Section A: Section 1: TITLE OF THE PROJECT

The section aims at a clear identification of the project including its applications and their limits. Particular attention should be paid to the use of wording that may induce fears or unrealistic promises.

A1. Project title

Establishment of hepatic-patient derived organoids

A2. Acronym of the project (if any)

A3. Type of organoid

- [x] Organoid for basic research
- [ ] Factoroid (organoid for bioproduction)
- [ ] Organoid for pre-clinical research
- [ ] Organoid for clinical research
- [ ] Organoid for clinical use
- [ ] Others

A4. Specify

Describe the usage of your planned organoid

A5. Name of the organoid (avoid minigut, minibrain, synthetic brain…)

Avoid minigut, minibrain, synthetic brain…

Hepatocellular carcinoma Organoids (HCCO)

A6. Purpose of the project

Describe the aim of the project including appropriate regulatory documents Compliance: brief description of compliance procedures, upload requested documents in next question.

A7. Upload appropriate regulatory documents

Section B: Section 2: SOURCE MATERIAL

Critical elements for this section are: 1) stem cell metadata based on ATCC model (batch, structural, morphological and functional data, maintenance and preservation protocol), 2) collection declaration (declaration or authorization of activities for the conservation and preparation for scientific purposes of human body elements), mandatory for human samples, 3) monitoring of possible drifts of starting material, 4) regulatory documents and medical ethics if any (restrictions of use according to donor consent).

Section C: Does your research involve human material ?

C1. Does your research involve human material ?

- [x] Yes
- [ ] No

C2. Is the material obtained from volunteers ?

- [ ] Yes
- [x] No

C3. An informed consent has been obtained ?

- [x] Yes
- [ ] No

C4. Provide details of the informed content

C5. Is the volunteer a patient ?

- [ ] Yes
- [ ] No

C6. Is the genetic identity at arrival known ?

Yes ☐

No ☒

C7. If starting material is obtained from a biopsy, are descriptors known ?

Descriptors : gender, age, anatomical regions, diagnosis, viral status.....

Yes ☒

No ☐

C8. Please list descriptors

Gender, age, anatomical region, diagnostic, weight

C9. Does the promoter of the research have clinical data on the patient ?

Yes ☒

No ☐

C10. Is the laboratory authorized to prepare and conserve human body elements for scientific purposes ?

Yes ☒

No ☐

C11. Give details and references of the collection declaration

DC-2015-2565

Section D: Is the starting material a cell line ?

D1. Is the starting material a cell line ?

For example, indifferent origin, IPSCs, ATCC, ESC...

Yes ☐

No ☒

D2. **Is the genetic identity at arrival known?**

Yes ☐

No ☐

D3. **Please specify (DNA sequence, SNPs, PCR, STR, CGH array…)**

D4. **Is there a genetic quality control (karyotype, STR, PCR…)?**

Yes ☐

No ☐

D5. **Please describe the procedure for genetic quality control**

You may give an URL to a file describing the protocol

D6. **Is the cell line functionally validated ?**

For example, differenciation test for pluripotency of IPSCs, permeability tests for epithelial cells etc…

Yes ☐

No ☐

D7. **Please describe the procedure**

You can indicate an URL to a file describing the procedure

D8. **Is the cell identity validated after X passages?**

Yes ☐

No ☐

D9. **Specify the number of passages (X)**

D10. Are cell type markers identified ?

Yes ☐

No ☐

D11. List the different markers used

D12. Is the number of passages at arrival known ?

Yes ☐

No ☐

D13. Is the number of possible, or required, passages before genesis of organoids defined?

Yes ☐

No ☐

D14. Are the storage conditions known?

Yes ☐

No ☐

D15. Please describe preservation protocol (culture, freezing, thawing protocol, storage modalities)

D16. Does the material contain mutations (genetic disease)?

Yes ☐

No ☐

D17. Is the sanitary status known?

Yes ☐

No ☐

D18. **Please give details tests (mycoplasma, bacteriological, fungal …..)**

Section E: Is the starting material primary cells of patients?

E1. **Is the starting material primary cells of patients?**

Yes ☒
No ☐

E2. **Is the genetic identity at arrival known?**

Yes ☐
No ☒

E3. **Please specify (DNA sequence, SNPs, PCR, STR, CGH array…)**

E4. **Is there a genetic quality control (karyotype, STR, PCR…)?**

Yes ☐
No ☒

E5. **Please describe the procedure for genetic quality control**

You may give an URL to a file describing the protocol

E6. **Is the cell line functionally validated ?**

For example, differenciation test for pluripotency of IPSCs, permeability tests for epithelial cells etc…

Yes ☐
No ☐

E7. Please describe the procedure

You can indicate an URL to a file describing the procedure

E8. Is the cell identity validated after X passages?

- [ ] Yes
- [ ] No

E9. Specify the number of passages (X)

E10. Are cell type markers identified ?

- [x] Yes
- [ ] No

E11. List the different markers used

CK19 (bile ducts marker), CD44 (sinusoids and portal areas marker), CD90 (cancer stem cell marker) by RT-qPCR and IF staining

E12. Is the number of passages at arrival known ?

- [ ] Yes
- [x] No

E13. Is the number of possible, or required, passages before genesis of organoids defined?

- [x] Yes
- [ ] No

E14. Are the storage conditions known?

- [x] Yes
- [ ] No

E15. Please describe preservation protocol (culture, freezing, thawing protocol, storage modalities)

3 wells in 1mL in freezing solution (10%DMSO, 90% FBS) and frozen gradually, decreasing temperature in MisterFrosty (-1°/min) to -80°C before long-term storage at -150/196°C

E16. Does the material contain mutations (genetic disease)?

- [ ] Yes
- [ ] No

E17. Is the sanitary status known?

- [x] Yes
- [ ] No

E18. Please give details tests (mycoplasma, bacteriological, fungal …..)

Mycoplasma test

Section F: Are the cell culture conditions precisely described ?

F1. Are the cell culture conditions precisely described ?

- [x] Yes
- [ ] No

F2. Are the culture media well defined ?

- [x] Yes
- [ ] No

F3. Provide extensive culture conditions

Let provide : Composition of culture media, nature, origin and quantities of supplements used -e.g. glucose, serum, antibiotics, growth factors , media changes etc. or an URL pointing to a file with the media descriptions

Organoïd initiation Medium (Stemcell technologies) for 2 weeks prior to first passaging, then newly formed domes were incubated with complete organoïd medium (Advanced DMEM/F12 + 10mM HEPES, 50U/mL Penicillin/Streptomycin, 2mM L-Glutamine) supplemented with 50% (vol/vol) of Organoïd Growth Medium (Stemcell technologies). At Day 5 (after proliferation), Organoïds were differentiated during 10 days with addition of Organoïd Differentiation Medium (Stemcell Technologies).

F4. **Are the nature and treatment of the supports well described?**

Yes ☐

No ☐

F5. **Provide details of the culture supports**

F6. **Are the seeding conditions well described?**

Yes ☒

No ☐

F7. **Is the frequency of media changes defined ?**

Yes, every 2-3 days

F8. **Are O2/CO2 concentrations given?**

Yes ☐

No ☐

F9. **Provide details of the culture conditions**

You can provide an URL pointing to a file or a folder with the seeding conditions

3 wells from 1 well for a passage

Section G: What are the storage conditions of the lines or cells

G1. **Are the procedure of cell banking described?**

Yes ☒

No ☐

G2. Are there cell master banks?

Yes ☒

No ☐

G3. Describe the procedures for the cell master banks and drift controls

Master banks at the CRB with double certification ISO 9001 and ISO 20387

G4. Are there cell working banks?

Yes ☒

No ☐

G5. Describe protocols and drift control for working banks

G6. Are storage conditions indicated?

Yes ☒

No ☐

G7. Describe freezing and thawing protocol

2-3 wells in 1mL in freezing solution (10%DMSO, 90% FBS) and frozen gradually, decreasing temperature in MisterFrosty (-1°/min) to -80°C before long-term storage at -150/196°C

G8. Are the storage modalities given?

Yes ☒

No ☐

G9. Please specify storage modalities

Long-term storage at -150/196°C

Section H: Section 3: MANUFACTURING OF THE ORGANOID

Critical elements in this section are : 1) Differentiation protocol and organoid generation (table of differentiation factors, timelines, culture protocols, purification protocols, if necessary, maintenance and preservation protocols), 2) Design and development of master organoid bak and working organoid bank, 3) Monitoring of the possible drift of organoids (genetic, proteic post translationnal modifications, metabolism, others biomarkers).

Section I: Does the project include 2D differentiation ?

I1. Does the project include 2D differentiation ?

Yes ☐

No ☒

I2. Provide details on culture media

Give informations about nature, origin, supplements used (e.g. growth factors glucose, serum, antibiotics, CO2/O2 concentrations …

I3. Describe sequence and duration of differentiation treatments

I4. Are culture supports treated ?

Yes ☐

No ☐

I5. **Describe treatment of support, seeding conditions and frequency of media changes**

I6. **Is there a quality control for the differentiation process ?**

Yes ☐

No ☐

I7. **Provide details (e.g. morphology, material homogeneity, max and min confluence, proliferation, functional test, monitoring of markers, possibly sorting, mortality rate)**

Section J: Does the project includes generation of (3D) organoids ?

J1. **Does the project include generation of (3D) organoids?**

Yes ☒

No ☐

J2. **Provide details on culture media, nature, origin, supplements used (e.g. growth factors glucose, serum, antibiotics, CO2/O2 concentrations …)**

Organoïd initiation Medium (Stemcell technologies) for 2 weeks prior to first passaging, then newly formed domes were incubated with complete organoïd medium (Advanced DMEM/F12 + 10mM HEPES, 50U/mL Penicillin/Streptomycin, 2mM L-Glutamine) supplemented with 50% (vol/vol) of Organoïd Growth Medium (Stemcell technologies). At Day 5 (after proliferation), Organoïds were differentiated during 10 days with addition of Organoïd Differentiation Medium (Stemcell Technologies). Ambient CO2/ 5% O2

J3. **Describe sequence and duration of differentiation treatments**

After day 5, change media to Organoid differentiation medium for 10 days

J4. **Are culture supports treated?**

Yes ☒

No ☐

J5. **Describe support treatments, seeding conditions and frequency of media changes**

Treated Costar (ref 3548) 48 wells plates. Media changes every 2-3 days.

J6. **Is there a quality control for the differentiation process?**

Yes ☒

No ☐

J7. **Provide details (e.g. morphology, material homogeneity, max and min confluence, proliferation, functional test, monitoring of markers, possibly sorting, mortality rate)**

Monitoring markers with RT-qPCR

Section K: Does organoid generation make use of matrices ?

K1. **Does organoid generation make use of matrices ?**

Yes ☒

No ☐

K2. Describe the nature of the matrix (matrigel, hydrogels, hyaluronic acid, human decellularized matrix etc.)

Matrigel Growth Factor Reduced basement membrane Matrix (Corning)

K3. Give matrix concentration

100% Matrigel (concentration depends on lot number)

K4. Let provide detail preparation method (temperature, polymerization time, drop or layer structure, etc.)

48 well plate 25µL drop per well or 24-well plate 50µL drop per well and 10 minutes polymerization at 37°C

K5. Give seeding density per matrix volume unit

3 wells from 1 well for a passage; 3 wells in a a cryovials

K6. Specify volume and number of drops of matrix per unit area in the culture medium

48 well plate 25µL drop per well or 24-well plate 50µL drop per well

K7. Specify amount of medium depending on the size of the well

For 48 well plate 250µL or 24-well plate 500µL of medium per well

K8. Describe matrix dissociation method for organoid recovery

Mechanical dissociation using cold Gentle Cell Dissociation Reagent (GCDR, stemcell technologies). Matrigel domes were pipetted 20 times in GCDR in order to fragment organoids and dissolve Matrigel. Cold DPBS was added to dilute at 1:2 and then centrifugated 5min

K9. Describe method of dissociation of organoids for their expansion

Mechanical dissociation using cold Gentle Cell Dissociation Reagent (GCDR, stemcell technologies). Matrigel domes were pipetted 20 times in GCDR in order to fragment organoids and dissolve Matrigel. Cold DPBS was added to dilute at 1:2 and then centrifugated 5min. 3 wells from 1 well for 1 passage.

Section L: Does the culture take place on solid 3D support ?

L1. Does the culture take place on solid 3D support (example: mineral support for bones, support for liquid-gas interfaces) ?

- [ ] Yes
- [x] No

L2. Describe preparation method of the 3D solid support (composition of the medium to be freeze-dried, freeze-drying conditions)

L3. Give details on seeding method

L4. **List biocompatible materials used (PDMS, COC, Silicon, etc.)**

L5. **Indicate chip design (provide a map)**

L6. **Give the physical characteristics of the chip**

Section M: Does the organoid grow in suspension (self-organization) ?

M1. **Does the organoid grow in suspension (self-organization)?**

Yes ☐

No ☒

M2. **Specify type of container**

M3. **Describe nature and protocol of the agitation**

M4. **Indicate nature and concentration of matrices**

Section N: Does the culture include multiple cell types ?

N1. **Does the culture include multiple cell types ?**

Yes ☐

No ☒

N2. **Describe the sequence of co-culturing and adaptation of co-culture media**

N3. **Indicate the proportions of cell types**

Section O: Section 4: ORGANOID CHARACTERIZATION

The detailed characterization is project dependent and should be carried out in line with the proposed use of organoid (research, bioproduction, preclinical and clinical studies); however, some standards emerge: 1) omics for structural characterisation, 2) imaging for morphology, 3) specific functional readouts depending of the foreseen use of organoid

Section P: Is there morphological/structure characterization ?

P1. **Is there morphological/structure characterization ?**

Yes ☒

No ☐

P2. **Describe appearance, size, shape [circularity, tubularity, regularity of contour (budding)]**

Proliferation state: round organoids with cystic growth appearance and defined contours, size between 20-100µm
Differentiation state (Day 15): presence of pseudogland-like structure in HCCO, round organoids more compact and opaque, size between 50-200µm

P3. **Evaluate opacity/refringency.**

Differentiation state, yes opaque and less during proliferation state

P4. **Quantify intra and inter-organoid homogeneity**

P5. **Develop expected morphological, architectural and ultrastructural features, organization of cell types (identity, proportions, distribution)**

Morphologic features similar to the tissue of origin with the presence of pseudogland-like structure in HCCO

Section Q: Is there molecular characterization ?

Q1. **Is there molecular characterization ?**

Yes ☒

No ☐

Q2. **Give elements of genomics, transcriptomics, metabolomics, proteomics**

Proliferation state: CD90, CD44 by RT-Qpcr at Day 5, GATA4, CK19,Ki67, F-Actin by IF
Differentiation state: CYP3A4, Albumin, Ki67, CD44 and LGR5 by RT-qPCR at Day 15, Decrease of GATA4 and Ki67, increase of Album by IF

Q3. **Indicate expected specific molecular markers, epigenetic characteristics**

Section R: Is there functional characterization ?

R1. **Is there functional characterization ?**

- [ ] Yes
- [ ] No

R2. **What are qualitative and (if possible) quantitative functional characteristics**

R3. **If treatments are done, detail treatment protocol , response to treatments (pharmacological, chemical, physical, hormonal, etc.), and evaluation (quantitative or qualitative)**

Section S: Are traceability and organoid drift evaluated ?

S1. Are traceability and organoid drift evaluated ?

Yes ☒

No ☐

S2. Describe how traceability of components is evaluated (batches, suppliers etc., environments, complements)

Yes, traceability of every components (batches number, expiration dates, etc)

S3. Indicate criteria for traceability of conditioned media (drift of cells used for conditioning, control of lines as for those at the origin of the organoid), control of at least one of the growth factors)

Yes, traceability of every components used for conditioned media (batches number, expiration dates, etc)

S4. Describe qualitative drift criteria (morphological, structural, functional, molecular, etc.) specific to each organoid. Specify indices if applicable

Morphological and structural by HES, IF markers and RT-qPCR markers

S5. How is robustness evaluated (same starting cells, same organoid). Specify indices if applicable

Section T: Section 5: USE OF ORGANOIDS

The critical element in this section is the robustness of the preparation and characterization of the organoid. To anticipate further use of organoids from basic to development of innovative applications (for instance, the use of Good Laboratory practices will facilitate the transition from basics to preclinical research).

T1. What is the domain of application of the organoid ?

- [x] Basic research
- [ ] Bioproduction
- [ ] Preclinical research (Pharmacology, toxicology, ...)
- [ ] Clinical research (personalized, predictive and regenerative medicine, transplantation….)
- [ ] Other

T2. Are GLP (good laboratory practice) required for organoid production?

- [ ] Yes
- [ ] No

T3. Give details for GLP

T4. Are GMP (good manufactory practice) required for organoid production ?

- [ ] Yes
- [ ] No

T5. Give details for GMP

T6. **Indicate functional similarity criterion between the organoid and the mimicked organ (battery of controls to be performed with target values)**

T7. **Is the organoid used for preclinical development of a drug candidate (IND file) ?**

- [ ] Yes
- [ ] No

T8. **Indicate the number of usable passage for drug candidate**

T9. **Is the organoid used to define predictive signatures of responses (companion test)?**

- [ ] Yes
- [ ] No

T10. **Indicate the number of usable passages for companion test**

T11. **Is the organoid used to validate a care protocol (specific patient) on a cohort: choice of a therapy**

- [ ] Yes
- [ ] No

T12. **Indicate the number of usable passages for care protocol**

T13. **Is the organoid used for Domain 1: Care protocol (specific patient) ? (validation of the protocol of use of the organoid for the orientation of the therapeutic choice)**

- [ ] Yes
- [ ] No

T14. **Specify process for GMP certification, total traceability of the components, qualification of the components**

T15. **Give criterion of similarity between the organoid and the biopsy**

T16. **Is the organoid used, for Domain 2: Use in regenerative medicine (same as cell and tissue therapies) ?**

- [ ] Yes
- [ ] No

T17. **Specify process for GMP certification, total traceability of the components, qualification of the components**

T18. **Specify functionality criteria, safety (Derivation of biological material and evaluation of the risk of cancer)**

T19. Specify others usages of organoid

Section U: END OF SURVEY

This is the last section of this survey.

You can use the "Resume later" button at the top-right of the screen to save your answers and come back to this form later.

If you are done, you can press the "Submit" button, you will then be able to print your answers.

Your answers were registered.

Dont forget to print your answers.