Health by Advanced Therapies

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Roadmap of WP2
Mission driven basic science and technology development

Public

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1. Deliverable's description

This document describes the roadmap of Work Package 2 (WP2) of RESTORE: The Definition of the Needs and Strategy of Mission-Driven Basic Science and Technology Development for Advanced Therapies. The purpose of this document is to outline the major challenges in basic research and technology in different areas pertaining to Advanced Therapies, as identified by RESTORE, and the proposed strategies by which RESTORE aims to overcome the identified barriers.

Objectives

To build a road map to navigate the complex landscape of realising Advanced Therapeutic Medicinal Products (ATMPs) as a standard of care across Europe. RESTORE aims to become a trusted source for those involved in all stages of ATMP development. As such, we have divided the road map into 3 main topic areas, which may include further sub-topics:

I. Foundational Research (new targets and new indications)
II. Preclinical Models and Technologies (focusing on human-on-chip)
III. Manufacturing Technologies (including product characterisation and automation)

Within these areas, RESTORE proposes strategies that aim to push forward advances in Advanced Therapies themselves, as well as strategies that will increase the pace of translation and development of ATMPs as commercial products by standardisation of protocols across the EU.
The road map is a constantly evolving document that we are working on and improving continuously as RESTORE develops

Road map: Mission driven basic science and technology development

2. Foundational Research (new targets and new indications)

2.1 Support of Endogenous Regeneration

2.1.1 State of the art

- In principle, there are 2 ways of pursuing regenerative medicine: i) support of endogenous regeneration, and, if this is not feasible, ii) replacement strategies
- Support of endogenous regeneration can be addressed by direct support (recruitment, stimulation, reprogramming) of parenchymal stem/progenitor cells (growth factors etc.), and indirectly by reshaping undesired inflammation/immunity (immunoregulation/immune reconstitution) or modelling extracellular matrix (ECM code)
- Tools of advanced therapies for the endogenous regeneration approach (please note, there exist also non-ATMP approaches not outlined here):
    - cell therapy (MSC, MSC-like cells, Treg, Mreg …+/- gene modification) or cell-derivatives (extracellular vesicles/exosomes),
    - combined products (e.g. cells + scaffold, theragnostic cellular devices for release of growth factor combinations),
    - *in vivo* gene therapy (delivery of growth factors, RNAi…)
    - Combination of ex vivo pre-grown micro tissues (tissue engineering) with in situ formation of larger tissue structures in the local environment together with additional support of endogenous regeneration ("triggered" endogenous regeneration)

Current indications: acute trauma (to prevent chronification), cardiovascular diseases, wound healing problems, stroke, immune diseases, organ transplantation and GvHD, immune reconstitution for severe viral infection in immunocompromised patients, cancer (cancer immunotherapy => see WG cancer), progressive loss of insulin secreting cells (T1 and T2 diabetes), diseases with altered immune response and fibrosis (i.e. lung fibrosis), Neurodegenerative diseases characterized by altered immune response (e.g. MS) and demyelination.

- Many trials were running with MSC/MSC-like cells but also other approaches (Treg, Teff, EV, gene therapy…) but few products approved so far – Why?
    - Poor product quality and characterization, high variability
    - Poor scaling-up capacities for later stage clinical trials
    - Insufficient funding at academic centres and start-up companies which frequently did drive the trials
    - Many data of trials are not published yet
    - Missing mechanistic side studies to understand failure/success

2.1.2 Major challenges and roadblocks to be addressed

- lack of efficacy in later stage trials because of
    - poor understanding on mechanisms of "failed" endogenous regeneration, particularly in aged population

    - missing biomarkers to identify patients where endogenous regeneration is still feasible and which approach might be the best one
    - improved product supply chain and delivery
    - the need to identify inflammatory biomarkers to both define the right time point of the therapy and also to monitor and predict response to treatment
    - poor biological understanding of niche environment
    - poor understanding of the correlation between inflammation and tissue degeneration, and inflammation and tissue regeneration
    - Imprecise concepts in biomaterial development for improved delivery, mechanic stress response etc.
    - lack of predictive preclinical *in vitro* and *in vivo* models – currently used models has several limitations
    - Lack of consistent manufacturing
    - lack of understanding of efficacy mediated by multiple cell populations either in a suspension or structured in a 3D pattern
    - Lack of standardization of biological components (cells, biomolecules, extracellular matrices due to inter-individual differences).
    - Lack of understanding of pathologies and disease to define efficient therapeutic products.
- Many developments academic-driven => limited resources for phase IIb/III
- Non-homogenous cell products (e.g. MSC)
- Problems with scaling-up manufacturing
    - inherent in cell sourcing
    - related to the rapid aging of cell progenitors \ stem cell *in vitro*
    - on-site production in a closed environment GMP-Compliant
- Need for next-generation products (more effective, more targeted, better cost effectiveness)
- Need for exploring new sources of therapeutic cells that overcomes the current limitations regarding the limited functionality and survival of cells (i.e. undifferentiated cells from cord blood, thymus, paediatric origin)
- novel imaging modalities to assess stem cell related disease or repair
    - necessity for a long term biodistribution assay which should be sensitive and not diluted by cell duplication
    - real time imaging on cell-cell interaction in the niche
- logistic challenges such as alternatives to cryo-shipping (hypothermic storage of cells)

2.1.3 Overall Goals

To develop safe and highly effective approaches to support endogenous regeneration for the pre-emptive or curative treatment of chronic diseases with high health-economic impact

2.1.4 Scope- Where can RESTORE make a difference

Long-term vision (8-10 years):

- less chronic diseases after acute injury or early life diseases
- To dramatically reduce incidence of chronic disease after acute events, like acute kidney injury, major trauma, stroke, traumatic brain injury, treatment of neurodegenerative pathological conditions, myocardial infarction, diabetes …
- Cure or at least deliver sustainable therapeutic efficacy of chronic immune diseases
- To sustainably reshape undesired immune response in autoimmunity and transplantation allowing weaning chronic immunosuppression and preventing chronic organ failure
- Reduce severe late complications in chronic diseases, like T2 diabetes, cardiovascular diseases

- To improve sustainable the outcome of chronic diseases with high unmet medical need
- *Restitutio ad integrum* after successful cancer therapy (see WG Gene/Cell/Tissue replacement strategies)
- To support repair processes/regeneration of affected tissue after successful treatment of cancer
- Reverse degeneration
- To leverage current understanding of senescence and cell reprogramming/ rejuvenation for the development of therapeutic programs supporting endogenous regeneration at the tissue and organ level
- To reverse degenerative processes of chronic inflammation e.g. after successful treatment of autoimmune diseases such as multiple sclerosis, psoriasis, lung diseases…)
- To reduce, or prevent, the development of chronic diseases after early-life insults.
- To promote regeneration processes led by the activation of progenitor cells or dedifferentiating cells.
- To establish a new paradigm in the treatment of a wide range of human diseases by exploring the effector capacity of immune cells (i.e CAR T cells, NK cells) or homeostatic and suppressive skills of regulatory cells (Treg, MSC).
- To Develop Personalised Medicine (PM) for an optimised strategy for prevention, diagnosis and treatment of disease for each individual person, based on his or her unique characteristics.
- To provide a "holistic" view on regeneration by combining knowledge gathered from different organ systems and with different technologies (cells, materials, etc...). Learning from successful examples with the goal to apply principles to other organs.
- To learn from organ and tissue development to identify targets (cells, factors, ECM) and time points for a therapy that aims at preventing failure of regeneration.

Short-term (next 3-5 years):

- Better understanding of “failure” of natural endogenous regeneration (senescence, fibrosis, scar formation, organ failure) to identify therapy targets and patients
- Definition and development of “master” products applicable by minor manipulation for defined medical indications (like MSC, PLX, Treg, cell-derived EV ....)
- Strategy for off-the-shelf products (immunogenicity issue)
- strategy to maximize efficacy of cell derivatives (i.e. microvesicles/secretome) and combinations with biomaterials
- Smart manufacturing of “master” (“blueprint” backbone products) cell products
- Potency markers relevant for the mode-of-action of the master cell products
- Biomaterials for composite advanced therapy product approaches
- AI tools for matching patients and products
- Standardized Tools for therapy response monitoring (PK/PD, safety, MoA, surrogate Biodistribution)
- Innovative manufacturing approaches with reduced cost dependent on the source (allogeneic, autologous) as central or on-site approach
- Scalable processes for advanced therapy products

2.1.5 Expected key deliverables for 4-5 years

- Biomarker platform available for the translational hub network:
    - to identify patients who have a high benefit from a special therapy
    - that allow for the determination which time point should inflammation be “targeted” in order to observe a therapeutic effect- in other words determination of window for treatment
    - for monitoring the response to treatment
- Application of state-of-the-art tools for the identification of cell subpopulations involved in successful and impaired endogenous healing to define new targets

- Novel, more relevant, potency markers for “master” cell products which can be used with minimal modifications for many applications, e.g. Treg, MSC
- TRL up to Clinical PoC trials with >10 next-generation products for endogenous regeneration with higher efficacy and low toxicity (next-generation Treg such as Car-Treg, targeted manipulated MSC [like] …), transitory gene therapy (local growth or other supportive factors delivery)
- PoC of applied Artificial Intelligence (AI) for >3 AT products
- Tools to non-invasively monitor *in vivo* bio-distribution of cell therapies in the clinic over extended periods of time
- Development of >2 composite product approaches for endogenous regeneration
- Definition of optimal molecular approaches for inducing in situ trans differentiation, e.g. pancreatic alpha to beta cells (=> link to WG “gene, call and tissue replacement” on replacement of irreversibly injured tissue)
- Development of tools of synthetic biology focused on regeneration that comprise engineered cells that will sense the local condition and respond by production of desired growth factor combination suited for selected therapeutic application
- White paper on trends and needs of endogenous regeneration

2.2 Gene, cell and tissue replacement

2.2.1 State of the art

As stated by Dunbar (2018) "After almost 30 years of promise tempered by setbacks, gene therapies are rapidly becoming a critical component of the therapeutic armamentarium for a variety of inherited and acquired human diseases."

The two essential tools that are currently widely used with success in clinical studies are vectors derived from adeno-associated viruses (AAVs) and lentivirus. Although these tools are the basis of several recently approved gene therapies, there is still much to do to fully exploit their potential for treating diseases that are currently without any specific treatment.

In addition to a rapid change in the vectors used for gene therapy based on gene addition, the Advanced Therapy Medicinal Products (ATMPs) field is also undergoing a transition from gene addition to editing approaches, which must, however, still be validated in clinical trials. These draw on a fast-expanding arsenal of CRISPR/Cas and other sequence-specific targeting technologies, and allow gene, base, epigenome and even RNA editing, with a plethora of potential therapeutic applications. Genome and epigenome editing technologies (not only based on CRISPR/Cas) are still mostly in the pre-clinical phase, but the first trials are already ongoing, and they are expected to play an increasing role in the field of gene therapy.

2.2.2 Major challenges and roadblocks to be addressed

Gene and cell therapy (transversal activities)

- Definition of mechanisms leading to immune-rejection/short-term stability of cells transduced or edited both *ex vivo* and *in vivo*
- Development of assays to predict genotoxicity of the editing reagents
- Further improvements to the safety of delivery vehicles and reagents for gene editing approaches
- Development of biomarkers to monitor safety and efficacy of ATMPs
- Harmonization of release testing and quality controls, both in process and on final gene/cell product
- Reduction of cost for GMP-compliant cell products
- Development of disease models (including organoids) and definition/further characterization of disease mechanisms (including gene discovery)

Gene and cell therapy (*in vivo* applications)

- Targeting the vector (both viral and non-viral, the latter for editing reagents) to specific tissues/cell types/differentiation stages at efficiencies compatible with clinical use
- Immunogenicity of the gene therapy components (including transgenes and delivery tools)
- Development of assays to monitor long-term toxicities and therapeutic efficacy
- Development of new vectors for the delivery of large genes
- Lack of effective strategies to target compromised organs (e.g. advanced fibrosis)
- Paucity of immune-compatible donors for cell/tissue transplantation (development of universally transplantable products)
- Standardization of "master cell" products

Gene and cell therapy (*ex vivo*)

- Improve cell/tissue engineering strategies (e.g. develop stealth gene transfer/editing procedures, further optimize culture conditions to preserve cell fitness/genomic integrity, increase gene transfer/editing, *ex vivo* expansion and selection of correctly-edited cells)

- Optimizing cell source for *ex vivo* gene and cell therapy applications (advanced purification of highly potent cell subsets, such as HSC or stem-like T cell subsets; alternative sources, such as immuno-compatible allogeneic cells or pluripotent-cell derived products for "off-the shelf" manufacturing)
- Reduce the need for potentially genotoxic recipient conditioning to allow efficient engraftment of *ex vivo* engineered cell therapy products
- Novel transplantation routes to allow local engraftment, e.g. in brain and lung
- Promote *in vivo* engraftment and/or expansion of transplanted engineered cells
- Determining the optimal timing and cell dose for gene and cell therapy
- Determining the optimal "cell dose"
- Determining the appropriateness of sequential cell product injection

2.2.3 Overall Goals

To develop safe and highly effective cell and gene therapy approaches to prevent and/or treat conditions with a high, unmet clinical need and for which current technologies could be transformative. To expand the breadth of gene therapy by including autoimmune diseases, viral infections, metabolic diseases, chronic degenerative diseases, neurodegenerative diseases, age-associated diseases and cancer.

2.2.4 Scope- Where can RESTORE make a difference

Short-term vision (next 3-5 years)

1. Safety and immunogenicity of gene and cell therapy:

- Harmonize tools and assays to monitor efficiency, safety (including off-target), immune response, etc.
- Dissecting biological consequences to gene transfer/editing in relevant models
- To further elaborate predictive models (both *in vitro* and *in vivo*) taking advantage of data from on-going/closed clinical trials
- To develop strategies to circumvent pre-existing immunity against gene therapy vectors
- To identify the most suitable cell type for gene and cell therapy applications, develop advanced purification and expansion methods compatible with clinical translation, and optimize their engraftment and long-term repopulation potential

2. New clinical applications:

- Foster clinical translation of novel ATMPs (including bridging approaches) for disease indications in which the risk/benefit ratio is high and standard gene therapy strategies (e.g. viral-based) are not applicable
- Understand why current and past clinical trials (including gene disruption in HSPCs) failed or succeeded in only a number of patients (responders/non-responder analysis)
- Implement/develop strategies to monitor long-term gene therapy patients (cross-border patients' registries, possibly based on ERN registries for genetic disorders, such as RADeep/PKDeep https://www.eurobloodnet.eu/radeep)
- Identify new groups of diseases for which the currently available cell & gene therapy approaches could work with minimal improvement/development
- Improve specificity and efficiency of delivery systems (viral and non-viral) to relevant cell types/tissues/target organs, also opening to those not yet within the reach of gene therapy
- Develop disease models that more accurately recapitulate disease pathophysiology
- Introduce autonomous and externally controllable therapeutic and sensing organoids able to interface with the external environment

3. Costs

- Identify underlying reasons that are stopping cell/gene therapy in reaching the clinic for specific indications where pre-clinical results are promising
- Devise a strategy for lowering the costs of cell/gene therapy clinical trials
- Streamline the regulatory process
- Fill the gap between academy and CMO by investing in new facilities for the production of ATMPs

Long-term vision (8-10 years):

- To develop new gene therapy tools characterized by a higher efficiency, safety and specificity profile for both *ex vivo* and *in vivo* applications
- To develop strategies to decrease toxicity associated with gene and cell therapy procedures (e.g. host conditioning, toxicity/immunogenicity of the delivery vehicles etc.)
- To expand the therapeutic potential of ATMPs to multifactorial and acquired diseases (including viral and chronic diseases), and enable early therapeutic intervention
- To engineer cells/tissues in order to impart new biological therapeutic functions
- To develop novel business models to foster translation of ATMPs and increase its sustainability

2.2.5 Expected key deliverables for 4-5 years

Safety and immunogenicity of gene and cell therapy

- Development of more efficient, safer and less immunogenic gene delivery vehicles
- Harmonized tools and assays to monitor efficiency, safety and immune responses to gene and cell therapy products
- Improved protocols of cell isolation, culture and expansion for both gene and cell therapy applications
- Predictive models of immune response and safety of gene transfer/editing

New clinical applications

- Biomarkers to identify responders vs. non-responders in clinical trials
- Pre-clinical PoCs and phase I/II clinical trials for diseases not currently treated with standard GT approaches
- Novel disease models

Costs

- Establishment of expert-consensus guidelines concerning the standardization of manufacturing/QC/release/GMP/toxicity studies of current gene therapy products to speed up clinical translation of ATMPs
- New academic facilities for ATMP development

2.3 Cell, tissue and organ replacement

2.3.1 State of the art

- Human pluripotent stem cell (hPSC)-derived cell- and tissue- products have therapeutic potential to treat a variety of diseases and first clinical studies have been initiated to test feasibility, safety and efficacy.
- The most advanced indications are Parkinson's disease, retinopathies, type I diabetes, myocardial infarction/heart failure, skeletal muscle disorders, paraplegia and several immunological or oncological diseases.
- The development of treatment strategies for such complex acute or degenerative human diseases has proven to be challenging based on existing animal models, making it difficult to devise and define the exact drug candidates, i.e. the definite phenotype of the target cells to be administered.
- Consequently, existing differentiation protocols are still evolving and have not reached maturity and consensus in many cases. Cell line-dependent as well as inter-experimental variability are not fully understood and still require major attention.
- Therefore, most studies have been initiated based on single cell lines and allogeneic transplantation settings, which allows for better control of the manufacturing process but implies extensive immunosuppression in patients.
- Many ongoing studies promise to deliver the desired proof of concept in man, thereby triggering the community to address the urgent challenges facing the industrialization of induced pluripotent stem cell (iPSC)-derived cell manufacturing
- Several companies in the US have taken the lead, Japan and China have started investigator-initiated trials – Europe is currently lagging behind.
- Tissue engineering products (TEP) were already approved for human use in Europe for treating cornea (Holoclar) and cartilage (Spherox), but two products were already withdrawn from the market (ChondroCelect and MACI).

2.3.2 Major challenges and roadblocks to be addressed

- What makes a cellular therapy successful? Among the most important points are: Medical need, efficacy, durability, affordability/competitiveness, scalability. Some of these characteristics also outline the major challenges currently faced by cellular therapies.
- While the medical need is well documented for a number of potential applications of iPSC-derived cells, the efficacy is often difficult to predict. One reason is a still poor understanding of the underlying cause(s) of a disease. Unlike monogenic diseases, many indications for iPSC-based therapies are idiopathic, i.e. the causative mechanisms are not fully understood, some include autoimmunity aspects and most of them develop over many years before they become apparent.
- A second challenge towards efficacy and durability of a cell therapy is the appropriate delivery and required functional integration of the transplanted cells into the host environment. There is a need to balance the plasticity of a cell with its maturity (i.e. which stage of maturation is best for transplantation), which is again often poorly understood – also because of missing adequate (animal) models.
- Furthermore, the route of administration is often unclear, including the questions of whether cells should be transplanted as single cells, as clumps, in scaffolds, encapsulated or even as printed organ-like structures / organs, and what tooling is necessary for delivery. Likewise, it is unclear how should cells be matured and administered or engineered to efficiently reach broadly distributed points of action, such as in Alzheimer's disease, or electrophysiological and mechanical coupling, such as in heart muscle repair. Finally, also early stage markers indicating success or failure of treatment are often missing.

- There is still no consensus on how transplant rejection should be avoided: by immunosuppression, by tolerance-induction, by cell engineering or by using matched or autologous cells.
- In terms of affordability of a cellular product, challenges are mainly related to the manufacturing process. Due to the complexity of a cell as compared to small molecules or biologicals, the manufacturing process needs to be highly reproducible, including all incoming materials and reagents. And as cells need be produced in large quantities / batch size to treat widespread diseases with allogeneic cells, scalability of the manufacturing process is a must for success. When considering matched or autologous scenarios, an out- or up-scalable process needs to be in place to serve many patients in parallel. Though autologous cells would be the gold standard (in case of no known genetic predisposition), they are currently more difficult to be adopted because manufacturing costs are prohibitively high.
- Finally, challenges are also posed by regulations. While the interaction with regulatory bodies is typically very supportive, standards are varying between different European countries hampering cross-border collaborations and studies. This concerns preclinical data as well as manufacturing procedures, release criteria for cells and scaffolds and medical devices. While some countries have more relaxed requirements for phase I trials, others have standards comparable to those for marketed products, making it challenging to initiate trials in a competitive manner on an international level.

To summarize, tissue engineered and composite products have difficulties in reaching the market by the high development and production cost, the complexity of the technologies, difficulties in manufacturing processes and regulatory barriers.

2.3.3 Overall Goals

To develop technologies and strategies for the production, administration and functional integration of cell therapy products enabling safe, functional, effective, affordable cell- and organ-replacement therapies from both adult and from pluripotent stem cells including tissue engineering for cell replacement to cure major widespread diseases caused by selective cell or organ loss and to demonstrate safety in phase 1 trials and efficacy in phase 2 trials.

2.3.4 Scope- Where can RESTORE make a difference

Long-term vision (8-10 years):

- RESTORE offers the unique possibility to set up a network of cross-sectoral and multidisciplinary teams, which follow an expertise-driven, consolidated vision enabling ground-breaking and sustained progress in cell therapies for widespread diseases.
- Only 10 years ago we learned how to reprogram somatic cells into pluripotent stem cells. In the past, it typically took about 20 years to translate revolutionary findings into clinical practice. Thus, in ten years from now, we will envision that:
    - cellular approaches are routinely used to substitute damaged (or exhausted) cells or tissues
    - cells are reprogrammed *in vivo* to substitute neighbouring cells
    - whole organs will be fully or partially substituted by *in vitro* generated, pre-organized mixtures of cells resembling functional entities.
- We suggest that the outlined goals will be achieved in three parallel but highly interactive tracks, gathering groups and projects to work on:
    - Track a) the optimization of current cell replacement strategies and tools. The results will build the bases for track b) and c) but also have a central role on their own for therapies where grafting of single or few cell types in defined and accessible regions are deemed sufficient e.g. in treatment areas aiming at replacing dopaminergic neurons, beta cells, hepatocytes, chondrocytes, cardiomyocytes and skeletal muscle cells.

    - Track b) *in vivo* reprogramming and trophic support & modulation of cells to substitute or support locally damaged cells or prevent/counteract the induction of senescence in response to treatment and inflammation. Track b) will build on the results of track a) in terms of efficient directed differentiation, but will also have its very own challenges in directing vehicles to the right cells and delivering the correct payload to achieve targeted reprogramming or correct integration/differentiation/proliferation of transplanted/manipulated/endogenous cells. Vehicles will be "master" products applicable by minor manipulation such as viral vectors, or engineered cells or even biomaterial scaffolds.
    - Track c) *in vitro* generation of organs or organoids to reconstitute functional units and types of diseases which cannot be addressed by one or few cell types and need to have a functional organization before being transplanted (e.g. kidney, heart, liver, pancreas).
- In addition, there will be an overreaching organizational level that reflects clinical procedures and regulatory issues and preparing and steering the selection of indications and clinical phase 1 trials (clinical track). People will be recruited from the groups of the three tracks.

Short-term (next 3-6 years):

- In line with our long-term vision and starting from multiple indications we will define five indications where medical need, scientific understanding, technical solution and commercial viability are best (e.g. neurodegenerative diseases, such as Parkinson's, Huntington's or Alzheimer's disease, developmental mental disabilities, diabetes, kidney, liver, skin-, cardiac-, bladder-, bone-, cartilage diseases, incontinence, local muscular dystrophies, cutaneous wounds, fibrosis, neonatal and chronic lung disease).
- For the five selected indications we will have a sufficient medical understanding of the disease and the functional requirements to restore or substitute affected cell types and organs. This includes a good understanding of "failure" of natural endogenous regeneration to identify therapy targets and patients.
- Robust protocols for manufacturing of cells, tissues and / or organs will be available for the above-mentioned diseases
- *In vitro* tissue engineered and composite products that can restore tissue function upon implantation in specific organs
- "Master" products applicable by minor manipulation for specific medical indications will be defined and developed (like Microglia, MSCs, PLX, Tregs, EV-derived cells etc.) including delivery of growth factors for the treatment of chronic diseases (e.g. in ophthalmology and dermatology)
- Strategies for off-the-shelf products (immunogenicity issue) as addressed in WG "Manufacturing: pluripotent stem cells" will be implemented

2.3.5 Expected key deliverables for 4-5 years

- Track a)
    - Fully automated, GMP-compliant process, combining short-term iPSC expansion (see WG "Manufacturing: pluripotent stem cells") and PSC differentiation to 10E10 cells per batch, further up- and out-scalable
    - GMP batch of cells for phase 1 trial in five therapy indications
    - Safety switch enabling controlled removal of transplanted cells on demand
    - Superior, organ-specific delivery tools and engraftment strategies
- Track b)
    - Payload (transcription factors, growth factors, enzymes etc.) for treatment of selected disease defined
    - Vehicles and delivery route defined
- Track c)

  - Biomaterials for composite advanced therapy product approaches available. This includes electro-conductive or bioactive biomaterials compliant with the ECM to overcome endogenous regeneration impairment (skin regeneration, including diabetic foot ulcer, CNS spinal cord injury, including reconnection of axons and promotion of remyelination, coupled with external electromagnetic stimulation, cartilage and bone regeneration)
  - Master templates with a repertoire of selectable material-tissue-cell combinations to treat many tissue/organs, including decellularized and repopulated tissues
  - Functional (physiologically responsive) small size constructs for well-defined patient groups with communal diseases and congenital disorders in new-borns
  - Standardized manufacturing procedures to generate functional tissues to repair organs (skin, cartilage, cornea/retina, cardiac patch, burns, chronic wounds, DMLA/Glaucoma, cardiac and skeletal muscle, auxiliary kidney tissue to compensate loss of function to keep patients out of dialysis, brain microtissue to repair traumatic brain injury and prevent neurodegeneration etc.). Set up in stages starting from pre-vascularized functional constructs, to simple organ, developed to clinical grade standard to regulatory approved platform for 3D tissue generation
  - Development of master templates with a repertoire of selectable materials-tissue-cell combinations to treat many tissue/organs
  - Regulatory approved platform for 3D tissue printing
  - New composite products that avoid rejection (growth factors + biomaterials)
  - Universal scaffold for advanced therapies approved by FDA/EMA

- Clinical track:
  - Recognized sites with manufacturing permission for at least five different cell therapy products
  - Study outline for clinical phase 1 trials for at least five diseases in at least five European countries
  - Standardized Tools for therapy response monitoring (PK/PD, safety, MoA, imaging markers, surrogate)
  - Harmonized animal models for five indications

2.4 Clinical applications and early product development – Cancer

2.4.1 State of the art

- cancer immunotherapy is a breakthrough approach of this decade
- complete and durable responses in some, late-stage patients, but majority of cancer patients is refractory or relapsing to such approaches
- approval of checkpoint inhibitors for different cancer types, including solid tumours
- tumour-specific, in particular neoantigen-specific T cells are key effectors
    - patient-specific immune response
- approval of two gene engineered cell therapies for haematological B cell malignancies: chimeric antigen receptor (CAR) T cells recognizing CD19
    - personalized, living drug

- further CAR T cell approaches and other immune cell based therapies are in the centre of intensive development efforts
    - e.g. TCR engineered T cells and tumour infiltrating lymphocytes (TILs), as well as NK, NKT or g/d T cell approaches towards allogeneic, off-the shelf products and many combination options, like with oncolytic virotherapy or vaccination

2.4.2 Major challenges and roadblocks to be addressed

- Limited understanding of immuno-oncology mechanisms
    - refractory or relapsing versus responding patients
    - critical design elements and principles of cellular products, processes and treatment
        - e.g. role of key transcription factors and key chromatin related factors
    - certain side-effects
- Efficacy, especially for solid tumour
    - Targets are not available and/or not sufficient
        - Limited number of good targets with high specificity, selectivity for tumour available
        - Neoantigen target space difficult to resolve and utilize for cellular immunotherapy
        - Antigen escape mechanisms leading to relapse, incl. antigen/epitope downregulation (or MHC downregulation for TCRs)
    - Function
        - Limited persistence/memory formation and exhaustion of T cells leading to relapse
        - Insufficient extravasation and trafficking of CAR T cells to the tumour
        - Insufficient infiltration of T cells, immunosuppressive environment
        - *for in vivo viral therapy/gene eng: limited targeting capacity and immune responses*
- Safety
    - On target / off tumour side-effects
    - Immunotoxicity of cells and treatment (including conditioning)
        - e.g. cytokine release syndrome, neurotoxicity, infections
- *Generation and availability of cell product (WG manufacturing. Somatic and gene-modified cells, WG implementation of new Advanced Therapies into clinical routine)*
- *Fast and easy translation of innovation (WG regulatory. Science and early health technology assessment)*
    - *incl. lack of means for assessing success at early stages and in a quantitative manner*
    - *or lack of standardisation and difficulties in comparing data across centres*
- *Broad application of cell products and therapy (WG implementation of new Advanced Therapies into clinical routine)*

- *incl. high cost of CAR T cell therapy, which may not be affordable to wide application by health care systems, and reimbursement models*

2.4.3 Overall Goals

- *Restore Europe to be a competitive place for innovation of advanced therapies*
- Better cell products and immunotherapies for cancer through quality by evidence-based design
- *Enable broad application of these complex products for patient care*
- Science-based product/therapy design and efficient personalized patient stratification based on integration of omics and clinical data
- Enhance efficacy, especially for solid cancer
- Reduce side-effects of cells and treatment
  - incl. synthetic biology-based safety regulators using autonomous or physician prescribed intervention to temporary decrease the harmful effects and logical-gated cells to increase cancer selectivity
- *Improve availability and delivery of cell products*
- Develop NextGen cell products and therapy strategies
  - patient-specific versus off the shelf, combination therapies

2.4.4 Scope- Where can RESTORE make a difference

Short-term (next 3-5 years):

Develop a European pipeline for novel advanced therapy products, especially for solid cancer and identify efficient biomarkers/ stratification strategies to select for optimal therapies and to monitor on a personalized way the response

- High-dimension System Analysis of immuno-oncology mechanisms in pre-clinical models, cell products and patients
  - incl. *in silico* tumour models
  - non-invasively track and quantify CAR-T biodistribution in the clinic
  - considering differences in paediatric and adult patients, type of cancer and genotype
- to identify target/candidate mechanisms for improved cellular function and combination therapies
  - incl. delineate the role of key transcription factors and chromatin related factors
- to identify efficient biomarkers/ stratification strategies to select for optimal therapies and to monitor on a personalized way the response
- Discover new targets on cancer and/or microenvironment for CAR, TCR or KAR based approaches, especially for solid tumours
  - including tools to explore and utilize neoantigen space.
  - based on large/strong network for *in silico* analysis
  - increased selectivity for cancer signature molecules based on synbio information processing platform
- Development of strategies for multi-antigen targeting to prevent antigen escape, as well as microenvironment targeting approaches
  - incl. combinations of MHC-dependent and -independent approaches
  - incl. multiplex / multifunctional targeting constructs that can also target immunosuppressive cells in solid tumours

- Development of systems for temporal and/or spatial control of cell function
    - incl. preclinical testing of these systems *in vitro* and *in vivo*
        - tissue engineered models for solid tumours and organotypic slices made from human tumours
        - new types of safety switches that will enable suppression of the CRS while maintaining memory T cells
- Development of strategies for improved engraftment
- Improve cell therapy product generation and delivery
- Feasibility / Comparison of patient-specific versus off the shelf product options
    - incl. identify the best cell subpopulation and source for product generation, like CAR T cells
    - Develop a just in time production pipeline for neo-epitope-specific, patient-derived TCR / T cell product production
    - Test autologous, allogeneic or universal iPSC-derived immuno-oncology products, iPSC-CAR-T, -CAR-NK or -DC
    - Develop universal or customized therapeutic off the shelf cells to increase affordability and enable rapid therapeutic application, e.g. based on NK, iNKT or gamma delta T cells
- Develop a pipeline of promising, systemic approaches for combination therapies with advanced cell products
    - incl. systemic oncolytic virotherapies or vaccination.

Success of cellular immunotherapies is determined by a multitude of influencing factors and thus needs a "system view". Therefore, although some of the separate aspects are also addressed by other grant programs, RESTORE would be a unique opportunity to integrate these important pieces towards the

Long-term vision (10 years)

- Superior, gene engineered cell products and combination therapies for cellular immunotherapy of solid tumours
    - utilizing the neoantigen space
    - with optimised and extended function (beyond specificity)
    - targeting and/or modifying the microenvironment
    - with high health-economic impact
- Decentralized production units and network for cellular therapies against cancer in Europe
- Biomarker analysis platform for optimal selection and adaption of therapies of solid tumours ("driver-assistant-system")

3. Preclinical Models and Technology (New Targets and New Indications)

Given the heterogeneous and complex nature of advanced therapies medicinal products (ATMPs), including cell-based therapies (e.g. somatic cell therapies, pluripotent stem cell (PSC)-derived therapies, and adoptive CAR-T cell immunotherapies), *in vivo* gene therapies and tissue-engineered products, assessing their safety and preclinical efficacy can pose some unique challenges. In this context, standard preclinical and toxicology programmes that are requested to support the clinical translation of conventional drugs (e.g. small molecules and biologics) may not apply to ATMPs. Instead a science-driven, risk-based approach has been suggested and endorsed by regulators as more appropriate to help defining product-specific concerns (e.g. immunogenicity, tumourigenicity, toxicity, efficacy/potency, dose/dosing schedule etc.) and relevant strategies to assess them. A major question that is often raised is whether data obtained from *in vivo* models are relevant and predictive of human responses. Indeed, assessing a human-derived product containing living cells in an animal species can present major limitations including the likely induction of xenogeneic immune responses to the human cells, potential differences in human versus animal cell behaviour, lack of human disease-specific microenvironment, as well as differences in the biodistribution profile. Several strategies have been implemented to address these challenges including the use of animal models with specific features (e.g. immunodeficient animals, animal models of disease/injury, humanised models, zebrafish models etc.) as well as the use of animal-derived equivalent products presenting the same futures of the human counterpart. In addition, there has been an increasing urge to develop innovative *in vitro* and *ex vivo* technologies to replace and/or complement *in vivo* systems while providing robust platforms to address specific questions. In this space, the development of human microphysiological systems (MPS) has been suggested as a promising approach to help overcoming some of the key limitations of *in vivo* models.

3.1 Preclinical model systems: *in vitro* and *in vivo*

3.1.1 State of the Art

Current preclinical *in vivo* and *in vitro* model systems for the evaluation of human advanced therapies, such as cell and gene therapy for chronic disorders, are poorly predictive. Laboratory animals are systemic organisms but do not reflect the pathophysiology of disease occurring in patients. The phylogenetic distance between animals and humans is a factor, which causes differences in crucial biological pathways. The different size and metabolic rate of the animals compared to humans is another relevant factor, as they influence the speed and extent of repair for the tissues affected. Moreover, xenogeneic transplantation of human cells into animal models requires immunosuppression, which impacts on organ function and bias experimental results.
On the other hand, conventional *in vitro* assays based on human cells, 3D models or organoids from diseased or healthy tissues do not reflect the systemic organ arrangement of a human being.

In vivo model systems

Due to a lack of alternatives, small and large preclinical animal models with indication-specific fit-for-purpose are still in use nowadays for informed decision-making by pharma, biotech and vaccine industries along the development cycle of advanced therapies. Furthermore, laboratory animal models are in use to satisfy regulatory and in particular safety requirements for the initiation of clinical trials in man for each new type of advanced therapy in a product-specific context of use. Due to ethical considerations, the 3R principles have been implemented in the European Union since 2009 and strengthened legally in 2013 by a ban on animal testing for cosmetic product authorization. These principles require the reduction, refinement and replacement (3Rs) of animal testing. Therefore, any

further improvement of *in vivo* models for the development and authorization of advanced therapies within RESTORE is solely focused on the implementation of two of the 3R principles: refinement and reduction of relevant animal models. These approaches provide room to optimise a given best-in-class small or large animal model for a selected advanced therapy product development purpose or an advanced therapy product-specific authorization of clinical trials. One possible ethically acceptable refinement approach, for example, might be the improvement of the humanization status of a given animal model to increase the power of prediction by using maximum the same numbers of animals, currently used per therapy assessment. Another approach might be the link with veterinary research institutions and an increased use of the pet population for advanced therapy studies, for example, on the capacity of regeneration of cell constructs.

In vitro model systems

Human microphysiological systems (MPS) are microfluidic devices capable of emulating human biology *in vitro* at the smallest biologically acceptable scale. The application of pulsatile physiological fluid flow is used for human-like nutrition of the tissues and organoids. The creation of microenvironmental biomolecular gradients and relevant mechanical cues, for example, shear stress, is another major aspect of these systems, differentiating them from the conventional static cell and tissue cultures. Therefore, MPS are generating human-relevant data by emulating human, instead of animal biology, on a chip at a systemic level. Due to these features, novel preclinical models based on such MPS aim to produce data currently generated in Phase 1 and 2 clinical trials on patients. Consequently, MPS may replace animal models in the two categories of use in the future:

1) Indication-specific decision-making fitting an advanced therapy product development purpose, and
2) advanced therapy product-specific regulatory authorization of clinical trials.

The first microfluidic MPS entered the academic scene more than a decade ago. Since that time, the scientific landscape has changed dramatically, with an increasing output of more than a thousand papers generated by about 360 research groups in 2018. Simultaneously, a vibrant supplier industry has been established (Zhang *et al*. Lab Chip 2017). Finally, the first qualified assays, such as the human bone marrow assay to assess lineage-specific toxicity for internal decision-making, have been adopted by the pharmaceutical industry.

Human MPS can be broken down into three distinct types: Single-organ systems, multi-organ systems and “body-on-a-chip” systems (Marx *et al*. ALTEX 2016). Single-organ systems are plates or chips emulating single tissue or organ function. They are designed to improve the predictability of aspects of safety and efficacy testing of a drug candidate on a heterologous single tissue or organ model level at early stages of development. Single-organ systems are also of major interest to generate “cancer on the dish” models. Multi-organ systems emulate systemic interactions of two or more organ models within one system to enable the evaluation of an adverse outcome pathway and mode of action data generation from their crosstalk. By contrast, body-on-a-chip systems are envisioned to mimic the physiological interaction of a minimal number of organs capable of emulating holistic organismal functionality. Multi-organ and body-on-a-chip systems have the potential to provide fully functional human model systems to predict the potency, efficacy and safety of the therapies advanced within the RESTORE programme.

Comparison of *in vivo* and *in vitro* models applied within RESTORE

The novel MPS-based models to be developed within RESTORE will be selected to fit defined industrial decision-making and authorization purposes for the advanced therapy products developed in the relevant WPs of RESTORE. Simultaneously, the best-in-class animal model in use to fit the defined purpose for each advanced therapy product selected by RESTORE will be refined according to the 3R principles. The resulting MPS-based *in vitro* model and the respective animal model will

then be used to evaluate the specific advanced therapy product. Each of the resulting MPS-based and animal datasets will finally be separately compared with the outcome of the respective phase 2 clinical trial. This strategy will enable direct comparison of the power of prediction of the *in vitro* and *in vivo* model for the same purpose.

3.1.2 Major challenges and roadblocks to be addressed

- Emulation of organismal homeostasis (body-on-a-chip models)
  - Blood perfusion
  - Integration of resident and systemic immune competence
  - Innervation of organ models, where fitting the purpose
  - Acquisition of a relevant functionality and multi-organ effects
  - Implementation of online monitoring tools for tissue live imaging
  - Identification of a pool of markers for specific monitoring of cell fate, activity etc. as commented in WG gene, cell and tissue replacement strategies
  - Implementation of histology and immune histology analysis in addition to the on-chip trials.
  - Exploration of optimized low sample volume micro assay platforms, such as Luminex, ELISA, ELISPOT, FACS for daily in-process controls
  - Integration of a relevant number of autologous organ models into *in vivo* data, especially in the field of organ replacement where a comparison with animal models is required
  - Capillary vascularization of organ equivalents
  - Implementation of enterohepatic circulation

- Creation and development of chronic disease models
  - De novo induction/integration into healthy body-on-a-chip models (tumour models including (oligo)(micro) metastatic cancer, autoimmune diseases, diabetes, organ transplant models, e.g. GvHD, 'aged' body-on-a-chip, inflammatory models, neurodegenerative diseases, cigarette smoke-induced lung disorders and wound repair)
  - Genetically encoded disease modelling using patient-based cells and tissues or patient-derived IPSC to create a patient-specific body-on-a-chip model carrying the disease gene(s)
  - Genetically engineered disease models, for example, disease gene KOs created by CRISPR and matched against non-KO body-on-a-chip controls

- Establishment of assay formats
  - Advanced therapy delivery route, mode and frequency
  - Engraftment mode and route
  - Product quantification tools
  - Product-specific potency, efficacy and safety readouts
  - Assay qualification and data reporting
  - Large scale production of required cells for the assays and high throughput-compatible screening capabilities

- Automation and data management
  - Automated facilities, robots including online imaging modalities and bedside labs to ensure a throughput of on-chip clinical trials of the size of phase 2 clinical trials in humans
  - Data mining, standardization, processing and computational modelling
  - Patient data integration and protection mechanisms
  - Informed decision-making exploring machine learning/artificial intelligence
  - Microphysiological System-based networks and their organisation in externally monitored, controllable and autonomous molecular, cellular and organismal communication systems
  - Disease-mimicking networks

3.1.3 Overall Goals

Establish and prove 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.
Refine laboratory animal models, which are in use to the RESTORE-defined indications and their respective regulatory contexts of use to generate data with higher predictive power (e.g. small or large size animal models with similar to human pathologies and/or with mechanisms of repair/regeneration congruent with humans) and/or with a smaller number of animals used.

3.1.4 Scope – Where can RESTORE make a difference

WG Preclinical model systems aims to enable health through advanced therapies by introducing a cutting-edge "patient-on-a-chip" platform leading to a paradigm shift in drug development. The development speed for new therapies and drugs will increase by a factor of two, whilst the development costs are supposed to decrease fivefold, making the advanced therapies accessible and beneficial for each patient. WG Preclinical model systems supports RESTORE in two stages: mid- and long-term.

<u>Mid-term targets (next four to seven years)</u>

- Create autologous miniaturized single or multi-organ chip models emulating aspects of selected chronic pathologies for fit-for-purpose advanced therapy testing, such as:
  - human on-chip "solid tumour-in-an-organ" models, multi-organ "chain" models to emulate metastatic tumour invasion into distant organs and tumour mimicking immunocompetent primary and metastatic tumour microenvironments, including an organ-specific vascularized connective tissue bed for the purpose of immunotherapy treatments based on T cells and NK-cells.
  - innate and adoptive on-chip immune response, for example, for the purpose of evaluating transplantation therapy testing monitored by secretion of inflammatory cytokines (IFNg, TNFa), T cell proliferation or humoral immunoglobulin responses
  - chronic kidney disease on-a-chip with the purpose of potency testing for the regenerative potential of kidney precursor cells
  - organ damage and reconstruction/regeneration on-a-chip, for example, for the purpose of protecting or repairing traumatic or neurodegenerative brain injury
  - inflammation of joints on-a-chip to emulate aspects of juvenile Idiopathic arthritis and rheumatoid arthritis fitting the purpose to understand the survival niche of resident proinflammatory antigen-specific T cells. As part of this: cartilage on a chip to study cartilage pathology in genetic disorders
  - human neurodevelopmental disease models on-a-chip using genetically engineered neural progenitors and their differentiated neuronal offspring and patient-derived material for advanced therapy testing for autism and/or schizophrenia
  - larger scale human single organ bioreactor models for purposes which need a relevant experimental size of the human damaged organ counterpart, for example, cartilage/bone or heart muscle implants
  - skeletal muscle on a chip to study muscle disorders and to develop therapeutic options
  - *ex vivo* models such as a vascularised chorioallantoic membrane on a chip
- Refine established small and/or large animal models, with the aim of improving their predictive power and/or reducing the number of animals used in such models. A number of approaches such as accounting for the experience of the adaptive immunity, comparison to patient samples, using more than one read out method or providing control parameters to replace control might flank the refinement efforts.

Long-term goals (seven to ten years):

- Engineer chip platforms enabling the long-term maintenance of individual "patient-on-a-chip" equivalents, their exposure to best-in-class RESTORE therapies and their monitoring and data processing at clinical trial phase 2 throughput
- Create autologous miniaturized "patient-on-a-chip" equivalents emulating a selected chronic pathology (immune-oncology, autoimmunity, diabetes, transplant rejection, including graft versus host disease

(GvHD), chronic wounds, cardiovascular toxicity, cardiac diseases, such as heart failure, and diabetic cardiac disease or other fields) taking gender and age issues into account
- Align the chip exposure regimen with patients' real exposure regimen based on Quantitative *In vitro* to *In vivo* Extrapolation (QIVIVE)
- Involve an artificially intelligent "PI" next to the clinical trial PI for monitoring and decision-making
- Prove the concept in a head-to-head role model case study with a RESTORE patient cohort and the best-in-class RESTORE animal model for the selected context of use, for example, immune-oncology, CAR T cell therapy

3.1.5 Expected Key deliverables for 4-5 years

Deliverables over four to seven years will be defined for the following objectives by the adaptation of existing multi-organ chip tools to selected purposes for advanced therapies, expedited in RESTORE:
- patient-derived tumours on-a-chip
- integration of tumour organoids into vascularized chip organ models in their natural connective tissue bed,
- multi-organ "chain" models to emulate metastatic tumour invasion into distant organs
- integration of naïve and adapted immunity on-a-chip
- on-chip immune response
- muscle on a chip
- cartilage on a chip
- Vascularised tissue beds on a chip
- tissue ageing and degeneration on-a-chip, for example, neurodegeneration in Alzheimer's and Parkinson's diseases, heart muscle maturation as well as (disease-specific) degeneration
- tissue transplantation models on-a-chip, including GvHD
- potency testing for selected indications

Impact statement:

Reaching the WP6 long-term goals of the RESTORE programme will have a paradigm-shifting impact particularly on advanced therapy development and on the entire drug development life cycle in general. The development speed for new therapies and drugs will increase by a factor of two, whilst the development costs are supposed to decrease fivefold, making the advanced therapies affordable and accessible for every patient.

In addition, at least one MPS-based disease model will be validated at a regulatory authorization level using the drug development tool pathway of the FDA for voluntary validation. Its predictive power will be compared to that of the best-in-class animal model for the respective product authorization purpose.

During the execution of the RESTORE programme, the WP6 mid-term deliverables will have an initial impact on the current advanced therapy landscape. Some novel solutions and tools will be established in the following areas:
- Development of a platform for the real-time measurement of biomolecules (e.g. by surface plasmon resonance) in-line with the microfluidic MPS
- Improvement of gene-editing technologies to establish disease models or evaluate immune escape mechanisms
- Organ regeneration mechanisms, including using decellularized matrices and 3D printing tools
- Optical imaging readouts from the blood flow and the interstitial part of the organs
- Inclusion of microbiome to enable research in gut, lung and skin models

These developments will allow the predictive assessment of particularly the safety and potency aspect of the advanced therapies already at the single-/multi-organ MPS level.

Furthermore, a number of MPS-based models and assays will be established at this level as qualified scientific methods for industrial decision-making along the advanced therapy product development cycle. Those data can already become part of the IND/IMPD submissions for advanced therapies in four to seven years.

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4. Manufacturing Technologies (Including Product Characterisation and Automation)

4.1 Manufacturing – Somatic and gene-modified cells

4.1.1 State of the art

- 4 gene-modified cell products in EU with market authorisation – Strimvelis, Kymriah, Yescarta and Zalmoxis. First three are autologous and Zalmoxis is allogeneic (on a 1 batch/patient basis).
- One non-gene modified product with MA – Alofisel, expanded human allogeneic mesenchymal adult stem cell.
- Multiple auto and allo products in clinical trials
- Most production processes are performed using manual, operator dependent and poorly controlled procedures. Miltenyi Prodigy system available for closed, automated processing of autologous therapies. Some users use a combination of Prodigy and other system in order to increase manufacturing flexibility
- Allogeneic products (e.g. MSC and MSC-like cells) can be produced in large batches, but currently there is a gap in supporting downstream automated, controlled and large-scale technologies.
- Cellular products mostly rely on end-of-process release testing and have limited in-process controls

4.1.2 Major challenges and roadblocks to be addressed

- Limited EU-wide harmonization of manufacturing and quality requirements
- Limited automated and scalable manufacturing technologies
    - Manufacturing processes are manual and operator-depended
    - Heterogeneity in cell product (variability of cell culture conditions) due to poor control over the process
    - Platforms are small in scale and have poor integration with other units of operations in the process
    - No solutions for industrial scale multi-marker cell isolation / selection technologies
    - Limited solutions for downstream processes
    - Limited GMP solutions for next generation gene-edited cell therapies
- Limited information on product quality during the process and up to the patient
    - Limited assays to determine product quality during the process
    - Limited tools for online, in-lime and at-line sterile and automated sampling
    - Limited on-line capability to analyze process data in computerized systems
    - Include a phrase such as: Lack of mechanistic understanding of product potency
    - Limited ability to connect process performance and patient outcome
- Limited supply chain and logistics capabilities
    - Raw material (cells, cytokines, plasmid DNA, synthetic nucleotide analogues, viral vectors transfection reagents etc) quality not sufficient for clinical use
    - Undefined raw materials
    - Limited ability to test incoming raw materials
    - Limited availability of suitable raw materials
    - Limited storage and shipment solutions for cellular products

4.1.3 Overall Goals

1. Enable Europe to be a competitive place for innovation of advanced therapies manufacturing for fast and easy translation into clinical evaluation and up to product approval
2. Enable the broad application of cell products for patient care by developing manufacturing processes for good, potent and affordable cellular products accompanied by innovative methods for timely delivery of cellular products from and to the patients
3. Establish a comprehensive analysis platform from starting material, manufacturing process, cell product to patient treatment for efficient iterative improvements in cell-based products

4.1.4 Scope – Where can RESTORE make a difference

Long-term vision (8-10 years):

- Develop "complete solution" platforms that are fully automated, controlled and GMP compliant in order to enable the production of different cell types, in different scales for different indications
- Develop analytical tools that can provide surrogate monitoring of critical quality attributes and could be used online or with short response time in order to enable the fast delivery of cellular products to patients
- Increase process control and product understanding by the development of AI systems that can connect process data (from raw materials to process performance and up to patient outcome) and effect the control and continuous improvement of the process.
- Develop self-adaptive bioprocesses using forward predictive control
- Develop agile technologies that will support next-generation large scale gene edited cellular products manufacturing
- Establish quality control standards for cellular products w/o gene modification and a battery of proposed tests to ensure comparability across locations and over time. All focused on the main claim of the functionality (MoA) of the cellular product
- Develop tools and technologies that allow for temperature-controlled storage in any location (i.e. not only in specialized Cryo-hubs) and fast shipment of cellular products for any location in the EU.

Short-term vision (next 3-5 years)

- Increase the understanding regarding the effects of raw materials and supply chain on the potency and reproducibility of different cellular products
- Promoting the definition of a standard for the quality of raw materials intended for clinical use
- Increase the understanding on process parameters effects on the potency and reproducibility of different cellular products
    - Development of AI tools and application to enable the modelling and correlation of process effects on product outcome
    - Smart cGMP, stability and scalability and correlation to patient's response
    - Development of smart monitoring tools that could detect and correct process/product deviation in real time and so increase process repeatability
    - Using multi-omics analysis to provide a more accurate view of cell physiology
- Develop different units of operation that could be connected to one another (i.e. large-scale purification, cell expansion, concentration and wash) and will allow continuous manufacturing by closing automation gaps existing in cellular products manufacturing
- Develop process-relevant scaled-down tools allowing the generation of high numbers of good quality data that could be used for rapid process understanding and at the same time provide high volumes of data
- Develop application that will support the leap for next generation products by integrating gene modification process to known large- and small-scale cellular product manufacturing

- Increase the understanding on the effect of supply chain parameters on the potency and stability of different cellular products
    - Development of temperature-controlled storage and shipment tools and infrastructure, suitable for clinical sites, in order to maximise space and protect the quality of cellular products in the supply chain
    - Develop tools and formulations to increase cellular product storage stability

4.2. Manufacturing – Tissue engineered and composite products

4.2.1 State of the art

- Range of synthetic, natural and decellularized biomaterials usable for tissue engineered and composite products
- Advanced *in vitro* and *ex vivo* culture systems (chorioallantoic membrane, bioreactors, embedded sensors for chemo-mechanical online monitoring, embedded micro-nano actuators for physical-chemical stimulation)
- Tools for advanced therapies:
    - Cells for autologous and for allogeneic therapies (MSC, MSC-like cells, Placenta, Umbilical cord, PSCs, Treg, Mreg, insulin-producing cells, iPSCs);
    - Cell-derivatives (extracellular vesicles/exosomes), secretome;
    - Combined products (scaffold + biologics);
    - Combined products (cells + scaffold + biologics);
    - *In vivo* gene therapy (delivery of genes, growth factors)
    - Materials with specific biological effects
- Current indications: skin, cartilage, bone, muscle, nucleus/spine, cornea, vascular grafts, oesophagus, trachea, bladder, neurological lesions and cardiovascular constructs like engineered heart tissues and myocardial/epi-cardial patches (with indications of ischemic diseases and heart failure), beta cell replacement
- Many trials with MSC/MSC-like cells but also other approaches (iPSCs, fetal- or placenta-derived MSCs, Treg, Teff, EV, gene therapy) but a limited number of products available for the clinical application (except in cartilage and nucleus)
- Intelligent polymers for endogenous tissue restoration (ETR)
- Advanced fabrication procedures (bio-printing, scaffold manufacturing, organoid and its combinations)
- Externally controllable and fully autonomous therapeutic and sensing organoids and associated interfaces, including but not being limited to optogenetics and radiofrequency-mediated genetics

4.2.2 Major challenges and roadblocks to be addressed

- Source of starting biomaterial (natural, synthetic, decellularized matrix)
- Engineered biomaterials for the design of artificial stem cells niches
- The approval process for any novel biomaterial or modifications of any existing approved biomaterial takes substantial time and frequently fail in clinical trials
- Xenogeneic decellularized matrix and the associated safety issues
- Reproducibility of the starting material independently of the source and potency
- Definition of “master” products applicable with few modifications for many indications (like heart valves, vessels, cartilage, bone, muscle, spine, brain injury, neurodegenerative diseases, engineered myocardium)
- Immunomodulatory properties, potential or induction of materials
- Development of composite tissue scaffold (e.g. bone-cartilage-bone) and tissue gradient and transition zones
- Capability and capacity to fabricate functioning and perfused soft human tissue in large scale
- Implant/transplant-integration of product into recipient-site
- Universal biomaterials (applicable for most tissues)
- Biomaterials for specific roles such as non-viral gene therapies
- Biomaterials safety and quality control strategies implemented by design
- Target structure (2D, 3D, specific morphologies copying main signals of the ECM)

- In process control and standardized biomaterial tests for manufacturing
- Stability/kinetics and safety of biomaterials biodegradation process
- In-depth characterization of master products, enabling safety definition and QC specifications
- Scale up / automation / scale out and undertaking product comparability studies
- Personalized medicine approaches (patient-specific geometry/size/cells and impact of ageing)
- Monitoring upon implantation (e.g.: to visualize remodelling over time, and ensure continuous functionality)
- Decrease immunogenicity/inflammatory/foreign body reaction and increased biocompatibility initially and throughout material integration and degradation processes
- Immune-active biomaterials eliciting positive immune responses, strategies in immune-engineering using pure biomaterial based strategies
- "Off-the-shelf" concepts and cell-free concepts
- Translation of the results from animal to human, also by preclinical multi-centre trials for efficacy
- Regulatory-hurdles: sometimes two quality guidelines relevant for one product type (e.g. ISO 13485, ISO10993, and EU-GMP)
- Risk-benefit analysis when failure of the product can lead to the death of the patient (e.g.: cardiovascular products)
- Patient-to-patient variation (autologous concept)
- Stability (storage/transport) of final product
- Aseptic production process (GMP – class A in B)
- Logistic/Shipping
- Packaging and terminal "sterilization" of the final product
- Development of "GMP" devices such as 3D printers, stereo-lithography, melt electrowriter
- "Growing" composite products grafted in paediatric patients
- Development of generalized integrated in-silico models for effective regeneration of tissue and organs
- Development of effective and generalized design techniques for 'master' products and components (multi-functional biomaterials, optimized scaffolds etc.)
- Develop strategies to reduce the cost of ATMPs without compromising its efficacy

4.2.3 Overall Goals

To develop safe and highly effective approaches to support tissue and organ regeneration for the pre-emptive or curative treatment of chronic diseases with high health-economic impact

4.2.4 Scope – Where can RESTORE make a difference

Long-term vision (8-10 years):

- Off-the-shelf EMA and FDA approved biomaterial scaffolds able to be applied in many clinical conditions
- Develop technology enabling to produce in hours, personalized scaffolds for advanced therapies particularly for trauma care (intra operation treatment: no GMP required)
- Automated and certified technology for autologous cell isolation and expansion (design of closed system: lower GMP requirements)
- Automated technology for cell seeding in both off-the-shelf and also in personalized therapies
- EMA approved bio-printing technology enabling to manufacture centimetre-scale advanced therapies ready for implantation in patients
- Certified and off-the-shelf cells (allogeneic, placenta-derived) for universal advanced therapy products
- Workflow for clinical image-to-product (3D printing, casting…) cellularised soft tissues.
- Multi-cue materials (e.g. architecture, mechanics, GFs, cells) that provide an optimal environment for endogenous regenerative processes in a space- and time-specific manner

Short-term vision (next 3-5 years):

- Definition of minimal functional requirements for successful advanced therapies (Quality by Design concept)
- Establish protocols to validate delivery, survival, tracing and if needed recovery of tissue engineering and composite products
- A better understanding of "failure" of advanced therapy products in patients (e.g. using in-silico-models)
- Feedback from the advanced therapies already in the clinic
- Definition and development of "master" products applicable by minor manipulation for defined medical indications
- Strategy for the design and GMP manufacturing off-the-shelf products (immunogenicity issue -> foetal cells/gene edited iPSCs)
- Smart and standardised (SOPs) manufacturing of master cell and composite products
- Decellularization/recellularization strategies
- Generation of bioinks from decellularised organs
- Potency markers and identity markers relevant for the mode-of-action and potency of the master tissue engineering and composite products
- Biomaterials, bioinks and scaffolds for composite advanced therapy products
- Standardized tools for therapy response monitoring
- GMP-manufacturing of synthetic materials and also being compliant to bio-printing technology
- Development of generalized design, modelling and fabrication techniques of composite and multifunctional master smart tissues
- Development of surface coatings for multi-functional biomaterials (i.e. for delivery of genes and growth-factors, surface modification of scaffolds)
- Development of tissue engineering and composite products as disease models for drug efficacy and safety screening as well as studies of the pathogenesis.
- To prepare the regulatory framework for prospective application

4.2.5 Expected key deliverables for the next 4-5 years

- IT strategy to standardize manufacturing of ATMPs
- Smart cGMP, stability and scalability and correlation to patient's response
- Engineering a synthetic stem cell niche
- TRL up to Clinical PoC trials with x (e.g. 10) next-generation tissue engineering and composite products
- PoC of applied master tissue engineering and composite products
- Potency markers for tissue engineering and composite products
- Development of 5 tissue engineering and composite products for 3 defined applications (e.g. bone, tendon, cartilage)
- Bio-printing of fully functional implantable tissues ready for clinical trials
- Definition of design priorities for synthetic materials on the macro- and microscale as well as the control of the time-dependency behaviour of a resorbable scaffold

4.3 Manufacturing – *in vivo* gene therapy and gene editing

4.3.1 State of the art

- ***In vivo* genetic engineering** holds potential to tackle a broad spectrum of inherited and acquired diseases. Genetic engineering includes:
  - gene therapy: direct delivery of a gene therapy medicinal product to patients either *in situ* in anatomically defined locations or systemically to reach organs or tissues such as (but not limited to) the central nervous system (CNS), peripheral nervous system (PNS), liver, muscles and lungs
  - gene editing: precise gene correction that allows silencing, activating or recoding loci of interest in the genome, also exploiting base editors
  - tumour cell elimination: genetic engineering to enhance the effectiveness of oncolytic vectors.
- The focus of RESTORE is on genetic engineering for therapy or prevention of disease (no genetic enhancement) and limited to modification of somatic cells (no germline) for functional repair or for sustained and regulated expression of therapeutic gene products.
- Potential areas of intervention: genetic diseases, by adding functional genes or replacing dysfunctional genes, correcting or disrupting mutated disease-causing genes through pre-natal, post-natal or adult intervention; endogenous regeneration by delivering factors for *in vivo* tissue protection/engineering; cancer, by direct/indirect tumour cell elimination (*but not the primary focus of WG Manufacturing: in vivo gene therapy*).
- Commercial products: Luxturna and Zolgensma
- There are >300 clinical trials testing *in vivo* genetic engineering, the vast majority rely on gene addition, only two trials are based on gene editing strategies so far.
- In terms of delivery vehicles, viral vectors are the most used (e.g. AAV, LV, oncolytic vectors) while non-viral vectors (e.g. nanoparticles), or vector-free approaches (RNA, proteins) are currently at an earlier stage of development.

4.3.2 Major challenges and roadblocks to be addressed

- Efficacy
  - Delivery efficiency of non-viral vs viral-mediated approaches
  - Cargo capacity as an impediment to the delivery of large payloads, such as large HDR donors or large or complex transgenes, such as photoreceptors, ion channels or multi-subunit enzymes
  - Effect of aging on delivery efficiency, tissue response and therapeutic efficacy
  - Suboptimal control of expression of the gene product(s) in time (onset and persistence) and space (biodistribution of vector and/or transgenes; cell type/stage of cell differentiation, i.e. progenitors vs. differentiated cells/tissue specificity)
  - Limited efficiency of gene editing methods
  - Limitation of current animal models/human systems to measure/predict efficacy
  - Oncolytic viruses: choice of the most appropriate transgenes and viruses for the selected tumours; limited indirect (immunological) effects on tumour cells (checkpoints, lack of TCR specificities…)
  - Limited efficiency in crossing the Blood Brain Barrier

- Safety

  - Acute and long-term toxicity related to the delivery system (e.g. genomic integration), to the transgene (expression/overexpression), to other components of the GT product
  - Effects on the biology of the target cells
  - Off-target effects related to both vector load and limited specificity and off–target expression of the transgene or gene editing machinery
  - Unclear danger, limited detection of gene editing-mediated off-target effects or integration of persistent episomal DNA

- Immunogenicity: innate or adaptive immune responses to any of the components of the gene therapy product may impact in different ways on the efficacy and safety of the approach (e.g. potential for re-administration) – **potential interaction with WG Manufacturing: *ex vivo* gene delivery/editing, WG endogenous regeneration**

- Manufacturing **- partial overlap with WG Manufacturing: *ex vivo* gene delivery/editing**
  - Scaling-up and standardize pre-GMP/GMP manufacturing (low yield vs. high costs)
  - Reduce release time and cost related to post-production quality control
  - Control of all steps of production up to fill and finish, supply chain/stability
  - Medical devices for delivery

4.3.3 Overall Goals

To develop safe, highly effective, and sustainable (feasibility and costs) approaches for *in vivo* gene therapy/gene editing to be able to: i) become standard of care for rare/orphan diseases, including those with pre- or perinatal onset (urgent medical need) and ii) broaden the range of possible applications for the curative treatment of multisystemic/multifactorial diseases and acute/chronic tissue degeneration (CNS, PNS, liver, muscles, lungs) and cancer (high health-economic impact).

4.3.4 Scope- Where can RESTORE make a difference

Long-term vision (8-10 years):

- To develop "once-in-a-lifetime", approaches to cure genetic and/or degenerative diseases that currently have unsatisfactory/no therapeutic option and/or to modify the disease course of fatal diseases with high unmet medical need
- To generate new and optimize current *in vivo* gene therapy/gene editing platforms that could be applied to several diseases, including rare diseases or those with higher prevalence
- To reduce manufacturing costs to make these therapies more available for patients and public Health Systems
- To reshape undesired immune response related to gene transfer by exploiting targeted immune modulatory approaches thus avoiding/reducing chronic immunosuppression
- To support tissue repair/regeneration by transient/stable expression of protective /differentiation factors either as a stand-alone approach (e.g degenerative diseases) or in concomitance/after correction of the main pathology (e.g. genetic diseases)

- Short-term (next 3-5 years):
- Identify the most appropriate *in vivo* delivery system(s) and route of administration for the desired application (gene therapy vs. gene editing) and indication
- Improve the efficiency of gene editing methods
- Improve the efficiency/safety of non-viral delivery approaches (e.g. nanoparticles for the delivery of the gene editing machinery)
- Optimize delivery system design in order to improve vector/transgene biodistribution, specific cell/tissue tropism, control of gene expression and reduction of off-target effects, decrease immunogenicity of vectors/Cas proteins (escape of innate and adaptive immunity)

- Develop novel (multi-scale hybrid) in-silico models of non-viral delivery for efficiency and safety evaluation and optimization
- Establish harmonised efficacy and safety standards and assays (e.g. for T-cell responses, for detecting off-target effects in gene-edited cells) and clinically relevant biomarkers validated for response to therapy
- Standardize and enhance tools/models (including human 3D tissue models and organoids) to measure/predict efficacy, evaluate/minimize acute and long-term toxicity and effect on target cell biology
- Develop and validate new synthetic biology tools aimed at regulating selected gene expression based on e.g. liquid phase separation of transcription factors, combinatorial targeting to decrease off-target effects, small molecule, optogenetics, and ultrasound-mediated targeting
- Develop safe and robust immune modulatory approaches to control immune response related to *in vivo* genetic engineering
- Scaling-up manufacturing of vectors (production, purification, stability) and analytics of products (bioreactors, costs of GMP-grade goods, cell line engineering to increase productivity and decrease costs)
- Smart cGMP, stability and scalability and correlation to patient's response

4.3.5 Expected Key deliverables for 4-5 years

- Pre-clinical PoC completed for 6-8 AT products
- Phase I/II Clinical trials for 3-4 new AT products
- *In vitro* and *in vivo* models that allow the measurement/standardization of the new AT approaches.
- Placements of academic staff/students with industrial partners
- Training seminars or workshops on ATMP aspects (technological, regulatory, patent-landscape, legal) organised by RESTORE (including by remote attendance of live transmission)
- Establishment of expert-consensus guidelines concerning manufacturing, efficacy and safety assessment of ATMP for *in vivo* genetic engineering, to be implemented in conjunction with manufacturing European Vector Cores and companies, with the additional value of generating new patents of European origin.
- Coordination and sharing of resources/expertise of a team of experts for each target system/organ/disease of interest

4.4 Manufacturing – Pluripotent stem cells and adult stem cells

4.4.1 State of the Art

During the last 10 years several major technological breakthroughs have fundamentally changed the field of cell therapy. These include the reprogramming technology to induce pluripotent stem cells (iPSC) from somatic cells, advanced gene editing technologies such as CRISPR/Cas9 and the ability to generate complex, tissue-like organoids combining different sources and types of stem- and progenitor- cells. These technologies have already generated a major stimulus for translational research in personalized medicine and pharmacology. Early clinical trials have been started for treating retinal disorders, heart failure, diabetes and Parkinson's disease, initially using human embryonic stem cells (hESC) and more recently iPSC-derived progenies. The latter eliminates the ethical issues raised by utilizing hESCs and clinical trials have started as early as 2013 in Japan for treating age-related macular degeneration using autologous iPSC-derived retinal pigment epithelial cells followed by allogeneic RPE. More recently cell therapy protocols have been initiated by injection of allogeneic iPSC-derived mesenchymal progenies (MSCs) for treating graft-versus-host-disease (GVHD) and iPSC-derived dopaminergic progenitors for Parkinson's disease. First-in-man clinical trials using allogeneic clinical grade iPSC lines are planned in the near future in France for cancer immunotherapy and for chronic ischemic heart disease and in Italy and other countries for beta cell replacement in type 1 diabetes.

Several challenges remain for the extended use of these cells in the clinic, especially regulatory compliant industrial cell manufacturing, robust starting material characterization, development of reproducible differentiation protocols to generate standardized ATMPs in cancer and chronic diseases and also the development of potency tests.

RESTORE presents a unique opportunity to build a competitive European infrastructure through international collaboration to support innovation in next generation advanced cell therapies from an accessible global cGMP iPSC bank in Europe. Disruptive projects will address identified blocks to improve the production of innovative cell therapies using iPSCs. European standards for best practice in iPSCs and derivatives manufacturing, expansion, differentiation, storage and shipping poised to have a big impact on European iPSC research and healthcare.

4.4.2 Major challenges and roadblocks to be addressed

Pluripotent stem cells:

- Define European rules for consent forms and data access of donated patient material for iPSCs generation; how to deal with information of the genome (QC) of the donated material?
- What patent issues do we have to face when using specific reprogramming factors/vectors/methods?
- cGMP manufacturing process for safe iPSCs generation, expansion and differentiation with less variability through robust automation, use of closed platforms with increasing in process control.
- Define safe iPSCs and iPSC-derived products through aligned characterization specifications: accurate and rapid quality control assays to reduce release time and controlled costs.
- Need harmonized quality controls and European and International Standards
- Standardization of differentiation and cell maturation
- Risk of Immunogenicity: Immune rejection (allogeneic ATMPs), autoimmunity (autologous ATMPs)

- Needs methods to assess the safety for regulatory authorities and patients: Assessing residual pluripotent stem cells in PSC-derived products and the tumourigenicity potential of cell-based therapies, with the ultimate goal to improve their safe application into the clinic. Exploring biological impact and cell transformation linked to genomic alterations and induced off targets after genome editing.
- Engraftment & homing issues
- Scaling-up production strategies for clinical trials (large vs. small scale)

Adult and neonatal stem cells:

- Expansion and self-renewal strategies remains a challenge for immunotherapy, multilineage hematopoietic engraftment from adult HSCs and for gene therapy using adult stem cells and committed progenitors in allogeneic or autologous context.
- What are the lessons from clinical trials using adult MSCs (link WG Endogenous regeneration) from different source of tissue, different potential and clinical context (autologous vs. allogeneic).
- Need harmonization standards and predictive value of existing tools and technologies to improve the clinical applications.
- Use neonatal stem cells as higher proliferative potential and relevant models for characterization of the developmental stage of iPSC-derived and foetal cells.

4.4.3 Overall Goals

The global aim is to accelerate the delivery and implementation of novel ATMPs from iPSCs or adult stem cells in cancer, injury and chronic diseases by promoting interactions between academic organizations and private companies. The major mission is to bridge the gap between scientific discovery (proof of concept) and its early translation into medicines and market.
The development of functional and safe ATMPs from adult stem cells and iPSCs (derived cells and organoids) will be focused on defined unmet medical needs (link WG "Endogenous regeneration", WG "Gene, cell and tissue replacement" and WG "Cancer") around following medical axes:

- Immune intervention strategies:
  - Induction of tolerance: Production of regulatory immune populations from the same iPSC-Master Cell Banks: Tregs, iMSCs, etc. Boosting cell function by drugs, antibodies. Developing small molecules targeting innate and adaptive arms of the patient's immune system (link with WG endogenous regeneration). Design scaffold protecting the cell graft from immune-recognition (link with WG Manufacturing: Tissue engineered & composite products)
  - Breaking tolerance: Production of engineered universal immune cells for adoptive immunotherapy and onco-vaccine strategies (CAR-T, NK, dendritic cells)

- Tissue replacement and repairing strategies in organ failure:
  - Repopulation of stem cells / differentiated depleted cells: Production of stem cell/ cells / organoid (link with WG "Endogenous regeneration", WG "Gene, cell and tissue replacement" and WG "Cancer"), methods of delivery and implementation (scaffold)

  - Correction of genetic defect for replacement therapies.

Specific aims:

To define the best cell source (i.e. adult stem cell vs. iPSCs, autologous / allogeneic, haplo-identical super-donors, universal) and "living biological drug products" regarding each clinical indication, expected biological action and stage of the disease (preventive vs curative).
To develop tools and techniques for process improvement, identify defects, minimize variability, understand and manage risk where possible.
To establish early position on manufacturing and regulatory processes yielding harmonized international quality standards.

4.4.4 Scope – Where can RESTORE make a difference

Long-term vision (8-10 years)

The ability to address new disruptive concepts regarding biological systems of interest: Introduction of new techniques from methods and equipment in other sectors (WG engineered-biomaterials, micro-manufacturing techniques, 3D bio-printing, bioreactors, sensors)

The ability to transfer the technologies developed in multimodal pilot units on pharmaceutical manufacturing sites for industrialization.

The ability to promote standardized scalable ATMPs from an accessible European bank of allogenic cGMP iPSC lines. To promote clinical trials with iPSC-derived cells using universal donors for a large market access high demand.

The ability to generate robust, reproducible, well-scalable protocols for transplantable iPSC-derived organoids / tissues and for *in vitro* analytic pre-clinical models and stem cells biology evaluation (link WG "Preclinical model systems")
The ability to use autologous iPSCs for clinical use for some clinical indication (chronic disease using small amount of cells)

The ability to prepare the regulatory environment for smoother application.

Short-term (next 3-5 years).

The ability to identify and select normal HLA homozygous donors for a large bank of very well characterized cells and informative registry data in order to generate iPSC lines.

The ability to produce 'super-donor' iPSCs that cover the majority of patients in Europe by combining different areas in European and worldwide countries. To generate universal and safe iPSC lines invisible to the immune system and with cell death-switch capacity.

The ability to assess the immunogenicity, tumourigenicity, and to control the differentiation characteristics and potential of each IPSC in several lineages.

The ability to validate harmonized assays, international standards and to obtain a global regulation of the products: to implement international standards maximizing global acceptability, leading to industry access to global markets with patient health benefiting from these next advanced therapies.

The ability to obtain long term self-renewal procedure in a safe (regulatory acceptable) manner

4.4.5 Expected Key deliverables for 4-5 years

ACCESS TO EUROPEAN IPSC LINES FOR CLINICAL USE

- Robust, reliable supply chain and database for safe clinical grade iPSC lines in Europe manufactured from a matched donor with their specifications and compliance according to internationally accepted quality criteria (GAIT). World-wide logistic distribution to private and public organizations for clinical use (link to WG Implementation of new Advanced Therapies into clinical routine) including ethical, regulatory and medico-economic aspects.
- Universal engineered iPSC line to evade the immune radar:
    - Focus on gene editing strategies of safe iPSC lines or progenitor populations with suitable tools (AAV recombination, Zn fingers, CRISPR/Cas9 approaches- link WG Manufacturing: *in vivo* gene therapy, WG Manufacturing: *ex vivo* gene delivery/editing).
    - Focus on different type of immune silent cells for different purposes (HLA II null for example macrophages, HLA I null for platelets and diabetes, ..) and suicide gene insertion to remove undifferentiated residual IPSCs in the final product and to kill as safety mechanism the cell therapy product *in vivo*
- Gene editing strategies to improve functionality of final cell product. What would be regulatory view on genetic insertion in the final cell therapy product?
- Robust automation and improved reprogramming process
- Comparability assays between iPSC lines vs. passages, vs. Master Cell Banks, vs. batch products: key critical quality attributes (CQAs), International standards and suitable assays.
- Robust assays for starting material characterization including differentiation propensity (into various cell types) of each iPSC line of interest. Assays in process controls and product release.

IPSC-DERIVED-ATMPS:

- Robust and reproducible directed differentiation procedures (homogenous cells / organoids) for pre-clinical models (WG “Preclinical model systems: *In vitro* and *in vivo*”) and clinical use: improve differentiation yield and maturation, developmental status / comparison with adult cells/ tissue (open source epigenetic, genomic proteomic, transcriptome from bulk and single cell databases). New methods of organoid generation and expansion from adult stem cells and iPSC-derived committed progenitors: to improve purification and expansion techniques, identification and isolation of initiating organoid committed progenitors (multiple potential). New methods for the generation of complex tissues using multiple precursors from different lineages in a specific organ/tissue.
- Design engineered iPSC-derived cells with inducible biological functions, produce. New living drug products and combination therapies.
- Implement early regulatory processes, ensuring the safety and efficiency of iPSC-derived cells and organoids, in order to facilitate their market development through reimbursement policies in Europe for the benefit of patients
- Suitability standards for analytical tests, QC of major steps of differentiation and expansion,
- Potency assays evaluating functionality, mode of action and reducing variability of the products and to identify and define "surrogate" markers of function.
- Identify cells (donor, iPSCs and final products) with potential safety risks: Genetic burden (WES / single cell RNA sequencing, gene arrays CNV) and novative signature and algorithm to interpret the massive genomic data (i.e. mutation / expression of oncogene, losing function of key genes in cell and tissue of interest) with their biological impact. Sensitive tumour assays (Organoids, Animal Models WG “Preclinical model systems: *In vitro* and *in vivo*”), Residual undifferentiated PSC- suicide gene strategies. Immunogenicity assays: Humanized mouse models/ Organoid systems (WG preclinical model systems),
- Strategies improving engraftment/ specific homing and biodistribution.

BIOPRODUCTION PROCESS OF iPSC AND ATMPs

- Cost effective full automation strategies with multi-layered workflows for cGMP iPSC derivation (from donor to safe iPSC selection, banking, data EU registry and distribution) and iPSC expansion (Master cell Banks/ data registry of homogenous qualified batches link to initial iPSC stock and to the product).
- Incorporation of automated processing for flexible Scale-out/ Scale-up strategies cGMP iPSC Bank stock (and research iPSCs from same cGMP batch), Master-cell bank size (diverse dose requirements for different targets).
- 2D Culture vs. 3D bioreactors using 3D artificial microenvironment design for stem cell fate control, autologous vs. allogeneic products (adult stem cells, iPSCs).
- Product comparability at different scales and methods (2D/3D) of production and across sites
- Robust and reproducible cell culture system: improve the bio-production processes with closed cell system culture & GMP-compliant digital tracking of all process equipment to reduce batch-to-batch variability. Producing a-cellular products from iPSC derivatives or adult stem cells with defined biological potential. Improving expansion, differentiation, cryochain of storage and supply, shipment condition.
- Hardware/Software systems and data analysis (WG Big Data/AI) to improve rationalization, bioengineering yields and standardization: upstream screening test, biological digital sensors, imaging, digitization, computerization, data mining, e-learning, bioinformatics and algorithms.
- Scalable procedure for ''Master" iPSC-derivative products (cells/ organoids) applicable for many indications: concept of a universal product for a broad type of disease or one cell/ intermediate progenitors ("pre-product") with multi-faceted functionality after small modification.
- Methods to detect genetic instability and possible cell transformation early post-treatment

4.5 Ex vivo gene delivery and editing

4.5.1 State of the Art

Ex vivo gene therapy enables precise and cost-effective payload delivery to target cells. The concepts and technologies for *ex vivo* gene therapy are based on decades of experience in immunotherapy and bone marrow transplantation. Today, *ex vivo* gene therapy is routinely used for gene modification of lymphocytes or hematopoietic stem cells with applications in cancer immunotherapy and hematopoietic gene therapy. The first *ex vivo* ATMPs have reached the market. Strimvelis and Zalmoxis (respectively a CD34-based cell product to treat a primary immune deficiency and a T cell product to treat GvHD) were approved in Europe in 2016 followed by Kymriah and Yescarta approved in the USA in 2017 and subsequently in European countries (two CD19 CAR-T cell products indicated in B cell leukaemia and lymphoma). Currently, based on clinicaltrials.gov, there are at least 30 ongoing *ex vivo* hematopoietic gene therapy studies and more than 300 ongoing CAR-T cell studies. The majority of the *ex vivo* gene therapy studies are still based on a single gene transfer. However, novel modalities are emerging that rely on genome editing technologies as well as multiplexed approaches combining gene transfer and gene inhibition. In addition, research efforts exist to genetically engineer cell types other than CD34+ cells or T cells, such as gene-modified NK cells, or B cells, as well as other stem cells, such as MSC, for novel ex vivo gene therapies.

In cell-based gene therapy, target human cells are isolated from the patient or from an allogeneic donor and the cells are cultured and genetically modified *ex vivo* through non-viral or viral vector-mediated gene transfer. Success requires the safe administration of the gene in target cells to reproducibly obtain a gene-modified cell product of high quality, the efficient engraftment of the manufactured cells in the patient and the appropriate therapeutic levels of cells in specific tissues.

The current state of the art is to use gammaretroviral and lentiviral vectors as delivery systems for *ex vivo* gene modification of isolated primary cells (Figure 1). The current processes are usually manual, but automated platforms such as the Miltenyi Prodigy, enable cell selection, transduction and expansion in a closed system. However, there are several manufacturing challenges across this workflow that can limit the translation of candidate *ex vivo* products into commercial products.

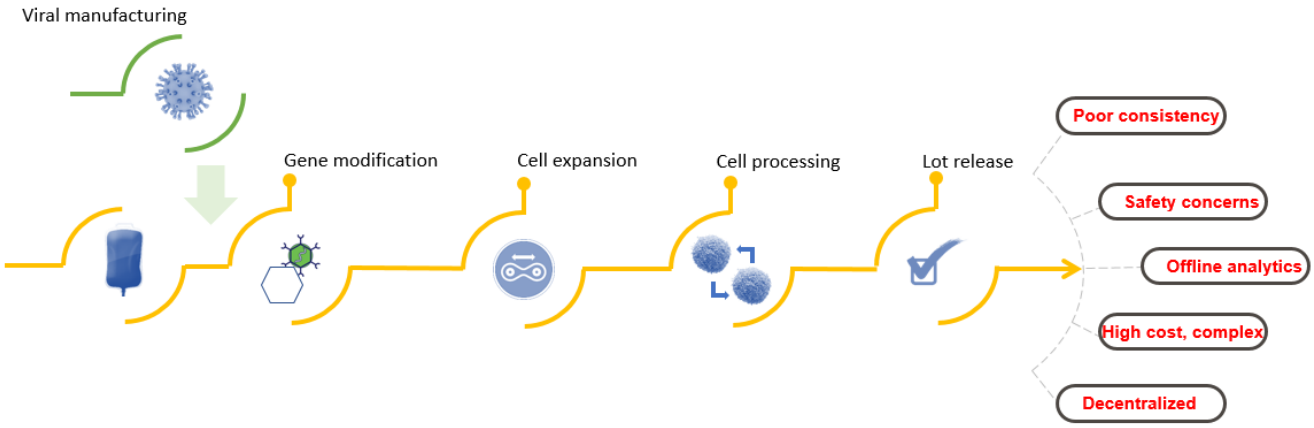

Figure 1: Current state of the art for ex vivo gene modification

4.5.2 Major challenges and roadblocks to be addressed

The high cost, time effort and logistical challenges to manufacture and deliver patient-specific cell therapy products severely limit the number of patients that can be included in clinical trials within a reasonable timeframe. While proof-of-concept could be obtained from relatively small phase I/II studies for monogenic diseases, genetically engineered cell therapies applied to complex diseases

such as cancer require much higher throughput at sustainable costs. This applies to multiplexed approaches where the optimal gene combinations must be tested experimentally in patients.
In parallel, there continues to be steady progress in the advancement of allogeneic cell therapeutics, and these are expected to emerge as commercial ATMP products over the next ten years (Figure 2).

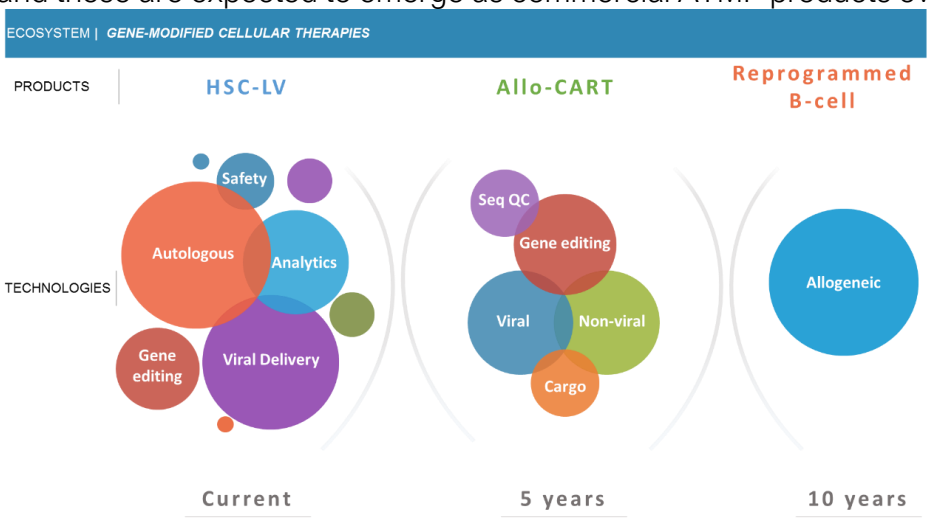

Figure 2: Predicted evolution of gene-modified cellular therapies over the next 10 years

As these therapies progress toward clinical application, establishing robust commercial scale manufacturing solutions will be essential. The greatest challenges facing the translation of *ex vivo* gene therapy at present are the unknowns and emerging technologies such as the fast-developing field of gene-editing. Added to this is the bottleneck of efficient delivery and the lagging auxiliary fields governing manufacturing and analytics on the one hand, and safety and regulatory issues on the other. Broken down into their key elements, these challenges can be addressed under four strategic priority areas concerning standardization, Smart QC, Cargo and Combinatorial (Figure 3).

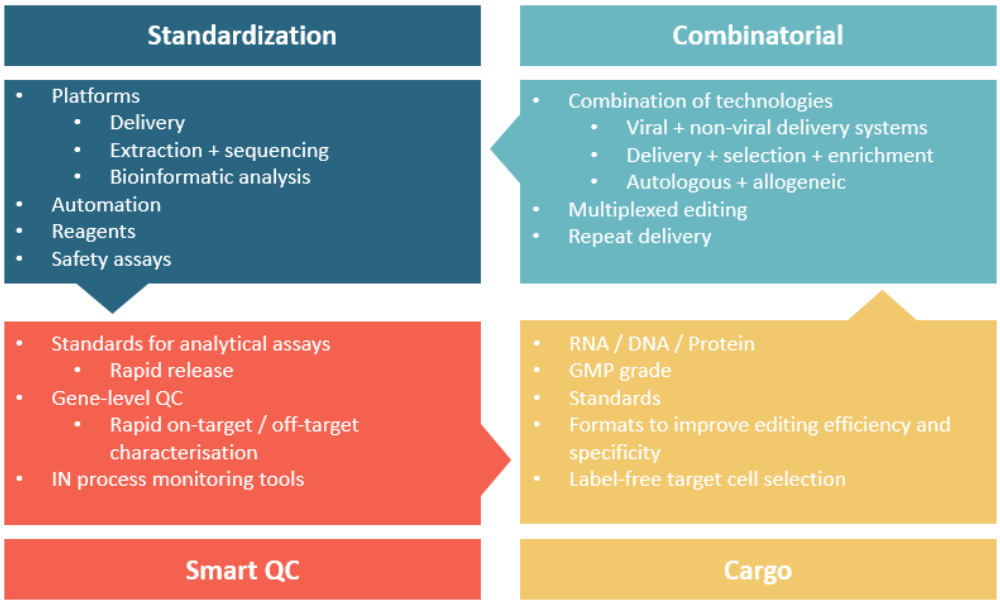

Figure 3: Four strategic priority areas identified as areas where growth is needed to support the scientific and commercial maturation of gene-modified cell therapies.

The next step is to develop a roadmap of the technical milestones and for this the approach has been to expand on the strategic priorities identified within the context of our ten-year vision for gene-modified cell therapies (Figure 4).

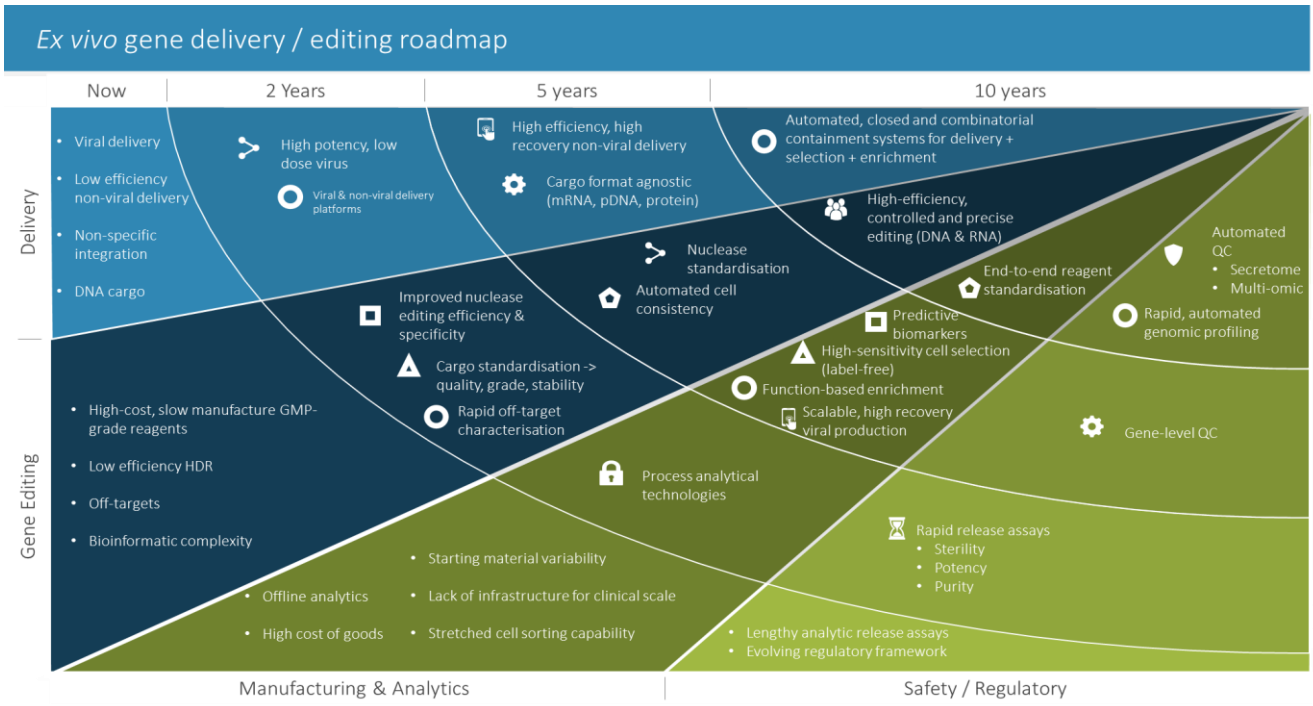

Figure 4: Roadmap capturing key technology initiatives needed to support the next-generation of gene-modified cell therapies

4.5.3 Overall Goals

Our goal will be to develop cost-effective (non-viral platforms) and broadly applicable (allogeneic cell platforms) strategies to overcome the manufacturing limitations currently impacting the translation of candidate ex vivo therapies into commercial products.

4.5.4 Scope – Where can RESTORE make a difference

The overarching deliverable will be the intensification of novel processes and transfer into GMP. This will involve:

- Identification of foundation projects

3-5 YEARS

- Analytical and manufacturing
    - Increase the understanding on the effects of raw materials and supply chain on the quality of cellular products.
        - Define quality standards that will form manufacturing guidance for “master” products (mRNA transfer, CAR-constructs, CRISPR/Cas system…)
        - Improved assays to predict gene transfer; improvement of strong *in silico* analysis platforms
        - Closed system manufacturing
            - Local transduction hubs that operate in a standardized manner
        - Development of smart monitoring tools that could detect process/product deviation in real time and so increase process reputability

- Delivery
    - Optimized LV gene transfer protocols using transduction enhancers and shortened ex vivo culture times
        - Rapid recovery

        - Reproducible and predictable levels of gene transfer
    - Local transduction hubs that operate in a standardized manner
    - Increase the understanding of the benefits of non-viral delivery platforms including mechanism of action for chemical-based approaches.
        - Non-viral delivery systems to support gene editing applications and construct optimization within clinical trial setting

- Gene Editing
    - Achieve therapeutic levels of gene disruption in cells of interest
    - Establish editing approaches of choice (disruption, precise HDR-based repair, base editing, epigenome editing, targeted integration etc.) for major classes of mutations or targets
    - Increase the understanding of cell response and functional consequence (i.e. p53 disruption in HSCs)
    - Optimize targeted integration with a focus on:
        - Cell fitness following ex vivo manipulation
        - Efficiency -> low / oligoclonal populations of gene-corrected cells
    - Develop reliable assays to measure genotoxicity to gene editing procedures (DNA damage, cellular sensing to viral DNA templates and nucleases)
    - Study the overall impact of gene editing procedures on genome stability (nuclease specificity, translocation assays, mutational burden)

- Safety / Regulatory
    - Gene-level QC
    - Development of smart monitoring tools that could detect process/product deviation in real time and so increase process reputability
    - Initial safety data for gene-corrected cells in humans
        - Relevance of off-targets
        - Tailored assays to predict product characteristics

<u>8-10 YEARS</u>

- Analytical and manufacturing
    - Increase manufacturing efficiency and throughput whilst bringing down costs
- Delivery / Gene Editing
    - Integration of *ex vivo* expansion, gene engineering, cell selection and enrichment
    - Targeted epigenome editing
    - Efficient therapeutic translation of NHEJ-based disruption

- Safety / Regulatory
    - Extended test assays for genetically engineered products
        - Identify “at risk” starting material such as clones carrying pro-leukemic mutations
        - Identify patient-specific polymorphisms/states that result in differential efficiency in genetic engineering (i.e. susceptibility to LV transduction or HDR efficiency)
        - Identify patient-specific polymorphisms/states that result in differential response to *ex vivo* culture with the possibility to personalize manufacturing conditions.
        - Personalized patient sequencing