Health by Advanced Therapies

D 2.7

Completion of selection and description of regulatory acceptable “contexts of use” for advanced animal vs human-on-chip model developments

Public

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Deliverable description

The current deliverable represents part of the activities done within the Working group 6 "Preclinical model systems: *in vitro* and *in vivo*".
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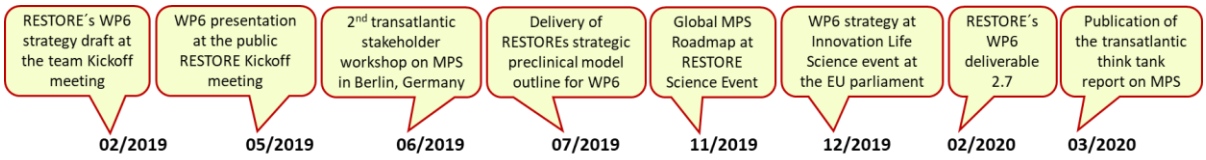
Introduction

RESTORE`s working group (WG) 6 activities of the Phase 2 programme focussed on the development of a 10-year roadmap towards microphysiological system-based human organ-on-a-chip and body-on-a-chip models enabling ever more predictive preclinical context-of-use assays to evaluate safety and efficacy of advanced cell therapies. The execution of this roadmap should substantially reduce the unacceptably high therapy attrition rate in clinical trials due to low predictive power of current preclinical in vivo and in vitro approaches. Within RESTORE and on a global scale the eight steps, illustrated below, have been carried out to finally deliver this European roadmap for the concerted development of cutting-edge MPS-based regulatory acceptable context-of-use assays to deliver advanced cell therapies for patients at increased speed and substantially reduced costs.



The ultimate ten-year goal of WG6 of the RESTORE programme is the establishment of a human artificially "intelligent", automated, high content "Patient-on-a-Chip" platform for predictive testing of advanced cell therapies by emulating RESTORE-defined chronic diseases at an individual patient level to a degree, comparable to that of the clinical trial phase 2 environment of the respective patient population. Predictive power of such a platform should be compared head to head with phase 2 clinical trial data and retrospectively be weighed against best in class animal models used in the selected indication.

A broad range of context-of-use assays based on singe- and multi-organ MPS models are expected to be developed within a short-term 4-year period and a mid-term stage from year 5 to 7 to create a basis for a final step towards holistic organismal "Patient-on-a-Chip"-based assays. The latter development will last from year 8 to 10 of the RESTORE programme.

Financing and execution of the 2021-2030 roadmap, outlined below, is Europe's unique opportunity to shift the drug development paradigm towards curing chronic diseases in unmet

medical need areas by affordable advanced cell therapies. Finally, the RESTORE programme is Europe´s last chance to regain a leading position in delivering advanced therapies for patient´s benefit in comparison to US and China.

The European 2021-2030 roadmap towards a paradigm shift in advanced therapy development

RESTORE's cell therapy development will be supported by three stages of MPS-based assay establishment in the frame of WG6: a first 4-year short-term stage, a second mid-term stage lasting from year 5 to 7 and, finally, a third long-term stage lasting from year 8 to 10. Here we defined those contexts-of-use, which will have the highest social and economic mid-term and long-term impact for our society. With regard to assay priority RESTORE´s selection has been defined by three criteria:

i. Urgency of unmet medical needs for a respective indication,
ii. Ethical aspects and social impact of a given disease, and
iii. Level of human biology necessary to be emulated to support the expected mechanism of action of the particular advanced cell therapy under evaluation.

1. Prioritized contexts-of-use assays for the short-term stage (2021 – 2024)

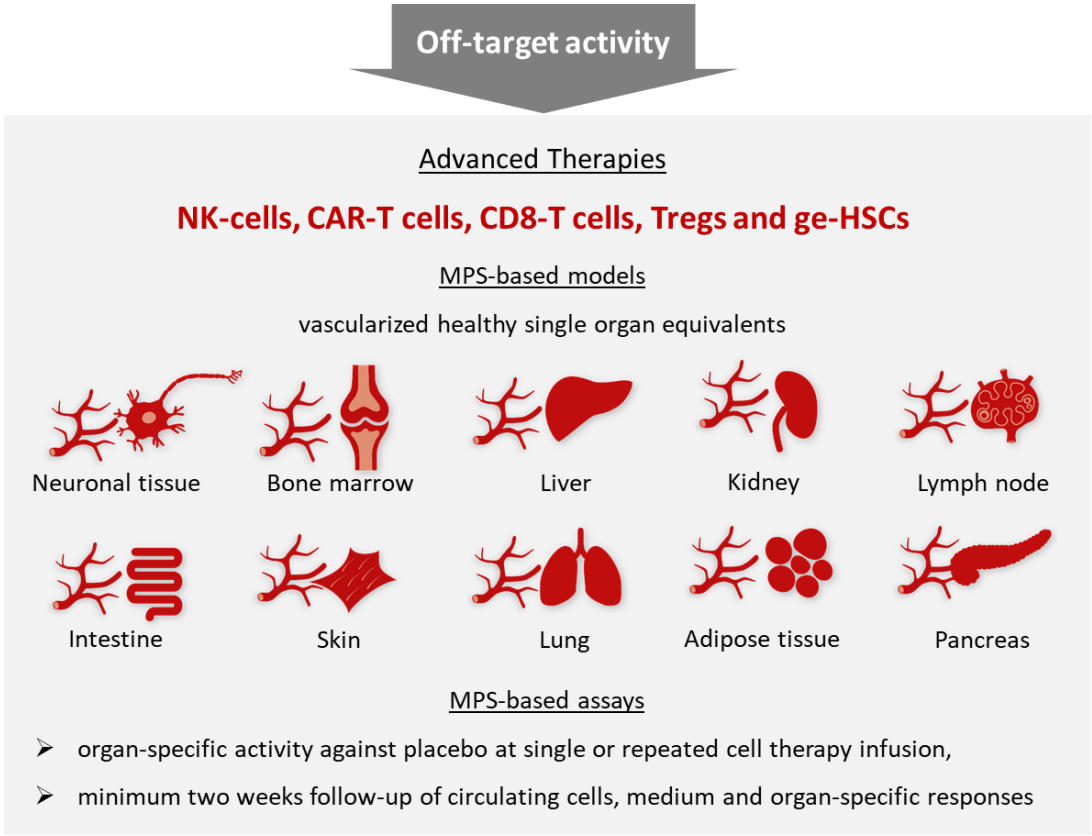
The short-term programme will focus on the establishment of ten robust off-target activity test assays based on ten healthy vascularized single-organ models.

Off-target activity is the biological activity of a cell therapy that is different from and not at the site of its intended biological target. It most commonly contributes to side effects, such as unwanted toxicity. For approved CAR-T cell therapies some of them have been recently reviewed (Zheng et al., 2018).

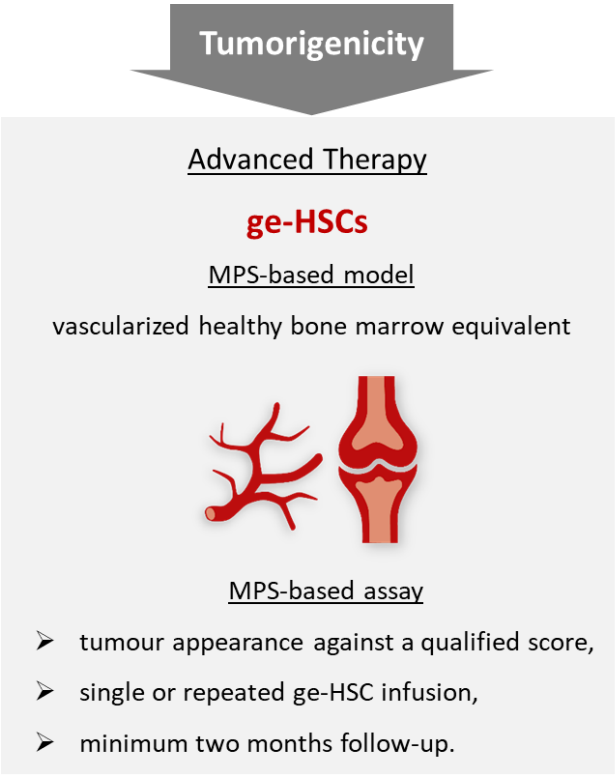
Here, we focus on those ten organ models, which in the long-term stage of the programme are capable to constitute minimal organismal homeostasis, when collectively being integrated into a single "Body-on-a-Chip" model. To meet physiological conditions at off-target sites a human-like network of endothelial cell-covered blood capillaries supplying nutrients and cells to each organ at relevant flow rates is essential. All five advanced therapies under evaluation - natural killer (NK) cells, CAR-T cells, CD8-T cells, regulatory T cells (Tregs) and gene edited haematopoietic stem cells (geHSCs) – need to cross the endothelial barriers to home into their target sites. To ensure later integration into higher order multi-organ chips, the same downscale factor has to be applied for each of the ten selected MPS-based fully vascularized functional organ model established at this first stage.

The models to be established within the first four years (2021-2024) of development are indicated in the schemes below. The cell therapies selected for evaluation within a defined context are highlighted. Organ icons present the MPS-based organ models to be established for a given assay. Finally, the schemes sketch basic assay parameters.

Due to the human nature of the selected advanced cell therapies off-target cell homing and organ-specific activity are quite species-specific. Adverse effects, such as cytokine storms, might be caused by product impurities constituted by other cells than the effective cell population or mismatches in histocompatibility, for example.



Simultaneously, a tumorigenicity assay should be established to accelerate risk assessment for tumour induction by gene-corrected haematopoietic stem cells, which are supposed to cure rare genetic diseases. The scheme below illustrates, that the



model background for such a context of use is the vascularized bone marrow, also included in the off-target activity assays. New read-outs and follow-up timelines need to be developed for such a tumorigenicity assay on the basis of such a long-term stable MPS-based model. The major challenge here is to define and qualify the right scoring background against tumorigenic reference cell lines or cells at a comparable and pathophysiologically relevant microenvironment. Due to its exceptional importance such an assay should ideally be developed with the direct scientific advice and input of EMA and relevant regional regulatory agencies.

Summarizing, the first 4 years of RESTORE´s WG6 should establish 11 assay formats on healthy single-organ models for five different advanced cell therapies which results in 55 context-of-use assays for a single exposure regimen. The number of assays will double with repeated exposure schemes for each therapy due to the different context-of-use. The established vascularized single-organ portfolio provides the basis to go for disease-related models in the next stage.

2. Prioritized contexts-of-use assays for the mid-term stage (2025 – 2027)

Potency, efficacy and "safficacy" assays should be developed at this stage based on the transition of some of the established healthy organ models into diseased organs and the integration of single-organ models into multi-organ arrangements for selected prioritized advanced therapy indications.

Potency is a measure of pharmaceutical drug activity expressed in terms of the amount required to produce an effect of given intensity. For living cell therapies, potency assays are targeting quantifiable parameters reflecting target site responses of the diseased organ. These assays are still in their infancy for advanced therapies, which hampers batch release procedures and

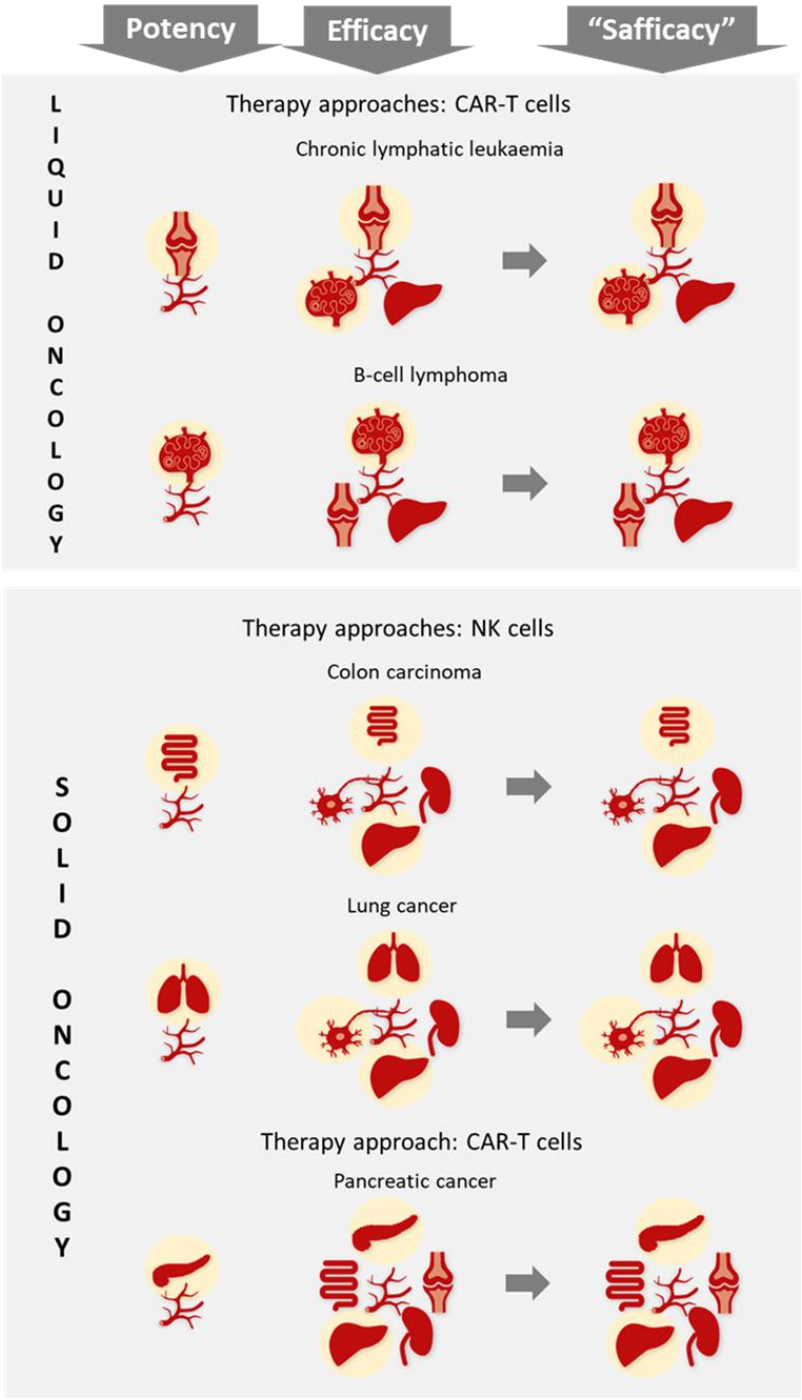
batch-to-batch consistency analyses in GMP-manufacturing. It furthermore complicates dose-finding approaches and, therefore, delays cell therapy approval processes.

Efficacy is both, the maximum response achievable from a pharmaceutical drug in qualified test settings, and the capacity to generate a sufficient therapeutic effect or beneficial change in clinical settings. For cell therapies, efficacy is exclusively dedicated to the target sites within diseased organs and tissues also these sites can be spread around patient´s body, for example, in case of metastatic malignant cancer.

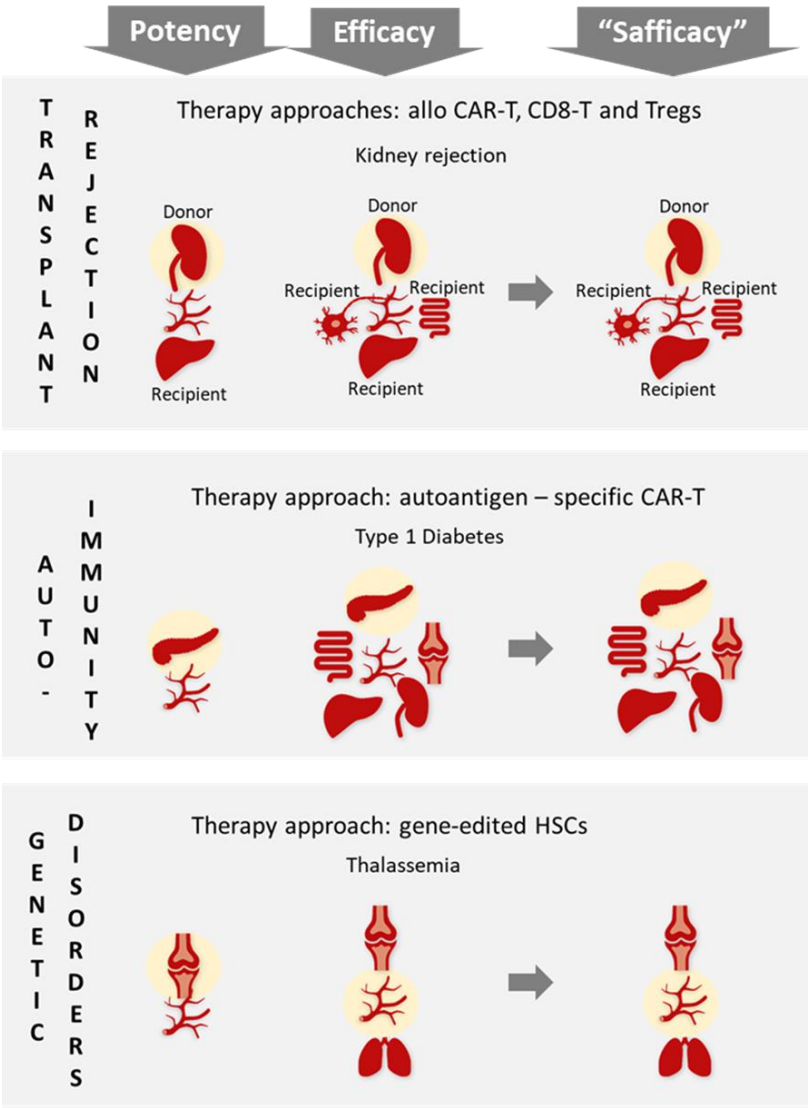
"Safficacy" is an artificial term combining the terms safety and efficacy. It was first used in connection with a qualified microfluidic multi-organ chip platform interconnecting a target human tumour tissue with a healthy human skin equivalent (Huebner et al., 2018). Such systems simultaneously provide read-out options for both, off-target safety in organs, not affected by the disease, and efficacy at target sites. For the five advanced cell therapies, evaluated in RESTORE such qualified preclinical assays might add scientific and regulatory value and speed up their development cycle.

The following schemes are summarizing the MPS-based models and the respective assays to be developed for the targeted five advanced therapies at their selected indications within the highlighted diseases areas at the mid-term stage. All models are supposed to be vascularized to provide a physiological transport background for the systemically applied cell therapies. Organ models are again represented by icons. Organ models highlighted in yellow are the diseased once, which contain the target sites. The others don´t.

The oncology portfolio consists of 5 potency and 5 efficacy assays to be established (2 of each for liquid and 3 of each for solid tumour therapies). The potency assays are based on vascularized single-organ disease models containing the target sites whilst the efficacy assays are already based on disease-specific vascularized multi-organ combinations, which consider secondary metastatic target sites and those non-affected organ models, which contribute to the long-term performance of the particular MPS-based disease model. The 5 "Safficacy" assays of the programme use essentially the same multi-organ model background but add safety read outs derived from non-affected organs to the context-of-use. Indications have been selected considering chronic course, unmet need and number of affected patients.



In addition to the oncology field, kidney transplant rejection, Type 1 diabetes autoimmunity and Thalassemia, a monogenetic congenital disease, have been selected for potency, efficacy and "safficacy" assay developments due to their urgent unmet medical need, chronic nature of the underlying diseases and their ethical and social impact for our society. The schemes below illustrate the models and assays targeted for these indications at mid-term stage. Again, except of the kidney rejection potency assays, which requires a two-organ combination (a donor and a recipient organ model) the other potency assays are based on vascularized single organ models and improve towards multi-organ arrangement at efficacy assay level. Finally, "safficacy" assay development again uses the efficacy model background.



Summarizing, the mid-term stage (2025-2027) of RESTORE´s WG6 aims to establish 7 assay formats on the level of vascularized single-organ disease models and 16 assay formats on the level of vascularized multi-organ disease models incorporating unaffected organs. All 23 models support single or repeated dose infusion regimens of the advanced therapy products in use.

3. Prioritized context-of-use for Europe´s "Patient-on-a-chip" platform (2027-2030)

The third and final stage of the RESTORE programme of WG6 will last three years and should implement at least one qualified regulatory acceptable assay format of holistic organismal complexity into one of the five advanced therapy product approval processes. The assay should be capable to identify a personalized therapeutic window for any of the five advanced therapy products in combination with standard care in any of the selected indications.
A therapeutic window is the range of doses of a pharmaceutical drug, which optimize between efficacy and toxicity, achieving the greatest therapeutic benefit without resulting in

unacceptable side effects or toxicity. It is established in cohorts of patients of the same gender with comparable age and disease backgrounds.

A (hypothetical) personalized therapeutic window optimizes between efficacy and toxicity at the level of an individual patient. Nowadays such personalized therapeutic windows cannot be assessed for any individual patient due to lack of a statistically relevant number of biological repeats of that particular patient for analysis.

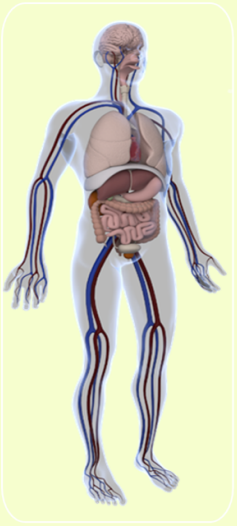
The decision, which context-of-use assay to become RESTOREs preclinical role model based on the established “Patient-on-a-Chip” platform will be done at the end of the preceding development of stage two. The establishment of such an organismal on-chip platform should follow latest strategies of the MPS-community to first establish a MPS-based human universal physiological template (UPT), which represents a minimal self-contained homeostasis of a healthy “Body-on-a-Chip” model (Dehne and Marx, 2020).

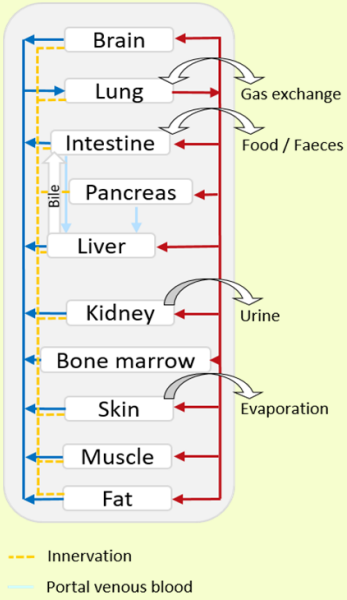
Subsequently, such UPTs can be developed into various disease models (Marx et al., 2020 in press) and thereby translate into disease-specific pathophysiological templates (PPTs). Mechanisms occurring naturally during development of human pathophysiology, such as tumour induction, integration of an HLA-mismatching organ model into a recipient organ background, exposure to an autoantigen, or use of organ models from donors with congenital diseases can be explored. These will allow translating a UPT into a PPT or generating a MPS-based organismal disease model from the scratch within the RESTORE WG6 programme. The scheme below is adopted from the publication of Dehne and Marx, 2020 and illustrates the organ set and arrangement to generate a UPT. It, furthermore describes the basic disease model and assay requirements at a glance. The assay should support oral, topical and systemic exposure of drugs and cell therapies and, therefore, should enable comparative testing of various combination therapies at automated high content throughput.

Identification of a personalized therapeutic window

Advanced Therapy plus standard-of-care

NK-cells, CAR-T cells, CD8-T cells, Tregs or ge-HSCs in combination with relevant standard-of-care single- or multi-drug therapies





MPS-based model

personalized healthy UPT and disease-specific PPT of each donor at statistically representative numbers of biological repeats

MPS-based assay

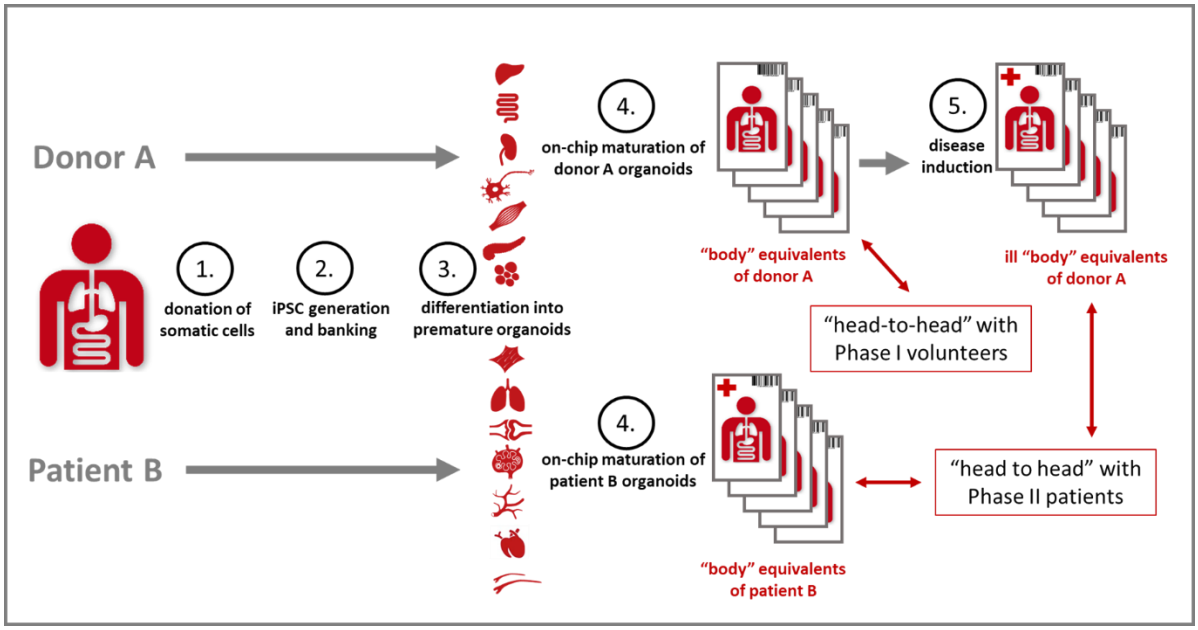
Quantitative on-target efficacy and organ-specific and systemic off-target activity at single or repeated dose combination therapy schedules derived from statistically representative of biological repeats of PPTs against placebo PPTs and therapy exposed UPTs of the particular donor,
Follow-up time line should match phase 2 clinical trial targets.

The basic biological functions required for the generation of the illustrated organismal homeostasis of a UPT include the intake of oxygen, the intake and processing of nutrients for energy provision, whole blood generation and distribution, systemic regulation through innervation, first line immune defence and excretion. Therefore, the minimal set of organ models required to enable a self-sustained organismal homeostasis are:

1. Circulatory – including a cardiovascular and a bone marrow model for blood transport and haematopoiesis
2. Respiratory – including a lung model for blood oxygenation
3. Digestive – including an intestinal and a liver model for absorption, metabolism and protein production
4. Endocrine – including pancreatic islets for glucose regulation via insulin
5. Urinary – including a kidney model for excretion
6. Integumentary – including skin and adipose models for barrier generation and storage of substances
7. Musculoskeletal – including muscle for metabolic homeostasis
8. Nervous – including a brain model and nerve projections for the generation and processing of neuronal signals and innervation
9. Immune – including innate immunity for first line defence

Finally, the platform becomes donor- or patient-specific and, therefore, personalized, when all integrated organ models can be generated from an autologous cell source. The differentiation

of induced pluripotent stem cells from a donor or patient into the different organ models is envisioned to become a possible solution, as recently highlighted by the European Investigative Toxicology Leaders Forum (Beilmann et al., 2019). The figure below is adopted from this publication and sketches the leaders forum hypothetical roadmap towards “clinical trials” on a chip envisioning minute personalized “body” equivalents assembled on chips and derived from cells of individual healthy donors or patients under ethically acceptable conditions.

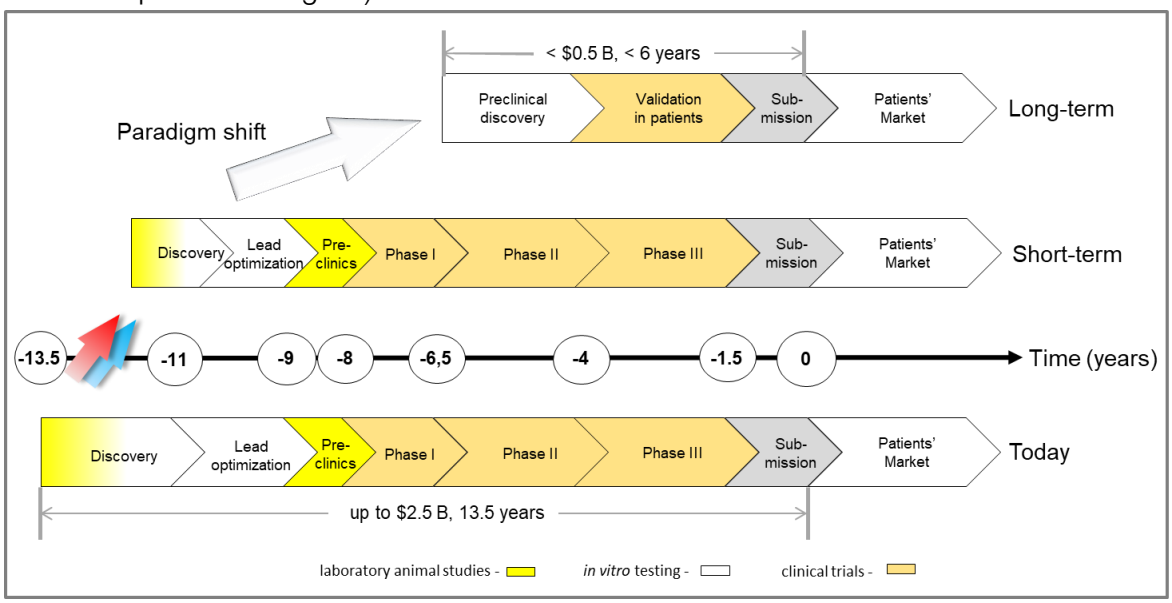


A first proof of concept for such a strategy has been recently provided within a physiology-based human 4-organ chip setting combining intestine, liver, kidney and neuronal models, collectively differentiated from induced pluripotent stem cell source of a single donor (Ramme et al., 2019).

Impact statement

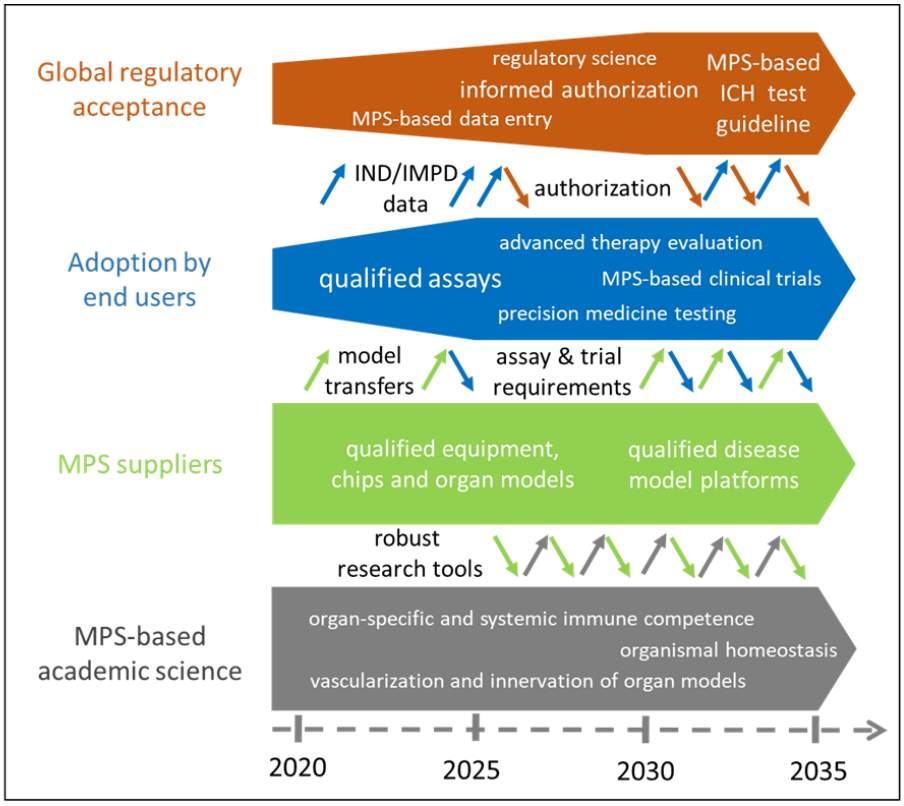
RESTOREs first short-term stage of WG6 will result in highly demanded assays in the context of off-target activity assessment introducing a portfolio of vascularized single human organ models. Execution of the second mid-term stage of RESTORE´s WG6 programme will generate MPS-based context-of-use assays predicting potency, efficacy and safety aspects of the selected five types of advanced therapy products at single-and multi-organ disease model level. It is envisioned to qualify a number of stage one and stage two MPS-based assays for industrial portfolio decision-making along the advanced therapy product development cycle. Data generated by such qualified assays can already become part of the IND/IMPD submissions for European advanced therapy products within four to seven years.

Executing the long-term RESTORE programme of WG6 will have a paradigm-shifting impact on advanced therapy development and on the entire drug development life cycle in general. The first stakeholder workshop of the MPS community held in Berlin in 2015, has estimated a reduction of the drug development costs by factor five at half of the time spent, if human "Body-on-a-Chip" platforms will generate regulatory accepted preclinical assays at organismal homeostasis (Marx et al., 2016). The figure below is adopted from this open-access publication and illustrates how significant MPS-based approaches and tools can shift the current drug testing paradigm (at the top of the figure) compared to current drug development phases (at the lower part of the figure).



No matter which of the indications and which product will be selected for the role model qualification, it definitely will support one of the most advanced cell therapies, will enable to solve an urgent unmet medical need and will have a major impact on ethical and social aspect of modern health care in our European community.

A global roadmap towards patient's benefit and animal's welfare has been recently sketched by the 2nd MPS stakeholder workshop, a transatlantic think tank on toxicology held in June 2019 in Berlin, Germany. It envisions "Patient-on-a-Chip" platforms to materialize way after 2030 as illustrated in the figure below adopted from the respective publication (Marx et al., 2020 in press). Brown, blue, green and grey arrows represent the influence of academia, MPS suppliers, end users and regulators, respectively, on other stakeholders in the process of development, transfer, use and data assessment of MPS-based models and assays.



The current global situation provides an unique and unprecedented opportunity for Europe to become THE Nr. 1 place for the development, pharmaceutical adoption and regulatory acceptance of "Patient-on-a-Chip" platforms. Consequently, Europe can become the leading driver for a paradigm shift in drug and cell therapy development in our century. Such a paradigm shift bears the potential to make curative advanced cell therapies affordable and accessible for every patient in the future. It furthermore will affect the pharmaceutical drug landscape with the same impact. Healthy human "Body-on-a-Chip" and "Patient-on-a-Chip" tools will enable the next ground-breaking basic discoveries in human life science and will generate the next generation of Nobel price laureates in medicine. The impact of such platforms on laboratory animal reduction is expected to be the largest since laboratory animals exist. Finally, "Patient-on-a-Chip" platforms will make the foreseeably largest social and ethical impact on restoring the innovation power of the global health care system in the 21st century. Europe should not miss this last window of opportunity.

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