Study title: Development and validation of (bio)sensors for the identification of pathogens.

Acronym: ECLIPSE

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Prot. nr. of the approval: 88/2024/Sper/AOUBo

1.1 Study rationale

The goal of the ECLIPSE project is to develop novel devices that are capable to identify pathogens; the new diagnostic tools should be reliable and accurate, but also portable, rapid, inexpensive, and easy-to-use to allow their employment as screening tests. In this study we propose an initial evaluation of new platforms that will be developed within the ECLIPSE project, these novel platforms will combine interdisciplinary elements as follows:

1) the use of electrochemiluminescence (ECL) as a highly sensitive transduction mechanism for the realization of easy-to-use, portable and inexpensive devices;

2) the development of nano- and biotechnological structures to increase sensitivity by signal amplification;

3) the development of recognition strategies that offer high affinity and selectivity, thus leading to high reliability.

The platforms developed within the ECLIPSE project exhibit the potential to become a game changer in European countries, becoming a cornerstone for rapid testing and reliable diagnosis of infections; these newly developed platforms are expected to overcome the current limitations of molecular testing (high cost, time required and need for well-equipped laboratories) and rapid testing (high number of false-negative results). In addition, the newly developed platforms may have important applications in low-income countries, countries that will benefit from a simple and inexpensive approach to detect the many infectious diseases that affect millions of people each year.

1.2.1 Extent and evaluation of current knowledge directly linked to the scientific question(s) to be answered by the clinical study

The recent COVID-19 pandemic has revealed the need to develop tests that are accurate, rapid, and inexpensive for the diagnosis of infectious diseases. This problem is relevant not only for viruses, but also for bacteria and parasites: the identification of pathogens at very low concentrations by simple and accurate methods is still largely unsatisfied because these microorganisms are structurally complex and are incorporated in complex and diverse biological samples, which can create relevant interferences in pathogens' detection. Direct diagnostic approaches, such as microscopic examination, culture and molecular testing are carried out in equipped laboratories and require long waiting times to obtain the results. Recently developed point-of-care (POC) tests are a group of technologies that miniaturize tests into portable devices such that they can be performed both in well-equipped laboratories and outside the conventional laboratory setting. The present study aims to explore the feasibility and adaptability of newly developed platforms, with the aim of showing their versatility on different models of infectious agents: a virus (SARS-CoV2), a bacterium (*Pseudomonas aeruginosa*) and a protozoan parasite (*Leishmania infantum*). The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) is the respiratory-transmitted virus responsible for the disease named COVID-19 that has been spreading globally since late 2019. The pandemic emergency from SARS-CoV-2 clearly showed the need for new diagnostic tests that are rapid, reliable and easy-to-use. *Pseudomonas aeruginosa* is a Gram-negative bacterium and is a major cause of infection in immunocompromised hospitalized patients. In these patients, *P.aeruginosa* causes nosocomial and ventilator-associated pneumonia as well aś bacteremia and sepsis. *P.aeruginosa* has become a major threat in the nosocomial setting because of its intrinsic resistance to several antimicrobial agents and the emergence of multi-drug resistance (MDR) strains; these factors are leading to high infection severity and increased mortality in infected patients. The management of *P. aeruginosa* infections focuses on early diagnosis and timely initiation of antimicrobial therapy. Finally, leishmaniasis is an infectious disease caused by a protozoan of the genus *Leishmania* and is one of the most prevalent parasitic diseases in the world. Clinically, there are several forms of the disease, including visceral leishmaniasis, which is fatal if not treated promptly. An increase in autochthonous cases of visceral leishmaniasis caused by *L. infantum* has recently been observed in the Emilia-Romagna region, northeastern Italy. As this is a neglected disease, resources invested in the control of this parasitic infection are scarce and diagnostic tools are often outdated and insensitive.

1.2.1.1 Outcomes (efficacy, safety) of completed and number of ongoing clinical studies utilising the same intervention in the same indication (including review of public registers)

ECLIPSE is a multicentric early feasibility study for the initial evaluation of newly developed platforms for the detection of three pathogens and has no outcomes in terms of efficacy and safety.

1.2.1.2 Level of evidence related to the mechanism of action of the intervention in the planned clinical

study population
Not applicable

1.3 Objective(s) of the clinical study (*Please differentiate between primary and secondary objective(s))*
The primary objective of the study is an initial evaluation (feasibility) of newly developed platforms for the detection of three selected pathogens, *i.e.*, SARS-CoV2, *P. aeruginosa*, and *L. infantum* in order to identify novel diagnostic tools that are simple to use, inexpensive, highly sensitive, and selective.
The secondary objective of the study is to evaluate the newly developed platforms using biological samples that are spiked with *P. aeruginosa* strains exhibiting different characteristics in terms of antimicrobial resistance.

1.4 Characteristics of the study population *(size, age group, sex distribution, inclusion and exclusion criteria; all items with justification)*
Overall, a total of 200 patients will be enrolled in the clinical study. For the retrospective phase, after obtaining informed consent, biological samples from 20 patients that tested positive for *L. infantum* at IRCCS Azienda Ospedaliero-Universitaria di Bologna (IRCCS AOUBO) will be analyzed.
In addition, samples obtained from 30 patients testing positive for SARS-CoV2 as well as 30 patients testing negative for the virus and stored in a repository by Personal Genomics (Verona) will be included. Regarding SARS-CoV2, the samples were collected during the COVID-19 pandemic emergency in routine diagnostic path. The informed consents were collected according to Guidelines 03/2020 - European Data Protection Board (EDPB); specific authorization for research use was explicitly indicated in the form (in compliance with art. 6, 1 comma, lett. a) GDPR). All the samples that will be employed in ECLIPSE activities by Personal Genomics have been completely anonymized.
For the prospective phase, patients' enrolment will be carried out at IRCCS AOUBO. We will enrol 30 patients testing positive *P.aeruginosa* and 50 negative patients for *P. aeruginosa* (including 20 Pseudomonas-negative patients whose respiratory samples will be used to perform bacterial spiking and/or DNA spiking); in addition, 10 Leishmania- positive and 30 Leishmania-negative patients will be enrolled.
The inclusion criteria will be as follows;

1. obtaining informed consent,
2. age ≥18 years,
3. patients who meet one of the following conditions: positive patients for SARS-CoV2 infection (group 1), negative patients for SARS-CoV2 infection (group 2), positive patients for *P. aeruginosa* infection (group 3), negative patients for *P. aeruginosa* infection (group 4), positive patients for *L. infantum* infection (group 5), negative patients for *L. infantum* infection (group 6).

There will be no exclusion criteria.
1.4.1 Details on sample size and power calculation
The optimal sample size (statistical power) was chosen according to a criterion of minimizing measurement uncertainty and minimizing false positives and false negatives. Since the measurements in question involve statistical methods applied to chemical analysis (chemometrics), the criterion of optimizing the p-values associated with the statistical parameters of significance tests (in particular, t-TEST and F-TEST or ANOVA) was chosen. For example, in the case of the t-Student parameter for accuracy testing, having fixed the typical significance of analytical measurements-which is 5 percent for confidence intervals (2 sigma approach) and 0.3 percent for detection limits (3 sigma approach)-it is shown that beyond a few dozen measurements this parameter does not improve further. Therefore, in analytical chemistry we set at least 20 measurements for a calibration model and 30 measurements for direct observations. Spiking measurements will be performed to show the dependence of the signal depending on the different *Pseudomonas* strain.

1.5 Design of the clinical study *(controlled / uncontrolled; randomised; open / blinded; parallel group / cross over / other, including innovative trial designs e.g. for personalised medicine, small study populations, or adaptive platform trials; please justify the appropriateness of the selected design)*
This is an early feasibility study involving an initial evaluation of newly developed platforms for the detection of three pathogens (SARS-CoV2, *P. aeruginosa* and *L. infantum*). Furthermore, this study is retrospective and prospective, and multicentric. The results obtained by employing the newly developed

platforms will be compared with the results of gold standard tests performed according to the diagnostic care procedure.
The study will include 6 cohorts of patients, as follows; SARS-CoV2 -positive patients (group 1), enrolled at Personal Genomics (Verona-based center) as retrospective cohort; SARS-CoV2 -negative patients (group 2), enrolled at Personal Genomics, retrospective cohort; *P. aeruginosa*-positive patients (group 3), enrolled at IRCCS AOUBO, prospective cohort; *P. aeruginosa* negative patients (group 4), enrolled at IRCCS AOUBO, prospective cohort; *L. infantum*-positive patients (group 5), enrolled at IRCCS AOUBO, retrospective and prospective cohort. Retrospective specimens will include leftover specimens collected for routine diagnostic testing, *i.e.*, archived specimens collected in the past and stored for extended periods in repositories. Samples will be obtained from patients that tested positive or negative to SARS-CoV2 (groups 1 and 2, respectively). The analyses will be carried out using the newly developed platforms at the Personal Genomics Laboratory. It should be noted that for the retrospective cohort of patients in groups 1 and 2, the patients' data have been anonymized by Personal Genomics. The retrospective phase will also involve samples obtained from patients that tested positive or negative for *L. infantum* (groups 5 and 6). Upon obtaining informed consent, an aliquot of peripheral blood samples that was previously collected according to standardized diagnostic care procedures and stored in a freezer at -80°C at the Unit of Microbiology (IRCCS AOUBO) will be used for the purpose of the study without going to affect any diagnostic investigations. The prospective phase will involve leftover samples that are obtained from for standard care procedures for the diagnosis of *P. aeruginosa* or *L. infantum* infection. EDTA-peripheral blood is routinely sampled for the diagnosis of visceral leishmaniasis, while respiratory samples are routinely collected for the diagnosis of *P. aeruginosa* infection. Clinical samples will be collected for the study purposes after performing the diagnostic process and after obtaining informed consent by the enrolled patients. The leftover samples will be stored at -80°C and will be analyzed by employing the newly developed platforms provided in the study without affecting any diagnostic findings. The sample storage and analysis with the newly developed devices will be carried out at the Unit of Microbiology, IRCCS AOUBO.

1.6 Type of intervention *(medicinal product / advanced therapy medicinal product / medical device / in vitro diagnostic medical device / surgical or other invasive procedure / other medical intervention, including, e.g., counselling)*
Not applicable, it is not an interventional study.

1.7 Description and timing of study procedures
In this clinical study, patients are recruited because they need to undergo diagnostic tests for presumed infection with one of the pathogens under investigation, independent of the study itself. Their diagnostic and therapeutic pathway is not altered. The patients' involvement ends with the sample collection, which is performed following the gold-standard diagnostic procedures. Only leftovers from the diagnostic care procedure will be used, and included in the study only after obtaining informed consent. The results obtained with the new platform will not affect therapeutic decisions, as the sole purpose of the study is to compare the analytical outcomes of the new platforms with those of the standard hospital procedure. No follow-up analyses are planned. The timing of the study will be as follows;

- Patients' recruitment and identification of samples.
- *P. aeruginosa* detection in the selected respiratory samples with the newly developed platforms
- Analysis of SARS-CoV-2 in the selected respiratory samples with the newly developed platforms
- Analysis of *L. infantum* in the selected peripheral blood samples with the newly developed platforms

2. Preparedness status

2.1 Development of the clinical study protocol
Please describe how the below aspects have been or will be addressed in developing the clinical study protocol:

2.1.1 Scientific advice from regulatory and health technology assessment bodies
The consortium includes chemists, molecular and cellular biologists, parasitologists, virologists, microbiologists, infectious disease specialists, data scientists, electronic engineers, and private partners

from European Member States joining in an EU Project.
The inter- and trans-disciplinary consortium is aimed at building innovative platforms to identify three selected pathogens in biological samples capable to have high grade sensitivity in a portable format for on field application. Standard operating procedures (SOPs) of all applicable research processes will be made available. The results of the experimental procedures and consequently the assessment of the proposed innovative platforms will be evaluated by a panel of experts enrolled by the EU Commission to monitor the outcome of the ECLIPSE project.

2.1.2 Clinical efficacy, safety, and methodological guidelines (including guidelines on statistics)
Not relevant in terms of efficacy and safety with respect to the methodological guidelines applied for clinical trials.

2.1.3 Involvement of citizens / patients, carers in drawing up the clinical study protocol
The involvement of patients or carers is not expected in drawing up the clinical study protocol.

2.2 Regulatory intelligence to ensure timely regulatory approval and ethics clearance of the clinical study in all jurisdictions where its implementation is planned
Please provide information on the following regulatory and ethics aspects

2.2.1 How the consortium will ensure access to regulatory expertise necessary to get advice on, and management of, regulatory affairs activities in all concerned jurisdictions?
SOPs will be written to detail all the steps to be performed from the sampling to the laboratory work. SOPs will be detailed, clear and concise, so that staff performing the procedure will be able to do so by following the SOPs and comparison of the results should be made. During the clinical study, periodic technical meetings will be organised to discuss protocols and identify critical issues to be tackled and managed. As registration of the newly developed platforms as in vitro diagnostic device is not part of the current study, no further regulatory activities are requested.

2.2.2 How the consortium will ensure access to ethics expertise necessary to get advice on current proceedings and documentation requirements of all concerned ethics committees?
The local data protection officers of UNIBO and IRCCS AOUBO have been involved in the preparation of the clinical study protocol and will supervise the implementation of the study to support all the required authorization and verification phases. The dedicated Service supporting medical departments of UNIBO will also assist the Principal Investigator (PI) of the clinical study for the ethics submission, having the necessary expertise to get advice on proceedings and documentation requirements. The PI in collaboration with his delegates will ensure that the study will be performed in full conformity with the protocol and ethical requirements set by the current version of the Declaration of Helsinki, the ICH Guidelines for Good Clinical Practice and the local national IRB/EC requirements, in order to assure the protection of the rights, safety, and well-being of study subjects and the transparency and credibility of study data. The study protocol, Informed Sheet, and Informed Consent sheets will be finalized by UNIBO in collaboration with the IRCCS AOUBO clinical site according to local IRB/EC requirements to be submitted for approval to Ethics Committee. The study will not be initiated until all local regulatory and ethical requirements are met. The PI of the clinical study in collaboration with his delegates will be responsible for reporting to the appropriate Regulatory and Ethical authorities all changes in research activity, including protocol amendment(s), study reporting and termination notification. In particular, the protocol amendments will not be implemented without prior Ethics Committee approval, or when the relevant competent authority has raised any grounds for non-acceptance.

2.3 How the scientific and operational governance of the clinical study will be ensured?
The clinical study is promoted by UNIBO that will collaborate to the study approval and verification of the operative procedures for the patients enrolment and sample collection.

2.3.1 Please give details about the sponsor(s) (name, type of entity, seat or country of residence).
The project is entirely funded by EU under the Horizon Europe EIC Pathfinder Open Programme; the sponsor of the project is the coordinator UNIBO.

2.3.2 Please describe the composition, the role and the functioning of the planned board(s), governing bodies.
Not applicable.

3. Operational feasibility

3.1 Please describe how the availability of the intervention(s) (including comparators) is secured throughout the entire implementation phase *(give details on manufacturing, packaging / labelling operations, storage, logistical, import/export issues, etc.)*
Not relevant.

3.2 Please describe how the study population will be recruited
Please give details on the recruitment strategy, monitoring of progress and potential mitigation measures
1_IRCCS AOUBO: the study population will be recruited during hospitalization. The patient will undergo sampling (peripheral blood in EDTA for diagnosis of leishmaniasis and respiratory samples for diagnosis of P. aeruginosa infection) during the routine diagnostic care procedures. Leftovers from the diagnostic care procedures will be selected to be included in the study only after obtaining informed consent.
2_ Personal Genomics: for SARS-CoV2 the samples were collected during the COVID-19 pandemic emergency in routine diagnostic path. All the samples that are employed in ECLIPSE activities have been completely anonymized

3.2.1 How many clinical sites will contribute to the recruitment of the study population in which countries? Are these clinical sites part of an established clinical trial network? Please also describe the selection criteria of the clinical sites.
Clinical sites are in Italy and include IRCCS AOUBO (1.1. AE) and Personal Genomics (P9) as partner of the ECLIPSE consortium.

3.2.2 Will recruitment of the study population be of competitive nature between the clinical sites? *(Please describe how underperformance of individual clinical sites in recruitment will be managed.)*
Not applicable

3.2.3 What evidence supports the ability of the individual clinical sites to recruit the required number of study participants within the planned timeline *(e.g. documented performance in previous clinical studies of similar complexity targeting very similar study population)?*
Analysis of positive and negative clinical specimens received by IRCCS AOUBO (1.1. AE) between January 1, 2023 and December 31, 2023 was performed and the results were as follows;
- 190 bronchoalveolar lavage specimens tested positive for *P.aeruginosa* during 2023 and more than 600 samples tested *P.aeruginosa*-negative during the same period;
-20 peripheral blood samples tested positive for *L.infantum* during 2023 and more than 50 samples tested *L.infantum* negative during the same period.
This evaluation clearly displays the ability of IRCCS AOUBO (1.1. AE) to recruit the requested number of study participants and the related clinical samples.

3.3 Please describe what additional supply *(e.g. an electronic device for remote data capture, a specific instrument for administering the investigational product, etc.)* **is necessary to carry out the required study procedures and how this supply will be made available to the clinical sites**
The device used for this clinical study is shown in the Figure 1; the disposable cartridge will be made available by CSEM, according to the diagrams shown later (Figure 2; the chip will be further implemented) and in collaboration with KIT, UNIBO, UNIME and PG; the other equipment is laboratory equipment owned by UNIBO, with the most important part being the SP-150e potentiostat from BioLogic. The system is portable and will be used at the partners' sites.

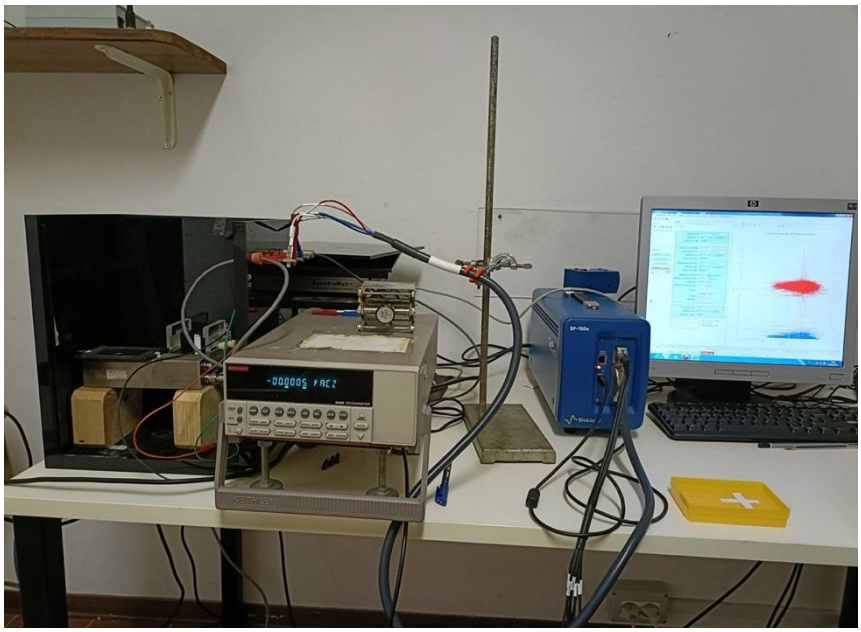

Figure 1: set-up of the device that will be used in the clinical study.

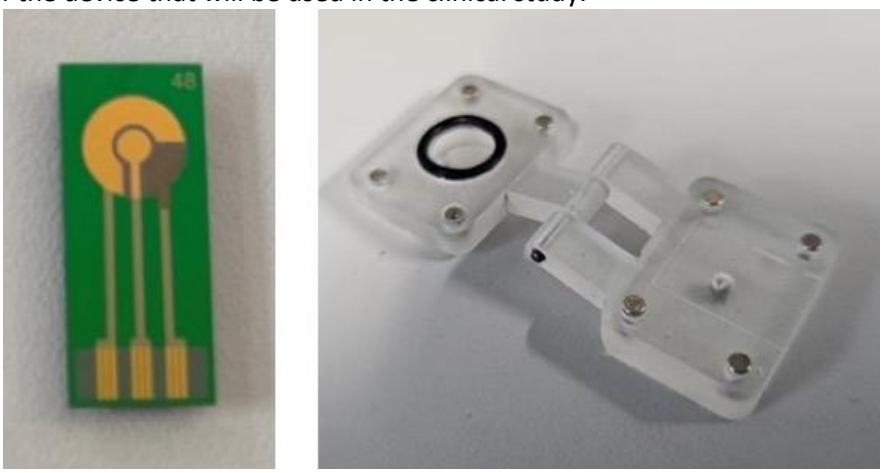

Figure 2: The current version of the chip and its holder

3.4 Please provide plans on data management aspects (data standards, type of data capture, verification of data, central data collection, cleaning, analysis, reporting, security)

ECLIPSE clinical study will produce quantitative, numerical data from measurements on patient's specimens in order to evaluate the performance of the newly developed platforms and to compare them with gold standard techniques.

Data and sample collection and their management will be conducted in compliance with the principles of the declaration of Helsinki (1996), the principles of GCP, GDPR directives and the Italian privacy law, according to the indications of the study protocol and as approved by the local regulatory authorities.

The IRCCS-AOUBO (P1.1) will be in charge of the secure collection of data from patients with *Leishmania infantum* or *Pseudomonas aeruginosa* infections. The collection will be carried out accordingly to the standard protocols defined within the IRCCS AOUBO. They will be standardised and harmonised to allow interoperability and comparison. Data quality assurance will be performed to ensure data inconsistency or missing information are considered and acted upon, to guarantee data precision and accuracy.

Data will be primarily collected in the storage space of the IRCCS AOUBO, hence they will be pseudonymized by data controllers and shared with the UNIBO team which will keep them on its cloud storage space. In all cases, data will be accessible through institutional passwords modified periodically and protected by regularly updated antiviruses. None of the project data will be left inadvertently available. All the research materials stored in computers are subject to back up regularly (according to each institution's regulations) to safeguard them from accidental losses.

Data Management aspects are reported in the periodically updated Data Management Plan of ECLIPSE project, following the EU guidelines.

3.5 Please give details on how reporting obligations (regarding study initiation, safety of study participants, ethical concerns, quality issues, integrity of data, study results) to regulatory bodies and ethics committees will be met.

The Sponsor in collaboration with the recruiting centres (P1.1-IRCCS AOUBO) as well as the local PI (P9-Personal Genomics) will be responsible for the approval by the Ethic Committee and verification of the compliance with GDPR for sample usage. The PIs of the clinical study in collaboration with their delegates will be also responsible for the supervision of the study conduct and for reporting to the appropriate Regulatory and Ethical authorities all changes in research activity, including protocol amendment(s), periodic reporting and preparing a final report on the results of the study. The PIs with their delegates will be also responsible for the conduct of the clinical study according to GCP principles, for data quality and data management. No study-specific informed consent will be necessary in regard with the retrospective part of the study for SARS-CoV2 clinical specimens. In fact, the samples were collected during the COVID-19 pandemic emergency in routine diagnostic path. The informed consents were collected according to Guidelines 03/2020 - European Data Protection Board (EDPB). The form contained the specific authorization for research use as explicitly indicated (in compliance with art. 6, 1 comma, lett. a) GDPR). All the samples that are employed in ECLIPSE activities at Personal Genomics have been completely anonymized. Regarding *Leishmania*-positive and *Leishmania*-negative retrospectively collected clinical samples, study specific informed consent will be collected at IRCCS AOUBO. For the prospective part of the sample's selection, study-specific informed consent will be obtained, with the assurance that all collected data will be pseudonymized and handled confidentially, and no individual will be identifiable in quotes or in the results. Following the ethics approval of the study, the sponsor of the clinical study will register it on the platform Clinicaltrials.gov. The integrity of data and the study results will be monitored and reported by the PI of the clinical study (communication of study initiation, annual monitoring, communication of study completion).

3.6 Please list all items of the sponsor's responsibilities *(e.g. monitoring clinical sites, meeting regulatory obligations, data management, etc.)* **that will be supported by entities that are not part of the sponsor's organisation. Please describe how the sponsor will ensure oversight of these activities.**

The data management plan will ensure the correct acquisition, validation and storage of the clinical data. The Sponsor will supervise this study, overlooking the correct and timely achievement of the regulatory obligations, while the clinical sites will share equal responsibility in the finalisation of the experimental design and the implementation of the clinical activities.

3.7 What are the plans for major study milestones and what evidence supports its feasibility?

Please describe a realistic plan (based on prior experience) detailing the time necessary for (i) compiling the required regulatory and ethics submission package, (ii) receipt of regulatory and ethics approval, (iii) initiation of clinical site(s), (iv) completion of recruitment of the study population, (v) final assessment of all study participants, (vi) analysis and reporting of the study results.

Key milestones (months referred to the ECLIPSE project)

- Study initiation package - M29
- Receipt of regulatory and ethics approval - M29
- First patient recruited - M29
- Midterm recruitment report - M34
- Recruitment closure - M40
- Report on the status of posting results - M42