Lignée ORGAPRED N°HN-107

Le MIAOU (**M**inimal **I**nformation **A**bout an **O**rganoid and its **U**se):
Eléments descriptifs permettant à l'homme de l'art de reproduire une expérience de fabrication, de caractérisation et d'étude fonctionnelle d'organoïdes

Le MIAOU sert à identifier les informations présentes (la réponse Oui/Non est la plus importante) et à évaluer la qualité de leur description pour la reproductibilité.

A) SOURCE MATERIAL MATERIEL SOURCE

Informed consent obtained Consentement adapté au but de la recherche	Yes
Collection declaration Déclaration de collection- CODECOH*	Yes
Descriptors: gender, age, anatomical region, diagnostic, viral statut Descriptif : genre, âge, région anatomique, diagnostic, statut viral	Yes
Clinical data on the patient Tableau clinique du patient	Yes

** : gestion de la COnservation D'Éléments du COrps Humain*

Primary cell of patient (and healthy subjects) and tumors Cellule primaire de patient (et sujets sains) et tumeurs

Genetic identity at arrival (example: DNA sequence, snips, digital PCR, STR, CGH array) Identité génétique à réception	Yes, STR on tumor and organoid sample for quality control
Genetic quality control (example : Karyotype, STR, digital PCR) Contrôle de qualité génétique	Yes, STR on tumor and organoid sample for quality control
Functional quality (example: differentiation test for pluripotency of IPSCs, permeability tests for intestinal epithelial cells...) Qualité fonctionnelle	Yes, organoid etablishment
Cell identity after X passages identité cellulaire après X passages	STR analysis on tumor DNA and on organoid DNA at passage 8.
Cell type marker (example : marker name, detection method, target value) Marqueur de type cellulaire	IHC specific markers on tumor fragment: p53 / p40 / p63 / p16 / Ki67
Number of passages at arrival Nombre de passages à la réception	0, cells directly obtained from patient's tumor
Number of possible or required passages before genesis of organoids Nombre de passages possibles ou requis avant genèse des organoïdes	0, considered established at passage 3 and above
Storage conditions Protocole de conservation	Between 100 and 1000k cells in 2 mL cryovials, resuspended in 1000 µL in freezing solution (10% DMSO, 90% FBS) and frozen gradually decreasing temperature

	(1°C/min in CoolCell) to -80°C before long-term storage at -196°C.
Mutations if genetic disease Mutations si maladie génétique	Mutation status of 19 major genes frequently mutated in HNSCC available.
Contamination tests (mycoplasma, bacteriological, fungal) Tests contamination	Absence of bacterial or fungal contamination
Method of tissue dissociation (production of single-cell material or tissue substructures - example: intestinal crypt) Méthode de dissociation du tissu	1h enzymatic and mechanical dissociation of tumor fragments with Tumor Dissociation Kit, human (Miltenyi) and 100 µm filtration.

Storage conditions of the lines or cells **Conditions de conservation des lignées ou cellules**

Master banks, (description of protocols, drift control) Banques mères	BRC of the BACLESSE Centre, NF S96-900 certified
Daughter banks(description of protocols, drift control) Banques filles….	No
Storage: freezing and thawing protocol Conservation : protocole de congélation et de décongélation	Freezing: between 100 and 1000k cells in 2 mL cryovials, resuspended in 1000 µL in Recovery Cell Culture Freezing Medium (Gibco) and frozen gradually decreasing temperature (1°C/min in CoolCell) to -80°C before long-term storage at -150°C. Thawing: Fast thawing at 37°C, transfer in fresh culture media before centrifugation and matrix seeding.
Storage modalities Modalités de conservation	Long-term storage at -150°C.

B) MANUFACTURING OF THE ORGANOIDS FABRICATION DE L'ORGANOIDE

Culture conditions of cells **Conditions de culture des cellules**

Composition of culture media, nature, origin and quantities of supplements used (e.g. glucose, serum, antibiotics, growth factors etc. .....) Composition des milieux	Culture in an enriched medium [Advanced DMEM (Gibco) supplemented with 100 UI/mL of penicillin and streptomycin (Gibco), 1% GlutaMAX (Gibco), 1X B27 (Gibco), 10 mM Nicotinamide (Sigma-Aldrich), 1.25 mM N-Acetyl-L-Cysteine (Sigma-Aldrich), 100 µg/mL Primocin (InvivoGen), 10 µM Y27632 (Interchim), 10 ng/mL FGF-10 (PeproTech), 500 nM A-83–01 (PeproTech), 50 ng/mL EGF (PeproTech), 5 ng/mL FGF-basic (PeproTech), 1 µM PGE2 (PeproTech), 1 µM Forskolin (PeproTech), 0,3 µM CHIR99021 (Biogems), 50% Wnt3a/RSPO3/Noggin-conditioned medium

	(L-WRN, ATCC), and 10% RSPO1-conditioned medium (Cultrex HA-R-Spondin-1-Fc 293T, Amsbio)].
Nature and treatment of the supports Nature et traitement des supports	24-well plate Costar: culture treated 6-well plate Nunc: culture treated, Nunclon Delta
Seeding conditions Conditions d'ensemencement	10 000 to 15 000 cells per matrix drop (mix of 70% BME2 and 30% culture media v/v)
Frequency of media changes Fréquence des changements de milieu	Twice a week
CO2 / O2 Concentration	Ambient O_2 and 5% CO_2

Generation of organoids (3D): specificities **Génération des organoïdes (3D) : spécificités**

Matrix culture *Culture en matrice*

Nature of the matrix (matrigel, hydrogels, hyaluronic acid, human decellularized matrix etc.) Nature de la matrice	Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2
Matrix concentration Concentration de la matrice	70% matrix in 30% media (v/v)
Preparation method (temperature, polymerization time, drop or layer structure, etc.) Modalité de préparation	50 µL drop of matrix and 30 minutes polymerization at 37°C
Seeding density per matrix volume unit Densité d'ensemencement	10 000 to 15 000 cells per matrix drop
Volume and number of drops of matrix per unit area in the culture medium	50 µL drop per well in 24-well plate, 10 drops of 50 µL per well in 6-well plate
Amount of medium depending on the size of the well Quantité de milieu en fonction de la taille du puits	24-well plate: 500µL 6-well plate : 2000µL
Matrix dissociation method for organoid recovery Méthode de dissociation de la matrice pour la récupération des organoïdes	Mechanical dissociation using cold Advanced DMEM media (Gibco) supplemented with 100 UI/mL of penicillin and streptomycin (Gibco), 1% GlutaMAX (Gibco), 1% BSA (Panreac)
Method of dissociation of organoids for their expansion Méthode de dissociation des organoïdes pour leur expansion	Enzymatic dissociation using TrypLE Express Enzyme (1X) solution, 5-15 minutes incubation at 37°C with gentle agitation every 5 minutes, cold Adv DMEM media (1% BSA, see above) is then add to stop the reaction before centrifugation, numeration and seeding.

Culture including multiple cell types *Culture incluant de multiples types cellulaires*

Sequence of co-culturing and adaptation of co-culture media Séquence des mises en co-culture	N.A.
Proportion of cell types Proportion des types cellulaires	N.A.

C) ORGANOID CHARACTERIZATION CARACTÉRISATION DES ORGANOIDES

The detailed characterization is project dependent, however some standards emerge

Morphology *Structure*	
Appearance, size, shape [circularity, tubularity, regularity of contour (budding)] Aspect, taille, forme	Round organoids with defined contours
Opacity/réfringency Opacité/réfringence	Opaque
Intra and inter-organoid homogeneity Homogénéité	Heterogeneity in shape and size Heterogeneous expression of some IHC markers
Expected morphological, architectural and ultrastructural features, organization of cell types (identity, proportions, distribution) Particularités morphologiques	N.A.

Molecular Characteirsation *Caractérisation moléculaire*	
Elements of genomics, transcriptomics, metabolomics, proteomics, Eléments de génomique, transcriptomique, métabolomique, protéomique	voir la fiche RIBBON de ce modèle disponible sur https://ribbon.unicaen.fr
Expected specific molecular markers, epigenetic characteristics Marqueurs moléculaires	IHC specific markers on organoids: p53 / p40 / p63 / p16 / Ki67

Function *Fonction*	
Qualitative and (if possible) quantitative functional characteristic Caractéristique fonctionnelle	N.A.
Response to treatments (pharmacological, chemical, physical, hormonal...) the treatment protocol, and evaluation (quantitative or qualitative) of the response are described Réponse aux traitements	Protocol: when PDTO reached the size of 75–150 µm in diameter, they were collected and resuspended in PDTO treatment medium (PDTO culture medium lacking primocin, Y-27,632 and N-acetylcysteine) with 2% BME2. 200 PDTO per well were seeded in 100 µL in white, clear-bottom 96-well plates (Greiner) previously coated with a 1:1 mixture of organoid treatment medium and BME2. For radiotherapy response assays, PDTO were irradiated in microtubes using the CellRad irradiator (FAXITRON Bioptics) prior

	to plating, and medium was replaced after 5 days. For chemotherapy response assays, drugs were prepared in 2% BME2/organoid treatment medium and added 1 hour after PDTO plating. PDTO morphology was monitored using IncuCyte S3 (Sartorius). After 7 days (chemotherapy) or 10 days (radiotherapy), ATP levels were quantified using CellTiter-Glo 3D assay (Promega), and luminescence was measured with the GloMax reader (Promega). Cell viability values were normalized to control. Two independent biological replicates were realized in most cases and treatment sensitivity was determined based on the earliest available passage of each PDTO model. **<u>Evaluation of treatment response for:</u>** Cisplatin = sensitive Radiotherapy = resistant

Traceability, organoid drift *Traçabilité, dérive des organoïdes*	
Traceability of components (batches, suppliers etc.. environments, complements) Traçabilité des composants	Yes, traceability of every components (batches number, expiration dates …)
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) Traçabilité des milieux conditionnés	Traceability of every components (batches number, expiration dates …) used for conditioned media production. No systematic growth factors quantification in conditioned medium.
Drift criteria (morphological, structural, functional, molecular....) specific to each organoid. Specify indices if applicable Critères de dérive	Morphological, response to treatments, IHC markers, STR
Robustness criterion (same starting cells, same organoid). Specify indices if applicable Critère de robustesse	No

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D) USE OF ORGANOIDS UTILISATION DES ORGANOIDES

Organoid for basic research *Organoide en recherche fondamentale*

	No

Organoid in preclinical research (pharmacology, toxicology, ...) *Organoïde en recherche préclinique (pharmacologie, toxicologie, …)*

Functional similarity criterion between the organoid and the mimicked organ (battery of controls to be performed with target values) Similarité fonctionnelle	Patient clinical data are available to assess concordance with organoid-based tests.
Number of usable passages Applicable for: Preclinical development of a drug candidate (IND file) using organoids Nombre de passages exploitables	More than 10
Number of usable passages Applicable for: Definition of predictive signatures of responses (companion test) Nombre de passages exploitables	More than 10
Number of usable passages Applicable for : Validation of a care protocol (specific patient) on a cohort: choice of a therapy Validation d'un Protocole de soin	To be defined

Organoid in clinic (personalized, predictive and regenerative medicine, transplantation)
Organoïde en clinique

GMP certification, total traceability of the components, qualification of the components for Domain 1: Care protocol (specific patient) (validation of the protocol of use of the organoid for the orientation of the therapeutic choice) - Criterion of similarity between the organoid and the biopsy Certification GMP	No
GMP certification, total traceability of components, qualification of components for Domain 2: Use in regenerative medicine (same as cell and tissue therapies) - Functionality criteria, safety (Derivation of biological material and evaluation of the risk of cancer) Certification GMP	No