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"Cholangioids to define the genetic factors involved in atypical primary sclerosing cholangitis"

(English title: "Cholangioids to dissect the genetic drivers of atypical Primary Sclerosing Cholangitis")

Acronym: CILIA

FRRB Project #4976687

Protocol version number: v1.0
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Promoter:IRCCS Foundation Ca' Granda Maggiore Hospital Polyclinic,
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Principal Investigator:Prof. Luca Valenti

FLOWCHARTS

	Screening	Intervention
Period	(-t1)	(t1)
Enlistment		
informed consent		
inclusion/exclusion criteria		
Administration of the intervention		
Biological sample collection: blood sampling and/or liver biopsy/resection		

LIST OF ABBREVIATIONS

aPSC:Atypical primary sclerosing cholangitis THERE
IS:Ethics Committee
THERE:Informed Consent
CRF:Case Report Form, data collection form
GCP:Good Clinica Practice, good clinical practice
GWAS:Genome-wide association studies HLA:
Human leukocyte antigen IBD:Inflammatory
bowel disease iPSC:Induced Pluripotent Stem
Cells PBMC:peripheral blood mononuclear cells

RESPONSIBILITY (role of the promoter and collaborators)

Promoter: IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico
Coordinating Center: SC Transfusion Medicine
PI of the study: Prof. Luca Valenti
Scientific Director of the FRRB Project: Dr. Alessandro Cherubini

Structure	Participant Name	Role and functions in the study
SC Transfusion Medicine, IRCCS Foundation Ca' Granda Maggiore Hospital Polyclinic	Dr. Alessandro Cherubini Prof. Luca Valenti Dr. Daniele Prati	Recruitment (LV) and Sample characterization; Isolation and generation of organoids, spheroids and tissues; Characterization of organoids by gene and protein expression Data Analysis

Internal collaborations

Structure	Participant Name	Role and functions in the study
SC General Surgery and Liver Transplantation, IRCCS Foundation Ca' Granda Hospital Maggiore Polyclinic	Dr. Daniele Dondossola	Reporting of eligible patients and characterization of samples
SC Scientific Direction IRCCS Foundation Ca' Granda Maggiore Hospital Polyclinic	Dr. Stefano Gatti	Support in cell isolation protocols

External collaborations (biological sample analysis, data analysis, diagnostic procedures, etc.)

Institution	Operational unit	Participant Name	Role and functions in the study
IRCCS Foundation	SC Gastroenterology	Prof. Pietro Invernizzi	Scientific support in

Saint Gerard of the Dyers - Monza			how much expert center in rare liver diseases such as sclerosing cholangitis primitive

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1. INTRODUCTION

1.1 Background and rationale

Primary sclerosing cholangitis (PSC) is a rare, progressive and often fatal disease of the intrahepatic or extrahepatic bile ducts, with an estimated prevalence in Western countries of 1/10,000. [1,2]. Biliary disease in PSC is represented by cholestasis, chronic inflammation of the bile ducts, the small tubes through which bile passes, progressive concentric fibrosis around the bile ducts[2]. This results in an obstruction to the passage of bile, which can lead to the development of cirrhosis with complications related to portal hypertension, cholangitis and often progress to bile duct cancer (cholangiocarcinoma). The only curative therapy in patients with PSC is liver transplantation, since no drug has been shown to be effective in preventing disease progression. The etiology is most likely multifactorial immune-mediated, where the onset of PSC is triggered by environmental factors in a genetically susceptible host[2]Genome-wide association studies (GWAS) have identified variations at the human leukocyte antigen (HLA) complex on chromosome 6 and several other loci, but these explain only a small part of the heritability of PSC.[3.4]In most cases, PSC occurs in men in their 30s and 40s who have inflammatory bowel disease (IBD).[5], suggesting a key role of altered intestinal permeability and inflammation. However, approximately 30% of patients do not present colonic inflammation, which is consistent with the heterogeneity of the disease. Preliminary data obtained in our laboratory analyzing a cohort of Italian individuals with atypical PSC (aPSC), identified a suggestive enrichment of rare variants in genes involved in cilia morphogenesis (e.g. CEP120 and AHI1). These data are consistent with previous findings, showing the correlation between gene variants involved in ciliopathies, including the DCDC26 gene, and chronic cholestatic disorders that can mimic PSC.

Primary cilia are organelles present on the outer membrane of ductal cells, called cholangiocytes. These organelles function as antennas that detect stimuli from bile and transmit information to cells by regulating various signaling pathways involved in secretion, proliferation and apoptosis.[6] . Therefore, the alteration of primary cilia plays an important role in the de-differentiation of cholangiocytes and therefore in the development of cholangiopathies, in the invasion of inflammatory cells and in the fibrotic process. However, to date little is known about the contribution of genetic variants to the severity and progression of PSC, perhaps also due to the lack of a reliable model of bile duct.

Recently, three-dimensional cell cultures, called organoids, have been proposed as a revolutionary tool in the field of cell biology, as they are able to mimic the corresponding organ in vivo.[7]Organoids can be derived from either induced pluripotent stem cells (iPSCs) or tissue-resident adult stem cells.[8]. Compared to conventional 2D cultures and animal models, organoids allow to reproduce the genetic background of the patient in the model, recapitulating in vitro structures and functions similar to in vivo tissues. For this reason, organoids have been exploited in different applications, including drug discovery and testing, precision medicine and cell therapy[9–11]. However, organoids still show several limitations to model liver diseases. Indeed, they are only able to recapitulate the hepatic epithelial component, cholangiocytes and/or hepatocytes and above all they lack the 3D hepatic microenvironment, such as stromal and immune cells, which play an important role in the pathogenesis of several liver diseases.

The present study is part of a project funded by the Regional Foundation for Biomedical Research (FRRB) whose general objective is to generate three-dimensional models of primary sclerosing cholangitis (PSC), called assemblyloids, and to study the cellular and molecular mechanisms through which genetic variants associated with genes involved in ciliopathies accelerate the progression of PSC. Our hypothesis is that the loss of function of cilia in cholangiocytes may represent a link between cellular senescence, development of inflammation, fibrosis and finally liver cancer. The variants related to ciliopathies could lead to an incomplete maturation of cholangiocytes with consequent malfunction that can therefore lead to a chronic inflammation of ductal cells and therefore to a persistent and uncontrolled activation of stromal cells and infiltration of immune cells.
Furthermore, the generation of assemblyloids capable of reproducing native tissue as faithfully as possible will provide a new in vitro model for testing new pharmacological approaches aimed at correcting genetic mutations for improved precision medicine.

2. OBJECTIVE/S/I HYPOTHESIS OF THE EXPERIMENTATION

2.1 Primary objective

The primary objective of the study is to explore the behavior of genetic variants involved in ciliopathies in the onset and progression of aPSC by analyzing in three-dimensional models called assemblyloids:

- Morphology of primary cilia
- Differentiation of cholangiocytes
- Activation of fibrogenesis
- Infiltration of immune cells

2.2 Secondary objective

Using assemblyloids as a templatein vitroto test new pharmacological approaches, such as extracellular vesicles containing the CRISPR/Cas9 genome editing system. This model more faithfully reproduces the counterpartin vivowill allow to have a more faithful answer than using the conventional 2D model, organoids or animal model.

3. STUDY DESIGN

3.1 Study design

This is a non-pharmacological interventional study, with collection of biological material, single-center, non-profit.

3.2 Inclusion criteria

Patients will be included:

- Aged between 18 and 90 years old
- Of both sexes

- willingness to sign informed consent for the study;

Additional criteria group 1
- patients with a confirmed diagnosis of aPSC

Additional criteria group 2
- patients with presumed aPSC who are candidates for liver biopsy

Additional criteria group 3
- non-aPSC patients scheduled for the following procedures:
- liver resection for hepatocellular carcinoma or other liver lesions (including secondary lesions from other neoplasms and benign focal lesions, which will allow obtaining healthy starting liver tissue);
- post-transplant healthy liver biopsies;
- cholecystectomies.

Additional criteria group 4
- patients previously genotyped in the study "Impact of whole exome sequencing on the clinical management of patients with non-alcoholic fatty liver disease and cryptogenic liver disease – Ricerca Finalizzata 2016, project code RF-2016-02364358" found to be carriers of gene variants associated with ciliopathies.

3.3 Exclusion criteria

Patients who do not meet the above study criteria and with the following criteria will be excluded:
- positivity for chronic viral hepatitis (HCV-RNA and/or HBsAg);
- positivity to other liver diseases such as autoimmune and viral hepatitis (hepatitis B and C), hereditary hemochromatosis, alpha-1-antitrypsin deficiency, Wilson's disease.

4. PROCEDURES RELATING TO THE STUDY

4.1 Enrollment and collection of biological samples

Patient enrollment in the study will be coordinated by the Transfusion Medicine Unit at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico.

Specifically, patients with a confirmed diagnosis of aPSC (group 1) in the context of scheduled clinical visits will be subjected to a blood sample of approximately 7 cc.

From patients with presumed aPSC who are candidates for liver biopsy to confirm the diagnosis of aPSC (group 2), any liver biopsy waste tissue will be collected. In addition, these patients will be required to have an additional peripheral blood sample of 7 cc.

Finally, after informed consent, in collaboration with the SC General Surgery and Liver Transplantation of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, samples of waste tissue will be collected (approx. 1 cm^3) starting from patients not affected by aPSC (group 3) who for clinical needs will undergo liver resection for hepatocellular carcinoma or other liver lesions, cholecystectomy or post-transplant whole liver biopsies. This procedure will not entail any additional risk for the patient compared to the usual routine, nor will it reduce the availability of material for standard pathological analyses. Finally, a blood sample (approximately 7 cc) will be required, in addition to those already foreseen during regular clinical checks, in order to isolate PBMC cells from which macrophages will be generated.

In general:

- The liver samples collected in the study will allow us to isolate cells (ovalocytes – liver progenitor cells) to generate organoids and stromal cells.
- The blood samples will allow us to 1) isolate PBMC cells from which macrophages and iPSCs will be generated; 2) isolate DNA from PBMC in order to identify any genetic variants related to ciliopathies.

Given the rarity of the pathology under study, in order to reach the number of samples planned in the study, during visits already scheduled for routine, patients affected by aPSC previously genotyped in the context of the study promoted by the Foundation (group 4) may be enrolled for a blood sample equal to 7 cc in order to isolate peripheral blood mononuclear cells to be reprogrammed to iPSC.

Following gene sequencing, a group of patients carrying the gene variants related to ciliopathies and a group not carrying these variants will be identified.

All cell types and genetic material obtained will be biobanked at the biobank of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico at the end of the study, following the signing of the informed consent obtained at the time of patient enrollment.

4.2 Isolation and culture of organoids

Biopsy fragments or fragments obtained from surgical resections will be transported in Celsior perfusion solution at 4°C and will be processed at the Translational Medicine laboratory, SC Transfusion Center of the IRCCS Ca' Granda Foundation Ospedale Maggiore Policlinico within 24 hours of collection to isolate liver organoids. The tissues will be mechanically fragmented into small pieces avoiding reducing them to single cells; this will increase the efficiency of organoid formation. Then the fragments will be further subjected to enzymatic digestion with a solution containing Collagenase and DNase at 37°C. The cell clusters obtained from enzymatic digestion will be included in reduced growth factor Matrigel. Once the Matrigel has polymerized, the complete culture medium for liver organoids will be added: Advanced DMEM/F12 supplemented with 1% N2 and 1% B27 (both from GIBCO), 1.25 mM N-

Acetylcysteine (Sigma), 10 nM Leu-Gastrin (Sigma), 50 ng/ml EGF (Peprotech), 1 -g/ml RSPO1 (Peprotech), 100 ng/ml FGF10 (Peprotech), 25 ng/ml HGF (Peprotech), 10 mMNicotinamide (Sigma), 5 -M A83.01 (Peprotech) and 10 -M Forskolin (Peprotech). In order to increase the efficiency of organoid isolation, 25 ng/ml Noggin (Peprotech), 100 ng/ml Wnt3a (peprotech) and 10 -M Rock inhibitor Y27632 (Peprotech) will be added to the medium for the first few days. The organoids will then be differentiated towards a hepatocyte phenotype.

4.3 Isolation and culture of stromal cells

A part of the biopsy fragments or those obtained from surgical resections, once digested to obtain the epithelial cells that will form the organoids, will be processed to obtain the stromal cells.
The liver sample will first be perfused with perfusion buffer (0.5 -M EGTA, 2 mM HCl2, 20 mM HEPES, 1 mg/ml pronase, 0.25 mg/ml collagenase type IV). Stromal cells will then be isolated by separation on a density gradient generated with OptiPrep solution (StemCell Technologies). Stromal cells will be cultured on plastic in DMEM medium containing 10% FBS and we will characterize their activation status and the purity of the cell population, by flow cytometry and quantitative Real Time PCR (RT-qPCR), evaluating the presence of specific cellular markers such as CD90, CD29 and CD73[12].

4.4 Isolation and culture of peripheral blood mononuclear cells (PBMC)

PBMC cells will be isolated from blood samples by density gradient using SepMate tubes (StemCell Technologies). The isolated cells will be cultured in RPMI 1640 containing 10% FBS and then differentiated into macrophages for 6 days by adding 50 ng/mL M-CSF to the culture medium.

4.5 Generation of induced pluripotent stem cells (iPSCs)

A portion of the PBMCs will be cultured in medium composed of StemPro™-34 SFM (ThermoFisher Scientific) containing 100 ng/mL SCF, 100 ng/mL FLT-3, 20 ng/mL IL-3 and 20 ng/mL IL-6 and then reprogrammed to iPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming kit (ThermoFisher Scientific). The obtained iPSCs will be maintained in Essential 8™ medium (ThermoFisher Scientific) on vitronectin-coated plates and their ability to differentiate to all three germ layers meso-, endo- and ectoderm will be assessed by RT-qPCR and immunofluorescence.

4.6 Generation of assemblyloids

In order to obtain the assemblyloids, both organoids and stromal cells and macrophages isolated directly from liver tissue or differentiated from iPSCs, will be dissociated into single cells with enzymatic digestion using trypsin. The cells will then be included in reduced growth factorMatrigel in different ratios in order to mimic the physiological conditions present in the liver tissue. Once the Matrigel has polymerized, the culture medium appropriately formulated to allow the survival of both ductal cells, stromal cells and immune cells will be added: Advanced DMEM/F12 supplemented with 1% N2 and 1% B27 (both from GIBCO), 1.25 mM N-Acetylcysteine (Sigma), 10 nM Leu-Gastrin (Sigma), 50 ng/ml EGF

(Peprotech), 25 ng/ml HGF (Peprotech) and 2% FBS (GIBCO). The assemblyloids will be grown in suspension using a shaker in order to increase the diffusion of nutrients and oxygen and promote the correct organization of the cells inside the matrix drop similar to that which would be found in the organ.in vivo.

4.7 Characterization of assemblyloids

The assemblyloids will be characterized in order to confirm their ability to mimic the complexity of the organ in vivo, both at the molecular level by single cell RNA-sequencing using the Chromium technology (10x Genomics) present at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and at the morphological level by immunohistochemical analysis.

4.8 Genetic analysis

DNA will be extracted by phenol-chloroform from peripheral blood and genetic variants of interest will be determined by TaqMan assay on an ABI 7500 Fast apparatus (Life Technologies, Carlsbad, CA, USA).

4.9 RNA isolation

RNA will be extracted from cultured organoids, stromal cells, macrophages and assemloids using Trizol reagent (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. 1 µg of total RNA will be reverse transcribed using the VILO kitrandom hexamers synthesis system (Life Technologies, Carlsbad, CA). Gene expression will be assessed by quantitative real-time PCR (RT-qPCR) using an ABI 7500 Fast thermal cycler (Life Technologies, Carlsbad, CA) and SYBR Green chemistry (Fast SYBR Green Master Mix; Life Technologies, CA). All reactions will be performed in triplicate.

4.10 Characterization of the role of cilia in the progression of sclerosing cholangitis primitive

In order to define the role of genetic variants involved in ciliopathies in the progression of aPSC, assemblyloids will be characterized at the molecular level by cutting edge technologies including single cell RNA-sequencing (scRNA-seq), using the Chromium 10x instrument present in the Foundation, which will allow us to understand how the variants influence the different cell populations at the molecular level. The morphology of primary cilia will be studied by electronic and confocal microscopy with instruments present in the Foundation. Finally, the role of genetic variants on the progression of the disease will be studied, looking at collagen deposition and fibrosis formation, by confocal microscopy and macrophage activation and infiltration, with functional assays and confocal microscopy.

4.11 Use of liver assembloids as a model to test new pharmacological approaches

Considering the ability of the assembloids to morphologically and physiologically reproduce the native tissue, they will be used as an in vitro model to test new pharmacological approaches such as engineered extracellular vesicles to carry the CRISPR/Cas9 genome editing system. Once generated, the assembloids will be treated with isolated extracellular vesicles at the Translational Medicine laboratory of the SC Transfusion Center of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico for 72h. The genetic editing will be evaluated

by TaqMan assay on ABI 7500 Fast apparatus as described in point 4.8.

5. ENDPOINT

5.1 Primary Endpoint

Exploration and description of the behavior of genetic variants associated with ciliopathies on the aPSC phenotype within the variant carrier group compared to the non-carrier group.

5.2 Secondary Endpoints

Testing of new pharmacological approaches based on extracellular vesicles containing the CRISPR/Cas9 genome editing system to correct mutations involved in ciliopathies using assemblyloids as a modelin vitro.

6. DURATION / TIMELINE OF THE STUDY

Study start month and year: 12/2024
Enrollment close month and year: 12/2026
Study end month and year: 12/2027

PROJECT GANTT

Intervention/months	1-6	7-12	13- 18	19-24	25-30	31-36
Enlistment						
Informed consent						
Inclusion/exclusion criteria						
Isolation of primary cells						
iPS Generation						
Assembloid Generation						
Gene expression analysis						
Morphological analysis by mycoscopy						
Functional analyses of the role of cilia in primary sclerosing cholangitis						
Using Assemblloids to Test EVs as a Novel Pharmacological Approach						

7. STATISTICAL ANALYSIS

7.1 Sample size

Since the disease is rare and to the inferential, but it is only descriptive sample size. Those patients carrying the variant to achieve this goal we set ourselves in point 4.1, given that as generation of organoids is

the inside of the primary endpoint are not and exploratory, for this reason it is not that we will be able to collect however and 6 samples of non-carrier patients we are going to recruit at least 20 patients for each indicated by previously published works 30% starting from biopsies [13,14].

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at least 6 samples from

of the variant. For each group indicated

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7.2 Data analysis

Statistical analyses will be carried out using the JMP software (SA, Cary, NC) and the scRNA-seq analyses will be performed using the Seurat package of the R software (https://www.r-project.org), with a pipeline already standardized within our laboratory. The results will be represented by descriptive statistics: categorical variables will be reported as frequency and percentage, while continuous variables will be reported as mean ± SD or median (interquartile range), depending on the distribution. If deemed appropriate, graphs will be used to integrate the descriptive analyses.

8. ADVERSE EVENTS

The project does not involve the administration of drugs or other substances or ad hoc invasive interventions outside of normal clinical practices. Therefore, no adverse events are expected.

9. RISK/BENEFIT ASSESSMENT

The study does not foresee an immediate benefit for patients, but the results of this trial will have the potential to lead to a decoding of the mechanisms underlying the development of PSC, resolve the temporal order of events that regulate its evolutionary trajectory, understand its progression, characterization and improve the therapeutic management of patients.

10. STUDIO MANAGEMENT

10.1 Data collection and management

Each participant, at the time of enrollment, will be assigned a unique code. The de-identification of the data will be done in such a way that the people who access the database will not be able to trace the identity of the subjects in any way. Only local experimenters will be able to trace the identity of the enrolled subjects.

The data needed for the study will be recorded in a specific eCRF in a Data Management System validated according to national regulations, provided by the Scientific Direction of the Foundation. The platform used will be RedCap (Research Electronic Data Capture).

The REDCap Consortium is composed of >1000 institutional partners worldwide (research institutions, universities, ministries etc). The consortium supports a secure web application (REDCap) designed exclusively to support data acquisition for research studies. The REDCap application allows users to create and manage online databases quickly and securely, and is currently in use for more than 110,000 projects with approximately 150,000 users covering numerous areas of research interest across the consortium.

Through REDCap, for this study the following will be implemented: a) user-level identification, with specific restrictions based on the role in the study b) real-time data integrity validation and control c) patient de-identification before data export d) centralized data storage with daily backup, a secure server within the Foundation's IT structure.

10.2 Regulatory aspects and ethical considerations

10.2.1 Approval by the Competent Authority

In accordance with applicable regulations, the principal investigator must obtain approval from the appropriate Competent Authority prior to initiating the clinical study.
This study will be conducted in accordance with the ICH/GCP (International Conference of Harmonization/Good Clinical Practice) rules and all applicable laws, including the Helsinki Declaration of June 1964, as amended by the last World Medical Association General Assembly in Seoul, 2008.

10.2.2 Ethics Committee Approval

The investigator must ensure that the protocol has been reviewed and approved by the local independent Ethics Committee (EC) before starting the study.
The CE must also review and approve the informed consent (IC) form and all written information received from the patient prior to enrollment in the study.
Should it be necessary to modify the protocol and/or the IC during the study, the investigator will be the guarantor and therefore the person responsible for ensuring the review and approval of such modified document as requested by the CE.

The content of these changes will be implemented only after the CE has approved them. Until then, it will be necessary to refer to the previous version of the document already approved.

10.2.3 Informed consent (IC)

The investigator or other personnel designated by him have the task of informing the subjects about all aspects and procedures of the study.
The process for obtaining informed consent must comply with applicable regulatory procedures. The investigator (or designated staff member) and the subject must date and sign the informed consent form prior to the patient initiating any study-related procedures. The subject will receive a copy of the IC dated and signed by both parties; the original copy will be retained in designated study archives. Neither the investigator nor designated staff member should in any way coerce or influence a subject to participate or continue to participate in the study. A subject's decision to participate in the study must be completely voluntary. The investigator and designated staff member should emphasize to the subject that he or she may withdraw consent at any time without penalty or loss of any benefits to which the subject may be entitled.
Written or oral information relating to the study, including the written consent form, must not contain any language that forces the subject to waive (even apparently) his or her legal rights, or that would exonerate the investigator, the institution or the

sponsor from liability for negligence.

10.3 Duties of the experimenter

In accordance with applicable local regulations, the investigator must submit periodic reports regarding the progress of the study at his/her site to the CE and notify the CE of study closure. Periodic reports and closure notification are part of the investigator's responsibilities.

10.4 Study monitoring

In accordance with applicable regulations and good clinical practice (GCP), the monitor must visit or contact the center periodically. The duration, nature and frequency of such visits / contacts depend on the recruitment frequency, the quality of the documents held by the center and their adherence to the protocol.

Through these contacts, the monitor must:
• monitor and evaluate the progress of the study
• examine the collected data
• conduct source document verification
• identify each problem and related solutions

The purposes of the monitoring activity are to verify that:
• the rights and well-being of the subject are respected
• the study data are accurate, complete and verifiable from the original documents
• the study is conducted in accordance with the protocol and any approved amendments, GCP and applicable regulations

The experimenter must:
• give the monitor direct access to all relevant documentation
• dedicate part of his time and his staff to the monitor to discuss the monitoring results and any other possible aspects.

The monitor must also contact the center prior to the start of the study to discuss the protocol and data collection procedures with the staff.

10.5 Study quality assurance

As the sponsor, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico may, at its discretion, carry out a quality control of the study. In this case, the investigator must allow the monitor direct access to all relevant documentation and dedicate part of his or her time and personnel to the reviewer to discuss the results of the monitoring and any other aspects of the study.
In addition, Regulatory Authorities may conduct inspections. In this case, the investigator must allow the inspector direct access to all relevant documentation, and dedicate part of his time and personnel to the inspector to discuss the monitoring results and any other aspects of the study.

10.6 Closing of the study

At the time of study closure, the monitor and the experimenter must activate a series of procedures:

- review all study documentation
- reconcile study data
- reconcile all clarifying reports.

10.7 Document storage

In accordance with current national regulations, the investigator must keep a copy of all documentation and store it in a dry and safe place after the study has been closed.

10.8 Disclosure of information regarding scientific discovery

10.8.1 Confidentiality

The investigator and other personnel involved in the study must handle all information relating to the study (including the protocol, data obtained and all documentation generated during the study) and must not use such information, data or reports for purposes other than those described in the protocol. These restrictions do not apply to:

1) information that becomes publicly available, not due to negligence on the part of the investigator or his staff;

2) information that requires confidential disclosure to CE for the sole purpose of evaluating the study;

3) information that must be disclosed in order to obtain appropriate medical care for a study subject.

10.8.2 Publications and Intellectual Property Rights on the Results of the Study

SINGLE-CENTER STUDY that includes collaborations:

Publications:

Foundation as a Prom studio, even in the case of results visibility proportional to the actual contribution provided in the form in aggregate or common the identification of the subject involved. Each magazine or publication has the role and the participation of the Ce

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ification containing the results and data of the collaborating institution and the Foundation, under study and the role covered. The data may therefore be anonymized, so as not to permit the identification of the person to whom the data refer.

one of the results of the collaborating center

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God, will have to indicate the proportional number to the
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or be published in any manner whatsoever

Intellectual Property Rights

The Parties acknowledge that for the to be used and shared by each party that right of access and use,

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management of collaboration in the field of ti, nformation,know-how,inventions (br and remains the exclusive owner even if n non-exclusive and free, for the sole purposes of the real

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of the study will be able to whether or not it is possible) and grant the other a ization of the activities

object of the study and limited use m entity for the duration of the same. It remains I know that this right of
does not include the ownership of the uninterrupted to sublicense to third parties.
the Promoter, except in compliance g entity, data and results generated within the framework of specific The Studio will be staffed by
with the legislation. And agreements between the Promoter and the centre/centre And collaborators.

11. INDEMNITY AND COMPENSATION IN CASE OF DAMAGES

In case of unwanted events or any damages that may arise from participation in research, our Institute's Insurance Policy also extends to cover subjects participating in research projects.

12. AMENDMENTS TO THE PROTOCOL

In case of changes in the study protocol, the amendment procedure will be activated in advance at the local independent CE. The CE will have to verify and approve again the CI form and all written information received from the patient before enrollment in the study.

13. FINANCIAL AGREEMENTS

The costs of study procedures exceeding normal clinical practice will be entirely covered by funds deriving from the EarlyCareerAward II Edition – Regional Foundation for Biomedical Research funding.

14. DISCLOSURE ON CONFLICTS OF INTEREST

The experimenters declare no conflicts of interest.

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