Interreg Co-funded by the European Union
North-West Europe
STEP4NAMs

# D.2.1.1: Pilot Rationale

# Rationales for Each of the Four STEP4NAMs Pilot Studies

| | |
|---|---|
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# Content

# Document summary

*Table 1 Document summary.*

| Category | Input |
|---|---|
| Project number | NWE0400563 |
| Project title | STEP4NAMs |
| Project full title | Step up the use for new approach methodologies to replace animal testing |
| Work package | WP2 Demonstrating the efficiency of NAMs across the (Bio)Pharma & MedTech sectors |
| Activity | A2.1 Joint setup of pilot studies |
| Deliverable | D2.1.1 Pilot rationale |
| Author(s) | Prof. Michael Madden (Pilot IB), Dr Bairbre McNicholas (Pilot IA), Dr. Mihai Lomora (Pilot IA), Dr. Sugunapriyadharshini Sundararaman (Pilot IA), Dr Hanna Vuorenpää (Pilot II), Dr Matthieu GOBIN (Pilot IV), Dr. Joaquin Penide (Pilot III) |
| Organization | University of Galway (responsible partner), 3R Tuebingen, University of Luxembourg, ATU |

*Table 2 Dissemination level.*

| Dissemination level | Abbreviation | Selection [X / - ] |
|---|---|---|
| Public | PU | X |
| Restricted to other programme participants | PP | |
| Restricted to a group specified by the consortium | RE | |
| Confidential, only for members of the consortium | CO | |

# Revision History

*Table 3 Revision history.*

| Version number | Author (Partner abbreviation) | Description | Date |
|---|---|---|---|
| **V01** | MM (UGal) | First version of document | 2025-11-26 |
| **V02** | ML & SS (UGal) | LOC project information | 2025-12-16 |
| **V03** | MJ (STERN) | Final Review and Formatting | 2026-03-30 |

# Abbreviations

*Table 4 Abbreviations.*

| Abbreviation | Description |
|---|---|
| AIS | Acute Ischemic Stroke |
| ALI | Acute Lung Injury |
| ARDS | Acute Respiratory Distress Syndrome |
| AT | Animal testing |
| BP | BioPharma |
| D | Deliverable |
| ECHA | European Chemical Agency |
| ECVAM | European Union Reference Laboratory for Alternatives to Animal Testing |
| EMA | European Medical Agency |
| ICU | Intensive Care Unit |
| KPI | Key performance indicator |
| LoC | Lung-on-Chip |
| LOI | Letter of intent |
| MedTech | Medical Technology |
| MDR | Medical Device Regulation |
| MDTA | Material and Data Transfer Agreement |
| MO | Midbrain Organoids |
| NAM | New Approach Methodology |
| NWE | North-West-Europe |
| OoC | Organ-on-Chip |
| PD | Parkinson's Disease |
| QC | Quality Control |
| R&D | Research and Development |
| SME | Small- and mid-sized enterprise |
| SOP | Standard Operating Procedure |
| WP | Work package |
| 3R | Replacement, Reduction and Refinement |

# Project partner

*Table 5 Project partner.*

| Abbreviation | Description | Country |
|---|---|---|
| Uni Tuebingen | 3R-Center for In vitro Models and Alternatives to Animal Testing | Germany |
| ATU | Atlantic Technological University | Ireland |
| STERN | BioRegio STERN Management GmbH | Germany |
| BOM | Brabant Development Agency | Netherlands |
| Eurasante | Eurasanté | France |
| Biovia | Biovia | Belgium |
| IQ | InnovationQuarter | Netherlands |
| MPR | Medicen Paris Region | France |
| Galway | University of Galway | Ireland |
| UL | University of Luxembourg | Luxembourg |

# 1. Overview

New Approach Methodologies (NAMs) have the potential to substantially reduce reliance on animal testing while improving the clinical relevance, efficiency, and ethical robustness of pre-clinical research in the biopharmaceutical and medical technology sectors. The STEP4NAMs project addresses these barriers through the coordinated development and validation of four complementary pilot studies, each focusing on a distinct class of NAMs: digital twins, organ-on-chip systems, advanced bench-top simulation models, and patient-derived organoids. Together, these pilots are designed to generate practical validation evidence, transferable methodologies, and best-practice guidance. In this deliverable, the scientific, technical, and strategic justification for each pilot study is demonstrated. For each pilot, this document outlines the purpose and scope, methodological approach, relevance to regulatory and industrial validation needs, and expected contribution to the overall objectives of STEP4NAMs. The rationales demonstrate how the pilots collectively support SMEs, researchers, regulators, and other stakeholders in building confidence in NAMs and accelerating their uptake across the North-West Europe (NWE) region.

# 2. Pilot I: Validation of a Multi-Organ Digital Twin System and Microfluidic Lung-on-Chip Model

## 2.1 Purpose and Scope of Pilot

This pilot study will develop and validate a coupled lung–kidney digital twin capable of real-time simulation of these organ function in critically ill patients. Building on established in-silico models of respiratory mechanics, gas exchange, and renal hemodynamics, the project will integrate these submodels into a unified framework. The digital twin will leverage patient-specific Intensive Care Unit data to personalize simulations, enabling in-silico evaluation of ventilator settings, fluid management strategies, and novel therapeutics.

The primary objective is to demonstrate that a lung–kidney digital twin: (1) can accurately reproduce patient trajectories of blood gases, urine output, and hemodynamics; (2) is suitable for bedside decision support to serve as a predictive platform for pharmaceutical and biotech R&D.

Scope includes model adaptation, software integration, data-driven calibration, and performance evaluation. Deliverables will encompass a validated coupling framework, user-interface prototypes, regulatory-compliant documentation, and a pilot deployment package for small- and medium sizer enterprise (SME) partners.

In addition, a second pilot study led by the University of Galway aims to develop and validate an ICU-informed, patient-derived microfluidic lung-on-a-chip platform capable of accurately modelling both healthy and acute lung injury (ALI) conditions. Building on established principles of lung endothelial–epithelial barrier biology, air–liquid interface physiology, and microfluidic perfusion, the project will integrate these elements into a unified, clinically relevant non-animal in-vitro framework. The model will leverage patient serum-derived samples and ICU-relevant conditions to enable physiologically meaningful evaluation of inflammatory responses and therapeutic efficacy.

The primary objective is to demonstrate that the lung-on-a-chip platform:

(1) can reproducibly recapitulate ALI-associated barrier dysfunction, inflammation, and injury-driven phenotypes; and (2) is suitable as a predictive preclinical tool for personalized medicine, supporting SMEs (short-term) and pharmaceutical and biotechnology R&D (long-term) by improving translation and reducing late-stage clinical trial failures.

## 2.2 Methodology of Pilot

In this pilot study, we will develop and validate a coupled lung–kidney digital twin tailored to critically ill patients by integrating pre-existing, peer-reviewed organ models and patient data. First, we will adapt an established in silico model of respiratory mechanics and gas exchange and incorporate published, physiologically based mathematical models of renal filtration, reabsorption, and hemodynamics. These submodels will be connected within a modular software framework so that changes in ventilation, perfusion, acid–base status or fluid balance in one organ appropriately influence the other. Partnership with the Department of Computer Science to ensure computational performance suitable for bedside use.

To personalize and validate each patient's digital twin, we will assemble a de-identified dataset of ICU patients that includes demographics, laboratory values, ventilator settings, and hemodynamic measurements. Advanced machine-learning technique will be employed to tune the model parameters until the twin accurately reproduces each patient's observed trajectory. We will then evaluate physiological accuracy by comparing model predictions of pulmonary, renal, and hemodynamic markers against real measurements; assess computational performance and test predictive utility by simulating common interventions (for example, adjustments in ventilator settings, fluid challenges, or contrast-agent dosing).

Upon successful validation, the lung–kidney digital twin will be offered as an in silico testbed for novel drug candidates and medical devices. Pharmaceutical and biotechnology partners will be able to use it to assess organ-level effects of therapies in a virtual critical-care environment before proceeding to animal or human trials, thereby reducing development time, cost, and ethical burden.

In the LOC pilot study, we will develop and validate a patient-informed, microfluidic lung-on-a-chip platform tailored to model both healthy and acute lung injury (ALI) conditions. Building on existing state-of-the-art lung-on-chip designs and our laboratory expertise in disease-specific microfluidic models, we will extend current systems beyond short-term experiments to enable long-term, physiologically relevant culture. The platform will integrate epithelial–endothelial barrier architecture, controlled perfusion, and patient-derived inputs to improve the

predictive value of preclinical drug testing, particularly for compounds that have previously failed in clinical trials.

First, we will design a membrane-based lung-on-chip using commercially available microfluidic devices. A porous separation membrane will recreate the air–liquid interface, dividing an air-exposed epithelial compartment from a perfused endothelial compartment. Human alveolar epithelial cells will be cultured at the air–membrane interface, while human pulmonary microvascular endothelial cells will be seeded on the opposite side of a collagen-coated membrane to represent the basal lamina and vascular endothelium. Model complexity will be optimized by tuning cell types, seeding densities, and extracellular matrix composition to ensure barrier integrity, physiological media flow, and long-term viability.

Short-term shear stress studies will define optimal perfusion parameters, followed by integration with a long-term perfusion system supporting up to three-week cultures. Barrier function, cellular health, and injury responses will be assessed using fluorescence markers, permeability assays, and metabolic readouts, enabling translational evaluation of lung injury and therapeutic responses.

Patient serum derived samples will be incorporated alongside available ICU clinical data to personalize the model and assess its ability to reproduce disease-relevant phenotypes. Physiological accuracy and translational relevance will be evaluated by comparing chip-based responses to known clinical trajectories and therapeutic outcomes, with a particular focus on drugs that have previously failed in clinical trials.

Upon successful validation, this lung-on-chip platform will serve as a predictive preclinical test-bed for SMEs and potential pharmaceutical and biotechnology partners. By enabling drug assessment in a patient-relevant, human-based micro physiological system, the platform has the potential to reduce reliance on animal models, lower development costs, and improve the success rate of therapies entering clinical trials. Ultimately, this approach offers a more ethical, scalable, and clinically meaningful strategy for personalized medicine in acute lung injury.

## 2.3 How the Pilot will improve NAM validation

This pilot study will provide education on data collection for digital twin systems, use of proxy data, theory behind digital twin development (physics or data-driven), and potential applications of digital twin technology, with real-world examples from clinical studies. The LOC pilot will involve validation of R&D equipment (microfluidic chips, perfusion systems) currently under development. The validation of technical outputs is expected to assist the SMEs so that equipment

meets the market needs for future commercialization. Once this the LoC model has been tested and validated, further validation of drugs will be beneficial to inform to confirm the benefit of NAMs as an aid for clinical trials.

## 2.4 Expected Outputs

- Integrated lung–kidney software prototype (open modular framework).
- Validation report comparing predictions with real-world ICU data.
- Best practice case study for inclusion in the NAM Validation Manual.
- Training modules on digital twin data collection, calibration, and clinical use cases.
- Regulatory-compliant documentation (CE/EMA Class IIa software medical device).
- A pilot deployment package for SME and pharma partners to enable preclinical in silico testing
- SME engagement report with pathways for commercialization and regulatory acceptance.
- Model ALI using commercial chips, patient serum, barrier integrity, shear stress, and perfusion (Construction of LoC platform).
- Validate microfluidics equipment and LoC for market readiness and drug testing (through SME collaboration).
- Test Parkinson's drugs in patient-specific organoids for adaptive modelling.
- Provide validated LoC systems for preclinical drug testing and reproducibility.
- Outline regulatory strategies, market alignment, and translational adoption pathways.
- Document definitions, principles, scientific validity, and stakeholder involvement.

# 3. Pilot II: Multi-site Functional Validation of Organ-on-Chip Models

## 3.1 Purpose and Scope of Pilot

Pilot Study II pre-validates established Organ-on-Chip (OoC) test systems through a multi-site study conducted in collaboration with industry partners. Within this study, OoC systems will be evaluated based on their functionality, long-term stability, and predictive accuracy. The objective is to generate robust and reproducible data that will support the broader acceptance of OoC technologies as alternatives to animal testing.

In Pilot Study II, three distinct OoC systems are tested across three independent sites. Submissions will be assessed with priority given to STEP4NAMs partner regions and the North-West Europe (NWE) region, while applications from other European countries will also be considered.

Aligned with the objectives of the STEP4NAMs project, pilot II seeks to strengthen stakeholder confidence in OoC technology. The focus is on SMEs and pharmaceutical companies, demonstrating the reliability and relevance of OoCs as advanced test systems. The study aims to identify and disseminate best practices for OoC pre-validation and contribute directly to the development of training materials and the NAM Validation Manual.

## 3.2 Methodology of Pilot

In addition to the 3R-Center Tübingen, two industrial partners providing their respective OoC test systems will participate in the pre-validation study. Following interviews with these technology providers, additional partners classified as OoC end-users will be recruited to serve as the third testing site for each OoC system.

The study will be implemented in three key phases across three independent testing sites:

1. Assessment of basic functionality and long-term stability

Evaluation of OoC models to determine operational robustness and sustained performance over time.

2. Evaluation of dose-response relationships
   Testing with six well-characterized reference compounds to assess the sensitivity and dynamic range of the OoC systems.

3. Validation of predictive accuracy
   Analysis using six blinded compounds provided by industry partners to confirm the predictive reliability of the models.

Test compound selection will follow national guidelines and be coordinated with the SAB and industry partners. All study protocols will be standardized to minimize testing site variability and to ensure reproducible assessment of the OoC test systems in different laboratories.

### 3.3 How the Pilot will improve NAM validation

Pilot study II generates robust datasets and practical insights into the functional pre-validation of OoC test systems across multiple testing sites. By systematically assessing key parameters, the study identifies best practices, common challenges, and critical success factors in OoC pre-validation. The findings will be compiled into clear, evidence-based guidance documents developed in close collaboration with the Strategic Advisory Board (SAB). These guidelines and best-practice documents will form a core component of the NAM Validation Manual, providing structured recommendations to support functional characterization and pre-validation. Ultimately, this work will contribute to the broader acceptance of OoC technologies as human-relevant alternatives to animal testing.

### 3.4 Expected Outputs

Pilot Study II is designed to facilitate the industrial adoption of OoC test systems as alternatives to animal testing. The study supports SMEs by benchmarking their technologies across three independent sites. Participating SMEs will gain access to unique data on the stability, reproducibility, and predictive power of their OoC test systems – data that would otherwise be unattainable.

The study will demonstrate the technology readiness of the tested OoC platforms, thereby enabling assessment of market readiness and customer confidence among end-users. One of the key outcomes is the strengthening of market position of partner SMEs through independent pre-validation data on their products. Finally, by generating robust, multi-site datasets, the study aims to lay the groundwork for regulatory acceptance of OoCs internationally to be utilized by professionals in industry, academia, and governments.

# 4. Pilot III: Validation of Bench-Top Simulation Systems for Testing Neuro-Thrombectomy Technologies

## 4.1 Purpose and Scope of Pilot

Pilot III focuses on the validation of clinically relevant bench-top test and simulation systems for endovascular technologies. The MET Technology Gateway at ATU has an established track record in developing advanced anatomical test systems that replicate the mechanical properties, geometry, and physiological conditions of the human body. These models enable innovators to evaluate device performance under realistic and ethically robust conditions.

The overarching aim of Pilot III is to develop standardised procedures for validating bench-top simulation systems used in the assessment of mechanical neuro-thrombectomy technologies for the treatment of acute ischemic stroke (AIS). By actively engaging with SMEs developing medtech solutions, the pilot seeks to help stakeholders optimise device design, enhance performance evaluation, reduce dependence on animal studies, and ultimately accelerate R&D timelines while improving cost-effectiveness.

Aligned to the objectives of the Step4NAMs project, this pilot study intends to enable SME's to conduct more effective design verification testing and gain a far greater insight into the challenges faced in treating the target disease:

- Improve clinical relevance of in-vitro models over animal models
- Reduce the number of animals used in pre-clinical studies
- Product Development: Accelerate the design process
- Allow companies to perform repeatable and validated bench top tests for their medical devices

The study seeks to develop knowledge on replicating clot-blood vessel wall adhesions using 3D-bio-printed materials. The goal is to establish best practices for the validation of bench-top simulation for the testing of neuro-thrombectomy technologies through tailored training activities and the NAM validation manual.

## 4.2 Methodology of Pilot

The planned activities of the pilot study include:

- Translate neurovascular medical data into engineering data.
- Manufacture models using a range of processes, including 3D printing and bioprinting and injection molding.
- Protocols to validate geometrical and mechanical compliance.
- Develop industry guidelines for the fabrication of clot analogues and the necessary steps to characterise each clot type.
- Incorporate thin-walled models into a simulation system replicating blood flow rate/pressure/temperature.
- Acute Ischemic Stroke will be created by releasing various clot analogues within the cerebral flow simulator.

The proposed pilot will allow for the collaboration with 2-4 medtech partners who will play a role in:
Informing the design and user requirement specification of the bench-top testing system.
Review and approve the final design of the bench-top system in advance of fabrication.
Review and approve validation protocols.
Conduct device performance tests and provide feedback.

## 4.3 How the Pilot will improve NAM validation

As part of this pilot, MET researchers will identify the specific types of evidence required for regulatory approval of neurothrombectomy devices, with particular attention to pre-clinical testing strategies that demonstrate safety and efficacy. Using this regulatory insight, together with medical data from the scientific literature and real patient cases, the team will translate clinical information into engineering specifications. These specifications will define the mechanical properties, anatomical features, physiological characteristics, and geometrical parameters that must be captured to ensure the models accurately represent the clinical environment faced by interventionists during treatment.

Once these clinically relevant parameters are established, they will be verified through targeted validation tests, providing robust evidence that the bench-top systems accurately reproduce real-world conditions.

## 4.4 Expected Outputs

The primary outcome of this pilot is the development of a robust validation process for bench-top test systems, enabling companies to demonstrate that their models are clinically relevant and aligned with the best available published evidence. A validated process will help ensure that bench-top systems replicate real endovascular interventions as accurately as possible, supporting the development of devices that are safer, more effective, and better prepared for subsequent animal or clinical trials when required.

For several types of pre-clinical tests and for the generation of regulatory technical documentation, these in-vitro systems are expected to surpass traditional animal models in clinical relevance, reproducibility, and suitability for iterative device development. By establishing clear validation criteria and demonstrating the superior fidelity of these systems, the pilot aims to provide medtech developers with stronger, more reliable pathways for testing, refining, and derisking innovative endovascular technologies.

# 5. Pilot IV: Validation of Parkinson's Disease Organoid

## 5.1 Purpose and Scope of Pilot

In this pilot study, our main objective is to offer a case study to help leverage the relevance and applicability of organoids and increase the confidence in NAMs for drug development and testing. Specifically, we aim to predict the failure of promising Parkinson´s disease (PD) drug candidates effective in pre-clinical studies but failing in human clinical trials.

## 5.2 Methodology of Pilot

The pilot design is based around a drug validation study using PD patient-derived midbrain organoids which will be subjected to a battery of different drugs and importantly, we intend to integrate 2-4 different drug candidates/compounds provided by stakeholders (SME from BP sector) from the NWE region. The integration of our targeted sector in the design of the pilot study will ensure that the outcome will be tailored to be easily taken up by other stakeholders thus maximizing the pilot output potential.

The planned activities of the pilot study include:

1. Generation and treatment of the PD midbrain organoids (3 age-matched PD and control cell lines)
2. Evaluation of the impact of drugs using high content imaging on key PD disease-associated hallmarks (e.g. loss of dopaminergic neurons and mitochondrial metabolism)
3. Determination of the predictive value (refute or prove pre-clinical tests) of the PD midbrain organoid model using cross-validation bioinformatical tools

## 5.3 How the Pilot will improve NAM validation

By demonstrating the potential of organoids in the context of pre-clinical drug testing, we anticipate more potent uptake of this disease model in the BP and related sectors. This particular pilot study will showcase the potential and value of pre-clinical testing in organoids compared to animal models. Additionally, our comprehensive case study will support NAM adoption by demonstrating that organoids for pre-clinical testing offer a cheaper and more efficient drug testing platform.

## 5.4 Expected Outputs

The main readout of the pilot study will be the molecular and cellular characterization of PD hallmarks in treated brain organoids in order to define the efficacy of drugs in diminishing or rescuing the PD phenotype. The experimental setup is designed to showcase the potential and value of *in vitro* organoid-based pre-clinical testing in predicting failure of compounds without the unnecessary use of rodents. Thus, we will generate an extensive dataset and protocol framework to help make data-driven decisions for which drugs have the potential to advance to clinical testing. Interestingly, by providing a comprehensive dataset, secondary data analysis will be possible to companies centered around machine learning-based models that aim to predict the efficacy of drugs before clinical testing.
To conclude, we expect to demonstrate that the use of organoids for pre-clinical testing will ultimately benefit SMEs and Big Pharma by offering a faster and more efficient testing platform.