Immune-based Stratification and Evolutionary Biomarkers In Oral potentially malignant disorders

V1.3 of 28/05/2025

Coordinating Investigator: Pr. Pierre SAINTIGNY, MD, PhD

Sponsor: Centre Léon Bérard, Lyon, France
Coordinating Centre: DMT-DRCI / Centre Léon Bérard

Sponsor ID: ET23-414

Site Number: **Participating site:**

GENERAL

- **Fundings**:
  - CA21140 – INTERCEPTOR
  - PREV-BIO21-008 ISEBIO
  - SIRIC LyriCAN+ INCa-DGOS-INSERM-ITMO cancer_18003

- **Regulatory approvals: Ethic committee / MR-004 approval at CLB : 04/01/2024** (French regulations)

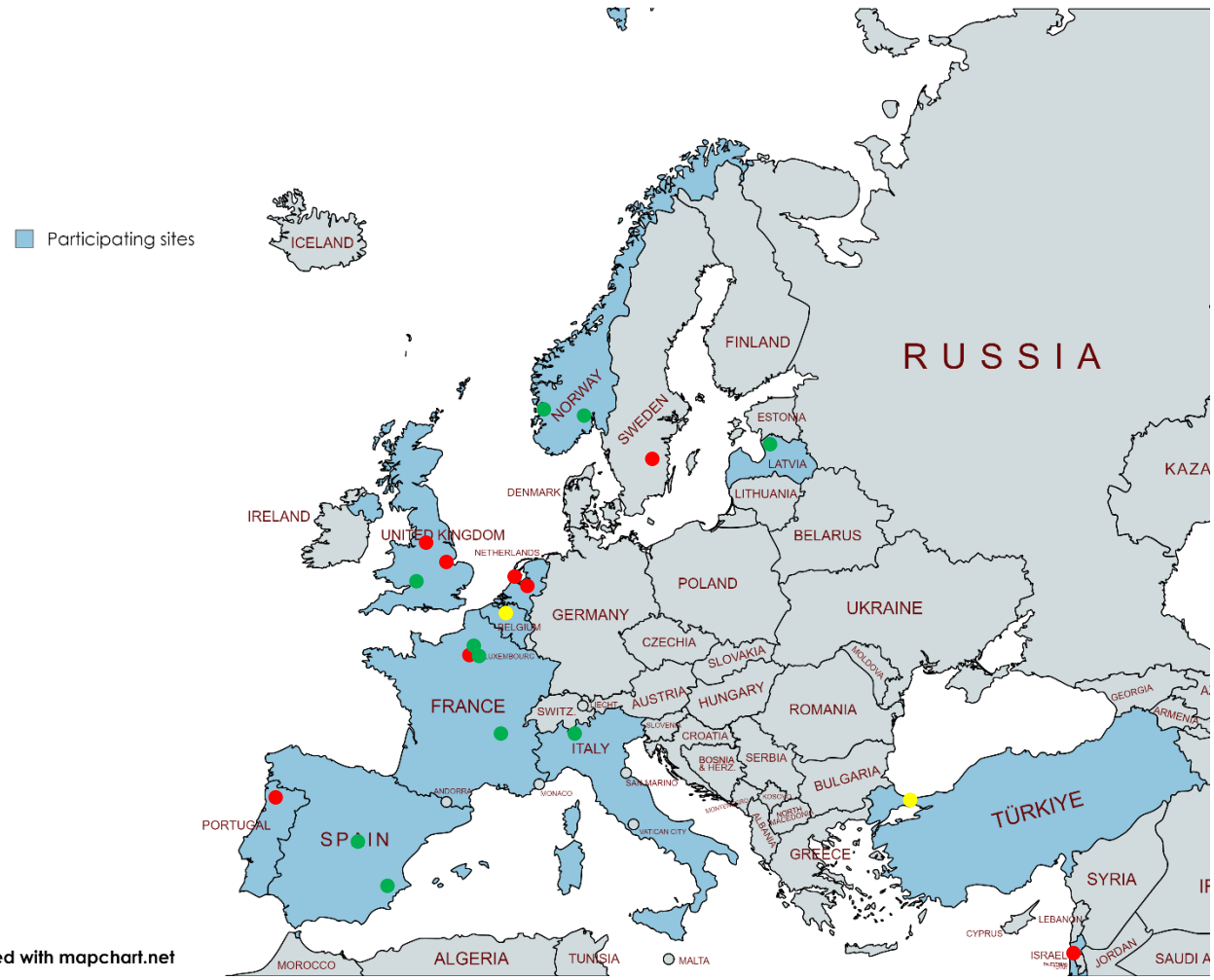
- **Non-interventional, multicentric, retrospective cohort**: RNIPH, MR-004 (French regulations)

- Sample size: **246 patients (minimum)**

- **Expected participating sites (20)**: Hospices Civiles de Lyon (**France**); APHP – Hôpital Pitié-Salpêtrière (**France**); Institut Gustave Roussy (**France**); Institut Curie (**France**); Antwerp University Hospital (**Belgium**); Barzilai University Medical Center (**Israel**); Humanitas Cancer Center (**Italy**); Riga Stradiņš University (**Latvia**); Rijnstate hospital (**The Netherlands**); UMC - University Medical Centers - Cancer Center Amsterdam (**The Netherlands**); Haukeland University Hospital (**Norway**); Institute of Oral Pathology (**Norway**); Faculty of Medicine University of Porto (**Portugal**); University Complutense of Madrid (**Spain**); University of Murcia (**Spain**); Istanbul University (**Turkey**); University of Bristol (**United-Kingdom**); University of Liverpool (**United-Kingdom**); University of Sheffield School of Clinical Dentistry (**United-Kingdom**)

- **Estimated total duration**: 36 months
  - Inclusion period: **6 months**

PARTICIPATING SITES



Created with mapchart.net

- : Opened sites
- : Non-opened sites
- : Central review partners

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

BACKGROUND AND RATIONAL

- **Oral squamous cell carcinomas (OSCC)**:
  - **8th** most common cancer occurrence worldwide
  - **50%** of patients with locally advanced OSCC die from recurrent disease
  - Diagnosis at **late stage**

- **Oral Potentially Malignant Disorders (OPMD)**:
  - Most frequent: oral leukoplakia, erythroplakia or erythroleukoplakia
  - Oral cancer risk (7,7 to 22,0 %)

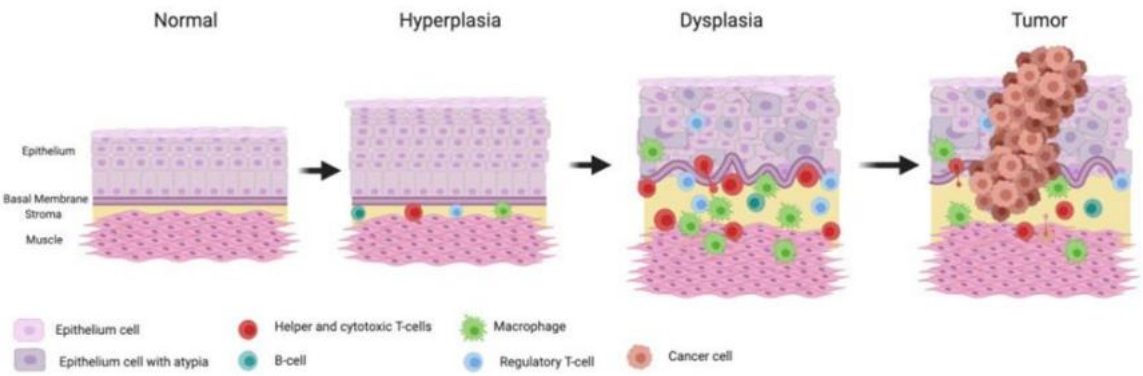


Figure 1. Schematic histological changes of the oral mucosa during malignant transformation.

Prospectively validated biomarker for oral cancer:
- Loss of heterozygosity (LOH)

HYPOTHESIS

- **Gene expression profile** and **genetic alterations** may **improve** oral cancer **risk assessment** in patients with oral leukoplakia, erythroplakia or erythroleukoplakia (E-/L)

- **ISEBIO** aims at testing this hypothesis in a **multicentric retrospective pan-European cohort**

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

PRIMARY OBJECTIVE

PRIMARY OBJECTIVE	PRIMARY ENDPOINTS
To **improve stratification** of patients with oral leukoplakia, erythroplakia or erythroleukoplakia (E-/L) by **refining oral cancer-risk assessment**	**3 strategies:** • Genomic analysis • Transcriptomic analysis • IHC- based markers on FFPE sections ***Biological biomarkers***: Copy-number alterations (including loss of heterozygosity (LOH)), targeted point mutations, gene expression signature

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

SECONDARY OBJECTIVES

LONG TERM OBJECTIVES	LONG TERM ENDPOINTS
• To **determine** whether **novel therapeutic targets** can be leveraged for **immuno-prevention strategies**	• Maybe new data will be available
• To **reduce the incidence** of OSCC	• No data will be provided from this study

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

PROGRESSOR/NON PROGRESSOR RATIO

Number of cases expected:

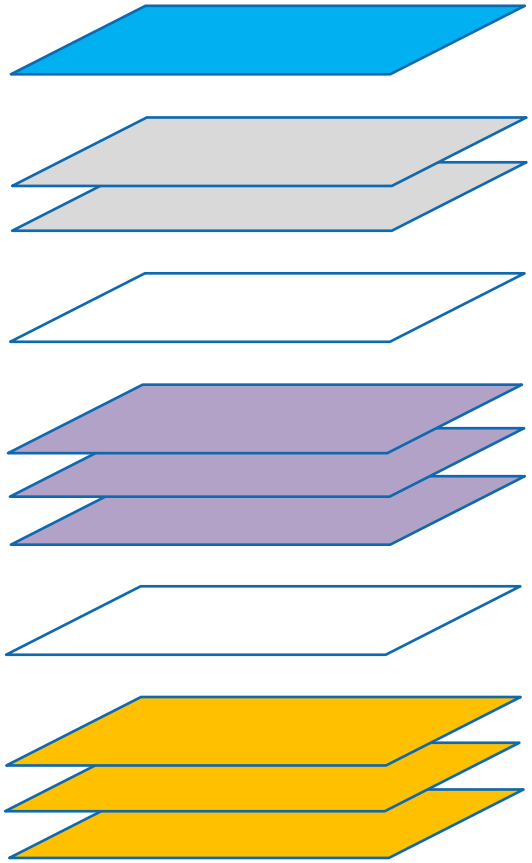
BEWARE: The **ratio 2/3** and **1/3** is very **important**

246 patients estimated

- 168 (2/3) non-progressors
- 78 (1/3) progressors

Ex of total number of patients	Non-progressors (68%)	Progressors (32%)
5	4	1
10	7	3
30	20	10
50	34	16
100	68	32

SECTIONS' SLIDES SUMMARY



H&E staining	**1 section of 3µm**
RNA	**2 sections of 10µm** **Whole section scraping**
Unstained section	1 section of 4µm
IHC CK13 and CK17	**3 sections of 3µm**
Unstained section	1 section of 4µm
DNA	**3 sections of 10µm** **Macrodissection of the epithelial layer**

- **IHC :** CK13 and CK17
- **DNA sequencing :** Agilent OneSeq Target Enrichment approach
  - whole-genome "backbone" (300kb, CNAs)
  - targeted sequencing panel (435kb panel based on the 62 HNSCC driver genes
- **RNA sequencing :** 3'Tag-Seq (Lexogen)

OVERFLOW (1/2)

MR-004 authorization for French Centres
Ethic approvals for other Centres

1. SCREENING

Documents

2. CONSENT / NON OPPOSITION

Patient Information Sheet

3. SAMPLES COLLECTION

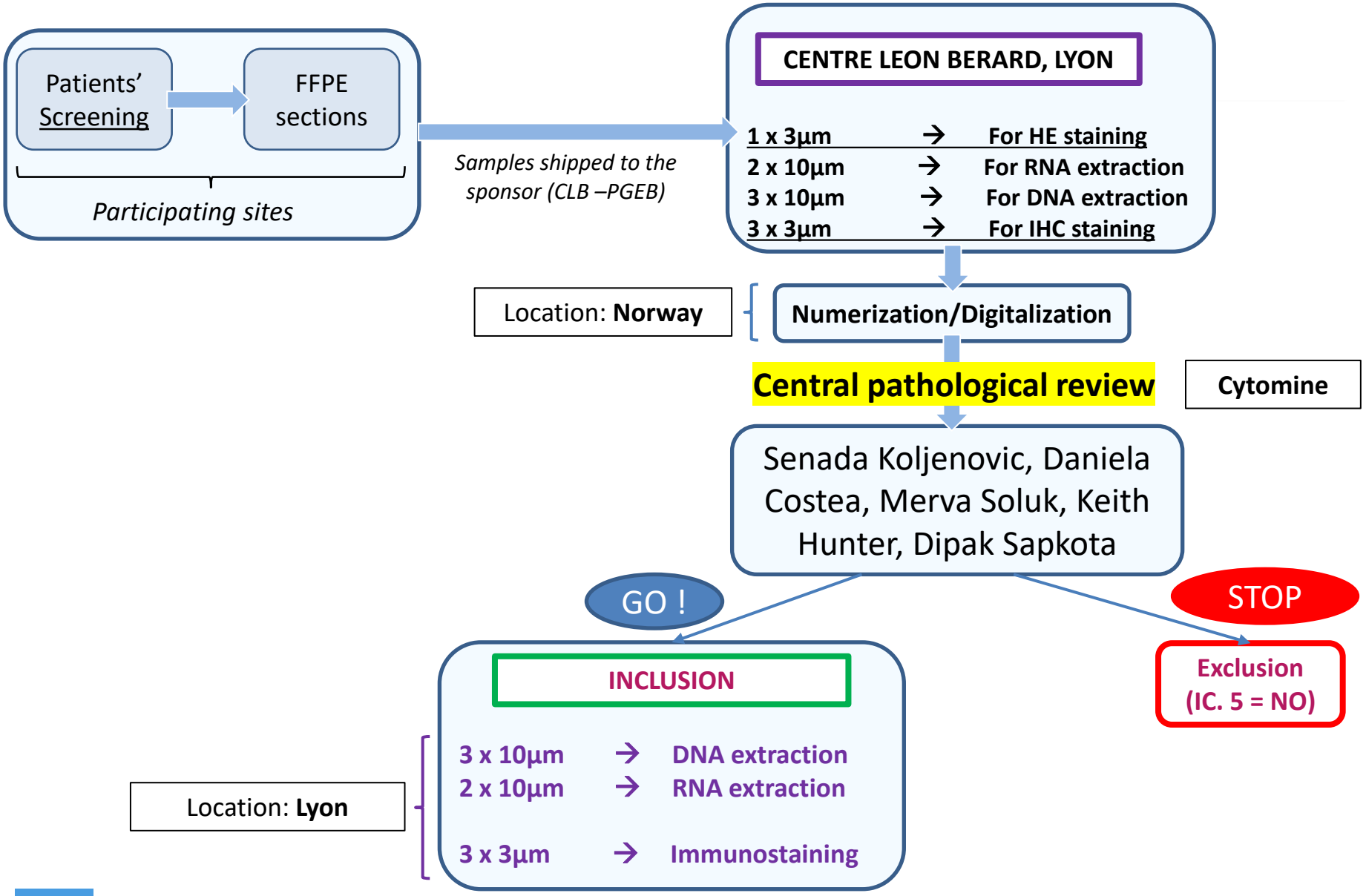
- FFPE sections
- Samples **less than 20 years**
- To be shipped to **PGEB** at Centre Léon Bérard (Appendix 1 & 2 – Laboratory Manual)
- Shipment assured by: **FedEx**
  - Please, send by email the following information: quantity of package, weight of each package, dimensions of each package
  - Then, a label with the necessary information will be sent by email and fixed on the package before the shipment

Samples form (Appendix 1 – Laboratory Manual)

Shipment form (Appendix 2 – Laboratory Manual)

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

OVERFLOW (2/2)


Patients' Screening
FFPE sections
Participating sites
Samples shipped to the sponsor (CLB –PGEB)
CENTRE LEON BERARD, LYON
1 x 3µm → For HE staining
2 x 10µm → For RNA extraction
3 x 10µm → For DNA extraction
3 x 3µm → For IHC staining
Location: Norway
Numerization/Digitalization
Central pathological review
Cytomine
Senada Koljenovic, Daniela Costea, Merva Soluk, Keith Hunter, Dipak Sapkota
GO !
STOP
Exclusion (IC. 5 = NO)
INCLUSION
3 x 10µm → DNA extraction
2 x 10µm → RNA extraction
3 x 3µm → Immunostaining
Location: Lyon

INCLUSION CRITERIA

→ Patients **must ALL meet** 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 (E-/L)

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

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

EXCLUSION CRITERIA

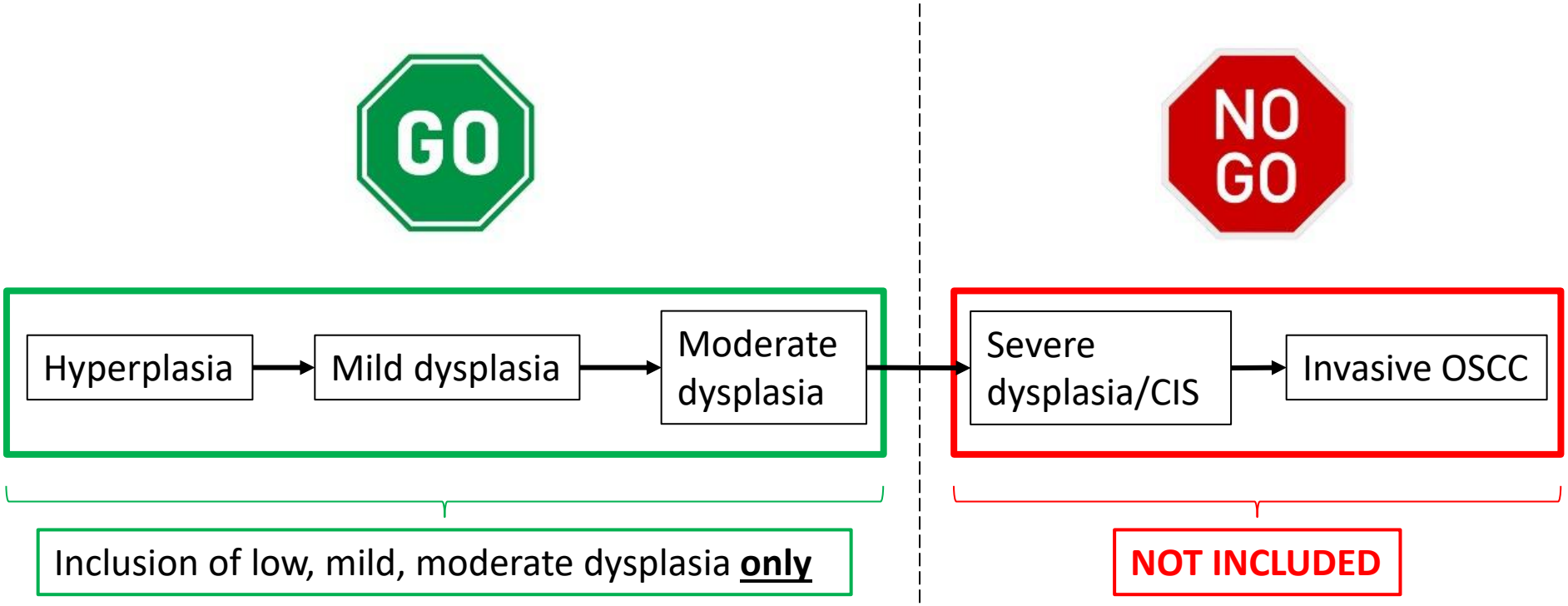
→ Patients **must NOT meet ANY** of the following criteria:

E1. Patients who developed OSCC within 6 months after initial diagnosis of oral leukoplakia, erythroplakia or erythroleukoplakia (E-/L)

E2. Patients with less than 2 years follow-up AND 2 years without previous Oral Cancer (OC) at the time of oral leukoplakia, erythroplakia or erythroleukoplakia (E-/L)

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

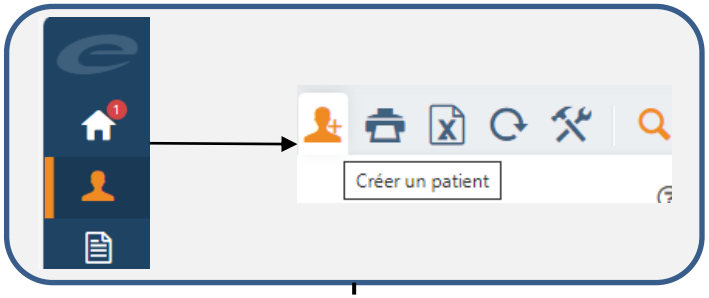
INCLUSION & EXCLUSION CRITERIA



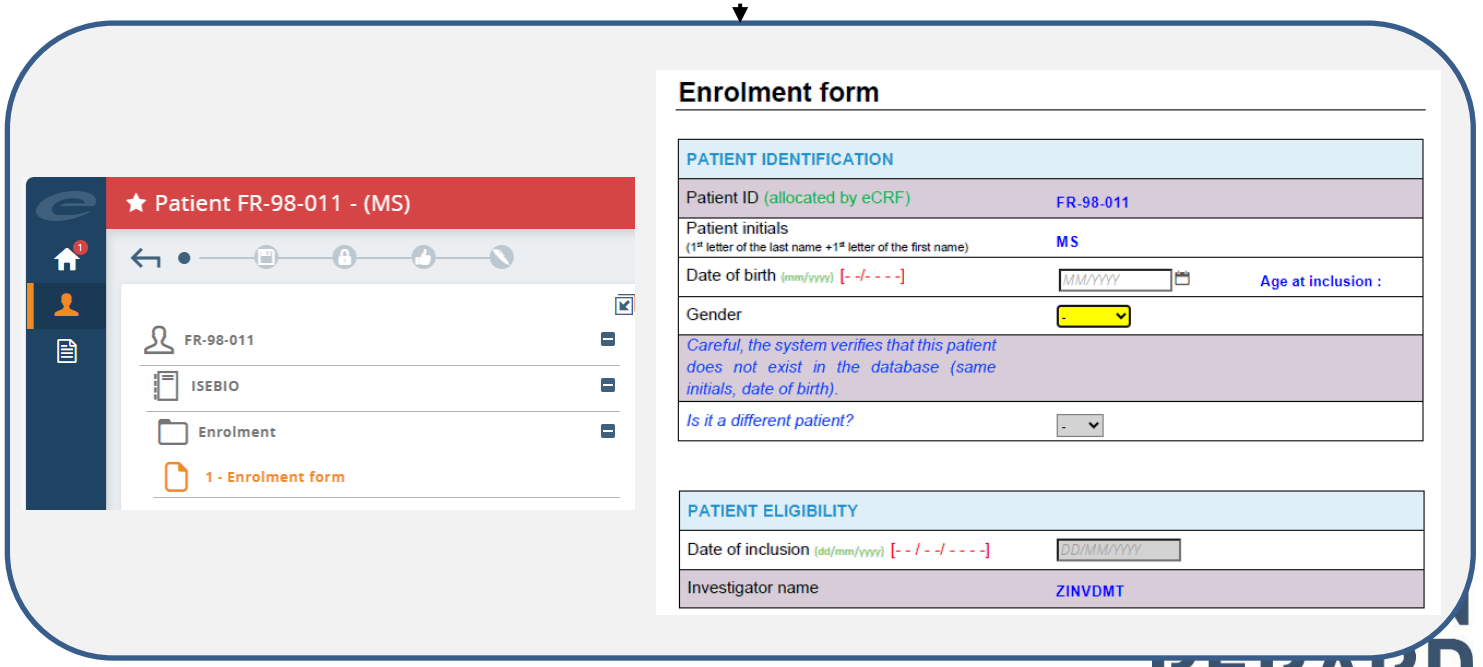
CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

eCRF: DEMONSTRATION (1/3)

Step 1: PATIENT REGISTRATION

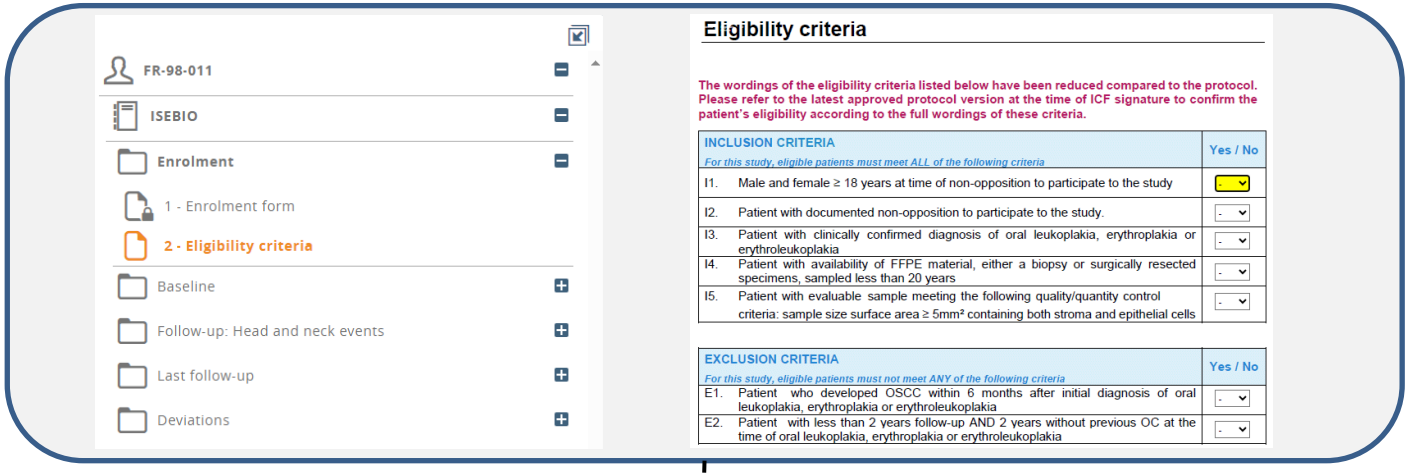


Step 2: ENROLMENT FORM

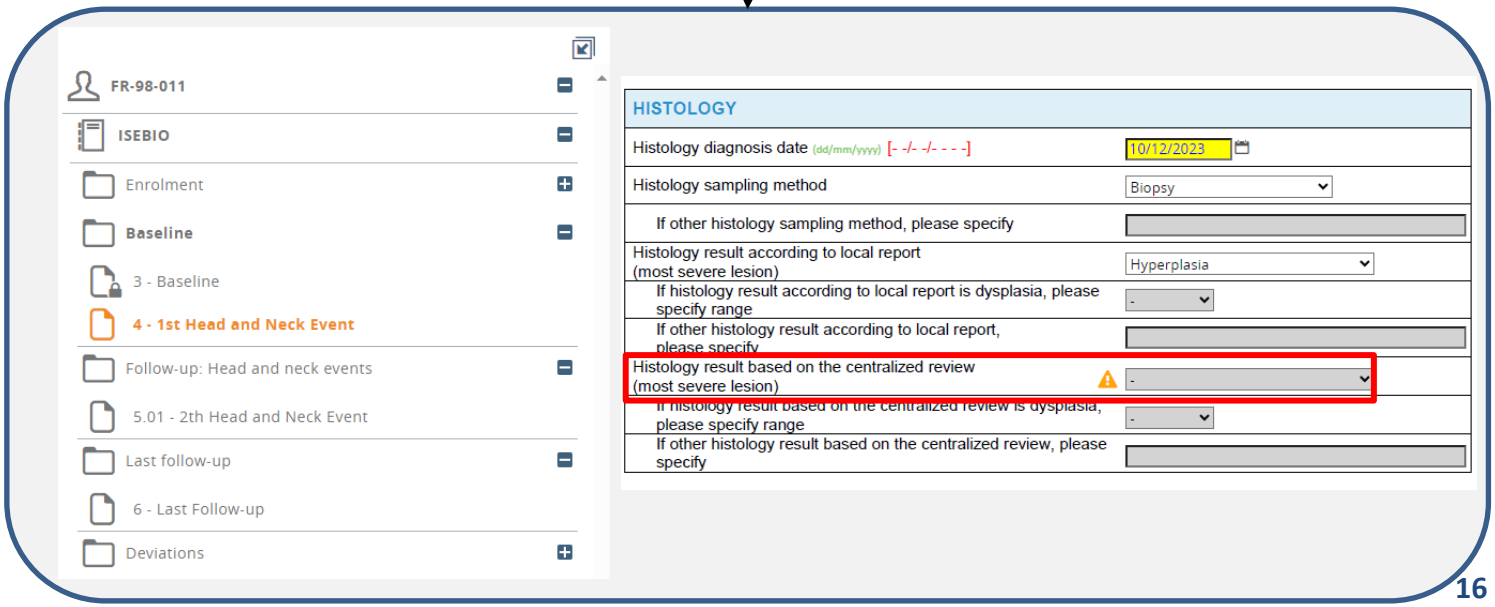


eCRF: DEMONSTRATION (2/3)

Step 3: ELIGIBILITY CRITERIA

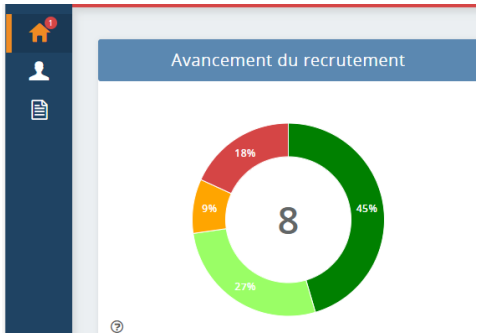


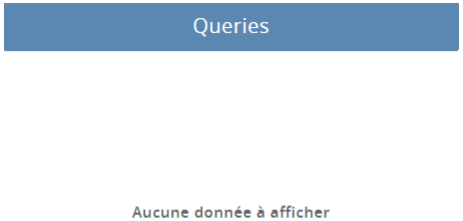
Step 4: OTHER PARTS

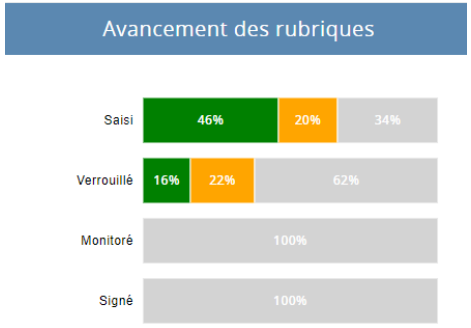


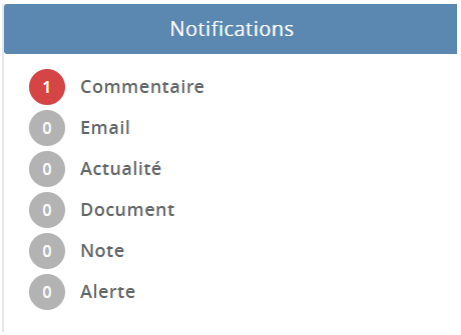
eCRF: DEMONSTRATION (3/3)

- **Important tools**:
  - To **lock** the pages
  - **Dashboard**



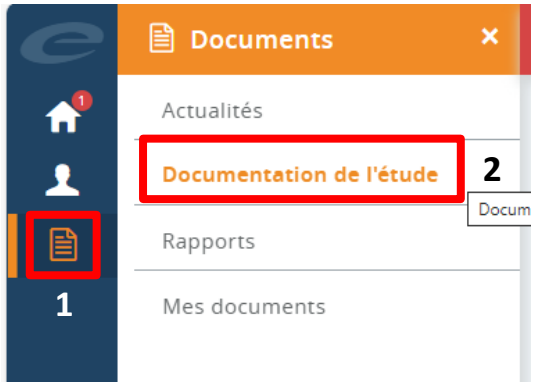






INVESTIGATOR MASTER FILE

- Investigator Master File: **HYBRID** STRUCTURE
  - Common documents:
    - Last version available on ENNOV CLINICAL
    - ENNOV CLINICAL's User guide available
  - Specific site documents:
    - Please, keep them on a paper master file



MONITORING PLAN

- **Online monitoring**
- After the central pathological review

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

GOOD CLINICAL PRACTICE

MAIN SPONSOR'S RESPONSIBILITIES	MAIN INVESTIGATOR'S RESPONSIBILITIES
• To insure the suitability of resources involved in conducting the clinical study; • To ensure that the clinical study is conducted in compliance with the current versions of the protocol and in accordance with the regulation in force; • To adequately monitor the conduct of the clinical study in order to verify that the rights, safety and well-being of subjects are protected, that the reported data are reliable and robust, and that the conduct of the research is in compliance with the requirements of the regulation and laws in force; • To declare the start and end of the trial and communicate a summary of the results to the appropriate Centre Léon Bérard MR004 Review Board within the requested time limits; • To draw up a final report, to inform all investigators about the results and to communicate a summary of the results to the appropriate Centre Léon Bérard MR004 Review Board within requested time limits.	• To demonstrate the suitability of the research site with the conduct of the study; • To identify investigators to assist in the conduct of the research. Qualification of the investigators will be documented in a current curriculum vitae and other relevant documents; • To provide to persons duly appointed by the Study data processor documentation and personal data strictly necessary to the study monitoring, quality control and auditing; • To collect subject's written patient 's non-opposition in his medical record after having given her/him (orally and in writing) the required and necessary to his/her decision-making information; the investigator have to ensure that the patient has understood the information; • To complete eCRF for each enrolled patient, to validate collected data in the eCRF and if necessary correct them following the procedures set up by the Study data processor to ensure traceability.

DATA COLLECTION

- eCRF: https://clb-lyon.ennov.com/CSOnline/

- E-learning: https://elearning-clinical.ennov.com/chamilo/
  - Followed by a short test
  - Send by email the certificate to the Project Manager

- eCRF User Guide available: V1.0 of 22/01/2024
  - Specific point: Password expires **every 3 months**

- Connexion information: Send by email
  - **Nominative** and **strictly personal**

- **If any problems, please contact :**
  - **Project Manager DMT, Marine Benaissa**
    💻: marine.benaissa@lyon.unicancer.fr
  - **Project coordinator/CRA, Al Mostafa Mouhammad**
    💻: mouhammad.almostafa@lyon.unicancer.fr

CONTACTS

- **Project Manager DMT, Marine Benaissa**

☎: +33 (0)4.78.78.20.24 - 💻: marine.benaissa@lyon.unicancer.fr

- **Investigator coordinator, Pr Pierre Saintigny, MD, PhD**

💻: Pierre.SAINTIGNY@lyon.unicancer.fr

- **Project coordinator/CRA, Al Mostafa Mouhammad**

💻: mouhammad.almostafa@lyon.unicancer.fr

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

DOCUMENTS VERSION

- **Protocol**: V1.0 of 12/12/2023
- **Synopsis**: V1.0 of 12/12/2023
- **Laboratory Manual**: V1.0 of 16/01/2024
- **Inclusion eCRF platform**:
  - eCRF: https://clb-lyon.ennov.com/CSOnline/
  - E-learning: https://elearning-clinical.ennov.com/chamilo/
  - **ENNOV CLINICAL User Guide**: V1.0 of 22/01/2024

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

THANKS FOR YOUR ATTENTION

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD