ulises
Cancer Curing and Vaccination

Deliverable 5.1
Report on establishment of optimal collection, processing, storing and analysis of biological materials from patients

1.1. D5.1 Report on establishment of optimal collection, processing, storing and analysis of biological materials from patients WP 5: Validation; T 5.1: Collection and characterization of biological samples (Months: 1-30 / Partners: IVO, IDIBAPS, FFIS)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 899708.

2. Technical references

Project Acronym	ULISES
Project Title	Immunological incompatibility as a basis for cancer curing and vaccination
Project Coordinator	Cristina Fillat IDIBAPS coordinatormail@partner.eu
Project Duration	October 2020 – September 2023 (36 months)

Deliverable No.	D5.1
Dissemination level*	PU
Work Package	WP 5: Validation
Task	T5.1: Collection and characterization of biological samples.
Lead beneficiary	IVO
Contributing beneficiary/ies	IDIBAPS; FFIS
Due date of deliverable	30 September 2021
Actual submission date	29 October 2021

* PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

v	Date	Beneficiary	Author
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4. Collection, processing, storing and analysis of biological materials from patients

4.1. Introduction

Pancreatic ductal adenocarcinoma (**PDAC**) has the **lowest rate of survival amongst all cancers across Europe** [1]. In the **EU alone**, it is responsible for over **95,000 deaths every year** and **life expectancy** at the time of diagnosis is **less than 5 months** [2]. Recent estimates have indicated that the number of pan-European deaths caused by pancreatic cancer overtook breast cancer fatalities in 2017, placing the condition as the third leading cause of cancer-related deaths [2]. There have been few advancements in patient outcomes over the last four decades with mortality rates having seen no improvement and just **3% of patients diagnosed with pancreatic cancer surviving beyond five years** (Max Roser and Hannah Ritchie (2015) - "Cancer". Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/cancer' [Online Resource]). The **incidence of PDAC is increasing by 0.5%to 1.0%per year**, and it is projected to become the second-leading cause of cancer-related mortality by 2030 [3].

General facts for PDAC are summarized in Table 1.

The **pathophysiology of PDAC** is characterized by **complex multistep genetic alterations**. In the precancerous state, **pancreatic intraepithelial neoplasias** (PanINs) acquire cumulative genetic insults resulting in instigating oncogenes that are responsible for the initiation and maintenance of PDAC, including *KRAS, CDKN2A, TP53*, and *SMAD4* [4]. Collectively, these genomic alterations contribute to multifaceted defects in tumor suppressor mechanisms resulting in dysregulated growth signaling and inflammation, which are key aspects of PDAC. Additionally, about 10% to 15% of PDACs acquire variants in the *SWI/SNF* chromatin remodeling genes related to large-scale structural genomic aberrations [5].

Recent advances in molecular pathology and classification of PDAC have revealed **two molecular biotypes**: ***'basal-like'*** and ***'classical'*** based on RNA transcriptional analyses [6]. The classical subtype of PDAC is characterized by a higher level of differentiation, fibrosis, and inflammation, whereas the basal-like subtype is associated with a poorer clinical outcome and loss of differentiation. Platinum-based therapy and poly (ADP-ribose) polymerase inhibitors may be effective for patients with pathogenic variants in *BRCA1/2* and *PALB2* [5].

Clinical Significance of Immune Tumor Microenvironment

PDAC cells exist in an impenetrable network, also known as the **tumor microenvironment** (**TME**), which comprises immune cells, cytokines, metabolites, fibroblast, and desmoplastic stroma rich in hyaluronan. Among solid tumors, **PDAC is an immunologically "cold" tumor**, characterized by sparse T-cell infiltrates. In contrast to immunologically "warm" tumors such as melanoma, with high neoantigen load and infiltrates. **The immunosuppressive TME helps PDAC cells evade host immune surveillance** promoting carcinogenesis. The TME of PDAC is characterized by **limited infiltration of CD8+ T** cells and an abundance of myeloid derived suppressor cells, tumor-associated macrophages, tumor associated neutrophils, and regulatory T cells. Additionally, the extracellular matrix,

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characterized by distinctive desmoplasia stemming from cancer-associated fibroblast, matrix metalloproteinases, and hyaluronan, may promote the immunosuppressive characteristic of the TME. These multifaceted compartments are viewed as responsible, in part, for the resistance to most single-agent therapeutic approaches [3].

Table 1. Main clinical manifestations of PDAC. Adapted from [3].

General facts	Approximately 60 000 diagnoses per year in the US
	Incidence of about 1% over lifetime
	The tenth to eleventh leading cause of cancer in the US
	Third leading cause of cancer-related mortality
	5-year survival (all-comers), 10%
	Median age at diagnosis, 71 years
	Male/female incidence ratio: 1.3/1.0
	50% of patients present with metastatic disease (AJCC stage IV)
	30% of patients present with locally advanced disease (AJCC stage III)
	20%of patients present with localized resectable disease (AJCC stage I and II)
	Most common causative germline alterations: *BRCA2, BRCA1, ATM,PALB2*
	Common sites of metastasis: liver, lymph node, lung, and peritoneum
	Rare sites of metastasis: skin, brain, and leptomeninges
Lifestyle Risk Factors	Tobacco
	Excess alcohol consumption (chronic pancreatitis)
	Obesity (body mass index >30), metabolic disorders, low levels of physical activity
	Diet: high fat, polyunsaturated fats, processed meats
Genetic Risk Factors[a]	Hereditary breast and ovary cancer syndrome (*BRCA1/2, PALB2*; 5%-9%)
	Ataxia-telangiectasia (*ATM*; approximately 3%-4%)
	Familial atypical multiple mole and melanoma syndrome (*CDKN2A, p16*; <1%)
	Lynch syndrome (*MLH1, MSH2, MSH6, PMS2, EPCAM*; <1%)
	Hereditary pancreatitis (*PRSS1, SPINK1*; <1%)
	Peutz-Jeghers syndrome (*STK11*; <1%)

[a]Percentages indicate the frequency per 100 unselected patients diagnosed with pancreas cancer.
Abbreviation: AJCC, American Joint Committee on Cancer.

Activated fibroblasts promote tumor cell growth, and inhibit effector T cells through several different mechanisms, including deposition of extracellular matrix to prevent T cell trafficking, cytokine excretion to suppress cell activity T and the induction of immunosuppressive myeloid cells directly with cytokines or indirectly through the recruitment of suppressor B cells. Some of the immune infiltrates, including suppressor B cells and myeloid cells, also produce growth factor ligands or cytokines to directly stimulate tumor growth. Metabolite changes in the tumor

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microenvironment also contribute to the inhibition of T cell activation [7]. Moreover, cancer cells express classical human leukocyte antigen class I antigens (HLA-I) being able to be recognized and eliminated by cytotoxic T cells. Reduction or loss of HLA-I antigens represent other mechanism of escape from antitumor immunity [8].

Novel Therapies for Pancreatic Cancer

A **subgroup of 10% to 15%** of individuals with PDAC have **DNA damage repair gene alterations other than *BRCA***. Novel combination strategies evaluating targeted agents and immune therapy combinations are undergoing testing for patients with PDAC associated with impaired DNA damage repair [5]. **Single-agent PD-1 blockade** has US Food and Drug Administration approval for mismatch repair deficiency in any tumor. **Mismatch repair deficiency occurs in approximately 1% of individuals with PDAC** and is defined by either germline or somatic alterations or loss in mismatch repair deficiency genes, such as *MLH1* and *MSH2* [9]. There are multiple drugs targeting the epithelial component, signaling pathways, metabolism, and the TME of PDAC. Single or combination immune checkpoint blockade inhibitors, such as durvalumab and tremelimumab, are ineffective for PDAC [10].

As a whole, **the characterization of the TME** in PDAC patients and the identification of biomarkers for diagnosis, prognosis and predictive response to treatments is key to guide patient management and therapeutic decisions.

With this background and in the context of ULISES project, in the FIVO is being carried out the identification, collection, processing and storing of sufficient biological material to demonstrate the proof of concept of the ULISES project. Additionally, FIVO will contribute to the characterization of PDAC samples by addressing the inflammation and immune contexts that support our understanding of the ULISES approach. Secondarily, these data might lead to the definition of a panel of potential biomarkers that might be clinically relevant in the management of PDAC patients.

The **purpose** of this Deliverable (D5.1) is to describe the **collection, processing, storing and analysis processes of human biological samples derived from PDAC patients**. These samples will be of great value to demonstrate and validate different tasks from other WPs, such as the delivery of nanoparticles including their different cargos. These nanoparticles will be tested in established and commercial cell lines, primary cell cultures and organoids from PDAC and finally in xenografted humanized mice. Cytotoxicity over non-cancer cells will also be tested.

4.2. FIVO Biobank

Biobanking constitutes the pillar around which most of the research activities of FIVO are sustained. Prospective collection of human samples (tissues, blood, urine) is actively performed according to all the ethical and legal standards and following the international guidelines for the use of biological resources for biomedical research [11].

FIVO Biobank belongs to different **cooperative initiatives** including:

- **Valencia Network of Biobanks** (RVB) **(**http://grupos.fisabio.san.gva.es/web/rvb/rvb;jsessionid=30BCC5B607102BDBD474016A896E9157**)**,

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- the **Spanish Platform of Biobanks** to promote biomedical research (RNBB) (http://www.redbiobancos.es/Default.aspx?i=10), and also
- the **OECI-TuBaFrost consortium** (http://www.tubafrost.org/).

Since 2013 the FIVO Biobank is accredited by the Valencia Government of Health and it is registered in the **National Registry of Biobanks** (from the Instituto de Salud Carlos III) with **ID B.0000773** (**https://biobancos.isciii.es/ListadoBiobancos.aspx?id=B.0000773**).

In May 2015, the **Quality Management System of FIVO Biobank was certified** with the **ISO 9001:2008** (Register ID: ES-0222/2015) for the activities of *reception*, *processing*, *storage,* and *supply* of human biological materials in the context of biobanking **(Annex 1).** Currently, and in line with our compromise with quality, we are adapting all our biobanking processes to obtain the accreditation following the rule **ISO 20387:2018 for Biotechnology – Biobanking – General requirements for biobanking**. We plan to have the accreditation audit during Q1 2022.

The workflow of the biobanking activities related with the Ulises proposal can be depicted in **Figure 1.** All these activities are distributed in **three well-defined clinical scenarios**: *medical treatment*; *clinical biobanking*; and *medical research*. Each of these scenarios are defined with specific procedures and that are discussed below.

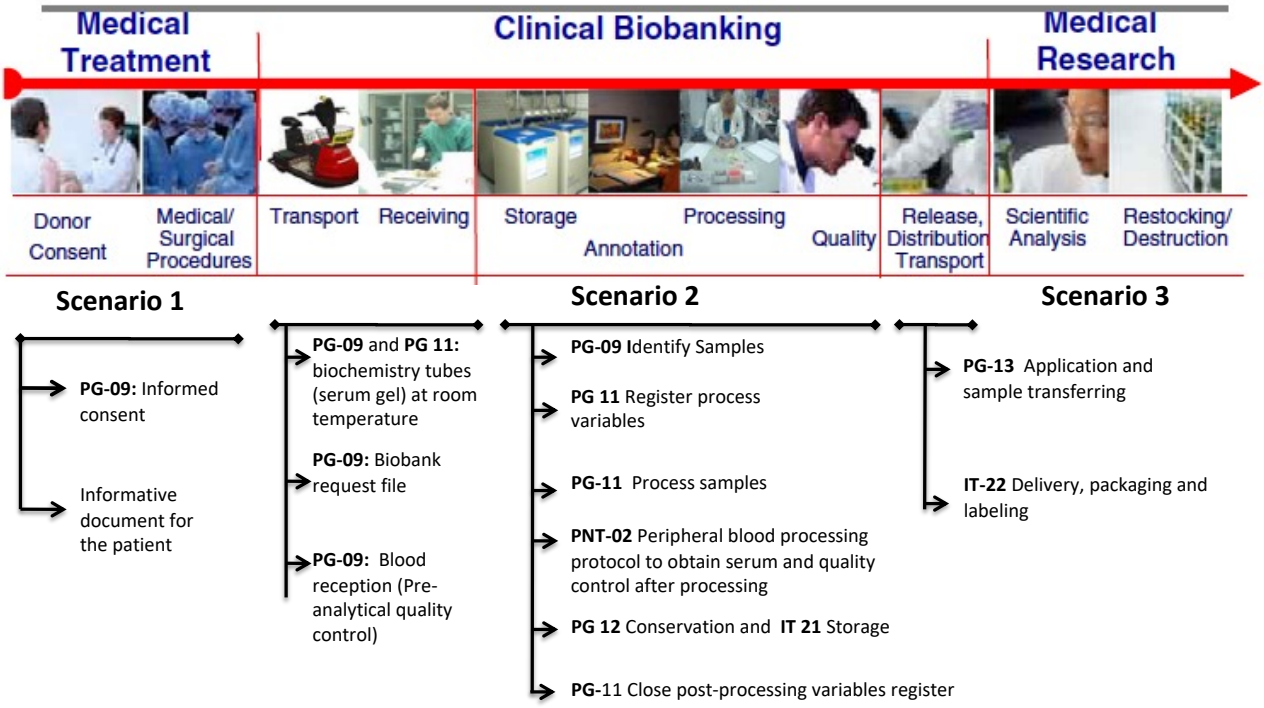

Figure 1. Workflow of the biobank activities at IVO. From the medical treatment scenario to the medical research. PG, refer to general processes of the biobank; IT, to technical instructions and PNT, to standard operating procedures.

4.2.1. Scenario 1: medical treatment

Before starting any sample collection within the context of a research project, the Institutional Ethical Committee must *approve* the proposal. In the case of the ULISES, the proposal was presented at the FIVO's Ethical Committee in September 2020 and get its approval in October 2020 (**Annex 2**).

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Once the research project has been authorized, the machinery for collecting biological samples can start. At this point, biobanking activities are defined by the **Specific Procedures (SP) 6 to 8** [**SP-006:** *Collection of peripheral blood*; **SP-007:** *Collection of tissues* (fresh frozen; fixed and paraffin embedded); and **SP-008**: *Primary cell cultures establishment*].

Patients potentially candidates to be included in the ULISES proposal are identified by clinicians and give their consent for collecting biological samples for the FIVO biobank. **Annexes 3** and **4** show the *Patient Information Sheet (PIS)* and the *Inform Consent Sheet* (ICS) for patients respectively.

Inclusion criteria of patients for the ULISES proposal:

- Adults only (> 18 yrs.).
- ECOG 0-1
- Operable disease or tumor accessible by echoendoscope biopsy
- Any type of pancreatic cancer histological subtype

All other patients that do not meet all these criteria will not be recruited for the ULISES proposal.

Once the individuals give their consent for collecting material a copy of the consent and samples of blood and tissue are sent to the Biobank with a specific *Biobank Sheet Request* (**Annex 5**).

4.2.2. Scenario 2: Clinical biobanking

A Lab Manual has been internally defined to coordinate clinicians, pathologists, laboratory staff and biobank technicians for an optimal collection of biospecimens. Briefly:

A. General instructions

Three types of samples are required:

- **Fresh tissue samples** from the patient referring to surgical specimens and/or diagnostic biopsies: pieces of approximately 10-20 mg are required for cell culture and generation of patient-derived xenografts (PDXs). The samples will be collected in DMEMF12 medium, which will be periodically supplied to the operating room. A part of the sample will be processed immediately after its collection for the generation of primary cell cultures. The pieces destined to the generation of PDXs will be preserved in the middle of freezing in NUNC freezing vials and stored at -80 until use. It is critically important that the specimens are provided as quickly as possible (no more than 2 hours after devascularization), in the collection medium (DMEMF12) on ice.
- **Formalin-fixed paraffin-embedded (FFPE) tissue** samples obtained at diagnosis, as well as from follow-up biopsies, if progression is produced or accessible. These samples will be necessary for histopathological review and genetic studies by next generation sequencing (NGS) and genomic profiling for immune components. The histopathological review is considered an added value to the diagnostic information of the original site. It is not intended, in any way, to be used as a substitute for the diagnostic report prepared by the local pathologist. It should be seen as a complementary pathological diagnosis of the patients included in the study. These studies will be critical to verify the quality of

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the material from which further studies will be carried out. **Figure 2** depicts the use of the tissues after the surgical intervention.

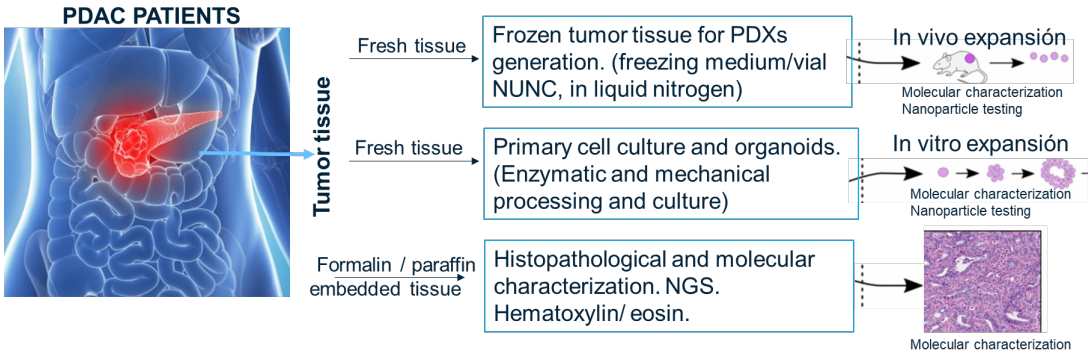

Figure 2. Work flow for human PDAC tissue samples. PDXs and primary cell cultures represent excellent preclinical platforms for drug testing (ULISES nanoparticles) and molecular characterization.

- **Peripheral blood samples.** Collection of the sample at the time of diagnosis, follow-up, progression and / or resection of the tumor. These samples will be used for liquid biopsy analysis (**Figure 3**):
  - **Two 7 ml EDTA tubes**, for carrying out a hemogram and genotyping for HLA.
  - **Two STRECK CELL-FREE DNA BCT®**-10 ml to isolate for cfDNA.
  - A **serum** tube for biochemical and cytokine analysis.

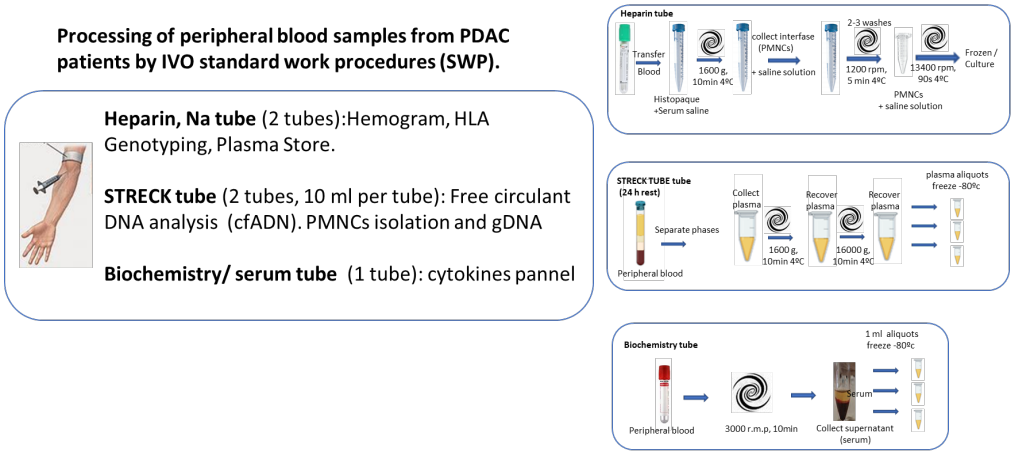

Figure 3. Work flow for human PDAC blood samples. These biospecimens will be used for HLA genotyping; peripheral blood mononucleated cells and cfDNA isolation; cytokine and other serum biomarker analysis.

The *Biobank Sheet Request* (**Annex 5**) will be used to document the details of the samples taken and confirm adequate communication with the laboratory. Each biobank sheet request must be completed at the time of sample collection and processing with the appropriate details. The original sheet will be kept for being sent along with the sample. In the case of FFPE tissue samples, the biobank sheet request is accompanied by a copy of an anonymous report from the local Pathologist (pathology report).

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B. Detailed description of the protocols

- **Fresh frozen tissue destined to PDX** (Fig.2):
  1. Select an area of the tumor without necrosis (it will ensure the success of the engraftment).
  2. Section the chosen tumor area with a scalpel. Up to 5 fragments of 10-20 ± 2 mg will be frozen. 1ml of **freezing medium** (DMEM-F12 culture medium with 10% DMSO and 40% FBS) is added to each freezing vial (NUNC® tubes).
  3. Subsequently, when reimplanting tissue fragments, thaw the vial and wash twice with saline to eliminate DMSO remnants.
  4. During the entire reimplantation process it is important that the fragments are immersed in saline solution.

- **Fresh tissue: Primary cell culture** (Fig.2)
  Two different strategies will be optimized:

  A) Mechanical strategy
  1. Process tumor material directly after surgery (within 2 hours of collection).
  2. Wash the tissue of blood debris with a sterile PBS buffer or DMEM F12 medium, gently.
  3. Cut the collected samples with a scalpel into Petri dishes and resuspend in the appropriate culture medium.
  4. Immediately transfer part of the suspended material into 75 cm2 culture flasks. Replace the medium daily for the first 3 days, taking care not to discard the non-adhered fragments.
  5. Replace completely the medium twice a week. The mean time to obtain confluence in both the Petri dish and the culture flask is approximately 14 days.

  B) Enzymatic strategy
  1. Process tumor material directly after surgery (within 2 hours of collection).
  2. Wash the tissue of blood debris with a sterile PBS buffer or DMEM F12 medium, gently.
  3. Cut the collected samples with a scalpel into Petri dishes. Proceed with enzymatic digestion with a solution including: 10 mg Collagenase II (ThermoFisher); 40 mg Dispase II (ThermoFisher) *10.5 μ M Y-27632 **; add to 8 mL of medium for digestion.

     * Dispase will produce a single cell suspension much smoother and more effective than trypsin, collagen or other proteolytic enzymes; it will not harm cells collected for subculture or bioassay. Recommended for recovering cells grown on basement membrane for matrix BD Matrigel ™
     ** 10.5 μ M Y-27632 in complete medium at 37°C with gentle shaking up to 1 hour. * (maintenance and self-renewal). It improves the survival of human embryonic stem cells (ES) when they dissociate into individual cells by preventing dissociation-induced apoptosis (anoikis), thus increasing their cloning efficiency [12]; improves the formation of embryoid bodies by means of forced aggregation protocols [13]; increases the survival of cryopreserved individual human embryonic stem cells after thawing [14].
  4. Disaggregate the tissue, seed in primary culture medium (DMEM / F12 with 6% FBS and supplements) Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 (Ham), (ThermoFisher). For 500 ml of medium: 30 mL FBS (ThermoFisher); 5.5 ml Penicillin/Streptomycin (ThermoFisher); 500 μ l EGF Human recombinant (100 μ g / 1 ml dH20) (ThermoFisher); 2 ml Bovine Pituitary Extract (ThermoFisher); 20 ml Hydrocortisone (1 mg dissolved in 1 ml ETOH plus 19 ml dH2O) (Sigma); 70ul

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Insulin Human Recombinant (ThermoFisher); Glutamax 1x (ThermoFisher) (alternative to L-glutamine improves health cells).

- **Tissue blocks** (Fig.2)
  Formalin fixed and paraffin-embedded (FFPE) tissues obtained from diagnostic, follow-up and specimen biopsies from tumor resection when possible. These samples will be necessary for the histopathological review and the genetic study by NGS (genetic and genomic profiling).
  A representative FFPE block from each tumor will be devoted for the ULISES project. The sample will preferably come from a primary tumor with more than 50% tumor content to avoid as far as possible samples with a large necrotic extension. FFPE will be accompanied by an H&E-stained section indicating the area of tumor and the percentage of tumor cell content.
  The following documentation will be also required: a copy of the local pathologist's report; and the corresponding *Biobank Sheet Request* (**Annex 5**).
  Patients are identified only with the protocol number, its inclusion code, and the number of the original FFPE block (number of biopsy).

- **Peripheral blood** (Fig. 3)**:**
  - **10 mL STRECK tubes:** to obtain cfDNA for liquid biopsy studies.
  - **EDTA tubes (2).** Whole blood is collected in EDTA (anticoagulant) tubes which better preserves the morphology of blood cells. One of the tubes will be destined to hemogram and HLA genotyping.
  - **Biochemistry/serum tube,** dry tube without anticoagulant. Leave at room temperature in an upright position until the clot coagulates, and retraction of the clot begins (30-40 minutes after obtaining the sample). Centrifuge at 2500-3000 rpm (5-10 min) to separate the serum from the clot. This separation is carried out within 2 hours after taking the sample to avoid deterioration of the sample due to contact between the cells.
  - A series of quality parameters will be considered to accept the sample before processing. These parameters include: intensity of the color; time from blood extraction to processing; integrity and tightness of the extraction tube; identification of the patient/donor; identification of the sample.

  For blood sample collection the following material is required:

  - STRECK collection tubes (10 ml) for venipuncture (it allows the isolation of high quality free circulating DNA for a wide range of applications, and allows for proper sampling, transport and storage; cfDNA and gDNA remain stable for up to 14 days from 6^{O} C to 37^{O} C).
  - EDTA collector tubes.
  - Biochemistry collecting tubes.
  - Tubes containing gels or separators will not be used.

  - **Blood sample processing:**

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1. Preferably, blood should be obtained at the **time of diagnosis**, at **follow-up**, and at **tumor progression and/or resection**. It is recommended that for a possible proteomic study of blood serum, the time elapsed between blood extraction and freezing at -80^{O}C should not exceed 45 min. Hence, samples are processed directly following venipuncture.
2. The person responsible for carrying out this procedure, with knowledge of the surgical intervention scheduling, will coordinate with the qualified personnel for blood extraction, ensuring that the blood collection tubes are properly identified and guaranteeing correct collection of the sample.
3. Serum or plasma is separated as soon as possible, the maximum limit being two hours. Any deviation to the standard operating procedure is properly reflected in the Quality Management System of the biobank as an incidence.
4. *Verification and identification*: verification of patient's information, always maintaining the privacy and ethics framed in the Data Protection Law, is of mandatory compliance for ensuring the correct relationship between the blood collection tubes (duly labeled) with the patient's information.

- **STRECK Tube Processing**
    1. Obtain two 10 ml-STRECK BCT tubes of venous blood at the previously specified times.
    2. Gently invert the STRECK BCT tubes 5-6 times to ensure proper mixing. Record the time of collection of the sample in the *Biobank Sheet Request*.
    3. Write the protocol number and the ID number of the patient in the study on the tube.
    4. Store samples at room temperature until processed.
    5. Within 24-48 hours after the last extraction, process the samples as indicated below:
        - Centrifuge the STRECK tubes at 1600xg, 10 min at 4^{O}C.
        - Collect the supernatant in a new tube, taking care not to drag the deposited pellet.
        - Centrifuge the new tubes at 16000xg, 10 min at 4^{O}C.
        - Collect the supernatant in tubes suitable for freezing at -80^{O}C.
        - Freeze at -80^{O}C until it is sent to the central laboratory and keep it at that temperature upon arrival until its use. Please avoid shipments on Fridays.
    6. Any deviation from these procedures should be recorded in the *Biobank Sheet Request*.

- **EDTA Tube Processing**
    1. Obtain two EDTA tubes of venous blood at the previously specified times.
    2. Gently invert tubes 5-10 times to ensure proper mixing. Record the time of collection of the sample in the *Biobank Sheet Request*.
    3. Write the protocol number and the ID number of the patient in the study on the tube.
    4. Store samples at room temperature until processed. If the blood count is not performed in 2-3 hours, the blood should be refrigerated at 4 ^{O} C

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(refrigerator). The red blood cell count, hemoglobin, and hematocrit remain unchanged if the blood is refrigerated for about 24 hours.

- **Biochemistry Tube Processing (serum)**
  1. Draw blood by collecting it in the identified biochemistry blood collection tube (without anticoagulant). These tubes contain particles that act as clotting activators.
  2. Immediately after extraction, gently invert the tube to promote coagulation.
  3. Transport it to the laboratory for processing in a time not exceeding 45 min. after extraction, maintaining the safety guidelines for the transport of biological material established by the center.
  4. Serum Centrifuge the blood tubes (without anticoagulant) at 1500g for 15 minutes. The upper fraction or supernatant after centrifugation with a clear and transparent appearance, and a yellowish color, corresponds to blood serum.
  5. During the centrifugation time, prepare 4-6 cryo-vials for the storage of the serum, duly labeled, and identified.
  6. After centrifugation, carefully aspirate the supernatant (upper phase) with the help of a sterile Pasteur pipette, if possible, completely in a single aspiration, and aliquot 0.5ml of this aspirate (corresponding to blood serum) in each one of the cryovials properly labeled and identified.
  7. Then store the cryo-vials in cryo-storage boxes and store them in a -80^{O}C freezer.
  8. Register the location of the sample saved in the Biobank Management Information System.

All collected samples are introduced in the Information Management System (IMS) that FIVO Biobank shares with the Valencia Network of Biobanks (RVB). This IMS is constituted by three different modules (Figure 5.2): module for management of donors; module of laboratory; and a module for management of requests. Each module is independent and the profile for accessing is untransferable.

4.2.3. Scenario 3: Medical research

Once the samples are stored and registered they are ready to be used by researchers (from the same institution or from external researchers). The processes of access and deliver of the samples are defined in the **GP-13** and **TI-22**.

GP-13: Access rules and assignment for the use of biological samples for research

This procedure defines the process to request samples to the biobank, and how the biobank evaluates the request and provides the samples to the requestors. Briefly, researchers may access to the biological material of the biobank by applying through an application form. This application form, together the information regarding the research to be carried out with the samples, will be evaluated by two external committees (Scientific and Ethic). If the evaluation is favorable

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then the samples are shipped to the requestor with a Material Transfer Agreement that will be signed by both parts (biobank and researcher).

TI-22: Technical instruction for packaging, labeling and transport of the biological samples for research

This IT establishes the rules for packaging and labeling of the containers in which the samples are placed for the shipment and deliver to the requestors. This document also describes the conditions in which each type of sample must be transported.

4.3. Recruited PDAC patients for the ULISES proposal

Up to September the 30th 2021, a total of 19 PDAC patients have been recruited for the ULISES project (**Table 2**). As established in our biobanking standard operating procedures, biological specimens including tissue, mononucleated peripheral blood cells, serum, and plasma have been collected. In patients #6, #10, #13 the surgery was no possible and only biopsies guided by echoendoscope were obtained (**Figure 4**). Six PDAC primary culture have been successfully stablished and other 4 are ongoing. Clinical data were obtained from the electronic medical record.

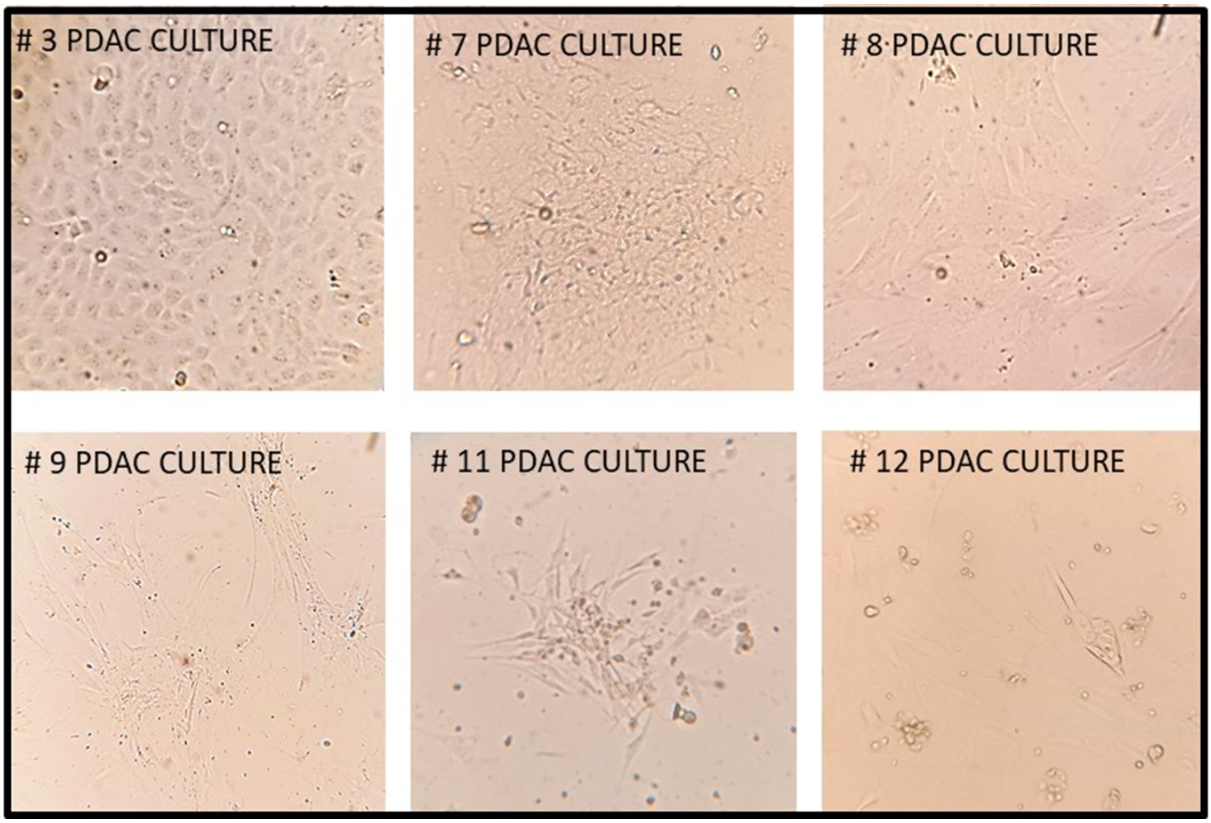

Figure 4. Representative images of PDAC primary cell cultures. These biospecimens will be used for HLA genotyping; peripheral blood mononucleated cells and cfDNA isolation; cytokine and other serum biomarker analysis.

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Table 2. Relationship of patients recruited for the ULISES project up to September the 30th 2021.

Collection data	Patient/intervention type/ Sex	Tissue	Primary culture	Success	Sent to PDX	Surgical Intervention	Hystological Type	Pathological stage
21/01/2021	#1 biopsy M	✓	-	?	✓	Endos-high digestive echoendoscopy	Malignant neoplasm of pancreas, unspecified (C2)	TNM 8th edition (pT2N2)
10/02/2021	#2 surgery M	✓	✓	-	✓	cephalic duodenopancreatectomy	Infiltrating NOS pancreatic ductal adenocarcinoma	TNM 8va edición (pT2N0)
17/02/2021	#3 surgery M	✓	✓	✓	✓	cephalic duodenopancreatectomy	Biliary-type adenocarcinoma (extrahepatic cholangiocarcinoma)	TNM 8va edición (pT2N0)
01/03/2021	#4 surgery F	✓	✓	-	Outstanding	Duodenum pancreatectomy	Infiltrating NOS pancreatic ductal adenocarcinoma	
03/03/2021	#5 surgery M	✓	✓	-	Outstanding	?	?	?
10/03/2021	#6 no surgery M	-	-	-	-	Biopsy	Ductal proliferation with atypia where adenocarcinoma cannot be ruled out	Bile duct hyperplasia with mild focal atypia
15/03 2021	#7 biopsy M	✓	✓	✓	Outstanding		Indetermine if the tumor is primary or metastatic	Primary pancreatic adenosquamous and squamous. Immunotherapy and lung carcinoma
17/03/2021	#8 surgery F	✓	✓	✓	Outstanding	Corporocaudal pancreatectomy and splenectomy	Infiltrating NOS pancreatic ductal adenocarcinoma	TNM 8va edición (pT2N1)
31/30/2021	#9 surgery M	✓	✓	✓	Outstanding	Biopsy	Ductal Adenocarcinoma with gastric infiltration	?
07/04/2021	#10 no surgery F	-	-	?	-	No surgeon	Malignant neoplasm of pancreas, unspecified (C2)	?
14/06/2021	#11 surgery ?	✓	✓	✓	Outstanding	Surgeon		?
18/06/2021	#12 surgery ?	✓	✓	✓	Outstanding	Surgeon		?
01/07/2021	#13 no surgery M	-	-	-	-	No surgeon		
30/07/2021	#14 biopsy F	✓	✓	On going	Outstanding	Biopsy	Adenocarcinoma of head of pancreas	TNM 4
16/08/2021	#15 biopsy M	✓	✓	On going	Outstanding	Biopsy	Neuroendocrine	?
20/09/2021	#16 biopsy M	✓	✓	On going	Outstanding	Biopsy		?
23/09/21	#17 surgery M	✓	✓	On going	Outstanding	Surgeon		?
27/09/21	#18 biopsy M	✓	✓	On going	Outstanding	Biopsy	?	?
27/09/21	#19 biopsy M	✓	✓	On going	Outstanding	Biopsy	?	?

PDAC Culture Characterization by Flow Cytometry

A flow cytometry approach was optimized for characterizing PDAC cells. For this propose the following biomarkers were considered:

- **Folate receptor (FR)**, as one of the targets of the ULISES project. It is overexpressed in a wide range of tumors [15].

- **The transcription factor PDX 1** (pancreatic and duodenal homeobox 1), also known as insulin promoter factor 1, is a transcription factor in the ParaHox gene cluster. In vertebrates, Pdx1 is necessary for pancreatic development, including β -cell maturation, and duodenal differentiation [16].

- **Cytokeratin 19 (CK19)**, as proof of concept of epithelial digestive/pancreatic source and tumor feature. Belongs to the family of keratins, the vast majority of adenocarcinomas in pancreas are CK19 positive [17]. It is associated with poor outcome [18].

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°899708.

- **CD47**. CD47 is an immunoglobulin that is overexpressed on the surface of many types of cancer cells. CD47 forms a signaling complex with signal-regulatory protein α (SIRP α), enabling the escape of these cancer cells from macrophage-mediated phagocytosis. In recent years, CD47 has been shown to be highly expressed by various types of solid tumors, including PDAC, and to be associated with poor patient prognosis in various types of cancer. A growing number of studies have since demonstrated that inhibiting the CD47-SIRP α signaling pathway promotes the adaptive immune response and enhances the phagocytosis of tumor cells by macrophages [19]. This biomarker is still being optimized for its use un flow cytometry.

- **CD44 and CD24**. As cancer stem cell markers [20]. CD44 specific receptor for hyaluronic acid, promoting migration in normal cells and highly expressed in almost every cancer cell in its standard or variant form [21]. CD24 is a small cell surface protein molecule anchored by glycosyl-phosphotidyl-inositol in a wide variety of cancer cells. It is heavily glycosylated and functions in cell-cell and cell-matrix interactions [22]. The presence and proportion of this population in PDAC cultures could be important connotations for tumor behaviour, prognosis and treatment response, and could be important implications in the doses and uptake of nanoparticle carrier designed in the context of this project [20, 23].

Representative examples of flow cytometry results are shown in **Figures 5**-**8**.

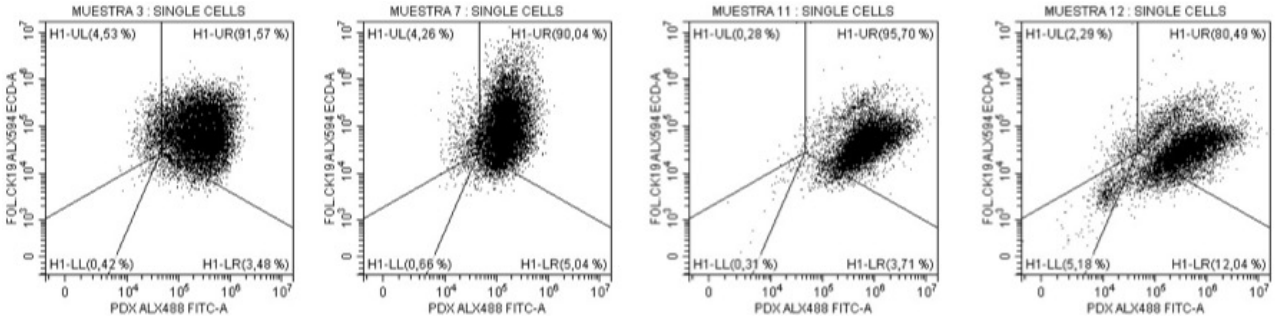

Figure 5. Analysis of double staining for PDX and RF in PDAC samples (#3, #7, #11, #12). Approximately between 80-95% of the cells derived from cultures are PDX+RF+.

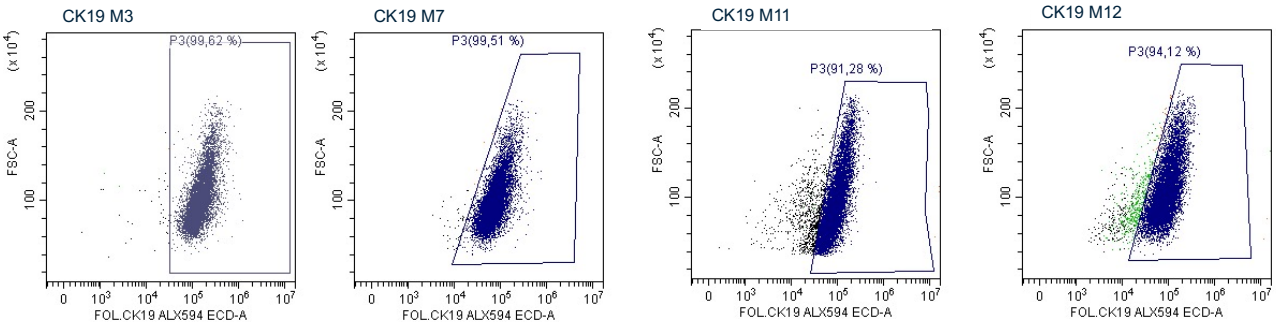

Figure 6. Analysis of CK19 in PDAC samples (#3, #7, #11, #12). >90% of cells from the primary PDAC cell cultures express CK19.

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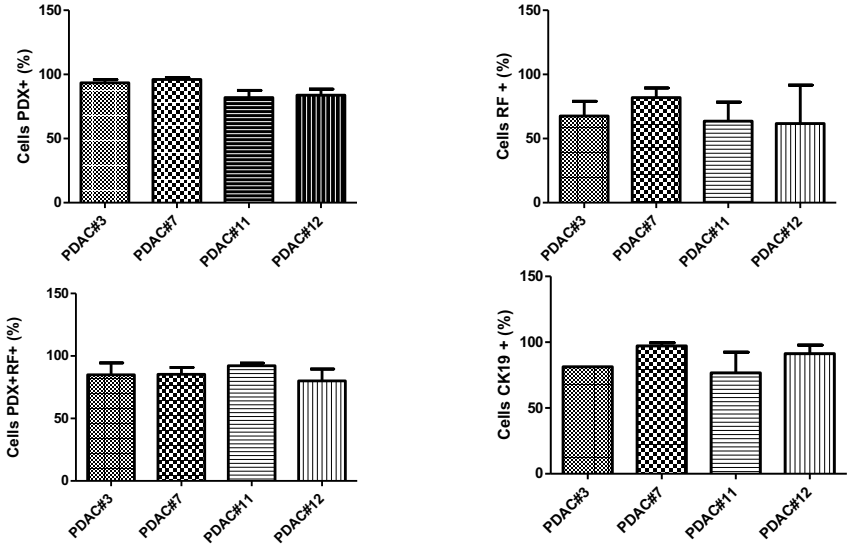

Figure 7. Histograms reflecting the different expresssion levels of PDX, FR and CK19 in PDAC (#3, #7, #11, #12). Most of the cells in cultures are positives for PDX, RF, CK19.

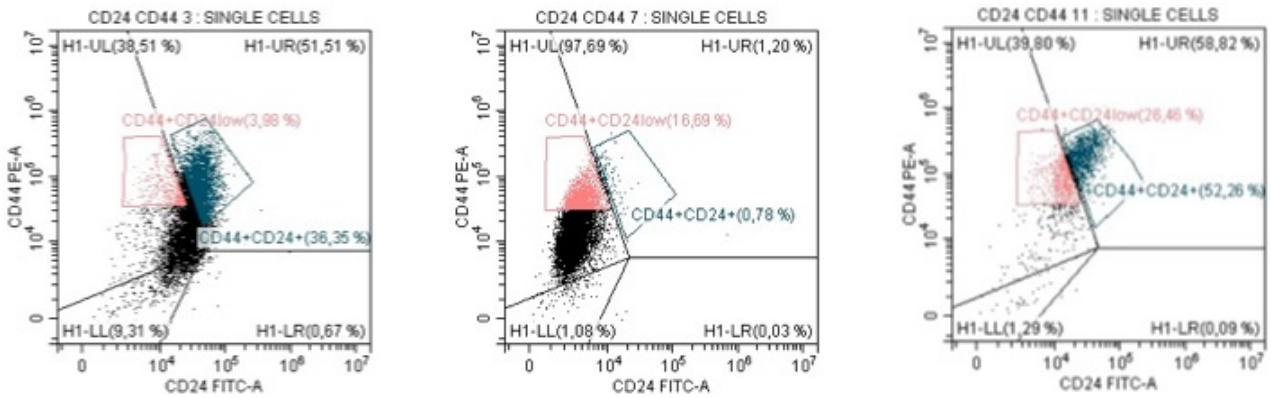

Figure 8. CD44 and CD24 as Cancer Stem Cells (CSCs) Markers in PDAC cultures (#3, #7 and #11). Proportion of CSCs (CD44+/CD24+) in our primary cultures is variable being 36.35% in #3; 0.78% in #7; and 52,26% in #11.

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5. Conclusions

- The collection of biological samples has been established according the plan scheduled and it is plenty operative.

- All the processes destined for the collection of bioresources for the ULISES project are being carried out in the context of the biobanking activities and are certified with the ISO 9001:2015.

- The Etical Committee approval was reached in October, 2020.

- Primary cultures from PDAC patients are being produced with a success rate of around 60% (10 primary cultures from 16 PDAC tissue samples).

- Characterization of these tumor cells have been optimized for flow cytometry.

- This collection of bioresources will constitute a valuable basis for the in vitro and in vivo validation of the ULISES approach.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°899708.

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7. Annexes

7.1. Annex 1: ISO 9001:2008 Certificate of the FIVO Biobank

IQNet

THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE

IQNet and
AENOR
hereby certify that the organization

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BIOBANCO.
CL PROFESOR BELTRAN BAGUENA, 8
46009 - VALENCIA

for the following field of activities

Reception, processing, storage and supply of human biological materials in the context of biobanking.

has implemented and maintains a

Quality Management System

which fulfills the requirements of the following standard

ISO 9001:2008

First issued on: 2015-04-15 Validity date: 2018-04-15

Registration Number: **ES-0222/2015**

AENOR Asociación Española de Normalización y Certificación

IQNet

AENOR

Michael Drechsel
President of IQNet

Avelino BRITO
Chief Executive Officer

IQNet Partners*:
AENOR *Spain* AFNOR Certification *France* AIB-Vincotte International *Belgium* ANCE *Mexico* APCER *Portugal* CCC *Cyprus*
CISQ *Italy* CQC *China* CQM *China* CQS *Czech Republic* Cro Cert *Croatia* DQS Holding GmbH *Germany*
FCAV *Brazil* FONDONORMA *Venezuela* ICONTEC *Colombia* IMNC *Mexico* Inspecta Certification *Finland* IRAM *Argentina*
JQA *Japan* KFQ *Korea* MIRTEC *Greece* MSZT *Hungary* Nemko AS *Norway* NSAI *Ireland* PCBC *Poland*
Quality Austria *Austria* RR *Russia* SII *Israel* SIQ *Slovenia* SIRIM QAS International *Malaysia*
SQS *Switzerland* SRAC *Romania* TEST St Petersburg *Russia* TSE *Turkey* YUQS *Serbia*
IQNet is represented in the USA by: AFNOR Certification, CISQ, DQS Holding GmbH and NSAI Inc.
* The list of IQNet partners is valid at the time of issue of this certificate. Updated information is available under www.iqnet-certification.com

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7.2. Annex 2: Approval of the ULISES proposal by the FIVO's Ethical Committee

Comité Ético de Investigación de la Fundación Instituto Valenciano de Oncología (CEI-FIVO)

INFORME DEL COMITÉ ETICO DE INVESTIGACION DE LA FUNDACION INSTITUTO VALENCIANO DE ONCOLOGIA

D. Carlos J. Andrés Blasco, Secretario del Comité Ético de Investigación de la Fundación Instituto Valenciano de Oncología (CEI-FIVO),

CERTIFICA

Ha tenido entrada en la secretaría del **Comité Ético de Investigación de la Fundación Instituto Valenciano de Oncología (CEI-FIVO)**, las respuestas al dictamen favorable condicionado del Proyecto de Investigación relacionado a continuación:

- Título: **"Immunological incompatibility as a basis for cancer curing and vaccination".**
- Nº Expediente CEIm: **2020-10**
- Código: **ULISES**
- Promotor: **Cristina Fillat, Instituto de Investigaciones Biomédicas Agust Pi i Sunyer (IDIBAPS)**
- Investigador Principal FIVO: **Jose Antonio Lopez Guerrero**
- Investigadores Colaboradores FIVO: **Antonio Fernandez Serra, Marta Ramirez Calvo, Ana Calatrava Fons, Isidro Machado Puerto, Rafael Estevan Estevan, Ricardo Yaya Tur y Marcos Melian Sosa.**
- Centros participantes incluidos en la solicitud: **Fundación Instituto Valenciano de Oncología (FIVO).**

Tras ser valorada y examinada la documentación, tomando en consideración las siguientes cuestiones:

- La pertinencia del estudio teniendo en cuenta el conocimiento disponible, así como los requisitos legales aplicables, y en particular la Ley 14/2007 de Investigación Biomédica, el Real Decreto 1716/2011, de 18 de noviembre, por el que se establecen los requisitos básicos de autorización y funcionamiento de los biobancos con fines de investigación biomédica y del tratamiento de las muestras biológicas de origen humano y se regula el funcionamiento y organización del Registro Nacional de Biobancos para investigación biomédica, Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica y Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales.
- Los principios éticos de la Declaración de Helsinki (Octubre 2013 Fortaleza, Brasil)

DICTAMEN PROYECTO DE INVESTIGACION, Página 1

Gregorio Gea, 31 CP 46009 – VALENCIA

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°899708.

Comité Ético de Investigación de la Fundación Instituto Valenciano de Oncología (CEI-FIVO)

- La idoneidad del protocolo en relación con los objetivos del estudio, justificación de los riesgos y molestias previsibles para el sujeto, así como los beneficios esperados.

CONSIDERA QUE

El Comité Ético de Investigación de la Fundación Instituto Valenciano de Oncología (CEI-FIVO), en su reunión del **09/09/2020 (acta nº 08/20)** decidió emitir dictamen favorable condicionado, y tras subsanación por parte del promotor de la condición establecida procede a emitir un **DICTAMEN FAVORABLE** con relación:

- Investigador Principal FIVO: **Jose Antonio Lopez Guerrero**
- Investigadores Colaboradores FIVO: **Antonio Fernandez Serra, Marta Ramirez Calvo, Ana Calatrava Fons, Isidro Machado Puerto, Rafael Estevan Estevan, Ricardo Yaya Tur y Marcos Melian Sosa.**
- Centros participantes incluidos en la solicitud: **Fundación Instituto Valenciano de Oncología (FIVO).**

DOCUMENTO	VERSIÓN Y FECHA (DD/MM/AAAA)
PROTOCOLO	FORMULARIO CONVOCATORIA HORIZON 2020
RESUMEN PROTOCOLO	V 1.3 DE 19/10/2020
HIP/CI	V 1.2 DE 25/09/2020

Y HACE CONSTAR QUE:

- El CEI FIVO cumple los requisitos legales vigentes (Ley 14/2007 de Investigación Biomédica).
- El CEI FIVO tanto en su composición como en sus Procedimientos Normalizados de Trabajo (PNTs), cumple las Normas de Buena Práctica Clínica (CPMP/ICH/135/95).
- Durante la evaluación de este estudio, existe el quórum suficiente para tomar decisiones de acuerdo a nuestros Procedimientos Normalizados de Trabajo (PNTs).
- A la fecha de aprobación del estudio, la composición del CEI FIVO es la que consta en el Anexo I

Lo que firmo en Valencia, a 27 de octubre de 2020.

Secretaria CEIm Fundación IVO

Fdo. Carlos J. Andrés Blasco

Gregorio Gea, 31 CP 46009 – VALENCIA

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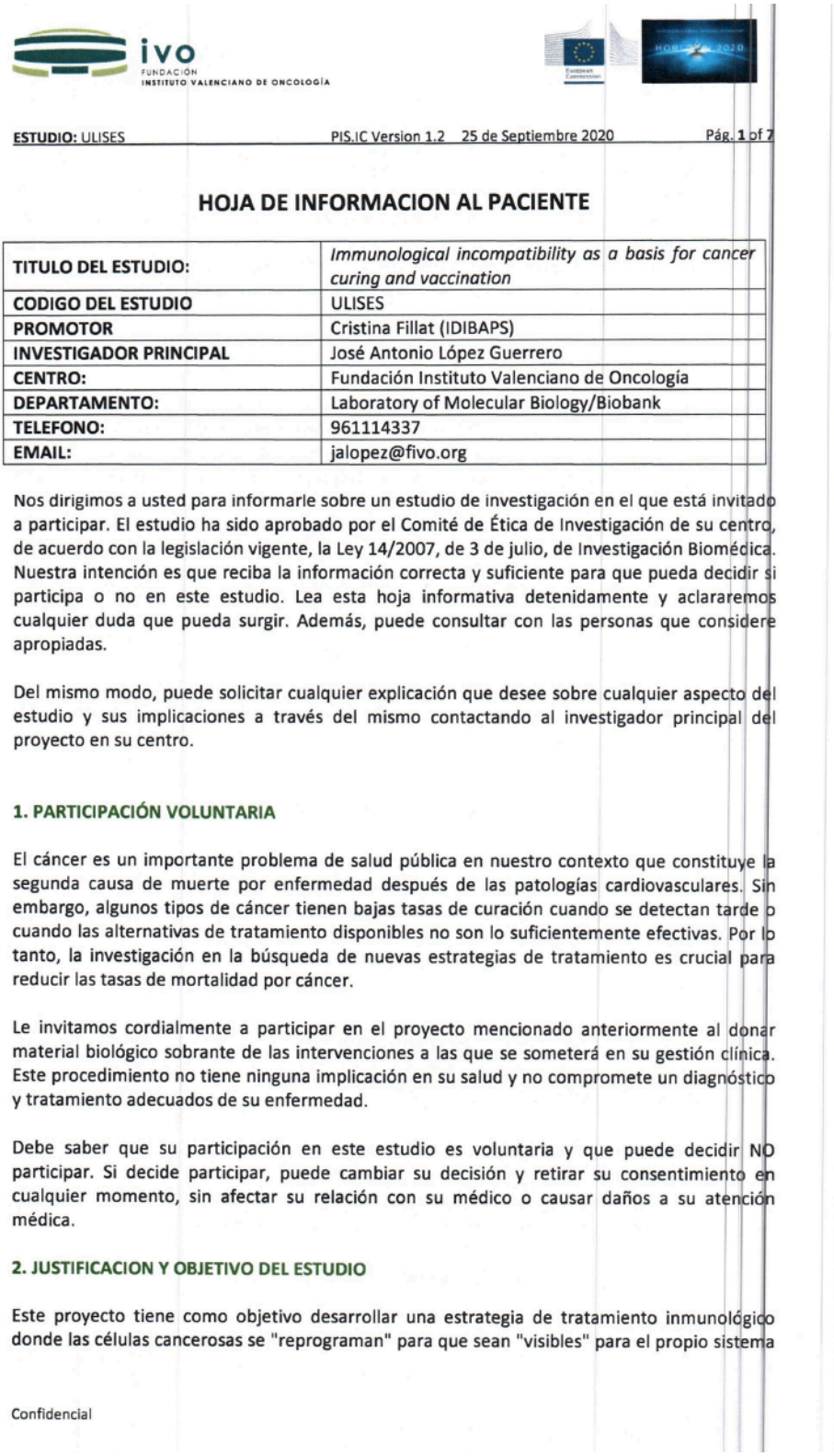
7.3. Annex 3: ULISES Patient Information Sheet



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ESTUDIO: ULISES PIS.IC Version 1.2 25 de Septiembre 2020 Pág. 1 of 7

HOJA DE INFORMACION AL PACIENTE

TITULO DEL ESTUDIO:	*Immunological incompatibility as a basis for cancer curing and vaccination*
CODIGO DEL ESTUDIO	ULISES
PROMOTOR	Cristina Fillat (IDIBAPS)
INVESTIGADOR PRINCIPAL	José Antonio López Guerrero
CENTRO:	Fundación Instituto Valenciano de Oncología
DEPARTAMENTO:	Laboratory of Molecular Biology/Biobank
TELEFONO:	961114337
EMAIL:	jalopez@fivo.org

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que está invitado a participar. El estudio ha sido aprobado por el Comité de Ética de Investigación de su centro, de acuerdo con la legislación vigente, la Ley 14/2007, de 3 de julio, de Investigación Biomédica. Nuestra intención es que reciba la información correcta y suficiente para que pueda decidir si participa o no en este estudio. Lea esta hoja informativa detenidamente y aclararemos cualquier duda que pueda surgir. Además, puede consultar con las personas que considere apropiadas.

Del mismo modo, puede solicitar cualquier explicación que desee sobre cualquier aspecto del estudio y sus implicaciones a través del mismo contactando al investigador principal del proyecto en su centro.

1. PARTICIPACIÓN VOLUNTARIA

El cáncer es un importante problema de salud pública en nuestro contexto que constituye la segunda causa de muerte por enfermedad después de las patologías cardiovasculares. Sin embargo, algunos tipos de cáncer tienen bajas tasas de curación cuando se detectan tarde o cuando las alternativas de tratamiento disponibles no son lo suficientemente efectivas. Por lo tanto, la investigación en la búsqueda de nuevas estrategias de tratamiento es crucial para reducir las tasas de mortalidad por cáncer.

Le invitamos cordialmente a participar en el proyecto mencionado anteriormente al donar material biológico sobrante de las intervenciones a las que se someterá en su gestión clínica. Este procedimiento no tiene ninguna implicación en su salud y no compromete un diagnóstico y tratamiento adecuados de su enfermedad.

Debe saber que su participación en este estudio es voluntaria y que puede decidir NO participar. Si decide participar, puede cambiar su decisión y retirar su consentimiento en cualquier momento, sin afectar su relación con su médico o causar daños a su atención médica.

2. JUSTIFICACION Y OBJETIVO DEL ESTUDIO

Este proyecto tiene como objetivo desarrollar una estrategia de tratamiento inmunológico donde las células cancerosas se "reprograman" para que sean "visibles" para el propio sistema

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inmunitario del paciente, que las verá como "no pertenecientes al cuerpo" y las atacará. Esta estrategia constituirá un tratamiento "natural", ya que el propio sistema inmunitario del paciente se utilizará para atacar las células cancerosas reduciendo significativamente el tiempo de tratamiento a pocas semanas y produciendo efectos secundarios mínimos o casi nulos. Además, esta "reprogramación" conducirá a una "memoria inmunológica" que evitará futuras recaídas (efecto similar a la vacuna) a través de los linfocitos generados alrededor del microambiente tumoral por el sistema inmune.

3. DESCRIPCIÓN DEL ESTUDIO.

Como el objetivo es reprogramar las células tumorales por ser incompatibles con el sistema inmune del huésped, sus muestras se utilizarán para diferentes propuestas. Se analizarán muestras de sangre para conocer su perfil inmunológico innato. Además, serán necesarias muestras de tejido para definir el inmunofenotipo del tumor y para aislar las células tumorales para generar cultivos primarios y organoides para validar la estrategia terapéutica ULISES. Cuando sea posible, estas células también se injertarán en ratones para validar la eficacia del tratamiento en condiciones in vivo.

Se espera que entre 50 y 60 pacientes sean reclutados para la recolección de muestras.

4. ACTIVIDADES DEL ESTUDIO

La duración de la propuesta ULISES es de 3 años y su participación no modificará las intervenciones habituales y el manejo clínico de su enfermedad porque solo se recolectará material biológico sobrante.

5. RIESGOS Y ENFERMEDADES DERIVADAS DE SU PARTICIPACIÓN EN EL ESTUDIO

Dado que la donación implica muestras sobrantes (tejido y sangre) de las intervenciones que ha sufrido, el procedimiento propuesto no supone ningún riesgo adicional para su salud ni compromete un diagnóstico y tratamiento adecuados de su enfermedad.

6. BENEFICIOS

Se le solicita que done material sobrante de muestras de tejido y sangre recolectadas en el contexto de su proceso de diagnóstico o terapéutico, sin perjuicio de que se le atienda adecuadamente.

El/La paciente no recibirá ninguna compensación económica ni otros beneficios materiales directos por donar sus muestras en este estudio. Sin embargo, si la investigación realizada fuera exitosa, contribuiría a mejorar el manejo clínico de los pacientes que comparten la misma enfermedad o la relacionada con la suya.

La donación de sus muestras le permitirá a usted o su familia su uso asistencial, cuando se solicite.

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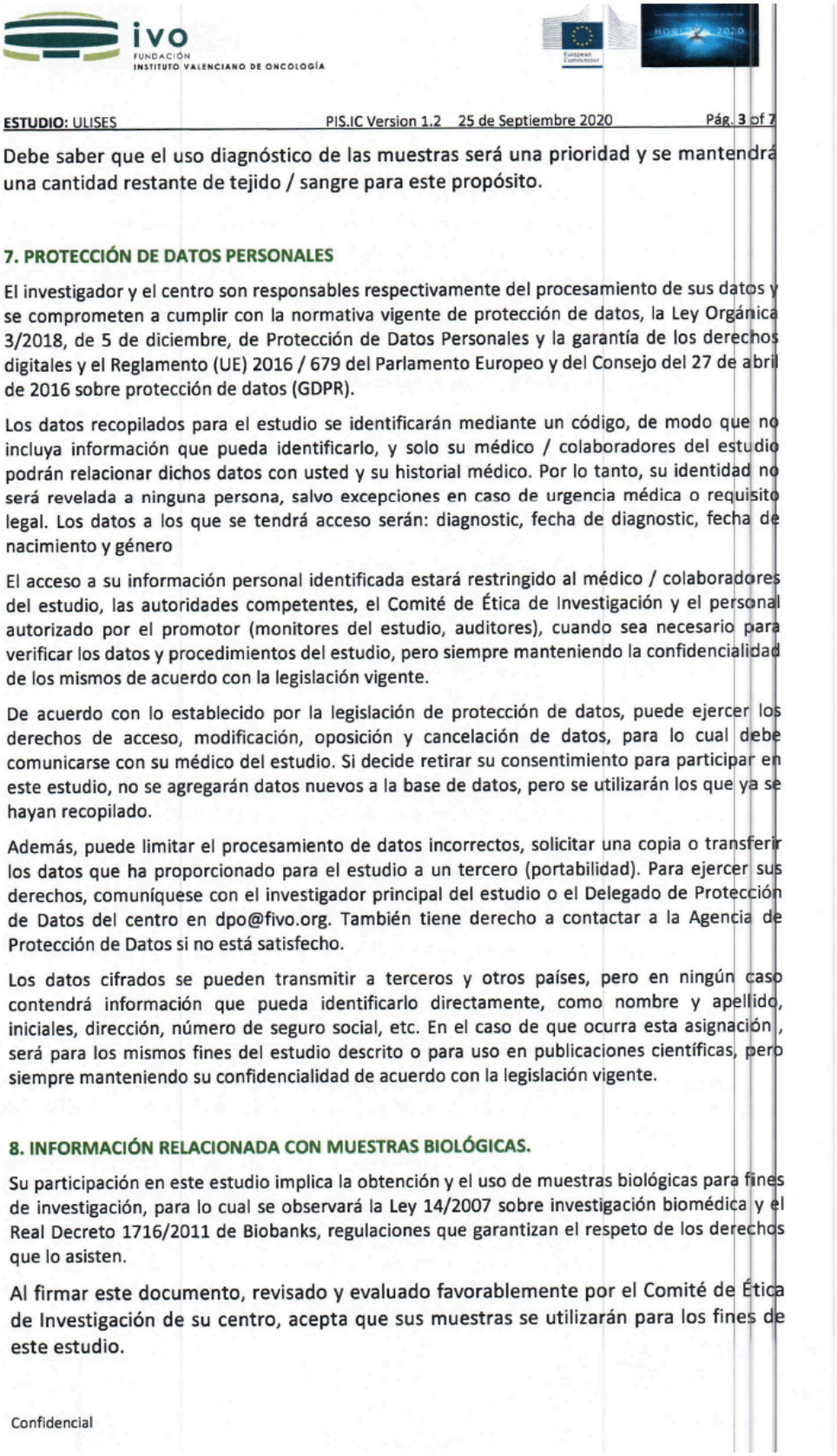

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Debe saber que el uso diagnóstico de las muestras será una prioridad y se mantendrá una cantidad restante de tejido / sangre para este propósito.

7. PROTECCIÓN DE DATOS PERSONALES

El investigador y el centro son responsables respectivamente del procesamiento de sus datos y se comprometen a cumplir con la normativa vigente de protección de datos, la Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y la garantía de los derechos digitales y el Reglamento (UE) 2016 / 679 del Parlamento Europeo y del Consejo del 27 de abril de 2016 sobre protección de datos (GDPR).

Los datos recopilados para el estudio se identificarán mediante un código, de modo que no incluya información que pueda identificarlo, y solo su médico / colaboradores del estudio podrán relacionar dichos datos con usted y su historial médico. Por lo tanto, su identidad no será revelada a ninguna persona, salvo excepciones en caso de urgencia médica o requisito legal. Los datos a los que se tendrá acceso serán: diagnostic, fecha de diagnostic, fecha de nacimiento y género

El acceso a su información personal identificada estará restringido al médico / colaboradores del estudio, las autoridades competentes, el Comité de Ética de Investigación y el personal autorizado por el promotor (monitores del estudio, auditores), cuando sea necesario para verificar los datos y procedimientos del estudio, pero siempre manteniendo la confidencialidad de los mismos de acuerdo con la legislación vigente.

De acuerdo con lo establecido por la legislación de protección de datos, puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual debe comunicarse con su médico del estudio. Si decide retirar su consentimiento para participar en este estudio, no se agregarán datos nuevos a la base de datos, pero se utilizarán los que ya se hayan recopilado.

Además, puede limitar el procesamiento de datos incorrectos, solicitar una copia o transferir los datos que ha proporcionado para el estudio a un tercero (portabilidad). Para ejercer sus derechos, comuníquese con el investigador principal del estudio o el Delegado de Protección de Datos del centro en dpo@fivo.org. También tiene derecho a contactar a la Agencia de Protección de Datos si no está satisfecho.

Los datos cifrados se pueden transmitir a terceros y otros países, pero en ningún caso contendrá información que pueda identificarlo directamente, como nombre y apellido, iniciales, dirección, número de seguro social, etc. En el caso de que ocurra esta asignación , será para los mismos fines del estudio descrito o para uso en publicaciones científicas, pero siempre manteniendo su confidencialidad de acuerdo con la legislación vigente.

8. INFORMACIÓN RELACIONADA CON MUESTRAS BIOLÓGICAS.

Su participación en este estudio implica la obtención y el uso de muestras biológicas para fines de investigación, para lo cual se observará la Ley 14/2007 sobre investigación biomédica y el Real Decreto 1716/2011 de Biobanks, regulaciones que garantizan el respeto de los derechos que lo asisten.

Al firmar este documento, revisado y evaluado favorablemente por el Comité de Ética de Investigación de su centro, acepta que sus muestras se utilizarán para los fines de este estudio.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°899708.

8.1 PROCEDIMIENTOS PARA OBTENER MUESTRAS, DESACUERDOS Y POSIBLES RIESGOS

Se obtendrán muestras durante el seguimiento habitual de su enfermedad o proceso.

- Se obtendrá una muestra de sangre de 7 ml al comienzo del estudio durante el procedimiento de diagnóstico de su enfermedad. Para la mayoría de las personas, los pinchazos con agujas para la extracción de sangre no son un problema. Sin embargo, a veces pueden causar sangrado, hematomas, molestias, infecciones y / o dolor en el punto de recolección de sangre. También puede sentirse mareado.

- Muestras de tumor: se le pedirá que done una porción de la muestra de tumor de una biopsia o intervención quirúrgica que se realizó durante el proceso de atención.

Las muestras se asociarán con un código que solo puede ser relacionado con su identidad por personal autorizado, de la misma manera que se explicó previamente con los datos obtenidos durante el estudio.

Los datos derivados del uso de estas muestras serán tratados de la misma manera que el resto de los datos obtenidos durante este estudio con respecto a la protección de datos.

Las muestras y los datos asociados se mantendrán bajo las condiciones de seguridad apropiadas y se garantiza que los sujetos no pueden ser identificados a través de medios considerados razonables por personas distintas a las autorizadas.

Algunos datos adicionales o muestras pueden ser necesarios. En ese caso, su médico se comunicará con usted para solicitar su colaboración nuevamente. Se le informará de los motivos y se le solicitará nuevamente su consentimiento.

8.2. LUGAR DE ANÁLISIS Y ALMACENAMIENTO DE MUESTRAS

Durante el desarrollo del estudio, sus muestras serán analizadas en el Laboratorio de Biología Molecular de la Fundación Instituto Valenciano de Oncología y posteriormente almacenadas únicamente en el Biobanco FIVO. Parte de este material podrá ser analizado en el CONSORCI INSTITUT D´INVESTIGACIONS BIOMEDIQUES AUGUST PI I SUNYER (IDIBAPS), la FUNDACION PARA LA FORMACION E INVESTIGACION SANITARIAS DE LA REGION DE MURCIA y la UNIVERSITAT POLITECNICA DE VALENCIA, que forman parte del consorcio ULISES

Durante este proceso, la persona responsable de las muestras será el investigador principal del estudio en su centro.

8.3. IMPLICACIONES DE LA INFORMACIÓN OBTENIDA AL ANALIZAR LAS MUESTRAS

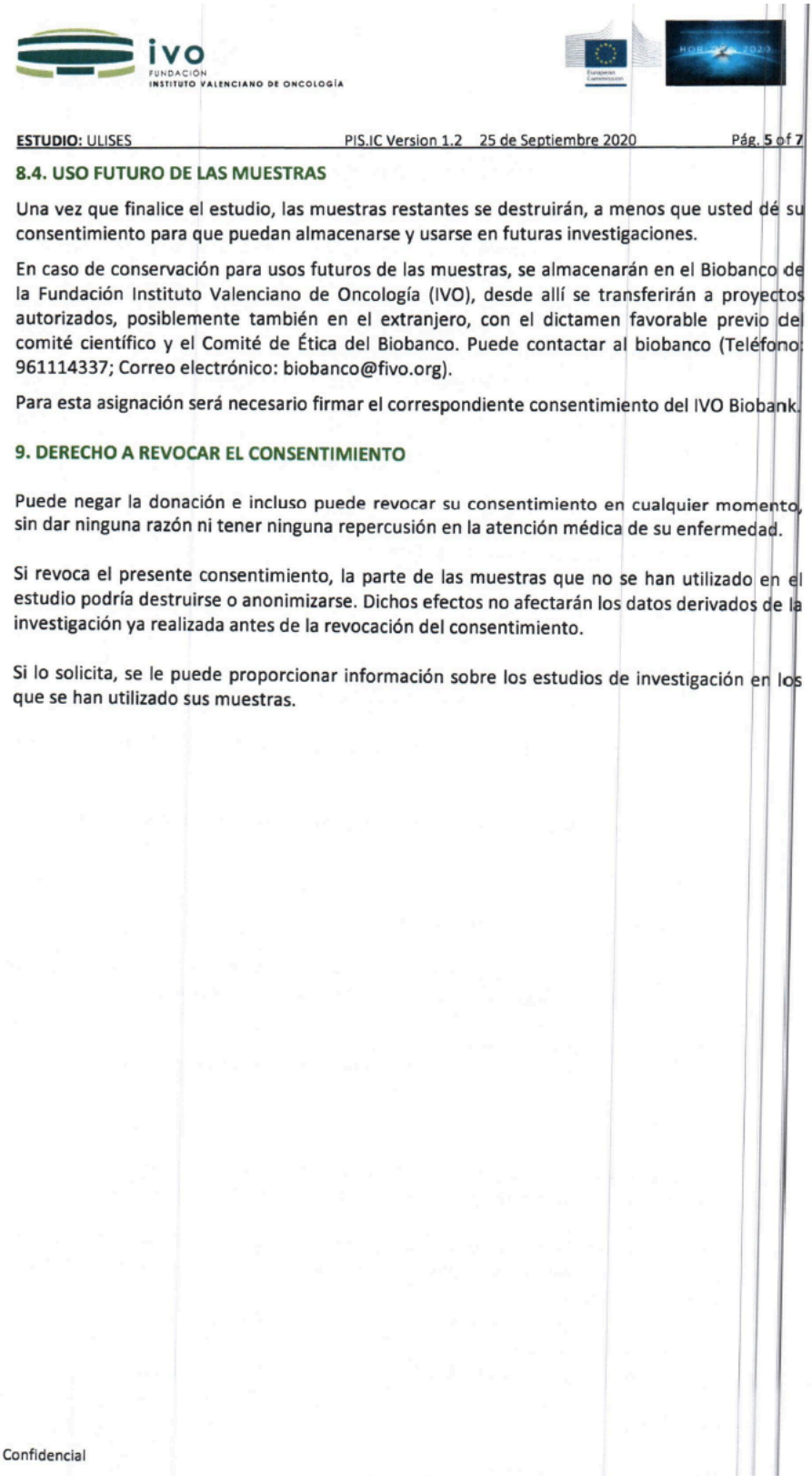
Si lo solicita, puede recibir información sobre los resultados generales de este estudio.

En caso de que este estudio obtenga datos que podrían ser clínicamente o genéticamente relevantes para usted, para su salud o la de su familia, puede solicitar que su médico del estudio se los comunique.

Sin embargo, si expresa su negativa a ser informado, pero de acuerdo con el criterio del médico responsable, la información obtenida es necesaria para evitar daños graves a su salud o la de sus parientes biológicos, se informará a un pariente cercano o un representante, después de consultar al Comité de Ética del Cuidado del Centro. La comunicación de esta información será realizada por profesionales que puedan explicar adecuadamente su relevancia y las opciones que puedan surgir. En caso de información genética clínicamente relevante, puede recibir el consejo genético obligatorio.

Confidencial

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°899708.



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FUNDACIÓN
INSTITUTO VALENCIANO DE ONCOLOGÍA

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8.4. USO FUTURO DE LAS MUESTRAS

Una vez que finalice el estudio, las muestras restantes se destruirán, a menos que usted dé su consentimiento para que puedan almacenarse y usarse en futuras investigaciones.

En caso de conservación para usos futuros de las muestras, se almacenarán en el Biobanco de la Fundación Instituto Valenciano de Oncología (IVO), desde allí se transferirán a proyectos autorizados, posiblemente también en el extranjero, con el dictamen favorable previo del comité científico y el Comité de Ética del Biobanco. Puede contactar al biobanco (Teléfono: 961114337; Correo electrónico: biobanco@fivo.org).

Para esta asignación será necesario firmar el correspondiente consentimiento del IVO Biobank.

9. DERECHO A REVOCAR EL CONSENTIMIENTO

Puede negar la donación e incluso puede revocar su consentimiento en cualquier momento, sin dar ninguna razón ni tener ninguna repercusión en la atención médica de su enfermedad.

Si revoca el presente consentimiento, la parte de las muestras que no se han utilizado en el estudio podría destruirse o anonimizarse. Dichos efectos no afectarán los datos derivados de la investigación ya realizada antes de la revocación del consentimiento.

Si lo solicita, se le puede proporcionar información sobre los estudios de investigación en los que se han utilizado sus muestras.

Confidencial

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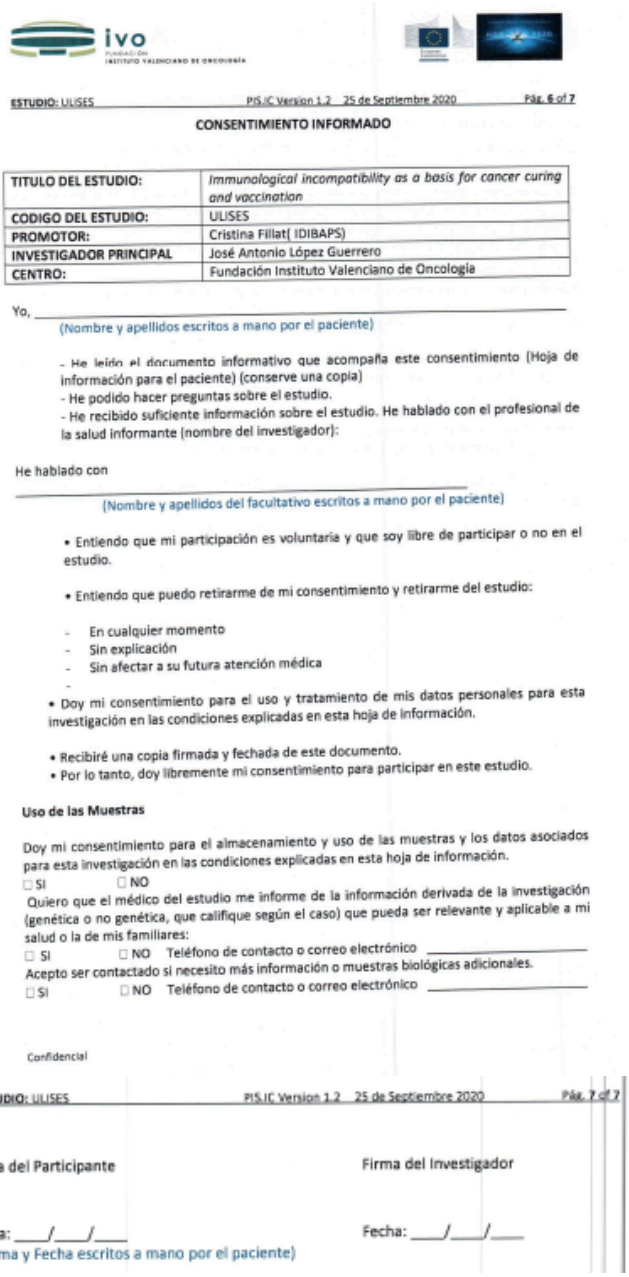
7.4. Annex 4: Informed consent form



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CONSENTIMIENTO INFORMADO

TITULO DEL ESTUDIO:	*Immunological incompatibility as a basis for cancer curing and vaccination*
CODIGO DEL ESTUDIO:	ULISES
PROMOTOR:	Cristina Fillat(IDIBAPS)
INVESTIGADOR PRINCIPAL	José Antonio López Guerrero
CENTRO:	Fundación Instituto Valenciano de Oncología

Yo, _____
(Nombre y apellidos escritos a mano por el paciente)

- He leído el documento informativo que acompaña este consentimiento (Hoja de información para el paciente) (conserve una copia)
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio. He hablado con el profesional de la salud informante (nombre del investigador):

He hablado con
_____
(Nombre y apellidos del facultativo escritos a mano por el paciente)

- Entiendo que mi participación es voluntaria y que soy libre de participar o no en el estudio.
- Entiendo que puedo retirarme de mi consentimiento y retirarme del estudio:
  - En cualquier momento
  - Sin explicación
  - Sin afectar a su futura atención médica
  -
- Doy mi consentimiento para el uso y tratamiento de mis datos personales para esta investigación en las condiciones explicadas en esta hoja de información.
- Recibiré una copia firmada y fechada de este documento.
- Por lo tanto, doy libremente mi consentimiento para participar en este estudio.

Uso de las Muestras

Doy mi consentimiento para el almacenamiento y uso de las muestras y los datos asociados para esta investigación en las condiciones explicadas en esta hoja de información.
☐ SI ☐ NO
Quiero que el médico del estudio me informe de la información derivada de la investigación (genética o no genética, que califique según el caso) que pueda ser relevante y aplicable a mi salud o la de mis familiares:
☐ SI ☐ NO Teléfono de contacto o correo electrónico _____
Acepto ser contactado si necesito más información o muestras biológicas adicionales.
☐ SI ☐ NO Teléfono de contacto o correo electrónico _____

Confidencial

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Firma del Participante

Fecha: ___/___/___
(Firma y Fecha escritos a mano por el paciente)

Firma del Investigador

Fecha: ___/___/___

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7.5. Annex 5: Biobank Sheet Request

FORMULARIO DE REGISTRO DE MUESTRA DE BIOBANCO (FRM)		**ESTUDIO: Zoraida**	**Sponsor:**		
		Site Nº:			
		Investigator:			

Por favor, guardar una copia e insertar el original en la caja

Para ser rellenado por el personal local

CONTENIDO DEL ENVÍO

Tejido Fresco

Paciente#	Indicar la fecha de recogida

Tejidos fijados en formalina y embebidos en parafina (FFPE)

Paciente#	ID del bloque	Fecha de recogid	Solicitud de retorno del bloque(Sí/No)

Sangre periférica de diagnóstico, seguimiento, progresión o prostatectomía radical

Paciente#	Indicar la fecha de recogida

La presentación de este formulario cumplimentado supone la confirmación de que todos los individuos disponen de un consentimiento informado para el análisis de muestras biológicas de tumor y sangre

Nombre del personal local	**Fecha y firma**

A cumplimentar por el Personal del Laboratorio Central

Por favor, guardar el original en el laboratorio central

Fecha de recepción:

Por favor, seleccione:

1) Material recibido DAÑADO [__]/NO DAÑADO [__]

2) Material recibido como se indica anteriormente por el personal local: YES [__]/NO [__]

Si NO, especificar: 1) _____

Nombre del personal del Laboratorio Central	**Fecha y firma**

Por favor, enviar el formulario cumplimentado y firmado por email a: zandreu@fivo.org

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