phase of a dynamic process between the malignant transformation and
249 surveillance of the immune system. One hypothesis is that malignancy will develop in the presence of
250 an immunosuppressive microenvironment. Another hypothesis is that OPMD do not elicit a sufficient
251 immune response, and that for two main reasons: (i) OPMD highly resemble ‘self’ and are not detected
252 as non-self by the immune system; (ii) OPMD barely induce local tissue-damage and therefore
253 insufficiently release the immune-attracting damage associated molecular patterns.

254 Overall, while promising, our knowledge of the complex and dynamic nature of the OPMD IME
255 remains incomplete which might explain the failure of immunoprevention strategies [63,64]. Thus,
256 further characterization of the dynamic changes immune response during oral carcinogenesis is
257 required [51,52], especially differences between OPMD that subsequently transformed into OSCC and
258 those that did not.

259

260 Oral Microbiome

261 The study of the potential contribution of the microbiome in the carcinogenesis of different cancer types
262 including OSCC is emerging [65]. Regarding the very few studies which have reported the microbiome
263 composition associated with OPMD, results are heterogeneous and difficult to compare because of
264 diversity in microbiota and methodological heterogeneity [66,67]. Briefly, it was suggested that the
265 microbiota may contribute to tumorigenesis, both directly (production of microbial genotoxin inflicting
266 DNA damages), and indirectly through its interplay with the immune system (stimulation of chronic
267 inflammation alters the immune responses and aberrant immune responses facilitate dysbiosis,
268 especially in aging context) [68]. Moreover, the dysregulation by the microbiome of some physiological

269 activities that are critical for oral carcinogenesis (nitrogen transport, response to stress, interspecies
270 interactions, Wnt pathway modulation, and amino acid and lipid biosynthesis) were identified using
271 the 4-NQO mice model [69]. Overall, the understanding of the role of the oral microbiome in
272 carcinogenesis is still an area of investigation [67].

273

274 Early diagnosis of OPMD

275 The early detection of OPMD serves the purpose of secondary prevention of oral cancer [70].
276 Examination of the oral cavity (visual inspection and palpation) is the conventional method for
277 identifying and monitoring OPMD. However, clinical recognition of OPMD is challenging [5]. Thus,
278 methods to enhance the early detection of OPMD are required [4,5,71].

279

280 In 2008, the International Agency for Research in Cancer (IARC) has published a digital manual to help
281 physicians in this aim (<https://screening.iarc.fr/index.php>). Furthermore, non-invasive *in-vivo* optical
282 imaging provides unique opportunities for real-time diagnosis of oral pre-malignancies. These
283 techniques are mainly autofluorescence imaging (AFI), targeted fluorescence imaging (TFI), high-
284 resolution microendoscopy (HRME), narrow band imaging (NBI), Raman spectroscopy (RS) (Table 1)
285 [72,73].

286 Using AFI, altered and dysplastic tissues appear darker compared to the healthy surrounding tissue
287 (autofluorescence loss). AFI devices displayed superior accuracy levels in the identification of OPMD
288 compared to clinical examination [74]. AFI devices evaluated for early diagnosis of OPMD are practical
289 and cost-effective but suffer from low specificity [5]. Moreover, mucosa with hyperkeratinization such
290 as some oral leukoplakia can demonstrate increased autofluorescence when compared to normal
291 mucosa which limits the ability to detect malignant change within such lesions [75]. TFI utilizes a
292 targeting fluorescence probe which can specifically target some elements by approved antibodies
293 (targeted *immune*-fluorescence imaging). However, the lesion heterogeneity could decrease the TFI
294 sensitivity.

295 NBI visualizes the angiogenic patterns within and surrounding lesions. NBI as an endoscopic system is
296 widely available and easy to use [5,76,77]. Moreover, the neoangiogenesis-related morphological
297 changes, especially the abnormal intraepithelial capillary loops (ICPL) patterns, have been widely
298 reported [5,75]. Unfortunately, IPCL patterns characterization is subjective and the visualization of
299 microvessel architecture may be affected by various factors. Artificial intelligence may make the
300 prediction of malignant transformation more objective and with greater accuracy [26].

301 HRME is cost effective, non-invasive and provides real-time high-resolution microscopic images (in situ
302 “optical biopsy”) [78]. HRME has demonstrated high sensitivity and specificity. However, HRME is not
303 commercially available, its contrast agent is not yet approved, and the field of view is limited [5].

304 RS is a non-destructive vibrational spectroscopic technique [79–81]. Raman spectra represent the overall
305 molecular composition of the tissue, and can be used to distinguish healthy tissue from (pre-)malignant
306 tissue [5]. RS is a promising tool for early diagnosis/biopsy guidance and follow up (optical biopsy) of
307 OPMD but required further development [82].

308
309 Other imaging techniques to detect OPMD are Optical coherence tomography, Elastic scattering
310 spectroscopy, Diffuse reflectance spectroscopy, confocal laser endomicroscopy and confocal reflectance
311 microscopy, but they are not widely developed [5,83]. Vital staining (toluidine blue, Methylene blue,
312 Rose Bengal and Ludo’s iodine) are sensitive, simple, rapid, efficient and low-cost techniques [5,75,84]
313 but false positive results are frequent, and their application is not without issues.

314 In summary, the previously described techniques are promising with high sensitivity to detect OPMD
315 but suffer from poor specificity. This is not only due to inherent limitations of the techniques, but also
316 to the lack of a good histological gold standard, which renders the development of predictive algorithms
317 based on optical methods very difficult [5,75,84]. To overcome the technical part of the problem,
318 combination of techniques, *e.g.*, combining AFI and HRME are interesting [85,86]. Further investigations
319 (large randomized clinical trial with long follow-up) are needed.

320

321 Preclinical models

322 *In vitro tissue culture models*

- 323 • 2D culture of cell lines

324 There are many reports of cell lines being established from OPMD biopsies (Table 2). These OPMD cell
325 line model systems recapitulate the key characteristics of the clinical lesions closely and have been used
326 to study the early stages of oral cancer and malignant transformation of oral keratinocytes *in vitro* [87–
327 94]. However, the major limitation of cell line models is that these cells fail to grow *in vivo* thereby
328 prohibiting to study the involvement of the oral microenvironments.

- 329 • 3D culture of organotypic co-culture

330 In this method, keratinocytes are cultured at an air-to-liquid interface on a fibroblast-containing
331 collagen type I matrix. While several refinements have been proposed to overcome the major limitations
332 of the classically used collagen-based connective tissue equivalent (deficit of complex structural
333 heterogeneity and collagen fiber crosslinking present in mature connective tissue, induction of artificial
334 epithelial invasion by lose of biostability over a long period of culture and lack of a well-defined
335 continuous basement membrane between the epithelium and connective tissue)[95], to date, most
336 organoids lacked vasculature, fibroblasts and immune cell components that are known to influence
337 malignant transformation, which make them not a true representation of *in vivo* transformation of
338 OPMD to OSCC.

339 Recently, to mimic the oral mucosal complexity, progress has been achieved in designing more complex
340 tissue engineering techniques in organotypic co-cultures that includes the incorporation of blood
341 capillaries to the cell surface [96], culturing oral keratinocytes with fibroblasts [97], immune cells [98],
342 and oral microbiota [99,100]. As protocols and analysis methods continue to improve, these 3D
343 techniques will become more accessible within the said field.

344

345 *In vivo rodent models*

- 346 • Carcinogen-induced models

347 Several agents, including coal tar, cigarette smoke, benzo[a]pyrene (B[a]P), 3-methylcholanthrene, 7,12-
348 dimethylbenz(a)anthracene (DMBA) and 4-nitroquinoline-1-oxide (4-NQO) have been used to induce
349 OSCC in rodent models. In particular, the 4-NQO-induced oral carcinogenesis murine model closely
350 resembles human OSCC in terms of pathogenesis, pathological changes, host immune activity, and
351 molecular levels, thus making this model widely acceptable to study OSCC, especially for the
352 identification of biomarkers for early diagnosis and the transformation of the epithelium [61]. The major
353 limitations of the carcinogen induced models are (i) the requirement of prolonged animal and
354 carcinogen handling making them laborious and time-consuming, (ii) the resulting tumors do not
355 recapitulate the tumors in patients, and (iii) it is not possible to study specific gene alterations in the
356 development and malignant transformation process.

357 • Genetically engineered mouse models (GEMMs)

358 GEMMs that allows oncogene activation and/or tumor suppressor inactivation solely in stratified
359 epithelia of the oral cavity under the control of inducible promoters are extensively used to study OPMD
360 [101]. While promising, there are still several barriers to their full application in understanding the
361 OPMD malignant transformation. The main limitation is that these models do not reflect human oral
362 pathogenesis in terms of the degree of gene expression during the transformation process. Secondly,
363 these models have low specificity to form premalignant lesions by gene activation or inactivation and
364 appear in sites other than the oral cavity. Thirdly, the introduction of exogenous genes or the knockout
365 of endogenous genes in GEMM will occur in almost every cell which does not recapitulate the normal
366 oral microenvironment of OPMD. Lastly, the potentially induced changes or disruptions to the oral
367 microbiome limit the use of GEMMs for understanding the relationship of oral microbiome and OPMD.

368

369 Prevention strategies

370 *Current clinical management of OPMD*

371 To date, there is a general consensus for the most appropriate management of OPMD [75]. Primary
372 prevention remains the first management measure. In all cases, tobacco and alcohol consumption

373 cessation is required to limit the risk of malignant progression, as well as the screening of whole upper
374 aero-digestive tract mucosa for OPMD [20]. Furthermore, the histological assessment of the biopsy,
375 especially the grading of dysplasia, should be performed both at baseline and in case of clinical
376 modifications (macroscopic, clinic) because of its high prognostic value [12]. Surgical resection is
377 applied when possible and certainly indicated for OPMD with moderate or severe dysplasia [20]. When
378 surgery is not feasible (patient not operable or surgery excessively mutilating), the two available options
379 are either destruction of the lesion (cryosurgery, carbon dioxide laser, photodynamic therapy) and/or
380 the close surveillance with repeated biopsies. Finally, a recent Cochrane database review, indicates no
381 useful medical treatments to prevent OPMD malignant transformation [17].

382

383 *Systemic strategies to prevent malignant transformation of OPMD*

384 Treatment of the lesion and prevention of malignant transformation of OPMD may improve patient
385 outcome [11]. Hence, inhibitors that eradicate the lesion, or chemopreventive agents that prevent the
386 malignant transformation of OPMD must be developed. Several systemic agents have been tested such
387 as bleomycin, vitamin E, retinoids, beta carotene, lycopene and mixtures of tea [31,75,102]. However,
388 these agents showed limited benefits. Although they caused macroscopic regression of OPMD,
389 recurrences occurred frequently after discontinuation of treatments, and they were not shown to
390 prevent OPMD malignant transformation [11,17].

391 It has been proposed to leverage premalignant biology for precision-based and more specifically
392 immune-based cancer prevention [103,104]. Unfortunately, targeted therapies have failed to prevent
393 malignant transformation of OPMD [31]. On the other hand, the IME is an attractive therapeutic target
394 [51,52]. The development of multimodal immune-prevention strategies to halt OSCC progression,
395 including immune check point inhibitors, vaccines, adjuvants activating the innate immune system and
396 combination with some chemopreventive agents that impact positively the tumor IME, is an interesting
397 option [105]. In recent clinical trials evaluating PD-1- and PD-L1 targeting monoclonal antibodies

- 398 (pembrolizumab and avelumab) patients with OPMD at high-risk of oral cancer development based on
399 LOH status have been enrolled (NCT02882282 and NCT04504552), but the results are still awaited.

400 **Conclusion and Discussion**

401 Given the knowledge gaps in OPMD clinical management, classification, and risk stratification as well
402 as the lack of standardized procedures for biospecimen collection (*i.e.*, mucosal biopsy; oral brushes;
403 saliva), the lack of efficient, acceptable and approved interventions to treat the whole cancerization field
404 and the lack of a network of cooperating centers for clinical research in this area, several European
405 experts in the field give their opinions and perspectives.

406 Joint efforts of academic teams and societies, clinical cancer research organizations, biotechs and
407 pharmaceutical companies should be engaged to decipher the full temporal spectrum of the disease that
408 may evolve to OSCC. There is need to define standardized procedures for sample collection, to refine
409 OPMD classification and improve patients' stratification. A biologically-driven classification of OPMD
410 may identify clusters with actionable biology, allowing to develop prevention strategies that treat the
411 entire field of cancerization.

412 There is a critical need for standardized protocols for the clinical screening and diagnosis of OPMD, in
413 particular to encourage systematic biopsies, and for patient follow-up and treatment. Minimally
414 invasive technologies for OPMD detection should be prioritized [106]. For pathological diagnosis, the
415 current gold standard, we should (i) develop standardized histopathological criteria encompassing the
416 various histological appearances for reliable diagnosis of OPMD; (ii) implement validated
417 immunohistochemical and molecular biomarkers; (iii) incorporate artificial intelligence for diagnostic
418 support; and (iv) develop and implement objective detection techniques as well as non-invasive
419 alternatives to biopsies (buccal brushes, saliva, buccal rinses, optical techniques) [83,107–110].

420 Prospective population-wide studies of longitudinal disease trajectories to interrogate the general
421 medical histories of patients with cancer represent a recently developed concept to improve healthcare
422 monitoring and reduce costs. Analysis of national or regional data hubs (*e.g.*, clinical data warehouses,
423 cancer registries, social security databases, hospital electronic medical records...) may identify disease
424 associations occurring prior to OSCC diagnosis.

425 Electronic health (eHealth) interventions and patient-reported outcome tools (PROMs) dedicated to
426 patients with OPMD to monitor disease progression, to identify early signs of transformation, to
427 monitor lifestyle and psychological impact of being at risk (uncertainty, anxiety and depression) [111]
428 should be developed and evaluated. This may spare unnecessary visits and exams, while providing the
429 best possible care.

430 Finally, there is a need to evaluate the socio-economic impact of preventive medicine and to perform
431 generalizable health technology assessment, a network of Centers gathering cost- and patient-related
432 data should be built. Eventually, the aim here would be to decrease the economic burden of OSCC.

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792 **Figure legends and tables**

793

794 **Table 1.** Main *in-vivo* optical imaging methods that could be used as an adjunct to conventional oral
795 examination in oral premalignant disorders screening are autofluorescence imaging (AFI), targeted
796 fluorescence imaging (TFI), high-resolution microendoscopy (HRME), narrow band imaging (NBI),
797 Raman spectroscopy (RS). For each method, basic principles, advantages and inconvenient are
798 described as well as references.

799

800 **Table 2.** Available cell lines to study oral premalignant disorders. (PMID: PubMed identification
801 Member; ISSN: International Standard Serial Number).