UNIVERSIDADE FERNANDO PESSOA

PHOTODYNAMIC THERAPY FOR THE TREATMENT OF ORAL LEUKOPLAKIA: SYSTEMATIC REVIEW

Terapia Fotodinâmica para o Tratamento da Leucoplasia Oral: Revisão Sistemática

Dissertação de Mestrado Integrado
Em Medicina Dentária

Shaï Odelia Mazal Bokobza

Orientadores:

Dra. Filipa Manuel Moreira Aroso Pinto de Oliveira

Dra. Helena Maria Gomes Ferreira das Neves

Junho 2025

PHOTODYNAMIC THERAPY FOR THE TREATMENT OF ORAL LEUKOPLAKIA: SYSTEMATIC REVIEW

Terapia Fotodinâmica para o Tratamento da Leucoplasia Oral: Revisão Sistemática

Dissertação de Mestrado Integrado

Em Medicina Dentária

Shaï Odelia Mazal Bokobza

Orientadores:

Dra. Filipa Manuel Moreira Aroso Pinto de Oliveira

Dra. Helena Maria Gomes Ferreira das Neves

Junho 2025

ACKNOWLEDGMENT

I would like to express my profound gratitude to the professors at the University Fernando Pessoa for imparting their knowledge, which has enabled me to acquire the necessary skills for advancing in my future dental career.

I extend my heartfelt to my research supervisors, Dra. Filipa Pinto Oliveira and Dra. Helena Neves, for their unweaving support, insightful guidance, and mentorship throughout the development of this thesis.

To my parents, I dedicate this work. Their constant love, steadfast, support and unwavering belief in me have been the solid foundation upon which I have built my academic and personal journey.

To my sister Cindy, who paved the way in the fields of science and health studies, and who has always been there for me during pivotal moments.

To mamie Haya, whose pride and love accompany me at every step of my life.

To Bibi and Paul, for the moments of joy and laughter shared, which have lightened my days.

To tata Patoune et tonton Eli, for their constant presence during the significant milestones of my life, offering support and warmth.

To Papy David, Mamie Denise & Papy Paul, I sincerely hope you are proud of me, wherever you are.

To Hillel, my better half, for this unweaving faith in me, his constant support, and his ability to always see the bright side of things.

To my clinical partners, Amélie, Mayane, Alex & Nathan, for their collaboration, support that have strengthened my confidence in my work.

To Maya, Luna, Sharon, David & Yossef my friends from before Porto and, I hope, for many years to come.

ABSTRACT

Background: Oral Leukoplakia is recognized as the most prevalent potentially malignant disorder, of the oral mucosa with significant variability in clinical presentation and risk of malignant transformation. Traditional treatment options often yield inconsistent results, and recurrence is frequent. Photodynamic Therapy has emerged as a non-invasive alternative with selective cytotoxicity, offering potential advantages and safety, efficacy, and aesthetic outcomes.

Objective: This systematic review aims to evaluate the clinical impact of photodynamic therapy on the treatment of oral leukoplakia, focusing on the lesion response, recurrence, malignant transformation, and adverse effects.

Methods: A comprehensive search was conducted across five databases (B-On, PubMed, Scielo, ScienceDirect, and Trip Database) using specific keywords and PRISMA guidelines. Twelve clinical studies met the inclusion criteria and were analyzed for protocol characteristics, patient demographics, and therapeutic outcomes. This systematic review is registered in PROSPERO with registration number CRD420251065255.

Results: Photodynamic therapy demonstrated favorable complete remission rates, particularly when using 5-aminolevolunic acid as the photosensitizer. Lesion type, light source, photosensitizer delivery, and number of sessions influenced treatment outcomes. Recurrence and malignant transformation were reduced in several studies. Photodynamic therapy was generally well-tolerated with mild and transient side effects.

Conclusion: Photodynamic therapy has a positive clinical impact on the treatment of oral leukoplakia, offering effective lesion control and a favorable safety profile. Its integration into oral medicine could benefit patients seeking minimally invasive and personalized therapeutic strategy, as photodynamic therapy is not only effective but also is generally painless, free of significant side effects, and without aesthetics compromise.

Keywords: Oral leukoplakia; photodynamic therapy; treatment.

RESUMO

Introdução: A leucoplasia oral é reconhecida como o distúrbio potencialmente maligno mais prevalente da mucosa oral, com apresentações clinicas variadas e risco significativo de transformação maligna. Os tratamentos convencionais apresentam resultados inconsistentes e alta taxa de recidiva. A terapia fotodinâmica surge com uma alternativa não invasiva com citotoxicidade selectiva, oferecendo vantagens em termos de segurança, eficácia e estética.

Objectivo: Avaliar o impacto clínico da terapia fotodinâmica no tratamento da leucoplasia oral, com foca na resposta da lesão, recidiva, transformação maligna e efeitos adversos.

Métodos: Foi realizada uma pesquisa abrangente em cinco bases de dados (B-On, PubMed, Scielo, ScienceDirect, e Trip Database), utilizando palavras-chaves especificas e as diretrizes PRISMA. Doze estudos clínicos foram incluídos e analisados quanto aos protocolos utilizados, características dos pacientes e desfechos terapêuticos. Esta revisão sistemática foi registada no PROSPERO com o número CRD420251065255.

Resultados: A terapia fotodinâmica demonstrou taxas favoráveis de remissão completa, especialmente com o uso do ácido 5-aminolevulínico com fotossensibilizador. O tipo de lesão, fonte de luz, forma de aplicação do fotossensibilizador e o número de sessões influenciaram os resultados. A recidiva e a transformação maligna foram reduzidas em vários estudos. A terapia fotodinâmica foi bem tolerada, com efeitos colaterais leves e transitórios.

Conclusão: A terapia fotodinâmica tem um impacto clínico positivo no tratamento da leucoplasia oral, oferecendo controle eficaz das lesões e um perfil de segurança favorável. A sua integração na medicina oral pode beneficiar pacientes que procuram estratégias terapêuticas minimamente invasivas e personalizadas, já que a terapia fotodinâmica é não apenas eficaz, mas também geralmente indolor, sem efeitos colaterais relevantes e sem comprometimento estético.

Palavras-chave: Leucoplasia oral; terapia fotodinâmica; tratamento.

Summary

Table of figures.

Table of Tables

List of abbreviations, acronyms and symbols.

AFL - Ablative Fractional Laser

ALA - 5-aminolevulinic acid

CNV - Copy Number Variation

CR - Complete Response

DNA - Deoxyribonucleic Acid

EBV - Epstein Barr Virus

HPD - Hematoporphyrin Derivative

HPV - Human papilloma Virus

LED - Light Emitting Diode

MT - Malignant Transformation

mTHPC- Temoporfin 5,10,15,20-tetra(m-hydroxyphenyl) chlorin

NR - No Response

OL - Oral Leukoplakia

OPMD - Oral Potentially Malignant Disorder

PD - Progressive Disease

PDR - Photodynamic Reaction

PDT - Photodynamic Therapy

PDL - Pulsed Dye Laser

PpIX - Protoporphyrin IX

PS - Photosensitizer

PR - Partial Response

PS - Photosensitizer

PVL - Proliferative Verrucous Leukoplakia

ROS - Reactive Oxygen Species

SD - Stable Disease

SR - Significant Response

WHO - World Health Organization

1 Introduction

Oral leukoplakia is the most common potentially malignant disorder affecting the oral mucosa, characterized by white patches that cannot be classified as any other definable disease. Its clinical significance lies in its potential to undergo malignant transformation, with rates varying depending on the lesion type, histological grading, and patient-related-risk factors such as tobacco or areca nut use. Despite decades of research, oral leukoplakia continues to present a therapeutic dilemma, as conventional treatment modalities, including surgical excision, laser ablation and pharmacologic approaches, often involve recurrence and limited efficacy in preventing progression to cancer.

Amid growing interest in non-invasive and tissue-preserving therapies, photodynamic therapy (PDT) has emerged as a promising option for managing oral leukoplakia. PDT utilizes a photosensitizer activated by light as a specific wavelength in the presence of oxygen, leading to the generation of reactive oxygen species that selectively destroy dysplastic cells. Its minimally invasive nature, repeatability, and favorable cosmetic outcomes have made PDT increasingly relevant in the treatment of oral potentially malignant disorders. However, reported outcomes across clinical studies vary significantly, raising critical questions about its true impact on disease control, recurrence, and malignant transformation.

This systematic review aims to explore and evaluate the impact of PDT on the treatment of oral leukoplakia. Specifically, it seeks to address the following research question: What is the impact of photodynamic therapy on the treatment outcomes of patients with oral leukoplakia? By synthesizing the findings of clinical trials, this review intends to clarify PDT's role in lesion resolutions, recurrence prevention, and safety profile. The expected contributions are both practical (supporting clinical decision making) and theorical (enhancing understanding of PDT's therapeutics mechanisms in the context of oral mucosal pathology).

1.1 Material and methods

In order to carry out this study, an online bibliographic search was performed across 5 different databases: B-On; PubMed; SCIELO; Science direct; Trip database. The following keywords used in the search: "photodynamic therapy"; "oral leukoplakia";

"treatment"; "clinical trial", connected by the Boolean connector "AND". No time restrictions were applied to this search.

The PICO (population, intervention, comparison, outcome) criteria was used to formulate the question addressed in this systematic review (cf. Table 1). Population: patients diagnosed with oral leukoplakia, Intervention: patients treated with photodynamic therapy; Comparison: oral leukoplakia before photodynamic therapy compared with oral leukoplakia after photodynamic therapy; Outcome: lesion response and lesion size after photodynamic therapy.

Table 1: Population, Intervention, Comparison, Outcome (PICO) strategy for question formulation.

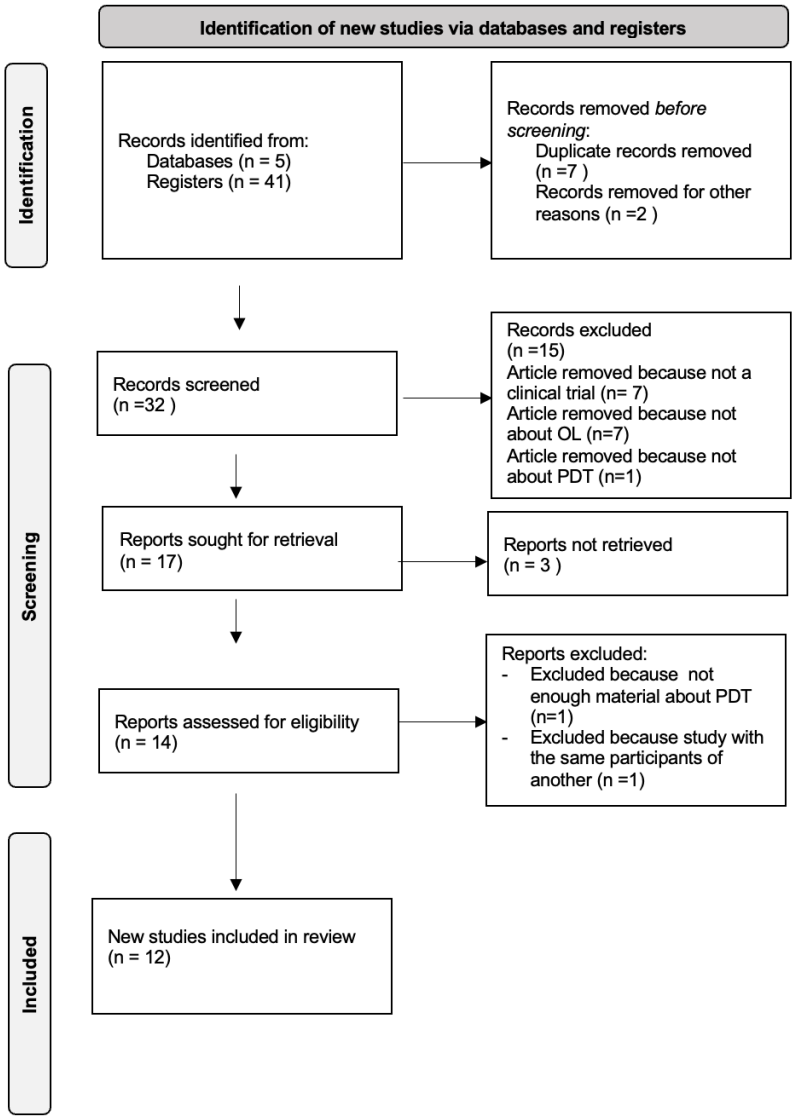
Parameter	Evaluation
Population (P)	Patients diagnosed with oral leukoplakia
Intervention (I)	Patients treated with photodynamic therapy
Comparison (C)	Oral leukoplakia before photodynamic therapy compared with oral leukoplakia after photodynamic therapy
Outcome (O)	Lesion response and size of the lesion after photodynamic therapy.

The research was subject to inclusion and exclusion criteria and studies were selected according to PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-analysis). The PRISMA 2020 flowchart is designed for new systematic reviews that only include databases and register searches. It outlines the process of identifying, screening and including studies in a systematic review.

The literature search was conducted between December 2024 and January 2025 using various combination of the search terms, to focus the results on the proposed topic. The inclusion criteria limited the search to articles published in English, involving patients diagnosed with oral leukoplakia and treated with photodynamic therapy. Exclusion criteria applied to articles that were not clinical trials or did not match the topic of interest.

A total of 41 records were identified across databases: B-On (12); PubMed (9); Scielo (0); Science Direct (12); Trip Databases (7). Before screening (7) duplicate records and (2) ongoing research were removed leaving (32) records for screening. Out of these, (15) records were excluded. This left, (17) reports for retrieval. Of these, (3) could not be retrieved. Of the (14) reports assessed for eligibility; The excluded articles were for specific reasons: (1) lacked sufficient information about photodynamic therapy and (1) used the same data as another study. In the end, 12 clinicals trials were included in this review (cf. Figure 1).

Figure 1: Flow chart Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA)



In the methodology, critical appraisal tool for the assessment of risk bias of the selected article has been done using the JBI Critical Appraisal Checklist for Case Series (cf. Table 2).

Table 2: Methodological evaluation of the selected articles- determining the risk of bias with JBI Critical Appraisal Checklist for Case Series.

Authors and publication date	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Yes %	Risk of bias	Overall Approval
Yanting Wang *et al.*- 2024	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	100%	Low	+
Jiali Ou *et al.* - 2022	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	100/%	Low	+
Yi-Lin Yao *et al.* - 2022	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	90%	Low	+
Ying Han *et al.*- 2019	YES	YES	YES	?	NO	YES	YES	YES	YES	YES	80%	Low	+
Kotya Naik Maloth *et al.* - 2016	YES	YES	YES	?	?	NO	YES	YES	YES	YES	70%	Low	+
Niranzena Panneer Selvam *et al.* -2015	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	90%	Low	+
Malgorzata Pietruska *et al.* –2013	YES	YES	YES	?	?	YES	YES	YES	YES	YES	80%	Low	+
Stuart J. WONG *et al.* - 2013	YES	YES	YES	?	?	NO	YES	YES	YES	YES	70%	Low	+
Aleksandra Kawczyk-Krupka *et al.* - 2012	YES	YES	YES	?	YES	NO	YES	YES	YES	YES	80%	Low	+
Gal Shafirstein *et al.* - 2011	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	90%	Low	+
Waseem Jerjes *et al.* - 2011	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	100%	Low	+
Alexander Kübler *et al.* - 1998	YES	YES	YES	?	?	NO	YES	YES	YES	YES	70%	Low	+

(Q: question; ?: unclear.)

This systematic review protocol was prospectively registered with PROSPERO (International Prospective Register of Systematic Reviews) under registration number CRD420251065255.

2 Development

2.1 Oral Leukoplakia

2.1.1 Definition and classification

Since the first description of oral leukoplakia (OL) in a scientific paper, multiple definitions have been suggested for this pathology overtime. Nowadays, oral leukoplakia is defined by the World Health Organizations (WHO) since 2007 as "a predominantly white plaque of questionable risk having excluded (other) known disease or disorders that carry no increased risk for cancer" (Warnakulasuriya et al., 2021). This definition can be coupled with the one presented by van de Waal in 2015: "a predominantly white patch or plaque that cannot be characterized clinically or pathologically as any other disorder; oral leukoplakia carries an increased risk of cancer development either in or close to the area of the leukoplakia or elsewhere in the oral cavity" (van der Waal, 2015).

OL is divided in two main clinical type (Parlatescu et al., 2014):

(i) The homogeneous leukoplakia that can be described as a flat white lesion, uniform, thin white area altering or not oral mucosa, which may exhibit crakes of the surface keratin, and cannot be rubbed off. Most homogeneous leukoplakias affect circumscribed area and have well demarcated borders, a smaller subset can present diffuse borders (Warnakulasuriya et al., 2021). This type of OL is mostly found in the lateral/ventral tongue and floor of the mouth (Woo, 2019).

(ii) The non-homogeneous leukoplakia, that includes speckled leukoplakia, a white and red lesion with a predominantly white surface (Warnakulasuriya et al., 2007). The nodular leukoplakia, a lesion with small polypoid outgrowths and rounded or white excrescences and finally verrucous leukoplakia which has a proliferative, elevated or corrugated surface appearance. This last type of leukoplakia has also a subtype known as proliferative verrucous leukoplakia (PVL) and can be characterized by an aggressive evolution and has a multifocal appearance, it can frequently cover wide aeras (van der Waal & Reichart, 2008). Those, non-homogeneous leukoplakias, typically present with more diffuse borders than homogeneous ones (Warnakulasuriya et al., 2021).

The grade of dysplasia is also an important factor to classify OL. A lesion is considered dysplastic when it reflects histological changes and are followed by the loss of uniformity or of the architecture of the epithelial cells. To help contribute of a diagnosis of epithelial dysplasia, a list of 13 histological changes (cf. Table 3) was made to homogenize the diagnostic criteria by the WHO and modified by Warnakulasuriya S, (Warnakulasuriya, 2001).

Table 3: Histological changes that may contribute to a diagnosis of epithelial dysplasia

1. Loss of polarity of the basal cells
2. The presence of more than one layer having a basaloid appearance
3. Drop-shaped rete-ridges
4. Increased nuclear-cytoplasmic ratio
5. Nuclear hyperchromatism
6. Enlarged nucleoli
7. Increased number of mitotic figures
8. Mitotic figures that are abnormal in form
9. The presence of mitotic figures in the superficial half of the epithelium
10. Cellular and nuclear pleomorphism
11. Irregular epithelial stratification
12. Loss of intercellular adherence
13. Keratinization of single cells or cell groups in the prickle cell layer

Source: Warnakulasuriya S. (2001). Histological grading of oral epithelial dysplasia: revisited. *The Journal of pathology*, *194*(3), 294–297. https://doi.org/10.1002/1096-9896(200107)194:3<294::AID-PATH911>3.0.CO;2-Q

Nevertheless, this type of grading is still considered subjective, and the conformity of histological interpretation decrease depending on the pathologist. One of the most used grading for epithelial dysplasia is the separation between non-dysplastic lesions and dysplastic lesion (mild or moderate epithelial dysplasia, severe dysplasia or carcinoma in situ) (Brouns et al., 2013).

Furthermore, OL is part of a larger group of oral diseases, the Oral Potentially Malignant Disorder (OPMD) and is the most studied. The definition of OPMD is “any oral mucosa abnormality that is associated with a statistically increased risk of developing oral cancer” (Warnakulasuriya et al., 2021). Moreover, the carcinogenic transformation of OL is multifactorial and is patient specific. However, the risk of malignant transformation of OL can be separate in two categories: high risk lesions and low risk lesions (Ribeiro et

al., 2010). Taking in consideration the gender (female patient has an increased risk of malignant transformation); location (increase risk when the lesion is observed in the lateral border of the tongue, gingiva, floor of the mouth and buccal mucosa), the type of leukoplakia can also be an indicator of the risk of malignant transformation (MT), for instance homogeneous OL have a lower risk of MT in comparation of non-homogeneous OL especially in case of a PVL (Narayan & Shilparshree, 2016). Nevertheless, epithelial dysplasia is still considered the "gold standard" to determine the risk of MT in this disorder (Parlatescu et al., 2014).

2.1.2 Incidence and prevalence.

The accurate incidence and prevalence are controversial due to lack of geographical and population stratification analysis (Zhang et al., 2023) and also due to the misdiagnosis and misclassification of leukoplakia (Auluck & Pai, 2005). In a study lead by Zhang et al. in 2023, the overall pooled estimated prevalence of OL is 3.41% with heterogeneity. Furthermore, this prevalence is not statistically different across continent, and worldwide the prevalence of OL is relatively consistent. In the same way the prevalence OL did not change, as the definition has been revised many times in the last decades. On the other hand, the characteristics of the population such as sex, age, habits have more impacts on the prevalence. OL can be often found in men and its prevalence increase with the age advancement (Zhang et al., 2023). Besides being one of the most studied OPMDs, OL is one with the higher risk of MT because of the different clinical presentations that this pathology presents. The MT rate of homogeneous OL is 9.5% and 49,5% for PVL (in comparison this rate for all the OPMDs is 7.5%) (Iocca et al., 2020).

2.1.3 Etiology and risk factors.

The etiology of oral leukoplakia is considered multifactorial, but the consumption of tobacco is one of the most frequently involved factors, in smoke or smokeless form (Sabashvili et al., 2018). Alcohol consumption can also be associated with OL, even though alcohol alone was not found to be associated with OL, it was found that alcohol can have a synergistic effect coupled with tobacco use in the development of OL (Abidullah et al., 2014). Betel quid/areca nut chewing habits are widely prevalent in South Asian population and their consumption led to a higher prevalence of OPMD such as OL (Warnakulasuriya et al., 2021). Sanguinaria used in mouth washes and toothpastes was

found to develop OL even after the interruption the usage of the product, the lesion doesn't disappear (Abidullah et al., 2014). Infection such as chronical candidiasis can be linked to OL, with the non-homogeneous type due to the presence of endogenous nitrosamines produced by some species of candida (Abidullah et al., 2014). Periodontal pathogenic microbes, nutritional deficiencies (such as iron deficiency anemia) are also described to be connected with OL (Serban et al., 2023). It was suggested in studies the correlations with human papilloma virus infection (HPV) and with Epstein Barr virus (EBV), even if this was not confirmed due to the lacks in the available data (Kumari et al., 2022).

2.1.4 Pathogenesis

A 2022 study published by Pratima Kumari (Kumari et al., 2022) showed the importance of the understanding of molecular pathogenesis in OL to minimize the chances of oncogenic transformation. Studies also shows that genetic anomalies, such as mutation, suppression or insertions of genes are correlated with OL and oral cancer. If we consider the level of copy number variation (CNV) at 3p, 9p,13q can be considered as a genetic marker for progressive type of epithelial dysplasia (Kil et al.,2016). The deletion of 3p14 and 9p21 can be seen in OL, and these genetic alteration in potential malignant tissues can be considered markers for cancer risk assessment (Kil et al.,2016).

Also, the DNA ploidy level can be an important indicator to determinate genetic stability and alteration in genomic sequence. Rahul Khanna showed in a study that for OL the aneuploidy rate (abnormal number of chromosome) of 20% while biopsies of normal mucosa were all diploid. Moreover, when there is evidence of histological dysplasia the aneuploidy rate was 38%, and without dysplasia were 14% (Khanna et al., 2010).

Furthermore, the expression of some proteins such as telomerase (enzyme which help cells from apoptosis), was found more elevated in case of OL and can indicate the progression to cancer (Thomas et al.,2020). Also, protein as p53, a tumor suppressor gene that regulate cell growth, apoptosis, differentiation and DNA are present in higher quantity in case of OL. The mutant P53, can lead to tumor progression and resistance to apoptosis, and also, mdm2, a protein with high tumorigenic potential can bind to P53 and inhibit its function. So, the positive rate of P53 and mdm2 show to have a positive correlation with the severity OL cancer (Cui et al., 2013).

OL can also lead to a modified response of the immune system and inhibit immune cells and includes immunes checkpoints like PD1/PD-L1. CD8+T cells and CD163+ macrophages are known to be important in the immune response. In presence of OL, there is an increased PD-L1 expression in dysplastic epithelial and inflating macrophage cells, that can suggest suppression of anti-tumor immunity. In the other hand, PD-L1 presence in subepithelial cells correlates negatively with CD8+ cell infiltration. Ultimately the PD-1/PD-L1 pathway plays a role in the early stages of oral cancer, and by the increase of PD-L1 there is an inhibition of CD8+ cell infiltration, inhibiting an anti-tumor immune response (Stasikowska-Kanicka et al.,2018).

2.1.5 Diagnosis

It is known that leukoplakias are not prone to symptoms in the beginning and even with larger sizes. It is mandatory a thorough inspection of the oral cavity by the dentist, in order to find these lesions. The gold standard for diagnosis is performing a biopsy and histopathological exam. Nowadays, accurately diagnosing OL and predicting its potential for malignant transformation remain a significant challenge in the clinical practice. The first temporary diagnosis especially depends on the experience and the skill of the practician, due to the lack of symptoms and performing the biopsy. Moreover, proper communication between clinicians and pathologist is important and as said before the histological classification of dysplasia is also challenging. To diagnose OL, clinician can use Certainty (C)-factor to assess the degree of correct identification (Cf. Table 4) (Brouns et al., 2013).

Table 4: Certainty (C)-factor of diagnosis of oral leukoplakia.

C_1	Evidence from a single visit, applying inspection and palpation as the only diagnosis means (Provisional clinical diagnosis), including a clinical picture of the lesion.
C_2	Evidence obtained by a negative result of elimination of suspected etiologic factors, e.g. mechanical irritation, during a follow-up period of 6 weeks (Definitive clinical diagnosis)
C_3	As C_2, but complemented by pretreatment incisional biopsy in which, histopathologically, no definable lesion is observed (Histopathologically supported diagnosis)
C_4	Evidence following surgery and pathologically examination of the resected specimen

Source: Brouns, E. R., Baart, J. A., Bloemena, E., Karagozoglu, H., & van der Waal, I. (2013). The relevance of uniform reporting in oral leukoplakia: definition, certainty factor and staging based on experience with 275 patients. *Medicina oral, patologia oral y cirugia bucal*, *18*(1), e19–e26. https://doi.org/10.4317/medoral.18756

First of all, a provisional diagnosis is established when a lesion observed during initial clinical examination cannot be definitively identified as either leukoplakia or another disease, in case of provisional diagnosis, certainty factor 1 is assigned (van der Waal, 2015).

Clinically, OL is a predominantly white patch/plaque that cannot be rubbed off and does not disappear or fade away on stretching (retracting) the tissue (Warnakulasuriya et al., 2021). If the suspected etiological factors are eliminated unsuccessfully or are absent, a definitive clinical diagnosis of leukoplakia is made, assigning certainty factor 2. When histopathological examination of an incisional biopsy did not show the presence of definable lesion, certainty factor 3 is assigned. In case of an excisional biopsy or surgical excision, performed after an incisional biopsy, certainty factor 4 is assigned based on histopathological examination of the surgical specimen. (van der Waal, 2015). As shown before, if following biopsy, no other disorder is confirmed the lesion is for now on characterized as leukoplakia with or without dysplasia (Warnakulasuriya et al.,2007). It is important to reach for a definitive and precise diagnosis for OL, and for that provisional and histopathological factors are taken into consideration together (Kumari et al., 2022).

As the definition of OL explain " having excluded (other) known disease or disorders" (Warnakulasuriya et al., 2021), the diagnosis of OL in considered exclusionary meaning that clinician have to excludes other white or white/ red lesions such as: white sponge naevus; frictional keratosis; biting of lip, commissures or cheeks (morsicatio buccorum); chemical or traumatic injury; oral liquen planus; acute pseudomembranous candidiasis, chronic hyperplastic candidiasis; leukoedema; skin graft; oral hairy leukoplakia; nicotine stomatitis (leukokeratosis nicotina palati or smokers palate) and uremic stomatitis (Warnakulasuriya et al., 2021). In terms of symptomology, OL is generally asymptomatic for the homogeneous subtype and can start to present with some discomfort can be found the non-homogeneous type.

2.1.6 Treatment

The need for treatment is primarily based on the nature of the OL lesions. The proposed treatment aims to prevent cancer development and assess the clinical and histological resolution of the OL. One of the possible choices is the "wait & see" approach, where OL

is closely monitored clinically and histologically through frequent visits and biopsies, without immediate treatment, this follow-up allows for the early detection of malignant transformation, enabling timely intervention if needed (Lodi & Porter, 2008).

Nevertheless, various interventions are available including surgical excision, with a scalpel, cryosurgery, PDT, laser surgery and vaporization (Kumari et al., 2022). Medical treatments, both topical and systemic, may also be considered with chemopreventive agents such as Vitamin A and retinoids, lycopene, systemic beta-carotene or local bleomycin (Lodi & Porter, 2008). It is also crucial to eliminate risk factors such as smoking and alcohol use, along with ensuring proper follow-up (Kumari et al., 2022).

Histopathologically identified white lesions with dysplasia (grades 1 and 2) or carcinoma in situ (grade 3) are recommended for excision, particularly in cases of moderate to severe epithelial dysplasia where the margins are unclear. (Kumari et al., 2022).
Even though, for the treatment of OL, most studies recommend complete removal of the lesion at a minimum, along with regular monitoring of patients, this do not reduce the risk of subsequent disease, nor does it lower the risk of malignant transformation (Lodi & Porter, 2008).

2.2 Photodynamic Therapy

2.2.1 History of PDT

Photodynamic therapy (PDT) is a relatively new and emerging non-invasive treatment method. However, the theorical description foundation and initial application can be traced back to ancient Egypt. For the Egyptians, the sun had healing powers, particularly for certain skin condition such as vitiligo, psoriasis or skin cancer (Stájer et al., 2020). Additionally, in the pivotal "Ebers Papyrus" (one of the most important and oldest medical texts from ancient Egypt), there is a description of the use of certain plants (such as parsnips and parsley), that were made into a powder and applied to depigmented skin lesion. After sun exposure, the result was skin pigmentation, producing an effect similar to a sunburn (Lima & Reis, 2023).

In today's practice, the use of sunlight or light as a therapeutic agent is known as phototherapy or heliotherapy. This last term "heliotherapy" comes from the Greek and the creation of this word is attributed to Hippocrates, often referred as the "father of medical science". After a trip do Egypt, he recommended sunlight for restorations of the health (Stájer et al., 2020).

In the western world, the theorical foundations of PDT were first established in the 1890s by Danish scientist Niels Finsen. He worked with a range of light sources, from low activity rays to ultraviolet radiation. His work in phototherapy gained international recognition and paved the way for the use of light sources as therapeutic modality for treating conditions such as lupus vulgaris and smallpox. In 1903, Finsen was awarded the Nobel Prize in Medicine in recognition of his contributions to this field (Møller et al., 2005; Grzybowski & Pietrzak, 2012).

Additionally, in 1900, German medical student Oscar Raab and his supervisor, Prof. Hermann von Trapeiner, observed that paramecia (a type of aquatic microorganism commonly used as a model at the time) which were incubated with the dye acridine orange, died faster when exposed to sunlight compared to those kept in the dark (Sharma et al., 2012). He then hypothesized that light might play a role in accelerating the chemical-biological reaction, and he further theorized that oxygen was necessary for the photosensitization process to occur (Stájer et al., 2020). Shortly after, von Trapeiner and Munich dermatologist Jesionek became the firsts to apply this discovery for a clinical use. They applied the xanthene dye eosin to a basal cell carcinoma on a patient's skin and

illuminated it with light, making the first use of PDT to treat a disease. In 1904, these researchers demonstrated the crucial role of atmospheric oxygen and its interaction with chemical substances and light to create this phenomenon introducing the term “photodynamic action” (Prazmo et al., 2016 & Aebisher et al., 2024).

PDT was revolutionized with a more complete understanding of its mechanism of action, following advancements in biochemistry and the study of porphyrin compounds. In 1913, Friedreich Meyer-Betz conducted an experiment on himself, receiving intravenous injection of 200mg of hematoporphyrin (a derivative of protoporphyrin IX, where the two vinyl groups have been hydrated). After exposure to light, he experienced severe pain and swelling, which was confined to the areas exposed to light. The photosensitivity persisted for months following the incident. He concluded that hematoporphyrin acted as a photosensitizing agent (Lee et al.,2020).

In 1955, Schwartz et al., also recognized hematoporphyrin as the most efficient photosensitizer. They found that this molecule targeted cancerous cells more effectively and produced better overall results. They highlighted that hematoporphyrin accumulates in cancerous tissues, which accelerated the development of novel porphyrin-based photosensitizers (PS) and their application in the treatment of malignant disorders (Lee et al., 2020).

In 1986, the International Photodynamic Association was founded, which has a worldwide expansion. As a result, PDT was approved by the FDA in 1999 for treating precancerous skin lesions on the face or scalp, and it has since been widely used for various cancer and diseases (Agostinis et al., 2011).

2.2.2 Theorical Basis of photodynamic therapy

Photodynamic Therapy (PDT) is a minimally invasive therapeutic approach that combines light of a specific wavelength, specially designed photosensitizing substances and dissolved oxygen in the cells. This modern therapy offers a promising perspective for the treatment of cancer, bacterial infection or other diseases. In light of the emerging problem of drug resistance, there is an urgent need to find alternatives therapies that can treat resistant diseases/ infections without the need of strong chemicals or antibiotics (Aebisher et al., 2024).

2.2.2.1 Photosensitizer

Photosensitizer (PS) are considered the key components of PDT. PS are chemical or dyes, whether natural, synthetic or hemi-synthetic that have the ability to absorb the light of a specific wavelength (Lima & Reis, 2023). For a photosensitizer to be effective in PDT therapy, it must meet several essential criteria:

(i) First, the PS must demonstrate thermostability, remaining stable during storage and application to maintain its therapeutic proprieties (Aebisher et al., 2024). Additionally, the PS must exhibit minimal to low toxicity in the dark, with significant cytotoxicity only when exposed to light (Lima & Reis, 2023).

(ii) The PS should not be prone to interactions with other chemicals in the body that may reduce its effectiveness or adversely affect the PDT process (Aebisher et al., 2024).

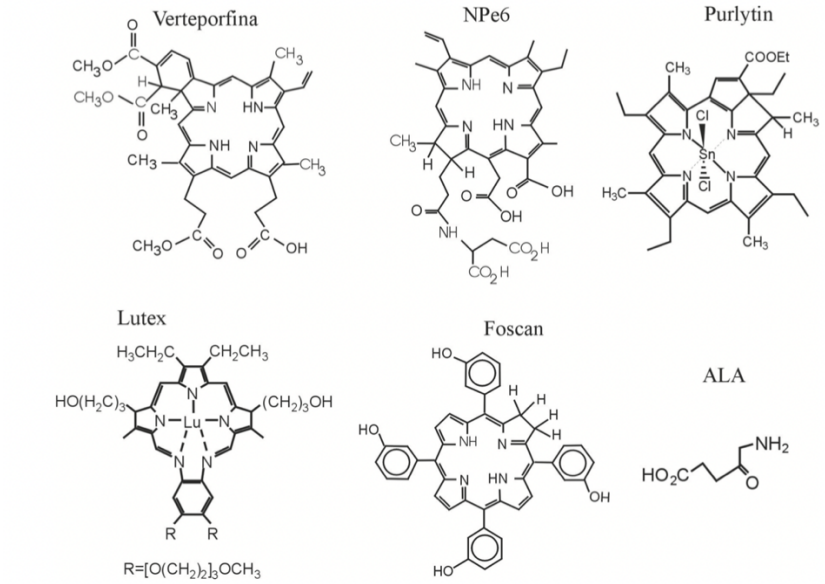
(iii) It also must be a single pure compound to allow quality control analysis, low manufacturing costs and good storage stability. The PS should have a high absorption peak between 600 and 800nm, as the optimal wavelength depends on the type of tissue and pathology. However, this peak generally does not exceed 800nm, as light penetration may be limited beyond this range, potentially reducing the therapy's effectiveness (Agostinis et al., 2011).

(iv) For the pharmacokinetics of the PS, the administration should be safe and painless, whether performed orally, topically or intravenously. Additionally, it should be highly soluble in the body's tissues (amphiphilic compound) to ensure efficient distribution and accumulation in the treatment area, enabling the PS to target and accumulate in abnormal tissues more effectively. Finally, it should permit a rapid clearance from the body to minimize the risk of toxicity to healthy tissues and reduce side effect, such as skin photosensitivity (Lima & Reis, 2023; Aebisher et al., 2024).

(v) High selectivity for tumor tissues allows precise destruction of targeted cells while minimizing damage to healthy tissues. Additionally, resistance to "photobleaching" (the loss of the PS's ability to absorb light due to its degradation during irradiation) is crucial. This resistance allows the use of higher energy light sources, which can result in deeper tissue penetration and greater therapeutic efficacy (Lima & Reis, 2023).

PS can be classified in function of their chemical structure and origins (cf. Figure 2): phthalocyanine dyes (aluminum disulphonated phthalocyanine, cationic zinc phthalocyanine, naphthalocyanine); porphyrins (Photofrin®, ALA (5-aminolevulinic acid) – pre-cursor of actual photosensitizer protoporphyrin IX), chlorins (temoporfin (Foscan®)), chlorin e6; xanthenes (erythrosine), monoterpenes (azulene) (Prazmo et al., 2016).

Figure 2: Chemical structures of Photosensitizer



Source: Prażmo, E. J., Kwaśny, M., Łapiński, M., & Mielczarek, A. (2016). Photodynamic Therapy As a Promising Method Used in the Treatment of Oral Diseases. *Advances in clinical and experimental medicine: official organ Wroclaw Medical University*, *25*(4), 799–807. https://doi.org/10.17219/acem/32488

The first generation of PS, introduced around 40 years ago, was a water-soluble mixture of porphyrins known as hematoporphyrin derivative (HPD), which later became Photofrin. Although still widely used, this PS has some disadvantages, including a long-lasting photosensitivity and a relatively low absorbance at 630nm. The second generation which includes porphyrin derivates and synthetics, was more effective in generating singlet oxygen. The third generation saw further modification, incorporating photobleaching capabilities, antibody conjugates or protein/receptor systems. These modifications enhance the selectivity of a PS to tumor tissues. By conjugating a PS with

an antibody, the targeting of healthy cells is reduced, while the affinity for tumor cells is increased (Prazmo et al., 2016).

2.2.2.2 Light Source

To activate the PS, light of a specific wavelength that matches the PS's absorption range is required (Aebisher et al., 2024). Various light sources can be used, such as lasers, Light Emitting Diodes (LEDs) or conventional lamps (the specifics characteristics of each will be discussed later), each with its own advantages and disadvantages. Despite the availability of various light sources, the choice should depend on factors such as of tumor location, the type of PS used, and the required dose (Kim & Darafsheh, 2020). Clinical efficacy is highly dependent on the accuracy of light delivery to the target tissue and its dose, which is determined by factors such as light fluence, light fluence rate, light exposure time and light delivery mode (single or fractionated) (Correia et al., 2021). The light fluence or dose exposure is the total energy of exposed light across sectional area of irradiated spot and expressed in J/cm^2. The light fluence rate is the incident energy per second across a sectional area of irradiated spot and is expressed as W/cm^2 (Dolmans et al., 2003). Finally, the light source should be capable of delivering wavelengths within the 600-800nm range, also known as the "therapeutic window" for PDT (Chen et al., 2019)

Lasers are commonly used for both superficial and interstitial PDT. A key propriety of lasers is their ability to generate monochromatic light with a very narrow bandwidth. They provide high optical power and can be tuned to emit a wavelength that specifically correspond to the absorption range of a specific PS. However, lasers can be expensive and requires high maintenance. Nowadays, diode lasers are the most wildly used light source for clinical PDT (Mang, 2004).

LEDs are semiconductor devices that generate light through electron-hole recombination. The light emitted by a LED is incoherent with broad spectral width and large beam divergence, which result in a lower absorption by the PS. However, LEDs offer advantages such as being low-cost, portable, and suitable for direct for endoscopic/ interstitial applications (Hempstead et al., 2015).

Lamps sources used for PDT include fluorescent, incandescent, metal halide, xenon arc and sodium arc lamps. These light sources offer several advantages, such as simple

design, low cost and a wide illumination range. However, while lamps can be optically coupled to light guides to focus the light onto the specific treatment site, significant coupling losses occur. Additionally, their broad spectral output can cause variations in light penetration depth. Shorter wavelengths, which have lower optical penetration, may activate the PS more effectively at shallow depths, potentially limiting the overall treatment depth. Another consideration is the risk of unintended photodynamic effects. Overhead surgical lighting used during intraoperative PDT can unintentionally activate the PS, as these lights often have a high intensity and spectral components within PS's Soret band (intense peak in the blue wavelength region of the visible spectrum). To decrease this risk, precautions should be taken to minimize exposure by controlling the intensity and duration of illumination, as well as employing optical filtering (Kim & Darafsheh, 2020).

Currently, no studies have shown a significant difference in the efficacy of PDT for treating OL when using lasers or LEDs. Nevertheless, lasers are commonly preferred due to their coherent and monochromatic light, which provides a more stable beam and facilitates precise light dosimetry calculations, ensuring optimal irradiation at a specific wavelength required for a given PS. However, LEDs serve as a more affordable and portable alternative when needed. (Saini & Poh, 2013).

2.2.2.3 Role of oxygen and mechanism of action

Oxygen is the last component needed for photodynamic reaction (PDR) to occur. The treatment begins with the administration of a PS (topically or intravenously). Over time, the PS selectively accumulates in tumor tissue during an incubation period. Once this phase is complete the lesion is exposed to light. Although, the PS itself does not directly interact with biomolecules, the absorbed light energy is transferred to molecular oxygen, leading to the formation of reactive oxygen species (ROS). These highly reactive molecules, including singlet oxygen ($^1O_2$), superoxide radicals (O2-.), hydroxyl radical (HO.), and hydrogen peroxide (H_2O_2), initiated a cascade of biochemical events that ultimately result in cellular damage and death (Aebisher et al., 2024).

The PDR begins when the PS absorbs light and enters an excited state. Initially, the PS transition from ground state to a short-lived singlet excited state. This state is unstable, and the PS can return to its ground state either by emitting energy in form of light (fluorescence) or by dissipating energy as heat (Donnelly et al., 2008). However, the PS

may also undergo intersystem crossing, a process in which it shifts into a more stable and longer-lived triplet excited state. This triplet state provides sufficient time for energy transfer to molecular oxygen, producing singlet oxygen through what is known as type II reaction. Singlet oxygen, is extremely reactive and interacts with biological molecules, leading to oxidative damage that disrupts cellular structures and function. A type I reaction may also occur if the excited PS interacts directly with cellular components, such as the cell membrane, leading to the formation of free radicals and other ROS that further contribute to oxidative stress and cellular destruction. While both type I and type II reactions can take place simultaneously, type II reactions are predominant in PDT. With singlet oxygen playing the central role in inducing cytotoxic effects (Correia et al., 2021).

The biological outcomes of PDT depend on multiple factors, including PS used, its location within cells, the total administered dose, the intensity and duration of light exposure, and the availability of oxygen. Since ROS have a short lifespan and high reactivity, they primarily affect cells in the immediate vicinity of PS accumulation. Tumor oxygenation plays a crucial role in PDT efficacy, well-oxygenated and superficial tumors tend to respond more effectively, whereas deeper or hypoxic tumors presents a greater challenge due to limited oxygen supply and reduced light penetration (Przygoda et al., 2023).

At a cellular level, PDT can induce different forms of cell death, depending on the extent of ROS production and the type of reactions that occur. High levels of ROS can cause necrosis, a rapid uncontrolled form of cell death that results in inflammation and damage to surrounding tissues. In contrast, lower levels of ROS typically trigger apoptosis, a programmed cell death mechanism that eliminates damages cells in a controlled manner without causing excessive harm to adjacent tissues. Apoptosis involves a series of biochemical events, which ultimately lead to cell destruction. Another possible outcome is autophagy, a self-digesting process in which cells break down and recycle damaged components. This mechanism can either contribute to cell survival by removing damaged organelles or lead to cell death when excessive. The extent to which necrosis, apoptosis or autophagy occurs depends on PDT parameters, including PS concentration, light fluence and oxygen availability (Allison & Moghissi, 2013; Kessel, 2008).

2.2.3 Use of Photodynamic Therapy nowadays

PDT is a minimally invasive procedure that is clinically used in the treatment of several oncologic, human diseases, such as skin, esophageal, head and neck, lung and bladder cancers (Plaetzer et al., 2013).

PDT offers several advantages over other conventional treatments. It is minimally invasive compared to surgical procedures, has no long-term side effects when used correctly, and can be performed on an outpatient basis. PDT selectively targets tumors through the preferential accumulation of PS and localized light activation, minimizing damage to healthy tissue. It also destroys tumor vasculature, can be repeated multiple times in the same area, leaves little to no scarring, and is often more cost-effective than other treatments. (Calixto et al., 2016)

However, PDT has limitations. Its effectiveness is restricted to the irradiated area. Sufficient tissue oxygenation is essential, meaning tumors with necrotic or dense tissue may not respond well. Additionally, visible light penetration is limited, making it challenging to treat deep-seated tumors without surgical assistance (Lange et al., 2021).

A standardized protocol exists for conventional topical PDT to optimize PS absorption and light penetration. The recommended steps for PDT treatment include (Ozog et al., 2016):

1. Cleansing: wash the treatment area with soap or water to remover dirt and oil
2. Residue removal: use a gauze soaked in acetone or alcohol to eliminate any remaining oil or debris
3. PS application: apply the PS evenly over the entire area, allowing it to dry before applying another layer
4. Incubation: let the PS absorb for 30 minutes to 4 hours
5. Activation: expose the area to the appropriate light source to activate the PS
6. Post-treatment cleaning: wash the treated area with soap and water to remove any residual PS
7. Sunlight Avoidance: Protect the area from direct sunlight for at least 48 hours to prevent unintended photoreactions.
8. Follow-up: repeat treatment, if necessary, after 2 to 3 weeks.

PDT has applications beyond oncology, offering promising treatments for various non-cancerous conditions. In dermatology, PDT is used to treat acne vulgaris, genital warts cause by HPV, and other sexually transmitted infections. Studies indicates that PDT results in lower recurrence rates compared to CO_2 laser therapy. It is also effective for managing psoriasis and vascular malformations, including venous, arteriovenous, capillary, and lymphatic abnormalities (Morton et al., 2020).

In dentistry, the antimicrobial proprieties of PDT play a significant role in improving oral health. In endodontics, PDT combined with conventional chemo-mechanical preparation has shown promising results in enhancing bacterial elimination and treatment success. PDT can also provide an effective method for plaque removal, reducing the risk of dental caries. In periodontology and implantology, PDT serves as a valuable adjunctive treatment for chronic periodontitis and peri-implantitis. Its potent antibacterial effects help control infections, improve tissue healing, and enhance overall treatment outcomes. By targeting harmful bacteria and efficient approach to managing oral infections and maintaining long-term oral health (Stájer et al., 2020).

In cardiovascular medicine, PDT has been explored for treating condition such as atherosclerosis and esophageal varices (Jerjes et al., 2011). Neurological disorders like Alzheimer's disease and prion disease may also benefit from PDT due to its potential neuroprotective and antimicrobial effects.

Additionally, PDT shows promise in addressing skeletal and autoimmune disorders, such as rheumatoid arthritis and synovitis, by targeting inflamed tissue and modulating immune responses. In gastroenterology, PDT has been investigated for condition like Crohn's disease, bacteria-mediated gastritis (colitis), offering a non-invasive approach to reducing inflammation and infection (Yoo et al., 2021).

PDT has also been studied for respiratory condition, including ventilator-associated pneumonia (Biel et al., 2011) and COVID-19, where its antimicrobial and anti-inflammatory proprieties may help manage infections and reduce complications (Almeida et al., 2020). As research continues, PDT's role in treating oncological and non-oncological disease is expanding, highlighting its versatility, and potential in various medical fields (Aebisher et al.,2024).

3 Results

3.1 General characteristics of the studies

A total of 12 clinical studies that investigates the use of PDT for the treatment of OL were includes in this review. The studies varied in design, including prospective studies (Jerjes et al. 2011; Yao et al., 2022), retrospective studies (Wang et al., 2024; Ou et al. 2022), pilot studies (Shafirstein et al., 2011; Selvam et al.,2015. One study was a phase I clinical trial (Wong et al., 2013), while others ranged from single-arm observational studies (Pietruska et al., 2014; Kübler et al.,1998) to comparative evaluations (Kawczyk-Krupka et al., 2012) and preliminary exploratory investigations (Han et al., 2019; Maloth et al., 2016). The studies were conducted in a total of 6 different countries, China (Wang et al., 2024; Ou et al. 2022; Yao et al., 2022; Han et al., 2019); India (Maloth et al., 2016; Selvam et al.,2015); Poland (Pietruska et al., 2014; Kawczyk-Krupka et al., 2012), USA (Wong et al., 2013; Shafirstein et al., 2011); United Kingdom (Jerjes et al. 2011) and Germany (Kübler et al.,1998).

The total number of participants across the studies was 480 participants, with individual study sample sizes ranging from 5 to 147 participants. Most studies included both male and female patients except for one. In the pilot study by Selvam et al. (2015), all participants were male. All participants were over 18 years of age.

Regarding lifestyle habits, most studies distinguished between smokers and non-smokers. However, four studies: Maloth et al. (2016); Wong et al. (2013); Shafirstein et al. (2011) and Kübler et al. (1998) did not report or assesses participants smoking status. Alcohol consumption was noted as a relevant factor in four studies: Wang et al. (2024); Ou et al. (2022); Yao et al. (2022); and Jerjes et al. (2011). Additionally, only the study by Ou et al. (2022) reported areca (betel) nut chewing as patient habits. Demographic information of participants can be found in Table 5.

Table 5: Demographic information of participants

Author / Date of publication	Country	N° of participants	Sex	Age (in years)	Race	Smokers	Alcohol	Areca nut chewing
Yanting Wang *et al.* - 2024	China	50	Male: 26/50 Female: 24/50	Min: 18 Max: 75	N/A	Yes : 27/50 No: 23/50	Yes: 32/50 No: 18/50	N/A
Jiali Ou *et al.* - 2022	China	71	Male: 69/71 Female: 2/71	Min: 18 Max:75	N/A	Yes: 71/71 No: 0/71	Yes: 37/71 No: 34/71	Yes: 20/71 No: 51/71
Yi-Lin Yao *et al.* - 2022	China	48	Male: 18/48 Female: 30/48	Min: 18 Max: 75	N/A	Yes: 14/48 No: 34/48	Yes: 6/48 No: 42/48	N/A
Ying Han *et al.* - 2019	China	29	Male: 11/29 Female:18/29	Mean: 56, 07 +/- 11,01	N/A	Yes: 5/29 No: 24/29	N/A	N/A
Kotya Naik Maloth *et al.* - 2016	India	13	N/A	Mean: 39.17 +/- 14,75	N/A	N/A	N/A	N/A
Niranzena Panneer Selvam *et al.*)-2015	India	5	Male: 5/5	Min: 35 Max: 49	N/A	Yes: 5/5 No: 0/5	N/A	N/A
Malgorzata Pietruska *et al.* – 2013	Poland	23	Male: 7/23 Female:16/23	Min: 21 Max: 79	N/A	Yes: 6/23 No: 17/23	N/A	N/A
Stuart J. WONG *et al.* - 2013	USA	11	Male: 6/11 Female: 5/11	Min: 48 Max: 74	N/A	N/A	N/A	N/A
Aleksandra Kawczyk-Krupka *et al.* - 2012	Poland	48	Male: 20/48 Female:28/48	Min: 32 Max: 75	N/A	Yes: 18/48 No: 30/48	N/A	N/A
Gal Shafirstein *et al.* - 2011	USA	23	Male: 13/23 Female: 10/23	Min: 37 Max: 79	N/A	N/A	N/A	N/A
Waseem Jerjes *et al.* - 2011	United-Kingdom	147	Male: 82/147 Female: 65/147	Min: 41 Max: 98	Caucasian: 96/147 Indian: 36/147 Middle Eastern: 7/147 Oriental: 6/147	Yes: 124/ 147 No: 23/147	Yes: 100/ 147 No: 47/147	N/A
Alexander Kübler *et al.* - 1998	Germany	12	Male: 11/12 Female: 1/12	N/A	N/A	N/A	N/A	N/A

N/A = Not applicable

Except for four studies, PDT was used as the sole therapeutic strategy for the management of OL. In the study by Kawczyk-Krupka et al. (2012), the efficacy of PDT was compared to cryotherapy. Maloth et al. (2016) evaluated PDT in comparison with conventional pharmacological treatment. Yao et al. (2022) assessed the outcomes of ablative fractional laser-assisted photodynamic therapy (AFL-PDT) versus ablative fractional laser (AFL) treatment alone. Additionally, Ou et al. (2022) used Waterlase-assisted photodynamic therapy as a modified approach to enhance treatment efficacy.

3.2 Lesions characteristics

A total of 546 lesions were diagnosed and treated across the studies included in this review. All studies reported a clinical and histological diagnoses of OL lesions.

Wang et al. (2024) investigated 50 lesions. The anatomical distribution was as follow: 40% (20/50) on the floor of the mouth, 28% (14/50) on the buccal mucosa; 10% (5/50) on the margin surface of the tongue; 8% (4/50) on the palate, 6% (3/50) on the gingiva; 4% (2/50) on the dorsal surface of the tongue and 4% (2/50) on the lips. Clinically, 68% (34/50) were classified as homogeneous and 32% (16/50) as non-homogeneous. 34%

(17/50) of the lesions showed no dysplasia; 40% (20/50) mild dysplasia and 26% (13/50) moderate to severe dysplasia.

Ou et al. (2022), examined 71 lesions. Most lesions, 67% (47/71) were located on the tongue, 25% (18/71) on the buccal mucosa and 8% (6/71) were on the lips. The clinical classification of the lesions was not reported. Regarding dysplasia, 41% (29/71) were mildly dysplastic, 46% (33/71) moderately dysplastic and 13% (9/71) were severely dysplastic.

Yao et al. (2022); studied 48 lesions. Of these, 42% (20/48) were present on the lateral margins of the tongue; 37% (18/48) on the buccal mucosa; 8% (4/48) on the gingiva, 6% (3/48) on the palate, 4% (2/48) on the dorsal surface of the tongue and 2% (1/48) on the floor of the mouth. The clinical classification of lesions was not provided. Histopathological evaluation revealed 4% (2/48) of lesions had no dysplasia; 56% (27/48) had mild dysplasia; 31% (15/48) moderate dysplasia and 8% (4/48) severe dysplasia.

Han et al. (2019) assessed 29 lesions. 31% (9/29) on the cheek, 7% (2/29) on the gum; 10% (3/29) on the dorsal surface of the tongue and 52% (15/29) on the ventral surface of the tongue. Clinically, 69% (20/29) of lesions were homogeneous and 31% (9/29) were non-homogeneous. Histological grading showed 35% (10/29) had no dysplasia; 41% (12/29) had mild dysplasia and 24% (7/29) had moderate to severe dysplasia.

Maloth et al (2016), evaluated 24 lesions. Most of them, 79% (19/24) were on the buccal mucosa; 13% (3/24) were on the tongue and 8% (2/24) were on the attached gingiva. Neither the clinical classification nor the histological grade of dysplasia of was reported.

Selvam et al. (2015); studied 5 lesions. Of these, 40% (2/5) were located on the buccal mucosa, 40% (2/5) on the gingiva and 20% (1/5) on the ventral surface of the tongue. Clinically, 60% (3/5) of the lesions were homogeneous, 20% (1/5) were verrucous leukoplakia and 20% (1/5) were erythroleukoplakia. Histopathologically, 60% (3/5) showed epithelial hypertrophy with hyperkeratosis; 20% (1/5) had moderate dysplasia and 20% (1/5) had mild dysplasia.

Pietruska et al. (2014); analyzed 44 lesions. Of these, 86% (38/44) are localized on the cheeks and lips and 14% (6/44) on the gums and tongue. 100% (44/44) of these lesions were classified as flat homogeneous leukoplakia. The grade of dysplasia was not reported.

Wong et al. (2013), 11 lesions studied. Of these, 82% (9/11) of the lesions were on the tongue; 9% (1/11) on the alveolar ridge and 9% (1/11) on the lip. The clinical

classification of the lesions was not specified. Histologically, 27% (3/11) showed no dysplasia; 54% (6/11) showed mild dysplasia; 9% (1/11) showed moderate dysplasia and 9% (1/11) showed severe dysplasia.

Kawczyk-Krupka et al. (2012) assessed 81 OL lesions. 53% (43/81) were located on the buccal mucosa, 14% (11/81) on the maxillary mucosa, 9% (7/81) on the mandibular mucosa, 9% (7/81) on the lips, 7% (6/81) on the floor of the mouth, 2% (2/81) on the palate, 2% (2%81) on the tongue and 4% (3/81) in the bucco-gingival sulcus. The clinical classification of the lesions was not reported in this study. Histopathological analysis showed that 93% (75/81) showed no dysplasia, while 7% (6/81) demonstrated low-grade dysplasia.

Sharfirstein et al. (2011) evaluated 24 OL lesions. Regarding location, 33% (8/24) were found on the buccal mucosa, 38% (9/24) on the tongue, 13% (3/24) on the alveolar ridge, and one lesion each (4% each) on the palate, retromolar trigone, floor of the mouth and bucco-gingival mucosa. The study did not report the clinical classification or the histopathological grade of dysplasia.

Jerjes et al. (2011) assessed 147 lesions of OL. The anatomical distribution was diverse: 17% (25/147) were located on the floor of the mouth, 22% (33/147) on the lateral tongue, 3% (5/147) on the dorsal surface of the tongue, 5% (8/147) on the ventral surface of the tongue, 12% (18/147) on the buccal mucosa, 4% (6/147) on the hard palate, 6% (9/147) on the upper alveolus, 8% (12/147) on the lower alveolus, 18% (27/147) in the retromolar area and 3% (4/147) in the maxillary tuberosity region. Clinically, 37% of the lesions (55/147) were classified as homogeneous leukoplakia, 50% (73/147) as non-homogeneous leukoplakia, and 13% (19/147) as erythroplakia. Histologically, 13% (19/147) showed mild dysplasia, 22% (33/147) moderate dysplasia, 43% (63/147) severe dysplasia, and 22% (32/ 147) were diagnosed as carcinoma in situ.

Kübler et al. (1998), examined a total of 12 OL lesions. The anatomical distribution showed that 42% (5/12) were located on the cheeks, 42% (5/12) in the vestibulum, and 17% (2/12) on the floor of the mouth. Clinically, 75% of the lesions (9/12) were classified as homogeneous and flat, while 25% (3/12) were non-homogeneous with verrucous appearance. Histopathological evaluation revealed that 50% (6/12) of the lesions showed no dysplasia, 33% (4/12) showed mild dysplasia and 2 lesions (8% each) showed moderate and severe dysplasia respectively. A summary of information on oral leukoplakia lesions can be found in Table 6.

Table 6 : Oral leukoplakia lesions characteristics

Author/ date of publication.	N° of lesions	Biopsy	Location	Classification	Grade of Dysplasia
Yanting Wang *et al.* - 2024	50	Yes	Floor of the mouth 20/50 Buccal mucosa 14/50 Margin surface of the tongue 5/50 Palate 4/50 Gingiva 3/50 Dorsal surface of the tongue 2/50 Lip 2/50	Homogeneous 34/50 Non-homogeneous 16/50	No dysplasia 17/50 Mild 20/50 Moderate to severe 13/50
Jiali Ou *et al.* - 2022	71	Yes	Tongue 47/71 Buccal 18/71 Floor of the mouth 6/71	N/A	Mild 29/71 Moderate 33/71 Severe 9/71
Yi-Lin Yao *et al.* - 2022	48	Yes	Margins surface of the tongue 20/48 Cheek mucosa 18/48 Gingiva 4/48 Palate 3/48 Dorsal surface of the tongue 2/48 Floor of the mouth 1/48	N/A	No dysplasia 2/48 Mild 27/48 Moderate 15/48 Severe 4/48
Ying Han *et al.*- 2019	29	Yes	Cheek 9/29 Gum 2/29 Dorsal surface of the tongue: 3/29 Ventral surface of the tongue 15/29	Homogenous 20/29 Non homogenous 9/29	No dysplasia 10/29 Mild dysplasia 12/29 Moderate to severe dysplasia 7/29
Kotya Naik Maloth *et al.* - 2016	24	Yes	Buccal mucosa 19/24 (PDT 9/12 & CT 10/12) Tongue 3/24 (PDT 2/12 & CT 1/12) Attached gingiva 2/24 (PDT 1/12 & CT 1/12)	N/A	N/A
Niranzena Panneer Selvam *et al.* -2015	5	Yes	Buccal mucosa 2/5 Gingiva 2/5 Ventral surface of the tongue 1/5	Homogenous leukoplakia 3/5 Verrucous Leukoplakia 1/5 Erythroleukoplakia 1/5	Epithelial hypertrophy with hyperkeratosis 3/5 Moderate dysplasia 1/5 Mild dysplasia 1/5
Malgorzata Pietruska *et al.* – 2013	44	Yes	Cheeks and lips 38/44 Gums and tongue 6/44	Oral homogeneous flat leukoplakia 44/44	N/A
Stuart J. WONG *et al.* -2013	11	Yes	Oral tongue 9/11 Alveolar ridge 1/11 Lip 1/11	N/A	No dysplasia 3/11 Mild 6/11 Moderate 1/11 Severe 1/11
Aleksandra Kawczyk-Krupka *et al.* - 2012	81	Yes	Buccal mucosa 43/81 Maxillary mucosa 11/81 Mandibular mucosa 7/81 Lips 7/81 Floor of the mouth: 6/81 Palate: 2/81 Tongue: 2/81 Bucco-gingival sulcus: 3/81	N/A	No dysplasia 75/81 Low grade dysplasia 6/81
Gal Shafirstein *et al.* 2011	24	Yes	Buccal mucosa:8/24 Tongue 9/24 Alveolar ridge: 3/24 Palate 1/24 Retromolar trigone 1/24 Floor of the mouth:1/24 Bucco gingival mucosa: 1/24	N/A	N/A
Waseem Jerjes *et al.* - 2011	147	Yes	Floor of the mouth 25/147 Tongue (lateral) 33/147 Tongue (dorsal) 5/147 Tongue (ventral) 8/147 Buccal mucosa 18/147 Hard palate 6/147 Upper alveolus 9/147 Lower alveolus 12/147 Retromolar area 27/147 Tuberosity 4/147	Homogenous leukoplakias 55/147 Non-homogenous leukoplakias 73/147 Erythroplakia 19/147	Mild dysplasia 19/147 Moderate dysplasia 33/147 Severe dysplasia 63/147 Carcinoma in situ 32/147
Alexander Kübler *et al.* - 1998	12	Yes	Cheeks: 5/12 Vestibulum: 5/12 Mouth floor: 2/12	Homogeneous, flat: 9/12 Non-homogeneous, verrucous: 3/12	No dysplasia: 6/12 Mild: 4/12 Moderate: 1/12 Severe: 1/12

N/A = Not Applicable

3.3 Treatment protocol and response

In the study conducted by Wang et al. (2024), PDT using 5-aminolevulinic acid (ALA) at a concentration of 20%, was applied to patients with OL. Treatment consisted of a 3 to 4 sessions, spaced 7 to 14 days apart, depending on the healing progress of the lesions. The protocol involved local anesthesia with lidocaine, followed by topical application of 20% ALA using a cotton piece, which was then covered with rice paper and plastic wrap, and secured in place with sterile gauze under pressure for two hours. Illumination was performed in a dark environment using a 365nm He-Ne laser, with a fluence of 150-300mW/cm^2 and an irradiation time of 300 seconds. Out of the 50 treated lesions, 52% (26/50) of the patients experimented local oral pain or ulcers as side effects. Regarding clinical response, complete remission (CR) was achieved in 6 cases (12%), partial remission (PR) in 28 cases (56%), while 16 lesions (32%) showed no response (NR). Recurrence occurred in 1 out of 6 CR lesions (16,7%), 13 out of 28 PR lesions (46,4%) and 2 out of 16 NR lesions (12,5%). Malignant transformation was reported in 2 out of the 50 treated lesions (4%).

In the study by Ou et al. (2022), a 20% concentration of ALA was applied in gel form and assisted with Waterlase technology. No local anesthesia was used. After salivary isolation, the ALA gel was applied directly to the lesion, covering a margin of approximately 2-3mm beyond visible area, and left in place for 3 hours. Illumination was performed using red light at a wavelength of 635nm, with an output power of 120J/cm^2 and a fluence rate of 80 mW/cm^2. Each spot (2 cm in diameter) was irradiated for approximately 25 minutes, with adjacent spots overlapping by 3-5mm to ensure full coverage. Out of 71 treated lesions, CR was observed in 60 cases (84,5%) and PR in 11 cases (15,5%). Recurrence was recorded in 4 patients (5,6%) and new lesions appeared in 5 patients (7%).

Yao et al. (2022) investigate the use of 20% ALA gel in combination with ablative fractional laser (AFL) pre-treatment. The protocol involved local anesthesia and removal of the stratum corneum with AFL before applying the ALA gel. The treated area was then covered with glutinous rice paper, plastic wrap, and gauze to ensure proper isolation and absorption for 3 hours. Red light illumination at 630 nm was delivered at a dose of 180J/cm^2 using 100 light sources, applied to 1 cm diameter spots for 5 minutes each, with 3mm overlaps between adjacent spots. Among the 48 lesions treated, CR was achieved in 30 cases (62,5%), PR in 12 (25%), and no response in 6 cases (12,5%). After three years of follow-up, recurrence occurred in 18 patients (37,5%), distributed as follows: 9

of 30 CR cases (30%), 6 of 12 PR cases (50%), and 3 of 6 NR cases (50%). Malignant transformation was reported in 4 cases (8,3%), affecting 2 CR and 2 NR patients.

In the study by Han et al. (2019), PDT using 20% ALA gel was administered over an average of 4.08±1.73 sessions. Patients received local anesthesia prior to the application of the gel, which was left on the lesion for 2 hours before laser illumination. A He-Ne laser with a wavelength of 632nm was used, with a fluence of 500mW/cm^2 and a total light dose ranging between 90 and 180 J/cm^2. Irradiation was applied for 3 minutes with 1-minute intervals between each stage. Out of 29 treated patients, 19 (65,5%) reported local ulcers or oral pain. The clinical response showed CR in 16 patients (55,2%), PR in 9 (31%), and NR in 4 cases (13,8%). Among the 25 patients who were followed up, recurrence was observed in 3 cases (12%)

Maloth et al. (2016) evaluated PDT protocol involving ALA, prepared by dissolving 50mg of ALA powder in 1mL of water. The solution was applied directly to the lesion, avoiding margin of 5-6mm, and allowed to incubate for 30 minutes. Illumination was delivered using blue LED light at a wavelength of 420nm, with a fluence over 500mW/cm$^{2.}$ The treated area, including the lesion and its margin, was irradiated for 10 minutes with fractionated exposure (3-minutes intervals). In the PDT treated group (n=12), CR was achieved in 2 patients (16,7%), PR in 8 patients (66,7%) and NR in 2 patients (16,7%). The outcomes in the control group (n =12) treated with conventional therapy (CT) showed: PR in 2 patients (16,7%) and NR in 9 patients (75%), no complete remission was observed.

In the study conducted by Selvam et al. (2015), PDT was performed using 10% ALA emulsion. Treatment consisted of 6 to 8 sessions with 1-week intervals between each session. The ALA emulsion was applied topically to the lesion and covered with gauze, allowing an incubation period of 3 hours. Illumination was delivered using a xenon lamp with a wavelength of 630±5mm, a power output of 0,1 W and a total dose of 100J/cm^2 per session. Irradiation lasted a total of 1000 seconds, divided into 3-minutes intervals interspersed with short rests. Among the 5 treated patients, 2 (40%) reported experiencing a mild burning sensation during light application. In terms of clinical outcomes, CR was achieved in 2 cases (40%), PR in 2 cases (40%), and NR was observed in 1 case (20%). No recurrence was reported during a 12-month follow-up period.

In the study by Pietruska et al. (2013), PDT was conducted using Chlorin-e6 as a the PS. The gel formulation was applied over the lesion as well as the adjacent healthy mucosa

for one hour prior to illumination. The treated area was then covered sequentially with a sheet of nonwoven fabric, a polyethylene sheet, and multiple layers of sterile gauze. A total of 10 sessions were performed. Illumination was carried out using the Haemato LS PDT 660 semiconductor laser, with a wavelength of 660nm and a superficial light energy density of 90J/cm^2. Out of the 44 treated lesions, CR was observed in 12 cases (27,3%), PR in 22 cases (50%) and NR in 10 cases (22,7%). No pain or discomfort was reported during the procedures. Data on recurrence were not provided in this study.

In the clinical trial by Wong et al. (2013), oral administration of ALA was explored for PDT in patients with OL. A single dose of ALA powder dissolved in 50mL of water was given 3-4 hours before light treatment. Among the 11 participants: 1 received 60mg/kg, 6 received 30mg/kg and 4 received no ALA. Illumination was performed using pulsed dye laser (PDL) with a wavelength of 585nm and 10mm spot size. Light doses ranged from 2 to 8 J/cm^2 in 2 J/cm^2 increments, with 4 patients per dose cohort. Several side effects were observed, including elevated liver enzymes, skin photosensitivity, and gastrointestinal symptoms. Although no CR or PR was recorded at 3 months, one patient exhibited a notable 50% reduction in lesion size. Histologically hyperplasia was classified as mild in 6 patients (54,5%), moderate in 4 (36,4%) and severe in 1 case (9,1%). No dysplasia was found in 6 patients (54,5%) and mild in 5 (45,5%). 3 participants experienced malignant progression during the follow-up period.

Kawczyk-Krupka et al. (2012) conducted a comparative study on the efficacy of PDT using two different concentrations of ALA: 20% and 10%. Patients were divided in two groups: group 1 received 20% ALA with illumination by Diomed laser (630nm), while group 2 was treated with 10% ALA and an argon-pumped dye laser (635 nm). Local anesthesia was administered prior to treatment. The ALA was applied topically with a 4mm safety margins and left to incubate for 2 hours. A single illumination dose of 100J/cm^2 was delivered, covering the lesion and a surrounding 10-20% safety margin, with a total treatment time of approximately 15 minutes at low fluorescence rate. The number of sessions varied between 2 and 12, with follow-ups every two weeks and treatment repeated if needed. Mild adverse effects were observed in a minority of cases, including erythema in 9 patients (18,75%), edema in 9 patients (19,75%), while 30 (62,5%) reported no side effects. Additionally, 30% of patients required analgesics. In terms of therapeutic outcomes, group 1 (n=52 lesions) CR was achieved in 40 cases (76,9%), PR in 8 cases (15,4%) and NR in 4 cases (7,7%). Group 2 (n=29 lesions) showed

CR in 24 cases (82,2%), PR in 2 cases (6,9%), and NR in 3 cases (10,3%). Overall, for all 48 patients, CR was observed in 35 cases (72,9%). Recurrence was noted in 11 lesions from group 1 (21,2%) and 2 lesions from group 2 (6,9%), totaling 13/81 lesions (16%).

In the study by Shafirstein et al (2011), PDT was applied to 17 patients using ALA solution either topically or through intralesional injections. For the topical treatment, ALA was applied for 1,5 hours, while for the intralesional injections, ALA was injected directly into the lesion. Local anesthesia was used if necessary. Illumination was performed with a PDL at a wavelength of 585nm, delivering a light dose of 6 to 8J/cm^2. The irradiation involved pulses of 1,5ms, with intervals of 1 to 3 seconds, and a 7-mm diameter spot. Side effect included sensitivity, soreness, swelling, a burning sensation, taste modification and occasional ulceration. In terms of therapeutic response, 7 patients (41,2%) achieved significant response (SR), 9 patients (52,9%) PR, and 1 patient (5,9%) NR.

In the study conducted by Jerjes et al. (2011), ALA (for thin mild moderate dysplasia) or mTHPC (for thicker mild-moderate dysplasia, severe dysplasia and carcinoma in situ) was used as PS, with respective illimitation via 628nm and 652nm diode lasers. Patients received either topical ALA (60mg/kg) 3-4 hours before illumination or intravenous mTHPC (0,1mg/kg) 96 hours prior. Light doses ranged from 100-200 J/cm^2 for ALA and 20J/cm^2 for mTHPC. Pain management included NSAIDs and opiates. Mild to moderate treatment-site pain was reported by 85,7% of patients (127/147), and 15% experienced photosensitivity (22/147). At the 3-years follow-up, CR was observed in 111 patients (75,5%), PR in 23 patients (15,6%), stable disease (SD) in 5 patients (3,4%) and progressive disease (PD) in 8 patients (5,4%). At 5 years, CR increased to 116 patients (78,9%), PR decreased to 11 patients (7,5%), SD remained stable and PD increase to 11 patients (7,5%). The final outcome showed 119 patients (81%) in CR, 12 patients (8,2%) in PR, 5 patients (3,4%) with SD and 11 patients (7,5%) with PD. Recurrence occurred in 17 patients (11,6%)

In the study by Kübler et al. (1998), a single session of PDT therapy was performed using 20% ALA cream applied topically to the lesion. The cream was left on for 2 hours, with an additional 3-5g of ALA applied every 30 minutes during the incubation period. The lesion was then covered with gauze. Illumination was performed using a monochromatic red light delivered by an argon-dye laser at a wavelength of 630nm, with a light dose of 100mW/cm^2 and 100J/cm^2, for a total irradiation time of 1000 seconds. 5 patients (41,7%) reported a mild burning sensation, which immediately reduced after the laser treatment.

In terms of response, CR was achieved in 5 patients (41,7%), PR in 4 patients (33,3%) and NR in 3 patients (25%). Additionally, one patient who initially achieved PR was retreated, and after the second session, the lesion disappeared. Recurrence data was not specified.

A summary of information on the PDT protocol and outcomes can be found in table 7.

Table 7 : Photodynamic therapy treatment protocol and Outcomes

Author and date of publication	PS	Associated with other treatment	Photoactivator tool and protocol timing	Side effect	Follow-up period	Response	Recurrence	MT
Yanting Wang *et al.* - 2024	ALA 20%	NO	N° of session: 3-4 with an interval of 7-14 days between each, depending on the healing **Protocol:** -Local anesthesia (lidocaine) - Surface cover with a cotton piece dipped with 20% 5-ALA, covered by a rice paper and a plastic wrap and fixed with sterile gauze under pressure for 2h. -Illumination parameter He-Ne laser of 365nm, fluence at 150-300mW/cm^2, irradiation time of 300s - Irradiation in a dark environment.	Oral pain local/ ulcers 26/50	2 years	CR 6/50 PR 28/50 NR 16/50	At 2 years 16/50 CR lesion 1/6 PR lesion 13/28 NR lesion 2/16	2/50 Location: Floor of the mouth At 3 and 18 months after treatment
Jiali Ou *et al.* - 2022	ALA 20%	Waterlase (YSGG)	N° of session: N/A **Protocol** -Without local anesthesia. -Application of ALA 20% dissolved in gel, placed over the lesion with a margin of +/- 2-3mm from the surrounding normal area for 3h (after salivary isolation). Illumination parameter: red light; wavelength of 635nm, output power 120J/cm^2, fluence at 80mW/cm^2 applied for +/- 25min/illuminated spot. Irradiation equally and homogeneously onto a spot of 2cm diameter. Overlap of the adjacent spot by 3-5mm.	N/A	1 year	CR 60/71 PR 11/71	4/71 New lesions 5/71	5/71 Epithelial dysplasia and carcinoma in situ in other part of the oral cavity (especially on the tongue)
Yi-Lin Yao *et al.* - 2022	ALA 20%	Ablative fractional laser (AFL)	N° of session: 1 **Protocol** -Local anesthesia, removal of the stratum corneum with AFL. -Application of 20% ALA gel on the lesion then covered with glutinous rice paper plastic wrap and gauze saliva isolation for 3h. -Illumination parameter: red light, wavelength of 630nm, light dose of 180J/cm^2, fluence rate of 100 sources. -Irradiation: spot of 1cm diameter for 5min, overlapped with adjacent spots by 3 mm to cover the entire lesion	N/A	3 years	CR 30/48 PR 12/48 NR 6/48	At 3years 18/48 9/30 CR 6/12 PR 3/6NR. Location: Especially on the gingiva and palate	4/48 2/30 CR 2/6 NR Location: Tongue margin 3/4 Gingiva 1/4
Ying Han *et al.* - 2019	ALA 20%	NO	N° of session: 4,08+/- 1,73. **Protocol:** -Local anesthesia -20% ALA gel applied on the lesions 2h before laser illumination. -Illumination parameter: He-Ne laser, wavelength of 632nm, fluence at 500mW/cm^2 light dose of 90-180 J/cm^2 -Irradiation: 3min therapy with 1 min intervals between each stage.	Local ulcer and oral pain 19/29	3 months	CR 16/29 PR 9/29 NR 4/29	3/25	N/A
Kotya Naik Maloth *et al.* - 2016	ALA	NO	N° of session 12 **Protocol** - Application of 5-ALA (50mg of ALA powder mixed with 1mL of water) on the lesions (except for a margin of 5-6nm around them). Incubation period of 30min. Illumination parameter: LED blue light, wavelength of 420nm and fluence >500mW/cm^2 -Irradiation: the lesion and the 5-6mm surrounding area were illuminated with a spot size of 1cm2 for 10 min (with 3 min fractionization)	N/A	4 weeks	PDT CR 2/12 PR 8/12 NR 2/12 CT PR 2/12 NR 9/12	N/A	N/A

Niranzena Panneer Selvam *et al.* -2015	ALA 10%	NO	N° of session 6-8 (with a 1-week interval) **Protocol** -Application of an emulsion of 10% ALA and a gauze place over the lesion (incubation for 3h) -Illumination parameter: Xenon Lamp, power 0,1W, wavelength of 630 +/- 5nm, total dose 100J/cm^2 per session. -Irradiation: 3 min for a total of 1000s interspersed by several 3 min rests.	Mild burning sensation at light application 2/5	1 year	CR 2/5 PR 2/5 NR 1/5	No recurrence at 12 months	None
Malgorzata Pietruska *et al.* –2013	Chlorin e6	NO	N° of session: 10 **Protocol** Gel of Chlorin-e6 applied on the lesion and surrounding healthy mucosa for one hour before illumination and then cover with a sheet of nonwoven fabric, a polyethylene sheet and few layers of sterile gauze. -Illumination parameter: semiconductor laser Haemato LS PDT 660; wavelength of 660nm, superficial light energy density of 90J/cm^2	No discomfort or pain	N/A	CR 12/44 PR 22/44 NR 10/44	N/A	N/A
Stuart J. WONG *et al.* - 2013	ALA	NO	N° of session: 1. **Protocol** -ALA dose 1/11 at 60mg/kg and 6/11 at 30mg/kg and 0mg/kg 4/11 ALA powder was administered by mouth as a single dose dissolved in 50 ml of water 3–4 h before light treatment. Illumination parameter: PDL 585 nm A laser spot size of 10 mm was used with a specific laser setting (Joules) to achieve the desired fluence (Joules/cm^2). Dose escalation cohorts were planned with 4 participants per cohort – each corresponding to a light dose from 2 to 8 J/cm2 by increments of 2 J/cm^2 .	ALT elevation AST elevation Urine color change Skin photosensitivity Nausea Pruritus Palpitations Nausea Skin Photosensitiviy Vomiting	3 months	At 3 months No clinical response (CR or PR) neither a progression of the lesion. But 1 patient, have a decrease of 50% in one lesion and significant reduction in the mucosal prominence of the other lesion Hyperplasia: Mild 6/11 Moderate 4/11 Extensive 1/11 Dysplasia None: 6/11 Mild 5/11	N/A	3/11 Location: 1/3 alveolar ridge 2/3 oral tongue
Aleksandra Kawczyk-Krupka *et al.* - 2012	ALA 20% or ALA 10%	NO	N° of session: between 2 and 12 **Protocol** Two groups: Group 1: 20% ALA and Diomed laser 630nm wavelength. Group 2: 10% ALA and argon-pumped dye laser 635nm wavelength Local anesthesia. -Application of ALA with margins of 4mm from the surrounding normal tissue, incubation period 2h. Illumination parameter: single dose of 100J/cm^2. Irradiation: Include at least a 10-20% margin around the lesion in the field of irradiation. Treatment time about 15min at low fluorescence rate. 30% of patients received analgesic drugs. Patient reviewed at 2 weeks interval and the treatment were repeated if necessary	Eritema 9/48 Edema 9/48 No adverse effect 30/48	Between 1 and 119 months (10 years)	Response (by location) Group 1 CR 40/52 PR 8/52 NR 4/52 Groupe 2: CR 24/29 PR: 2/29 NR 3/29 Total: CR 35/48	Groupe 1: 11/52 Groupe 2: 2/29 Total: 13/48	None

Gal Shafirstein *et al.* - 2011	ALA	NO	N° of sessions: 8 (separated by 6 to 8 weeks) **Protocol** local anesthesia if necessary Application topically an ALA solution for 1.5 hours (for 17 patients) or administration of ALA intralesional injection. Illumination parameter: PDL of 585nm wavelength; light dose 6 to 8J/cm^2 Irradiation: pulses of 1,5ms at intervals of 1 to 3 sec, and 7-mm diameter sport size.	Sensitivity, Soreness, Swelling Burning feeling, Taste modification Ulceration	1 year	SR 7/17 PR/9/17 NR 1/17	1/17 Location: Retromolar trigone	None
Waseem Jerjes *et al.* - 2011	ALA or mTHPC *	NO	N° of session: 1 to 5 **Protocol** -Under local or general anesthesia Application of 60mg/kg 5-ALA cream topically 3-4h prior to tissue illumination. Illumination parameter 628nm diode laser used for illumination. Core diameter of 400um Light delivered at 100 or 200J/cm^2 per site. or mTHPC administered 0,1mg/kg intravenously 96h prior to treatment. Illumination parameter 652nm diode laser used for illumination. Core diameter of 400umLight delivered at 20J/cm^2. NSAISs and opiates for pain control	Mild to moderate pain at the treatment site 126/147 Mild to moderate skin photosensitivity reaction 22/147	Mean of 7,3 years	At 3-year CR 111/147 PR 23/147 SD 5/147 PD 8/147 At 5 years CR 116/147 PR 11/147 SD/ 9/147 PD 11/147 Final outcome CR 119/147 PR 12/147 SD 5/147 PD 11/147	17/147 (11M/6F)	At 5 years: 11/147
Alexander Kübler *et al.* - 1998	ALA 20%	NO	N° of session: 1 **Protocol** 20% ALA cream applied to the lesion for 2 hours and covered with gauze (every 30min another 3-5g of ALA cream with applied to the lesion). Illumination parameter: monochromatic red light delivered by an argon-dye laser at 630nm, 100mW/cm^2 and 100J/cm2, 1000 sec) Irradiation: Irradiation for 1 hour	5 patients described a mild burning sensation and vanish immediately after laser treatment.	Between 6 to 16 months	CR 5/12 PR 4/12 NR 3/12 One PR patient was retreated, after which the lesion disappeared	None	None

ALA= 5-aminolevulinic acid
mTHPC= temoporfin 5,10,15,20-tetra(m-hydroxyphenyl) chlorin
PDL= Pulsed dye laser
SR: significant response.
CR= complete response
PR= partial response
MT= Malignant Transformation
N/A= Not applicable
NR= no response
SD= Stable disease
PD= progressive disease.

*5-ALA applied for thin mild-moderate dysplasia) and mTHPC (for thicker mild-moderate dysplasia, severe dysplasia and carcinoma in situ)

4 Discussion

The reviewed literature on PDT for the treatment of OL demonstrates a wide range of clinical protocols and clinical responses and safety profiles across various protocols and patients populations. Overall, several studies have reported promising CR rates; for example, Ou et al. (2022) achieved 82,3% CR with low recurrence in patients with mild to moderate dysplasia, and Wang et al. (2024) demonstrated effective outcomes particularly in homogeneous lesions. Conversely, Wong et al. (2023) did not meet their primary endpoint regarding optimal dosing or toxicity.

The clinical outcomes seem closely linked to specific treatment parameters. For instance, Ou et al. (2022), reported that using Waterlase technology (which combines water, air, and laser energy) appears to enhance PS absorption, resulting in higher CR rates and a relatively low recurrence rate. Similarly, the results of Wang et al. (2024) suggest that topical ALA-PDT is particularly effective for homogeneous OL lesions and that the buccal mucosa may act as a protective factor against recurrence. The effective management of OL in studies by Yao et al. (2022), with the association between PDT and AFL supports the notion that modifications in light delivery and pre-treatment techniques (such as AFL to remove the stratum corneum) can improve outcomes by facilitating deeper and more uniform PS penetration. Overall, these data indicate that the efficacy of PDT is multifactorial, with PS concentration, incubation time, light wavelength and energy dose all contributing to treatment success.

In term of PS, while ALA is the most wildly used PS in PDT for the treatment of oral leukoplakia, several studies have also evaluated alternative PS, such as Clhorin-e6 and mTHPC, to address certain limitation of ALA. ALA is favored primarily because of its safety profile, ease of topical application, and its selective uptake by dysplastic cells that convert ALA into the photosensitive molecule protoporphyrin IX (PpIX). These characteristics enable ALA-PDT to produce localized tissue necrosis with minimal systemic toxicity and scarring. For instance, many studies reported minimal adverse events with topical ALA application, and the lack of systemic photosensitivity enabled patients to resume their daily activities immediately following treatment. These are extremely important benefit when compared to others photosensitizer.

In contrast, other PS offer distinct advantages and challenges. Pietruska et al. (2013) utilized Chlorin-e6 in gel formulation and reported a considerable reduction in OL lesion

size. Although effective, Chlorin-e6 based PDT requires a longer incubation time and has been tested in smaller patient cohorts; hence, further research is needed to validate its long-term efficacy and safety. Additionally, Jerjes et al. (2011) investigated the use of mTHPC, administered intravenously, primarily in cases of thicker lesions or those exhibiting severe dysplasia and carcinoma in situ. mTHPC offers the benefit of deeper tissue penetration when compared with topical ALA; however, its systemic administration is associated with higher risk of photosensitivity reactions and cumulative toxicity, making it less attractive for repeated treatments or for managing superficial lesions.

The choice between ALA and other photosensitizers is ultimately influenced by lesion characteristics such as thickeners, degree of dysplasia and anatomical site. For superficial lesions with a thin epithelial layer, ALA is highly effective owing to its rapid cellular uptake and conversion to PpIX. Conversely, for lesions that are thicker or exhibit higher dysplasia, PS with deeper penetration (such as mTHPC) might be more beneficial despite the need for stringent management of systematic side effects. As research continues, the development of novel PS formulations and improved delivery vehicles may allow for tailored PDT approaches that optimize the advantages of each PS while minimizing their limitations.

In summary, while ALA remains the cornerstone for PDT in OL therapy due to its convenience and safety, alternative PS such as Chlorin- e6 and mTHPC show potential in addressing specific clinical challenges. Direct comparisons in well-designed, head-to head trials and further investigations into optimized formulations are essential to fully delineate the roles of different PS in personalized PDT protocols of OL.

As said before, the choice of the light source plays crucial role in the clinical outcomes of PDT for OL, as reflected in the diverse results across studies. Diode lasers (630nm) (Kawczyk-Krupka et al.,2012) was associated with high CR rates, of 76.9%, indicating their efficiency in activating ALA, and achieving therapeutic depth. Similarly, the He-Ne laser, employed in studies such as Han et al. (2019) and Wang et al. (2024), yielded moderate CR rates (12- 55,2%), though often accompanied by higher rates of local side effects like pain or ulcers. xenon lamps, as used by Selvam et al. (2015) produced satisfactory responses (CR 40%), with minimal reported discomfort, suggesting they offer a gentler alternative for illumination. Pulsed dye lasers (PDL) featured in Wong et al. (2013) and Sharfirstein et al. (2011), delivered lower light doses (2-8J/cm^2) and were

linked to more modest therapeutic responses but unique side effects, including systemic photosensitivity and hepatic changes in systematic ALA use. Red light LEDs and broad-spectrum sources, such as those used by Ou et al. (2022) and Yao et al. (2022), also showed promising results (CR rates up to 84,5%) while maintaining a favorable safety profile. These findings highlight that monochromatic laser sources tend to achieve deeper penetration and higher efficacy but may increase adverse effects, while non-laser sources may offer improved tolerability at the potential cost of slightly reduced therapeutic modality.

When compared with other OL treatment, many of the current findings are consistent with earlier evidence that PDT offers a minimally invasive alternative that combines therapeutic efficacy with favorable patient tolerance to conventional treatments such as surgery, radiotherapy, and chemotherapy. Maloth et al. (2016) demonstrated that ALA-mediated PDT achieved a complete or partial response in 83,4% of patients, significantly outperforming conventional therapies, which showed no complete responses and a high proportion of non-responders. Kawczyk-Krupka et al. (2012) further supported PDT's effectiveness, reporting complete remission in 35 patients (comparable to the 33 cases observed in cryosurgery), while highlighting the superior tolerability of PDT. Patients treated with PDT reported significantly less post treatment pain and fewer adverse effect, although treatment time and the number of sessions required were higher compared to cryotherapy. Nowadays, surgical excision remains the gold standard, yet findings from Sundberg et al. (2019) highlight its limitations, with recurrence rates approaching 49% after five years. Furthermore, specific clinical factors such as non-homogeneous leukoplakia and snuff use were found to significantly increase recurrence risk, regardless of surgical margins. Notably, recurrence was also linked to a higher risk of malignant transformation. These findings underscore the importance of individualized treatment strategies. PDT emerges as a strong alternative, particularly for patients where surgery poses greater risks or when minimizing invasiveness is a priority. While surgery and cryotherapy remain valid options, PDT offers a balance of therapeutic efficacy and patient tolerance and may be especially valuable in managing lesions with lower recurrence or malignant transformation.

Several unexpected outcomes warrant attention. Wong et al. (2013) did not observe significant clinical responses despite the absence of major toxicity, suggesting that's factors such as suboptimal dosing or individual pharmacokinetic variability may have

played a role. Moreover, the persistent recurrence observed in some studies, such as in Yao et al. (2022) where lesions on the gingiva or palate were predisposed to relapse, may reflect differences in tissue architecture or inadequate PS penetration in regions with a thicker mucosal barrier.

A major limitation across the studies is the lack of standardized PDT protocols. Variability in the PS used and their different concentrations; ALA concentrations (ranging from 10% to 20%), differences in incubation periods (from 30 minutes to several hours), and the use of various light sources and wavelengths compromise the ability to make a direct inter-study comparison. Additionally, small sample sizes studies (Selvam et al., 2015) reduce the statistical power and generality of the findings. In some cases, inconsistent reporting of adverse events and follow-up durations further limits a comprehensive evaluation of long-term efficacy, particularly in terms of recurrences rates and malignant transformation.

The findings suggest that PDT, particularly when using 5-aminolevulinic acid (ALA) as the photosensitizer, demonstrates promising clinical outcomes in terms of lesion resolution. Complete remission rates ranged widely across studies, with minimal side effects, most commonly mild to transient. Importantly, several studies documented a decrease in recurrence and malignant transformation rates, reinforcing PDT's role as a potentially preventive strategy in the malignant progression of OL.

These results are consistent with PDT's therapeutic mechanism, which enables the selective destruction of dysplastic tissues while sparing adjacent healthy mucosa. This characteristic is particularly valuable in the oral cavity, where functional and aesthetic preservation is critical. Furthermore, PDT's repeatability allows for flexible management of persistent or recurring lesions without the cumulative damage often associated with repeated surgical interventions. From a clinical perspective, this review reinforces the value of PDT as a minimally invasive, patient-friendly option that can be integrated into standard oral medicine practice. It is especially suitable for patients who are medically compromised, have lesions in cosmetically sensitive areas, or for whom surgery is contraindicated. Furthermore, the ability to perform PDT in an outpatient setting with minimal downtime enhances patient's compliance and satisfaction.

Moreover, while ALA-based PDT appears most common due to its safety and practicality, other photosensitizer such as mTHPC have also shown potential for deeper tissue penetration in more advanced or thicker lesions, although with increased systematic risks.

This implies a need for stratifying PDT approaches based on lesion characteristics, including thickness, histopathological grade, and anatomical location.

4.1 Limitations of this study

The mains limitations of this studies are that the methodology, the photosensitizer use, and protocols are different making the comparison difficult. Direct comparisons in well-designed, head-to-head trials and further investigations into optimized formulations are essential to fully delineate the roles of different PS in personalized PDT protocols of OL.

Nevertheless, this review also underscores the heterogeneity in the treatment protocols and reporting standards across the included studies. Variables such as photosensitizer concentration and type (ALA, chlorin e6 and mTHPC), light source and wavelength, fluence, incubation time, and number of treatment sessions all vary widely and significantly influence therapeutic outcomes. For instance, the use of pre-treatment modalities like ablative fractional lasers or Waterlase was associated with improved photosensitizer penetration and higher CR rates. These protocol-specific variations suggest that treatment customization is essential, and that standardization of clinical protocols would greatly enhance reproducibility and comparative analysis.

Another limitation was the short-term follow-up of time that varied from 4 weeks (Maloth et al. 2016) to 10 years (Kawczyk-Krupka et al. 2012). This variability complicates the interpretation of PDT's long-tern efficacy and underscores the necessity for standardized follow-up protocols to accurately assess its impact on recurrence and malignant transformation rates.

4.2 Future Prospects

In terms of research implication, this review identifies several gaps that warrant further investigation. Future studies should aim to establish standardized treatment parameters and outcomes reporting frameworks. Randomized controlled trials with larger sample sizes and longer follow-up periods are essential to conclusively determine PDT's long-term effectiveness in preventing recurrence and malignant transformation. Additionally, innovations in photosensitizer delivery systems and light sources may further improve efficacy and patient comfort.

The current evidence found in this systematic review suggests several indications for future investigation:

(i) Standardization of protocols: studies with randomized controlled trials can be relevant to establish the optimal ALA concentrations, incubation time and illumination parameter that maximize efficacy while minimizing side effects.

(ii) Adjunctive and combination therapies: further exploration of adjunctive techniques, such as the use of Waterlase technology and AFL pre-treatment, enhance the absorption of PS and improve the overall treatment outcome.

(iii) Improved PS delivery: development of more adhesive carriers or gels that's remains in situ on the oral mucosa is needed to prevent premature removal by saliva and ensure optimal PS absorption.

(iv) Long term follow-up: More extensive long-term studies with larger sample size are essential to accurately assess recurrence rates and the impact of PDT on malignant transformation, particularly in patients with severe dysplasia.

(v) Safety and toxicity Assessments: Although most studies report minimal systemic toxicity, further research should refine dosing strategies to avoid adverse events like liver enzyme elevation, ensuring safety without compromising efficacy.

5 Conclusion

This systematic review aimed to evaluate the impact of photodynamic therapy for the treatment of oral leukoplakia, a condition of considerable clinical relevance due to the fact of being relatively common and due to its potential for malignant transformation. This review highlights PDT as a viable and often effective therapeutic approach for the management of OL, offering an alternative to traditional invasive techniques that are often associated with discomfort, scarring, and variable recurrence rates.

In conclusion, photodynamic therapy has a significant and positive impact on the treatment of oral leukoplakia. It offers an effective, non-invasive option for lesion resolution, with the potential to reduce recurrence and malignant transformation, thereby contributing to better patient outcomes. While further research is needed to optimize and standardize its application, the current evidence supports the integration of PDT into the therapeutic arsenal for oral leukoplakia, particularly in personalized, lesion-specific management strategies.

6 Bibliography

Abidullah, M., Kiran, G., Gaddikeri, K., Raghoji, S., & Ravishankar T, S. (2014). Leuloplakia - review of a potentially malignant disorder. *Journal of clinical and diagnostic research : JCDR*, *8*(8), ZE01–ZE4. https://doi.org/10.7860/JCDR/2014/10214.4677

Aebisher, D., Czech, S., Dynarowicz, K., Misiołek, M., Komosińska-Vassev, K., Kawczyk-Krupka, A., & Bartusik-Aebisher, D. (2024). Photodynamic Therapy: Past, Current, and Future. *International journal of molecular sciences*, *25*(20), 11325. https://doi.org/10.3390/ijms252011325

Aebisher, D., Szpara, J., & Bartusik-Aebisher, D. (2024). Advances in Medicine: Photodynamic Therapy. *International journal of molecular sciences*, *25*(15), 8258. https://doi.org/10.3390/ijms25158258

Agostinis, P., Berg, K., Cengel, K. A., Foster, T. H., Girotti, A. W., Gollnick, S. O., Hahn, S. M., Hamblin, M. R., Juzeniene, A., Kessel, D., Korbelik, M., Moan, J., Mroz, P., Nowis, D., Piette, J., Wilson, B. C., & Golab, J. (2011). Photodynamic therapy of cancer: an update. *CA: a cancer journal for clinicians*, *61*(4), 250–281. https://doi.org/10.3322/caac.20114

Allison, R. R., & Moghissi, K. (2013). Photodynamic Therapy (PDT): PDT Mechanisms. *Clinical endoscopy*, *46*(1), 24–29. https://doi.org/10.5946/ce.2013.46.1.24

Almeida, A., Faustino, M. A. F., & Neves, M. G. P. M. S. (2020). Antimicrobial Photodynamic Therapy in the Control of COVID-19. *Antibiotics (Basel, Switzerland)*, *9*(6), 320. https://doi.org/10.3390/antibiotics9060320

Auluck, A., & Pai, K. M. (2005). (Mis)Interpretations of leukoplakia. *Journal (Canadian Dental Association)*, *71*(4), 237–238.

Biel, M. A., Sievert, C., Usacheva, M., Teichert, M., Wedell, E., Loebel, N., Rose, A., & Zimmermann, R. (2011). Reduction of Endotracheal Tube Biofilms Using Antimicrobial Photodynamic Therapy. *Lasers in surgery and medicine*, *43*(7), 586–590. https://doi.org/10.1002/lsm.21103

Brouns, E. R., Baart, J. A., Bloemena, E., Karagozoglu, H., & van der Waal, I. (2013). The relevance of uniform reporting in oral leukoplakia: definition, certainty factor and staging based on experience with 275 patients. *Medicina oral, patologia oral y cirugia bucal*, *18*(1), e19–e26. https://doi.org/10.4317/medoral.18756

Calixto, G. M., Bernegossi, J., de Freitas, L. M., Fontana, C. R., & Chorilli, M. (2016). Nanotechnology-Based Drug Delivery Systems for Photodynamic Therapy of Cancer: A Review. *Molecules (Basel, Switzerland)*, *21*(3), 342. https://doi.org/10.3390/molecules21030342

Chen, Q., Dan, H., Tang, F., Wang, J., Li, X., Cheng, J., Zhao, H., & Zeng, X. (2019). Photodynamic therapy guidelines for the management of oral leucoplakia. *International journal of oral science*

Correia, J. H., Rodrigues, J. A., Pimenta, S., Dong, T., & Yang, Z. (2021). Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions. *Pharmaceutics*, *13*(9), 1332.

Cui, J. J., Han, X. L., & Wang, W. M. (2013). Expression and significance of p53 and mdm2 in patients with leukoplakia cancer. *Asian Pacific journal of tropical medicine*, *6*(10), 831–834. https://doi.org/10.1016/S1995-7645(13)60147-9

Dolmans, D. E., Fukumura, D., & Jain, R. K. (2003). Photodynamic therapy for cancer. *Nature reviews. Cancer*, *3*(5), 380–387. https://doi.org/10.1038/nrc1071

Donnelly, R. F., McCarron, P. A., & Tunney, M. M. (2008). Antifungal photodynamic therapy. *Microbiological research*, *163*(1), 1–12. https://doi.org/10.1016/j.micres.2007.08.001

Grzybowski, A., & Pietrzak, K. (2012). From patient to discoverer--Niels Ryberg Finsen (1860–1904) --the founder of phototherapy in dermatology. *Clinics in dermatology*, *30*(4), 451–455.

Han, Y., Xu, S.,n, J., Wang, X., Liu, X., Hua, H., Wang, X., & Liu, H. (2019). Primary Clinical Evaluation of Photodynamic Therapy With Oral Leukoplakia in Chinese Patients. *Frontiers in physiology*, *9*, 1911. https://doi.org/10.3389/fphys.2018.01911

Hempstead, J., Jones, D. P., Ziouche, A., Cramer, G. M., Rizvi, I., Arnason, S., Hasan, T., & Celli, J. P. (2015). Low-cost photodynamic therapy devices for global health settings: Characterization of battery-powered LED performance and smartphone imaging in 3D tumor models. *Scientific reports*, *5*, 10093. https://doi.org/10.1038/srep10093

Iocca, O., Sollecito, T. P., Alawi, F., Weinstein, G. S., Newman, J. G., De Virgilio, A., Di Maio, P., Spriano, G., Pardiñas López, S., & Shanti, R. M. (2020). Potentially malignant disorders of the oral cavity and oral dysplasia: A systematic review and meta-analysis of malignant transformation rate by subtype. *Head & neck*, *42*(3), 539–555. https://doi.org/10.1002/hed.26006

Jerjes, W., Upile, T., Hamdoon, Z., Mosse, C. A., Akram, S., & Hopper, C. (2011). Photodynamic therapy outcome for oral dysplasia. *Lasers in surgery and medicine*, *43*(3), 192–199. https://doi.org/10.1002/lsm.21036

Jerjes, W., Upile, T., Hamdoon, Z., Mosse, C. A., Akram, S., Morley, S., & Hopper, C. (2011). Interstitial PDT for vascular anomalies. *Lasers in surgery and medicine*, *43*(5), 357–365. https://doi.org/10.1002/lsm.21058

Kawczyk-Krupka, A., Waśkowska, J., Raczkowska-Siostrzonek, A., Kościarz-Grzesiok, A., Kwiatek, S., Straszak, D., Latos, W., Koszowski, R., & Sieroń, A. (2012). Comparison of cryotherapy and photodynamic therapy in treatment of oral leukoplakia. *Photodiagnosis and photodynamic therapy*, *9*(2), 148–155. https://doi.org/10.1016/j.pdpdt.2011.12.007

Kessel D. (2019). Apoptosis, Paraptosis and Autophagy: Death and Survival Pathways Associated with Photodynamic Therapy. *Photochemistry and photobiology*, *95*(1), 119–125. https://doi.org/10.1111/php.12952

Khanna, R., Agarwal, A., Khanna, S., Basu, S., & Khanna, A. K. (2010). S-phase fraction and DNA ploidy in oral leukoplakia. *ANZ journal of surgery*, *80*(7-8), 548–551. https://doi.org/10.1111/j.1445-2197.2009.05196.x

Kil, T. J., Kim, H. S., Kim, H. J., Nam, W., & Cha, I. H. (2016). Genetic Abnormalities in Oral Leukoplakia and Oral Cancer Progression. *Asian Pacific journal of cancer prevention : APJCP*, *17*(6), 3001–3006.

Kim, M. M., & Darafsheh, A. (2020). Light Sources and Dosimetry Techniques for Photodynamic Therapy. *Photochemistry and photobiology*, *96*(2), 280–294. https://doi.org/10.1111/php.13219

Kübler, A., Haase, T., Rheinwald, M., Barth, T., & Mühling, J. (1998). Treatment of oral leukoplakia by topical application of 5-aminolevulinic acid. *International journal of oral and maxillofacial surgery*, *27*(6), 466–469. https://doi.org/10.1016/s0901-5027(98)80040-4

Kumari, P., Debta, P., & Dixit, A. (2022). Oral Potentially Malignant Disorders: Etiology, Pathogenesis, and Transformation Into Oral Cancer. *Frontiers in pharmacology*, *13*, 825266. https://doi.org/10.3389/fphar.2022.825266

Lange, N., Szlasa, W., Saczko, J., & Chwiłkowska, A. (2021). Potential of Cyanine Derived Dyes in Photodynamic Therapy. *Pharmaceutics*, *13*(6), 818. https://doi.org/10.3390/pharmaceutics13060818

Lee, C. N., Hsu, R., Chen, H., & Wong, T. W. (2020). Daylight Photodynamic Therapy: An Update. *Molecules (Basel, Switzerland)*, *25*(21), 5195. https://doi.org/10.3390/molecules25215195

Lima, E., & Reis, L. V. (2023). Photodynamic Therapy: From the Basics to the Current Progress of *N*-Heterocyclic-Bearing Dyes as Effective Photosensitizers. *Molecules (Basel, Switzerland)*, *28*(13), 5092. https://doi.org/10.3390/molecules28135092

Lodi, G., & Porter, S. (2008). Management of potentially malignant disorders: evidence and critique. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *37*(2), 63–69. https://doi.org/10.1111/j.1600-0714.2007.00575.x

Maloth, K. N., Velpula, N., Kodangal, S., Sangmesh, M., Vellamchetla, K., Ugrappa, S., & Meka, N. (2016). Photodynamic Therapy - A Non-invasive Treatment Modality for Precancerous Lesions. *Journal of lasers in medical sciences*, *7*(1), 30–36. https://doi.org/10.15171/jlms.2016.07

Mang T. S. (2004). Lasers and light sources for PDT: past, present and future. *Photodiagnosis and photodynamic therapy*, *1*(1), 43–48. https://doi.org/10.1016/S1572-1000(04)00012-2

Møller, K. I., Kongshoj, B., Philipsen, P. A., Thomsen, V. O., & Wulf, H. C. (2005). How Finsen's light cured lupus vulgaris. *Photodermatology, photoimmunology & photomedicine*, *21*(3), 118–124. https://doi.org/10.1111/j.1600-0781.2005.00159.x

Morton, C. A., Szeimies, R. M., Basset-Séguin, N., Calzavara-Pinton, P. G., Gilaberte, Y., Haedersdal, M., Hofbauer, G. F. L., Hunger, R. E., Karrer, S., Piaserico, S., Ulrich, C., Wennberg, A. M., & Braathen, L. R. (2020). European Dermatology Forum guidelines on topical photodynamic therapy 2019 Part 2: emerging indications - field cancerization, photorejuvenation and inflammatory/infective dermatoses. *Journal of the European Academyof Dermatology and Venereology : JEADV*, *34*(1), 17–29. https://doi.org/10.1111/jdv.16044

Narayan, T. V., & Shilpashree, S. (2016). Meta-analysis on clinicopathologic risk factors of leukoplakias undergoing malignant transformation. *Journal of oral and maxillofacial pathology : JOMFP*, *20*(3), 354–361. https://doi.org/10.4103/0973-029X.190900

Ou, J., Gao, Y., Li, H., Ling, T., & Xie, X. (2022). Application of 5-aminolevulinic acid-mediated Waterlase-assisted photodynamic therapy in the treatment of oral leukoplakia. *Scientific reports*, *12*(1), 9391. https://doi.org/10.1038/s41598-022-13497-3

Ozog, D. M., Rkein, A. M., Fabi, S. G., Gold, M. H., Goldman, M. P., Lowe, N. J., Martin, G. M., & Munavalli, G. S. (2016). Photodynamic Therapy: A Clinical Consensus Guide. *Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al.]*, *42*(7), 804–827. https://doi.org/10.1097/DSS.0000000000000800

Parlatescu, I., Gheorghe, C., Coculescu, E., & Tovaru, S. (2014). Oral leukoplakia - an update. *Maedica*, *9*(1), 88–93.

Pietruska, M., Sobaniec, S., Bernaczyk, P., Cholewa, M., Pietruski, J. K., Dolińska, E., Skurska, A., Duraj, E., & Tokajuk, G. (2014). Clinical evaluation of photodynamic therapy efficacy in the treatment of oral leukoplakia. *Photodiagnosis and photodynamic therapy*, *11*(1), 34–40. https://doi.org/10.1016/j.pdpdt.2013.10.003

Plaetzer, K., Berneburg, M., Kiesslich, T., & Maisch, T. (2013). New applications of photodynamic therapy in biomedicine and biotechnology. *BioMed research international*, *2013*, 161362. https://doi.org/10.1155/2013/161362

Prażmo, E. J., Kwaśny, M., Łapiński, M., & Mielczarek, A. (2016). Photodynamic Therapy As a Promising Method Used in the Treatment of Oral Diseases. *Advances in clinical and experimental medicine : official organ Wroclaw Medical University*, *25*(4), 799–807. https://doi.org/10.17219/acem/32488

Przygoda, M., Bartusik-Aebisher, D., Dynarowicz, K., Cieślar, G., Kawczyk-Krupka, A., & Aebisher, D. (2023). Cellular Mechanisms of Singlet Oxygen in Photodynamic Therapy. *International journal of molecular sciences*, *24*(23), 16890. https://doi.org/10.3390/ijms242316890

Ribeiro, A. S., Salles, P. R., da Silva, T. A., & Mesquita, R. A. (2010). A review of the nonsurgical treatment of oral leukoplakia. *International journal of dentistry*, *2010*, 186018. https://doi.org/10.1155/2010/186018

Sabashvili, M., Gigineishvili, E., Jikia, M., & Chitaladze, T. (2018). Role of tobacco in the development of oral leukoplakia and oral cancer. Dentistry, 08(06). https://doi.org/10.4172/2161-1122.1000495

Saini, R., & Poh, C. F. (2013). Photodynamic therapy: a review and its prospective role in the management of oral potentially malignant disorders. *Oral diseases*, *19*(5), 440–451. https://doi.org/10.1111/odi.12003

Selvam, N. P., Sadaksharam, J., Singaravelu, G., & Ramu, R. (2015). Treatment of oral leukoplakia with photodynamic therapy: A pilot study. *Journal of cancer research and therapeutics*, *11*(2), 464–467. https://doi.org/10.4103/0973-1482.147703

Serban, A. E., Nicolae, C., Parlatescu, I., & Tovaru, S. (2023). Oral Leukoplakia. A Five-Year Follow-Up Study. *Maedica*, *18*(4), 645–650. https://doi.org/10.26574/maedica.2023.18.4.645

Shafirstein, G., Friedman, A., Siegel, E., Moreno, M., Bäumler, W., Fan, C. Y., Morehead, K., Vural, E., Stack, B. C., Jr, & Suen, J. Y. (2011). Using 5-aminolevulinic acid and pulsed dye laser for photodynamic treatment of oral leukoplakia. *Archives of otolaryngology--head & neck surgery*, *137*(11), 1117–1123. https://doi.org/10.1001/archoto.2011.178

Sharma, S. K., Mroz, P., Dai, T., Huang, Y. Y., St Denis, T. G., & Hamblin, M. R. (2012). Photodynamic Therapy for Cancer and for Infections: What Is the Difference?. *Israel journal of chemistry*, *52*(8-9), 691–705. https://doi.org/10.1002/ijch.201100062

Stájer, A., Kajári, S., Gajdács, M., Musah-Eroje, A., & Baráth, Z. (2020). Utility of Photodynamic Therapy in Dentistry: Current Concepts. *Dentistry journal*, *8*(2), 43. https://doi.org/10.3390/dj8020043

Stasikowska-Kanicka, O., Wągrowska-Danilewicz, M., & Danilewicz, M. (2018). CD8+ and CD163+ infiltrating cells and PD-L1 immunoexpression in oral leukoplakia and oral carcinoma. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*, *126*(9), 732–738. https://doi.org/10.1111/apm.12881

Sundberg, J., Korytowska, M., Holmberg, E., Bratel, J., Wallström, M., Kjellström, E., Blomgren, J., Kovács, A., Öhman, J., Sand, L., Hirsch, J. M., Giglio, D., Kjeller, G., & Hasséus, B. (2019). Recurrence rates after surgical removal of oral leukoplakia-A prospective longitudinal multi-centre study. PloS one, 14(12), e0225682. https://doi.org/10.1371/journal.pone.0225682

Thomas, G., Tr, S., George S, P., Somanathan, T., Sarojam, S., Krishnankutti, N., Sreedharan, H., & Ankathil, R. (2020). Prognostic Implications of DNA Repair, Ploidy and Telomerase in the Malignant Transformation Risk Assessment of Leukoplakia. *Asian Pacific journal of cancer prevention : APJCP*, *21*(2), 309–316. https://doi.org/10.31557/APJCP.2020.21.2.309

van der Waal I. (2015). Oral leukoplakia, the ongoing discussion on definition and terminology. *Medicina oral, patologia oral y cirugia bucal*, *20*(6), e685–e692. https://doi.org/10.4317/medoral.21007

van der Waal, I., & Reichart, P. A. (2008). Oral proliferative verrucous leukoplakia revisited. *Oral oncology*, *44*(8), 719–721.

Wang, Y., Tang, H., Wang, K., Zhao, Y., Xu, J., & Fan, Y. (2024). Clinical evaluation of photodynamic therapy for oral leukoplakia: a retrospective study of 50 patients. *BMC oral health*, *24*(1), 9. https://doi.org/10.1186/s12903-023-03791-5

Warnakulasuriya S. (2001). Histological grading of oral epithelial dysplasia: revisited. *The Journal of pathology*, *194*(3), 294–297. https://doi.org/10.1002/1096-9896(200107)194:3<294::AID-PATH911>3.0.CO;2-Q

Warnakulasuriya, S., Johnson, N. W., & van der Waal, I. (2007). Nomenclature and classification of potentially malignant disorders of the oral mucosa. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *36*(10), 575–580. https://doi.org/10.1111/j.1600-0714.2007.00582.x

Warnakulasuriya, S., Kujan, O., Aguirre-Urizar, J. M., Bagan, J. V., González-Moles, M. Á., Kerr, A. R., Lodi, G., Mello, F. W., Monteiro, L., Ogden, G. R., Sloan, P., & Johnson, N. W. (2021). Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. *Oral diseases*, *27*(8), 1862–1880. https://doi.org/10.1111/odi.13704

Wong, S. J., Campbell, B., Massey, B., Lynch, D. P., Cohen, E. E. W., Blair, E., Selle, R., Shklovskaya, J., Jovanovic, B. D., Skripkauskas, S., Dew, A., Kulesza, P.,

Parimi, V., Bergan, R. C., & Szabo, E. (2013). A phase I trial of aminolevulinic acid-photodynamic therapy for treatment of oral leukoplakia. *Oral oncology*, *49*(9), 970–976. https://doi.org/10.1016/j.oraloncology.2013.05.011

Woo S. B. (2019). Oral Epithelial Dysplasia and Premalignancy. *Head and neck pathology*, *13*(3), 423–439. https://doi.org/10.1007/s12105-019-01020-6

Yao, Y. L., Wang, Y. F., Li, C. X., Wu, L., & Tang, G. Y. (2022). Management of oral leukoplakia by ablative fractional laser-assisted photodynamic therapy: A 3-year retrospective study of 48 patients. *Lasers in surgery and medicine*, *54*(5), 682–687. https://doi.org/10.1002/lsm.23534

Yoo, S. W., Oh, G., Ahn, J. C., & Chung, E. (2021). Non-Oncologic Applications of Nanomedicine-Based Phototherapy. *Biomedicines*, *9*(2), 113. https://doi.org/10.3390/biomedicines9020113

Zhang, C., Li, B., Zeng, X., Hu, X., & Hua, H. (2023). The global prevalence of oral leukoplakia: a systematic review and meta-analysis from 1996 to 2022. *BMC oral health*, *23*(1), 645. https://doi.org/10.1186/s12903-023-03342-y