

Lignée 3D-ONCO N°22COL03-TO

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

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

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

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| Genetic identity at arrival (example: DNA sequence, snips, digital PCR, STR, CGH array) Identité génétique à réception | Yes, mutation analysis on a panel of regions of interest, MSI status |
| Genetic quality control (example : Karyotype, STR, digital PCR) Contrôle de qualité génétique | Yes, |
| Functional quality (example: differentiation test for pluripotency of iPSCs, permeability tests for intestinal epithelial cells...) Qualité fonctionnelle | Yes, organoid establishment |
| Cell identity after X passages identité cellulaire après X passages | Material available analyses will be done later |
| Cell type marker (example : marker name, detection method, target value) Marqueur de type cellulaire | Yes, IHC specific markers on tumor fragment: H&E |
| 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 | Yes, 1 FFPE block 1 snapfrozen fragment |
| Mutations if genetic disease | TP53 (c.303_304dup) |

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| Mutations si maladie génétique | |
| Contamination tests (mycoplasma, bacteriological, fungal) Tests contamination | Yes, absence of contaminants (bacterial, mycoplasma, fungi) |
| Method of tissue dissociation (production of single-cell material or tissue substructures - example: intestinal crypt) Méthode de dissociation du tissu | Yes, 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

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| Master banks, (description of protocols, drift control) Banques mères | Yes, BRC of the LEON BERARD Centre, NF ISO20387 et ISO9001 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 500 and 5000 organoids in 2 mL cryovials, resuspended in 1000 µL in freezing solution (10% DMSO, 10% FBS, 80% medium) and frozen gradually decreasing temperature (1°C/min in CoolCell) to -80°C before long-term storage at -196°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 -196°C. |

B) MANUFACTURING OF THE ORGANOIDS FABRICATION DE L'ORGANOIDE

Culture conditions of cells Conditions de culture des cellules

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| 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 : <ul style="list-style-type: none"> - 100 UI/mL of penicillin and streptomycin (Gibco) - 50 µg/mL Primocin (InvivoGen) - 1% HEPES (Gibco) - 1% GlutaMAX (Gibco) - 1.25 mM N-Acetyl-L-Cysteine (Sigma-Aldrich) - 1X B27 (Gibco) - 1 µM SB202190 (Sigma) - 5 mM Nicotinamide (Sigma-Aldrich), - 5 µM Y27632 (Miltenyi) - 500 nM A-83-01 (Miltenyi) - 10 nM PGE2 (Tebu Bio) |
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| | <ul style="list-style-type: none"> - 10 nM GASTRIN (Sigma) - 50 ng/mL EGF (Miltenyi) - RSPO1-conditioned media (Cultrex HA-R-Spondin1-Fc 293 T, Amsbio) - NOGGIN- conditioned media (Home made cell line) |
| Nature and treatment of the supports Nature et traitement des supports | <ul style="list-style-type: none"> - Untreated plates or dishes - Ultra Low Attachment 96-well plate |
| Seeding conditions Conditions d'ensemencement | 50µL matrix drop (mix 1:1,5 of cell suspension and matrix v/v) |
| Frequency of media changes Fréquence des changements de milieu | Twice a week |
| CO2 / O2 Concentration | Yes, ambient O ₂ and 5% CO ₂ |

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

Matrix culture Culture en matrice

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| Nature of the matrix (matrigel, hydrogels, hyaluronic acid, human decellularized matrix etc.) Nature de la matrice | Corning™ Matrice Matrigel™ (reference : 354234) for amplification |
| Matrix concentration Concentration de la matrice | Mix ratio 1:1,5 of cell suspension and matrix (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 | Between 10 000 – 20 000 cells per matrix drop |
| Volume and number of drops of matrix per unit area in the culture medium | 50 µL stack per well in 96-well plate 30 µL drop per well in 48-well plate 50 µL drop per well in 24-well plate 10 drops of 50 µL per well in 6-well plate or 60mm petri dish |
| Amount of medium depending on the size of the well Quantité de milieu en fonction de la taille du puits | 50 µL stack per well in 96-well plate 30 µL drop per well in 48-well plate 50 µL drop per well in 24-well plate 50 µL drop per well in 12-well plate 10 drops of 50 µL per well in 6-well plate or 60mm petri dish |
| 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: <ul style="list-style-type: none"> - 100 UI/mL of penicillin and streptomycin (Gibco) - 1% GlutaMAX (Gibco) - 1% BSA (PAN Biotech) |

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| 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 Advanced DMEM media (1% BSA, see above) is then add to stop the reaction before centrifugation, numeration and seeding. |
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Culture including multiple cell types *Culture incluant de multiples types cellulaires*

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| 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

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| <i>Morphology</i> <i>Structure</i> | |
| Appearance, size, shape [circularity, tubularity, regularity of contour (budding)] Aspect, taille, forme | Yes, round organoids with defined contours and budding, between 50-300 µm |
| Opacity/réfringency Opacité/réfringence | Yes, opaque |
| Intra and inter-organoid homogeneity Homogénéité | Yes, in shape and size Heterogeneity for some IHC markers |
| Expected morphological, architectural and ultrastructural features, organization of cell types (identity, proportions, distribution) Particularités morphologiques | N.A. |

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| <i>Molecular Characterisation</i> <i>Caractérisation moléculaire</i> | |
| Elements of genomics, transcriptomics, metabolomics, proteomics, Éléments de génomique, transcriptomique, métabolomique, protéomique | Yes, NGS |
| Expected specific molecular markers, epigenetic characteristics Marqueurs moléculaires | Yes, same as the parental tumor |

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| <i>Function</i> <i>Fonction</i> | Specific to each organoid |
| Qualitative and (if possible) quantitative functional characteristic Caractéristique fonctionnelle | N.A. |

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| Response to treatments (pharmacological, chemical, physical, hormonal...) the treatment protocol, and evaluation (quantitative or qualitative) of the response are described Réponse aux traitements | N.A. |
| <i>Traceability, organoid drift</i> <i>Traçabilité, dérive des organoïdes</i> | |
| 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, IHC markers |
| 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 *Organoïde en recherche fondamentale*

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

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| Functional similarity criterion between the organoid and the mimicked organ (battery of controls to be performed with target values) Similarité fonctionnelle | Yes, - Clinical data available - Correlation between tumor and organoids markers |
| Number of usable passages Applicable for: Preclinical development of a drug candidate (IND file) using organoids Nombre de passages exploitables | Yes, more than 10 |
| Number of usable passages Applicable for: Definition of predictive signatures of responses (companion test) Nombre de passages exploitables | Yes, 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*

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| 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 |

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