SYNOPSIS

TITLE	**Immune-based Stratification and Evolutionary Biomarkers In Oral potentially malignantdisorders**
ACRONYME	**ISEBIO**
STUDY DATA PROCESSOR	Centre Léon Bérard, Lyon, France
INDICATION & SAMPLE SIZE	Oral squamous cell carcinomas (OSCC) represent the most common site of all head & neck squamous cell carcinomas. OSCC may be preceded by an oral potentially malignant disorder (OPMD), associated with hyperplasia and/or dysplasia. Oral leukoplakia, erythroplakia or erythroleukoplakia (E-/L) is the most frequent OPMD. We have previously reported the immunological/classical classification of E-/L which is expected to yield an approximate 50%-50% split of samples. E-/L samples from 246 patients are expected in this study, including 168 non-progressors and 78 progressors.
PARTICIPATING SITES	**20 sites from 12 European countries will participate to this study:** Antwerp University Hospital (Belgium) ; Centre Léon Bérard (Lyon, France), Hospices Civils de Lyon (Lyon, France), Institut Curie (Paris, France), Hôpital Pitié Salpêtrière (Paris, France), Institut Gustave Roussy (Villejuif, France), Barzilai University Medical center (Ashkelon, Israel), Humanitas Cancer Center (Milano, Italy), Riga Stradiņš University (RSU) (Riga, Latvia), UMC - University Medical Centers - Cancer Center Amsterdam an Rijnstate hospital (The Netherlands); University of Murcia and the Facultad de Odontología Universidad Complutense de Madrid (Spain); Institute of Odontology (Gothenberg, Sweden); Istanbul University (Istanbul, Turkey); Institute of Odontology Haukeland University Hospital (Bergen, Norway), Institute of Oral Pathology (Oslo, Norway), Faculty of Medicine, University of Porto (Porto, Portugal), The University of Liverpool Cancer Research Centre (Liverpool, UK), School of Clinical Dentistry (Sheffield, UK). NB: This study will involve additional sites abroad.
PHASE	This project will include a large retrospective cohort of patients identified within the partners of the COST funded Action INTERCEPTOR (CA21140). In France, this cohort study qualifies as a non-interventional study and will follow the French MR-04 rules. This approach will be validated by the Data Protection officer from CLB using specific processes already in place at Centre Leon Bérard for project relying on MR-04 rules.The participating site will be entirely responsible for obtaining the necessary regulatory and ethics approvals as well as to consent patients, in accordance with applicable law.
OBJECTIVES & ENDPOINTS	The project aims at improving stratification of patients with E-/L by refining oral cancer-risk assessment. This will be achieved through the genomic and transcriptomic analysis of formalin-fixed paraffin-embedded samples of E-/L from a retrospective multicentric European cohort of 246 patients with available clinical follow-up. Molecular profiles will allow to accurately determine copy-number alterations [including loss of heterozygosity (LOH)], targeted point mutations and gene expression signatures. Based on the classifier that will be developed, we will estimate if immune-based stratification can improve risk prediction by tailoring biomarker use to specific patient profiles. Our cohort has been adequately powered to assess if the proposed biomarkers outperform current standards.
EXPERIMENTAL PLAN	We will assemble a pan-European cohort including patients who did develop (progressors, n=78) or did not develop (non-progressors, n=168) OSCC during their

	follow-up. Standard H&E section of all samples will be centrally reviewed by expert pathologists of the consortium. Genomic analysis will be performed using the Agilent OneSeq Target Enrichment technology. Starting material for genomic analysis requires two 10µ epithelial layer macrodissected to enrich for epithelial, pre-neoplastic cells followed by DNA extraction, library prep and sequencing. Transcriptomic analysis will be performed using the HTG Molecular Diagnostics technology. Starting material for transcriptome analysis using HTG Transcriptome Panel (19,398 targets) requires five 4µ sections for scraping of the epithelial layer and the underlying stroma, sample lysis, library prep and sequencing. Both approaches are routinely used at Centre Léon Bérard.
ELLIGIBILITY CRITERIA	**<u>INCLUSION CRITERIA</u>** For this study, eligible patients must meet ALL of the following criteria I1. Male and female ≥ 18 years at time of non-opposition to participate to the study I2. Patient with documented non-opposition to participate to the study. I3. Patient with clinically confirmed diagnosis of oral leukoplakia, erythroplakia or erythroleukoplakia I4. Patient with availability of FFPE material, either a biopsy or surgically resected specimens, sampled less than 20 years I5. Patient with evaluable sample meeting the following quality/quantity control criteria: sample size surface area ≥ 5mm² containing both stroma and epithelial cells **<u>EXCLUSION CRITERIA</u>** For this study, eligible patients must not meet ANY of the following criteria E1. Patients who developed OSCC within 6 months after initial diagnosis of oral leukoplakia, erythroplakia or erythroleukoplakia E2. Patients with less than 2 years follow-up AND 2 years without previous OC at the time of oral leukoplakia, erythroplakia or erythroleukoplakia
STUDY TIMETABLE	Study start : Q1 2024 Inclusion Period : 6 months Sample management : 6 months Study Duration : 36 months